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Dedication

This dissertation is dedication to my parents, Barbara and Dennis Dean, to my husband,
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Abstract

Metabolomics is the study of small molecules (metabolites) <1500 Da. It combines analytical and biochemical techniques to help understand disease pathogenesis. Metabolites also change dynamically in response to disease and hence corresponds closely to host phenotype. They can ultimately present information related to host and disease metabolism. Liquid chromatography tandem mass spectrometry (LCMS/MS) is a technique most used for metabolomics because it is the most sensitive, it has high statistical power, large software availability to analyze data output, and has a high structure annotation confidence. It also can analyze several sample types including tissues, biofluids, and cells. Combining LCMS/MS with a technique called ‘spatial metabolomics or chemical cartography’ can address knowledge gaps in the realm of infectious diseases. Spatial metabolomics/chemical cartography integrates analytical, biochemical, and computational techniques to study metabolites in 3D space. It allows the mapping of metabolites onto 3D organs to determine pathogen and metabolite location, tropism, and distribution. Ultimately, it helps to understand the relationship between local tissue metabolic perturbation and clinical disease symptoms and gives insight to disease pathogenesis. Chagas disease is a neglected tropical disease that is severely understudied and understood, yet affects millions of people worldwide. The influenza virus is one of the most concerning viral based diseases due to its high infection rate. LCMS/MS based spatial metabolomics can be implemented to study these diseases to reveal novel information about how host and disease/disease causing agents interact in the body. In this dissertation, I present fundamental and translational applications of LCMS/MS-based metabolomics in infectious diseases. The data presented here supports the use of spatial metabolomics/chemical cartography in infectious diseases to further understand disease pathogenesis and how infection, whether viral or parasitic, affects host metabolism.

Introduction

Metabolomics is one of the many ‘omics’ that has helped propel the world of science. It is the study of metabolites, which are small molecules usually <1500 Da, using analytical and biochemical techniques [1]. Since metabolites are the products of many metabolic pathways, they are direct markers of changes in biochemical pathways [2]. This can ultimately give insight to host chemical changes upon infection [2]. In addition, metabolites are the final product of mRNA and protein expression, as well as protein activity [3]. They also change dynamically in response to disease [4,5]. As such, metabolites correlate information closely linked to phenotype [6]. These comprehensive aspects of metabolites can provide understanding between host and disease metabolism which could aid in the development of new treatment options for many of the world's less understood diseases.

Metabolomics is not the only ‘omic’ to contribute to the realm of understanding host and disease interaction. Genomics: the study of the genome (all DNA molecules), transcriptomics: the study of the transcriptome (all RNA molecules), and proteomics: the study of the proteome (proteins) has all made significant impacts in the scientific world. The human genome project for example has provided the opportunity for faster diagnoses of several diseases. It has led to advances in technology and medicine such as rapid DNA sequencing which can be used for less invasive gene testing, for specific diseases such as the BRAC1 gene indicative of hereditary breast cancer [7]. Along with the human genome project came the ability to study the RNA present in the human body, the transcriptome [8]. Specifically, RNA-sequencing, which is a rapid tool using high throughput sequencing techniques to help understand a cell’s transcriptome [8,9], has led to several clinical RNA-based tests such as using viral RNA for detection of human immunodeficiency virus (HIV), influenza virus, ebola virus, and dengue virus [9]. Proteomics is

a complementary technique that can target the presence of a particular protein to understand its overall function in metabolism. Additionally, proteins are currently being used as biomarkers for diseases including liver, testicular, and colon cancer [10].

The different ‘omic’ techniques, including metabolomics have all addressed several concerns relating to health and disease. They can all work together integratively or independently to reveal useful information needed to understand host response to disease. Metabolomics, however, does correlate most closely to overall phenotype and has extensive capabilities of understanding overall metabolism which drives the ability for the human body to thrive. Metabolomics has already made an impact in the realm of biomarker discovery, human health and disease, animal health and disease, drug discovery and development, food, and the environment [11,12]. What makes metabolomics so advantageous is its ability to analyze several sample types including tissues, biofluids, soil, water, or fumes, which allows the technique to be used both on *in vivo* and *in vitro*-derived samples [11].

In the context of infectious diseases, metabolomics has shown the ability to discriminate between patients with active tuberculosis (TB) and those without TB in a pilot study [13]. Specifically, metabolites found in the plasma such as resolvins and *Mtb*-derived glycolipids were increased in TB positive patients and have biomarker potential [13]. Metabolomics has also been applicable to other infectious diseases such as influenza virus for understanding disease progression and response, respiratory syncytial virus for disease pathogenesis and biomarker discovery, *Pseudomonas aeruginosa* infection for biomarker of disease severity and novel therapeutic design, and *Aspergillus fumigatus* for biomarker discovery [14–17].

Due to the diversity of metabolomics and its applications, a wide range of instruments are used, which allows the technique to be utilized in multiple ways based on specific sample type.

The main types of instrumental analysis used for metabolomics are nuclear magnetic resonance (NMR) and mass spectrometry (MS) based techniques. NMR can analyze all sample types and utilizes a magnet to determine metabolite structure based on its nuclear spin interaction.

Unfortunately, NMR is not as sensitive as MS-based techniques, which is why MS techniques are far more commonly used for metabolomics. However, NMR can elicit information such as structure information more readily and accurately than MS techniques, especially with regards to stereochemistry, which is not usually able to be elucidated by MS.

MS has emerged as one of the most utilized and prime techniques used in metabolomics, due to its strong molecular identification capabilities and high detection sensitivity. MS instruments used commonly to analyze these samples include gas chromatography mass spectrometry (GCMS), matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI), secondary ion mass spectrometry (SIMS), desorption electrospray ionization (DESI) MSI and, liquid chromatography mass spectrometry (LCMS). These techniques mostly do not preserve samples but are indeed the most sensitive when analyzing metabolite presence. GCMS analyzes gases or liquid-based solutions which are heated through a capillary tube at high temperatures and volatilized. While GCMS has high sensitivity, its main disadvantage is that metabolites must be volatile in order to be analyzed. It is thus well suited to gas-phase samples. Non-volatile metabolites need to be derivatized to increase their volatility and enable GCMS analysis.

MALDI-MSI is a technique that uses a laser beam to ionize an analyte co-crystallized with a small molecule matrix. The laser is rastered across the surface to provide spatial information, and the ions developed are detected by a mass analyzer. SIMS utilizes secondary ions developed from a primary beam that bombards a surface to generate ions. These ions are

then sent to a mass spectrometer for analysis. Unfortunately, both MALDI-MS and SIMS have lower sensitivity and lower rates of metabolite detection. DESI-MSI releases a charged solvent onto a sample which desorbs analytes from the surface of a sample to a mass analyzer. This technique preserves the sample and actually does not require sample preparation but does have lower spatial resolution.

LCMS analyzes solutions and unlike GCMS, metabolites do not need to be volatile. Samples are first separated based on polarity via LC, then by mass/charge ratio in the MS. Interestingly, LCMS can detect both polar and less-polar metabolites based on extraction type. Biofluids such as urine, blood, and saliva, tissues and cells can be analyzed by LCMS. Additionally, using liquid chromatography tandem mass spectrometry (LCMS/MS) facilitates increased metabolite identification via metabolite fragmentation patterns. However, this technique is indeed destructive, and samples cannot be used for other analysis. LCMS/MS is the technique that is most popular because it is the most sensitive, it has high statistical power, large software availability to analyze data output, and has a high structure annotation confidence.

The mentioned techniques can be combined with other chemical techniques for further understanding of disease pathogenesis. ‘Spatial Metabolomics’ or ‘chemical cartography’ integrates biochemical, analytical, and computational techniques to study small molecules in 3D space. It allows the mapping of metabolites onto 3D organs to determine pathogen and metabolite location, tropism, and distribution. Ultimately, it helps to understand the relationship between local tissue metabolic perturbation and clinical disease symptoms and gives insight to disease pathogenesis.

LCMS/MS based spatial metabolomics is uniquely positioned to address several gaps in knowledge pertaining to infectious diseases. Combining (LCMS/MS) and spatial metabolomics

enhances the capabilities of metabolomics due to its wide range of capabilities such as the use of different sample usage, and large software analysis and compatibility, in addition to those already mentioned. It has already unlocked a novel pathway to understanding acute Chagas Disease (CD), [18,19] which led to a novel drug treatment for acute CD [19]. In addition, LCMS/MS based metabolomics has the capability to be applied to infectious diseases like leishmaniasis and non-communicable diseases like different forms of cancer which bridges the gap between what is currently known in research and what is still to be discovered [20,21].

Infectious diseases are becoming a worldwide concern due to increased transmission, increased migration, antibiotic and antiviral resistance, and lack of research. Specifically, parasitic and viral based diseases are in need of novel therapeutics and diagnostics. Of the many parasitic diseases, CD, one of several neglected tropical diseases, is in need of further research [22,23]. CD is a vector borne disease transmitted by the triatomine bug (kissing bug) and caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*) [22,23]. It can also be transmitted by blood transfusions, congenital transmission, and infected food and water. CD is endemic to the Americas and infects 7-8 million people worldwide, including 300,000 in the United States due to migration [22–25]. The disease has two stages: acute and chronic. The acute stage is usually asymptomatic but if symptoms are seen, they include rashes and fever. In addition, during the acute stage, parasite load is increased [22,23,26]. The chronic stage of the disease however presents with more severe symptoms including cardiomegaly, cardiac arrhythmias, apical aneurysms, megacolon, and megaesophagus. A minority of patients (20-30%) will progress to the chronic stage of CD however, diagnostic rates are low due to decreased to no parasitemia present in the blood [22,23,26]. Current treatments for CD include benznidazole and nifurtimox. Unfortunately, these treatments cause severe side effects and do not prevent or reverse already

developed cardiac or gastrointestinal tract disease [27,28]. CD disease pathogenesis is not fully understood [18,19,23,29]. It was thought to be an autoimmune disease, but recent research has clearly shown the impact of parasite persistence on disease pathogenesis [30]. Several autoimmune responses contribute to overall damage as a result of *T. cruzi* infection such as cytokine release, CD8⁺ T cell-mediated cytotoxicity, and pro-inflammatory response as well as parasite persistence [18]. Several parasite-host factors play a role in clinical disease outcomes including *T.cruzi* parasite strain, parasite load and tissue tropism, mode of infection and host genetic background [31,32]. A holistic understanding of how parasite persistence and which molecular pathways are involved in disease pathogenesis could lead to novel treatments and diagnostics of CD.

Due to the unique tropism of parasite disturbance in the heart and gastrointestinal tract of infected specimens, spatial metabolomics presents a unique opportunity to study and further understand host and parasite interaction in CD. Using LCMS/MS based spatial metabolomics Hossain *et al*, identified a novel role of metabolism in Chagas Disease (CD) pathogenesis in mice [19]. Specifically, the authors noted specific changes in metabolic perturbation and parasite disturbance in organ specific regions of the gastrointestinal tract. During the acute stage of CD infection, there were significant increases in chemical disturbance in the distal small intestine which also was a site of high parasite load. Interestingly, during the chronic stage of the disease, chemical disturbance was decreased in the small intestine and increased in the esophagus and colon. Increased perturbations in the esophagus and colon during the chronic stage matches clinical symptoms of megacolon and megaesophagus thus indicating a biochemical explanation of the symptoms [19]. Specific chemical families were affected by infection, including acylcarnitines and phosphatidylcholines. Identification of carnitine metabolism specifically

linked to CD led to the implementation of carnitine supplementation treatment in mice during the acute stage. Notably, this reduced cardiac stress in mice infected with *T.cruzi* and decreased metabolic perturbations induced by infection in the heart and plasma [19]. Ultimately, this study was the first to reveal a novel mechanistic understanding of localized CD symptoms and disease tolerance.

Spatial metabolomics has also been implemented to study Alzheimer's disease by identifying decreased gangliosides in the right hemisphere of the brain in mice with Alzheimer's disease when compared to control [33]. In an *in vitro* study of ovarian cancer, it was revealed that norepinephrine is released by the fallopian tubes to the ovary which advances invading cancer cells and encourages metastasis [34]. Spatial metabolomics provides a stepping stone to understanding disease pathogenesis for future drug development and discovery, host-directed therapeutics, prognostics, and diagnostics. Specifically, this can be achieved through LCMS/MS based spatial metabolomics by analyzing cross sectioned *T. cruzi*-infected tissue, blood plasma, saliva, and urine.

Researchers have also become interested in host directed therapeutics to help treat viral based diseases such as the influenza virus (IAV). IAV is a global public health issue due to its ever-increasing infections yearly [35]. There are over 1 billion infections and 300,000-500,000 deaths each year, with significant increases in pandemic years [35]. Vaccines are one of the main preventative methods used but they fail to take into consideration the IAV's large strain diversity. Other issues related to IAV include vaccine uptake and manufacturing limitations. While antivirals are used for treatment of IAV infection, they are most effective in the early stage of the disease and several IAV strains have become drug resistant [36]. Given these concerns, it is evident that there needs to be advances in IAV treatment. In a 2012 study by Louie

et al, it was found that when influenza A(H1N1)pdm09-infected intensive care unit patients, those treated with a neuraminidase inhibitor had increased survival rates compared to untreated [37]. Unfortunately, 1 in 4 treated patients died [37]. This study highlights the importance of not only advances in IAV treatment but also the increased potential of host-directed therapeutics to help supplement antiviral treatment. However, in order for the development of novel IAV treatments, there needs to be a better understanding of its disease pathogenesis which is not completely clarified. Interestingly, the spatial dynamics of IAV infection in the lung allows interpretation between viral spatial distribution and disease severity. Using LCMS/MS based spatial metabolomics, we can anticipate novel findings to understand IAV disease pathogenesis.

This dissertation presents fundamental and translational applications of LCMS/MS-based metabolomics in infectious diseases. Specifically, I present data that supports the use of spatial metabolomics/chemical cartography in infectious diseases as a means to further understand disease pathogenesis and how infection, whether viral or parasitic, affects host metabolism. Each chapter presented here provides novel information regarding CD or IAV infection in the realm of host and disease interaction with hopes to lay a foundation for drug discovery and development, therapeutics, and biomarkers of treatment or disease presence. Ultimately, this dissertation presents a steppingstone to bridging the gap to understanding some of the world's most under-studied or still poorly understood diseases.

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Chapter 1: Spatial metabolomics identifies localized chemical changes in heart tissue during chronic cardiac Chagas Disease

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Allocation of Contribution

This chapter is a published manuscript in the journal PLOS Neglected Tropical Diseases. Sample collection and raw data acquisition was performed by Dr. McCall, Dr. Jair L. Siqueira-Neto, Dr. James H. McKerrow, and Dr. Pieter C. Dorrestein at UCSD. My contribution to this chapter was the analysis of chronic-stage infection samples using statistical and computation software to understand changes in chronic cardiac metabolism. All data analysis, manuscript writing, and figures were developed by me and Gautham, a former McCall lab researcher who is a co-author on this publication. Specifically, I performed random forest analysis and generated figures 1 and 2. Gautham annotated the differential metabolites (SI tables 1-6) and identified acylcarnitines and phosphatidylcholines from the data, which I then used to generate figures 3 and 4. The introduction was written by Gautham, and the results and discussion sections written by me. We both contributed to writing the methods section.

1.1 Abstract

Chagas disease (CD), caused by the parasite *Trypanosoma cruzi*, is one of nineteen neglected tropical diseases. CD is a vector-borne disease transmitted by triatomines, but CD can also be transmitted through blood transfusions, organ transplants, *T. cruzi*-contaminated food and drinks, and congenital transmission. While endemic to the Americas, *T. cruzi* infects 7-8 million people worldwide and can induce severe cardiac symptoms including apical aneurysms, thromboembolisms and arrhythmias during the chronic stage of CD. However, these cardiac clinical manifestations and CD pathogenesis are not fully understood. Using spatial metabolomics (chemical cartography), we sought to understand the localized impact of chronic CD on the cardiac metabolome of mice infected with two divergent *T. cruzi* strains. Our data showed chemical differences in localized cardiac regions upon chronic *T. cruzi* infection, indicating that parasite infection changes the host metabolome at specific sites in chronic CD. These sites were distinct from the sites of highest parasite burden. In addition, we identified acylcarnitines and glycerophosphocholines as discriminatory chemical families within each heart region, comparing infected and uninfected samples. Overall, our study indicated global and positional metabolic differences common to infection with different *T. cruzi* strains and identified select infection-modulated pathways. These results provide further insight into CD pathogenesis and demonstrate the advantage of a systematic spatial perspective to understand infectious disease tropism.

1.2 Introduction

Chagas disease (CD) is a parasitic disease caused by the protozoan *Trypanosoma cruzi* and is one of the designated neglected tropical diseases [1]. *T. cruzi* is endemic to the Americas and infects 7-8 million people worldwide [1]. An estimated 300,000 infections have been recorded in the United States due to a large Latin American immigrant population and endemic transmission [2–4]. CD is primarily transmitted through triatomine insects of the *Triatoma* and *Rhodnius* genera [2,5]. Non-vectorial modes of transmission involve blood transfusion, transplacental transmission, and food and drink contaminated with *T. cruzi* [1]. The *T. cruzi* life cycle includes three main stages: epimastigotes, trypomastigotes and amastigotes. *T. cruzi* in the insect vector undergoes transformation from trypomastigotes to epimastigotes in the midgut, and then migrates to the hindgut and differentiates into infective trypomastigotes [1]. Upon triatomine defecation on the human host, the infective trypomastigotes enter the host through scratching or rubbing of the bite wound, or through eyes and mucosal surfaces [1]. Following mammalian host cell infection, trypomastigotes differentiate into amastigotes, which proliferate and subsequently transform into trypomastigotes [1].

CD has two disease stages: acute and chronic [1,2,5]. The acute stage, with high parasite load, is usually asymptomatic, or presents with non-specific symptoms (fever, malaise) [1,2,5]. A minority of infected individuals (20-30%) will then progressively develop clinical manifestations of chronic CD, including cardiomegaly, cardiac arrhythmias, apical aneurysms, megacolon, and megaesophagus [2]. In contrast to acute stage CD, chronic CD presents with low to no parasitemia [5]. *T. cruzi* infections are treated with either benznidazole or nifurtimox; however, these treatments cause significant adverse effects, to the point that up to 30% of treated individuals fail to complete the full treatment course [6,7].

CD was previously considered to have an autoimmune etiology, but parasite persistence has now conclusively been demonstrated to be required for disease pathogenesis [8]. Along with parasite persistence, chronic pro-inflammatory responses, including cytokine release and CD8+ T cell- mediated cytotoxicity, contribute to tissue damage [9]. A heterogeneity of interacting parasite-host factors, including *T. cruzi* strain, load and tissue tropism, host genetic background, and mode of infection, influence the clinical outcomes of the disease [10,11]. However, CD pathogenesis is not yet completely understood [2]. A holistic understanding of the molecular pathways involved in disease progression could help identify new drug development avenues.

Metabolites are the final products of mRNA and protein expression and of protein activity, thus providing information closely linked to phenotype [12]. Metabolic pathways are druggable. They also change dynamically in response to disease [13,14]. As such, an improved understanding of metabolism in CD may lead to new avenues for drug development and CD patient monitoring. Acute *T. cruzi*-infection affects *in vitro* and *in vivo* host metabolic pathways, including decreasing mitochondrial oxidative phosphorylation-mediated ATP production [9,15–17]. In addition, acutely *T. cruzi*-infected mice heart tissue and plasma showed significant changes in certain metabolic pathways, such as glucose metabolism (glucose levels elevated in heart tissue and lowered in plasma over time), tricarboxylic acid cycle (TCA) (decrease in select TCA metabolites in the heart tissue and a decrease in all detected TCA metabolites in plasma), lipid metabolism (increased long-chain fatty acids in the heart tissue and decreased long-chain fatty acids in plasma), and phospholipid metabolism (high accumulation of phosphocholine precursor metabolites in the heart) [15]. Prior analysis of hearts from acutely infected mice also showed that cardiac metabolite profiles reflected disease severity, with changes in cardiac acylcarnitines and glycerophosphocholines predictive of acute infection outcome [9].

Metabolomic analysis of chronic CD has been limited to serum and gastrointestinal tract samples [18,19]. Serum analysis demonstrated significant changes in amino acid and lipid metabolism, particularly acylcarnitines, sphingolipids, and glycerophospholipids [19]. Analysis of GI tract samples observed persistent metabolic perturbations in the oesophagus and large intestine in chronic CD, including infection-induced elevation of acylcarnitines, phosphatidylcholines and amino acid derivatives [18]. However, metabolic changes in the heart may differ from those in the circulation or in the GI tract [15]. It is therefore essential to perform metabolomic analysis of tissues collected from the heart in chronic CD.

Many sudden fatalities due to chronic cardiac CD are often attributed to apical aneurysms which occur at the bottom of the heart [20,21]. Importantly, parasite load is low, spatially heterogeneous and poorly correlated to the magnitude of tissue damage in chronic CD, including in clinical samples [22,23], indicating possible spatial disconnect between CD-induced metabolic alterations and tissue parasite load. We therefore focused on liquid chromatography-tandem mass spectrometry-based metabolomic analysis of transversely sectioned hearts from mice chronically infected with *T. cruzi* strains CL and Sylvio X10/4. These samples were previously collected but only previously analyzed with regards to parasite load and differences in overall metabolome between heart apex and heart base (infected and uninfected samples combined), without characterizing the impact of chronic infection on the heart metabolome [9]. In contrast, this study focuses on metabolic changes associated with chronic (90 and 147 days) *T. cruzi* strains CL and Sylvio X10/4 infection, compared to matched uninfected controls. Overall, we observed significant localized chemical differences associated with infection, with a disconnect between parasite localization and overall positional metabolic perturbations. Our data also showed infection-induced variations in acylcarnitine and glycerophosphocholine chemical families.

1.3 Methods

1.3.1 Ethics Statement

All vertebrate animal studies were performed in accordance with the USDA Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the University of California San Diego Institutional Animal Care and Use Committee (protocol S14187).

1.3.2 *In vivo* experimentation

All *in vivo* experimentation and sample collection were conducted and previously reported in [9]. Briefly, n=5 five-week-old male C3H/HeJ mice (Jackson Laboratories) were infected with 1,000 *T. cruzi* strain CL trypomastigotes, with n=7 matched uninfected controls; both groups were euthanized 90 days post-infection. N=5 five-week-old male C3H/HeJ mice (Jackson Laboratories) were infected with 1 million *T. cruzi* strain Sylvio X10/4 trypomastigotes, with n=5 matched uninfected control mice. N=4 of these *T. cruzi* Sylvio X10/4-infected animals survived to the endpoint, at 147 days post-infection, when they were euthanized along with matched controls. Euthanized mice were perfused with phosphate-buffered saline to clear any circulating trypomastigotes. The hearts were removed, transversely sectioned into four sections from the base to the apex of the heart (sections A to D in text below) and snap-frozen using liquid nitrogen, for a total of n=4 sections per animal. Each section was then used in its entirety for metabolite extraction and LC-MS analysis, so that we have complete and consistent spatial coverage of the heart tissue.

Although sample collection was reported in McCall *et al* [9], the authors only studied the acute impact (12 days post-infection) of Chagas disease on mouse hearts. This study, in contrast,

examines the impact of infection on the heart samples extracted on days 90 and 147 post-infection (chronic stage).

qPCR was performed in McCall *et al* [9]. Briefly, a Quick-DNA universal kit from Zymo Research was used to extract DNA from homogenized heart tissue sections, and a nanodrop was used to quantify DNA. 180 ng was used for qPCR analysis. ASTCGGCTGATCGTTTTTCGA and AATTCCTCCAAGCAGCGGATA primers were used to amplify parasite satellite DNA region [24] and TCCCTCTCATCAGTTCTATGGCCCA and CAGCAAGCATCTATGCACTTAGACCCC to amplify host TNF α [25]. The following parameters were used for amplification: initial denaturation for 10 min at 95°C, then 40 cycles of denaturation (95°C for 30s), annealing (58°C for 60s), and extension (72°C for 60s). PCR product formation was confirmed through melting curve analysis. Parasite burden in each heart section was determined using a standard curve developed from samples extracted from mouse heart tissue spiked with 2×10^7 *T. cruzi* epimastigotes [9].

1.3.3 Metabolite extraction and UHPLC-MS/MS

The two-step procedure for metabolite extraction was conducted as described in McCall *et al* [9], with “aqueous” and “organic” extracts referring to the metabolites recovered from the first 50% methanol extraction and the second 3:1 dichloromethane-methanol extraction, respectively. Dried samples were obtained and resuspended in 50% methanol spiked with sulfadimethoxine as internal standard. A checkmix solution with 6 known molecules was also run at the beginning and end of LC-MS analysis to monitor instrumental drift and showed only minor retention time shifts (S1 Fig). Both extracts were analyzed separately and were randomized according to infection type as well as position to prevent sample bias. Thermo Scientific UltiMate 3000 Ultra High Performance Liquid Chromatography was used to analyze

samples using a 1.7 μm C8 (50 x 2.1 mm) UHPLC column (Phenomenex) equipped with a C8 guard cartridge (Phenomenex). Chromatography was done with water + 0.1% formic acid (mobile phase A) and acetonitrile + 0.1% formic acid (mobile phase B), at a 0.5 mL/min flow rate (7.00 min aqueous extract and 10.5 min organic extract) with a 40°C column temperature. LC gradient for aqueous extract is as follows: 0-1 min, hold at 2% B; 1-1.5 min, increase to 40% B; 1.5-4 min, increase to 98% B; 4-5 min, hold at 98% B; 5-6 min, decrease to 2% B; 6-7 min, hold at 2% B. LC gradient for organic extract is as follows: 0-1 min, hold at 2% B; 1-1.5 min, increase to 60% B; 1.5-5.5 min, increase to 98% B; 5.5-7.5 min, hold at 98% B; 7.5-8.5 min, decrease to 2% B; 8.5-10.5 min, hold at 2% B.

MS/MS detection was conducted on a Maxis Impact HD QTOF mass spectrometer (Bruker Daltonics) [9]. Electrospray ionization was used to generate ions and MS spectra obtained in positive mode only. ESI-L Low concentration Tuning Mix (Agilent Technologies) was used for daily instrument calibration and Hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazene (Synquest Laboratories), m/z 922.009798, was used throughout the analysis as internal calibrant (lock mass). The following instrumental parameters were used for UHPLC-MS/MS: runtime: 0 to 7 min (aqueous extract), 0 to 10.5 min (organic extract); polarity: positive; exclusion: on; nebulizer gas pressure: 2 bar; capillary voltage: 4,500 Volts; ion source temperature: 200°C; dry gas flow: 9.0 L/min, spectra rate acquisition: 3/sec; TopN: 7 (aqueous extract), 10 (organic extract); mass range: 80 – 2,000 m/z ; active exclusion: after 4 spectra and release after 30s; ramped collision-induced dissociation energy: 10-50 eV. Mass ranges with common contaminants and lock masses were excluded (m/z 123.59-124.59, 143.50-144.50, 159.47-160.47, 182.49-183.49, 216.61-217.61, 309.83-310.83, 337.50-338.50, 359.50-360.50, 622.00-622.05, 921.50-925.50).

1.3.4 LC-MS/MS data analysis

Data processing was performed as previously reported using Optimus, July 21, 2016 version [9,26,27]. Optimus data processing parameters are as follows: (i) LC-MS feature detection: m/z tolerance: 20.0 ppm; noise threshold: 1000; half of MS/MS isolation window: 2.0 Da. (ii) Advanced FD settings: `commom_chrom_fwhm`: 20; `common_chrom_peak_snr`: 2.0; `mtd_reestimate_mtd_std`: enabled; `epd_width_filtering`: off; `epd_min_fwhm`: 1.5; `epd_max_fwhm`: 25.0; `ffm_report_summed_ints`: Enabled. (iii) Filter features: minimum occurrence rate: 0.01. (iv) Missing feature intensities: Enable imputation of missing features: Enabled. (v) Normalize features: Enable feature normalization using internal standards (sulfadimethoxine injection control): enabled.

Data was normalized to sulfadimethoxine internal standard peak, followed by total ion current (TIC) normalization in R. Random forest analyses were performed separately on the aqueous extract feature table and the organic extract feature table (both total ion current (TIC)-normalized), with both results jointly summarized in figures and tables. Principal coordinate analysis (PCoA) was performed on total ion current (TIC) normalized MS1 feature data table using the Bray-Curtis-Faith dissimilarity metric using QIIME1 [28], for both organic and aqueous extractions combined. The three-dimensional PCoA plots were visualized in EMPERor [29]. Three-dimensional data visualization was performed using 'ili' (<http://ili.embl.de/>) [27] using a three dimensional heart model from 3DCADBrower.com (<http://www.3dcadbrower.com/>).

Global Natural Products Social Molecular Networking (GNPS) was used to perform molecular networking according to the following parameters: precursor mass tolerance: 0.02 Da; fragment ion mass tolerance: 0.02 Da; cosine score: 0.7; minimum matched fragment ions: 4;

search analogs: do search; network TopK: 10; maximum connected component size: 100; minimum cluster size: 2; score threshold: 0.7; library search min. matched peaks: 4; max. analog search mass: 100; filter precursor window: filter; filter peaks in 50 Da window: filter; filter below Std. Dev: 0.0; min. peak intensity: 0.0; filter library: filter; filter spectra from G6 as blanks before networking: don't filter [30].

Metabolite annotation was based on selected libraries in the GNPS infrastructure: GNPS-COLLECTIONS-MISC, GNPS- EMBL-MCF, GNPS-FAULKNERLEGACY, GNPS-LIBRARY, GNPS-NIH-CLINICALCOLLECTION1, GNPS-NIH-CLINICALCOLLECTION2, GNPS-NIST14-MATCHES, HMDB, MASSBANK, MASSBANKEU, PNNL-LIPIDS, MONA. Direct MS2 spectral matches to these libraries enable metabolomics standard initiative level 2 annotation confidence [31]. All spectral matches were visually inspected and MS2 fragment annotation was performed using CFM-ID [32,33], HMDB [34,35] and LipidMaps [36]. Cytoscape 3.7.0. was used to visualize the molecular networks [37]. Molecular networks were used to annotate metabolites without a direct match to GNPS libraries, using sub molecular network chemical families and annotation propagation [30], enabling metabolomics standard initiative level 3 annotation confidence [31]. Given the fragmentation pattern for glycerophosphocholines (PCs) under our instrumental conditions, we cannot distinguish based on MS2 pattern or m/z between PC isomers, such PC O-16:0/18:1 and PC O-12:0/22:1. Therefore we reported both library spectral matches and all possible other possible annotations nomenclature based on LipidMaps nomenclature [36]. Lastly, annotations were filtered based on plausibility vis-à-vis the observed retention time.

Random forest analysis was performed in Jupyter Notebook using R with the number of trees set to 500. Random forest classifier cutoff was based on ranked variable importance score

of differential metabolites >1.3. FDR-corrected Mann-Whitney $p < 0.05$ for all positions was used as an alternate method to determine significant metabolite differences. Venn diagrams were used to visualize the unique and common metabolites differential between CL and Sylvio X10/4 infection, compared to uninfected samples, based on heart segment positions, random forest classifier for all positions, or FDR-corrected Mann-Whitney $p < 0.05$ for all positions, using <http://bioinformatics.psb.ugent.be/webtools/Venn/>. Effect size calculations were performed on total acylcarnitine and glycerophosphocholine levels in heart section A using Hedges' g , via <https://www.socscistatistics.com/effectsize/default3.aspx>.

PCs were selected through the following steps: 1) Collect all metabolite features annotated as PCs through matching to GNPS reference libraries, for each extract (including matches with identical m/z to reference libraries and analog matches, with mass differences representing for example PCs with different chain lengths or degree of saturation compared to reference libraries). 2) Extend these annotations using molecular networking, collecting all metabolites with shared fragmentation patterns to these library matches. This ensures that all candidate PCs with quality MS2 fragmentation patterns are selected. 3) Verify that the MS2 spectra contain the PC diagnostic peaks of m/z 86.10 (N,N,N-Trimethylethenaminium), m/z 125.00 (2,2-Dihydroxy-1,3,2-dioxaphospholan-2-ium) and m/z 184.08 (phosphocholine). 4) Verify m/z presence in LipidMaps [36]. 5) Subset feature abundance table to these m/z and retention times, to obtain a new feature table with the peak area of PCs in our samples. Specifically, there were a total of 396 PCs where 81 were short (400 – 599.99 m/z), 207 were mid-length (600 – 799.99 m/z), and 108 were long (>800 m/z). A similar process was used to sub-select acylcarnitines, monitoring presence of the 3-carboxyallylium +CH₂-CH=CH-COOH diagnostic peak at m/z 85.03). There were a total of 84 acylcarnitines, which were assigned to

short- (C2-C4), mid- (C5-C11) and long-chain (C12 and longer) categories using LipidMaps [36].

1.4 Results

The purpose of this study was to compare the metabolic impact of chronic CD in the mouse heart between divergent *T. cruzi* strains and between cardiac regions. To do so, we analyzed previously-collected positive mode LC-MS/MS data [9]. While this prior study focused on the impact of acute infection (12 days post-infection in mice) on the cardiac metabolite profile, here we specifically focused on the impact of 90 and 147day infection (chronic stage CD in mice) on the cardiac metabolite profile, which had not been studied before.

We observed a clear localized impact of *T. cruzi* infection on the overall metabolite profile (Figs 1, S2 and S3). As previously described [9], parasite burden was highest at the base of the heart (position A) for strain CL and central positions (position C) for strain Sylvio X10/4 (Fig 1A). PERMANOVA analysis indicated that the highest significant perturbation in the overall metabolite profile occurred at central positions for strain CL infection (PERMANOVA analysis of Bray-Curtis-Faith distance matrix $R^2=0.20813$, p-value=0.004 at position C) and at apical positions for strain Sylvio X10/4 infection (PERMANOVA $R^2=0.27923$, p-value=0.014 at position D) (Fig 1B). Strikingly, in both cases chemical disturbance was greatest at sites distinct from the site of highest parasite burden, which corroborates our observations in the context of chronic gastrointestinal *T. cruzi* infection in mice [18]. The localization of chemical disturbance also provides a molecular mechanism explaining the apical aneurysms observed in CD patients and the fact that these happen even though cardiac tissue parasite burden is low [22].

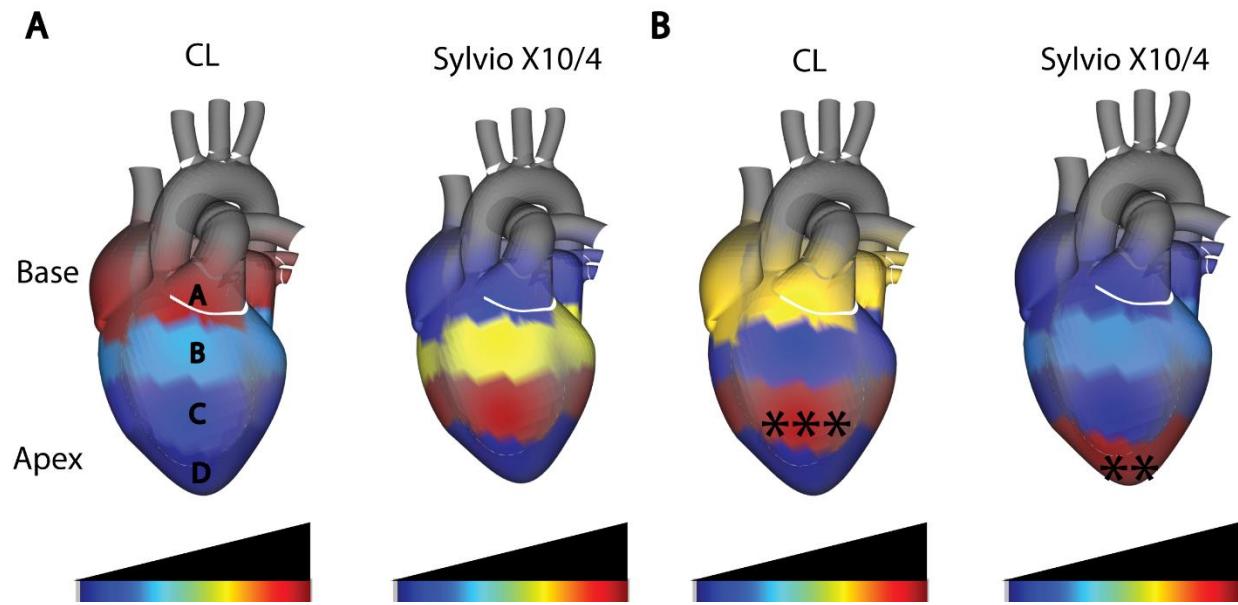


Fig. 1 Disconnect between sites of parasite persistence and metabolic alterations in chronic cardiac CD.

(A) Median cardiac parasite burden, as determined by qPCR. Parasite burden was highest at the heart base (position A) for strain CL and central heart segments (position C) for strain Sylvio X10/4, indicating parasite strain-specific differences in parasite tropism. (B) Statistically significant perturbations in the overall metabolite profile between uninfected and strain CL-infected mice (left), and between uninfected and strain Sylvio X10/4-infected mice (right). The highest significant metabolite perturbation was at central heart segments (position C) for strain CL (***, $p < 0.001$ by PERMANOVA) and at the heart apex (position D) for strain Sylvio X10/4 (**, $p < 0.01$ by PERMANOVA).

To identify the specific cardiac metabolites spatially perturbed by infection, initially we built a random forest classifier for each position, each strain and each extraction method, comparing to uninfected matched control samples (S1-S4 Tables). We first assessed the overlap between the top-ranked most differential metabolites by random forest for the two different strains, at each position, as described in Methods. Limited overlap of these significant metabolites was observed between strains (Fig 2A-D). To address the possibility that a given metabolite was modified by each strain at different positions, we also performed this analysis for all positions combined (Fig 2E and 2F, S5 and S6 Tables). Indeed, a higher overlap was observed between strains under these conditions, but still only representing a fraction of all differential metabolites. However, annotation of these differential metabolites using molecular networking through the GNPS platform [30] revealed that while differing in terms of m/z , many were part of the same chemical families, including acylcarnitines and glycerophosphocholines (S1-S6 Tables, S4 and S5 Figs).

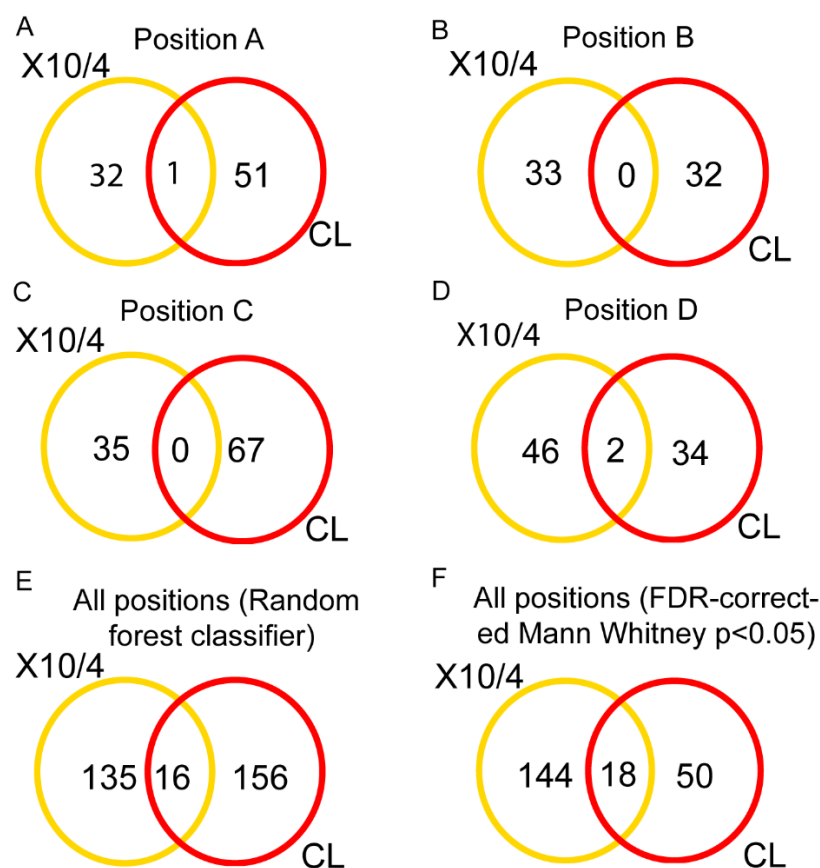


Fig. 2 Limited overlap of specific differential metabolites between strains.

Yellow and red circles represent differential metabolites between strain Sylvio X10/4-infected and matched uninfected controls, and between strain CL-infected and matched uninfected controls, respectively. Intersect are metabolites impacted by infection in both strains. (A-D) Differential metabolites for each strain, at given heart positions, as determined by random forest classifier, with variable importance score cutoff as described in Methods. (E) Metabolites impacted by infection with each strain, irrespective of position, as determined by random forest classifier, with variable importance score cutoff as described in Methods. (F) Metabolites impacted by infection with each strain, irrespective of heart position, using FDR-corrected Mann Whitney $p < 0.05$ cutoff.

Random forest classifier identified several acylcarnitines and glycerophosphocholines as impacted by infection (S1 - S5 Tables). Both chemical families play a major role in several biochemical pathways. Carnitine serves as a shuttling mechanism for fatty acids, in the form of acylcarnitines, from the cytosol into the matrix of the mitochondria for beta-oxidation [38]. Glycerophosphocholines are major components of lipid metabolism, cell membrane structure, and choline production, the latter of which is essential for select amino acid and neurotransmitter synthesis [39,40].

Total acylcarnitines in the central positions of the heart were decreased by strain Sylvio X10/4 infection compared to the uninfected group (Fig 3A and 3B, Mann-Whitney $p < 0.05$). A similar pattern was observed for total acylcarnitines following strain CL infection when compared to matched uninfected samples at the heart base (Fig 3A and 3B, Mann-Whitney $p < 0.05$). Normal levels and distributions of acylcarnitines in the heart are represented by uninfected samples (Fig 3). Previous studies demonstrated that acylcarnitines of different lengths were associated with infection outcome in acute (12 days post-infection) *T. cruzi* mouse models [9]. Therefore, we sought to understand how different length acylcarnitines were affected by chronic (90 and 147 days post-infection) infection. Acylcarnitines are classified based on the number of carbons in their fatty acid chain as short- ($\leq C4$), mid- ($C5 - C11$), and long-chain ($\geq C12$) acylcarnitines. In the case of CL strain infection, when compared to uninfected samples, short chain acylcarnitines were significantly decreased at the heart base ($p < 0.05$ Mann-Whitney, Fig 3C and 3D). Strain Sylvio X10/4 infection significantly decreased mid-chain acylcarnitine at all positions (Mann-Whitney $p < 0.05$, Fig 3E and 3F) and significantly decreased long-chain acylcarnitines at heart base, center and apex (Mann-Whitney $p < 0.05$, Fig 3E-HH).

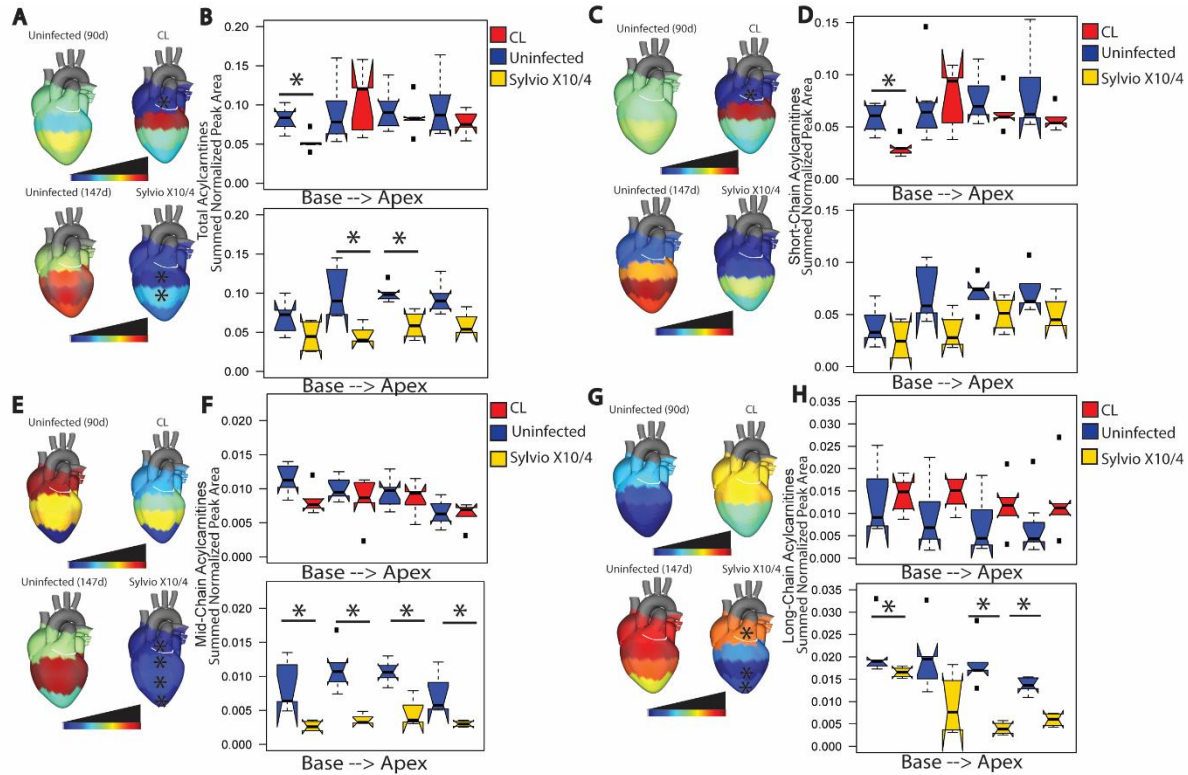


Fig. 3 Spatial impact of chronic *T. cruzi* infection on cardiac acylcarnitines.

Normal levels and distribution of acylcarnitines are represented by uninfected samples. (A, B) Differential total acylcarnitine distribution between uninfected and infected heart sections for both CL and Sylvio X10/4 strains. CL-infected mice showed statistically significant decreases in total acylcarnitine levels at heart base when compared to uninfected mice (*, $p < 0.05$ by Mann-Whitney test). (C, D) CL-infected mice showed statistically significant decreases (*, $p < 0.05$, by Mann-Whitney test) in short-chain acylcarnitine ($\leq C4$) at heart base. (E, F) Sylvio X10/4-infected mice showed statistically significant decreases in mid-chain acylcarnitines at all positions compared to uninfected mice. (*, $p < 0.05$ by Mann-Whitney test). (G, H) Sylvio X10/4-infected mice showed statistically significant decreases in long-chain acylcarnitines ($\geq C12$) at most heart positions compared to uninfected mice (*, $p < 0.05$ by Mann-Whitney test).

Select glycerophosphocholines were increased at specific sites upon infection. CL strain infection significantly increased total glycerophosphocholines, at central heart positions compared to uninfected samples (Mann-Whitney $p < 0.05$), as did strain Sylvio X10/4 at the heart apex (Mann-Whitney $p < 0.05$, Fig 4A and 4B). Further analysis based on glycerophosphocholine m/z was performed, because previous studies showed differences in glycerophosphocholine m/z range between fatal and non-fatal acute mouse infection [9]. Glycerophosphocholines were categorized into three mass ranges: short (400 – 599.99 m/z), mid (600 – 799.99 m/z), and long (> 800 m/z). Significantly increased glycerophosphocholines were observed for CL strain infection in short (m/z 401-599.99) glycerophosphocholines at heart center and apex (Mann-Whitney $p < 0.05$, Fig 4C and 4D). Sylvio X10/4 strain infection significantly increased short (m/z 401-599.99) and long ($m/z > 800$) glycerophosphocholines at the heart apex when compared to uninfected samples (Mann-Whitney $p < 0.05$, Fig 4C, 4D, 4G and 4H).

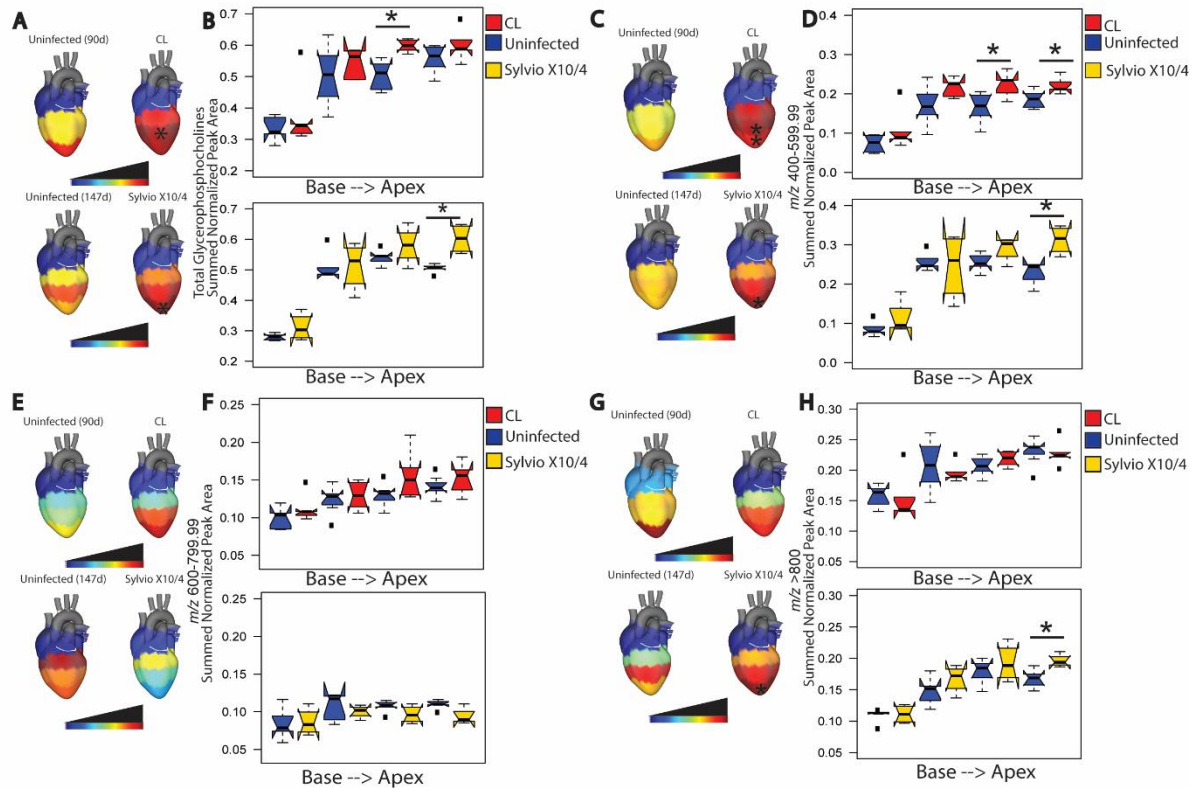


Fig. 4 Spatial impact of chronic *T. cruzi* infection on cardiac glycerophosphocholines.

(A, B) Differential total glycerophosphocholine distribution between uninfected and infected heart sections for both CL and Sylvio X10/4 strains. CL-infected mice showed statistically significant increases in total glycerophosphocholine levels at central heart positions when compared to uninfected mice (*, $p < 0.05$ by Mann-Whitney test). (C, D) Both infected strains showed statistically significant increases (*, $p < 0.05$, by Mann-Whitney test) for short glycerophosphocholines (m/z 400-599.99) at central and apical positions for strain CL and apical positions for strain Sylvio X10/4. (E, F) Mid-sized glycerophosphocholines (m/z 600-799.99) were not significantly affected by infection for both strains. (G, H) Sylvio X10/4-infected mice showed a statistically significant increase (*, $p < 0.05$ by Mann-Whitney test) in long glycerophosphocholines ($m/z > 800$) at apical positions.

1.5 Discussion

There are 7 *T. cruzi* discrete typing units infectious to humans (DTUs TcI - TcVI and Tcbat). These DTUs, while still currently considered the same species, nevertheless present significant genetic differences [41,42]. Select *T. cruzi* strains are also more virulent than others or require a greater dose to establish infection. The inocula used in this study for both strains are standard and based on their relative degree of acute-stage lethality [9,43]. Specifically, strain Sylvio X10/4 is a low virulence strain, necessitating a higher infectious inoculum to observe cardiac pathology at chronic timepoints [43]. In contrast, susceptible mice infected with 1000 CL trypomastigotes survive the acute stage to develop chronic cardiac symptoms, while mice infected with higher inocula only survive a few weeks [9]. Timing and magnitude of induced disease may also differ between *T. cruzi* strains. Ninety days timepoint for strain CL infection enables comparison with Hossain *et al*, which analyzed impact of infection on the gastrointestinal metabolome at 89 days post-infection [18]. In addition, the different doses and timepoints enabled us to find commonalities across infection systems, mimicking the clinical situation where patients do not know how long ago they were infected, or with which strain. Cross-strain, -dose and -timepoint comparisons are thus important to guide drug development.

However, pathogenic processes are overall similar in cardiac CD across *T. cruzi* strains, with accumulation of fibrosis and inflammation [42,44]. These similarities are reflected in the common metabolomic changes observed for strain Sylvio X10/4 (TcI) and strain CL (TcVI)-infected heart tissue in this study, including chronic infection-induced increases in glycerophosphocholines and decreases in acylcarnitines. These results concur with independent findings with regards to select acylcarnitines in the serum of CD1 mice chronically infected with *T. cruzi* strain Brazil (TcI) and to short-chain acylcarnitines and glycerophosphocholines in the

heart of BALB/c mice acutely infected with *T. cruzi* strain Y (TcII) [15]. They also concur with the negative relationship between mid-chain acylcarnitines vs fibrosis and disease progression-associated cytokine PDGF, and the positive relationship between glycerophosphocholines in mass ranges 400-499, 500-599 and over all mass ranges vis-a-vis of inflammation, fibrosis and progression-associated cytokines in the heart of BALB/c mice chronically infected with *T. cruzi* strain H1 (TcI) [45].

Differences in pathogenesis between strains may be due to differential strain tropism. Indeed, TcI strains tend to produce cardiomyopathy, while TcVI strains commonly produce megacolon and megaesophagus, although cardiomyopathy can still occur [46]. Our results indicate a disconnect between sites of highest parasite burden and sites of metabolic perturbation. Although parasite levels were highest in central heart segments following strain Sylvio X10/4 infection, we observed statistically significant perturbations in metabolism at the apex of the heart (Fig 1). Apical aneurysms are one of the major symptoms in chronic CD patients [47]. In addition, lateral heart wall damage is also common among chronic CD patients, in central regions of the heart [48], and we observed significant perturbations in cardiac metabolism at lower central heart positions in strain CL infection (Fig 1B) [48]. These results are consistent with clinical findings of low cardiac tissue parasite burden in CD in humans. Although parasite persistence is required for Chagas disease progression, nevertheless, cardiac tissue parasite load was not correlated with the magnitude of tissue damage, in patient-derived samples [22]. Based on these results, we propose a concept of spatial disease tolerance, whereby some tissue regions are more affected by infection, while others are less functionally affected. This is likely due to a combination of host and pathogen factors, given the differences we observe here between strain CL and strain Sylvio X10/4 infection in the same C3H mouse genetic background. Importantly,

the localization of maximal metabolic perturbation in acute strain Sylvio X10/4 infection was also the heart apex, indicating that the spatial course of disease may be set early in CD [9]. Likewise, host factors likely contribute, such as the higher production of antiparasitic but tissue-damaging IFN γ at the heart apex or specific cardiac regions being more prone to microvasculature disruptions [9].

Our results also highlight the importance of considering metabolic changes at the level of chemical families, beyond just individual metabolites. While there was little overlap of highly significant metabolite m/z at each position between strains, most differential annotatable metabolites were from these two chemical families. McCall *et al.* described these two chemical families as discriminatory compounds between fatal and non-fatal acute *T. cruzi* infected heart tissue [9]. Considering acute stage infection progresses into chronic stage infection, it is not surprising that changes in the relative abundance of these molecules are also observed in chronic CD.

Glycerophosphocholines have been linked to coronary heart disease due to production of lysophosphatidylcholines and choline [39,49]. Increased acylcarnitine levels have been linked to cardiovascular disease as well as cardiac symptoms in non-CD cardiac disease [50,51]. However, our results show the opposite pattern for acylcarnitines compared to non-infectious heart disease, highlighting the need to specifically study CD rather than extrapolate from other cardiac conditions. Indeed, this divergence was also observed at the level of gene expression profiles: upregulation of lipid metabolism gene expression was observed in heart samples of human cardiac CD patients compared to controls, while downregulation was seen in non-infectious dilated cardiomyopathy patient samples [52]. Higher lipid metabolism would increase acylcarnitine catabolism and thus decrease overall acylcarnitine abundance. Decreased carnitine

palmitoyltransferase and acetyltransferase levels, as observed by proteomic analysis of infected mouse heart tissue [53], may alternatively also contribute to the decreased acylcarnitine levels we observed. Interestingly, a few infection-perturbed metabolites were annotated as odd chain saturated fatty acids. While rare, odd chain saturated fatty acids have also been linked to protection from coronary heart disease, atherosclerosis and type II diabetes [54–56].

These results set a foundation for host-directed therapeutic development. CD may be particularly amenable to such treatment strategies, due to the contribution of host-mediated tissue damage to CD pathogenesis [1,8]. Indeed, we have previously shown that carnitine supplementation can be used to treat acute CD [18]. These findings also demonstrate a causal role in disease pathogenesis for the metabolic alterations observed in our studies. Our observation of decreases in cardiac acylcarnitines in chronic CD indicate that carnitine-based treatment regimens may also be useful to treat chronic CD. Importantly, the fact that acylcarnitines are affected in both chronic CL and Sylvio X10/4 infection suggests cross-strain applicability. Other studies have emphasized the impact of metabolism modulators on CD progression. High fat diet reduces parasite levels and increases survival in acute CD mouse models [57]. Treatment of acutely *T. cruzi* infected mice with metformin (a metabolic modulator used to treat diabetic patients) also led to an increase in overall survival rate and decreased blood parasitemia [58].

Due to the low parasite burden in chronic Chagas disease and instrumental limits of detection, we anticipate most if not all detected metabolites to be host-derived, supported by their detection in uninfected tissues. As such, this study is focused on the impact of *T. cruzi* infection on host metabolism. A further limitation is that many of the differential metabolites were not annotatable, as is usual in metabolomic studies [59]. Nevertheless, we were able to annotate

metabolites affected by chronic infection that make up important host biochemical pathways. We only analyzed samples in positive mode due to the greater availability of positive mode reference libraries, leading to better annotation rates (35.4% in positive mode vs 10.2% in negative mode [18]). A further limitation is that free fatty acids do not ionize well under our instrument conditions, so that we cannot link the changes in PCs to other lipids [60]. Lastly, effect size calculations on our total acylcarnitine and glycerophosphocholine data indicate that we were only adequately powered to detect large differences (Hedges' $g > 0.6-0.8$).

Overall, our study highlights the importance of identifying overall differences, but also positional metabolic differences associated with infection, and the need to study multiple *T. cruzi* strains. Results also show the strength of systematic chemical cartography in understanding disease tropism and how it differs from pathogen tropism. These results will serve as stepping stones for further CD drug development, something that is urgently needed.

1.6 Conflict Statement

The content is solely the responsibility of the authors and does not necessarily represent the official views of the funders.

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Chapter 2: Persistent biofluid metabolic alterations induced by *Trypanosoma cruzi* infection are not restored by antiparasitic treatment

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Allocation of Contribution

This chapter is currently still being written with intention to be submitted to ACS Infectious Diseases for publication. I will be the first author on this publication. I designed this project with help from Dr. McCall and performed all the *in vitro* cell culturing, animal experimentation, sample collection and data acquisition (with technical assistance from Dr. Ekram Hossain) for the timecourse analysis portion of this study. Contributions to data analysis of this portion of the study was done by Morgan Harris who created the feature table, performed normalization to the feature table, and created initial principal coordinate analysis. Michael Jimenez Sandoval also contributed to data analysis by performing longitudinal analysis of the biofluids and developing volatility plots. Miranda Schaefer performed some initial metabolite annotations. Yi Xiong and Dr. Pan Chongle performed GLMM analysis. I annotated GLMM output and generated Figures 6, 7, 8 and all supplemental information. The effects of benznidazole treatment portion of the study were carried out by Shelley Kane and Rebecca Ulrich vonBargen, with contributions to data analysis by Jarrod Roach and Sayre Barron. DNA and RNA extraction was carried out by me with help from Michelle Katemauswa. PCR experimentation was carried out by Kate Wheeler and London Klechka. The introduction, results, discussion, and methods were my main contributions to the writing with help from Jarrod Roach and Rebecca Ulrich vonBargen in the methods sections.

2.1 Abstract

Chagas Disease (CD), caused by *Trypanosoma cruzi* protozoa, is a complicated parasitic illness with inadequate medical measures for diagnosis and monitoring treatment success. Therefore, there needs to be advances in diagnostics for CD. To address this gap, we analyzed changes in the metabolome of *T. cruzi*-infected mice via liquid chromatography tandem mass spectrometry analysis of clinically-accessible biofluids: saliva, urine, and plasma. Urine was the most indicative of CD progression. Metabolites perturbed by infection in the urine include glutamic acid, kynurenate, adenosine, acylcarnitines, and phosphatidylcholines. Based on these results, we sought to implement urine as a tool for assessment of CD treatment success. Strikingly, it was found that mice with successful parasite clearance following benznidazole treatment had similar overall urine metabolome to mice that failed to clear parasites. These results match with clinical outcomes in which benznidazole treatment did not improve infection outcomes. Overall, this study provides insights into new small molecule-based CD diagnostic methods and a new approach to assess functional treatment response.

2.2 Introduction

With 6-7million people infected worldwide and 300,000 in the United States, Chagas Disease (CD) is progressively becoming a worldwide concern due to emigration of infected individuals ^{1,2}. This parasitic disease is caused by the protozoan *Trypanosoma cruzi*, transmitted by triatomine insects. It can also be transmitted through food and drink, organ transplants, blood transfusions or congenital transmission. The disease presents in three stages, acute, indeterminate and chronic. The acute stage presents with non-specific symptoms such as nausea and fever; however, patients quickly become asymptomatic within a few weeks. This is the indeterminate stage, which may last decades. 30-40% of infected people progress to symptomatic chronic-stage, with severe cardiac symptoms such as arrhythmias, cardiac failure, and apical aneurysms, and less commonly intestinal tract symptoms including megacolon and megaesophagus ^{1,3}. 55-65% of symptomatic patients die due to cardiac arrest, 25–30% due to heart failure and 10-15% due to a thromboembolic event ³.

Current diagnostic methods include several serological tests: enzyme-linked immunosorbent assays, indirect hemagglutination and indirect immunofluorescence ^{4,5}. These tests rely on the presence of anti-*T. cruzi* antibodies for diagnosis but suffer from low reliability due to the rate of false positives (0.3 – 3.2%) and false negatives (0.7 – 3.7%) ^{6,7}. Indeed, there must be a minimum of two positive test results to diagnose CD. These tests can also take decades to become negative after successful antiparasitic treatment, hampering drug development. In addition, there has been about 50% of actual CD cases missed in blood banks in Venezuela alone due to low specificity and sensitivity of current diagnostic methods ^{6,7}. Unfortunately, not all countries such as Venezuela, have adopted the two-test positive rule to confirm CD diagnosis which also contributed to high percentages of missed diagnosis in its blood bank ⁷. This is

concerning as misdiagnosed blood samples can be ultimately used for blood transfusions leading to more *T. cruzi* infections. Microscopy methods are available but are only useful in the acute stage of CD. In the chronic stage, parasite levels decrease drastically, so PCR techniques have to be used instead of microscopy. PCR utility is also limited by intermittent positive and negative test results for a given patient. Importantly, all of these diagnostic methods lack the ability to monitor disease progression and treatment success. In a survey of 155 CD experts around the world, 62 acknowledged early assessment of treatment response as a high need in research priorities in the CD community ⁸.

Several approaches have been tested to monitor CD progression and treatment success. Current treatments for CD include the antiparasitics benznidazole and nifurtimox. Unfortunately, these drugs have severe side effects and some patients do not continue treatment due to their severity. In addition, over 20% of patients are not cured by these standard treatments ⁹⁻¹³. Addressing treatment success is thus essential to prioritize follow-up treatment in these patient cohorts, and to guide drug development. Current treatment assessment methods include anti-*T. cruzi* antibody quantification or PCR detection of *T. cruzi* DNA. Challenges associated with these methods are that anti-*T. cruzi* antibodies can take decades to return to normal after treatment success, therefore not indicating the need to stop or continue treatment. PCR detection is only 60-70% sensitive as it is not consistently positive in patients with treatment failure. PCR thus cannot indicate treatment success but only treatment failure ^{8,14,15}. These issues are concerning as without knowledge of treatment assessment, a delay in treatment can lead to irreparable cardiac damage and continued unnecessary treatment can lead to unwanted severe drug adverse effects. While electrocardiograms may be useful for identifying heart dysfunction, they alone cannot diagnose chronic stage CD but simply cardiac disease ¹⁶. Furthermore, most

patients in the chronic stage of CD have irreversible damage, which highlights the importance of earlier diagnosis. Brain Natriuretic Peptide (BNP), a known biomarker of heart failure is increased in patients with CD. It identifies cardiac disease but does not necessarily diagnose parasitic infection or predict progression to severe CD from the indeterminate stage. Researchers have also tested magnetic resonance imaging (MRI) on CD patients to assess disease severity. The study was able to identify 84.6% of CD patients with myocardial fibrosis by MRI, thus is useful during asymptomatic or indeterminate periods ¹⁷. However, this method again does not indicate CD presence but only heart damage only after initial diagnosis.

Based on the lack of diagnostics for chronic CD for initial diagnosis and for determination of treatment success, there is a need for novel diagnostic methods for CD ⁸. Metabolomics can bridge this gap and provide useful information towards diagnostics of chronic CD presence and treatment success or failure.

Metabolomics is the integration of analytical and biochemical methods to study small molecules. Metabolites are the products of many metabolic pathways and are direct markers of changes in biochemical processes ¹⁸⁻²⁰. Using liquid chromatography tandem mass spectrometry (LCMS/MS) we studied mice infected with *T. cruzi* parasites and assessed 3 biofluids for metabolite biomarker discovery of infection and treatment success. We hypothesized that LCMS/MS based metabolomics would enable the discovery of CD biomarkers and give insight to chronic *T. cruzi* infection status and disease progression. Discovery of biomarkers throughout chronic *T. cruzi* infection would increase specificity and sensitivity of diagnosis and allow earlier treatment due to precise indicators of disease presence and severity. We also hypothesized that metabolites could represent novel biomarkers for early treatment assessment. We found that changes in the urine metabolome were most reflective of disease progression. Specific perturbed

metabolites include glutamic acid, kynurenate, adenosine, acylcarnitines, and phosphatidylcholines. Overall, this study provides insights to new small molecule-based diagnostic methods by providing potential biomarkers of disease progression and host response to treatment of *T. cruzi* infection.

2.3 Materials and Methods

2.3.1 Ethics statement

All vertebrate animal studies were performed under protocol number R17-035 and R20-027, approved by the University of Oklahoma Institutional Animal Care and Use Committee.

2.3.2 *In vitro* parasite cell culture

T. cruzi strain Sylvio X10/4 epimastigotes were obtained from ATCC (catalog number 50800) and placed in co-culture with C2C12 mouse myoblasts (ATCC catalog number CRL-1772). *T. cruzi* luciferase-expressing strain CL Brener was a kind gift of Dr. John Kelly, London School of Hygiene and Tropical Medicine and was likewise co-cultured with C2C12 cells. Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin were used to maintain cell and parasite cultures at 37°C with 5% CO₂.

2.3.3 *In vivo* experimentation - timecourse analysis

Male Swiss Webster mice were obtained and were either infected, uninfected, or injected with isoproterenol. Infected mice (n=15) were injected intraperitoneally with 500,000 culture derived *T. cruzi* trypomastigotes of strain Sylvio X10/4 parasites. N=15 mice were left uninfected. The remaining 15 mice were injected with 100 mg/kg isoproterenol subcutaneously as a chemically induced heart disease control group. Mice were monitored for 90 days.

Blood, saliva and urine collection once a week for 8 weeks and then every other week for 4 weeks. Blood was collected via the saphenous vein and centrifuged for plasma retrieval. Saliva was collected by intraperitoneal injection of pilocarpine hydrochloride as salivary gland stimulant (0.375 mg/kg) and a cotton swab placed in the mouth for 5 minutes ⁴¹. The cotton swab was placed into a small eppendorf with the bottom cut and then placed into a large eppendorf. Both eppendorfs with the swab were centrifuged for saliva retrieval. Urine was collected by placing mice in a single cage until urination. The urine was pipetted into an eppendorf. All samples were stored at -80°C until extraction.

Infected and uninfected mice (n=3 per group and timepoint) were euthanized at two different time points (12 days post infection, acute stage and 52 days post infection, mid-chronic stage). All remaining mice from all groups were euthanized at the 90 day endpoint which is the chronic stage of the disease. Isoflurane overdose was used for euthanization. Blood plasma and transversely sectioned (4 sections) heart tissue was collected at all euthanization time points. Phosphate-buffered saline was used to perfuse heart tissue to remove circulating parasites. Heart tissue was stored in RNAlater and used to monitor cardiac fibrosis via qRT-PCR and parasite burden via qPCR (see below).

2.3.4 *In vivo* experimentation - effects of benznidazole treatment

8-week old female BALB/c mice (Jackson) were obtained and assigned to 3 cohorts: infected (n=45), uninfected (n=13), or isoproterenol (n=12). Mice in the infected group were injected intraperitoneally with 1,000 luciferase-expressing *T. cruzi* trypomastigotes of strain CL Brener parasites while the uninfected control mice were mock-infected intraperitoneally with DMEM. Mice in the isoproterenol group were injected with a single 100 mg/kg dose of isoproterenol.

116-125 days post-infection (DPI), surviving infected mice were treated with 30 mg/kg benznidazole in solutol once daily via oral gavage. 160 DPI (35 days post-treatment), urine was collected from each surviving mouse in all 3 cohorts using the previously described method.

2.3.5 Parasite burden and cardiac fibrosis quantification

DNA and RNA extraction of heart tissue from the heart base was performed using ZYMO research Quick-DNA/RNA Miniprep Plus Kit (ZD7003) per manufacturer's protocol. mRNA was reverse transcribed into cDNA using Invitrogen High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor. cDNA, DNA, and RNA were stored at -20°C until analysis.

2.3.6 qPCR

After extraction, DNA was quantified using nanodrop and 180 ng was used for qPCR analysis on a Roche Lightcycler 96 as in McCall *et al* 2017², using the conditions in Table 1 and PowerUp SYBR Green master Mix (Fisher). Spiked 2×10^7 *T. cruzi* epimastigotes samples were used to generate a standard curve from mouse heart tissue and were used to determine parasite burden in each section. Primers used for analysis were ASTCGGCTGATCGTTTTTCGA (S = G or C) the otenand AATTCCTCCAAGCAGCGGATA for parasite amplification⁴² and TCCCTCTCATCAGTTCTATGGCCCA and CAGCAAGCATCTATGCACTTAGACCCC for normalization to mouse DNA⁴³.

2.3.7 qRT-PCR

qRT-PCR analysis was conducted on a Roche Lightcycler 96. cDNA samples were diluted 1:100 in water and were mixed with PowerUp SYBR Green master Mix (Fisher). Brain natriuretic peptide (cardiac disease biomarker) (Forward: AAGTCCTAGCCAGTCTCCAGA, Reverse: GAGCTGTCTCTGGGCCATTTC) and *Gapdh* primers (Forward:

GACTTCAACAGCAACTCCCAC, Reverse: TCCACCACCCTGTTGCTGTA were used for analysis and were added in 0.25 μ M concentration ⁴⁴. qRT-PCR parameters are as in table 1.

Table 1 qPCR and qRT-PCR parameters

Program	Description	Acquisition mode
Preincubation	1 cycle	
	95°C for 600s	none
3 Step Amplification	40 cycles	
	95°C for 30s	none
	58°C for 60s	none
	72°C for 60s	single
Melting	1 cycle	
	95°C for 60s	none
	55°C for 30s	none
	95°C for 1s	continuous

2.3.8 Metabolite extraction and LC-MS/MS data collection

Samples were extracted according to (Adapted from Dunn 2011 Nature Protocols) ⁴⁵. Briefly, all samples were extracted with 100% HPLC-grade methanol with internal control (0.5 μ M sulfachloropyridazine) to a final concentration of 50% methanol. Samples were then vortexed

and centrifuged at 16,000xg for 15 mins. The supernatant was then dried overnight in a Speedvac and stored at -80 °C until LC-MS/MS analysis. For LC-MS/MS data analysis, samples were resuspended in 150 µl HPLC-grade water + 0.5 µM sulfadimethoxine, sonicated for 5 min and centrifuged at 16,000xg for 15 mins. The supernatant was then transferred to a run plate. Pooled quality controls (QC) were made using 1 µl per well per sample type, and blanks made of resuspension solvent. Samples were analyzed in randomized order per sample type and 10 µl was injected for analysis. LC analysis was performed using Thermo Scientific Vanquish UHPLC. A Phenomenex 1.6 µm 100 Å Luna Omega Polar C18 column (50 × 2.1 mm) was used for separation. The LC column temperature was set to 40 °C and analysis was done using mobile phase A (water + 0.1% formic acid) and mobile phase B (acetonitrile + 0.1% formic acid) with a 0.5 mL/min flow rate (12.5mins). See below for LC gradient (Table 2). Sample analysis was conducted using a blank and pooled QC every 12 samples per sample type. Instrumental drift was monitored using a 6-mix solution of 6 known molecules at the beginning, between each sample type and at the end of analysis. Instrument calibration was done using Pierce LTQ Velos ESI positive ion calibration solution immediately prior to instrument analysis. A Q Exactive Plus (Thermo Scientific) high resolution mass spectrometer was used for MS/MS detection (Table 3) and ions were generated for MS/MS analysis in positive mode.

Table 2 LC gradient

Time(min)	Flow (ml/min)	%B	Curve
0.000	Run		
0.000	0.500	5.0	5
1.000	0.500	5.0	5
9.000	0.500	100.0	5
11.000	0.500	100.0	5
11.500	0.500	2.0	5
12.500	0.500	2.0	5
12.500	Stop Run		

Table 3 Q Exactive Plus (Thermo Scientific) instrument parameters

Run time	0 to 12.5 min
Polarity	Positive
Default charge state	1
Full MS	
Resolution	70,000

AGC target	1e6
Maximum IT	246 ms
Scan range	70 to 1050 <i>m/z</i>
dd-MS2	
Resolution	17,500
AGC target	2e5
Maximum IT	54 ms
Loop count	5
TopN	5
Isolation window	1.0 <i>m/z</i>
(N)ce/stepped (N)CE	NCE: 20, 40, 60
dd Settings	
Minimum AGC target	8.00e3
Intensity Threshold	1.5e5
Peptide match	preferred
Exclude isotopes	on

Dynamic exclusion	10.0s
Tune Data	
Spray Voltage	3800.00
Capillary Temperature	320.00
Sheath Gas	35.00
Aux Gas	10.00
Spare Gas	0.00
Max Spray Current	100.00
Probe Heater Temp	350.00
S-Lens RF Level	50.00
Ion Source	HESI

2.3.9 Data analysis

Data was obtained from LC-MS/MS analysis and converted to mzXML files using MSConvert⁴⁶. A feature table containing all metabolites for further analysis was developed using MZmine version 2.53 (Table 4). A three-fold blank removal was performed, and total ion current (TIC) normalization was performed in Jupyter notebooks. Principal coordinate analyses (PCoA), longitudinal volatility analyses, and PERMANOVA analyses were all performed in QIIME2 based on TIC normalized feature table^{47,48}. PCoA plots were visualized using EMPeror⁴⁹.

For comparison of treatment success vs failure, a parasite burden cutoff post-immunosuppression of value in the DMEM group + 3 standard deviations was used (20 parasite equivalents).

Feature-based molecular networking (FBMN) was performed using global natural product social molecular networking site (GNPS)^{50,51} based on the parameters in table 5.

Table 4 MZmine 2.53 parameters

Type	Parameter	Value
Feature detection	MS1 Mass Detection	Centroid
	MS1 noise level	4e5
	MS2 noise level	1e3
Chromatogram builder	Masses	
	Time span	0.01 s
	Min height	1.2e6
	m/z tolerance	10 ppm
Chromatogram deconvolution	Local minimum	
	Chromatographic Threshold	20%
	Retention Time (minutes)	0.05 ^a ; 1 ^b

	Minimum Relative Height	26% ^a ; 40% ^b
	Minimum Absolute Height	1.2e6 ^a ; 1.0e0 ^b
	Minimum Ratio of Peak Top/Edge	1.19 ^a ; 1.20 ^b
	Peak Duration Range	0.01 ^a ; 0.04 - 5.00 ^b
	<i>m/z</i> MS2	0.01
	RT MS2 (minutes)	0.1
Isotopic peaks grouper	<i>m/z</i> tolerance	10 ppm
	Retention Time Tolerance (minutes)	0.1
	Charge	3
	Lowest <i>m/z</i>	
Alignment	Join aligner	10 ppm
	Retention Time Tolerance (minutes)	0.3 ^a ; 0.1 ^b
	Weight <i>m/z</i>	1
	Weight RT	1
Feature list	Keep only with MS2 scan	
	Cut off first 20s	

	Cut off last 30s	
	Minimum peaks in a row	8 ^a ; 10 ^b
	Reset ID	

^a Value for timecourse-analysis

^b Value for treatment effect analysis. Where no footnote is provided, values were the same for both analyses.

Table 5 GNPS Parameters

Feature-Based Molecular Network	
Precursor Ion Mass Tolerance	0.02 Da
Fragment Ion Mass Tolerance	0.02 Da
Minimum Matched Fragment Ions	4
Maximum Connected Component Size (Beta)	100
Maximum shift between precursors	200 ^a ; 500 ^b
Min Pairs Cos	0.7
Network TopK	10
Library Search Min Matched Peaks	4
Search analogs	Do Search

Top results to report per query	1
Score Threshold	0.7
Maximum analog difference	200.0 Da ^a ; 100.0 Da ^b
Minimum Peak Intensity	0.0
Filter Precursor Window	Filter
Filter peaks in 50 Da Window	Filter
Filter Library	Filter Library
Normalization per file	Row Sum Normalization (Per file Sum to 1,000,000)
Aggregation Method for peak abundances per group	Mean
Run Dereplicator	Run

^a Value for timecourse-analysis

^b Value for treatment effect analysis. Where no footnote is provided, values were the same for both analyses.

Analysis for the GLMM model (Generalized Linear Mixed Model) was performed using the glmmTMB package (Version 1.1.2.3) in the R environment (version 3.6.2) ⁵². Days post-infection and treatment groups (infected, uninfected, isoproterenol treatment) were set as fixed

effectors while the mouse ID was set as the random effector. The family and link of the GLMM model were set to Gaussian and identity, respectively. The false discovery rate q-values for the experimental group for each metabolite were derived with the p.adjust function in the R environment.

2.3.10 Data Availability

Metabolomics data has been deposited in MassIVE, accession number MSV000085900 (<ftp://MSV000085900@massive.ucsd.edu>) and MSV000088436. GNPS feature-based molecular networking results can be accessed at

<https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=83f9b24689e245f2bf3511cdc29bc45d>

2.4 Results

***T. cruzi* infection perturbs the urine, saliva, and plasma metabolome**

Building on our prior work demonstrating that the cardiac metabolome differed between mild and severe *T. cruzi* infection², we assessed whether metabolome alterations were also observed over the course of experimental *T. cruzi* infection in clinically-accessible biofluids: saliva, urine and plasma. Control samples were also collected from mice treated with isoproterenol to induce cardiac hypertrophy independent of *T. cruzi*²¹. We confirmed that isoproterenol treatment induced persistent heart damage in this model via qRT-PCR assessment of *Bnp* gene expression (average $2^{-\Delta\Delta C_t}=2.71\pm0.62$ compared to *Gapdh* expression and to uninfected samples at the chronic stage of the disease (90 days post infection (DPI)); $p=0.029$, Student's T test, compared to uninfected samples). As expected, cardiac parasite burden was highest in the acute stage of infection and then decreased to low levels (Fig. 5A). *Bnp* gene

expression levels, in contrast, were only significantly affected at the chronic (90 DPI) timepoint in infected animals compared to uninfected animals (Table 6)

Table 6 Impact of infection on cardiac Bnp expression.

Timepoint	$2^{-\Delta\Delta C_t} \pm$ standard error	Student's T test p-value, two-tailed to uninfected samples
Acute: 12 DPI	2 ± 0.92	0.44
Midchronic: 52 DPI	2.98 ± 1.08	0.19
Chronic: 90 DPI	7.59 ± 4.5	0.047

The overall urine metabolome was the most impacted by infection, followed by saliva and plasma (Fig. 5B-E and Figure S1, PERMANOVA $p < 0.05$ $R^2 = 0.22$ at day 28 for plasma, all other timepoints non-significant; R^2 range for significant timepoints for saliva from $R^2 = 0.13$ at day 25 to 0.18 at day 18; R^2 range for significant timepoints for urine from 0.26 at day 10 to 0.44 at day 52). Strikingly, the overall impact of infection on the saliva metabolome was similar to the impact of isoproterenol treatment (both groups, PERMANOVA $p < 0.05$ to uninfected at day 11 and day 18), whereas the urine metabolome of infected mice diverged from both isoproterenol-treated and uninfected animals (Fig. 5B-C).

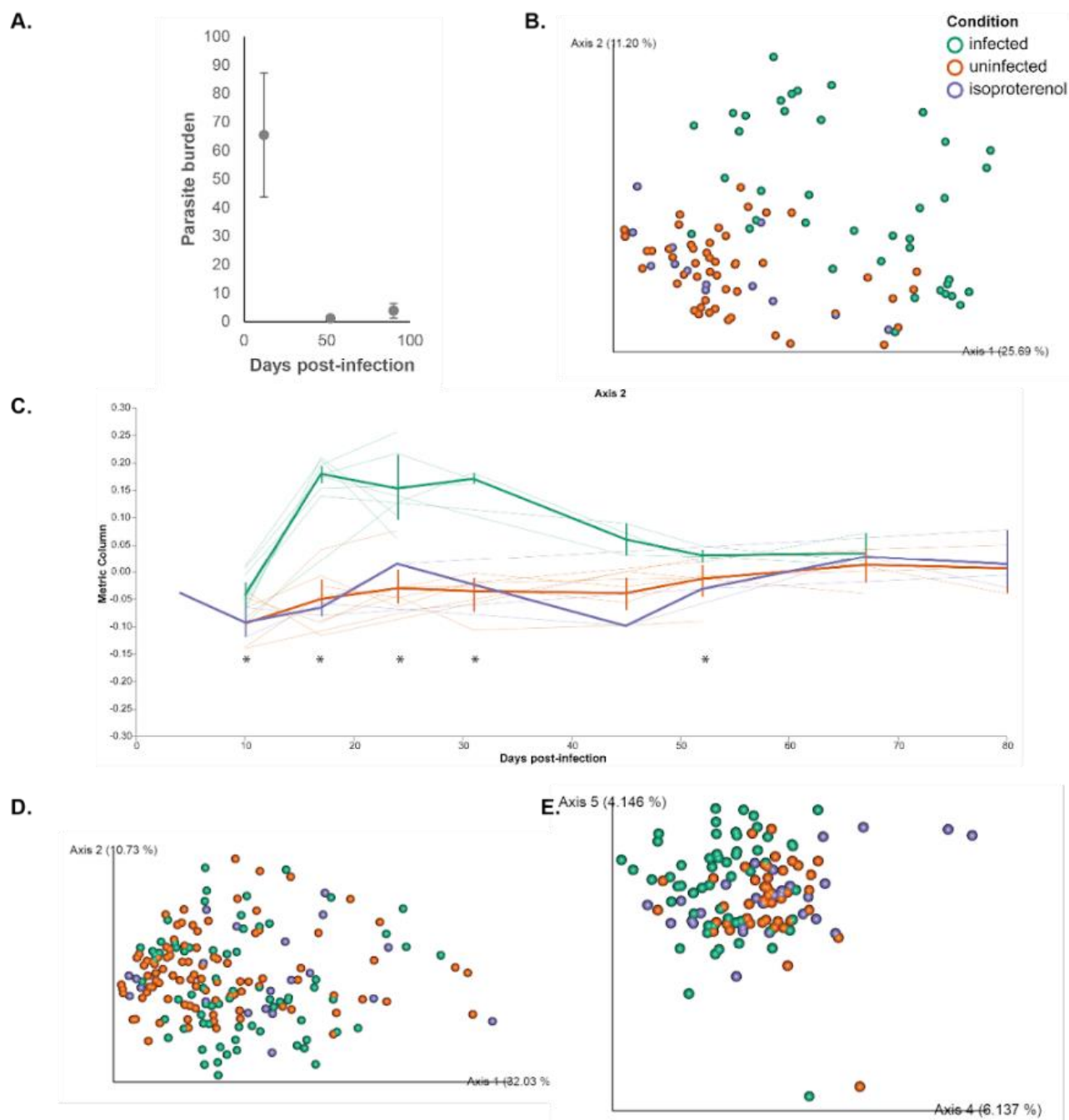


Fig. 5 *T. cruzi* infection impacts the urine metabolome more than the salivary and plasma metabolome.

(A) Parasite burden (parasite equivalent in 50gm tissue) at heart base decreases as mice transition from acute to chronic infection. (B) Urine sample principal coordinate analysis (all timepoints). (C) Urine sample volatility analysis showing separation between infected samples and both

uninfected and isoproterenol-treated samples over time. Thick lines indicate group mean and thin lines represent the trajectory of each individual mouse along principal component axis 2. *, PERMANOVA $p < 0.05$ for infected to uninfected animals. #, PERMANOVA $p < 0.05$ for isoproterenol-treated to uninfected animals. **(D)** Saliva sample principal coordinate analysis (all timepoints). **(E)** Plasma sample principal coordinate analysis (all timepoints).

Next, we assessed the specific metabolites perturbed in each biofluid over time using Generalized linear mixed models (GLMM) as described in the methods⁵². Based on GLMM analysis, urine revealed the most metabolites perturbed by infection (182 metabolites), followed by saliva (37 metabolites), then plasma (17 metabolites) (Table 7). However, there were very few metabolites perturbed by isoproterenol treatment across all biofluids (Table 7). We then sought to determine commonalities between biofluids and between infected and isoproterenol samples for a given biofluid via Venn Diagrams (Fig 6). Interestingly, there was limited overlap between biofluids for metabolites perturbed by *T. cruzi* infection or by isoproterenol treatment (Fig 6A-B). In addition, there was no commonality found in plasma and in urine between metabolites perturbed by infection and by isoproterenol (Fig 6C-D), and only 1 metabolite overlapped between infected and isoproterenol saliva samples. Some of the most differential metabolites amongst the biofluids were m/z : 204.123, retention time (RT): 0.39 min annotated as acetylcarnitine, m/z : 190.0498, RT 2.31 min annotated as kynurenate, m/z : 209.092, RT 0.65 annotated as kynurenine, m/z : 400.3417, RT 6.6 min annotated as palmitoylcarnitine, m/z : 166.0863, RT 0.63 min annotated as phenylalanine, and several phosphatidylcholines (Table S1). The one metabolite commonly perturbed by both interventions in saliva samples was omega-hydroxydodecanoate (m/z : 244.1904, RT: 4.84) and the common metabolite perturbed by infection in both saliva and urine was acetylcarnitine (m/z : 204.123, RT: 0.39) (Table 7).

To determine whether the limited commonality of metabolites identified was due to inherent heterogeneity between sample types, we developed Venn diagrams of all detected metabolites via molecular networking (Fig 7). Large commonality was found between biofluids in both infected and isoproterenol-treated samples (Fig 7A-B). The same trend was noticed in each individual biofluid as well between interventions (Fig 7C-E). This indicates that overall metabolites are mostly homogenous across biofluids (Fig 7), but significant differences in response to perturbations are observed between biofluids and between interventions (Fig 6).

Table 7 Number of significant metabolites perturbed by infection or by isoproterenol treatment in each biofluid over time

	Plasma	Saliva	Urine
Infected vs Uninfected	17	37	182
Isoproterenol vs Uninfected	7	4	6

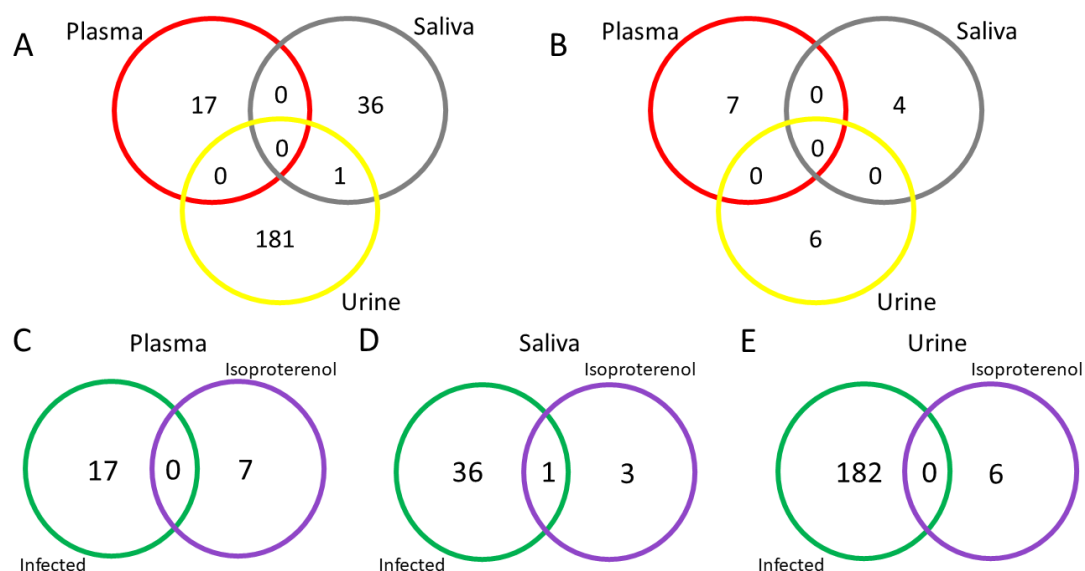


Fig. 6 Commonalities between biofluids and between infected and isoproterenol treated samples for a given biofluid based on GLMM output.

(A) Limited overlap of metabolites perturbed by *T. cruzi* infection between biofluids. (B) No overlap of metabolites perturbed by isoproterenol injection between biofluids. (C) No commonalities found between metabolites significantly perturbed by infection or isoproterenol treatment in plasma samples. (D) Limited commonality between significantly perturbed (FDR-corrected p-value <0.05) metabolites in saliva samples. (E) No commonalities found between significantly perturbed (FDR-corrected p-value <0.05) metabolites in urine samples.

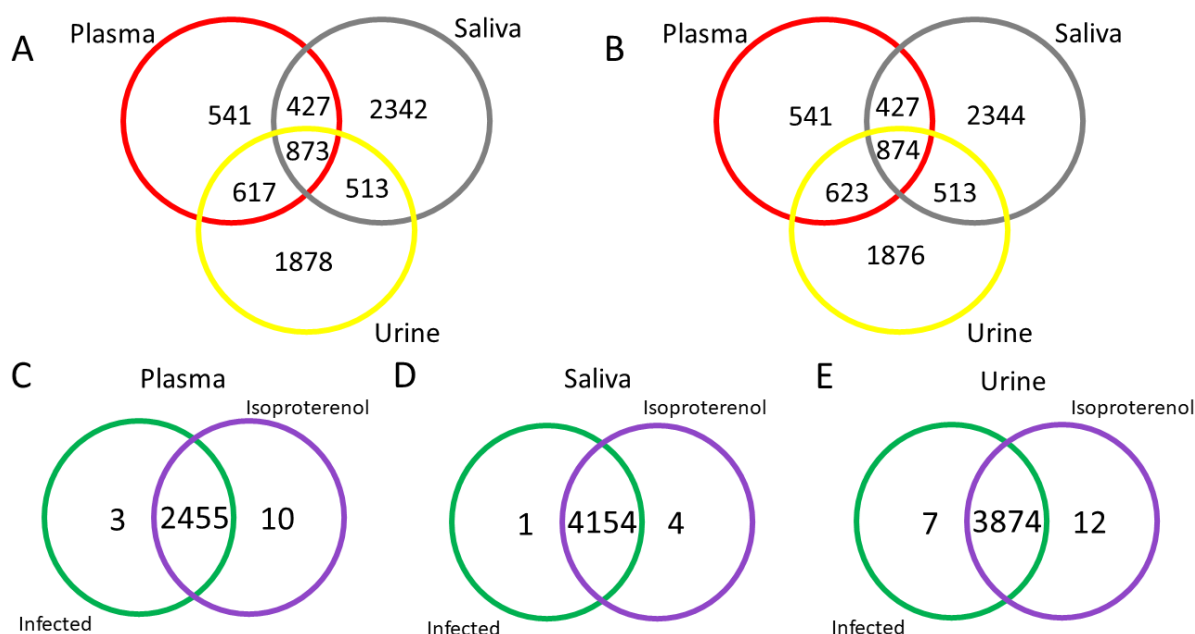


Fig. 7 Commonalities between biofluids in terms of metabolite occurrence regardless of timepoint.

(A) Large overlap of metabolites present in infected biofluids. (B) Substantial overlap of metabolites present in isoproterenol injection in biofluids. (C) Commonalities found between metabolites in infected or isoproterenol treated plasma samples. (D) Commonalities between metabolites infected or isoproterenol treated in saliva samples. (E) Commonalities found between metabolites in infected or isoproterenol treated urine samples.

Based on the significant metabolites identified by the GLMM models, we determined how different metabolites in each biofluid changed over time using boxplots. In the urine, acetylcarnitine increased in infected samples during the acute stage of CD and resolved in the chronic stage (Fig 8A). The same trend was seen for kynurenate, while a persistent increase in glutamic acid was observed throughout infection (Fig 8C&E). 4-hydroxynonenal and riboflavin was decreased by infection and then becomes comparable to uninfected in the chronic stage (Fig 8B&D). In isoproterenol treated samples Lyso-PC was increased until 52 days post-treatment,

after which it renormalized (Fig 8E). Other metabolites such as phenylalanine, o-acetyl carnitine, and kynurenine were increased during the acute and mid chronic stage of CD and decreased in the chronic stage in saliva and plasma infected samples (Fig S2 and S3). Isoproterenol treated samples had a similar trend as urine in the plasma for metabolite palmitoylcarnitine where it was initially increased and then decreased and comparable to uninfected by the chronic stage (Fig S3).

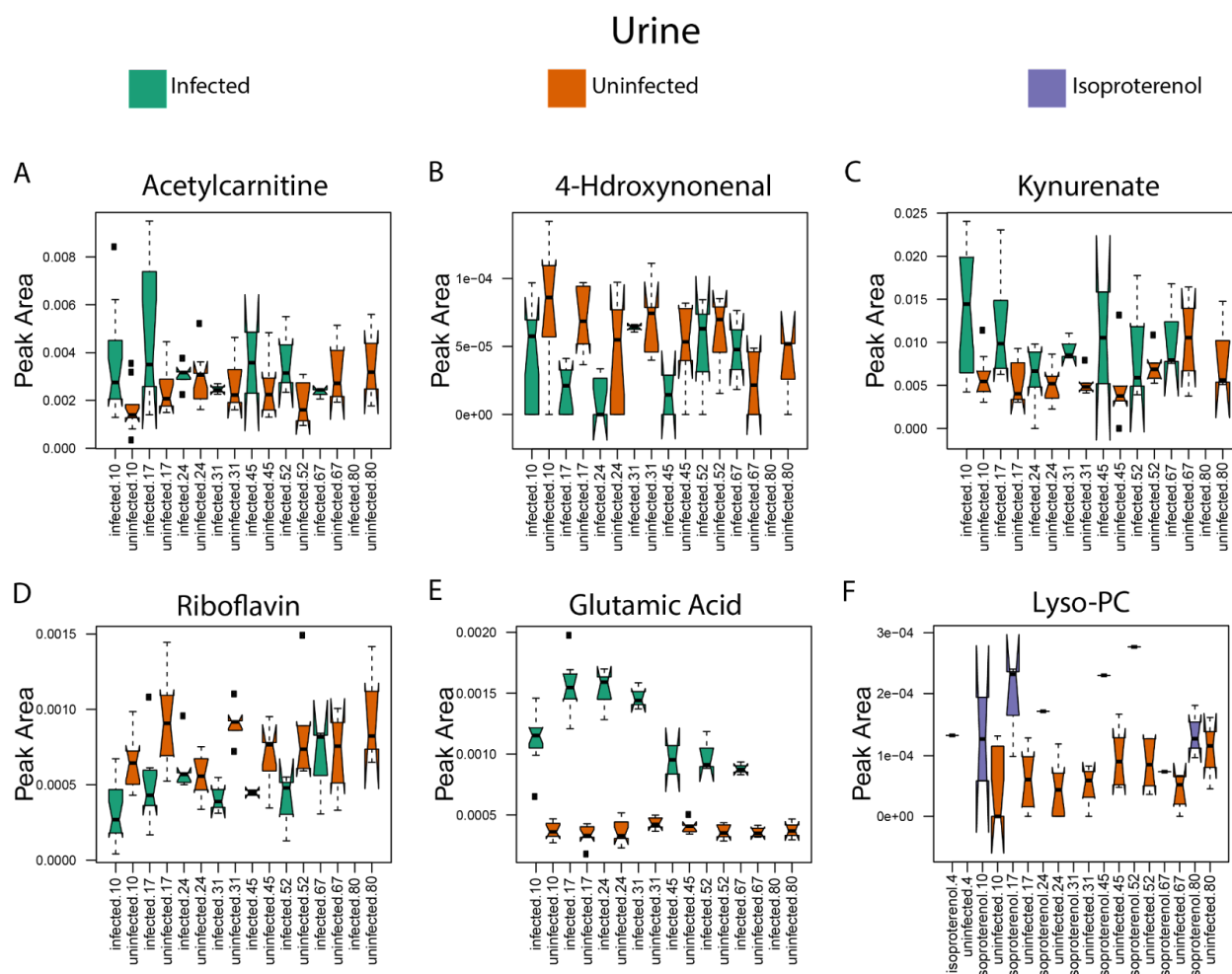


Fig. 8 Representative boxplots of significant metabolites perturbed by infection or isoproterenol treatment in the urine.

(A) Acetylcarnitine (m/z : 204.123, RT: 0.39 min) (B) 4-Hydroxynonenal (m/z :157.1222, RT: 4.06 min) (C) Kynurenate (m/z : 190.0498, RT 2.31 min) (D) Riboflavin (m/z : 377.1449, RT: 2.94 min) (E) Glutamic Acid (m/z : 221.0919, RT :0.52 min) (F) Lyso-PC (m/z : 496.3397, RT: 6.81 min)

Infection-induced metabolic alterations in the urinary metabolome are not restored by antiparasitic treatment

Having established that the urinary metabolome is perturbed by infection (Fig 5B), we then assessed whether this could be restored by successful antiparasitic treatment. To enable this analysis, we switched to a well-characterized model of antiparasitic treatment assessment, BALB/c mouse infection with luciferase-expressing strain CL Brener ^{22,23}. For comparison of treatment success vs failure, we defined a parasite burden cutoff post-immunosuppression of value in the uninfected group + 3 standard deviations was used (20 parasite equivalents). By this definition, 17 mice were deemed treatment successes and 25 were deemed treatment failures (Fig 9A). There was a clear and significant difference between the uninfected controls and the infected mice by principal coordinate analysis (whether treatment was successful or not, PERMANOVA $p < 0.05$, $R^2 = 0.08$, Fig. 9B), confirming our prior findings (Fig 5B). In contrast, there was no significant difference between the successfully treated mice and the failures at 35 days post-treatment (PERMANOVA $p > 0.05$, Fig. 9C). Likewise, no proportional correlation to post-immunosuppression parasite burden was observed (Fig. 9D).

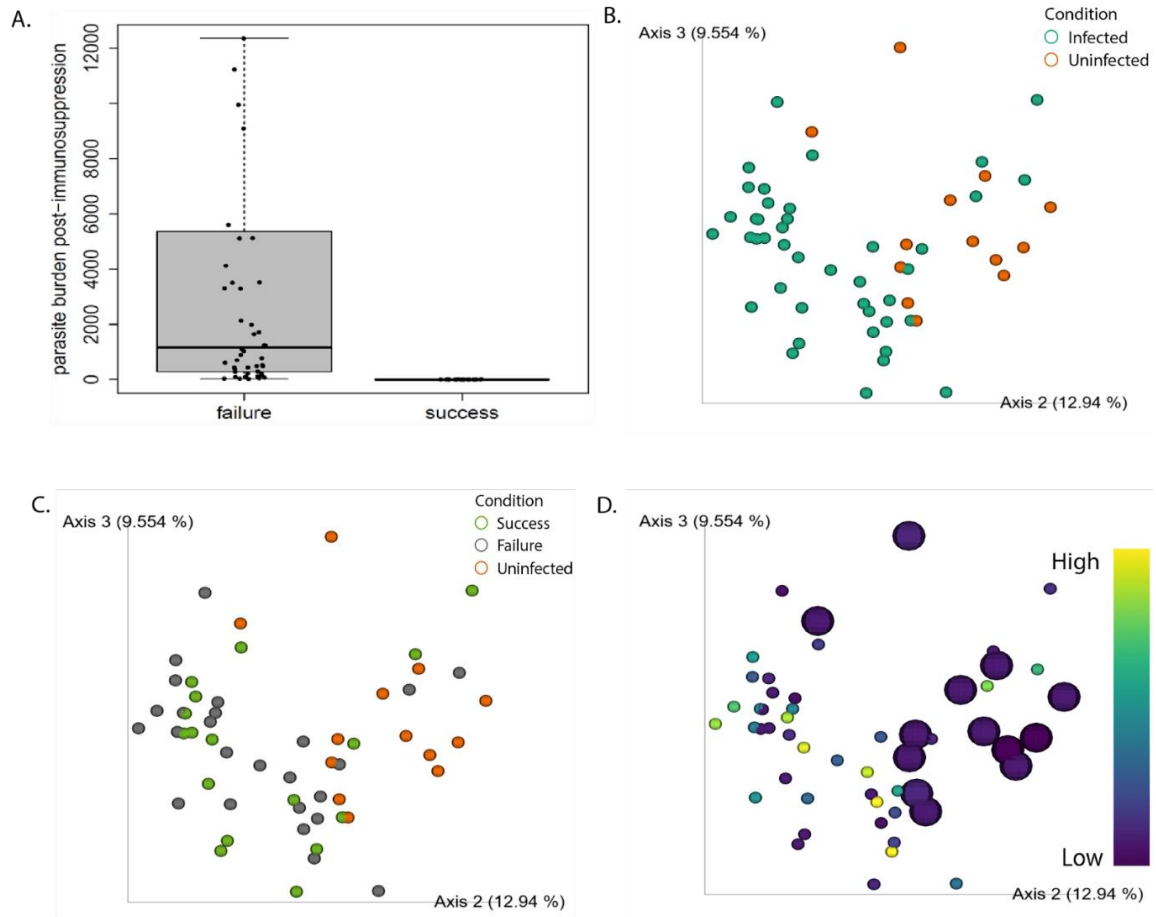


Fig. 9 Urine in mice that were successfully treated for *T. cruzi* infection shows no significant metabolic difference from urine in mice that were not successfully treated

(A) Parasite burden post-infection, treatment and immunosuppression in mice that were deemed failures versus those that were considered successes. (B) Urine sample principal coordinate analysis with mice that were infected and then treated (both successfully and unsuccessfully) versus our uninfected control mice. PERMANOVA $p < 0.05$ for treated mice versus uninfected mice, $R^2 = 0.08$. (C) Same PCoA analysis as in (B), recolored to compare successfully-treated mice, unsuccessfully treated mice and uninfected control mice. PERMANOVA $p > 0.05$ for successfully treated mice versus unsuccessfully treated mice, $R^2 = 0.03$. (D) Same PCoA analysis as in (B), recolored to display normalized parasite burden. No segregation by parasite burden was observed. Uninfected controls enlarged for contrast.

2.5 Discussion

Biofluids can tell us a vast amount of information about the human body. Blood circulates throughout the human body and interacts with all organs which allows absorption of metabolites into the plasma. Metabolites are excreted into the saliva due to salivary glands being surrounded by capillaries which help to filter blood. Kidneys filter blood, so urine will contain vast numbers of metabolites that have been passed throughout the body ²⁴. Hence urine can not only indicate kidney function but also systemic and cardiovascular health. These three biofluids all can provide useful information for diagnostic and prognostic purposes. Several blood-based diagnostics are used every day in the medical field, such as glucose detection for diabetes monitoring, measurement of alanine transferase for liver function, and creatinine for kidney function. Current urine diagnostics include dipsticks for pregnancy determination, albumin to creatinine ratio for kidney function, and measurement of bilirubin for liver damage. Saliva diagnostics are currently limited but their non-invasive and easy to acquire characteristic make them a highly desired sample type for future diagnostics ²⁵.

Building on prior work in the heart and in feces ^{2,26}, we analyzed urine, plasma and saliva biofluids with regards to metabolite biomarker discovery of *T. cruzi* infection and treatment success. We found that changes in the urine metabolome were most reflective of disease progression (Fig 5). Specifically, infected samples diverged from uninfected and isoproterenol-treated samples indicating that impacts of CD on urine metabolism diverge from general effects of cardiac damage. In addition, identification of several metabolites such as tryptophan related metabolites, amino acids, acylcarnitines and phosphocholines in the urine were present in prior work in the heart and feces of CD mice.

Interestingly, a proof-of-concept diagnostic test using urine called the Chunap test was used to determine parasite presence in congenital CD and in *T. cruzi*/HIV co-infected patients ^{27,28}. Both of these studies had increased specificity (91.7% *T. cruzi*/HIV co-infected patients, 95% congenital CD) and sensitivity (90% congenital CD) when compared to current blood-based PCR, microscopy and ELISA methods. *T. cruzi* parasite antigens and DNA have been previously detected in the urine of patients with acute, chronic, and congenital CD ²⁹⁻³¹. In guinea pigs, it was found that parasite antigens and DNA in urine are not associated with kidney injury or parasites in kidney but rather are an effect of heart and cardiovascular system ³². The renal system involvement of CD is limited: while parasites can be found in the kidneys, their effect on kidney function was mild to none ³². In addition, Lemos *et al.* identified changes in the kidney in mice infected with high dose (30,000) *T. cruzi* trypomastigotes during the acute stage of the disease ³³. However, alterations in kidney function were due to cardiac alterations of CD and the kidneys remained functional ³³. While kidney damage is seen in CD patients post treatment, there is little research assessing how this damage occurs. Heart failure could be contributing to kidney damage in CD, as is known in other cardiovascular diseases ³⁴. These studies signify that our results are not just an artifact of the mouse model and may be well translatable to humans.

Acylcarnitines and phosphatidylcholines have already been linked to CD in both heart tissue and plasma ^{2,37,38}. Our study identified L-carnitine, palmitoylcarnitine, acetylcarnitine (all increased in acute stage and resolved in chronic stage) and LPC 16:0 as significantly perturbed metabolites in the urine (Fig 8). Interestingly, acylcarnitines were decreased by chronic CD in heart tissue but increased in acute stage infection in the gastrointestinal tract ^{37,38}. Phosphatidylcholines were characteristically increased in both chronic CD in heart tissue and in acute CD in the gastrointestinal tract ^{37,38}. These match our results seen in different acylcarnitines

and phosphatidylcholines in the urine of infected mice (Fig 8). Acylcarnitine metabolism has been causally linked specifically to cardiac metabolism and disease severity in *T. cruzi* infected mice ^{37,38}. Lizardo *et al* also identified these classes of molecules as perturbed by *T. cruzi* infection in chronic CD mice as potential biomarkers of cardiac CD in serum ³⁹. Specifically, most acylcarnitines identified were decreased by infection, as seen in our study. Other molecules identified as potential biomarkers were citrulline, glutamine, spermidine and several sphingolipids ³⁹. These metabolites are not identified in our study and may be due to differences in sample type, sample preparation and data acquisition methods, and limits of detection, or had no statistical difference based on time-course analysis.

Based on these results, we sought to use the urinary metabolome to assess treatment success vs failure, using a mouse model with post-treatment immunosuppression as the gold standard of treatment assessment. Interestingly, during chronic CD, while benznidazole treatment did indeed clear parasites (Fig 9A), there was no difference in the overall urine metabolome between cured and not-cured urine samples (Fig 9B), so that the metabolome of successfully treated infected mice was not reset by antiparasitic treatment (Fig 9C). This contrasts with acute-stage treatment, which successfully restored the plasma metabolome ³⁸. This could be due to the use of different mouse and parasite strains ³⁸, but may also reflect the lack of improvements of clinical outcomes in late-stage patient treatment ⁹. As such, our method could represent a quick and efficient method to identify compounds with superior efficacy to benznidazole, though this will need to be validated in animal models and human systems.

One limitation is that we only assessed metabolic restoration at 35 days post-treatment. It is possible that metabolism gradually improves over time. Indeed, in a long-term study of benznidazole treatment, there were no longer any significant differences in cardiac inflammation

or left ventricle hypertrophy in benznidazole-treated mice when compared to uninfected mice at 378 days post-benznidazole treatment, though two treated mice remained above values in the uninfected group ⁴⁰. In contrast, the BENEFIT trial (clinical trial that evaluated benznidazole efficacy and safety in humans for use as a potential drug to reduce severe chronic cardiac CD outcomes in patients) followed patients 7 years post-treatment did not observe any improved clinical outcomes in treated patients, indicating that longer follow up may not show improvements ⁹.

2.6 Conclusion

Overall, this study demonstrated a new method to monitor CD progression and to rapidly assess treatment success vs failure in chronic CD, currently a difficult task. This shows promise but will need further development and validation prior to clinical implementation, especially in clinical cohorts. However, our results can serve as a steppingstone into further understating CD pathogenesis and addressing current diagnostic concerns.

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2.8 Conflicts of Interest

The authors declare no conflict of interest.

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Chapter 3: Chemical Cartography Approaches to Study Trypanosomatid Infection

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Allocation of Contribution

This chapter is a published manuscript in the Journal of Visualized Experiments (JoVE). This was a collaborative manuscript where I am first co-author alongside Jacob Haffner. My main contribution was writing the 3D modeling and 'ili plot generation portions of the protocol, writing about the background of Chagas Disease in the introduction, and providing alternative methods to for the project in the discussion. I also contributed to making figure 11.

3.1 Summary

This protocol describes the steps to generate a 3D model of metabolite distribution during trypanosomatid infection, including sample collection, metabolite extraction, an overview of liquid chromatography-tandem mass spectrometry data acquisition, 3D model generation, and finally, data visualization.

3.2 Abstract

Pathogen tropism and disease tropism refer to the tissue locations selectively colonized or damaged by pathogens, leading to localized disease symptoms. Human-infective trypanosomatid parasites include *Trypanosoma cruzi*, the causative agent of Chagas disease; *Trypanosoma brucei*, the causative agent of sleeping sickness; and *Leishmania* species, causative agents of leishmaniasis. Jointly, they affect 20 million people across the globe. These parasites show specific tropism: heart, esophagus, colon for *T. cruzi*, adipose tissue, pancreas, skin, circulatory system and central nervous system for *T. brucei*, skin for dermatropic *Leishmania* strains, and liver, spleen, and bone marrow for viscerotropic *Leishmania* strains. A spatial perspective is therefore essential to understand trypanosomatid disease pathogenesis. Chemical cartography generates 3D visualizations of small molecule abundance generated via liquid chromatography-mass spectrometry, in comparison to microbiological and immunological parameters. This protocol demonstrates how chemical cartography can be applied to study pathogenic processes during trypanosomatid infection, beginning from systematic tissue sampling and metabolite extraction, followed by liquid chromatography-tandem mass spectrometry data acquisition, and concluding with the generation of 3D maps of metabolite distribution. This method can be used

for multiple research questions, such as nutrient requirements for tissue colonization by *T. cruzi*, *T. brucei*, or *Leishmania*, immunometabolism at sites of infection, and the relationship between local tissue metabolic perturbation and clinical disease symptoms, leading to comprehensive insight into trypanosomatid disease pathogenesis.

3.3 Introduction

Trypanosomatid parasites consist of *Leishmania* species, African trypanosomes (*Trypanosoma brucei*), and American trypanosomes (*Trypanosoma cruzi*). *Leishmania* protozoa cause leishmaniasis, which includes self-healing and self-limited localized cutaneous leishmaniasis, mucocutaneous leishmaniasis in which the mucosal tissues of the mouth, nose, and throat become damaged, and visceral leishmaniasis with parasite tropism to the visceral organs causing fever and hepatosplenomegaly^{1,2}. *T. brucei* causes Human African trypanosomiasis (HAT), also known as sleeping sickness, mainly reported in African countries³. The clinical signs and symptoms include hepatosplenomegaly, fever, headache, musculoskeletal pains, lymphadenopathies, and anemia in the hemo-lymphatic stage when parasites localize to the bloodstream and lymphatics. This is followed by the meningo-encephalitic stage, where parasites localize to the central nervous system and cause sleep disturbance, behavioral alteration, and eventually fatal comas⁴. *T. cruzi* causes Chagas disease, endemic in the Americas. Infected individuals experience an initial acute stage, usually asymptomatic, with broad parasite tropism. About 10%–30% of infected individuals experience chronic stage symptoms after decades of infection, characterized by megaesophagus, megacolon, and cardiovascular complications^{5,6}. Metabolomics studies small molecular species (50–1,500 Da), including biological compounds from primary or secondary metabolism and externally-derived compounds such as drugs or food-derived molecules. In the context of host-pathogen interactions, metabolomics can explore the

impact of infection on host metabolite environments, crucial in accessing the effect of the pathogen on the host. It can also assess pathogen adaptations to the host nutritional and immunological environment⁷⁻⁹. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are common metabolomics tools used to identify, quantify, and characterize metabolites. This "omics" approach can also be applied to biomarker discovery and drug development^{10,11}.

Given the specific tissue tropism of trypanosomatid parasites, spatial metabolomics analyses can enable significant insight into the pathogenesis of the diseases they cause. Mapping the spatial distribution of metabolites revealed metabolites locally affected by chronic *Trypanosoma cruzi* infection in mouse heart tissue and acute and long-term *Trypanosoma cruzi* infection in the mouse gastrointestinal tract^{6,12,13}. Specifically, 3D chemical cartography demonstrated a disconnect between parasite persistence and metabolic alterations in the heart tissue of chronically *Trypanosoma cruzi*-infected mice. Metabolism was most perturbed in lower and apical segments of the heart, matching with sites of Chagas disease symptoms (cardiac apical aneurysms). Metabolite families perturbed by infection at specific cardiac sites and correlated to disease severity include acylcarnitines and glycerophosphocholines¹²⁻¹⁴. In the gastrointestinal tract, persistent metabolic alterations concurred with sites of Chagas disease symptoms: esophagus and colon. In contrast, metabolism is re-normalized at sites not associated with Chagas disease symptoms, such as the small intestine. Metabolites locally perturbed by infection in the gastrointestinal tract include acylcarnitines, glycerophosphocholines, kynurenine, tryptophan, and cholic acid. In addition, these analyses enabled the identification of a new metabolic mechanism of tolerance to Chagas disease⁶. Applying these methods to the study of cutaneous leishmaniasis revealed significant metabolic perturbations at the site of the lesion, but

also specific metabolic changes in lesion-adjacent, macroscopically healthy tissue. For example, glutamine was depleted at the lesion site, whereas glycerophosphocholines in the m/z (mass to charge ratio) 200–299, 400–499, 500–599, and 600–699 were significantly increased at the lesion site. PC (O-34:1) was only increased at lesion-adjacent sites¹⁵.

The goal of this manuscript is to demonstrate the steps necessary to generate 3D models of metabolite distribution ("chemical cartography") as applied to trypanosomatid parasite infection models (**Figure 10**). This approach builds on several critical advances in the context of metabolomics and metabolomics data processing, particularly the development of 'ili software to plot metabolomics data onto 3D models easily¹⁶.

3.4 Protocol

All animal experiments described were approved by the University of Oklahoma or the University of California San Diego Institutional Animal Care and Use Committee. All steps handling infectious material were performed inside a biosafety cabinet (class II, type A2) and according to local regulations.

1. Tissue collection

1.1. Infect appropriate trypanosomatid infection animal models, generally mice or hamsters.

NOTE: There is a considerable variety of mouse models for trypanosomatid infection, depending on the desired symptoms to be produced, speed of disease progression, disease severity, etc. The users can choose at their convenience.

1.1.1. For cutaneous leishmaniasis infection models, infect subcutaneously in the footpad or intradermally in the ear¹⁵.

1.1.2. For visceral leishmaniasis infection models, infect intravenously^{1,17}.

1.1.3. For Chagas disease and sleeping sickness infection models, infect intraperitoneally^{6,12,18,19}. Determine the parasite dose to use based on the parasite strain and planned time points.

1.2. Plan sectioning positions.

1.2.1. Plan to generate sections with a minimum of 10 mg of tissue per section. It is best to use 30–50 mg.

1.2.2. For Chagas disease infection models, plan to collect cardiac and gastrointestinal segments systematically.

1.2.3. For cutaneous leishmaniasis infection models, collect lesional tissue and lesion-adjacent samples.

1.2.4. For visceral leishmaniasis infection models, plan to collect spleen and multiple liver lobes. Additional tissue sites such as adipose tissue may also be of interest to collect.

CAUTION: Samples from infected animals must be handled under the appropriate, institutionally-approved biosafety protocol. This will generally involve personal protective equipment (PPE) requirements and only opening tubes and collecting samples inside a biosafety cabinet (class II, type A2).

1.3. Label and weigh tubes for homogenization.

1.3.1. Use the tube type appropriate for the available homogenization system. For a TissueLyser, use 2 mL microcentrifuge tubes.

1.3.2. Euthanize mice at the desired infection timepoints using isoflurane overdose as approved by institutional IACUC or according to IACUC-approved protocol.

1.3.3. Section tissue systematically as planned, with one section per tube.

1.3.4. Remember to wash sample collection equipment between samples with extraction solvent (50% methanol in this protocol).

1.4. Keeping tube lid open, immediately snap freeze samples in liquid nitrogen.

CAUTION: Do not close tubes until any liquid nitrogen entered the tube has wholly evaporated to prevent tubes from exploding as nitrogen expands. Take adequate steps to ensure that the skin

does not come in contact with liquid nitrogen (use cryo gloves and forceps to hold the tubes).

Wear a safety face shield.

1.5. Store the tubes on dry ice until all the desired samples have been collected.

1.6. Pause point: store samples at -80 °C until ready to do extractions.

1.7. Weigh tubes to determine tissue sample weight. Record in a spreadsheet.

1.7.1. Keep the samples frozen during the weighing process: keep tubes on dry ice, rapidly weigh, put back on dry ice right away. Do not allow the samples to thaw while weighing.

2. Metabolite extraction

NOTE: Only LC-MS grade liquids and reagents must be used throughout. This method was adapted from Reference²⁰.

2.1. Prepare all extraction solvents (LC-MS grade H₂O, LC-MS grade methanol, spiked with 4 µM of sulfachlorpyridazine, LC-MS grade dichloromethane: methanol spiked with 2 µM of sulfachlorpyridazine) in 1 L glass bottles, using dedicated glassware.

NOTE: Volume to be prepared should be calculated based on sample weights, considering 500 µL of water per 50 mg of sample, 500 µL of methanol spiked with 4 µM of sulfachlorpyridazine per 50 mg of sample and 1,000 µL of prechilled dichloromethane: methanol spiked with 2 µM of sulfachlorpyridazine per 50 mg of sample, increasing the calculated volume by 10% to allow for pipetting inaccuracy. Store extraction solvent at 4 °C at least overnight to pre-chill.

2.2. Perform water-based homogenization of the tissue samples as per the steps mentioned below.

2.2.1. Add one 5 mm stainless steel bead (see **Table of Materials**) to each of the 2 mL microcentrifuge tubes containing tissue samples, using a bead dispenser. Keep the tubes on ice.

2.2.2. Make one blank tube containing LC-MS grade H₂O that will go through all the steps and serve as an extraction blank. Use the average H₂O volume from sample extractions. Add chilled LC-MS grade H₂O to the frozen tissue samples.

2.2.2.1. Normalize water volume to tissue weight by adding 500 µL of water / 50 mg of sample, using the sample weights calculated at step 1.7.

CAUTION: If handling biohazardous samples, continue to follow the appropriate, institutionally-approved biosafety protocol. This will generally involve requirements for personal protective equipment (PPE) and opening tubes only inside the biosafety cabinet (class II, type A2).

2.2.3. Homogenize samples at 25 Hz speed for 3 min using a tissue homogenizer (see **Table of Materials**).

2.2.3.1. Close tubes tightly to avoid spilling the reagents during processing.

2.2.4. Collect about 1/10th of the homogenization volume for DNA extraction, qPCR, protein-based analyses, or other analyses (if desired). Store in a microcentrifuge tube or 96-well plate (depending on the volume collected) for up to 6 months at -80 °C, if DNA extraction experiments are to be performed on a different day. Longer storage durations may be possible but have not been tested.

2.2.4.1. Perform DNA extractions using any standard commercial kit for mammalian DNA extraction (see **Table of Materials**) from tissues as described in Reference¹³. Quantify

DNA yield and store the extracted DNA at -20 °C. Acceptable DNA quantity and quality for qPCR has been observed even up to 3 years later.

NOTE: This step can be performed on frozen homogenate on a subsequent day from metabolite extraction.

2.2.4.2. Perform qPCR as described in Reference¹³, using 180 ng of extracted DNA.

NOTE: This can be performed on DNA collected on a previous day and frozen at -20 °C.

2.2.4.2.1. In the case of studies on *T. cruzi* infection, use the following primers:

ASTCGGCTGATCGTTTTCGA and AATTCCTCCAAGCAGCGGATA to quantify parasite levels²¹ and the following primers to normalize to host DNA levels

TCCCTCTCATCAGTTCTATGGCCCA and CAGCAAGCATCTATGCACTTAGACCCC²²

(see **Table of Materials**).

NOTE: The recommended qPCR cycles are as follows: denature at 95 °C for 10 min; perform 40 cycles at 95 °C for 30 s, and then 58 °C for 60 s, and finally 72 °C for 60 s. Perform melting curve analysis as appropriate for the available thermocycler. Process data using the $\Delta\Delta C_t$ method²³ to obtain relative parasite load between sampling sites. Absolute quantification can be obtained by comparing sample-derived $\Delta\Delta C_t$ values to a standard curve generated from known amounts of parasites, spiked into uninfected tissue samples, and extracted as in steps 2.2 to

2.2.4.1.

2.2.4.3. Perform protein-based characterization of immune responses using multiplexed cytokine kits or standard commercial ELISA kits (see **Table of Materials**) as described in Reference¹³ on the stored homogenate.

2.2.5. Save at least half of the 500 μ L of the homogenization volume for metabolite extraction.

2.3. Perform aqueous metabolite extraction.

NOTE: Solvent selection can be adapted based on the chemical properties of metabolites of interest.

2.3.1. Add ice-cold LC-MS grade methanol spiked with 4 μ M of sulfachlorpyridazine to the homogenate to achieve a final concentration of 50% methanol with 2 μ M of sulfachlorpyridazine in water.

CAUTION: Methanol is flammable and hazardous. Use appropriate safety procedures, including handling inside a fume hood or biosafety cabinet.

2.3.2. Homogenize samples in a tissue homogenizer at 25 Hz speed for 3 min. Centrifuge samples at 16,000 $\times g$ at 4 $^{\circ}$ C for 10 min.

2.3.3. Collect an equal volume of supernatant into a 96-well-plate. Select the volume to match the smallest volume of methanol + water combined across all samples. This is the aqueous fraction.

2.3.3.1. Set aside any remaining aqueous homogenate supernatant at -80 $^{\circ}$ C as a backup.

2.3.4. Keep the solid residue on ice while collecting the supernatants.

2.3.5. Dry aqueous extraction supernatant until dry (~3 h or overnight). Use maximum speed and no heating.

2.3.6. Freeze the dried 96-well-plate at -80 $^{\circ}$ C.

2.4. Perform organic metabolite extraction.

NOTE: Solvent selection can be adapted based on the chemical properties of metabolites of interest.

2.4.1. Add 1,000 μL per 50 mg of the sample of prechilled dichloromethane: methanol spiked with 2 μM of sulfachlorpyridazine to the solid residue from step 2.3.4.

CAUTION: Use appropriate safety procedures when handling solvents, including handling inside a fume hood with a good flow rate.

2.4.2. Homogenize samples in a tissue homogenizer at 25 Hz speed for 5 min. Centrifuge the samples at 16,000 $\times g$ at 4 $^{\circ}\text{C}$ for 10 min.

2.4.3. Collect an equal volume of supernatant into a 96-well-plate. Select the volume to match the smallest volume of dichloromethane: methanol, across all samples. This is the organic fraction.

2.4.4. Store the pellet at -80 $^{\circ}\text{C}$ as a backup. Store the remaining organic extract at -80 $^{\circ}\text{C}$ as a backup. Air-dry the organic extract in a fume hood overnight.

2.4.5. Freeze the dried 96-well-plate at -80 $^{\circ}\text{C}$.

3. LC-MS data acquisition

3.1. Resuspend aqueous and organic extracts into 60 μL each of 50% methanol + 2 μM of sulfadimethoxine, and combine. Sonicate for 10 min; then, centrifuge for 10 min and transfer the supernatant to a clean 96-wellplate. Seal with zone-free plate seal and place the plate in an LC autosampler.

CAUTION: Use appropriate safety procedures when handling solvents, including handling inside a fume hood with a good flow rate.

3.2. Connect appropriate mobile phases to the LC system (see **Table of Materials**).

NOTE: For positive mode reversed-phase LC, authors recommend LC-MS-grade H₂O + 0.1% formic acid as mobile phase A and LC-MS-grade acetonitrile + 0.1% formic acid as mobile phase B with a flow rate of 0.5 mL/min and a 7.5 min LC gradient. Recommended gradient steps are as published in Reference⁶: 0–1 min, 2% B; 1–2.5 min, linear increase to 98% B; 2.5–4.5 min, hold at 98% B; 4.5–5.5 min, linear decrease to 2% B; 5.5–7.5 min, hold at 2% B.

3.3. Ensure that the instrument is clean. Calibrate MS in both positive and negative mode.

3.4. Perform MS performance evaluation as appropriate for the instrument.

3.5. Create MS run sequence.

3.5.1. Start with 2 blanks, 2 standards (6-mixes), and 5 pooled quality controls (QC) in a dilution series, beginning at 2 µL of injection volume and increasing stepwise to 30 µL injection volume.

3.5.2. Randomize the sample order.

3.5.3. After every 12 samples, run a blank, and then a pooled QC.

3.6. Connect C8 LC column (1.7 µm particle size, 100 Å pore size, 50 x 2.1 mm length x internal diameter) and monitor for leaks and excessive backpressure. Fix issues as per instrument standard operating procedure.

3.7. Start MS run sequence and collect data-dependent LC-MS/MS data.

NOTE: For a Q-Exactive Plus MS instrument, use Heated Electrospray Ionization and data-dependent MS2 acquisition (top 5) in positive mode, a resolution of 70,000 for MS1 and 17,500 for MS2, AGC Target of 1E6 for MS1 and 2E5 for MS2, maximum IT of 100 ms for both MS1

and MS2, scan range to 100–1500 m/z for MS1, and MS2 isolation window of 1 m/z . Set sheath gas to 35, aux gas to 10 and sweep gas to 0, spray voltage to 3.8 kV, capillary temperature to 320 °C, S-lens RF level to 50, and aux gas temperature to 350 °C.

3.7.1. Verify the data quality: check initial blanks (confirm lack of major peaks), standards (confirm the presence of expected peaks and symmetric peak shape), and QCs (confirm the presence of expected peaks, peak shape, and expected peak intensity).

3.7.2. Periodically monitor MS run during the run sequence.

3.8. Once the run is finished, check the data for any missed injections or other errors.

3.9. Store the LC column as recommended by the manufacturer. Remove and store samples at -80 °C.

3.10. Upload raw data to the data repository.

NOTE: MassIVE (massive.ucsd.edu) is recommended to enable the downstream link to molecular networking for metabolite annotations^{24,25}.

4. LC-MS data processing

4.1. Convert raw files to open format (.mzXML or .mzML) using MSConvert²⁶.

4.1.1. Upload raw data and mzXML or mzML data to the data repository.

4.2. Generate feature table. There are multiple tools to do so (MZmine, MS-DIAL, openMS, XCMS, etc.^{27–30}).

NOTE: MZmine is recommended because it is free, open-source, and can directly import mzXML files after MSconvert, has graphical user interface options for processing data and

monitoring the impact of parameter selection, and can directly export to GNPS for molecular networking²⁴.

NOTE: Follow tool documentation and use parameters appropriate for the available instrument. Additional details can also be found in Reference³¹.

4.2.1. Export feature table.csv.

5. 3D model generation

5.1. Hand-draw 3D model de novo to scale as per the steps mentioned below.

5.1.1. Take a picture of the organ of interest.

5.1.2. Perform the following in SketchUp software (see **Table of Materials**) as mentioned below.

5.1.2.1. Delete the default picture of a man that appears when the software opens.

5.1.2.2. Click on **File > Import** to import a picture of the organs of interest.

5.1.2.3. Click on the **Lines** tool and select the **Freehand** option. Use the pencil tool to trace and draw the outlines of the organs of interest. Ensure to close the line by drawing all the way back to the starting point. Once the line has been successfully closed, the drawn area will automatically appear shaded.

5.1.2.4. Select the **Push/Pull** tool and pull up on the shaded area to convert the drawing from 2D to 3D.

5.1.2.5. Delete the organ picture: select the **Eraser** button, and then right-click on the picture and select **Erase**.

5.1.2.6. Export the file in .dae format: **File > Export > 3D Model**.

5.1.3. Improve the realism of the model as per the steps mentioned below.

5.1.3.1. Import the model into MeshLab software (see **Table of Materials**): open MeshLab and select **File > Import Mesh**. Select the .dae model generated at the previous step. A **Pre-Open Options** menu will pop up. Select **OK**.

5.1.3.2. Select **Wireframe** on the top menu. Then, select: **Filters > Remeshing, Simplification and Reconstruction > Subdivision surfaces: Midpoint**. Leave all values as default and select **Apply** twice. Visually inspect the wireframe view of the model to ensure it is finely gridded. Close the pop-up menu.

5.1.3.3. Export the model in .stl format: **File > Export Mesh As**, and then select **STL File Format (*.stl)** in the **Files of Type** dropdown menu. Click on **Save**. Select **OK** in the next pop-up menu.

5.1.3.4. Open the **Meshmixer** software (see **Table of Materials**). Click on the **Import (+)** button. Select the .stl file generated at the previous step. Use the **Sculpt > Brushes > Drag** and **Sculpt > Brushes > Inflate** tools to pull out the model's surfaces that need to be rounded out.

5.1.3.5. Once the model has the desired appearance, save it in .stl format: **File > Export**. Name the file as desired and select **STL Binary Format (*.stl)** in the **Save as type** dropdown menu. Click on **Save**. If a pop-up menu appears, click on **Continue**.

6. 'ili plot generation

6.1. Obtain coordinates for the positions in the 3D model that correspond to the sampling sites.

6.1.1. Open the 3D model from step 5.1.3.5 in the **MeshLab** software: **File > Import Mesh**.

Select the model generated in step 5.1.3.5. Click on **OK** in the **Post-Open Processing** pop-up window.

6.1.2. To obtain x, y, and z coordinates for each sampling spot: select the **PickPoints** tool, and then right-click at regularly spaced intervals across the 3D model surface. Once all the desired coordinates have been selected, click on the top-most **Save** button in the **Form** pop-up window.

NOTE: This will export the coordinates in .pp file format. This file can be opened in spreadsheet software.

6.1.3. In spreadsheet software: Open the .pp file generated in step 6.1.2. Adjust data display using **Data > Text to Columns > Delimited**. Click on **Next**, and then select **Space**, and click on **Finish**.

6.1.4. Reformat so that only numerical values remain in the spreadsheet cells by selecting **Home > Find & Select > Replace**. In the Find what box, enter: **y="**. Leave the Replace with box empty. Click on **Replace All**, and then on **OK**. Repeat for **x="** and for **z="** and for **" />**. Values are now ready for step 6.2.

6.2. Make the 'ili feature table. This method was adapted from Reference¹⁶.

6.2.1. In a spreadsheet software, build the feature table. Rows correspond to each position and columns to data.

NOTE: The first columns must be the position name (sample name), followed by x, y, and z coordinates of the sampling spots obtained at step 6.1.2 (with column headers x, y, z). The fifth column must be titled "radius".

6.2.2. Paste the appropriate metadata and metabolite feature abundance in the subsequent spreadsheet columns.

6.2.3. In column "radius", enter the desired size of the sampling spots to be visualized on the model. Determine the values for radius empirically: enter 1 as default, and then assess whether radius or coordinates need to be adjusted in step 6.3. Save the file in .csv format (**File > Save As**), and then select **CSV (Comma delimited) (*.csv)** in the dropdown menu. Name the file as desired. Click on **Save**.

6.3. Open the data in 'ili (software developed by¹⁶).

6.3.1. Open the 'ili website (ili.embl.de). Select **Surface**. Drag and drop the created 3D model into the browser window. Drag and drop the created feature table into the same browser window.

6.3.2. Use the legend at the bottom-right corner to project the desired data column on the 3D model. Ensure that the spots and radii selected in step 6.2.1 match the sampling sites. If necessary, adjust values in the feature table, or choose additional coordinates in the MeshLab (see step 6.1.2).

NOTE: Visualization can also be improved by selecting the **Spots > Border Opacity** and setting the slider to its maximal value.

6.3.3. Consecutively select each data column to assess the distribution of this metabolite feature on the 3D model.

NOTE: This approach can also be used to visualize only specific metabolite features of interest, for example, those with $p < 0.05$ or a certain fold change between infected and uninfected

samples, as determined in external statistical analysis tools. Features to visualize may also be selected outside of 'ili through machine learning approaches such as a random forest.

6.4. Perform linear/log data visualization as per the steps mentioned below.

6.4.1. Using the **Mapping** tab at the top right of the page, select **Linear** or **Logarithmic** in the **Scale** drop-down menu.

6.4.2. Set the same scale for all plots if visualizing multiple features.

NOTE: The website automatically chooses the minimum and maximum for each data column to display.

6.4.3. To set the scale manually, deselect the **Auto Min/Max** option and enter the desired scaling. All data will now be displayed within the same scale.

6.5. Change the color scale of the data (grayscale, blue-white-red, etc.).

6.5.1. Change the color scale to the desired color scheme in the **Mapping** menu using the **Color Map** drop-down menu. Ensure that the selected color scheme is color-blind-friendly.

6.6. To change the color of the 3D model or the background color, use **Color** and **Background** options under the 3D menu.

6.7. Hide 3D axes by unselecting the **Show the Origin** box under the 3D menu.

6.8. Save the image by screenshotting, using the snipping tool, or the shortcut key Ctrl + S for Windows or Linux and ⌘ + S on OS X.

3.5 Representative Results

The number of metabolite features obtained depends on the tissue type analyzed and data processing parameters. For example, this protocol has been used to analyze the spatial impact of *T. cruzi* infection on the gastrointestinal tract metabolome in a mouse model of *T. cruzi* infection. In our previous work, male C3H/HeJ were injected intraperitoneally with 1,000 CL + luc *T. cruzi* parasites^{32,6}. Animals were euthanized 12 or 89 days, post-infection, and a chemical cartography analysis of 13 contiguous segments of the gastrointestinal tract was performed as described in this protocol. This analysis led to a feature table of 5,502 features, which were then visualized into 3D using the steps described in this protocol. This approach enables the visualization of metabolite features in individual animals that are high at the site of high parasite load (kynurenine, **Figure 11B** vs. parasite load, **Figure 11A**), of metabolites with differential distribution across tissue regions (glutamine, **Figure 11C**) and metabolite features that are found at comparable levels across small and large intestines (LPE 16:0 **Figure 11D**). Kynurenine was selected for visualization because of its known relationship to inflammation and prior publications on the ability of kynurenine-derived metabolites to regulate the *T. cruzi* load³³. Random forest-based machine learning models had previously revealed an association between kynurenine levels and infection status⁶. Glutamine was selected for a visualization based on previous publications demonstrating a relationship between *in vitro* glutamine availability and *T. cruzi* drug sensitivity³⁴. Differential distribution was confirmed using logistic regression, $p < 0.05$. LPE 16:0 was selected after visual inspection of the data to discover metabolite features found at comparable levels across tissue sites.

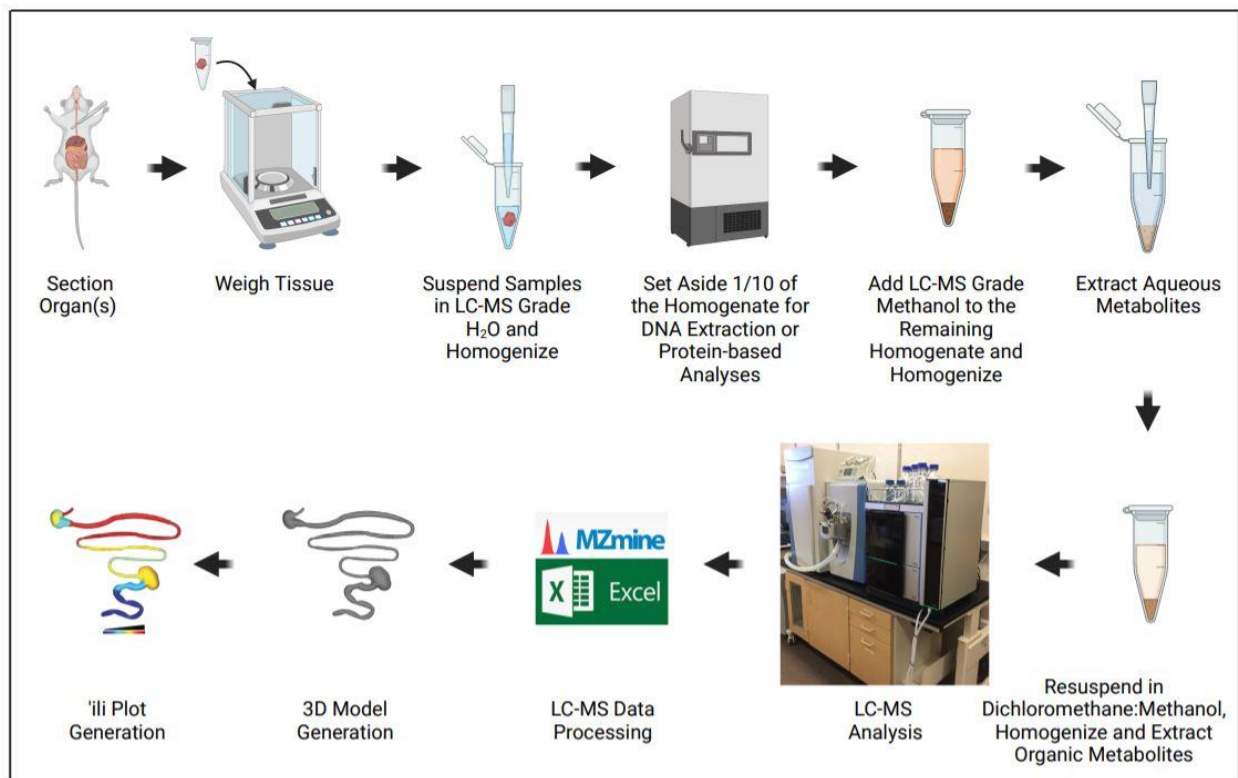


Fig. 10 Protocol overview. The illustration was created with BioRender.com.

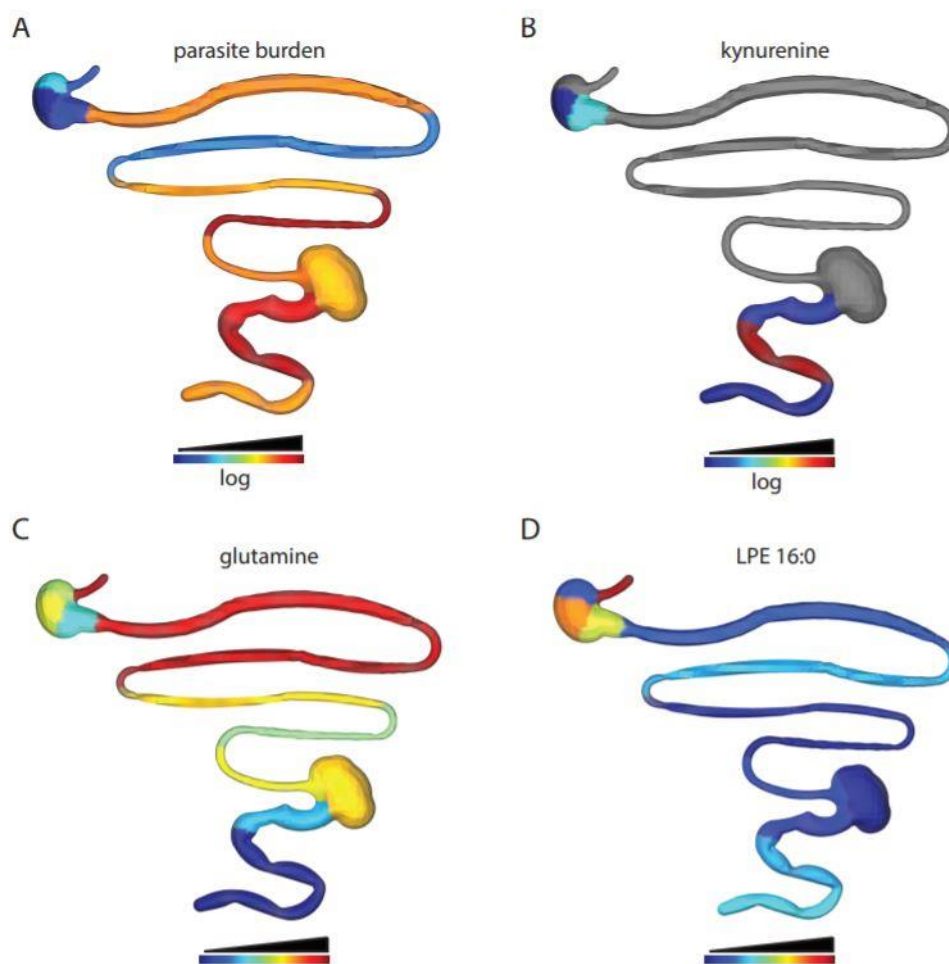


Fig. 11 Chemical cartography analysis.

Male C3H/HeJ were injected intraperitoneally with 1,000 CL+luc *T. cruzi* parasites³². Animals were euthanized 12 or 89 days, post-infection, and the gastrointestinal tract was collected and sectioned systematically (step 1)⁶. Metabolites were extracted as in step 2 and analyzed by LC-MS/MS. 3D model generation was performed using the SketchUp software (step 5), and data were plotted in 3D in step 6. **(A)** Parasite distribution in a specific mouse, 12 days post-infection. **(B)** Kynurenine metabolite distribution in the same mouse, 12 days post-infection. **(C)** Mean glutamine distribution across infected mice, 89 days post-infection. **(D)** Comparable levels of *m/z* 454.292 retention time 2.929 min, annotated as 2-hexadecanoyl-sn-glycero-3-

phosphoethanolamine (LPE 16:0), in the same mouse as in A and B in the small intestine and colon. Samples and data were generated in⁶.

3.6 Discussion

Understanding trypanosomatid infections is essential to guide novel drug development and treatment approaches. This chemical cartography method is uniquely poised to provide actionable insights into the relationship between metabolism and trypanosomatid disease pathogenesis, thus addressing this translational need.

Only LC-MS grade solvents are recommended during metabolite extraction and MS analyses, to lessen background contamination. Polymeric contamination³⁵, commonly derived from paraffin film and/or other plastics^{36–38}, must be avoided where possible. Parafilm, in particular, must never be used. These aspects are crucial since LC-MS data quality depends on the materials used during sample preparation and metabolite extraction. Data quality should be ensured before generating 'ili plots. In addition, generating these comprehensive spatial metabolomics maps requires the collection of all adjacent tissue samples and metabolite extraction from all collected samples to avoid gaps in these maps. Collection procedures, logistics of metabolite extraction and LC-MS analysis, and costs should thus be considered and planned accordingly.

This protocol can be modified to meet user needs in multiple ways. For example, the polarity and solubility of solvents used during metabolite extraction will influence what metabolites are detected³⁹. To maximize the diversity of detected metabolite features for untargeted chemical cartography analyses, combining multiple extraction steps and solvents is recommended. For example, this method utilizes dichloromethane, methanol, and water as extraction solvents because they enable accurate detection of nonpolar and polar molecules^{20,40}. However, these

solvents are not universally suitable for every MS experiment, and researchers should select extraction solvents based on the goals of their project. Likewise, different LC-MS/MS conditions can be used, such as replacing reversed-phase chromatography with normal phase chromatography. Alternative columns can also be used for reversed-phase data collection instead of C8 chromatography, though empirically, C8 chromatography is more robust to tissue lipids and has a lower clogging frequency. Conceptually, these protocols can also be applied to other mass spectrometry methods such as gas chromatography-mass spectrometry, etc.

An alternative approach is mass spectrometry imaging. Indeed, unlike mass spectrometry imaging approaches, liquid chromatography-mass spectrometry does not inherently preserve spatial information¹⁰. Chemical cartography approaches bridge this gap by including sampling location at the time of project conceptualization, in the sample metadata, and at data processing steps. A strength of this chemical cartography approach, unlike mass spectrometry imaging, is the ability to provide confident annotations (Metabolomics Standards Initiative level 1 or level 2 annotation confidence⁴¹), unlike mass spectrometry imaging where the bulk of applications rely on accurate mass only for annotation. Mass spectrometry imaging will enable fine-grained spatial mapping, sometimes down to the single-cell level, e.g.,^{42,43}. In contrast, chemical cartography approaches enable large-scale cross-organ mapping of metabolite distribution without requiring highly specialized whole-animal cryosectioning skills. Chemical cartography provides complementary evidence to the many spatial transcriptomic approaches being developed, e.g.,⁴⁴, with the advantage of focusing on the 'omics layer closest to the phenotype⁴⁵. Alternative methods for parasite load quantification include measuring bioluminescence at the time of sample collection⁶. Fine segments could also be collected to enable confocal or electron microscopy to assess localized parasite burden and tissue damage. The water homogenate, which

is used for cytokine quantification in this protocol and prior publications¹³, could also be used to quantify protein-based markers of tissue damage.

There are also multiple ways to obtain 3D models suitable to plot the resulting LC-MS data. In addition to the method suggested here, models can be purchased pre-made from various online vendors. Ensure that the terms of use match with the intended usage, especially concerning publication. Models for large organs can be generated de novo using 3D scanners according to scanner instructions. Alternatives such as MATLAB exist for generating and visualizing 3D models for chemical cartography⁴⁶, but they were primarily implemented before the development of 'ili¹⁶. MATLAB is a data analysis and programming tool suite offering a wide variety of applications across many fields. However, MATLAB is neither free nor open-source, and it requires familiarity with MATLAB interfaces, especially considering MATLAB was not developed for processing mass spectrometry data. This proposed method's alternatives, namely, SketchUp, Meshlab, and 'ili, are freely accessible, user-friendly, and offer similar functions as MATLAB for chemical cartography purposes.

This method is robust concerning sample preparation and metabolite extraction. Troubleshooting is most often necessary at the LC-MS data acquisition step. This is beyond the scope of this article. Readers are directed to excellent publications on LC-MS data acquisition troubleshooting, including^{20,47}. Likewise, the complexities of metabolite annotation are beyond the scope of this method's focus on 3D model generation. Useful references on this topic include^{24,25,48,49}.

While this method effectively explores disease pathogenesis, there are limitations to this approach, some of which are common across any metabolomics experiment. One such limitation is the low annotation rate of LC-MS features⁵⁰, which is contingent upon reference spectral

libraries' availability and quality. A further limitation is that this protocol does not preserve mRNA due to the incompatibility of RNA preservation reagents such as RNAlater with LC-MS/MS analysis. However, the protein quality is adequate for downstream analyses and thus can replace mRNA-based analyses.

A chemical cartography approach to infection pathogenesis directly reflects how bacterial, viral, or parasitic infections develop in organ systems and cause localized disease. Analyzing these regional subsamples and generating 3D models ultimately conveys how metabolites function across three-dimensional space, shedding light on these previously unrecognized spatial dimensions of molecular biology. Using this protocol, for example, metabolite localization was compared to *Trypanosoma cruzi* parasite load. Results clarified the relationship between the pathogen and host tissue and also demonstrated the metabolic dynamics of Chagas disease symptom progression⁶. Chemical cartography methods have also been applied to various topics, such as human-built environment interaction^{51–53}, the chemical makeup of organ systems like human skin⁴⁶ and lungs⁵⁴, and plant metabolism and environment interactions⁵⁵. Future applications can involve assessing localized disease tolerance and resilience, or the relationship between local metabolite levels, pathogen tropism, and disease tropism in models beyond trypanosomatid infection. This approach should also have broad applicability to expand current pharmacokinetics protocols, to assess the relationship between local tissue drug levels and drug metabolism vs. overall metabolic context, tissue damage, and pathogen clearance. Overall, chemical cartography allows unique explorations of metabolite distributions in various sample types, with applications, including disease pathogenesis, human health, human-environment interactions, and microbial dynamics.

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3.8 Disclosures

No conflicts of interest to report.

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Chapter 4: Spatial metabolomics reveals localized impact of influenza virus infection on the lung tissue metabolome

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Running Head title: Spatial metabolomics of IAV infection

Allocation of Contribution

This manuscript is currently pre-printed on Biorxiv and has undergone the peer review process at mSystems. Comments were received and the manuscript is currently being revised to be resubmitted. I am the first author on this manuscript. My contribution was the extraction of metabolites for LCMS analysis with technical assistance from Adwaita Parab, along with all analysis for data production (feature table production and data normalization) and statistical analysis (random forest analysis, variable importance list generation), with assistance for metabolite annotation, p-value calculation, venn diagram analysis and writing of the introduction by undergraduate London Klechka. I made and developed all figures and wrote the manuscript. Animal experimentation and sample collection was done by our collaborators at Oklahoma State University and LCMS data collection was done by Ekram Hossain.

4.1 Abstract

The influenza virus (IAV) is a major cause of respiratory disease, with significant infection increases in pandemic years. Vaccines are a mainstay of IAV prevention, but they are complicated by consideration of IAV's vast strain diversity, manufacturing and vaccine uptake limitations. While antivirals may be used for treatment of IAV, they are most effective in early stages of the infection and several virus strains have become drug resistant. Therefore, there is a need for advances in IAV treatment, especially host-directed, personalized therapeutics. Given the spatial dynamics of IAV infection and the relationship between viral spatial distribution and disease severity, a spatial approach is necessary to expand our understanding of IAV pathogenesis. We used spatial metabolomics to address this issue. Spatial metabolomics combines liquid chromatography-tandem mass spectrometry of metabolites extracted from systematic organ sections, 3D models and computational techniques, to develop spatial models of metabolite location and their role in organ function and disease pathogenesis. In this project, we analyzed plasma and systematically sectioned lung tissue samples from uninfected or infected mice. Spatial mapping of sites of metabolic perturbations revealed significantly lower metabolic perturbation in the trachea compared to other lung tissue sites. Using random forest machine learning, we identified metabolites that responded differently in each lung position based on infection, including specific amino acids, lipids and lipid-like molecules, and nucleosides. These results support the implementation of spatial metabolomics to understand metabolic changes upon IAV infection and to identify candidate pathways to be targeted for IAV therapeutics.

4.2 Importance

The influenza virus is a major health concern. Over 1 billion people become infected annually despite the wide distribution of vaccines, and antiviral agents are insufficient to address current clinical needs. In this study, we used spatial metabolomics to understand changes in the lung and plasma metabolome of mice infected with influenza A virus, compared to uninfected controls. We determined metabolites altered by infection in specific lung tissue sites and distinguished metabolites perturbed by infection between lung tissue and plasma samples. Our findings highlight the importance of a spatial approach to understanding the intersection between lung metabolome, viral infection and disease severity. Ultimately, this approach will expand our understanding of respiratory disease pathogenesis and guide the development of novel host-directed therapeutics.

4.3 Introduction

Influenza virus outbreaks are a continuous public health issue. Seasonal global epidemics caused by both influenza A viruses (IAV) and influenza B viruses cause 300,000-500,000 deaths each year (1). Vaccinations are the current method of prevention, but they fail to account for every possible viral strain. Antiviral drugs are used for treatment of IAV but are most effective within a short window during early infection. Additionally, it is believed that some strains have developed resistance to these drugs (2). One study indicated that while influenza A(H1N1)pdm09-infected intensive care unit patients treated with neuraminidase inhibitors have greater survival rates than untreated patients, one in four treated patients still die (3). These findings indicate a strong need for new treatments for IAV infection, and the potential for host-targeted therapeutics to supplement antiviral agents. Their development, however, necessitates an understanding of disease pathogenesis, which remains incompletely elucidated for IAV.

We used metabolomics to identify and analyze metabolites affected by IAV infection. Metabolomics is a method of analysis that focuses on small molecules involved in biological processes. This technique allows us to gain insight on the host chemical response to viral infection. A comprehensive understanding of the relationship between host and virus could aid in the development of more effective prevention and treatment options. Previous studies applied metabolomic methods to lung tissue and serum during IAV infection. These studies found that nucleosides such as uridine, lipids such as sphingosine, sphinganine, and amino acid metabolites such as kynurenine are increased during infection in lung tissue (4). Additionally, carbohydrates such as mannitol, myo-inositol and glyceric acid are decreased during active infection. However, location of IAV within the respiratory tract is dynamic. Viral localization and location of tissue damage within the respiratory tract also influences disease symptoms, disease severity and transmissibility of the infection (5, 6, 7, 8). Thus, a spatial perspective is necessary with regards to IAV and tissue metabolism. Spatial metabolomics is an approach that combines liquid chromatography-mass spectrometry (LC-MS) with 3D visualizations, leading to detailed spatial maps of metabolite distribution compared to pathogen load, tissue damage, or immune responses (9, 10, 11). This approach, when applied to other infectious diseases enabled the discovery of new treatments for these conditions (10). This method has been used to study the impact of cystic fibrosis on the local lung metabolome (12, 13), but had not previously been applied to IAV infection.

We therefore hypothesized that spatial metabolomics could provide new insights regarding IAV infection. Using this method, we analyzed the distribution of small (m/z 100-1,500) metabolites within the infected trachea and lung, in comparison to plasma samples and to uninfected animals. We identified changes in the lung metabolome and determined limited

overlap in metabolites perturbed by IAV infection between lung tissue and plasma. Additionally, we identified several metabolites altered by infection such as amino acids, lipids and lipid-like molecules, and nucleosides. Interestingly, these metabolites were differentially affected at each lung position. Ultimately, our study highlights the application of spatial metabolomics to understand IAV infection and further our understanding of respiratory disease pathogenesis, to guide the development of novel host-directed therapeutics.

4.4 Results

Viral distribution influences IAV transmissibility, viral reassortment and disease severity (5, 14, 15). While a few studies have investigated the changes in the metabolome during IAV infection, a spatial perspective of the metabolic disturbances is lacking (4, 16–18). We therefore used spatial metabolomics to identify candidate pathways and metabolites altered by infection in specific lung locations. Plasma, trachea and lungs were collected from IAV-infected mice at 3 days post-infection. Lungs were systematically sectioned into 11 segments (Fig. 1A), and all samples analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), followed by 3D reconstruction of metabolomics data. In addition, tissue homogenate bioluminescence was measured as an indicator of local viral burden in each tissue segment.

Using principal coordinate analysis (PCoA), we first compared lung and plasma metabolomes and found that the local lung tissue metabolome does not reflect the circulating metabolome (PERMANOVA <0.001 , Fig 12D). Furthermore, lung tissue overall was found to be greatly impacted by infection (PERMANOVA <0.001 , R^2 0.093931) (Fig 12E). A similar but non-significant, trend was seen between infected and uninfected plasma samples (Fig 12F).

Spatial analyses of lung tissue showed that viral load was largely localized to the lung tissue (positions 1-11) with minimal viral load in the trachea (position 12) (Dunn's test $p < 0.05$, FDR-corrected, for comparisons between trachea and all positions except left lung middle (position 3, Fig 12B)). Overall local impact of infection on the metabolome was quantified using PERMANOVA R^2 at each sampling site. Magnitude of metabolic perturbation was variable between tissue segments, with the highest degree of metabolic perturbation in two segments of the left lung (position 1 and position 4), and the right lung middle lobe (position 8), whereas the trachea metabolome was least affected (R^2 range from 0.05 in trachea to 0.18 at position 4; Fig 12C and Table S15). Thus, sites of highest and lowest metabolic perturbation match sites with high vs low viral load, respectively, enabling insight into the specific metabolites most affected by infection.

We next sought to determine whether the specific metabolites perturbed by infection differed between lung and plasma (Fig 12G-I). We used a random forest classifier, applied to plasma on the one hand, and to lung tissue on the other hand (all positions combined). After applying significance cutoffs (see Materials and Methods), this approach yielded a total list of 9 plasma metabolites and 374 lung tissue metabolites significantly perturbed by infection. There was strikingly limited overlap of infection-perturbed metabolites between lung tissue samples and plasma samples, indicating that both sites respond differentially to infection (Fig 12H) and concurring with overall PCoA analysis findings (Fig 12D). We then sought to assess whether the metabolites perturbed by infection were uniquely elicited by infection or found under both conditions but at differential levels. The majority of infection-perturbed metabolites were common to infected and uninfected samples but found at different levels, with a minority uniquely detected in uninfected samples only (Fig 12I). A similar trend was seen in infected and

uninfected plasma samples, where most infection-perturbed metabolites were present in both infected and uninfected samples, albeit at different levels (Fig 12G). This signifies that the effect of IAV infection on the metabolome is primarily on metabolite levels rather than induction of novel metabolites.

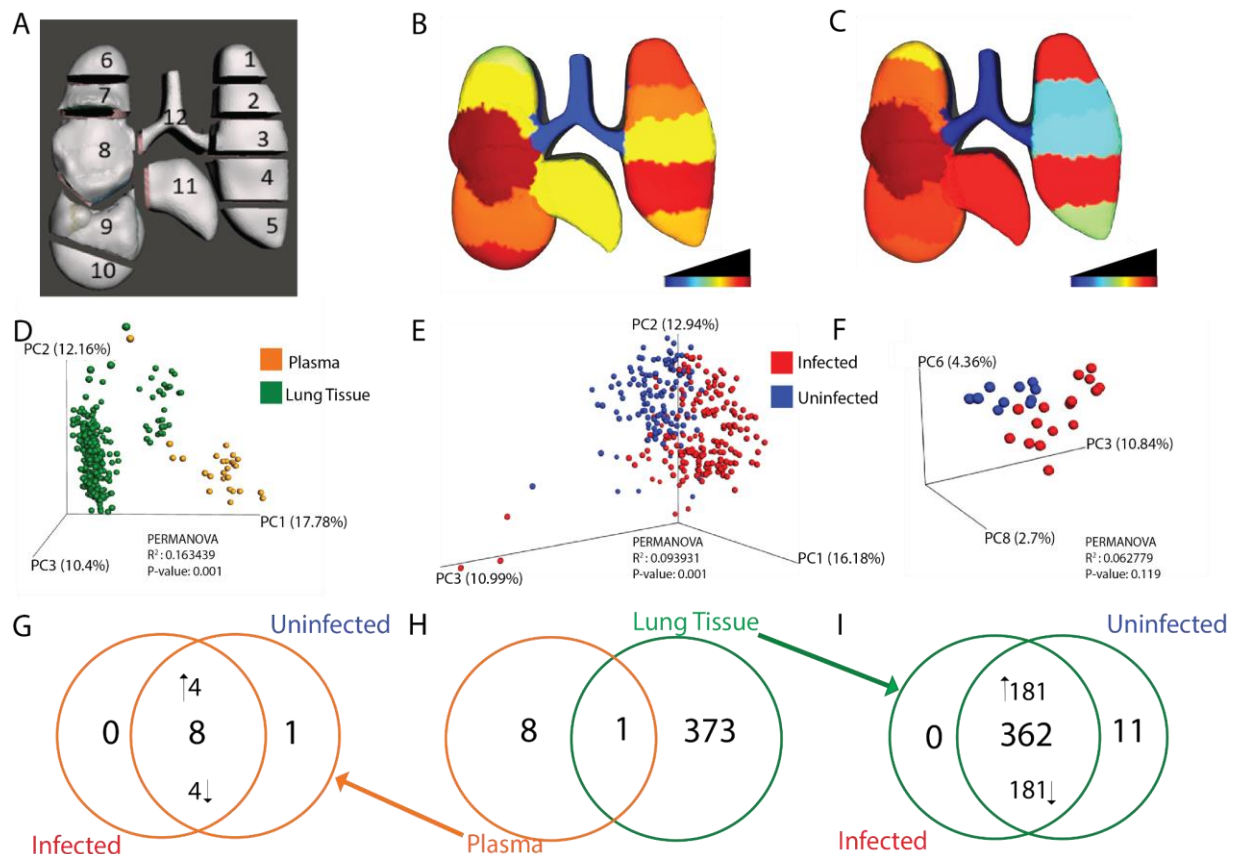


Fig. 12 Localized impact of IAV infection in lung tissue and plasma.

A: 3D model of lung tissue showing sampling positions. **B:** Median viral burden distribution of IAV. Lower infection levels were observed in the trachea (Dunn's test, FDR-corrected, $p < 0.05$ for comparisons between trachea and all positions except left lung middle (position 3)). **C:** Magnitude of metabolic perturbation compared to uninfected (PERMANOVA R^2). **D:** Principal coordinate analysis (PCoA) plot showing differences in overall metabolome by sample type (PERMANOVA p-value <0.001). **E:** Impact of infection on overall lung tissue metabolome

(PERMANOVA p-value <0.001). **F:** No significant impact of infection on the overall plasma metabolome by PCoA. **G-I:** Unique and common metabolites perturbed by infection **G:** Most metabolites perturbed by infection in plasma samples are found in both infected and uninfected samples, albeit at different levels. 4/8 metabolites were increased by infection and 4/8 were decreased by infection, while only one of the statistically significant metabolites was uniquely observed only in uninfected plasma samples. **H:** Overlap of metabolites perturbed by infection in plasma (orange) or lung tissue all positions combined (green). **I:** Most metabolites perturbed by infection in lung samples are found in both infected and uninfected samples, albeit at different levels (181/382 metabolites increased by infection and 181/362 decreased by infection, with 11 of the statistically significant metabolites uniquely detected in uninfected lung tissue).

Random forest classifier was also used to analyze the common and unique responses of each lung and tracheal tissue site to infection. A random forest model was built for each lung position and for plasma, classifying infected versus uninfected samples. Strikingly, most infection-impacted metabolites were only affected at one or a few tissue sites (Fig 13A). This is not due to divergence in overall metabolome between sites, as analysis of all metabolites, irrespective of abundance, revealed large commonality across lung tissue sites and plasma (Fig 13B). Likewise, infection-perturbed metabolites do not overlap appreciably with metabolites differing in abundance between lung and plasma (Fig S3). Very few perturbed metabolites were identified for position 12 (trachea), perhaps as a consequence of the low viral load at that site (Fig 12B).

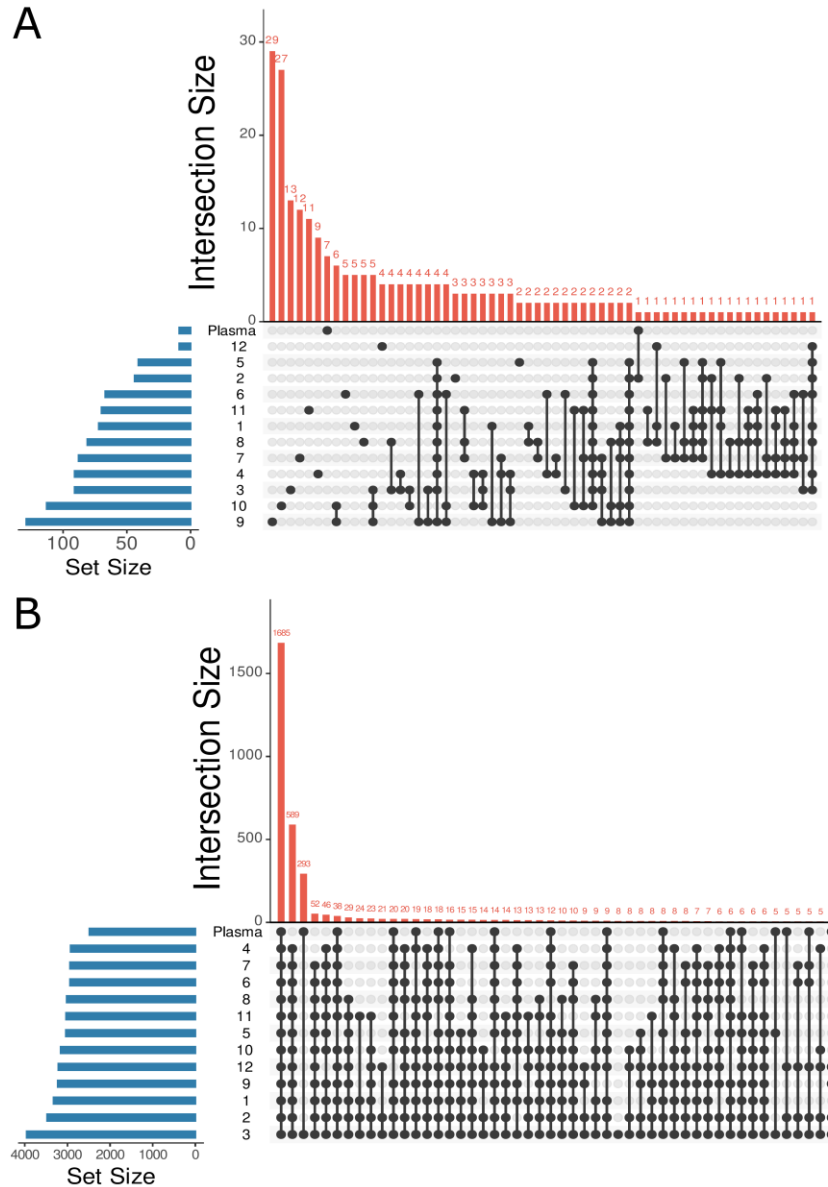


Fig. 13 Location-specific impact of IAV infection on metabolism.

A: UpSet plot (Number of intersections to show: 60) showing uniqueness of statistically significant infection-impacted metabolites in lung tissue and plasma perturbed, based on 13 random forest models analyzing each lung segment and plasma separately. B: UpSet plot (Number of intersections to show: 50) of total metabolites found in lung tissue and plasma showing large commonality across tissue sites and plasma. Position numbers as in Fig. 1A.

Metabolites perturbed by infection were annotated using molecular networking (19). Among these, carnitine, glutamine, kynurenine, and cytosine were found to be significantly and markedly perturbed by IAV infection and in a spatial manner (Fig 14, Fig S1). Glutamine, cytosine, and kynurenine were all significantly increased by IAV infection at all tissue sites except the trachea (Wilcoxon FDR-corrected p-value <0.05 at all sites except trachea) (Fig 14, Fig S1). The opposite trend was seen for carnitine which was decreased by infection at all tissue sites except the trachea (Wilcoxon FDR-corrected p-value <0.05 at all sites except trachea) (Fig 14, Fig S1). In contrast, these metabolites were not significantly affected by infection in the plasma.

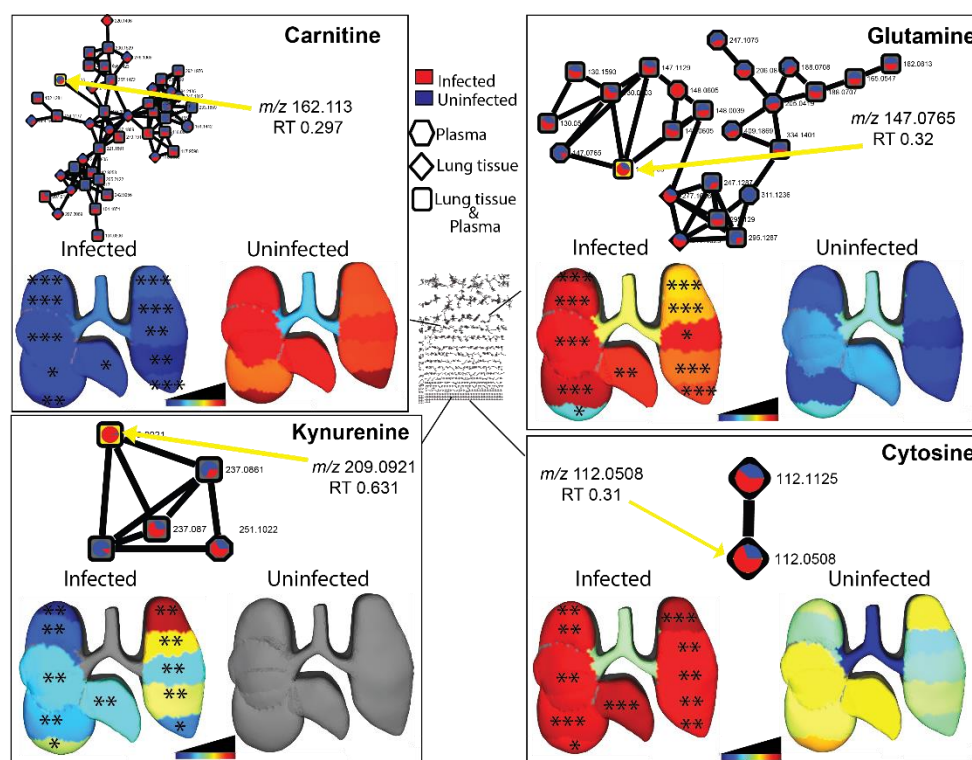


Fig. 14 Representative metabolites perturbed by IAV infection.

Molecular networks display summed peak area of metabolites in infected (red) and uninfected (blue) samples. Each connected node represents structurally-related metabolites, as determined

by molecular networking. 3D lung 'ili plots show median peak area of each displayed metabolite across tissue sites. Wilcoxon FDR-corrected p-values comparing matched infected and uninfected lung tissue sites * <0.05 ; ** <0.01 ; *** <0.001 .

Additional metabolites perturbed by infection in plasma and lung tissue include amino acids, acylcarnitines and nucleobases (Fig 15, Table S1, Table S2), with contrasting effects between lung tissue positions and sample types. For example, amino acids had dissimilar effects following IAV infection in plasma and lung tissue samples (Fig 15): methionine was decreased in infected plasma samples (Wilcoxon FDR-corrected p-value <0.001) while phenylalanine and L-citrulline were increased in infected lung tissue samples (Wilcoxon FDR-corrected p-value <0.05) (Fig 15 A, E-F). However, the dipeptide methionine-phenylalanine was decreased in infected lung tissue (Wilcoxon FDR-corrected p-value <0.05) (Fig 15K). Acylcarnitines (oleylcarnitine and palmitoylcarnitine) were significantly increased in infected plasma samples (Wilcoxon FDR-corrected p-value <0.001) compared to uninfected samples (Fig 15B-C). Guanine, S-adenosyl-homocysteine, spermidine, 4-hydroxynonenal, and hemin cation were all significantly increased upon infection (Wilcoxon FDR-corrected p-value <0.05) at select lung tissue sites (Fig 15F-H, G and L). Infection had the opposite effect on palmitamide, which was significantly decreased in infected tissue samples (Fig 15I). In contrast, none of these metabolites apart from phenylalanine were significantly affected by infection in the trachea.

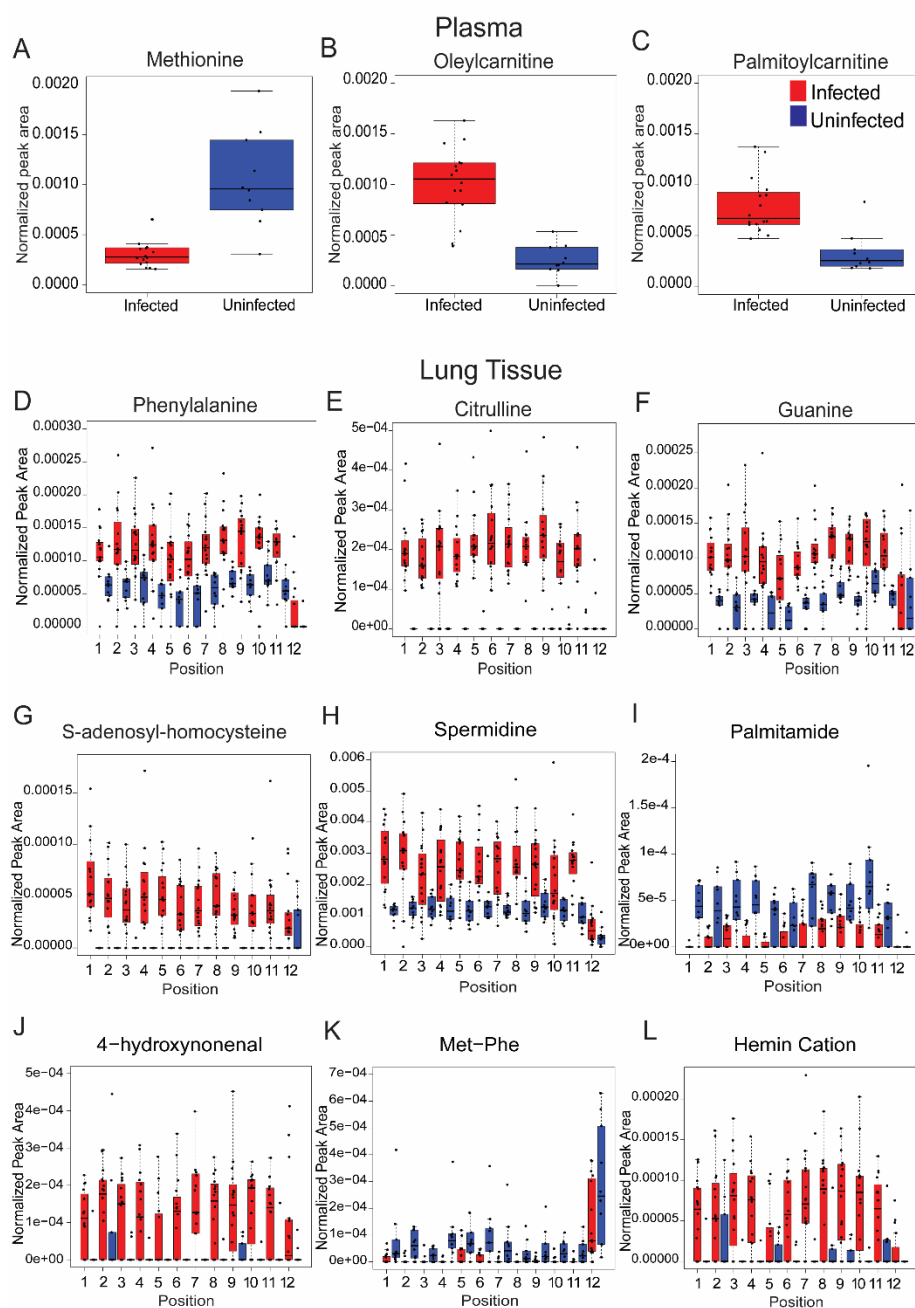


Fig. 15 Representative metabolites perturbed by IAV infection in plasma and lung tissue.

A-C: Statistically significant metabolites altered by infection in plasma (Wilcoxon FDR-corrected p-value <0.001). **A:** Methionine (m/z 150.058, RT 0.323 min). **B:** Oleyl L-carnitine (m/z 426.357, RT 2.982 min). **C:** Palmitoylcarnitine (m/z 400.341, RT 2.952 min). **D-L:**

Statistically significant metabolites altered by infection in lung tissue. **D:** Phenylalanine (m/z 166.042, RT 2.246 min) statistically different at all positions (Wilcoxon FDR-Corrected p-value <0.05) except positions 2, 5, 10 and 12. **E:** Citrulline (m/z 198.085, RT 0.309 min) statistically significant at all positions (Wilcoxon FDR-Corrected p-value <0.05) except position 12. **F:** Guanine (m/z 150.977, RT 0.291 min) statistically different at all positions (Wilcoxon FDR-Corrected p-value <0.05) except position 12. **G:** S-Adenosyl-homocysteine (m/z 385.212, RT 2.166 min) statistically significant at all positions (Wilcoxon FDR-Corrected p-value <0.05) except position 12. **H:** Spermidine (m/z 146.165, RT 0.287 min) statistically significant at all positions (Wilcoxon FDR-Corrected p-value <0.05) except positions 3, 10, and 12. **I:** Palmitamide (m/z 256.073 RT 2.563 min) statistically significant at all positions (Wilcoxon FDR-Corrected p-value <0.05) except positions 2, 5, 6, 7, 11, and 12. **J:** 4-Hydroxynonenal (m/z 139.112, RT 2.644 min) statistically significant at all positions (Wilcoxon FDR-Corrected p-value <0.05) except positions 1, 2, 3, 5, 11, and 12. **K:** Met-Phe (m/z 297.123, RT 2.444 min) statistically significant at all positions (Wilcoxon FDR-Corrected p-value <0.05) except positions 3, 4, 6, and 7. **L:** Hemin cation (m/z 616.358, RT 2.994 min) statistically significant at all positions (Wilcoxon FDR-Corrected p-value <0.05) except positions 3, 4, 9, and 10.

4.5 Discussion

Here, we generated spatial maps of the metabolic impact of IAV infection on the mouse lung and trachea. This approach revealed differential effects of infection across tissue sites and between lung and plasma, as well as differential viral burden between trachea and lung tissue. Based on random forest analysis and molecular networking, infection-impacted metabolites of biological significance were annotated as acylcarnitines, amino acids, phospholipids, and nucleotides, amongst others.

L-carnitine was significantly decreased by IAV infection at all lung positions except the trachea (Fig 14 top left). Likewise, short chain acylcarnitines such as CAR 2:0 and CAR 6:0 were also decreased in lung tissue (Table S3-14). In contrast, in both plasma and lung tissue, different long chain acylcarnitines were increased by infection, including oleyl carnitine (CAR 18:1) and palmitoylcarnitine (CAR 16:0) in plasma and CAR 14:1 and CAR 20:1 in lung tissue (Fig 4B-C, Table S1-14). Carnitines and acylcarnitines are key intermediates in energy production via fatty acid beta oxidation, and influenza virus replication is sensitive to fatty acid beta oxidation activity (20),(21). Likewise, these metabolic alterations may also be contributing to the differential responses to vaccination in obese vs lean animals (22).

Our study indicated that glutamine is increased upon IAV infection (Fig 14 top right). T cell proliferation and cytokine secretion relies heavily on glutamine presence (23). Thus, elevated glutamine levels may enable anti-viral immunity. IAV-infected cells are also more dependent on glutamine availability than uninfected cells for survival, suggesting a pro-survival effect of our observed elevated glutamine levels (24). Phenylalanine and citrulline were increased in infected lung tissue, in contrast with methionine, which was decreased in plasma (Fig 15D-E). Both phenylalanine and citrulline are involved in immune response by aiding in T-cell function (25). Phenylalanine aids in activation of T-cells while citrulline has downstream effects through the production of arginine which is used for T-cell growth and response (25–27). Upregulation of both amino acids suggest their role in overall immune response to IAV.

Nucleotides, cytosine and guanine were elevated by infection in all lung sites except trachea (Fig 14 bottom left and Fig 15F). Pyrimidine nucleotide biosynthesis is elevated upon IAV infection, and indeed its replication was dependent on pyrimidine biosynthesis (28–30).

Interestingly, cytosine was found discriminatory in the plasma between RT-PCR positive COVID patients and RT-PCR COVID negative patients (29).

Several of the metabolites annotated and identified as infection-impacted in the lung tissue and plasma in this study are congruent with prior studies of respiratory infection (4). Amino acids and nucleotides in particular were upregulated in lung tissue in other IAV studies as well as in *Mycobacterium tuberculosis* infection (TB) and respiratory syncytial virus (RSV) studies (4, 31–33). Phenylalanine was increased in mouse lung tissue in another IAV study and in the serum and lung of mice infected with TB, while L-citrulline is elevated in mouse lung tissue infected with RSV and in mice infected with TB (4, 17, 31, 32, 34). This coincides with our findings showing elevations of these amino acids in IAV-infected lung tissue (Fig 15D and E). Glutamine was identified in mouse lung tissue infected with TB and was elevated alongside other immune response amino acids, corresponding with our study (Fig 14 top right) (32, 34). Kynurenine, an infection-induced anti-inflammatory molecule, is consistently upregulated in mouse lung tissue of several respiratory infection studies including TB, RSV, and our IAV study (Fig 14 bottom left) (4, 31, 33, 34). Upregulation in cytosine in the lung tissue, oleylcarnitine in plasma, and several phospholipids in the lung tissue are also consistent with current literature (Fig 14 bottom right, Fig 15B and Table S1) (4, 17, 33).

Analysis of circulating metabolites has been performed in multiple studies on IAV vaccinology (35), (36). Our findings of differential impact of infection on lung and plasma metabolites (Fig. 12H, Table S1, Table S2) indicate that the metabolic changes observed in those studies may not be directly linked to lung metabolic patterns, and this discrepancy was not due purely to differences between plasma and lung overall (Fig. 13B). Likewise, the majority of COVID-19 metabolomic studies have relied on serum or plasma samples (37). By extension,

based on this study's results, they may not be relevant to the pathogenesis of COVID-19 in the lung, hampering translatability for drug development purposes. Thus, studies seeking to build on metabolomics to design new treatments should rely on analysis of the affected organ, rather than biofluids, even if the latter are more readily available.

We also observed differential impact of infection across lung sections, with the majority of infection-perturbed metabolites only significantly perturbed at one lung segment (Fig. 13A). The trachea in particular was especially divergent from the lung lobes in terms of viral burden (Fig. 12B), overall magnitude of infection-induced metabolic perturbations (Fig. 12C) and specific metabolic changes (Fig. 14, Fig. 15). Jointly, these results highlight the strength of our spatial perspective.

As with any untargeted metabolomics studies, a significant fraction of infection-impacted metabolites could not be annotated (~65%). The most commonly observed metabolite subclasses in our dataset overall were amino acids, peptides, and analogues, glycerophosphocholines, amines and fatty amides. Although this represents a broad diversity of metabolite classes, nevertheless complementary metabolite extraction or data acquisition methods could further expand this list. We further acknowledge that the mouse model of influenza virus infection and mouse-adapted influenza viral strain may not be the most representative of the functional lung alterations that would occur during human infection (38). Indeed, the predominance of lower respiratory tract metabolic alterations over changes in the trachea observed in this study may be a consequence of this model. A further limitation is that we focused on a single time point, in a lethal infection model. However, our findings serve as proof-of-concept of the applicability of our approach to study respiratory viruses, with future work applying this method in other biological systems.

We anticipate our findings to serve as a resource upon which the research community can build to study the impact of different disease modifiers on the relationship between spatial changes in the lung metabolome and disease severity, for example the impact of age, comorbidities or treatment. Our findings and our approach also serve as a framework to study how the metabolome is restored in a spatially-dependent fashion during recovery from respiratory infection, or it fails to recover during chronic disease in additional disease models, and to identify markers of treatment response and infection outcome. Overall, we anticipate our approach to be broadly applicable to many other respiratory infections, helping expand our understanding of respiratory disease pathogenesis and to drive the development of host-targeted therapeutic regimens.

4.6 Materials and Methods

4.6.1 *In vivo* infection

All vertebrate animal studies were performed under a protocol approved by Oklahoma State University Institutional Animal Care and Use Committee (protocol number VM20-36), in accordance with the USDA Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Female C57BL/6J mice were anesthetized with ketamine and xylazine intraperitoneally and then intranasally inoculated with PR8-Glu (39) (a generous gift from Dr. Peter Palese, Icahn School of Medicine at Mount Sinai, Department of Microbiology, New York, New York, USA) at 2×10^3 pfu/mouse in 25-50 μ l PBS. Controls were inoculated with PBS alone. Body weights were monitored daily. Three days later, mice were anesthetized with ketamine and xylazine and injected IV with a working solution of coelenterazine (GoldBio, St. Louis, Mo.) made according to standard protocol (40). Briefly, using a stock solution of 7.5 mg/ml in acidified alcohol, each

mouse was administered 98 µg substrate in PBS (13 µl stock in 137 µl PBS) into the retroorbital sinus. After substrate administration, the thoracic cavity was opened followed by exsanguination by cardiac blood collection for serum and by lung tissue harvesting. Upper trachea, cranial to tracheal bifurcation, was also collected. Subsequently, samples were submerged in coelenterazine 0.3 mg/ml in a 96 well plate. Once collected the tissue pieces were blotted off and flash frozen in liquid nitrogen for later analyses.

4.6.2 Metabolite extraction and UHPLC-MS/MS

A two-step metabolite extraction procedure was performed according to Want *et al.*(41) for both tissue and plasma samples. Samples were homogenized in LC-MS grade water with steel beads utilizing a Qiagen TissueLyzer at 25 Hz for 3 min, and 1 µl removed for luminescence analysis. Methanol was added for a final concentration of 50%, samples homogenized again for 3 mins and centrifuged at 16,000xg for 10 mins at 4°C. The supernatant (aqueous extract) was collected, dried overnight in a Speedvac and frozen at -80°C until LC-MS analysis. The pellet produced from centrifugation was collected for organic extraction via addition of 3:1 dichloromethane:methanol, homogenized for 5 mins and centrifuged for 10 mins at 4°C. The organic extract was air dried overnight and then frozen at -80°C until LC-MS analysis. Dried aqueous and organic extracts were resuspended in 1:1 methanol and water spiked with the internal standard sulfadimethoxine and combined. Samples were then sonicated, centrifuged, and the supernatant collected for analysis. A Thermo Scientific Vanquish UHPLC system was used for tissue and plasma analysis using a Kinetex 1.7µm C8 100 Å LC column (50 x 2.1mm). Chromatography was done with water + 0.1% formic acid (mobile phase A) and acetonitrile + 0.1% formic acid (mobile phase B), at a 0.5 mL/min flow rate (7.5 mins) with a 40°C column temperature. LC gradient can be found in Table 8. Data acquisition was performed

in random sample order, with a blank and pooled quality control every 12 samples. To monitor instrumental drift, a 6-mix solution with 6 known molecules was run at the beginning and end of LC/MS/MS analysis. Calibration of the instrument was also done immediately prior to instrument analysis using Pierce LTQ Velos ESI positive ion calibration solution. MS/MS detection was conducted on a Q Exactive Plus (Thermo Scientific) high resolution mass spectrometer (Table 9). Ions were generated for MS/MS analysis in positive mode.

Table 1 LC Gradient

Time(min)	Flow (ml/min)	%B	Curve
0.000	Run		
0.000	0.500	2.0	5
1.000	0.500	2.0	5
2.500	0.500	98.0	5
4.500	0.500	98.0	5
5.500	0.500	2.0	5
7.500	0.500	2.0	5
7.500	Stop Run		

Table 2 Q Exactive Plus (Thermo Scientific) instrument parameters

Runtime	0 to 7.5 min
Polarity	Positive
Default charge state	1
Full MS	
Resolution	70,000
AGC target	3e6
Maximum IT	246 ms
Scan range	100 to 1500 <i>m/z</i>
dd-MS2/dd-SIM	
Resolution	17,500
AGC target	1e5
Maximum IT	54 ms
Loop count	5
TopN	5
Isolation window	1.0 <i>m/z</i>
(N)ce/stepped (N)CE	NCE: 20, 40, 60
dd Settings	
Minimum AGC target	8.00e3
Intensity Threshold	1.5e5

Peptide match	preferred
Exclude isotopes	on
Dynamic exclusion	10.0s
Tune Data	
Spray Voltage (+)	3800.00
Capillary Temperature (+ or +/-)	320.00
Sheath Gas (+ or +/-)	35.00
Aux Gas (+ or +/-)	10.00
Spare Gas (+ or +/-)	0.00
Max Spray Current (+)	100.00
Probe Heater Temp (+ or +/-)	0.00
S-Lens RF Level	50.00
Ion Source	HESI

4.6.3 Luminescence analysis

Coelenterazine was prepared according to GoldBio standard protocols. Briefly, 1 mg coelenterazine was added to 1 mL of acidified methanol to make a stock solution. The stock solution was made to a final concentration of 1.5 μ M. A 1:10 solution of sample homogenate to coelenterazine was analyzed on a GloMax Explorer (Promega).

4.6.4 LC-MS Data analysis

Data analysis was performed using MZmine version 2.53, according to Table 10 parameters, to develop the feature table for further analysis. Blank removal with a 3-fold threshold was performed and Jupyter notebooks were used to perform total ion current (TIC) normalization. Principal coordinate analysis (PCoA) was performed on the TIC-normalized MS1 feature table using the Bray-Curtis dissimilarity metric in QIIME2 (42). Three-dimensional PCoA plots were developed and visualized using EMPeror (43). Lung three-dimensional model developed using Sketchup and Mesh lab, and modelling was completed using ‘ili (<http://ili.embl.de/>) (44).

Random forest analysis was conducted using R in Jupyter notebooks. Number of trees were restricted to 500 and random forest classifier cutoff was based on variable importance score of mean decrease accuracy >1. Lists were further restricted to FDR-corrected Mann-Whitney p-value less than 0.05 and fold change <0.05 or >2.0. Venn diagrams and UpSet plots were developed to quantify metabolite overlap within lung tissue positions and between lung tissue and plasma using Intervene Shiny App (45).

Global Natural Products Social Molecular Networking (GNPS) was used to perform feature-based molecular networking and MolNetEnhancer according to the parameters in Table 11. Cytoscape 3.8.2. was used to visualize all molecular networks. All reported annotations are at Metabolomics Standards Initiative confidence level 2 (specific metabolite name provided) or level 3 (metabolite family name provided only) (46). Lipids were annotated based on GNPS library or analog matches and using standard LipidMAPS nomenclature (47).

Table 3 MZmine 2.53 parameters

MS ¹	
Retention Time (min)	0-7.5
Noise Level	5E5
MS ²	
Retention Time (min)	0-7.5
Noise Level	1E3
Chromatogram Builder	
Mass list	Masses
Minimum Time span (min)	0.01
Minimum Height	1.5E6
m/z Tolerance (ppm)	10.0
Algorithm	Baseline cut-off
Chromatogram Deconvolution	
<i>m/z</i> Range for MS ² scan pairing (Da)	0.001
RT Range for MS ² Scan pairing (min)	0.2
Minimum peak height	1.5E6
Peak duration range (min)	0-3.5
Baseline level	5E5
Deisotoping	

<i>m/z</i> tolerance (ppm)	10.0
Retention time tolerance (min)	0.5
Monotonic shape	Yes
Maximum Charge	3
Representative Isotope	Most intense
Alignment	
<i>m/z</i> Tolerance (ppm)	10.0
Weigh for <i>m/z</i>	1
Weight for Retention time	1
Retention time tolerance (min)	0.5
Row filtering	
Minimum peaks in a row	3
Retention time (min)	0.2-6.5
Keep only peaks with MS ² scans	YES
Reset Peak No. ID	YES

Table 4 GNPS Parameters

Feature-Based Molecular Network	
Precursor Ion Mass Tolerance	0.02
Fragment Ion Mass Tolerance	0.02
Minimum Matched Fragment Ions	4
Maximum Connected Component Size (Beta)	100
Maximum shift between precursors	500
Library Search Min Matched Peaks	4
Search analogs	Do Search
Top results to report per query	1
Score Threshold	0.7
Maximum analog difference	100.0 Da
Minimum Peak Intensity	0.0
Filter Precursor Window	Filter
Filter peaks in 50 Da Window	Filter
Filter Library	Filter Library
Normalization per file	Row Sum Normalization (Per file Sum to 1,000,000)
Aggregation Method for peak abundances per group	Mean
PCoA	braycurtis

Run Dereplicator	Run
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4.6.5 Data availability: All metabolomics data is publicly available in MassIVE under accession number MSV000085389 (<ftp://massive.ucsd.edu/MSV000085389>).

MOLNet Enhancer link:

<https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=f9f194c9b723409f8927c84470f9a0c5>

Original GNPS link:

<https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=bb7a5f7fb32045208b6040908a1453f7>

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Dissertation Conclusions

The purpose of this dissertation was to highlight the effectiveness of LCMS/MS based metabolomics as a tool to further understand some of the world's less understood and understudied infectious diseases. In chapter 1, this method allowed the identification of two distinct chemical families altered by chronic *T. cruzi* infection in the heart of infected mice. Because of the identification of specific alterations in chronic cardiac CD, we determined a potential drug to be used to treat chronic cardiac CD which is in great need. In chapter 2, I emphasized the importance of novel diagnostics for CD. Using LCMS/MS based metabolomics, I identified potential urine-based biomarkers that can be used to help monitor CD disease progression. This non-invasive sample type can reshape current diagnostics methods which are all invasive. In addition, I implemented the technique in a proof-of-concept study to determine treatment success versus failure in benznidazole treated mice. Further work is being done to determine specificity and sensitivity of these potential biomarkers and their universality across multiple *T. cruzi* strains. Chapter 3 emphasizes step by step how spatial metabolomics is done and alludes to several studies it has been performed in and its success. In chapter 4, I used the same technique in IAV. Based on this study I was able to shed light on IAV pathogenesis by determining that circulating chemical profile is not reflective of the lung tissue. In addition, I identified several metabolites altered by infection and show that select positions of the lung respond differently to infection. Future work of this chapter is being done by the McCall lab by applying this technique to other infectious diseases such as the coronavirus which is the cause of the current pandemic.

This dissertation ultimately serves as a foundation for drug discovery and development, therapeutics, and biomarkers of treatment or disease presence. My hope is that it also a steppingstone to bridging the gap to understanding some of the world's most under-studied or still poorly understood diseases.

It is important to note that this dissertation was largely carried out and written in a global pandemic caused by the coronavirus disease (COVID-19). Because this pandemic occurred during the course of my research, it has only highlighted the importance of the need to understand infectious diseases. While there have been COVID outbreaks in the past and there have been decades of research done on the virus, there is still no effective treatment. We still don't fully understand its disease pathogenesis and there are several strains evolving from month to month. This adds an extra layer of complexity to the disease. Therefore, we need to continue to come up with novel ways that will effectively and efficiently help us research diseases like COVID-19 before we no longer have the resources too.

Unfortunately, we have come to the point that we can only live alongside most infectious diseases and sadly they may not be fully eradicated due to individual personal morals, politics, lack of financial and economic resources, and education amongst others. As a scientist and researcher, this dissertation is one of the many contributions I have made and will continue to make, not only to the scientific community, but to the world. It is a foundation to understanding the known and lead to advances into the unknown.

Appendix A: Chapter 1 Supporting Information

S1 Table. Annotated and unannotated metabolites perturbed by infection at position A, identified through random forest

classifier. * indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.

S1 Table. Annotated and unannotated metabolites perturbed by infection at position A, identified through random forest classifier.									
* indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.									
Top-ranking metabolites for <i>T. cruzi</i> strain CL that met the random forest cut-off criteria in descending order of variable importance									
<i>m/z</i>	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position A (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position A (CL vs uninfected 90 days)
503.307	145	aqueous	NA	NA	NA	NA	NA	0.594695151	0.530966952
241.155	26	aqueous	NA	NA	NA	NA	NA	0.456444776	0.504221396
298.275	213	aqueous	NA	NA	NA	NA	NA	0.129605115	0.175604238
343.296	177	aqueous	NA	NA	NA	NA	NA	0.253353722	0.128883009
296.259	187	aqueous	NA	NA	NA	NA	NA	0.272715174	0.125902588
320.256	206	aqueous	NA	NA	NA	NA	NA	0.022023425	0.078623652
252.233	190	aqueous	NA	NA	NA	NA	NA	0.372939278	0.131648425
336.251	190	aqueous	NA	NA	NA	NA	NA	0.17749415	0.076029815
250.095	66	aqueous	NA	NA	NA	NA	NA	0.199314049	0.394219535
316.285	191	aqueous	NA	NA	NA	NA	NA	0.178989857	0.118319978
211.145	151	aqueous	NA	NA	NA	NA	NA	0.329213216	0.300952612
320.256	217	aqueous	NA	NA	NA	NA	NA	0.035920607	0.086372244
204.124	24	aqueous	CAR 2:0 (Spectral Match to Acetyl-DL-carnitine)	1	7	0	1.96	0.59385512	0.467715934
256.229	193	aqueous	NA	NA	NA	NA	NA	0.419086334	0.456903336
278.248	179	aqueous	NA	NA	NA	NA	NA	0.326549246	0.087968293
331.166	58	aqueous	NA	NA	NA	NA	NA	0.436241611	0.663908046
380.21	258	aqueous	NA	NA	NA	NA	NA	0.87802152	0.616748749
327.234	214	aqueous	NA	NA	NA	NA	NA	0.07756311	0.578203196
634.249	187	aqueous	NA	NA	NA	NA	NA	0.581128056	1.525983325

248.151	47	aqueous	CAR 4:0:0 (Spectral Match to Acetyl-DL-carnitine)	0.82	4	44.03	7.25	0.19306014	0.474745429
280.264	206	aqueous	NA	NA	NA	NA	NA	0.176388841	0.121414103
523.359	212	aqueous	NA	NA	NA	NA	NA	1.981822665	3.182957714
931.451	189	aqueous	NA	NA	NA	NA	NA	0.056617687	0.070183096
170.118	147	aqueous	NA	NA	NA	NA	NA	0.394073761	0.273658863
209.093	53	aqueous	NA	NA	NA	NA	NA	2.201371405	7.567440512
546.354	219	aqueous	PC O-20:3 (Spectral Match to 1-Myristoyl-sn-glycero-3-phosphocholine)	0.91	13	56.06	2.56	2.120747423	4.628094744
366.374	314	organic	NA	NA	NA	NA	NA	1.168545783	0.27997189
768.554	519	organic	NA	NA	NA	NA	NA	0.674201972	0.327429678
280.265	177	organic	NA	NA	NA	NA	NA	0.537788917	0.160416287
511.521	218	organic	NA	NA	NA	NA	NA	0.109383746	0.032875399
284.239	155	organic	NA	NA	NA	NA	NA	0.951631368	0.274756316
269.09	26	organic	NA	NA	NA	NA	NA	3.412997876	6.013739244
280.265	250	organic	NA	NA	NA	NA	NA	0.609945963	0.281578577
537.395	389	organic	NA	NA	NA	NA	NA	2.583479789	0.499061093
625.515	243	organic	NA	NA	NA	NA	NA	0.197392956	0.191576551
802.574	299	organic	PC 36:2:0 (Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine)	0.99	18	41.98	26.9	1.044560554	1.435563261
401.355	255	organic	NA	NA	NA	NA	NA	0.751243923	1.944515313
754.572	399	organic	NA	NA	NA	NA	NA	1.221445835	1.969421558
250.132	136	organic	NA	NA	NA	NA	NA	0.696956799	0.359958578
566.274	152	organic	NA	NA	NA	NA	NA	1.881222558	2.008358464
797.621	393	organic	NA	NA	NA	NA	NA	2.303494943	1.712426335
857.569	223	organic	NA	NA	NA	NA	NA	1.067696313	0.655079892
606.377	191	organic	PC 22:2:0 (Spectral Match to 1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine)	0.98	15	68.06	0.82	1.698363604	1.180186534
856.564	222	organic	NA	NA	NA	NA	NA	1.1377682	0.597262751
496.341	201	organic	NA	NA	NA	NA	NA	1.174316426	6.133262311
809.563	308	organic	NA	NA	NA	NA	NA	1.120979325	2.220711579
810.525	252	organic	NA	NA	NA	NA	NA	0.729753972	0.687562688
850.632	417	organic	PC 41:5 or PC O-41:6:0 (Spectral Match to 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine)	0.99	11	68.06	0	1.014296722	0.530055951
660.554	234	organic	NA	NA	NA	NA	NA	1.092798649	0.152943874
508.377	187	organic	NA	NA	NA	NA	NA	3.830926104	2.124899945

802.56	380	organic	PC family member (Spectral Match to 1-Octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine) *	0.88	9	8.03	NA	1.252018571	1.913370954
Top-ranking metabolites for <i>T. cruzi</i> strains CL and Sylvio X10/4 that met the random forest cut-off criteria in descending order of variable importance									
<i>m/z</i>	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position A (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position A (CL vs uninfected 90 days)
562.389	219	organic	NA	NA	NA	NA	NA	2.031990521	2.308188266
Top-ranking metabolites for <i>T. cruzi</i> strain Sylvio X10/4 that met the random forest cut-off criteria in descending order of variable importance									
<i>m/z</i>	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position A (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position A (CL vs uninfected 90 days)
550.244	144	aqueous	NA	NA	NA	NA	NA	0.541230573	0.797393387
332.244	158	aqueous	CAR 10:0:O (Spectral Match to Palmitoylcarnitine)	0.87	4	68.1	2.71	0.172393354	0.74357277
248.15	30	aqueous	CAR 4:0:O2 (Spectral Match to Acetyl-DL-carnitine)	0.82	4	44.03	3.22	0.194880471	0.719601923
816.575	273	aqueous	PC O-40:8 (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.96	13	55.99	18.61	0.503269618	1.787370284
436.211	150	aqueous	NA	NA	NA	NA	NA	0.333186954	0.650965316
200.069	18	aqueous	NA	NA	NA	NA	NA	0.546417851	0.954823331
354.265	195	aqueous	NA	NA	NA	NA	NA	2.058267692	1.706105568
444.369	204	aqueous	CAR 18:0:O (Spectral Match to Oleoyl L-carnitine)	0.81	5	18.01	1.58	0.07938026	1.147417381
480.201	147	aqueous	NA	NA	NA	NA	NA	0.493561449	1.087065629
494.215	147	aqueous	NA	NA	NA	NA	NA	0.575857869	0.997660938
749.424	194	aqueous	NA	NA	NA	NA	NA	0.315441176	0.938067944
276.181	104	aqueous	NA	NA	NA	NA	NA	0.21682796	0.701658218
300.085	58	aqueous	NA	NA	NA	NA	NA	0.461558661	0.644552407
518.182	146	aqueous	NA	NA	NA	NA	NA	0.282710803	0.73335047
384.179	139	aqueous	NA	NA	NA	NA	NA	0.530749091	0.719988706
412.307	179	aqueous	CAR 16:2:O (Spectral Match to Lauroylcarnitine)	0.8	4	68.03	3.15	0.246340647	0.66482436
414.322	186	aqueous	CAR 16:1:O (Spectral Match to Palmitoylcarnitine)	0.85	7	13.98	1.45	0.230864525	0.558156672
260.187	148	aqueous	CAR 6:0 (Spectral Match to Acetyl-DL-carnitine)	0.96	5	56.06	5.38	0.245706519	0.588928695
299.201	171	aqueous	NA	NA	NA	NA	NA	2.15653622	1.421556551
302.207	157	organic	NA	NA	NA	NA	NA	0.387062481	1.26836379
442.354	175	organic	CAR 18:1:O (Spectral Match to Palmitoylcarnitine)	0.87	8	42.01	2.94	0.238358659	1.008777332
720.59	455	organic	NA	NA	NA	NA	NA	1.775442709	1.590526091
796.615	397	organic	NA	NA	NA	NA	NA	2.185744171	1.728787488

470.385	189	organic	CAR 20:1:O (Spectral Match to Palmitoylcarnitine)	0.87	8	70.04	2.13	0.135411785	0.521174237
570.349	202	organic	PC family member (Spectral Match to PAF C-16) *	0.99	18	45.98	NA	1.859193946	0.569340904
582.414	187	organic	NA	NA	NA	NA	NA	2.707283998	3.075774135
213.21	42	organic	NA	NA	NA	NA	NA	0.325333333	0.639621366
386.291	157	organic	CAR 14:1:O (Spectral Match to Lauroyl L-carnitine)	0.78	4	42.01	2.33	0.264585899	0.932151568
482.42	231	organic	CAR 22:1 (Spectral Match to Palmitoylcarnitine)	0.8	6	82.08	0.83	0.2744204	0.688301902
746.605	457	organic	PC family member (Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine) *	0.87	16	40	NA	1.68609124	1.37276495
360.275	153	organic	NA	NA	NA	NA	NA	0.184299287	0.965485176
454.389	250	organic	NA	NA	NA	NA	NA	0.337953845	1.06506658

S2 Table. Annotated and unannotated metabolites perturbed by infection at position B, identified through random forest classifier. * indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.

S2 Table. Annotated and unannotated metabolites perturbed by infection at position B, identified through random forest classifier.									
* indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.									
Top-ranking metabolites for <i>T. cruzi</i> strain CL that met the random forest cut-off criteria in descending order of variable importance									
<i>m/z</i>	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position B (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position B (CL vs uninfected 90 days)
556.443	228	aqueous	NA	NA	NA	NA	12.58	1.3125	0.252072
296.259	187	aqueous	NA	NA	NA	NA	NA	0.228585	0.039898
560.334	180	aqueous	NA	NA	NA	NA	NA	1.05836	1.812023
332.28	175	aqueous	NA	NA	NA	NA	NA	0.290813	0.029855
154.059	19	aqueous	NA	NA	NA	NA	NA	0.953964	7.862954
270.244	200	aqueous	NA	NA	NA	NA	NA	0.514964	0.08603
241.155	26	aqueous	NA	NA	NA	NA	NA	0.820536	0.620669
336.251	190	aqueous	NA	NA	NA	NA	NA	0.214577	0.064627
252.233	190	aqueous	NA	NA	NA	NA	NA	0.524686	0.076855
280.264	206	aqueous	NA	NA	NA	NA	NA	0.29916	0.036026
312.254	194	aqueous	NA	NA	NA	NA	NA	0.501089	0.08075
320.256	206	aqueous	NA	NA	NA	NA	NA	0.114979	0.021714
478.185	141	aqueous	NA	NA	NA	NA	NA	0.376556	0.51199
316.285	191	aqueous	NA	NA	NA	NA	NA	0.267304	0.050385
670.408	164	aqueous	NA	NA	NA	NA	NA	1.024351	1.414171
588.111	138	aqueous	NA	NA	NA	NA	NA	0.093809	0.254114
270.181	150	aqueous	NA	NA	NA	NA	NA	0.741517	1.400178
320.256	217	aqueous	NA	NA	NA	NA	NA	0.126656	0.022758
832.558	227	organic	PC 36:3/O3 (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.97	11	71.98	14.2	1.218046	1.504673
600.468	199	organic	NA	NA	NA	NA	NA	1.06453	2.467949
280.265	177	organic	NA	NA	NA	NA	NA	0.704212	0.117913
381.263	212	organic	NA	NA	NA	NA	NA	0.032047	1.67034
550.387	199	organic	LPC 20:1 or LPC O-20:2:O or PC O-20:1 (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.98	19	28.03	0.54	0.354028	0.589754
362.097	20	organic	NA	NA	NA	NA	NA	0.948276	0.491553
780.473	144	organic	NA	NA	NA	NA	NA	0.86712	0.740715

153.041	28	organic	NA	NA	NA	NA	NA	1.267689	1.953456
209.093	57	organic	NA	NA	NA	NA	NA	4.031316	2.107946
241.156	20	organic	NA	NA	NA	NA	NA	0.926558	0.432981
623.319	185	organic	NA	NA	NA	NA	NA	1.152041	0.645975
640.589	337	organic	NA	NA	NA	NA	NA	0.471197	0.248592
480.309	182	organic	NA	NA	NA	NA	NA	0.421359	0.527827
744.555	323	organic	PC 33:2 or PC O-33:3:O (Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine)	0.94	12	42.05	1.61	1.066837	0.247263
Top-ranking metabolites for <i>T. cruzi</i> strain Sylvio X10/4 that met the random forest cut-off criteria in descending order of variable importance									
m/z	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position B (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position B (CL vs uninfected 90 days)
182.082	32	aqueous	tyrosine	0.97	5	0	5	1.329797	0.682664
299.201	171	aqueous	NA	NA	NA	NA	NA	2.082799	1.854234
398.195	143	aqueous	NA	NA	NA	NA	NA	0.553474	0.539181
568.324	162	aqueous	NA	NA	NA	NA	NA	0.442155	1.026793
392.114	50	aqueous	NA	NA	NA	NA	NA	0.238332	0.964053
412.2	132	aqueous	NA	NA	NA	NA	NA	0.625535	0.665636
568.325	190	aqueous	NA	NA	NA	NA	NA	0.523092	1.038727
302.216	178	aqueous	NA	NA	NA	NA	NA	0.17251	1.618517
332.244	158	aqueous	CAR 10:0:O (Spectral Match to Palmitoylcarnitine)	0.87	4	68.1	2.71	0.108901	1.662846
336.224	197	aqueous	NA	NA	NA	NA	NA	2.074967	2.150649
480.343	212	aqueous	PC family member (Spectral Match to 1-(1Z-Hexadecenyl)-sn-glycero-3-phosphocholine) *	0.98	16	0	NA	3.698226	1.605736
492.2	144	aqueous	NA	NA	NA	NA	NA	0.740092	0.767561
534.319	181	aqueous	LPC 18:3:O (Spectral Match to 1-(9Z-Octadecenyl)-sn-glycero-3-phosphocholine)	0.98	17	11.96	0	0.695607	1.37961
264.17	136	aqueous	NA	NA	NA	NA	NA	1.705645	0.804868
386.291	175	aqueous	CAR 14:1:O (Spectral Match to Palmitoylcarnitine)	0.86	6	14.05	2.33	0.347334	1.423758
399.252	222	aqueous	NA	NA	NA	NA	NA	1.690399	0.162109
434.16	135	aqueous	NA	NA	NA	NA	NA	0.591587	0.65549
440.338	190	aqueous	CAR 18:2:O (Spectral Match to Palmitoylcarnitine)	0.85	5	40	2.27	0.075863	2.083664
412.307	179	aqueous	CAR 16:2:O (Spectral Match to Lauroylcarnitine)	0.8	4	68.03	3.15	0.110111	1.447753
520.34	216	aqueous	LPC 18:2 or LPC 18:3:O (Spectral Match to 1-(9Z-Octadecenyl)-sn-glycero-3-phosphocholine)	0.98	20	2.01	0.38	0.711253	1.216008
838.267	187	aqueous	NA	NA	NA	NA	NA	0.385417	0.831631
302.197	145	aqueous	NA	NA	NA	NA	NA	0.316465	0.796895
478.168	143	aqueous	NA	NA	NA	NA	NA	0.548547	0.848332
302.215	161	organic	NA	NA	NA	NA	NA	0.090615	1.343357
304.213	141	organic	CAR 8:0:OH (Spectral Match to Palmitoylcarnitine)	0.83	4	96.13	3.94	0.379713	1.134689

414.322	164	organic	CAR 16:1:O (Spectral Match to Palmitoylcarnitine)	0.83	6	13.98	1.44	0.049432	1.359945
332.244	146	organic	CAR 10:0:O (Spectral Match to Palmitoylcarnitine)	0.81	5	68.1	2.71	0.129804	1.303094
370.296	166	organic	CAR 14:1 (Spectral Match to Lauroyl L-carnitine)	0.87	5	26.02	2.16	0.049431	2.330736
480.552	384	organic	NA	NA	NA	NA	NA	6.991075	1.513876
734.45	189	organic	PC 32:8:O (Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.98	8	25.91	14.8	1.591373	0.838625
372.312	172	organic	CAR 14:0 (Spectral Match to Lauroyl L-carnitine)	0.91	5	28.03	3.22	0.10799	5.433971
153.043	34	organic	xanthine	0.94	5	0	6	6.344298	2.23169
440.338	168	organic	CAR 18:2:O (Spectral Match to Palmitoylcarnitine)	0.86	6	40	2.27	0.075315	1.691291

S3 Table. Annotated and unannotated metabolites perturbed by infection at position C, identified through random forest classifier. * indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.

S3 Table. Annotated and unannotated metabolites perturbed by infection at position C, identified through random forest classifier.									
* indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.									
Top-ranking metabolites for <i>T. cruzi</i> strain CL that met the random forest cut-off criteria in descending order of variable importance									
<i>m/z</i>	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position A (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position A (CL vs uninfected 90 days)
494.288	186	aqueous	LPC 15:2:O (annotated via networking)	NA	NA	NA	0.61	0.919233334	1.7564445
389.232	202	aqueous	NA	NA	NA	NA	NA	1.425835722	1.5773329
240.102	69	aqueous	NA	NA	NA	NA	NA	0.644371716	0.6544077
296.259	187	aqueous	NA	NA	NA	NA	NA	0.23920557	0.1131879
209.093	53	aqueous	NA	NA	NA	NA	NA	4.868035061	4.9059258
375.216	186	aqueous	NA	NA	NA	NA	NA	1.469633437	1.4966917
336.251	190	aqueous	NA	NA	NA	NA	NA	0.239971852	0.1326074
393.263	193	aqueous	NA	NA	NA	NA	NA	0.882696197	1.5899369
675.544	270	aqueous	NA	NA	NA	NA	NA	0.950404427	1.9371502
524.372	222	aqueous	LPC 18:0 or PC O-18:0 or LPC O-18:1:O (Spectral Match to 1-(9Z-Octadecanoyl)-sn-glycero-3-phosphocholine)	0.98	18	2.02	1.71	1.357819675	2.2910611
606.341	173	aqueous	NA	NA	NA	NA	NA	0.589057214	0.3535916
405.226	186	aqueous	NA	NA	NA	NA	NA	1.512345027	1.8626222
482.325	234	aqueous	PC family member (Spectral Match to 1-Myristoyl-sn-glycero-3-phosphocholine) *	0.96	12	14.01	NA	1.108244481	2.3799438
223.133	183	aqueous	NA	NA	NA	NA	NA	0.688021241	1.531623
332.28	175	aqueous	NA	NA	NA	NA	NA	0.299988628	0.1218912
510.319	183	aqueous	LPC 16:1:O (Spectral Match to 1-Myristoyl-sn-glycero-3-phosphocholine)	0.97	12	42.01	0	1.50553318	1.6004579
605.357	169	aqueous	NA	NA	NA	NA	NA	0.333432991	0.5200237
280.264	206	aqueous	NA	NA	NA	NA	NA	0.606244221	0.1780236
278.248	179	aqueous	NA	NA	NA	NA	NA	0.48201086	0.2454296
496.341	208	aqueous	PC family member (Spectral Match to HEPIC) *	0.99	17	14.02	NA	1.250405509	1.8429064

155.071	123	aqueous	NA	NA	NA	NA	NA	1.495390069	1.9722219
307.213	245	aqueous	NA	NA	NA	NA	NA	1.334809649	0.3752048
434.168	44	aqueous	NA	NA	NA	NA	NA	1.185774437	0.587599
496.34	214	aqueous	LPC 16:0 or PC O-16:0 or LPC O-16:1:O (Spectral Match to HEPC)	0.99	17	14.02	0.41	1.250538669	1.5797854
828.537	248	aqueous	PC 36:5;O3 (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycerol-3-phosphocholine)	0.98	15	67.96	1.81	1.011109553	3.9476397
300.29	195	aqueous	D-erythro-C18-Sphingosine	0.85	9	0	0	0.777822174	1.3905208
320.256	206	aqueous	NA	NA	NA	NA	NA	0.187804029	0.1201012
858.584	261	aqueous	PC 42:8 or PC O-42:9:O (Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycerol-3-phosphocholine)	0.99	16	71.98	19.45	0.924606909	3.7954075
250.095	66	aqueous	NA	NA	NA	NA	NA	0.571377894	0.4896209
407.242	190	aqueous	NA	NA	NA	NA	NA	1.34290053	1.5504688
421.221	177	aqueous	NA	NA	NA	NA	NA	1.622371754	1.8567698
496.304	189	aqueous	NA	NA	NA	NA	NA	1.007086982	1.6911602
829.674	242	aqueous	NA	NA	NA	NA	NA	1.382754995	2.0075728
166.088	180	aqueous	phenylalanine	0.96	5	0	6	1.963262888	0.74274
199.146	69	aqueous	NA	NA	NA	NA	NA	1.108521259	1.8273458
538.35	185	aqueous	LPC 18:1:O (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycerol-3-phosphocholine)	0.95	14	16	0.56	1.307475457	1.5404654
270.244	200	aqueous	NA	NA	NA	NA	NA	0.35655647	0.2411408
510.356	213	aqueous	PC family member (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycerol-3-phosphocholine) *	0.99	17	12	NA	1.684629322	2.7415514
494.567	279	aqueous	NA	NA	NA	NA	NA	1.86124199	5.3570011
482.406	237	aqueous	NA	NA	NA	NA	NA	1.080610284	0.3951072
316.285	191	aqueous	NA	NA	NA	NA	NA	0.197577705	0.2692392
332.331	219	organic	NA	NA	NA	NA	NA	1.91158283	0.4162421
552.403	215	organic	LPC 20:0 or LPC O-20:1:O or PC O-20:0 (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycerol-3-phosphocholine)	0.98	19	30.05	1.09	0.313514454	0.4799797
606.92	34	organic	NA	NA	NA	NA	NA	3.387130205	5.8557484
722.496	425	organic	PC family member (annotated via networking) *	NA	NA	NA	NA	2.587084352	3.0136847
528.307	179	organic	NA	NA	NA	NA	NA	0.679642247	0.5250737
304.301	177	organic	NA	NA	NA	NA	NA	1.816977619	0.26651
550.351	190	organic	LPC 19:2:O (Spectral Match to PAF C-16)	0.98	11	25.98	1.82	0.881240658	0.3646375
810.599	274	organic	NA	NA	NA	NA	NA	0.516416139	1.2731508
518.377	307	organic	NA	NA	NA	NA	NA	1.508646504	2.9872173
326.379	206	organic	NA	NA	NA	NA	NA	0.143505857	0.1993589

672.424	233	organic	PC 27:4;O (Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine)	0.8	5	60.13	0.74	1.182027188	0.6209303
297.28	302	organic	NA	NA	NA	NA	NA	0.763704686	0.4000966
326.379	231	organic	NA	NA	NA	NA	NA	0.208393969	0.2739089
359.221	161	organic	NA	NA	NA	NA	NA	1.285508244	1.4507024
616.325	153	organic	NA	NA	NA	NA	NA	1.561330149	1.3959733
686.439	211	organic	PC 28:4;O (Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine)	0.99	13	46.12	0.15	0.977647551	0.7717081
280.265	177	organic	NA	NA	NA	NA	NA	0.313380812	0.3955645
388.395	236	organic	NA	NA	NA	NA	NA	1.349952214	0.35082
798.546	266	organic	PC 36:4;O (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine) LPC 30:1 or LPC O-20:2;O or PC O-20:1 (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.95	10	37.97	22.9	1.116607322	1.2529278
550.387	199	organic	NA	0.98	19	28.03	0.54	0.517857245	0.623223
580.566	402	organic	Cer 37:1;O2 (annotated via networking)	NA	NA	NA	0.52	1.176120448	2.3586322
1049.63	178	organic	NA	NA	NA	NA	NA	0.681330787	0.4334224
782.57	336	organic	NA	NA	NA	NA	NA	1.140774306	1.3465717
812.516	244	organic	PC 39:10 or PC O-39:11;O (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.96	9	51.94	8	0.935778544	0.7621514
594.583	411	organic	Cer 38:1;O2 (Spectral Match to N-(1,3-dihydroxyoctadec-4-en-2-yl)tetradecanamide)	0.86	11	84.09	1.68	1.14361832	2.057899
646.614	417	organic	Cer 42:3;O2 (Spectral Match to Ceramide (18:1/18:0))	0.8	13	0.26	1.08	1.381578947	2.336887
Top-ranking metabolites for <i>T. cruzi</i> strain Sylvio X10/4 that met the random forest cut-off criteria in descending order of variable importance									
<i>m/z</i>	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position A (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position A (CL vs uninfected 90 days)
381.227	178	aqueous	NA	NA	NA	NA	NA	1.519695825	1.6452024
368.201	147	aqueous	NA	NA	NA	NA	NA	0.388828246	0.6027795
414.322	186	aqueous	CAR 16:1;O (Spectral Match to Palmitoylcarnitine)	0.85	7	13.98	1.45	0.088828664	2.2169772
390.206	149	aqueous	NA	NA	NA	NA	NA	0.483776208	0.7015617
258.112	146	aqueous	NA	NA	NA	NA	NA	4.903589928	0.9248519
175.12	19	aqueous	NA	NA	NA	NA	NA	1.732170355	0.7966164
257.117	31	aqueous	NA	NA	NA	NA	NA	0.721038172	0.9147418
258.171	145	aqueous	CAR 6:1 (Spectral Match to Acetyl-L-carnitine)	0.77	4	54.05	3.87	0.245779416	1.4505706
358.26	164	aqueous	CAR 12:1;O (Spectral Match to Palmitoylcarnitine)	0.84	9	42.08	3.35	0.175368312	1.5281096
388.307	181	aqueous	CAR 14:0;O (Spectral Match to Palmitoylcarnitine)	0.87	5	12.04	3.35	0.118186248	2.2631822
438.299	210	aqueous	NA	NA	NA	NA	NA	1.280767448	1.3538983
502.223	155	aqueous	NA	NA	NA	NA	NA	0.557267591	0.6964316

412.2	132	aqueous	NA	NA	NA	NA	NA	0.654344516	0.9005598
426.359	210	aqueous	CAR 18:1 (Spectral Match to Palmitoylcarnitine)	0.89	5	26.02	2.81	0.125238701	6.1709739
346.259	182	aqueous	NA	NA	NA	NA	NA	0.386392489	0.8546449
442.353	197	aqueous	CAR 18:1;O (Spectral Match to Palmitoylcarnitine)	0.88	8	42.01	0.68	0.094003566	3.294068
492.272	183	aqueous	NA	NA	NA	NA	NA	0.631857505	1.3085519
400.343	205	aqueous	CAR 16:0 (Spectral Match to Lauroyl L-carnitine)	0.92	5	56.06	2.25	0.210696157	7.2462111
398.327	198	aqueous	CAR 16:1 (Spectral Match to Lauroyl L-carnitine)	0.89	5	54.05	1.26	0.19195695	3.8057225
814.559	280	organic	PC O-40:9 (Spectral Match to 1,2-Dilindoleyl-sn-glycero-3-phosphocholine)	0.98	12	31.99	19	0.473208001	0.9391256
788.581	294	organic	NA	NA	NA	NA	NA	0.394569333	1.5950881
400.343	209	organic	NA	NA	NA	NA	NA	0.179979191	1.7404495
302.215	161	organic	NA	NA	NA	NA	NA	0.10119101	0.6845906
426.358	229	organic	NA	NA	NA	NA	NA	0.129074293	1.2065664
553.408	214	organic	NA	NA	NA	NA	NA	0.343986677	0.4919876
428.373	240	organic	NA	NA	NA	NA	NA	0.191184584	1.4490736
228.196	177	organic	NA	NA	NA	NA	NA	6.251256631	0.5827663
414.359	193	organic	NA	NA	NA	NA	NA	0.218621045	1.7394344
442.354	175	organic	CAR 18:1;O (Spectral Match to Palmitoylcarnitine)	0.87	8	42.01	2.94	0.050226837	1.4076464
786.534	426	organic	PC O-38:9 (annotated via networking)	NA	NA	NA	11.7	4.832369403	2.1934928
832.539	266	organic	PC 39:8;O (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.96	10	71.95	11.7	0.601031128	0.8798038
454.39	210	organic	CAR 20:1 (Spectral Match to Palmitoylcarnitine)	0.88	8	54.05	1.98	0.101851726	0.9412326
302.207	157	organic	NA	NA	NA	NA	NA	0.499834746	0.9291355
237.651	135	organic	NA	NA	NA	NA	NA	16.6664188	0.1710872
455.394	209	organic	NA	NA	NA	NA	NA	0.114725195	1.2232034

S4 Table. Annotated and unannotated metabolites perturbed by infection at position D, identified through random forest classifier. * indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.

S4 Table. Annotated and unannotated metabolites perturbed by infection at position D, identified through random forest classifier.									
* indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.									
Top-ranking metabolites for <i>T. cruzi</i> strain CL that met the random forest cut-off criteria in descending order of variable importance									
<i>m/z</i>	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position A (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position A (CL vs uninfected 90 days)
185.596	143	aqueous	NA	NA	NA	NA	NA	0.322938398	0.43112
332.28	175	aqueous	NA	NA	NA	NA	NA	0.363405998	0.15278
370.178	142	aqueous	NA	NA	NA	NA	NA	0.32745339	0.5475
1049.34	20	aqueous	NA	NA	NA	NA	NA	0.824167802	0.38
312.254	194	aqueous	NA	NA	NA	NA	NA	0.570524738	0.20258
186.113	149	aqueous	NA	NA	NA	NA	NA	0.738311058	1.2841
304.652	208	aqueous	NA	NA	NA	NA	NA	1.216857871	1.447
912.412	212	aqueous	NA	NA	NA	NA	NA	1.048798014	0.43046
348.275	169	aqueous	NA	NA	NA	NA	NA	0.967299051	0.63037
153.042	32	aqueous	xanthine	0.93	5	0	8	1.349940507	1.4588
166.088	180	aqueous	phenylalanine	0.96	5	0	6	0.484768013	0.79465
462.191	140	aqueous	NA	NA	NA	NA	NA	0.77570402	0.62319
524.372	222	aqueous	LPC 18:0 or PC O-18:0 or LPC O-18:1:O (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.98	18	2.02	1.71	2.151553376	1.49221
949.626	209	aqueous	NA	NA	NA	NA	NA	2.259937959	2.94623
199.146	69	aqueous	NA	NA	NA	NA	NA	1.13587514	1.58065
241.155	26	aqueous	NA	NA	NA	NA	NA	0.790130997	0.74115
548.371	216	aqueous	NA	NA	NA	NA	NA	1.287617471	1.49809
386.211	146	aqueous	NA	NA	NA	NA	NA	0.549047182	0.51846

296.259	187	aqueous	NA	NA	NA	NA	NA	0.422964248	0.2407
432.281	145	aqueous	NA	NA	NA	NA	NA	0.790330973	0.6002
496.34	222	aqueous	PC family member (Spectral Match to HEPC) *	0.99	17	14.02	NA	1.459404083	1.33696
540.31	158	aqueous	NA	NA	NA	NA	NA	0.610357704	0.53773
280.264	206	aqueous	NA	NA	NA	NA	NA	0.163662896	0.28729
584.43	200	organic	NA	NA	NA	NA	NA	1.663633819	1.91025
774.895	136	organic	NA	NA	NA	NA	NA	0.537419264	2.28174
368.28	158	organic	CAR 14:2 (Spectral Match to Lauroyl L-carnitine)	0.82	5	24	1.36	0.36473184	1.5321
509.405	212	organic	NA	NA	NA	NA	NA	0.850573713	1.60145
850.632	417	organic	PC 41:5 or PC O-41:6/O (Spectral Match to 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine)	0.99	11	68.06	0	0.795988341	0.455
451.341	214	organic	NA	NA	NA	NA	NA	1.506612091	0.66498
640.589	337	organic	NA	NA	NA	NA	NA	0.385970532	11.0213
506.531	422	organic	NA	NA	NA	NA	NA	0.079552609	0.15988
802.557	291	organic	PC family member (Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine) *	0.99	18	41.98	NA	1.147268603	0.84482
522.598	509	organic	NA	NA	NA	NA	NA	0.950385427	3.88025
750.542	377	organic	PC 32:0/O (Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine)	0.74	12	3.97	29.7	0.969177042	0.63497
Top-ranking metabolites for T. cruzi strains CL and Sylvio X10/4 that met the random forest cut-off criteria in descending order of variable importance									
m/z	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position A (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position A (CL vs uninfected 90 days)
368.201	147	aqueous	NA	NA	NA	NA	NA	0.409540101	0.46617
209.093	53	aqueous	NA	NA	NA	NA	NA	8.994161812	4.60867
Top-ranking metabolites for T. cruzi strain Sylvio X10/4 that met the random forest cut-off criteria in descending order of variable importance									
m/z	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Peak area fold change at position A (Sylvio X10/4 vs uninfected 147 days)	Peak area fold change at position A (CL vs uninfected 90 days)
304.213	149	aqueous	NA	NA	NA	NA	NA	0.13574775	1.6222
332.244	158	aqueous	CAR 10:0/O (Spectral Match to Palmitoylcarnitine)	0.87	4	68.1	2.71	0.175181124	1.1221
442.353	197	aqueous	CAR 18:1/O (Spectral Match to Palmitoylcarnitine)	0.88	8	42.01	0.68	0.149823628	1.60518
412.307	179	aqueous	CAR 16:2/O (Spectral Match to Lauroylcarnitine)	0.8	4	68.03	3.15	0.133319458	1.85743
232.155	115	aqueous	NA	NA	NA	NA	NA	0.320500632	1.4039
316.249	170	aqueous	CAR 10:0 (Spectral Match to Propionylcarnitine)	0.92	5	98.11	2.53	0.118304109	3.06445
147.077	24	aqueous	NA	NA	NA	NA	NA	1.512336077	0.96543
155.108	189	aqueous	NA	NA	NA	NA	NA	0.808243734	1.05562

172.985	26	aqueous	NA	NA	NA	NA	NA	0.526642675	0.97737
225.149	173	aqueous	NA	NA	NA	NA	NA	0.145695416	1.08652
248.15	30	aqueous	CAR 4:0;O2 (Spectral Match to Acetyl-DL-carnitine)	0.82	4	44.03	3.22	0.300751323	1.30245
251.201	207	aqueous	NA	NA	NA	NA	NA	0.597842221	0.95233
274.184	166	aqueous	NA	NA	NA	NA	NA	0.21881209	1.17927
412.2	132	aqueous	NA	NA	NA	NA	NA	0.650825695	0.80711
419.373	255	aqueous	NA	NA	NA	NA	NA	0.369498577	0.55627
468.39	227	aqueous	NA	NA	NA	NA	NA	0.429163902	0.32461
604.366	164	aqueous	NA	NA	NA	NA	NA	0.303719008	0.68368
700.528	309	aqueous	NA	NA	NA	NA	NA	1.60192419	1.02656
302.216	178	aqueous	NA	NA	NA	NA	NA	0.179621176	1.48079
316.285	191	aqueous	NA	NA	NA	NA	NA	0.240678883	0.29079
444.369	204	aqueous	CAR 18:0;O (Spectral Match to Oleoyl L-carnitine)	0.81	5	18.01	1.58	0.121177739	2.53176
260.187	148	aqueous	CAR 6:0 (Spectral Match to Acetyl-DL-carnitine)	0.96	5	56.06	5.38	0.136583244	1.62023
398.195	143	aqueous	NA	NA	NA	NA	NA	0.634937552	0.75584
508.376	215	aqueous	NA	NA	NA	NA	NA	2.091808969	1.44989
570.34	155	aqueous	LPC 22:5 or LPC O-22:6;O or PC O-22:5 (Spectral Match to 1-Arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine)	0.91	8	17.94	27	0.531768954	1.47694
704.524	287	aqueous	NA	NA	NA	NA	NA	0.808147175	0.78376
496.34	214	aqueous	LPC 16:0 or PC O-16:0 or LPC O-16:1;O (Spectral Match to HEPc)	0.99	17	14.02	0.41	1.45943503	1.3352
246.171	142	aqueous	CAR 5:0 (annotated via networking)	NA	NA	NA	4.06	0.421525006	1.02077
754.54	285	aqueous	NA	NA	NA	NA	NA	2.29230038	0.98642
508.34	212	aqueous	LPC 17:1 or LPC O-17:2;O (Spectral Match to 1-Heptadecanoyl-sn-glycero-3-phosphocholine)	0.86	8	2.02	0.39	1.699151136	0.91946
784.585	383	organic	PC family member (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine) *	1	20	24	NA	0.609209112	1.0474
400.343	187	organic	CAR 16:0 (Spectral Match to Lauroyl L-carnitine)	0.91	5	56.06	2.25	0.147228782	2.15702
622.614	438	organic	Cer 40:1;O2 (Spectral Match to N-[1,3-dihydroxyoctadec-4-en-2-yl]tetracos-15-enamide)	0.83	7	8.01	1.12	0.721542768	0.97042
584.335	161	organic	LPC 22:6;O or PC 22:5 or PC O-22:6;O (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.95	10	61.98	0.51	2.371138445	1.08357
302.207	157	organic	NA	NA	NA	NA	NA	0.653543422	0.95068
553.408	214	organic	NA	NA	NA	NA	NA	0.475	0.74729
147.078	19	organic	NA	NA	NA	NA	NA	2.503841752	0.13847
153.041	28	organic	NA	NA	NA	NA	NA	2.006539978	0.93404

388.307	160	organic	CAR 14:0;O (Spectral Match to Palmitoylcarnitine)	0.86	4	12.04	3.35	0.098155164	1.63434
400.343	209	organic	NA	NA	NA	NA	NA	0.18191294	1.99998
784.583	346	organic	PC 36:3 or PC O-36:4;O (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	1	20	24	2.68	0.600938566	1.23155
787.606	384	organic	NA	NA	NA	NA	NA	0.571769888	1.08829
774.564	296	organic	PC family member (Spectral Match to 1-Hexadecyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine) *	0.83	9	27.99	NA	0.566663494	0.912
450.359	190	organic	CAR 20:3 (Spectral Match to Oleoyl L-carnitine)	0.85	6	24	2.66	0.139213623	0.96538
504.515	404	organic	NA	NA	NA	NA	NA	0.083813685	0.21697
806.571	354	organic	PC family member (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine) *	0.98	14	45.99	NA	1.347456104	1.03502

S5 Table. Annotated and unannotated metabolites perturbed by infection at positions A-D, identified through random forest classifier. * indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.

S5 Table. Annotated and unannotated metabolites perturbed by infection at positions A-D, identified through random forest classifier.							
* indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.							
Top-ranking metabolites for <i>T. cruzi</i> strain CL that met the random forest cut-off criteria in descending order of variable importance							
<i>m/z</i>	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error
332.28	175	aqueous	NA	NA	NA	NA	NA
252.233	190	aqueous	NA	NA	NA	NA	NA
336.251	190	aqueous	NA	NA	NA	NA	NA
296.259	187	aqueous	NA	NA	NA	NA	NA
605.357	169	aqueous	NA	NA	NA	NA	NA
292.227	199	aqueous	NA	NA	NA	NA	NA
320.256	217	aqueous	NA	NA	NA	NA	NA
312.254	194	aqueous	NA	NA	NA	NA	NA
280.264	206	aqueous	NA	NA	NA	NA	NA
400.269	187	aqueous	NA	NA	NA	NA	NA
278.248	179	aqueous	NA	NA	NA	NA	NA
476.307	146	aqueous	LPC O-16:3 (Spectral Match to 1-Arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine)	0.83	8	76.1	13.65
270.244	200	aqueous	NA	NA	NA	NA	NA
931.451	189	aqueous	NA	NA	NA	NA	NA
464.206	147	aqueous	NA	NA	NA	NA	NA
189.124	74	aqueous	NA	NA	NA	NA	NA
432.281	145	aqueous	NA	NA	NA	NA	NA
524.372	222	aqueous	LPC 18:0 or PC O-18:0 or LPC O-18:1:O (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.98	18	2.02	1.71
370.178	142	aqueous	NA	NA	NA	NA	NA
316.285	191	aqueous	NA	NA	NA	NA	NA
407.242	190	aqueous	NA	NA	NA	NA	NA

380.113	41	aqueous	NA	NA	NA	NA	NA
465.21	148	aqueous	NA	NA	NA	NA	NA
343.23	204	aqueous	NA	NA	NA	NA	NA
482.325	234	aqueous	PC family member (Spectral Match to 1-Myristoyl-sn-glycero-3-phosphocholine) *	0.96	12	14.01	NA
250.095	66	aqueous	NA	NA	NA	NA	NA
634.187	180	aqueous	NA	NA	NA	NA	NA
238.155	148	aqueous	NA	NA	NA	NA	NA
251.103	147	aqueous	NA	NA	NA	NA	NA
722.504	224	aqueous	NA	NA	NA	NA	NA
200.632	80	aqueous	NA	NA	NA	NA	NA
268.108	33	aqueous	NA	NA	NA	NA	NA
689.559	279	aqueous	NA	NA	NA	NA	NA
199.146	69	aqueous	NA	NA	NA	NA	NA
462.191	140	aqueous	NA	NA	NA	NA	NA
618.338	166	aqueous	NA	NA	NA	NA	NA
428.374	217	aqueous	CAR 18:0 (Spectral Match to Palmitoylcarnitine)	0.86	5	28.03	1.4
185.596	143	aqueous	NA	NA	NA	NA	NA
189.077	142	aqueous	NA	NA	NA	NA	NA
616.177	186	aqueous	hemin cation	0.98	18	0	1
556.443	228	aqueous	NA	NA	NA	NA	12.58
204.124	135	aqueous	NA	NA	NA	NA	NA
1043.71	214	aqueous	NA	NA	NA	NA	NA
678.473	240	aqueous	PC 27:1; O (Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine)	0.97	9	54.08	3.83
344.281	182	aqueous	CAR 12:0 (Spectral Match to Valerylcarnitine)	0.92	6	98.11	4.36
323.258	229	aqueous	NA	NA	NA	NA	NA
758.503	160	aqueous	NA	NA	NA	NA	NA
349.235	235	aqueous	NA	NA	NA	NA	NA
806.57	299	aqueous	PC 38:6 or PC O-38:7;O (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.97	10	45.99	0.74
422.159	137	aqueous	NA	NA	NA	NA	NA
1047.74	222	aqueous	NA	NA	NA	NA	NA
349.069	30	aqueous	NA	NA	NA	NA	NA

510.356	213	aqueous	PC family member (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine) *	0.99	17	12	NA
552.403	236	aqueous	PC O-20:0 (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.99	19	30.05	1.09
237.149	189	aqueous	NA	NA	NA	NA	NA
574.279	166	aqueous	NA	NA	NA	NA	NA
221.094	68	aqueous	NA	NA	NA	NA	NA
224.633	138	aqueous	NA	NA	NA	NA	NA
331.267	198	aqueous	NA	NA	NA	NA	NA
664.309	147	aqueous	NA	NA	NA	NA	NA
548.371	216	aqueous	NA	NA	NA	NA	NA
446.196	147	aqueous	PC 12:4 (annotated via networking)	NA	NA	NA	4.93
376.262	191	aqueous	NA	NA	NA	NA	NA
749.424	194	aqueous	NA	NA	NA	NA	NA
452.241	153	aqueous	NA	NA	NA	NA	NA
318.241	212	aqueous	NA	NA	NA	NA	NA
748.528	297	aqueous	NA	NA	NA	NA	NA
1277.35	180	aqueous	NA	NA	NA	NA	NA
520.231	148	aqueous	NA	NA	NA	NA	NA
298.246	223	aqueous	NA	NA	NA	NA	NA
245.13	152	aqueous	NA	NA	NA	NA	NA
389.232	202	aqueous	NA	NA	NA	NA	NA
454.294	206	aqueous	LPC 13:0 or LPC O-13:1:O (annotated via networking)	NA	NA	NA	2.64
209.093	57	organic	NA	NA	NA	NA	NA
850.632	417	organic	PC 41:5 or PC O-41:6:O (Spectral Match to 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine)	0.99	11	68.06	0
504.515	404	organic	NA	NA	NA	NA	NA
506.531	422	organic	NA	NA	NA	NA	NA
332.281	155	organic	NA	NA	NA	NA	NA
296.259	162	organic	NA	NA	NA	NA	NA
417.358	223	organic	NA	NA	NA	NA	NA
234.116	133	organic	NA	NA	NA	NA	NA
534.302	160	organic	LPC 18:3:O (Spectral Match to 1-Arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine)	0.95	9	18.09	31.8
508.546	444	organic	NA	NA	NA	NA	NA

550.558	287	organic	NA	NA	NA	NA	NA
691.539	264	organic	NA	NA	NA	NA	NA
804.522	284	organic	PC family member (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine) *	0.97	10	43.94	NA
852.401	169	organic	NA	NA	NA	NA	NA
480.515	416	organic	NA	NA	NA	NA	NA
872.56	249	organic	PC 42:9;O (Spectral Match to 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine)	0.98	12	89.99	22.9
326.379	206	organic	NA	NA	NA	NA	NA
566.552	387	organic	Cer(d18:0/18:1) (Spectral Match to N-(1,3-dihydroxyoctadec-4-en-2-yl)tetradecanamide)	0.84	10	56.06	2.29
814.573	323	organic	NA	NA	NA	NA	NA
211.17	156	organic	NA	NA	NA	NA	NA
166.087	59	organic	NA	NA	NA	NA	NA
688.454	223	organic	PC 28:3;O (Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.99	9	18.08	1.16
652.411	190	organic	LPC 27:7;O or PC 27:6 or PC O-27:7;O (Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.95	7	54.12	21
228.196	177	organic	NA	NA	NA	NA	NA
543.383	236	organic	NA	NA	NA	NA	NA
774.895	136	organic	NA	NA	NA	NA	NA
296.259	181	organic	NA	NA	NA	NA	NA
262.239	145	organic	NA	NA	NA	NA	NA
686.439	211	organic	PC 28:4;O (Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine)	0.99	13	46.12	0.15
429.395	283	organic	NA	NA	NA	NA	NA
462.191	138	organic	NA	NA	NA	NA	NA
754.502	251	organic	PC family member (Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine) *	0.99	12	19.94	NA
764.58	302	organic	NA	NA	NA	NA	NA
429.378	206	organic	NA	NA	NA	NA	NA
880.587	322	organic	PC family member (Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine) *	0.99	11	93.98	NA
566.274	152	organic	NA	NA	NA	NA	NA
570.458	211	organic	NA	NA	NA	NA	NA
746.57	400	organic	NA	NA	NA	NA	NA
694.425	180	organic	LPC 30:7;O or PC 30:6 or PC O-30:7;O (Spectral Match to KDaA-PC)	0.93	15	26.01	27.6
556.303	187	organic	NA	NA	NA	NA	NA
137.047	30	organic	hypoxanthine	0.91	4	0	7

280.265	250	organic	NA	NA	NA	NA	NA
156.139	150	organic	NA	NA	NA	NA	NA
384.116	133	organic	succinyladenosine (annotated via networking)	NA	NA	NA	2.6
480.276	137	organic	LPC 14:2:O (annotated via networking)	NA	NA	NA	8.12
531.388	213	organic	NA	NA	NA	NA	NA
798.531	255	organic	NA	NA	NA	NA	NA
526.28	164	organic	NA	NA	NA	NA	NA
742.498	254	organic	PC 32:4:O (Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycerol-3-phosphocholine)	0.97	13	44.1	4.98
436.174	140	organic	NA	NA	NA	NA	NA
448.236	133	organic	NA	NA	NA	NA	NA
532.335	186	organic	NA	NA	NA	NA	NA
648.395	142	organic	NA	NA	NA	NA	NA
824.617	396	organic	PC 39:4 or PC O-39:5:O (Spectral Match to 1-Octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycerol-3-phosphocholine)	0.99	16	14.02	0.73
249.046	22	organic	NA	NA	NA	NA	NA
519.327	169	organic	NA	NA	NA	NA	NA
359.222	179	organic	NA	NA	NA	NA	NA
856.564	222	organic	NA	NA	NA	NA	NA
461.362	236	organic	NA	NA	NA	NA	NA
626.382	142	organic	NA	NA	NA	NA	NA
582.414	187	organic	NA	NA	NA	NA	NA
466.536	367	organic	NA	NA	NA	NA	NA
738.562	297	organic	NA	NA	NA	NA	NA
395.56	139	organic	NA	NA	NA	NA	NA
508.581	438	organic	NA	NA	NA	NA	NA
312.254	167	organic	NA	NA	NA	NA	NA
816.649	367	organic	PC family member (Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycerol-3-phosphocholine) *	0.99	15	30.04	NA
355.284	215	organic	NA	NA	NA	NA	NA
730.562	323	organic	NA	NA	NA	NA	NA
538.52	362	organic	Cer 34:1:O2 (Spectral Match to N-(1,3-dihydroxyoctadec-4-en-2-yl)tetradecanamide)	0.87	10	28.03	1.11
502.294	172	organic	NA	NA	NA	NA	NA
748.548	265	organic	NA	NA	NA	NA	NA

712.455	250	organic	PC family member (Spectral Match to 1,2-Ditetradecanoyl-sn-glycero-3-phosphocholine) *	0.74	6	33.95	NA
298.275	190	organic	NA	NA	NA	NA	NA
203.224	554	organic	NA	NA	NA	NA	NA
352.322	220	organic	NA	NA	NA	NA	NA
652.419	205	organic	NA	NA	NA	NA	NA
834.594	329	organic	NA	NA	NA	NA	NA
728.486	241	organic	PC 31:4:O (Spectral Match to 1-Octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine)	0.99	16	82.11	0.14
150.112	18	organic	NA	NA	NA	NA	NA
218.14	23	organic	CAR 3:0 (Spectral Match to acetyl-carnitine)	0.79	4	14.02	5.96
284.296	250	organic	NA	NA	NA	NA	NA
269.09	26	organic	NA	NA	NA	NA	NA
Top-ranking metabolites for <i>T. cruzi</i> strains CL and Sylvio X10/4 that met the random forest cut-off criteria in descending order of variable importance							
<i>m/z</i>	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error
209.093	53	aqueous	NA	NA	NA	NA	NA
320.256	206	aqueous	NA	NA	NA	NA	NA
193.61	145	aqueous	NA	NA	NA	NA	NA
421.221	177	aqueous	NA	NA	NA	NA	NA
258.171	145	aqueous	CAR 6:1 (Spectral Match to Acetyl-L-carnitine)	0.77	4	54.05	3.87
426.359	210	aqueous	CAR 18:1 (Spectral Match to Palmitoylcarnitine)	0.89	5	26.02	2.81
375.216	186	aqueous	NA	NA	NA	NA	NA
382.296	182	aqueous	NA	NA	NA	NA	NA
534.561	450	organic	NA	NA	NA	NA	NA
269.093	43	organic	NA	NA	NA	NA	NA
408.179	137	organic	NA	NA	NA	NA	NA
370.296	166	organic	CAR 14:1 (Spectral Match to Lauroyl L-carnitine)	0.87	5	26.02	2.16
809.587	347	organic	NA	NA	NA	NA	NA
291.148	142	organic	NA	NA	NA	NA	NA
470.385	189	organic	CAR 20:1:O (Spectral Match to Palmitoylcarnitine)	0.87	8	70.04	2.13
484.364	175	organic	NA	NA	NA	NA	NA
Top-ranking metabolites for <i>T. cruzi</i> strain Sylvio X10/4 that met the random forest cut-off criteria in descending order of variable importance							

m/z	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error
332.244	158	aqueous	CAR 10:0:O (Spectral Match to Palmitoylcarnitine)	0.87	4	68.1	2.71
412.307	179	aqueous	CAR 16:2:O (Spectral Match to Lauroylcarnitine)	0.8	4	68.03	3.15
442.353	197	aqueous	CAR 18:1:O (Spectral Match to Palmitoylcarnitine)	0.88	8	42.01	0.68
304.213	149	aqueous	NA	NA	NA	NA	NA
416.338	192	aqueous	CAR 16:0:O (Spectral Match to Palmitoylcarnitine)	0.87	4	16	2.4
440.338	190	aqueous	CAR 18:2:O (Spectral Match to Palmitoylcarnitine)	0.85	5	40	2.27
248.15	30	aqueous	CAR 4:0:O2 (Spectral Match to Acetyl-DL-carnitine)	0.82	4	44.03	3.22
414.322	186	aqueous	CAR 16:1:O (Spectral Match to Palmitoylcarnitine)	0.85	7	13.98	1.45
388.307	181	aqueous	CAR 14:0:O (Spectral Match to Palmitoylcarnitine)	0.87	5	12.04	3.35
260.187	148	aqueous	CAR 6:0 (Spectral Match to Acetyl-DL-carnitine)	0.96	5	56.06	5.38
398.195	143	aqueous	NA	NA	NA	NA	NA
368.201	147	aqueous	NA	NA	NA	NA	NA
400.343	205	aqueous	CAR 16:0 (Spectral Match to Lauroyl L-carnitine)	0.92	5	56.06	2.25
276.181	104	aqueous	NA	NA	NA	NA	NA
274.184	166	aqueous	NA	NA	NA	NA	NA
386.211	146	aqueous	NA	NA	NA	NA	NA
444.369	204	aqueous	CAR 18:0:O (Spectral Match to Oleoyl L-carnitine)	0.81	5	18.01	1.58
276.181	139	aqueous	CAR 6:0:O (Spectral Match to Acetyl-DL-carnitine)	0.94	5	72.06	1.81
302.197	145	aqueous	NA	NA	NA	NA	NA
302.216	178	aqueous	NA	NA	NA	NA	NA
175.12	19	aqueous	NA	NA	NA	NA	NA
468.368	198	aqueous	NA	NA	NA	NA	NA
412.2	132	aqueous	NA	NA	NA	NA	NA
584.335	182	aqueous	LPC 22:6:O or PC 22:5 or PC O-22:6:O (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.97	13	61.98	0.51
248.151	47	aqueous	CAR 4:0:O (Spectral Match to Acetyl-DL-carnitine)	0.82	4	44.03	7.25
570.34	155	aqueous	LPC 22:5 or LPC O-22:6:O or PC O-22:5 (Spectral Match to 1-Arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine)	0.91	8	17.94	27
148.061	27	aqueous	glutamine	0.84	4	0.99	4
568.325	190	aqueous	NA	NA	NA	NA	NA
240.102	69	aqueous	NA	NA	NA	NA	NA

150.059	27	aqueous	NA	NA	NA	NA	NA
614.31	174	aqueous	NA	NA	NA	NA	NA
316.249	170	aqueous	CAR 10:0 (Spectral Match to Propionylcarnitine)	0.92	5	98.11	2.53
528.293	157	aqueous	NA	NA	NA	NA	NA
262.166	43	aqueous	NA	NA	NA	NA	NA
220.136	160	aqueous	NA	NA	NA	NA	NA
398.327	198	aqueous	CAR 16:1 (Spectral Match to Lauroyl L-carnitine)	0.89	5	54.05	1.26
600.33	174	aqueous	PC 22:4:O (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.98	12	77.97	0.67
415.158	29	aqueous	NA	NA	NA	NA	NA
381.227	178	aqueous	NA	NA	NA	NA	NA
520.34	216	aqueous	LPC 18:2 or LPC 18:3:O (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.98	20	2.01	0.38
510.283	168	aqueous	NA	NA	NA	NA	NA
386.291	175	aqueous	CAR 14:1:O (Spectral Match to Palmitoylcarnitine)	0.86	6	14.05	2.33
670.408	164	aqueous	NA	NA	NA	NA	NA
405.226	186	aqueous	NA	NA	NA	NA	NA
384.179	139	aqueous	NA	NA	NA	NA	NA
838.267	187	aqueous	NA	NA	NA	NA	NA
228.135	138	aqueous	NA	NA	NA	NA	NA
329.221	189	aqueous	NA	NA	NA	NA	NA
714.626	151	aqueous	NA	NA	NA	NA	NA
390.206	149	aqueous	NA	NA	NA	NA	NA
232.155	115	aqueous	NA	NA	NA	NA	NA
227.091	144	aqueous	NA	NA	NA	NA	NA
476.277	185	aqueous	NA	NA	NA	NA	NA
416.153	33	aqueous	NA	NA	NA	NA	NA
412.21	147	aqueous	NA	NA	NA	NA	NA
526.294	202	aqueous	NA	NA	NA	NA	NA
478.293	202	aqueous	LPC 15:2 or LPC O-15:3:O (Spectral Match to 1-Heptadecanoyl-sn-glycero-3-phosphocholine)	0.7	9	32.06	0.42
383.242	187	aqueous	NA	NA	NA	NA	NA
719.57	280	aqueous	NA	NA	NA	NA	NA
146.119	87	aqueous	NA	NA	NA	NA	NA

482.361	210	aqueous	NA	NA	NA	NA	NA
259.095	28	aqueous	NA	NA	NA	NA	NA
302.207	157	organic	NA	NA	NA	NA	NA
386.291	157	organic	CAR 14:1:O (Spectral Match to Lauroyl L-carnitine)	0.78	4	42.01	2.33
414.322	164	organic	CAR 16:1:O (Spectral Match to Palmitoylcarnitine)	0.83	6	13.98	1.44
416.338	171	organic	CAR 16:0:O (Spectral Match to Palmitoylcarnitine)	0.84	5	16	2.4
332.244	146	organic	CAR 10:0:O (Spectral Match to Palmitoylcarnitine)	0.81	5	68.1	2.71
360.275	153	organic	NA	NA	NA	NA	NA
452.374	199	organic	CAR 20:2 (Spectral Match to Palmitoylcarnitine)	0.89	8	52.03	1.33
442.354	175	organic	CAR 18:1:O (Spectral Match to Palmitoylcarnitine)	0.87	8	42.01	2.94
454.39	210	organic	CAR 20:1 (Spectral Match to Palmitoylcarnitine)	0.88	8	54.05	1.98
452.373	244	organic	NA	NA	NA	NA	NA
468.369	179	organic	CAR 20:2:O (Spectral Match to Palmitoylcarnitine)	0.85	7	68.03	1.49
440.338	168	organic	CAR 18:2:O (Spectral Match to Palmitoylcarnitine)	0.86	6	40	2.27
388.307	160	organic	CAR 14:0:O (Spectral Match to Palmitoylcarnitine)	0.86	4	12.04	3.35
412.307	160	organic	CAR 16:2:O (Spectral Match to Lauroyl L-carnitine)	0.84	4	68.03	3.15
274.183	153	organic	NA	NA	NA	NA	NA
304.213	141	organic	CAR 8:0-OH (Spectral Match to Palmitoylcarnitine)	0.83	4	96.13	3.94
455.394	209	organic	NA	NA	NA	NA	NA
454.389	250	organic	NA	NA	NA	NA	NA
424.343	184	organic	CAR 18:2 (Spectral Match to Palmitoylcarnitine)	0.95	9	24	2.12
368.28	158	organic	CAR 14:2 (Spectral Match to Lauroyl L-carnitine)	0.82	5	24	1.36
456.406	229	organic	CAR 20:0 (Spectral Match to Palmitoylcarnitine)	0.83	4	56.06	2.85
372.312	172	organic	CAR 14:0 (Spectral Match to Lauroyl L-carnitine)	0.91	5	28.03	3.22
302.215	161	organic	NA	NA	NA	NA	NA
484.437	254	organic	CAR 22:0 (Spectral Match to Palmitoylcarnitine)	0.83	6	84.09	2.06
482.42	231	organic	CAR 22:1 (Spectral Match to Palmitoylcarnitine)	0.8	6	82.08	0.83
260.187	141	organic	CAR 6:0 (annotated via networking)	NA	NA	NA	5.38
426.359	193	organic	CAR 18:1 (Spectral Match to Lauroyl L-carnitine)	0.91	7	82.08	0.45
248.151	19	organic	NA	NA	NA	NA	NA
414.359	193	organic	NA	NA	NA	NA	NA

819.594	297	organic	NA	NA	NA	NA	NA
248.15	36	organic	CAR 4:0:O (Spectral Match to acetyl-carnitine)	0.88	6	44.03	3.22
400.343	187	organic	CAR 16:0 (Spectral Match to Lauroyl L-carnitine)	0.91	5	56.06	2.25
396.312	169	organic	CAR 16:2 (Spectral Match to Palmitoyl-carnitine)	0.78	4	4.03	3.03
806.57	345	organic	PC family member (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine) *	0.98	14	45.99	NA
472.401	202	organic	NA	NA	NA	NA	NA
400.343	209	organic	NA	NA	NA	NA	NA
438.323	163	organic	NA	NA	NA	NA	NA
450.359	190	organic	CAR 20:3 (spectral Match to Oleoyl L-carnitine)	0.85	6	24	2.66
512.468	276	organic	CAR 24:0 (Spectral Match to Oleoyl L-carnitine)	0.88	9	86.11	1.37
394.296	168	organic	NA	NA	NA	NA	NA
426.358	229	organic	NA	NA	NA	NA	NA
510.453	249	organic	CAR 24:1 (Spectral Match to Oleoyl L-carnitine)	0.84	6	84.1	2.55
580.362	187	organic	NA	NA	NA	NA	NA
444.369	185	organic	CAR 18:0:O (Spectral Match to Oleoyl L-carnitine)	0.88	13	18.01	1.57
203.224	133	organic	spermine isomer (annotated via networking) *	NA	NA	NA	4.92
480.406	214	organic	NA	NA	NA	NA	NA
398.328	179	organic	CAR 16:1 (Spectral Match to Lauroyl L-carnitine)	0.9	5	54.05	3.77
326.379	231	organic	NA	NA	NA	NA	NA
632.392	195	organic	PC 24:3:O (Spectral Match to 1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine)	0.74	8	42.05	0.32
770.591	321	organic	NA	NA	NA	NA	NA
646.614	417	organic	Cer 42:3:O2 (Spectral Match to Ceramide (18:1/18:0))	0.8	13	0.26	1.08
746.605	457	organic	PC family member (Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine) *	0.87	16	40	NA
618.377	207	organic	PC family member (Spectral Match to 1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine) *	0.97	11	56.06	NA
796.615	397	organic	NA	NA	NA	NA	NA
704.557	371	organic	NA	NA	NA	NA	NA
746.603	523	organic	NA	NA	NA	NA	NA
870.549	241	organic	PC 42:10:O (Spectral Match to 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine)	0.98	13	87.98	17.6
822.563	285	organic	PC 38:6:O (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.98	14	61.98	1.58
800.617	452	organic	PC family member (Spectral Match to 1-Octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine) *	0.95	11	9.98	NA
147.114	18	organic	lysine (annotated via networking)	NA	NA	NA	8.16

539.351	216	organic	NA	NA	NA	NA	NA
703.576	304	organic	NA	NA	NA	NA	NA
807.574	354	organic	NA	NA	NA	NA	NA
742.538	396	organic	PC family member (Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine) *	0.81	16	44.06	0.13
834.592	323	organic	NA	NA	NA	NA	NA
153.043	34	organic	xanthine	0.94	5	0	6
716.523	395	organic	NA	NA	NA	NA	NA
720.443	205	organic	PC 32:7 or PC O-32:8:O (Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.98	9	13.91	23.5
153.041	28	organic	NA	NA	NA	NA	NA
719.57	296	organic	NA	NA	NA	NA	NA
716.449	209	organic	PC family member (Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine) *	0.97	7	9.91	22.3
600.33	159	organic	PC 22:5:O (Spectral Match to PAF C-16)	0.95	9	75.96	0.67
800.617	416	organic	PC family member (Spectral Match to 1-Octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine) *	0.95	11	9.98	NA

S6 Table. Annotated and unannotated metabolites identified as perturbed by infection at all positions (FDR-corrected Mann Whitney $p < 0.05$). * indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.

S6 Table. Annotated and unannotated metabolites identified as perturbed by infection at all positions (FDR-corrected Mann Whitney $p < 0.05$).							
* indicate confidence level 3 annotations. All other annotations at level 2 confidence. NA, not annotated/not applicable.							
All statistically significant metabolites for T. cruzi strain CL							
m/z	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error
155.071	123	aqueous	NA	NA	NA	NA	NA
165.091	170	aqueous	NA	NA	NA	NA	NA
184.074	25	aqueous	phosphocholine (annotated via networking)	NA	NA	NA	0.54
185.596	143	aqueous	NA	NA	NA	NA	NA
189.124	74	aqueous	NA	NA	NA	NA	NA
211.145	151	aqueous	NA	NA	NA	NA	NA
218.14	39	aqueous	CAR 3.0 (annotated via networking)	NA	NA	NA	5.96
251.103	147	aqueous	NA	NA	NA	NA	NA
252.233	190	aqueous	NA	NA	NA	NA	NA
270.244	200	aqueous	NA	NA	NA	NA	NA
278.248	179	aqueous	NA	NA	NA	NA	NA
280.264	206	aqueous	NA	NA	NA	NA	NA
292.227	199	aqueous	NA	NA	NA	NA	NA
296.259	187	aqueous	NA	NA	NA	NA	NA
312.254	194	aqueous	NA	NA	NA	NA	NA
316.285	191	aqueous	NA	NA	NA	NA	NA
332.28	175	aqueous	NA	NA	NA	NA	NA
336.251	190	aqueous	NA	NA	NA	NA	NA
344.228	143	aqueous	NA	NA	NA	NA	NA

365.232	194	aqueous	NA	NA	NA	NA	NA
368.28	177	aqueous	NA	NA	NA	NA	NA
370.178	142	aqueous	NA	NA	NA	NA	NA
372.312	197	aqueous	CAR 14:0 (Spectral Match to Lauroyl L-carnitine)	0.91	4	28.03	3.22
379.21	214	aqueous	NA	NA	NA	NA	NA
380.113	41	aqueous	NA	NA	NA	NA	NA
382.296	182	aqueous	NA	NA	NA	NA	NA
384.275	171	aqueous	CAR 14:2:O (annotated via networking)	NA	NA	NA	1.56
388.255	144	aqueous	NA	NA	NA	NA	NA
396.312	192	aqueous	CAR 16:2 (Spectral Match to Lauroyl L-carnitine)	0.82	4	52.03	3.03
400.269	187	aqueous	NA	NA	NA	NA	NA
415.254	146	aqueous	NA	NA	NA	NA	NA
422.159	119	aqueous	NA	NA	NA	NA	NA
423.237	174	aqueous	NA	NA	NA	NA	NA
432.281	145	aqueous	NA	NA	NA	NA	NA
446.196	147	aqueous	PC 12:4 (annotated via networking)	NA	NA	NA	4.93
465.21	148	aqueous	NA	NA	NA	NA	NA
476.307	146	aqueous	LPC O-16:3 (Spectral Match to 1-Arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine)	0.83	8	76.1	13.65
478.185	141	aqueous	NA	NA	NA	NA	NA
482.325	234	aqueous	PC family member (Spectral Match to 1-Myristoyl-sn-glycero-3-phosphocholine) *	0.96	12	14.01	NA
520.231	148	aqueous	NA	NA	NA	NA	NA
524.372	222	aqueous	LPC 18:0 or PC O-18:0 or LPC O-18:1:O (Spectral Match to 1-(9Z-Octadecenoyl)-sn-glycero-3-phosphocholine)	0.98	18	2.02	1.71
537.34	219	aqueous	NA	NA	NA	NA	NA
605.357	169	aqueous	NA	NA	NA	NA	NA
616.177	186	aqueous	hemin cation	0.98	18	0	1
634.187	180	aqueous	NA	NA	NA	NA	NA
657.204	180	aqueous	heme (M+ACN+H adduct, Spectral Match to Hemin cation)	0.81	15	41.02	0
701.561	274	aqueous	NA	NA	NA	NA	NA
703.575	286	aqueous	NA	NA	NA	NA	NA
931.451	189	aqueous	NA	NA	NA	NA	NA
850.632	417	organic	PC 41:5 or PC O-41:6:O (Spectral Match to 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine)	0.99	11	68.06	0
All statistically significant for <i>T. cruzi</i> strains CL and Sylvio X10/4							

m/z	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error
193.61	145	aqueous	NA	NA	NA	NA	NA
209.093	53	aqueous	NA	NA	NA	NA	NA
209.093	57	organic	NA	NA	NA	NA	NA
250.095	66	aqueous	NA	NA	NA	NA	NA
320.256	206	aqueous	NA	NA	NA	NA	NA
320.256	217	aqueous	NA	NA	NA	NA	NA
368.201	147	aqueous	NA	NA	NA	NA	NA
375.216	186	aqueous	NA	NA	NA	NA	NA
381.227	178	aqueous	NA	NA	NA	NA	NA
383.242	187	aqueous	NA	NA	NA	NA	NA
389.232	202	aqueous	NA	NA	NA	NA	NA
400.343	205	aqueous	CAR 16:0 (Spectral Match to Lauroyl L-carnitine)	0.92	5	56.06	2.25
405.226	186	aqueous	NA	NA	NA	NA	NA
407.242	190	aqueous	NA	NA	NA	NA	NA
421.221	177	aqueous	NA	NA	NA	NA	NA
426.359	210	aqueous	CAR 18:1 (Spectral Match to Palmitoylcarnitine)	0.89	5	26.02	2.81
462.191	140	aqueous	NA	NA	NA	NA	NA
464.206	147	aqueous	NA	NA	NA	NA	NA
All statistically significant for <i>T. cruzi</i> strain Sylvio X10/4							
m/z	RT (sec)	Extract	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error
147.114	14	aqueous	NA	NA	NA	NA	NA
148.061	27	aqueous	glutamine	0.84	4	0.99	4
150.059	27	aqueous	NA	NA	NA	NA	NA
172.985	26	aqueous	NA	NA	NA	NA	NA
220.136	160	aqueous	NA	NA	NA	NA	NA
223.133	183	aqueous	NA	NA	NA	NA	NA
227.091	144	aqueous	NA	NA	NA	NA	NA
232.155	115	aqueous	NA	NA	NA	NA	NA
240.102	69	aqueous	NA	NA	NA	NA	NA

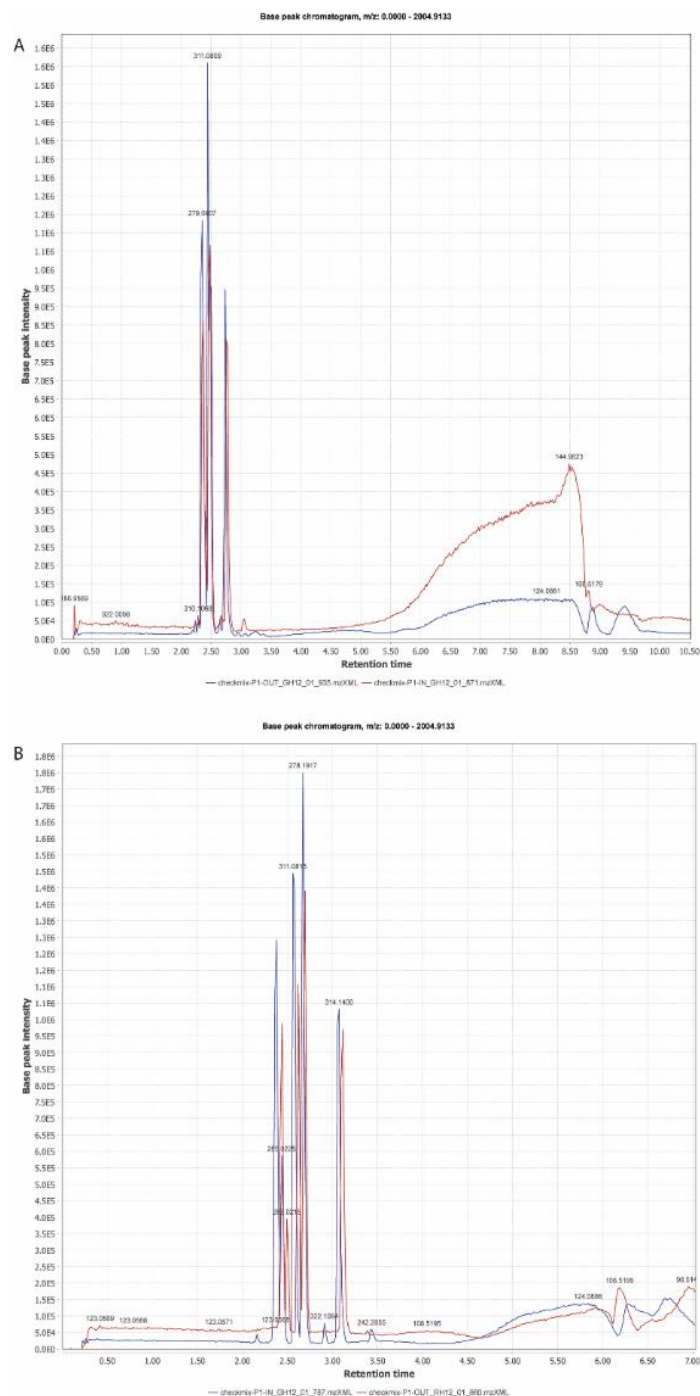
244.155	140	aqueous	NA	NA	NA	NA	NA
246.171	142	aqueous	CAR 5:0 (annotated via networking)	NA	NA	NA	4.06
248.15	30	aqueous	CAR 4:0:O2 (Spectral Match to Acetyl-DL-carnitine)	0.82	4	44.03	3.22
248.151	47	aqueous	CAR 4:0:O (Spectral Match to Acetyl-DL-carnitine)	0.82	4	44.03	7.25
257.117	31	aqueous	NA	NA	NA	NA	NA
258.171	145	aqueous	CAR 6:1 (Spectral Match to Acetyl-L-carnitine)	0.77	4	54.05	3.87
260.187	148	aqueous	CAR 6:0 (Spectral Match to Acetyl-DL-carnitine)	0.96	5	56.06	5.38
262.166	43	aqueous	NA	NA	NA	NA	NA
274.184	166	aqueous	NA	NA	NA	NA	NA
276.181	104	aqueous	NA	NA	NA	NA	NA
276.181	139	aqueous	CAR 6:0:O (Spectral Match to Acetyl-DL-carnitine)	0.94	5	72.06	1.81
286.202	154	aqueous	CAR 8:1 (annotated via networking)	NA	NA	NA	2.45
288.218	159	aqueous	CAR 8:0 (Spectral Match to Acetyl-L-carnitine)	0.87	4	84.09	3.82
299.201	171	aqueous	NA	NA	NA	NA	NA
302.197	145	aqueous	NA	NA	NA	NA	NA
302.216	178	aqueous	NA	NA	NA	NA	NA
304.213	149	aqueous	NA	NA	NA	NA	NA
316.249	170	aqueous	CAR 10:0 (Spectral Match to Propionylcarnitine)	0.92	5	98.11	2.53
332.244	158	aqueous	CAR 10:0:O (Spectral Match to Palmitoylcarnitine)	0.87	4	68.1	2.71
360.275	170	aqueous	NA	NA	NA	NA	NA
384.116	136	aqueous	Succinyladenosine (Spectral Match to Adenylosuccinic acid)	0.88	9	79.97	1.3
384.179	139	aqueous	NA	NA	NA	NA	NA
386.211	146	aqueous	NA	NA	NA	NA	NA
386.291	175	aqueous	CAR 14:1:O (Spectral Match to Palmitoylcarnitine)	0.86	6	14.05	2.33
388.307	181	aqueous	CAR 14:0:O (Spectral Match to Palmitoylcarnitine)	0.87	5	12.04	3.35
390.206	149	aqueous	NA	NA	NA	NA	NA
398.195	143	aqueous	NA	NA	NA	NA	NA
398.327	198	aqueous	CAR 16:1 (Spectral Match to Lauroyl L-carnitine)	0.89	5	54.05	1.26
412.19	137	aqueous	NA	NA	NA	NA	NA
412.2	132	aqueous	NA	NA	NA	NA	NA
412.21	147	aqueous	NA	NA	NA	NA	NA
412.307	179	aqueous	CAR 16:2:O (Spectral Match to Lauroylcarnitine)	0.8	4	68.03	3.15

414.322	186	aqueous	CAR 16:1:O (Spectral Match to Palmitoylcarnitine)	0.85	7	13.98	1.45
416.144	34	aqueous	NA	NA	NA	NA	NA
416.338	192	aqueous	CAR 16:0:O (Spectral Match to Palmitoylcarnitine)	0.87	4	16	2.4
420.144	139	aqueous	NA	NA	NA	NA	NA
424.269	185	aqueous	NA	NA	NA	NA	NA
424.343	203	aqueous	NA	NA	NA	NA	NA
428.374	217	aqueous	CAR 18:0 (Spectral Match to Palmitoylcarnitine)	0.86	5	28.03	1.4
436.211	150	aqueous	NA	NA	NA	NA	NA
440.214	141	aqueous	NA	NA	NA	NA	NA
440.338	190	aqueous	CAR 18:2:O (Spectral Match to Palmitoylcarnitine)	0.85	5	40	2.27
442.353	197	aqueous	CAR 18:1:O (Spectral Match to Palmitoylcarnitine)	0.88	8	42.01	0.68
444.369	204	aqueous	CAR 18:0:O (Spectral Match to Oleoyl L-carnitine)	0.81	5	18.01	1.58
462.191	148	aqueous	NA	NA	NA	NA	NA
468.368	198	aqueous	NA	NA	NA	NA	NA
476.17	140	aqueous	NA	NA	NA	NA	NA
478.151	139	aqueous	NA	NA	NA	NA	NA
478.168	143	aqueous	NA	NA	NA	NA	NA
480.201	147	aqueous	NA	NA	NA	NA	NA
480.343	212	aqueous	PC family member (Spectral Match to 1-(1Z-Hexadecenyl)-sn-glycero-3-phosphocholine) *	0.98	16	0	NA
492.272	183	aqueous	NA	NA	NA	NA	NA
508.376	215	aqueous	NA	NA	NA	NA	NA
510.283	168	aqueous	NA	NA	NA	NA	NA
528.293	157	aqueous	NA	NA	NA	NA	NA
534.319	181	aqueous	LPC 18:3:O (Spectral Match to 1-(9Z-Octadecenyl)-sn-glycero-3-phosphocholine)	0.98	17	11.96	0
552.33	169	aqueous	NA	NA	NA	NA	NA
568.324	162	aqueous	NA	NA	NA	NA	NA
568.325	190	aqueous	NA	NA	NA	NA	NA
570.34	155	aqueous	LPC 22:5 or LPC O-22:6:O or PC O-22:5 (Spectral Match to 1-Arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine)	0.91	8	17.94	27
570.341	173	aqueous	PC family member (Spectral Match to 1-(9Z-Octadecenyl)-sn-glycero-3-phosphocholine) *	0.96	13	47.99	NA
584.335	182	aqueous	LPC 22:6:O or PC 22:5 or PC O-22:6:O (Spectral Match to 1-(9Z-Octadecenyl)-sn-glycero-3-phosphocholine)	0.97	13	61.98	0.51
594.339	163	aqueous	NA	NA	NA	NA	NA
600.33	174	aqueous	PC 22:4:O (Spectral Match to 1-(9Z-Octadecenyl)-sn-glycero-3-phosphocholine)	0.98	12	77.97	0.67

614.31	174	aqueous	NA	NA	NA	NA	NA
137.047	30	organic	hypoxanthine	0.91	4	0	7
138.05	29	organic	NA	NA	NA	NA	NA
216.197	150	organic	NA	NA	NA	NA	NA
248.15	36	organic	CAR 4:0:O (Spectral Match to acetyl-carnitine)	0.88	6	44.03	3.22
248.151	19	organic	NA	NA	NA	NA	NA
260.187	141	organic	CAR 6:0 (annotated via networking)	NA	NA	NA	5.38
269.093	43	organic	NA	NA	NA	NA	NA
274.183	153	organic	NA	NA	NA	NA	NA
302.207	157	organic	NA	NA	NA	NA	NA
302.215	161	organic	NA	NA	NA	NA	NA
304.213	141	organic	CAR 8:0-OH (Spectral Match to Palmitoylcarnitine)	0.83	4	96.13	3.94
326.379	206	organic	NA	NA	NA	NA	NA
326.379	231	organic	NA	NA	NA	NA	NA
332.244	146	organic	CAR 10:0:O (Spectral Match to Palmitoylcarnitine)	0.81	5	68.1	2.71
358.26	150	organic	CAR 12:1:O (Spectral Match to Lauroyl L-carnitine)	0.81	4	13.98	3.35
360.275	153	organic	NA	NA	NA	NA	NA
368.28	158	organic	CAR 14:2 (Spectral Match to Lauroyl L-carnitine)	0.82	5	24	1.36
370.296	166	organic	CAR 14:1 (Spectral Match to Lauroyl L-carnitine)	0.87	5	26.02	2.16
372.312	172	organic	CAR 14:0 (Spectral Match to Lauroyl L-carnitine)	0.91	5	28.03	3.22
386.291	157	organic	CAR 14:1:O (Spectral Match to Lauroyl L-carnitine)	0.78	4	42.01	2.33
388.307	160	organic	CAR 14:0:O (Spectral Match to Palmitoylcarnitine)	0.86	4	12.04	3.35
396.312	169	organic	CAR 16:2 (Spectral Match to Palmitoylcarnitine)	0.78	4	4.03	3.03
398.328	179	organic	CAR 16:1 (Spectral Match to Lauroyl L-carnitine)	0.9	5	54.05	3.77
400.343	187	organic	CAR 16:0 (Spectral Match to Lauroyl L-carnitine)	0.91	5	56.06	2.25
400.343	209	organic	NA	NA	NA	NA	NA
412.307	160	organic	CAR 16:2:O (Spectral Match to Lauroyl L-carnitine)	0.84	4	68.03	3.15
414.322	164	organic	CAR 16:1:O (Spectral Match to Palmitoylcarnitine)	0.83	6	13.98	1.44
414.359	193	organic	NA	NA	NA	NA	NA
416.338	171	organic	CAR 16:0:O (Spectral Match to Palmitoylcarnitine)	0.84	5	16	2.4
424.343	184	organic	CAR 18:2 (Spectral Match to Palmitoylcarnitine)	0.95	9	24	2.12
426.358	229	organic	NA	NA	NA	NA	NA

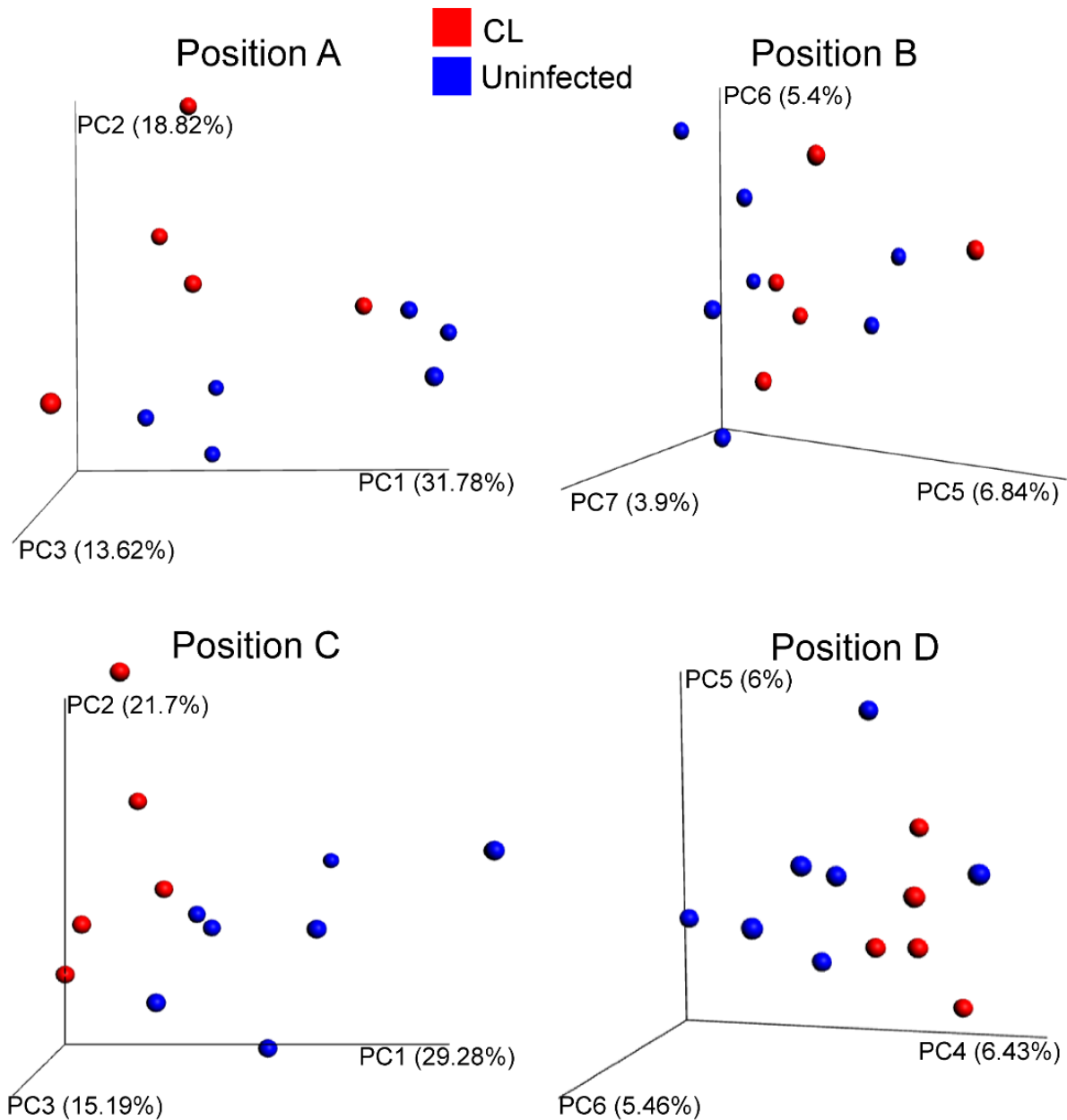
426.359	193	organic	CAR 18:1 (Spectral Match to Lauroyl L-carnitine)	0.91	7	82.08	0.45
428.373	240	organic	NA	NA	NA	NA	NA
428.374	205	organic	CAR 18:0 (Spectral Match to Lauroyl L-carnitine)	0.88	5	84.09	1.4
438.323	163	organic	NA	NA	NA	NA	NA
440.338	168	organic	CAR 18:2:O (Spectral Match to Palmitoylcarnitine)	0.86	6	40	2.27
442.354	175	organic	CAR 18:1:O (Spectral Match to Palmitoylcarnitine)	0.87	8	42.01	2.94
442.391	218	organic	NA	NA	NA	NA	NA
444.369	185	organic	CAR 18:0:O (spectral Match to Oleoyl L-carnitine)	0.88	13	18.01	1.57
450.359	190	organic	CAR 20:3 (spectral Match to Oleoyl L-carnitine)	0.85	6	24	2.66
452.373	244	organic	NA	NA	NA	NA	NA
452.374	199	organic	CAR 20:2 (Spectral Match to Palmitoylcarnitine)	0.89	8	52.03	1.33
454.389	250	organic	NA	NA	NA	NA	NA
454.39	210	organic	CAR 20:1 (Spectral Match to Palmitoylcarnitine)	0.88	8	54.05	1.98
455.394	209	organic	NA	NA	NA	NA	NA
456.406	229	organic	CAR 20:0 (Spectral Match to Palmitoylcarnitine)	0.83	4	56.06	2.85
468.369	179	organic	CAR 20:2:O (Spectral Match to Palmitoylcarnitine)	0.85	7	68.03	1.49
470.385	189	organic	CAR 20:1:O (Spectral Match to Palmitoylcarnitine)	0.87	8	70.04	2.13
472.401	202	organic	NA	NA	NA	NA	NA
480.406	214	organic	NA	NA	NA	NA	NA
482.42	231	organic	CAR 22:1 (Spectral Match to Palmitoylcarnitine)	0.8	6	82.08	0.83
484.364	175	organic	NA	NA	NA	NA	NA
484.437	254	organic	CAR 22:0 (Spectral Match to Palmitoylcarnitine)	0.83	6	84.09	2.06
510.453	249	organic	CAR 24:1 (Spectral Match to Oleoyl L-carnitine)	0.84	6	84.1	2.55
512.468	276	organic	CAR 24:0 (Spectral Match to Oleoyl L-carnitine)	0.88	9	86.11	1.37
513.336	207	organic	NA	NA	NA	NA	NA
541.368	228	organic	NA	NA	NA	NA	NA
569.398	249	organic	NA	NA	NA	NA	NA
580.362	187	organic	NA	NA	NA	NA	NA
596.342	177	organic	PC 23:6 (Spectral Match to PAF C-16)	0.98	11	71.97	12.2
600.33	159	organic	PC 22:5:O (Spectral Match to PAF C-16)	0.95	9	75.96	0.67
632.32	150	organic	NA	NA	NA	NA	NA
716.523	395	organic	NA	NA	NA	NA	NA

744.56	406	organic	PC 33:2 or PC O-33:3:O (Spectral Match to 1-Hexadecyl-2-(9Z-octadecenyl)-sn-glycero-3-phosphocholine)	0.82	13	2.01	8.33
800.617	416	organic	PC family member (Spectral Match to 1-Octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenyl)-sn-glycero-3-phosphocholine) *	0.95	11	9.98	NA
800.617	452	organic	PC family member (Spectral Match to 1-Octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenyl)-sn-glycero-3-phosphocholine) *	0.95	11	9.98	NA
816.59	344	organic	PC O-40:8 (Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine)	0.99	10	56.01	0.24
817.577	275	organic	NA	NA	NA	NA	NA
819.594	297	organic	NA	NA	NA	NA	NA
868.533	230	organic	PC 42:11:O (Spectral Match to 1,2-Dilinoleoyl-sn-glycero-3-phosphocholine)	0.98	14	85.96	18.1

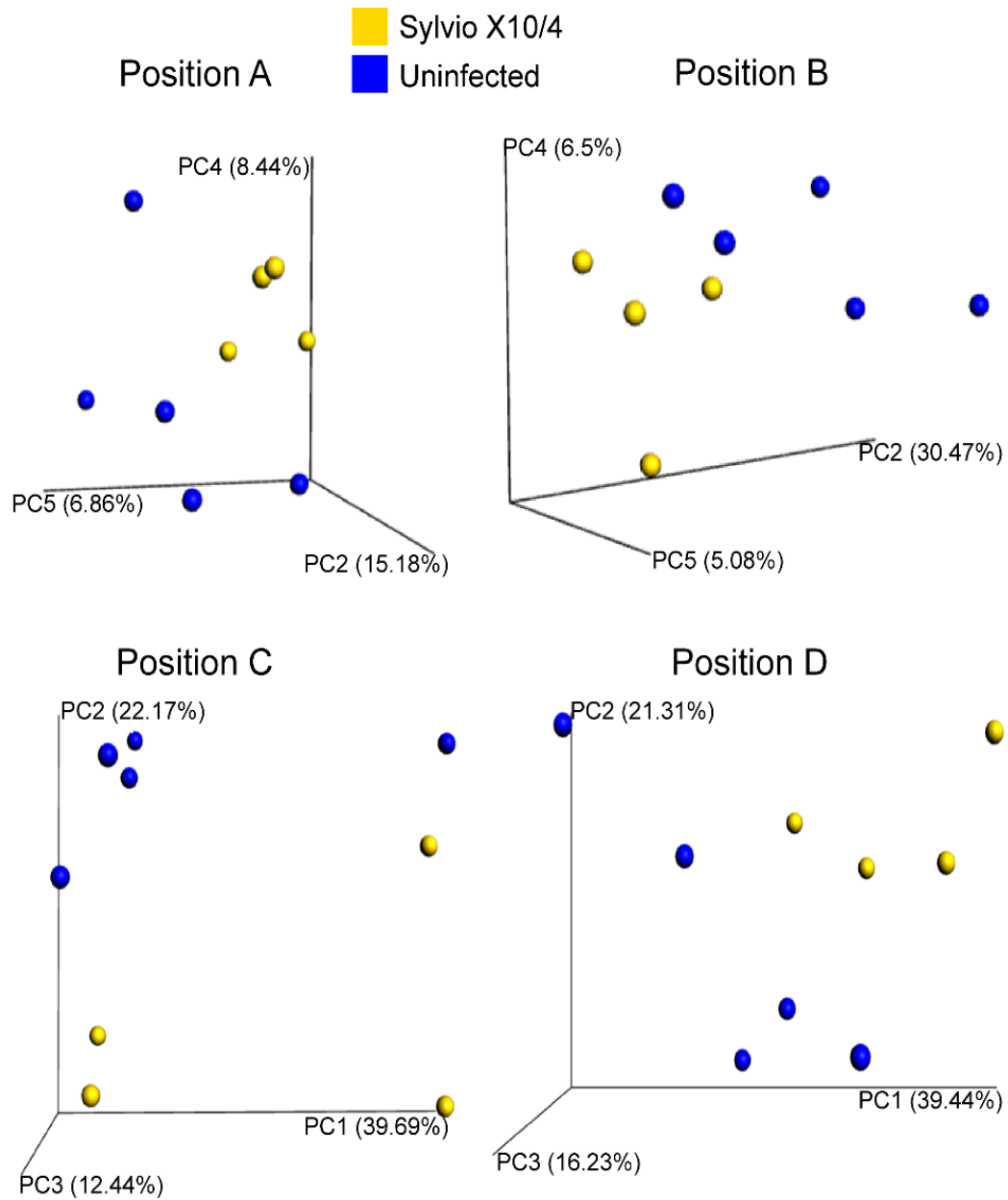


S1 Fig. Base peak chromatogram of checkmix solution used to monitor instrumental drift.

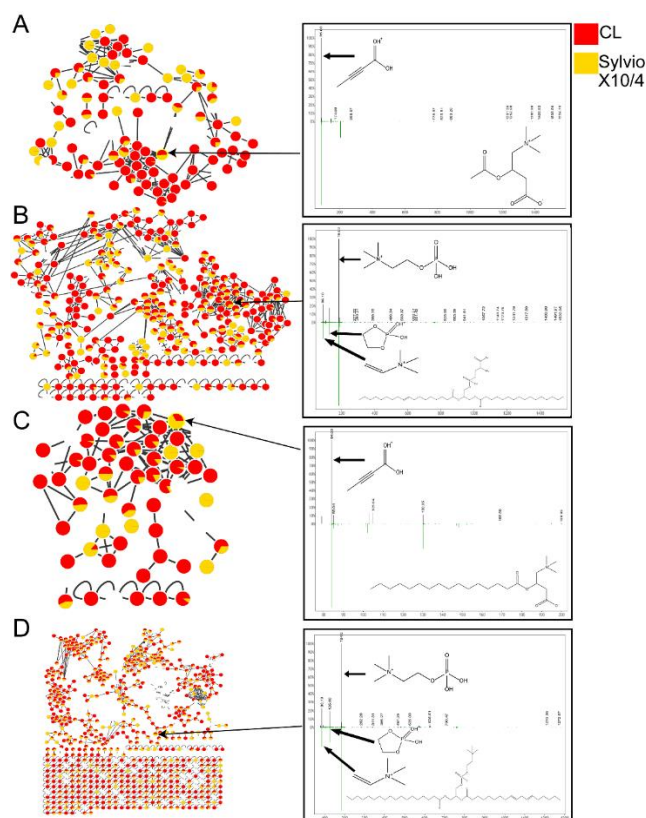
(A) Base peak chromatogram of organic extract checkmix solution at beginning (red) and end (blue) of LC-MS analysis. (B) Base peak chromatogram of aqueous extract checkmix solution at beginning (blue) and end (red) of LC-MS analysis.



S2 Fig. Principal coordinate analysis plot of *T. cruzi* strain CL infected (red) and uninfected (blue) heart tissue samples. Statistically different clustering found in position C (PERMANOVA p-value<0.05).

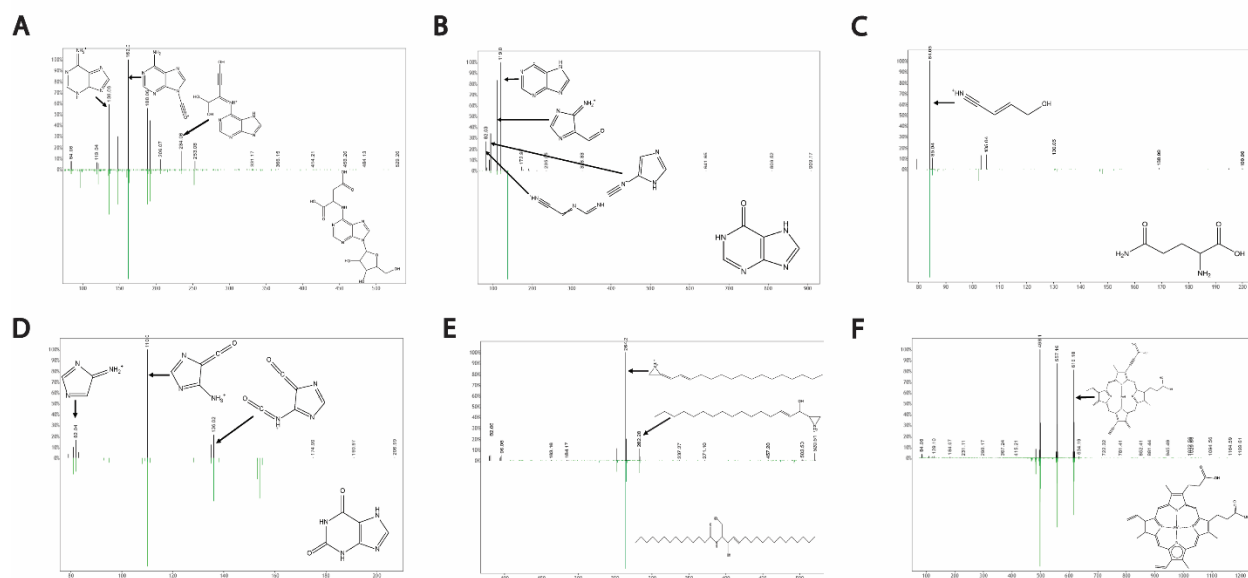


S3 Fig. Principal coordinate analysis plot of *T. cruzi* strain Sylvio X10/4 infected (gold) and uninfected (blue) heart tissue samples. Statistically different clustering found in position D (PERMANOVA p-value<0.05).



S4 Fig. Molecular subnetworks and mirror plot of aqueous and organic extract

acylcarnitines and glycerophosphocholines. Each pie chart is one metabolite colored by MS2 spectral count in CL-infected and Sylvio X10/4-infected samples where red is CL and gold is Sylvio X10/4. (A) Subnetwork of aqueous extract acylcarnitines with representative acylcarnitine mirror plot (acetylcarnitine, m/z 204.124). (B) Subnetwork of aqueous extract phosphocholines with representative glycerophosphocholine mirror plot (Spectral match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine reference library spectrum, m/z 772.549). (C) Subnetwork of organic extract acylcarnitines with representative acylcarnitine mirror plot (palmitoylecarnitine, m/z 424.343). (D) Subnetwork of organic extract phosphocholines with representative glycerophosphocholine mirror plot (Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine reference library spectrum, m/z 794.57).



S5 Fig. Representative GNPS mirror plots of annotated metabolites. (A) Mirror plot of m/z 384.116, RT 136s (top, black) to reference library spectrum (Succinyladenosine, bottom, green). (B) Mirror plot of m/z 137.047, RT 30s (top, black) to reference library spectrum (Hypoxanthine, bottom, green). (C) Mirror plot of m/z 148.061, RT 27s (top, black) to reference library spectrum (L-Glutamine, bottom, green). (D) Mirror plot of m/z 153.043, RT 34s (top, black) to reference library spectrum (Xanthine, bottom, green). (E) Mirror plot of m/z 538.52, RT 362s (top, black) to reference library spectrum (N-(1,3-dihydroxyoctadec-4-en-2-yl)tetradecanamide, bottom, green). (F) Mirror plot of m/z 657.204, RT 180s (top, black) to reference library spectrum (hemin cation, bottom, green).

Appendix B: Chapter 2 Supporting Information

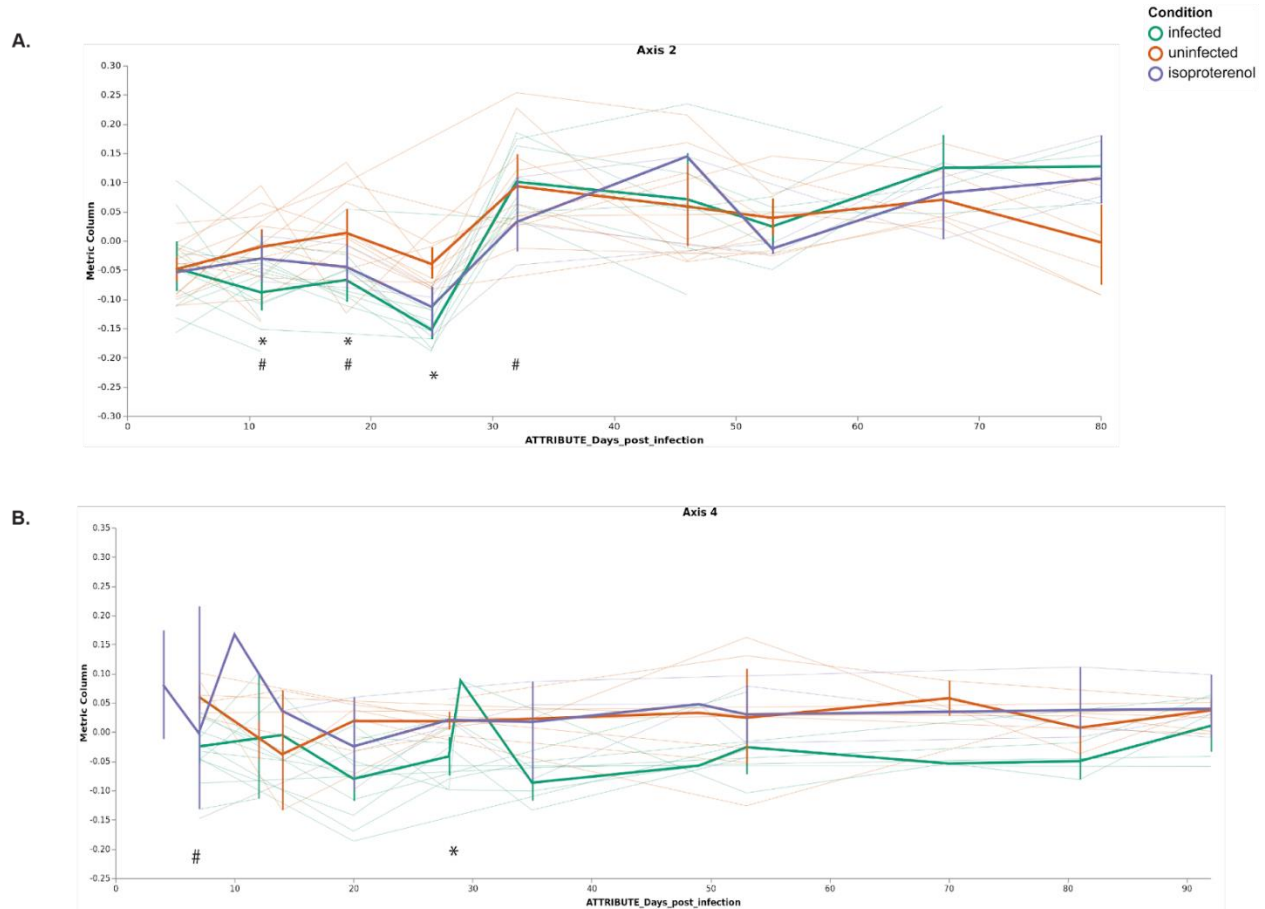


Fig S1: *T. cruzi* infection has minor impacts on the salivary and plasma metabolome.(A)

Saliva sample volatility analysis showing separation between uninfected samples and both infected and isoproterenol-treated samples at early timepoints along principal component axis 2.

*, PERMANOVA $p < 0.05$ for infected to uninfected animals. #, PERMANOVA $p < 0.05$ for isoproterenol-treated to uninfected animals. **(B)** Plasma sample volatility analysis showing limited differences between groups along principal component axis 4. *, PERMANOVA $p < 0.05$ infected vs uninfected. #, PERMANOVA $p < 0.05$ for isoproterenol-treated to uninfected animals.

Saliva

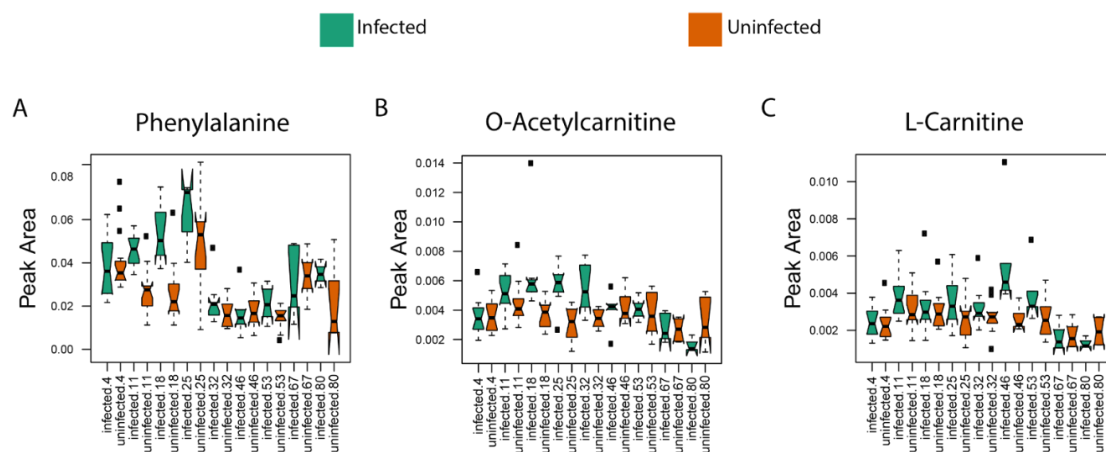


Fig S2: Representative boxplots of significant metabolites perturbed by infection treatment in the saliva. (A) Phenylalanine (m/z : 166.0863, RT: 0.63 min) (B) O-Acetylcarnitine (m/z : 204.123 , RT: 0.39 min) (C) L-Carnitine (m/z : 162.1124, RT: 0.3 min)

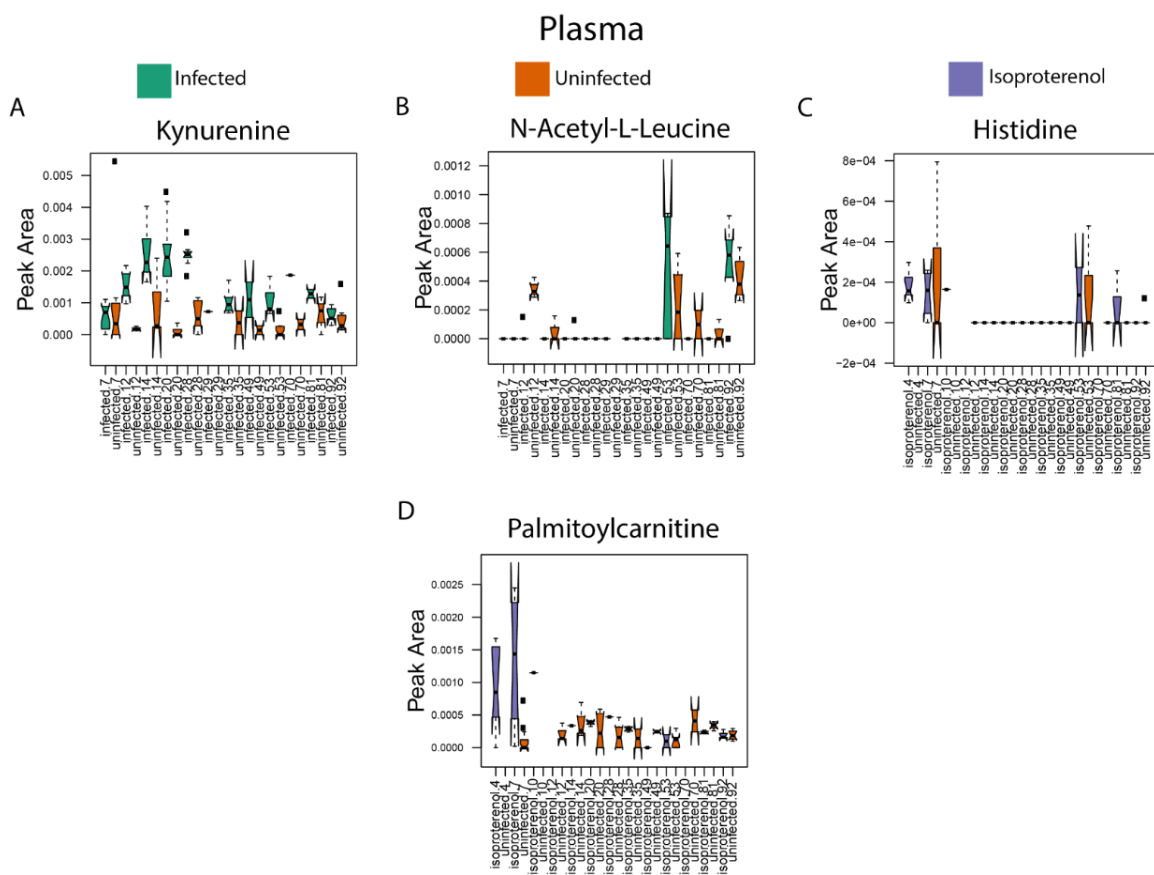


Fig S3: Representative boxplots of significant metabolites perturbed by infection or isoproterenol treatment in the plasma. (A) Kynurenine (m/z : 209.092, RT: 0.65 min) (B) N-Acetyl-Leucine (m/z 174.1125, RT: 2.77 min) (C) Histidine (m/z : 129.0659, RT: 0.31 min) (D) Palmitoylcarnitine (m/z : 400.3417, RT: 6.6 min)

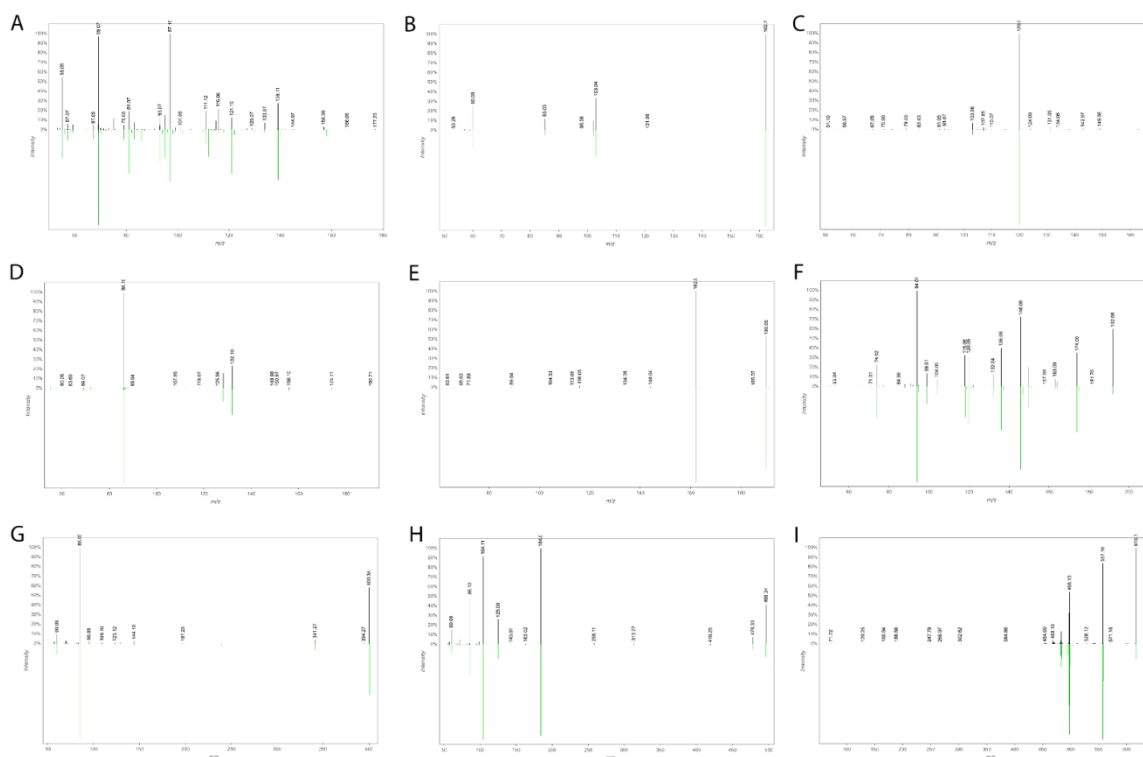


Fig S4: Representative mirror plots of metabolites perturbed by *T.cruzi* infection or isoproterenol treatment (A) Mirror plot of m/z 157.1222, RT 4.06 min (top, black) to reference library spectrum (4-Hydroxynonenal, bottom, green) (B) Mirror plot of m/z 162.1124, RT 0.3 min (top, black) to reference library spectrum (L-Carnitine, bottom, green) (C) Mirror plot of m/z 166.0863, RT 0.63 min (top, black) to reference library spectrum (Phenylalanine, bottom, green) (D) Mirror plot of m/z 174.1125, RT 2.77 min (top, black) to reference library spectrum (N-Acetyl-L-Leucine, bottom, green) (E) Mirror plot of m/z 190.0498, RT 2.31 min (top, black) to reference library spectrum (Kynurenate, bottom, green) (F) Mirror plot of m/z 209.092, RT 0.65 min (top, black) to reference library spectrum (Kynurenine, bottom, green) (G) Mirror plot of m/z 400.3417, RT 6.6 min (top, black) to reference library spectrum (Palmitoylcarnitine, bottom, green) (H) Mirror plot of m/z 496.3397, RT 6.81 min (top, black) to reference library spectrum (LPC 16:0, bottom, green) (I) Mirror plot of m/z 616.1758, RT 5.87 min (top, black) to reference library spectrum (Hemin cation, bottom, green)

Table S1: Annotation table of metabolites significantly perturbed by infection or isoproterenol treatment. NA, not applicable.

m/z	RT	Annotation	Cosine Score	No. of Shared Peaks	Mass Diff. to Library Reference	ppm error	Cluster Index	Analogue	AIC	Estimate	Std. Error	z-value	Pr. > z	qOrFDR	Group present in
76.0763	0.3	NA	NA	NA	NA	NA	222	No	-6.63E+02	-3.54E-03	1.19E-03	-2.97E+00	3.03E-03	2.33E-02	urine_inf_nif
77.0388	3.52	NA	NA	NA	NA	NA	2949	No	-1.01E+03	2.46E-05	6.62E-06	3.72E+00	2.00E-04	3.02E-03	urine_inf_nif
79.0217	0.4	NA	NA	NA	NA	NA	3691	No	-1.03E+03	-2.30E-04	5.40E-05	-4.26E+00	2.06E-05	5.14E-04	urine_inf_nif
80.9484	0.29	NA	NA	NA	NA	NA	103	No	-1.39E+03	6.67E-04	1.63E-04	4.10E+00	4.21E-05	1.42E-02	saliva_inf_nif
83.0221	0.29	NA	NA	NA	NA	NA	181	No	-2.08E+03	6.18E-05	1.80E-05	3.44E+00	5.88E-04	2.80E-02	saliva_inf_nif
83.0496	1.22	NA	NA	NA	NA	NA	2127	No	-1.18E+03	6.43E-04	6.27E-05	1.02E+01	1.19E-24	6.10E-22	urine_inf_nif
95.0859	3.66	NA	NA	NA	NA	NA	2369	No	-1.39E+03	6.41E-05	1.51E-05	4.23E+00	2.30E-05	5.36E-04	urine_inf_nif
101.0059	2.53	NA	NA	NA	NA	NA	4742	No	-8.87E+02	3.36E-05	6.30E-06	5.33E+00	9.70E-08	4.32E-06	urine_inf_nif
103.0545	3.52	NA	NA	NA	NA	NA	2079	No	-9.90E+02	9.64E-04	1.74E-04	5.53E+00	3.12E-08	1.68E-06	urine_inf_nif
113.0963	2.16	NA	NA	NA	NA	NA	2938	No	-1.04E+03	8.19E-05	2.45E-05	3.35E+00	8.14E-04	8.88E-03	urine_inf_nif
118.0653	3.52	NA	NA	NA	NA	NA	2223	No	-8.68E+02	1.70E-04	4.04E-05	4.21E+00	2.60E-05	5.45E-04	urine_inf_nif
120.0445	0.96	NA	NA	NA	NA	NA	2143	No	-1.19E+03	3.52E-04	6.31E-05	5.58E+00	2.37E-08	1.43E-06	urine_inf_nif
130.0499	4.82	NA	NA	NA	NA	NA	2511	No	-1.06E+03	-5.76E-05	1.75E-05	-3.30E+00	9.66E-04	1.03E-02	urine_inf_nif
134.06	1.83	Indoxyl Sulfate	0.944929	4	0	0	2415	No	-1.10E+03	3.40E-04	7.03E-05	4.84E+00	1.29E-06	4.27E-05	urine_inf_nif
144.0807	3.52	NA	NA	NA	NA	NA	2297	No	-1.35E+03	1.06E-04	1.82E-05	5.82E+00	5.72E-09	4.51E-07	urine_inf_nif
146.0447	2.53	NA	NA	NA	NA	NA	3381	No	-9.02E+02	3.03E-05	6.20E-06	4.89E+00	1.03E-06	3.64E-05	urine_inf_nif
146.0924	0.38	4-Guanidino-Butanoate	0.950592	6	0.000595	4.07338	3416	No	-8.68E+02	-1.01E-03	3.62E-04	-2.78E+00	5.49E-03	3.68E-02	urine_inf_nif
146.9803	11.15	NA	NA	NA	NA	NA	1752	No	-2.44E+02	2.65E-03	6.99E-04	3.79E+00	1.49E-04	1.60E-02	plasma_iso_nif
147.0763	0.44	NA	NA	NA	NA	NA	5569	No	-8.41E+02	2.17E-04	5.82E-05	3.73E+00	1.95E-04	2.98E-03	urine_inf_nif
149.1073	0.4	NA	NA	NA	NA	NA	2233	No	-9.56E+02	1.55E-04	4.29E-05	3.61E+00	3.05E-04	3.96E-03	urine_inf_nif
151.144	0.3	NA	NA	NA	NA	NA	2054	No	-7.38E+02	-2.26E-03	7.81E-04	-2.90E+00	3.79E-03	2.79E-02	urine_inf_nif
157.1222	4.06	4-Hydroxynonenal	0.759243	5	0.000198	1.26249	1351	No	-1.16E+03	2.31E-05	7.62E-06	3.03E+00	2.43E-03	2.09E-02	urine_inf_nif
160.0967	1.23	N-Isovaleroylglycine	0.888507	4	0.000305	1.90619	969	No	-3.67E+02	7.49E-03	1.07E-03	7.03E+00	2.11E-12	3.60E-10	urine_inf_nif
162.1124	0.3	L-Carnitine	0.994627	7	0.000397	2.44725	46	No	-1.62E+03	-7.48E-04	2.35E-04	-3.18E+00	1.45E-03	3.93E-02	saliva_inf_nif
164.0705	1.81	NA	NA	NA	NA	NA	2770	No	-1.14E+03	7.65E-05	1.42E-05	5.37E+00	7.75E-08	3.78E-06	urine_inf_nif

166.0863	0.63	DL-Phenylalanine	0.993306	6	0.003311	19.9368	43	No	-8.34E+02	-9.67E-03	2.87E-03	-3.37E+00	7.41E-04	3.12E-02	saliva_inf_nif
173.1172	1.86	NA	NA	NA	NA	NA	631	No	-1.24E+03	6.76E-05	1.81E-05	3.73E+00	1.94E-04	2.98E-03	urine_inf_nif
174.1125	2.77	N-Acetyl-L-Leucine	0.964249	5	0.000504	2.89203	1433	No	-3.82E+02	-2.60E-04	7.58E-05	-3.43E+00	6.09E-04	1.55E-02	plasma_inf_nif
175.0866	0.54	NA	NA	NA	NA	NA	527	No	-6.85E+02	-3.30E-05	6.52E-06	-5.06E+00	4.16E-07	1.68E-05	urine_inf_nif
176.0916	0.51	NA	NA	NA	NA	NA	2163	No	-1.12E+03	2.31E-04	6.47E-05	3.57E+00	3.59E-04	4.55E-03	urine_inf_nif
178.0498	2.73	NA	NA	NA	NA	NA	2921	No	-9.58E+02	-8.44E-05	1.74E-05	-4.84E+00	1.29E-06	4.27E-05	urine_inf_nif
180.0654	1.93	Hippuric acid	0.944185	4	0.000595	3.30486	966	No	-2.93E+02	-2.19E-02	5.31E-03	-4.12E+00	3.79E-05	7.61E-04	urine_inf_nif
183.0653	3.2	NA	NA	NA	NA	NA	1745	No	-1.57E+03	1.18E-04	3.58E-05	3.29E+00	9.96E-04	3.43E-02	saliva_inf_nif
190.0498	2.31	Kynurenate	0.991269	5	0.000198	1.04375	2080	No	-6.50E+02	-5.50E-03	1.30E-03	-4.24E+00	2.25E-05	5.36E-04	urine_inf_nif
190.1073	0.75	NA	NA	NA	NA	NA	2130	No	-9.85E+02	3.73E-04	6.47E-05	5.77E+00	7.93E-09	5.81E-07	urine_inf_nif
192.0688	1.08	NA	NA	NA	NA	NA	3345	No	-8.29E+02	-3.65E-05	1.24E-05	-2.93E+00	3.37E-03	2.56E-02	urine_inf_nif
192.0688	1.32	NA	NA	NA	NA	NA	2338	No	-9.84E+02	7.06E-05	2.31E-05	3.05E+00	2.26E-03	1.98E-02	urine_inf_nif
200.2373	5.42	NA	NA	NA	NA	NA	5865	No	-2.79E+02	2.77E-04	5.30E-05	5.23E+00	1.71E-07	7.26E-05	plasma_iso_nif
202.1073	1.42	NA	NA	NA	NA	NA	2826	No	-9.68E+02	2.59E-05	6.67E-06	3.88E+00	1.04E-04	1.75E-03	urine_inf_nif
203.0527	0.29	NA	NA	NA	NA	NA	60	No	-1.31E+03	3.41E-04	1.09E-04	3.13E+00	1.76E-03	4.19E-02	saliva_inf_nif
204.0688	0.65	NA	NA	NA	NA	NA	5098	No	-7.69E+02	8.74E-05	2.04E-05	4.29E+00	1.82E-05	4.66E-04	urine_inf_nif
204.0688	0.92	NA	NA	NA	NA	NA	2289	No	-1.27E+03	2.86E-04	3.48E-05	8.21E+00	2.14E-16	5.48E-14	urine_inf_nif
204.123	0.39	O-Acetylcarnitine	0.985943	6	0	0	83	No	-1.58E+03	-1.10E-03	2.98E-04	-3.69E+00	2.26E-04	2.14E-02	saliva_inf_nif
204.123	0.39	O-Acetylcarnitine	0.985943	6	0	0	83	No	-8.45E+02	-1.10E-03	3.90E-04	-2.83E+00	4.65E-03	3.29E-02	urine_inf_nif
206.1134	0.68	NA	NA	NA	NA	NA	3320	No	-1.36E+03	2.29E-05	5.43E-06	4.22E+00	2.48E-05	5.40E-04	urine_inf_nif
207.065	3.93	NA	NA	NA	NA	NA	2900	No	-9.61E+02	-9.32E-06	3.38E-06	-2.76E+00	5.83E-03	3.78E-02	urine_inf_nif
208.0855	3.66	NA	NA	NA	NA	NA	742	No	-9.98E+02	2.74E-05	7.00E-06	3.92E+00	8.89E-05	1.57E-03	urine_inf_nif
209.0784	2.87	NA	NA	NA	NA	NA	1799	No	-1.43E+03	5.10E-05	1.34E-05	3.81E+00	1.38E-04	1.77E-02	saliva_inf_nif
209.092	0.65	Kynurenine	0.969712	8	0	0	358	No	-1.16E+03	-8.17E-04	2.31E-04	-3.53E+00	4.08E-04	1.43E-02	plasma_inf_nif
211.1691	4.09	NA	NA	NA	NA	NA	2339	No	-1.14E+03	6.48E-05	2.14E-05	3.03E+00	2.48E-03	2.09E-02	urine_inf_nif
214.1071	2.55	NA	NA	NA	NA	NA	2301	No	-1.36E+03	6.06E-05	1.98E-05	3.07E+00	2.16E-03	1.91E-02	urine_inf_nif
215.1388	0.44	Pro-Val	0.96911	5	0.000214	0.992954	2465	No	-1.29E+03	-6.58E-05	1.80E-05	-3.65E+00	2.62E-04	3.58E-03	urine_inf_nif
219.0265	0.28	NA	NA	NA	NA	NA	249	No	-1.75E+03	4.03E-04	1.30E-04	3.11E+00	1.87E-03	4.19E-02	saliva_inf_nif
219.1701	0.32	NA	NA	NA	NA	NA	555	No	-6.66E+02	-1.87E-03	5.94E-04	-3.14E+00	1.70E-03	1.57E-02	urine_inf_nif
220.0636	3.49	NA	NA	NA	NA	NA	2170	No	-1.01E+03	1.17E-03	1.58E-04	7.41E+00	1.30E-13	2.66E-11	urine_inf_nif
220.1289	0.43	NA	NA	NA	NA	NA	2371	No	-1.26E+03	-1.75E-04	4.71E-05	-3.71E+00	2.05E-04	3.04E-03	urine_inf_nif

220.1289	1.25	NA	NA	NA	NA	NA	3253	No	-9.39E+02	8.07E-05	2.45E-05	3.29E+00	9.89E-04	1.03E-02	urine_inf_nif
222.0793	0.71	NA	NA	NA	NA	NA	2213	No	-1.30E+03	1.25E-04	3.05E-05	4.09E+00	4.35E-05	8.57E-04	urine_inf_nif
223.1075	0.45	NA	NA	NA	NA	NA	2837	No	-1.16E+03	3.38E-05	1.24E-05	2.73E+00	6.33E-03	3.94E-02	urine_inf_nif
230.102	0.93	NA	NA	NA	NA	NA	2643	No	-1.33E+03	2.80E-05	1.05E-05	2.67E+00	7.48E-03	4.33E-02	urine_inf_nif
231.1449	0.61	NA	NA	NA	NA	NA	4731	No	-7.81E+02	2.33E-05	8.69E-06	2.68E+00	7.34E-03	4.30E-02	urine_inf_nif
232.1289	0.55	NA	NA	NA	NA	NA	2187	No	-1.24E+03	-1.23E-04	3.40E-05	-3.63E+00	2.88E-04	3.79E-03	urine_inf_nif
238.0933	0.47	NA	NA	NA	NA	NA	2582	No	-1.21E+03	-7.07E-05	2.07E-05	-3.41E+00	6.40E-04	7.38E-03	urine_inf_nif
240.1591	3.22	NA	NA	NA	NA	NA	2334	No	-4.86E+02	3.91E-04	1.49E-04	2.63E+00	8.64E-03	4.86E-02	urine_inf_nif
240.1591	3.99	NA	NA	NA	NA	NA	2199	No	-1.20E+03	1.64E-04	3.90E-05	4.21E+00	2.53E-05	5.40E-04	urine_inf_nif
240.8388	0.27	NA	NA	NA	NA	NA	1614	No	-1.49E+03	1.09E-04	3.16E-05	3.46E+00	5.49E-04	2.80E-02	saliva_inf_nif
242.1383	3.5	NA	NA	NA	NA	NA	2185	No	-1.07E+03	3.23E-04	1.06E-04	3.03E+00	2.42E-03	2.09E-02	urine_inf_nif
243.1336	1.02	NA	NA	NA	NA	NA	2340	No	-1.35E+03	-5.12E-05	1.90E-05	-2.69E+00	7.05E-03	4.20E-02	urine_inf_nif
248.1128	1.25	NA	NA	NA	NA	NA	3325	No	-7.85E+02	-7.05E-05	2.24E-05	-3.14E+00	1.67E-03	1.55E-02	urine_inf_nif
251.0878	2.86	NA	NA	NA	NA	NA	5746	No	-2.65E+02	3.60E-05	9.54E-06	3.77E+00	1.62E-04	4.09E-02	urine_iso_nif
251.1386	2.88	NA	NA	NA	NA	NA	2452	No	-1.32E+03	-3.87E-05	1.18E-05	-3.29E+00	1.00E-03	1.04E-02	urine_inf_nif
252.0723	0.51	NA	NA	NA	NA	NA	3153	No	-1.14E+03	-4.45E-05	1.55E-05	-2.88E+00	4.03E-03	2.90E-02	urine_inf_nif
252.1202	3.17	NA	NA	NA	NA	NA	2472	No	-1.35E+03	3.08E-05	1.17E-05	2.63E+00	8.56E-03	4.85E-02	urine_inf_nif
254.1495	0.28	NA	NA	NA	NA	NA	2351	No	-1.04E+03	6.09E-05	1.78E-05	3.42E+00	6.36E-04	7.38E-03	urine_inf_nif
258.1695	4.42	NA	NA	NA	NA	NA	2617	No	-1.32E+03	2.39E-05	7.53E-06	3.17E+00	1.54E-03	1.46E-02	urine_inf_nif
258.1696	3.83	NA	NA	NA	NA	NA	2604	No	-1.31E+03	3.77E-05	1.35E-05	2.80E+00	5.09E-03	3.48E-02	urine_inf_nif
262.0915	0.45	NA	NA	NA	NA	NA	2737	No	-8.02E+02	-1.60E-04	5.46E-05	-2.93E+00	3.35E-03	2.56E-02	urine_inf_nif
264.0377	0.38	NA	NA	NA	NA	NA	2391	No	-6.66E+02	-7.60E-05	1.98E-05	-3.84E+00	1.25E-04	2.03E-03	urine_inf_nif
264.1634	3.12	NA	NA	NA	NA	NA	2876	No	-8.88E+02	2.12E-05	7.00E-06	3.02E+00	2.49E-03	2.09E-02	urine_inf_nif
266.1364	3.29	NA	NA	NA	NA	NA	2560	No	-8.29E+02	8.74E-05	2.92E-05	3.00E+00	2.74E-03	2.20E-02	urine_inf_nif
266.1377	3.17	NA	NA	NA	NA	NA	2383	No	-1.25E+03	8.14E-05	2.45E-05	3.32E+00	8.94E-04	9.65E-03	urine_inf_nif
268.0435	3.1	NA	NA	NA	NA	NA	2324	No	-1.29E+03	6.54E-05	2.41E-05	2.72E+00	6.62E-03	4.04E-02	urine_inf_nif
272.1488	3.77	NA	NA	NA	NA	NA	4837	No	-7.16E+02	1.08E-05	4.07E-06	2.66E+00	7.75E-03	4.44E-02	urine_inf_nif
274.1829	4.71	NA	NA	NA	NA	NA	2141	No	-1.14E+03	-2.81E-04	6.60E-05	-4.25E+00	2.11E-05	5.14E-04	urine_inf_nif
275.1228	0.65	NA	NA	NA	NA	NA	7218	No	-1.31E+03	-5.20E-05	1.88E-05	-2.76E+00	5.82E-03	3.78E-02	urine_inf_nif
277.2158	4.84	NA	NA	NA	NA	NA	1677	No	-2.49E+03	-3.50E-05	1.13E-05	-3.11E+00	1.88E-03	4.19E-02	saliva_inf_nif
279.1334	2.93	NA	NA	NA	NA	NA	2083	No	-9.89E+02	-7.39E-04	1.76E-04	-4.21E+00	2.52E-05	5.40E-04	urine_inf_nif

280.1289	1	Asn-Phe	0.871371	11	9.16E-05	0.326823	875	No	-1.39E+03	1.61E-03	4.24E-04	3.81E+00	1.40E-04	1.77E-02	saliva_inf_nif
280.165	0.3	NA	NA	NA	NA	NA	2188	No	-1.27E+03	5.92E-05	1.99E-05	2.97E+00	2.93E-03	2.31E-02	urine_inf_nif
281.1491	3.81	NA	NA	NA	NA	NA	2237	No	-1.25E+03	-8.71E-05	3.02E-05	-2.89E+00	3.91E-03	2.84E-02	urine_inf_nif
284.0986	0.51	Guanosine	0.982011	8	0.005615	19.7655	514	No	-1.16E+03	-5.50E-05	1.69E-05	-3.26E+00	1.12E-03	1.12E-02	urine_inf_nif
285.1305	3.59	NA	NA	NA	NA	NA	1058	No	-1.79E+03	7.92E-05	2.43E-05	3.26E+00	1.11E-03	3.56E-02	saliva_inf_nif
286.1644	3.86	NA	NA	NA	NA	NA	2471	No	-1.35E+03	4.89E-05	1.78E-05	2.75E+00	6.00E-03	3.84E-02	urine_inf_nif
294.1002	0.83	NA	NA	NA	NA	NA	5247	No	-2.64E+02	1.23E-05	2.26E-06	5.45E+00	4.98E-08	3.78E-05	urine_iso_nif
295.2263	4.84	NA	NA	NA	NA	NA	529	No	-2.03E+03	-2.09E-04	6.77E-05	-3.09E+00	2.02E-03	4.37E-02	saliva_inf_nif
297.0478	0.33	NA	NA	NA	NA	NA	3209	No	-8.55E+02	3.78E-05	1.34E-05	2.82E+00	4.75E-03	3.31E-02	urine_inf_nif
303.1003	2.63	NA	NA	NA	NA	NA	3436	No	-8.64E+02	-4.11E-05	1.17E-05	-3.52E+00	4.30E-04	5.31E-03	urine_inf_nif
304.7424	0.31	NA	NA	NA	NA	NA	772	No	-1.45E+03	1.27E-04	3.42E-05	3.72E+00	2.01E-04	3.63E-02	saliva_iso_nif
318.1541	2.76	NA	NA	NA	NA	NA	2813	No	-1.04E+03	2.30E-05	8.63E-06	2.67E+00	7.61E-03	4.38E-02	urine_inf_nif
325.2115	4.05	NA	NA	NA	NA	NA	3193	No	-1.00E+03	-2.77E-05	7.96E-06	-3.48E+00	5.09E-04	5.99E-03	urine_inf_nif
327.1002	2.29	NA	NA	NA	NA	NA	2730	No	-9.98E+02	7.57E-05	1.54E-05	4.92E+00	8.57E-07	3.25E-05	urine_inf_nif
327.1002	2.47	NA	NA	NA	NA	NA	2507	No	-1.11E+03	9.70E-05	2.01E-05	4.83E+00	1.35E-06	4.32E-05	urine_inf_nif
327.1545	1.14	NA	NA	NA	NA	NA	3284	No	-8.10E+02	-1.83E-05	6.49E-06	-2.82E+00	4.75E-03	3.31E-02	urine_inf_nif
330.1902	2.62	NA	NA	NA	NA	NA	1086	No	-7.93E+02	-8.07E-05	2.46E-05	-3.28E+00	1.05E-03	1.08E-02	urine_inf_nif
333.0509	3.26	NA	NA	NA	NA	NA	2697	No	-1.22E+03	5.56E-05	1.24E-05	4.47E+00	7.97E-06	2.15E-04	urine_inf_nif
333.0744	1.25	NA	NA	NA	NA	NA	5101	No	-7.71E+02	-2.19E-05	5.55E-06	-3.95E+00	7.91E-05	1.42E-03	urine_inf_nif
339.1404	0.39	NA	NA	NA	NA	NA	3310	No	-6.29E+02	-4.91E-05	1.39E-05	-3.52E+00	4.25E-04	5.31E-03	urine_inf_nif
351.2138	5.65	NA	NA	NA	NA	NA	3588	No	-1.85E+03	-7.16E-05	2.09E-05	-3.44E+00	5.92E-04	2.80E-02	saliva_inf_nif
353.2294	4.84	NA	NA	NA	NA	NA	579	No	-1.96E+03	-2.81E-04	7.01E-05	-4.01E+00	6.20E-05	1.42E-02	saliva_inf_nif
366.1752	3.32	NA	NA	NA	NA	NA	3004	No	-8.07E+02	-2.03E-05	7.56E-06	-2.68E+00	7.34E-03	4.30E-02	urine_inf_nif
369.2241	0.36	NA	NA	NA	NA	NA	5626	No	-1.52E+02	3.57E-05	8.39E-06	4.26E+00	2.02E-05	7.32E-03	saliva_iso_nif
375.114	0.52	NA	NA	NA	NA	NA	3278	No	-9.89E+02	-5.49E-05	1.09E-05	-5.06E+00	4.25E-07	1.68E-05	urine_inf_nif
377.1449	2.94	Riboflavin	0.935131	14	0.001099	2.91302	2115	No	-1.17E+03	2.71E-04	6.02E-05	4.50E+00	6.74E-06	1.92E-04	urine_inf_nif
395.2037	3.72	NA	NA	NA	NA	NA	4696	No	-6.83E+02	8.56E-05	2.72E-05	3.15E+00	1.62E-03	4.19E-02	saliva_inf_nif
395.2762	7.03	NA	NA	NA	NA	NA	145	No	-1.53E+02	1.49E-05	2.58E-06	5.77E+00	7.99E-09	1.21E-05	urine_iso_nif
398.2531	4.04	NA	NA	NA	NA	NA	2962	No	-9.88E+02	-3.54E-05	9.88E-06	-3.59E+00	3.35E-04	4.29E-03	urine_inf_nif
400.2687	4.35	NA	NA	NA	NA	NA	3172	No	-8.41E+02	-5.39E-05	1.86E-05	-2.90E+00	3.72E-03	2.77E-02	urine_inf_nif
400.3417	6.6	Palmitoylcarnitine	0.947114	6	0.000305	0.762288	315	No	-8.29E+02	-6.70E-04	1.92E-04	-3.49E+00	4.75E-04	3.27E-02	plasma_iso_nif

402.1501	0.65	NA	NA	NA	NA	NA	2635	No	-1.10E+03	1.47E-04	5.41E-05	2.72E+00	6.56E-03	4.04E-02	urine_inf_nif
413.141	2.37	N6-Threonylcarbamoyl adenosine	0.890542	9	0.001007	2.43761	2190	No	-1.22E+03	-1.29E-04	4.04E-05	-3.18E+00	1.45E-03	1.39E-02	urine_inf_nif
416.2637	3.48	NA	NA	NA	NA	NA	2880	No	-8.76E+02	-1.87E-05	6.19E-06	-3.03E+00	2.48E-03	2.09E-02	urine_inf_nif
421.8694	0.26	NA	NA	NA	NA	NA	2651	No	-1.81E+02	-2.06E-05	5.07E-06	-4.06E+00	4.94E-05	1.71E-02	urine_iso_nif
423.1978	3.37	NA	NA	NA	NA	NA	4653	No	-7.52E+02	-3.02E-05	1.14E-05	-2.66E+00	7.89E-03	4.50E-02	urine_inf_nif
427.1566	2.78	NA	NA	NA	NA	NA	3152	No	-1.16E+03	-3.08E-05	6.88E-06	-4.47E+00	7.95E-06	2.15E-04	urine_inf_nif
437.1779	5.02	NA	NA	NA	NA	NA	1332	No	-2.06E+03	5.21E-05	1.49E-05	3.51E+00	4.55E-04	2.65E-02	saliva_inf_nif
453.1434	3.1	NA	NA	NA	NA	NA	2669	No	-1.22E+03	3.48E-05	1.24E-05	2.82E+00	4.87E-03	3.38E-02	urine_inf_nif
474.2872	3.89	NA	NA	NA	NA	NA	570	No	-1.21E+03	-1.24E-04	3.89E-05	-3.19E+00	1.44E-03	2.73E-02	plasma_inf_nif
496.3397	6.81	LPC 16:0	0.974362	11	0.010681	21.5203	36	No	-4.12E+02	2.76E-02	8.77E-03	3.15E+00	1.63E-03	2.73E-02	plasma_inf_nif
496.3397	6.81	LPC 16:0	0.974362	11	0.010681	21.5203	36	No	-7.25E+02	-9.17E-05	1.86E-05	-4.92E+00	8.62E-07	4.36E-04	urine_iso_nif
497.1334	3.1	NA	NA	NA	NA	NA	2064	No	-8.86E+02	1.53E-03	3.95E-04	3.89E+00	1.02E-04	1.74E-03	urine_inf_nif
501.2306	2.46	NA	NA	NA	NA	NA	4298	No	-8.81E+01	2.43E-03	2.10E-04	1.16E+01	6.26E-31	4.54E-28	saliva_iso_nif
514.1599	3.1	NA	NA	NA	NA	NA	2868	No	-1.11E+03	2.83E-05	9.68E-06	2.93E+00	3.43E-03	2.59E-02	urine_inf_nif
516.8545	7.35	NA	NA	NA	NA	NA	3685	No	-2.37E+03	3.15E-05	1.00E-05	3.14E+00	1.67E-03	4.19E-02	saliva_inf_nif
519.1155	3.1	NA	NA	NA	NA	NA	2228	No	-1.26E+03	1.38E-04	3.76E-05	3.66E+00	2.47E-04	3.47E-03	urine_inf_nif
550.3871	7.77	LPC 20:1/LPC O-20:2,O/PC 20:1	0.881588	7	6.10E-05	0.110895	427	No	-9.01E+02	5.04E-04	1.53E-04	3.30E+00	9.50E-04	2.05E-02	plasma_inf_nif
603.8957	4.33	NA	NA	NA	NA	NA	504	No	-1.34E+03	2.58E-04	5.61E-05	4.60E+00	4.30E-06	3.01E-04	plasma_inf_nif
616.1758	5.87	Hemin cation	0.861494	5	0.004211	6.83473	443	No	-4.84E+02	-1.93E-02	4.85E-03	-3.99E+00	6.67E-05	3.11E-03	plasma_inf_nif
627.1898	4.38	NA	NA	NA	NA	NA	1481	No	-1.00E+03	9.12E-05	1.91E-05	4.78E+00	1.74E-06	1.63E-04	plasma_inf_nif
627.333	4.38	NA	NA	NA	NA	NA	1498	No	-8.70E+02	7.15E-05	1.85E-05	3.86E+00	1.13E-04	4.53E-03	plasma_inf_nif
656.4394	7.05	NA	NA	NA	NA	NA	931	No	-1.97E+03	1.30E-04	3.87E-05	3.36E+00	7.88E-04	3.14E-02	saliva_inf_nif
659.3776	4.09	NA	NA	NA	NA	NA	615	No	-7.17E+02	1.09E-04	3.28E-05	3.32E+00	8.88E-04	2.05E-02	plasma_inf_nif
669.709	4.12	NA	NA	NA	NA	NA	546	No	-1.09E+03	9.16E-05	2.91E-05	3.15E+00	1.65E-03	2.73E-02	plasma_inf_nif
678.4513	7.04	NA	NA	NA	NA	NA	1211	No	-1.97E+03	1.23E-04	3.80E-05	3.25E+00	1.15E-03	3.56E-02	saliva_inf_nif
722.4784	7.03	NA	NA	NA	NA	NA	1317	No	-2.02E+03	1.02E-04	3.09E-05	3.31E+00	9.36E-04	3.43E-02	saliva_inf_nif
731.5537	4.38	NA	NA	NA	NA	NA	618	No	-8.08E+02	1.02E-04	2.45E-05	4.17E+00	3.03E-05	1.70E-03	plasma_inf_nif
823.66	2.67	NA	NA	NA	NA	NA	1137	No	-2.14E+03	-2.84E-05	8.89E-06	-3.20E+00	1.39E-03	3.93E-02	saliva_inf_nif
172.0967	2.38	Analog of (3-Phenylpropionyl)glycine	0.920131	4	36.0003	172998	2056	Yes	-7.55E+02	4.28E-03	6.92E-04	6.18E+00	6.29E-10	5.94E-08	urine_inf_nif
178.0532	0.76	Analog of (3-Phenylpropionyl)glycine	0.850274	5	30.0438	144374	2095	Yes	-9.56E+02	1.39E-03	3.78E-04	3.67E+00	2.40E-04	3.41E-03	urine_inf_nif
212.1279	4.22	Analog of (3-Phenylpropionyl)glycine	0.744197	5	4.0309	19370.3	3974	Yes	-1.30E+03	8.63E-05	1.60E-05	5.39E+00	7.15E-08	3.66E-06	urine_inf_nif

428.1885	2.94	Analog of Analog of .beta.-D-Glucose	0.846737	4	45.0735	117650	2711	Yes	-6.17E+02	6.83E-05	2.09E-05	3.27E+00	1.07E-03	1.09E-02	urine_inf_nif
283.1032	0.62	Analog of 1-Methyladenosine	0.964153	5	0.983215	3485.1	2181	Yes	-1.16E+03	-2.09E-04	3.91E-05	-5.35E+00	8.77E-08	4.09E-06	urine_inf_nif
237.0866	0.5	Analog of 2-Quinolincarboxylic Acid	0.893934	4	63.0266	362097	87	Yes	-1.44E+03	-7.79E-05	1.12E-05	-6.97E+00	3.07E-12	4.50E-10	urine_inf_nif
385.3462	9.43	Analog of 3Beta-Hydroxy-23,24-Bisnorchol-5-Enic Acid	0.909235	14	0.132202	343.191	484	Yes	-9.79E+02	1.32E-03	2.67E-04	4.95E+00	7.41E-07	1.63E-04	plasma_inf_nif
308.1348	0.98	Analog of 5'-S-methyl-5'-thioadenosine	0.989726	5	10.0378	33673	2211	Yes	-5.87E+02	-5.42E-04	1.54E-04	-3.52E+00	4.38E-04	5.35E-03	urine_inf_nif
245.1381	3.59	Analog of 9-(2,3-dihydroxypropoxy)-9-oxononanoic acid	0.845161	9	18.0109	68443.7	917	Yes	-1.97E+03	1.15E-04	3.53E-05	3.24E+00	1.18E-03	3.56E-02	saliva_inf_nif
262.1281	0.42	Analog of Acetyl-DL-carnitine	0.822865	7	58.0051	284167	2588	Yes	-1.36E+03	-4.83E-05	1.33E-05	-3.64E+00	2.78E-04	3.75E-03	urine_inf_nif
293.1127	0.56	Analog of Adenosine	0.930996	5	25.0087	93279.8	2633	Yes	-9.51E+02	1.94E-05	5.75E-06	3.38E+00	7.30E-04	8.13E-03	urine_inf_nif
413.1232	0.65	Analog of Adenosine, 5'-S-methyl-5'-thio-	0.835956	4	115.03	385887	2430	Yes	-1.29E+03	-3.37E-05	1.24E-05	-2.71E+00	6.67E-03	4.04E-02	urine_inf_nif
241.0928	0.64	Analog of AICAR	0.776485	7	97.9772	288959	2358	Yes	-1.25E+03	-1.17E-04	3.00E-05	-3.90E+00	9.50E-05	1.65E-03	urine_inf_nif
297.1076	0.61	Analog of alpha.-L-Asp-L-Phe	0.783268	10	15.9946	56897.4	1886	Yes	-1.31E+03	-2.65E-05	9.51E-06	-2.79E+00	5.27E-03	3.55E-02	urine_inf_nif
354.1289	0.66	Analog of alpha.-L-Glu-L-Tyr	0.760929	7	43.0059	138228	3228	Yes	-9.60E+02	-3.29E-05	7.00E-06	-4.70E+00	2.63E-06	8.18E-05	urine_inf_nif
125.0962	3.61	Analog of Analog of (2R)-2-[(2R,5S)-5-[(2S)-2-hydroxybutyl]oxolan-2-yl]propanoic acid	0.852525	5	109.074	465789	2909	Yes	-9.08E+02	1.61E-05	5.15E-06	3.13E+00	1.75E-03	1.60E-02	urine_inf_nif
401.3411	9	Analog of Cholest-4,6-Dien-3-One	0.768661	8	18.0101	46983.2	468	Yes	-5.77E+02	8.11E-03	1.67E-03	4.85E+00	1.21E-06	1.63E-04	plasma_inf_nif
142.0862	1.25	Analog of Creatinine	0.78738	4	28.0202	245649	2658	Yes	-1.27E+03	6.30E-05	1.01E-05	6.22E+00	4.89E-10	5.57E-08	urine_inf_nif
188.0739	1.81	Analog of Cysteine	0.711501	4	66.0459	541236	2084	Yes	-8.60E+02	2.55E-03	4.00E-04	6.38E+00	1.81E-10	2.31E-08	urine_inf_nif
225.0346	0.3	Analog of Glucose	0.892866	5	45.9716	256734	89	Yes	-1.23E+03	7.34E-04	2.32E-04	3.16E+00	1.55E-03	2.73E-02	plasma_inf_nif
221.0919	0.52	Analog of Glutamic acid	0.803247	4	73.0319	493259	1441	Yes	-1.20E+03	-8.74E-04	4.80E-05	-1.82E+01	4.42E-74	4.53E-71	urine_inf_nif
350.101	3.1	Analog of Glutathione	0.783527	12	42.01	136356	2418	Yes	-1.27E+03	9.24E-05	2.63E-05	3.51E+00	4.43E-04	5.35E-03	urine_inf_nif
155.0814	0.4	Analog of Histamine	0.921088	4	42.9914	383544	3696	Yes	-6.92E+02	-1.47E-04	5.37E-05	-2.74E+00	6.23E-03	3.92E-02	urine_inf_nif
203.1026	0.48	Analog of Ile-Pro	0.934836	4	26.0524	113689	3451	Yes	-7.58E+02	-9.84E-05	3.67E-05	-2.68E+00	7.38E-03	4.30E-02	urine_inf_nif
290.1593	0.71	Analog of Lauroylcarnitine	0.785434	8	54.1207	157200	4042	Yes	-4.42E+02	-3.26E-04	8.09E-05	-4.03E+00	5.63E-05	1.71E-02	urine_iso_nif
298.2007	3.94	Analog of Lauroylcarnitine	0.913416	8	46.0793	133842	2408	Yes	-1.31E+03	5.65E-05	1.67E-05	3.38E+00	7.24E-04	8.13E-03	urine_inf_nif
304.1749	1.25	Analog of Lauroylcarnitine	0.74059	8	40.1051	116490	2361	Yes	-1.21E+03	-1.41E-04	5.23E-05	-2.71E+00	6.82E-03	4.11E-02	urine_inf_nif
343.222	3.64	Analog of Lauroylcarnitine	0.891165	8	1.05801	3073.12	2910	Yes	-9.82E+02	-3.79E-05	1.13E-05	-3.36E+00	7.67E-04	8.45E-03	urine_inf_nif
129.0659	0.31	Analog of L-HISTIDINE	0.950434	4	27.0141	173079	91	Yes	-2.93E+02	3.08E-04	7.06E-05	4.36E+00	1.33E-05	2.81E-03	plasma_iso_nif
494.324	6.31	Analog of LPC 16:1/LPC O-16:2;O	0.97123	10	2.005	4039.67	217	Yes	-1.11E+03	1.10E-03	3.14E-04	3.49E+00	4.83E-04	1.50E-02	plasma_inf_nif
538.387	8.16	Analog of LPC 19:0/LPC O-19:1;O	0.972994	11	42.058	84738.2	548	Yes	-7.99E+02	5.14E-04	1.50E-04	3.44E+00	5.89E-04	1.55E-02	plasma_inf_nif
164.0375	3.26	Analog of L-Phenylalanine	0.886717	6	44.0595	211726	2257	Yes	-1.28E+03	1.85E-04	4.18E-05	4.43E+00	9.46E-06	2.49E-04	urine_inf_nif
130.0499	4.57	Analog of L-Saccharopine	0.711319	4	129.079	498127	2488	Yes	-1.04E+03	-3.45E-05	1.18E-05	-2.92E+00	3.52E-03	2.63E-02	urine_inf_nif

238.1434	3.76	Analog of N-(8Z,11Z,14Z-Eicosatrienoyl)ethan- olamine	0.767302	5	32.9484	160571	2309	Yes	-8.33E+02	8.63E-05	2.38E-05	3.63E+00	2.85E-04	3.79E-03	urine_inf_nif
238.1434	3.61	Analog of N-(8Z,11Z,14Z-Eicosatrienoyl)ethan- olamine	0.794821	5	32.9484	160571	2120	Yes	-1.13E+03	2.96E-04	7.28E-05	4.07E+00	4.76E-05	9.20E-04	urine_inf_nif
281.0987	2.37	Analog of N6-Threonylcarbamoyl- adenosine	0.873358	7	132.043	319608	4661	Yes	-9.33E+02	-1.66E-05	5.83E-06	-2.85E+00	4.33E-03	3.08E-02	urine_inf_nif
103.0546	0.63	NA	NA	NA	NA	NA	200	Yes	-1.90E+03	-2.76E-04	7.76E-05	-3.56E+00	3.71E-04	2.65E-02	saliva_inf_nif
105.0338	1.93	NA	NA	NA	NA	NA	6452	Yes	-2.75E+02	-1.12E-02	3.49E-03	-3.23E+00	1.25E-03	1.23E-02	urine_inf_nif
109.1014	2.97	NA	NA	NA	NA	NA	450	Yes	-9.86E+02	4.60E-04	1.54E-04	3.00E+00	2.74E-03	2.20E-02	urine_inf_nif
120.081	0.63	NA	NA	NA	NA	NA	53	Yes	-1.22E+03	-2.85E-03	8.03E-04	-3.55E+00	3.89E-04	2.65E-02	saliva_inf_nif
127.1118	2.98	NA	NA	NA	NA	NA	1505	Yes	-5.67E+02	5.82E-03	1.93E-03	3.02E+00	2.53E-03	2.09E-02	urine_inf_nif
131.0492	3.53	NA	NA	NA	NA	NA	586	Yes	-6.43E+02	7.82E-03	1.31E-03	5.96E+00	2.59E-09	2.21E-07	urine_inf_nif
141.1273	3.64	NA	NA	NA	NA	NA	4830	Yes	-7.61E+02	2.67E-05	8.84E-06	3.02E+00	2.55E-03	2.09E-02	urine_inf_nif
143.1178	0.4	NA	NA	NA	NA	NA	2121	Yes	-1.11E+03	3.13E-04	1.14E-04	2.74E+00	6.10E-03	3.89E-02	urine_inf_nif
144.0841	1.1	NA	NA	NA	NA	NA	2062	Yes	-6.81E+02	4.94E-03	9.37E-04	5.27E+00	1.35E-07	5.76E-06	urine_inf_nif
148.0391	3.1	NA	NA	NA	NA	NA	2579	Yes	-8.85E+02	6.19E-05	2.21E-05	2.80E+00	5.12E-03	3.48E-02	urine_inf_nif
158.027	0.73	NA	NA	NA	NA	NA	838	Yes	-1.30E+03	-7.53E-05	2.45E-05	-3.07E+00	2.15E-03	1.91E-02	urine_inf_nif
158.0811	1.23	NA	NA	NA	NA	NA	650	Yes	-8.00E+02	1.10E-03	1.29E-04	8.54E+00	1.40E-17	4.78E-15	urine_inf_nif
174.1237	0.48	NA	NA	NA	NA	NA	297	Yes	-6.90E+02	-3.60E-03	9.52E-04	-3.78E+00	1.54E-04	2.43E-03	urine_inf_nif
183.1128	0.59	NA	NA	NA	NA	NA	2277	Yes	-1.36E+03	-4.99E-05	1.82E-05	-2.74E+00	6.14E-03	3.89E-02	urine_inf_nif
188.0917	0.73	NA	NA	NA	NA	NA	2161	Yes	-1.20E+03	2.31E-04	6.59E-05	3.50E+00	4.65E-04	5.54E-03	urine_inf_nif
193.1223	3.05	NA	NA	NA	NA	NA	2392	Yes	-1.36E+03	-3.46E-05	1.28E-05	-2.70E+00	7.01E-03	4.20E-02	urine_inf_nif
195.0764	0.96	NA	NA	NA	NA	NA	2096	Yes	-1.09E+03	6.72E-04	1.20E-04	5.60E+00	2.16E-08	1.38E-06	urine_inf_nif
196.0968	0.59	NA	0.832556	7	79.0062	287188	2714	Yes	-7.75E+02	-3.42E-04	1.19E-04	-2.88E+00	4.04E-03	2.90E-02	urine_inf_nif
206.0811	3.53	NA	NA	NA	NA	NA	569	Yes	-6.23E+02	9.12E-03	1.48E-03	6.18E+00	6.37E-10	5.94E-08	urine_inf_nif
207.1049	3.66	NA	NA	NA	NA	NA	2761	Yes	-1.06E+03	-2.84E-05	1.06E-05	-2.68E+00	7.38E-03	4.30E-02	urine_inf_nif
211.1691	3.39	NA	NA	NA	NA	NA	2797	Yes	-8.80E+02	4.88E-05	1.48E-05	3.30E+00	9.71E-04	1.03E-02	urine_inf_nif
224.1279	2.82	NA	NA	NA	NA	NA	2754	Yes	-7.06E+02	6.19E-05	2.09E-05	2.97E+00	3.02E-03	2.33E-02	urine_inf_nif
226.1435	2.94	NA	NA	NA	NA	NA	3947	Yes	-4.35E+02	2.25E-03	5.88E-04	3.83E+00	1.28E-04	2.04E-03	urine_inf_nif
226.1435	3.04	NA	NA	NA	NA	NA	3946	Yes	-4.31E+02	2.22E-03	5.48E-04	4.04E+00	5.32E-05	9.91E-04	urine_inf_nif
226.1435	3.17	NA	NA	NA	NA	NA	2057	Yes	-7.72E+02	2.58E-03	7.62E-04	3.39E+00	7.10E-04	8.09E-03	urine_inf_nif
226.1436	3.38	NA	NA	NA	NA	NA	3128	Yes	-4.01E+02	2.15E-03	7.79E-04	2.76E+00	5.75E-03	3.78E-02	urine_inf_nif
231.1411	4.38	NA	NA	NA	NA	NA	2345	Yes	-1.29E+03	-5.07E-05	1.68E-05	-3.02E+00	2.54E-03	2.09E-02	urine_inf_nif

244.154	3.34	NA	NA	NA	NA	NA	2193	Yes	-1.28E+03	8.65E-05	2.91E-05	2.97E+00	2.95E-03	2.31E-02	urine_inf_nif
246.1695	4.54	NA	NA	NA	NA	NA	2397	Yes	-1.21E+03	-1.27E-04	2.59E-05	-4.91E+00	8.90E-07	3.26E-05	urine_inf_nif
247.1073	3.5	NA	NA	NA	NA	NA	2105	Yes	-1.04E+03	5.47E-04	1.72E-04	3.19E+00	1.44E-03	1.39E-02	urine_inf_nif
248.1675	4.38	NA	NA	NA	NA	NA	2088	Yes	-1.05E+03	-3.51E-04	1.08E-04	-3.24E+00	1.20E-03	1.19E-02	urine_inf_nif
251.1022	1.79	NA	NA	NA	NA	NA	3365	Yes	-8.95E+02	-9.58E-05	1.68E-05	-5.68E+00	1.31E-08	8.98E-07	urine_inf_nif
257.1565	4.71	NA	0.710116	6	157.165	379330	3052	Yes	-1.00E+03	-2.70E-05	9.74E-06	-2.77E+00	5.57E-03	3.71E-02	urine_inf_nif
258.1696	3.99	NA	NA	NA	NA	NA	2440	Yes	-1.29E+03	6.38E-05	1.73E-05	3.69E+00	2.21E-04	3.24E-03	urine_inf_nif
270.079	2.14	NA	NA	NA	NA	NA	4798	Yes	-6.38E+02	-2.40E-05	8.73E-06	-2.75E+00	5.93E-03	3.82E-02	urine_inf_nif
278.1416	3.08	NA	NA	NA	NA	NA	2335	Yes	-1.19E+03	5.34E-05	1.96E-05	2.73E+00	6.34E-03	3.94E-02	urine_inf_nif
288.1622	3.54	NA	0.77469	9	41.9922	170582	2469	Yes	-1.30E+03	4.40E-05	1.20E-05	3.65E+00	2.61E-04	3.58E-03	urine_inf_nif
295.2264	6.01	NA	NA	NA	NA	NA	534	Yes	-1.88E+03	-1.39E-04	4.55E-05	-3.04E+00	2.33E-03	4.89E-02	saliva_inf_nif
307.201	4.57	NA	NA	NA	NA	NA	3148	Yes	-7.13E+02	-1.34E-04	3.38E-05	-3.97E+00	7.05E-05	1.29E-03	urine_inf_nif
308.2216	4.36	NA	NA	NA	NA	NA	919	Yes	-2.29E+03	-3.41E-05	8.60E-06	-3.96E+00	7.49E-05	1.42E-02	saliva_inf_nif
311.069	3.26	NA	NA	NA	NA	NA	2171	Yes	-1.21E+03	3.16E-04	6.96E-05	4.54E+00	5.53E-06	1.62E-04	urine_inf_nif
318.1363	3.09	NA	NA	NA	NA	NA	3042	Yes	-7.50E+02	-2.63E-05	8.78E-06	-3.00E+00	2.71E-03	2.20E-02	urine_inf_nif
325.0845	2.51	NA	NA	NA	NA	NA	2640	Yes	-1.13E+03	-7.27E-05	2.30E-05	-3.16E+00	1.58E-03	1.49E-02	urine_inf_nif
342.1178	0.65	NA	NA	NA	NA	NA	2675	Yes	-1.12E+03	3.26E-05	1.16E-05	2.81E+00	4.97E-03	3.42E-02	urine_inf_nif
348.274	4.84	NA	NA	NA	NA	NA	834	Yes	-2.28E+03	-8.79E-05	2.50E-05	-3.52E+00	4.29E-04	2.65E-02	saliva_inf_nif
359.123	1.95	NA	NA	NA	NA	NA	2553	Yes	-1.12E+03	-4.94E-05	1.79E-05	-2.76E+00	5.80E-03	3.78E-02	urine_inf_nif
373.2215	3.47	NA	NA	NA	NA	NA	3074	Yes	-8.75E+02	2.56E-05	8.56E-06	2.99E+00	2.78E-03	2.21E-02	urine_inf_nif
373.2216	3.6	NA	NA	NA	NA	NA	2482	Yes	-1.22E+03	5.58E-05	1.81E-05	3.08E+00	2.07E-03	1.86E-02	urine_inf_nif
411.1543	3.53	NA	NA	NA	NA	NA	2682	Yes	-1.01E+03	4.60E-05	1.19E-05	3.87E+00	1.11E-04	1.83E-03	urine_inf_nif
428.1885	3.04	NA	NA	NA	NA	NA	2641	Yes	-6.77E+02	6.37E-05	2.20E-05	2.89E+00	3.85E-03	2.82E-02	urine_inf_nif
434.2378	4.35	NA	NA	NA	NA	NA	2155	Yes	-1.15E+03	-1.32E-04	3.26E-05	-4.05E+00	5.09E-05	9.66E-04	urine_inf_nif
470.3504	7.38	NA	NA	NA	NA	NA	949	Yes	-2.35E+03	6.07E-05	1.90E-05	3.19E+00	1.41E-03	3.93E-02	saliva_inf_nif
492.3636	7.37	NA	NA	NA	NA	NA	1978	Yes	-2.38E+03	4.75E-05	1.57E-05	3.04E+00	2.39E-03	4.89E-02	saliva_inf_nif
590.4005	7.07	NA	NA	NA	NA	NA	1255	Yes	-1.91E+03	1.05E-04	3.07E-05	3.42E+00	6.33E-04	2.82E-02	saliva_inf_nif
612.4129	7.06	NA	NA	NA	NA	NA	1212	Yes	-1.95E+03	1.16E-04	3.72E-05	3.12E+00	1.84E-03	4.19E-02	saliva_inf_nif
834.6147	7.4	NA	NA	NA	NA	NA	3674	Yes	-2.19E+03	3.68E-05	1.11E-05	3.30E+00	9.61E-04	3.43E-02	saliva_inf_nif
376.1497	3.3	Analog of N-Acetyl-D-Tryptophan	0.776808	8	129.04	522195	2614	Yes	-1.26E+03	-8.23E-05	1.48E-05	-5.55E+00	2.78E-08	1.58E-06	urine_inf_nif
217.1181	0.55	Analog of N-Acetylproline	0.905105	4	59.0371	373461	2092	Yes	-1.06E+03	-3.83E-04	1.20E-04	-3.18E+00	1.45E-03	1.39E-02	urine_inf_nif

325.15	3.91	Analog of N-alpha-Acetyl-L-ornithine	0.707254	7	150.042	856854	5202	Yes	-7.55E+02	-9.40E-06	2.28E-06	-4.12E+00	3.76E-05	7.61E-04	urine_inf_nif
188.1281	3.68	Analog of N-Palmitoylglycine	0.744339	4	126.14	401377	2069	Yes	-9.89E+02	7.78E-04	1.84E-04	4.22E+00	2.40E-05	5.40E-04	urine_inf_nif
218.1386	0.52	Analog of O-Acetylcarnitine	0.985482	6	14.0156	68662.5	205	Yes	-1.84E+03	-9.09E-05	2.59E-05	-3.51E+00	4.56E-04	2.65E-02	saliva_inf_nif
226.18	4.84	Analog of Omega-Hydroxydodecanoate	0.773095	8	45.021	248516	153	Yes	-2.25E+03	-6.00E-05	1.60E-05	-3.75E+00	1.78E-04	1.93E-02	saliva_inf_nif
244.1904	4.84	Analog of Omega-Hydroxydodecanoate	0.797085	8	63.0314	347934	115	Yes	-2.18E+03	-1.36E-04	3.32E-05	-4.09E+00	4.37E-05	1.42E-02	saliva_inf_nif
244.1904	4.84	Analog of Omega-Hydroxydodecanoate	0.797085	8	63.0314	347934	115	Yes	-1.47E+03	-2.70E-04	6.89E-05	-3.92E+00	8.76E-05	2.11E-02	saliva_iso_nif
382.258	4.94	Analog of Palmitoylcarnitine	0.726184	8	18.084	45171.4	2836	Yes	-7.10E+02	-1.23E-04	3.34E-05	-3.67E+00	2.38E-04	3.41E-03	urine_inf_nif
566.3211	6.53	Analog of Phosphocholine family member	0.923331	8	21.9841	40387	233	Yes	-7.18E+02	2.14E-04	6.18E-05	3.46E+00	5.40E-04	3.27E-02	plasma_iso_nif
590.3212	6.53	Analog of Phosphocholine family member	0.799588	6	163.268	216654	1510	Yes	-2.44E+02	2.13E-04	5.61E-05	3.79E+00	1.51E-04	1.60E-02	plasma_iso_nif
173.092	0.33	Analog of PyroGlu-Pro	0.713767	4	54.011	237826	1421	Yes	-9.54E+02	2.41E-04	7.75E-05	3.11E+00	1.88E-03	1.71E-02	urine_inf_nif
243.1336	0.8	Analog of PyroGlu-Pro	0.895531	6	16.0306	70587.4	2893	Yes	-7.35E+02	2.14E-05	7.86E-06	2.72E+00	6.61E-03	4.04E-02	urine_inf_nif
355.1541	0.29	Analog of S-[5'-Adenosyl]-L-methionine	0.951293	5	43.9909	110213	2660	Yes	-1.18E+03	4.92E-05	1.06E-05	4.62E+00	3.75E-06	1.13E-04	urine_inf_nif
313.2369	5.8	Analog of Stearidonic acid	0.82944	11	7.9899	26175.2	6622	Yes	-1.70E+02	-1.29E-04	3.50E-05	-3.69E+00	2.23E-04	1.89E-02	plasma_iso_nif

Appendix C: Chapter 3 Supporting Information

Materials List			
Name	Company	Catalog Number	Comments
1.5 mL Eppendorf tubes	NA	NA	any brand, as available
1 L bottle, pyrex	NA	NA	any brand, as available
1 L graduated cylinder, pyrex	NA	NA	any brand, as available
2 mL SafeLock Eppendorf tubes	VWR	20901-540	use the appropriate tube model for the available tissue homogenizer
3D model (de-novo generated according to protocol steps, or purchased)	NA	NA	as appropriate for system under investigation
5 mm stainless steel bead	Qiagen	69989	
96 well plate	Fisher	3252449	
AATTCCTCCAAGCAGCGGATA primer	NA	NA	any brand, as available; published in Piron, M. et al. Development of a real-time PCR assay for <i>Trypanosoma cruzi</i> detection in blood samples. Acta tropica. 103 (3), 195–200 (2007).
analytical balance	NA	NA	any brand, as available
ASTCGGCTGATCGTTTTCGA primer	NA	NA	any brand, as available; published in Piron, M. et al. Development of a real-time PCR assay for <i>Trypanosoma cruzi</i> detection in blood samples. Acta tropica. 103 (3), 195–200 (2007).
benchtop centrifuge with microcentrifuge, falcon tube and 96-well-plate capacity	NA	NA	any brand, as available
biosafety cabinet	NA	NA	class II, type A2; any brand, as available
CAGCAAGCATCTATGCACTTAG	NA	NA	any brand, as available; published in Cummings, K.L., Tarleton, R.L. Rapid quantitation of <i>Trypanosoma cruzi</i> in host tissue by real-time PCR. Molecular and biochemical parasitology. 129 (1), 53–59 (2003).

ACCCC primer			
camera	NA	NA	any brand, as available. A cellphone camera is adequate for this protocol
chemiluminescent-capable imaging system	NA	NA	any system, as available
cotton balls	NA	NA	any brand, as available
cryogloves	VWR	97008-208	replace with any brand, as available
dissection scissors	NA	NA	any brand, as available
dry ice	NA	NA	any brand, as available
extra-length forceps	NA	NA	any brand, as available
flammable-grade refrigerator	NA	NA	any brand, as available
freezer storage boxes for microcentrifuge tubes	NA	NA	any brand, as available
fume hood	NA	NA	any brand, as available
high-resolution mass spectrometer	NA	NA	any brand, as available, such as ThermoFisher Q-Exactive Plus (catalog number 0726030)
ice bucket	NA	NA	any brand, as available
ili software	ili.embl.de	NA	
isoflurane	Covetrus	29405	
large tupperware	NA	NA	any brand, as available; large enough to comfortably contain mouse, cotton ball
LC-MS grade acetonitrile	Fisher Optima	A955-4	
LC-MS grade dichloromethane	Fisher Optima	D151-4	
LC-MS grade formic acid	Fisher Optima	A11750	
LC-MS grade methanol	Fisher Optima	A456-4	
LC-MS grade water	Fisher Optima	W64	
liquid chromatography column	Phenomenex	00B-4499-AN	may be changed to other brands and models as appropriate for the metabolites of interest
liquid chromatography column guard cartridge	Phenomenex	AJ0-8784	may be changed to other brands and models as appropriate for the metabolites of interest
liquid chromatography column guard cartridge holder	Phenomenex	AJ0-9000	may be changed to other brands and models as appropriate for the metabolites of interest
liquid nitrogen	NA	NA	any brand, as available
luciferin	Goldbio	LUCK-1G	
MeshLab software	https://www.meshlab.net/	NA	
Meshmixer software	https://www.meshmixer.com/	NA	
MS calibrant	NA	NA	appropriate one for available instrument

MS data processing software	NA	NA	multiple options available; authors recommend MZmine
MSConvert software	http://proteowizard.sourceforge.net/	NA	
Nanodrop	ThermoFisher	ND-ONE-W	other nanodrop models are also suitable
p1000 pipet tips	NA	NA	use the appropriate brand to fit available pipettors
p1000 pipettor	NA	NA	any brand, as available
p20 pipette tips	NA	NA	use the appropriate brand to fit available pipettors
p20 pipettor	NA	NA	any brand, as available
p200 pipette tips	NA	NA	use the appropriate brand to fit available pipettors
p200 pipettor	NA	NA	any brand, as available
personal protective equipment (gloves, lab coat, safety glasses/goggles; faceshield)	NA	NA	any brand, as available
Q-Plex Mouse Cytokine - Screen (16-Plex)	Quansys biosciences	110949MS	can replace with other protein-based cytokine assays such as other commercial cytokine ELISA kits
Quick-DNA Miniprep Plus Kit (200 preps)	Zymo	D4069	replace with any brand of mammalian DNA extraction kit, as available
real-time thermocycler	NA	NA	any brand, as available
salt shaker or tea infuser	NA	NA	any brand; to contain isoflurane-soaked cotton ball and prevent contact with mouse skin
SketchUp software	https://www.sketchup.com/	NA	
specimen forceps	NA	NA	any brand, as available
speedvac with microcentrifuge tube and 96-well-plate capacity	NA	NA	any brand, as available
spreadsheet software	https://www.microsoft.com/en-us/microsoft-365/excel	NA	can replace with other spreadsheet management software, as applicable
sulfachloropyridazine	Sigma	S9882-100G	
sulfadimethoxine	Sigma	S7007-10G	
Sybr green qPCR reaction mix	Fisher	A25780	can replace with other Sybr green qPCR reaction mixes, as desired

TCCCTCTCATCAGTTCTAT	NA	NA	any brand, as available; published in Cummings, K.L., Tarleton, R.L. Rapid quantitation of <i>Trypanosoma cruzi</i> in host tissue by real-time PCR. Molecular and biochemical parasitology. 129 (1), 53–59 (2003).
GGCCCA primer			
tissue homogenizer	NA	NA	any brand, as available; for example, Qiagen TissueLyser II, catalog number 85300, with TissueLyser Adapter Set (2 x 24), catalog number 69982
tissue samples	NA	NA	from appropriate infection model
TissueLyser single-bead dispenser	Qiagen	69965	
UHPLC	NA	NA	any brand, as available, such as ThermoFisher Vanquish (catalog number IQLAAAGABHFAPUMBHV)
ultra-low temperature freezer (-80)	NA	NA	any brand, as available
ultrasonic bath	NA	NA	any system, as available
wet ice	NA	NA	any brand, as available
zone-free sealing film	VWR	490007-390	

Appendix D: Chapter 4 Supporting Information

Table S1: Infection-perturbed metabolites in the lung (all positions combined). NA, not applicable.

Cluster Index	M/Z	RT	features	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
91	162.113	0.297	X162.113 0.297	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.000504	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
223	643.135	2.525	X643.135 2.525	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
236	221.105	2.547	X221.105 2.547	NA	NA	NA	NA	NA	NA	NA	NA	Xylenes	Benzenoids
291	788.542	3.343	X788.542 3.343	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
336	238.099	2.267	X238.099 2.267	Ketamine	Massbank:EA282604 Ketamine 2-(2-chlorophenyl)-2-(methylamino)-1-cyclohexanone	0.959255	8	0.000198	0.83	No	[M+H] ⁺	Halobenzenes	Benzenoids
380	391.284	3.623	X391.284 3.623	NA	NA	NA	NA	NA	NA	NA	NA	Benzoic acids and derivatives	Benzenoids
564	438.297	2.951	X438.297 2.951	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
575	467.277	3.202	X467.277 3.202	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
592	332.646	5.656	X332.646 5.656	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
619	115.054	2.251	X115.054 2.251	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
630	128.062	2.252	X128.062 2.252	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
635	358.208	2.219	X358.208 2.219	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
642	814.558	3.367	X814.558 3.367	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
649	172.591	2.548	X172.591 2.548	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
705	538.314	2.974	X538.314 2.974	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
709	313.131	5.657	X313.131 5.657	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
710	240.096	2.268	X240.096 2.268	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
730	221.186	0.271	X221.186 0.271	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
746	147.076	0.32	X147.076 0.32	Glutamine	Glutamine	0.972569	6	0.000504	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
747	160.133	0.303	X160.133 0.303	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
761	464.313	2.984	X464.313 2.984	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
779	218.139	0.499	X218.139 0.499	CAR 3:0	Propionylcarnitine	0.985552	5	0.000305	1.38	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
782	253.101	2.236	X253.101 2.236	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
797	704.522	3.673	X704.522 3.673	PC 30:1 or LPC 30:2;O or PC O-30:2;O	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycerol-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
805	420.899	2.095	X420.899 2.095	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

815	385.346	3.548	X385.346_3.548	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
835	508.376	2.982	X508.376_2.982	LPC O-18:1	Spectral Match to 1-(12-Octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.813677	5	0.000885	0.2	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
854	185.033	0.306	X185.033_0.306	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
857	295.226	3.017	X295.226_3.017	FA 18:3;O	Spectral Match to 13-Keto-9Z,11E-octadecadienoic acid from NIST14	0.763788	8	0.000793	2.71	No	[M+H] ⁺	Fatty amides	Lipids and lipid-like molecules
862	111.021	2.398	X111.021_2.398	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
874	700.571	3.709	X700.571_3.709	HexCer 34:1;O2 (annotated via network annotation propagation)	NA	NA	NA	NA	NA	Yes	[M+H] ⁺	Phosphosphingolipids	Lipids and lipid-like molecules
875	384.394	2.573	X384.394_2.573	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
877	295.066	0.357	X295.066_0.357	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
878	112.051	0.31	X112.051_0.31	Cytosine	Spectral Match to Cytosine from NIST14	0.829279	4	0.000206	1.84	No	[M+H] ⁺	no matches	no matches
886	220.118	0.659	X220.118_0.659	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
888	184.095	0.281	X184.095_0.281	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
895	441.738	2.143	X441.738_2.143	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
901	596.355	2.942	X596.355_2.942	PC 20:1;O2	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.949106	5	29.9728	1.34	Yes	[M+H] ⁺	no matches	no matches
904	810.525	3.34	X810.525_3.34	PC 36:6;O2 (annotated via network annotation propagation)	Spectral Match to N-Oleoyl-D-erythro-sphingosylphosphorylcholine from NIST14	NA	NA	NA	3.7	Yes	[M+Na] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
905	766.572	3.984	X766.572_3.984	PC O-36:5	Spectral Match to Eicosapentaenoyl PAF C-16 from NIST14	0.90891	9	0	3.26	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
910	146.165	0.287	X146.165_0.287	Spermidine	Massbank:PB000484 Spermidine N-(3-aminopropyl)butane-1,4-diamine	0.907869	6	0.000793	5.43	No	[M+H] ⁺	Amines	Organic nitrogen compounds
915	248.149	0.435	X248.149_0.435	CAR 4:0;O	Propionylcarnitine	0.923178	5	30.0101	0.81	Yes	[M+H] ⁺	no matches	no matches
917	200.069	0.278	X200.069_0.278	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
918	295.226	2.849	X295.226_2.849	FA 18:3;O	Spectral Match to 13-Keto-9Z,11E-octadecadienoic acid from NIST14	0.784467	9	0.000702	2.71	No	[M+H] ⁺	Fatty amides	Lipids and lipid-like molecules
923	772.547	3.474	X772.547_3.474	PC 34:3;O	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.934941	6	37.9772	2.2	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
929	504.306	2.958	X504.306_2.958	LPE 20:3 or LPE O-20:4;O or PE O-20:3	Spectral Match to 1-(9Z-Octadecenyl)-sn-glycero-3-phosphoethanolamine from NIST14	0.75305	10	23.9982	4.96	Yes	[M+H] ⁺	no matches	no matches
931	610.37	2.946	X610.37_2.946	PC 21:1;O2	Spectral Match to 1-Palmitoyl-2-glutaryl phosphatidylcholine from NIST14	0.927889	8	0.000793	2.46	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
933	244.093	0.307	X244.093_0.307	cytidine (annotated via network annotation propagation)	3'-CMP - 60.0 eV	NA	NA	NA	1	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
948	678.505	3.633	X678.505_3.633	PC 28:0 or LPC 28:1;O or PC O-28:1;O	Spectral Match to 1,2-Ditetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.002686	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102_0.311	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
964	828.537	3.257	X828.537_3.257	PC 36:5;O3	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.937119	9	93.9668	1.81	Yes	[M+H] ⁺	no matches	no matches
966	838.556	3.34	X838.556_3.34	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
976	220.089	2.267	X220.089_2.267	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

979	207.014	0.281	X207.014_0.281	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
981	262.165	0.512	X262.165_0.512	CAR 5:0;O	Acetyl-Carnitine	0.939864	5	58.0417	0.38	Yes	[M+H] ⁺	no matches	no matches
982	403.284	3.151	X403.284_3.151	NA	NA	NA	NA	NA	NA	NA	NA	Fatty amides	Lipids and lipid-like molecules
993	399.25	3.063	X399.25_3.063	NA	NA	NA	NA	NA	NA	NA	NA	Phosphate esters	Organic acids and derivatives
1009	209.092	0.631	X209.092_0.631	Kynurenine	Kynurenine	0.940758	8	0.000107	0.51	No	[M+H] ⁺	Carbonyl compounds	Organic oxygen compounds
1011	462.78	2.966	X462.78_2.966	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1015	176.103	0.321	X176.103_0.321	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1019	265.212	0.265	X265.212_0.265	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1020	660.422	3.07	X660.422_3.07	PC 26:3;O	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.949795	5	94.0404	2.27	Yes	[M+H] ⁺	no matches	no matches
1025	449.266	3.234	X449.266_3.234	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1031	427.741	2.93	X427.741_2.93	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1033	746.532	3.459	X746.532_3.459	PC 32:2;O	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.940517	7	11.9619	1.34	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1048	198.085	0.309	X198.085_0.309	Citrulline	Spectral Match to L-Citrulline from NIST14	0.876088	5	9.16E-05	0.46	No	M+Na	no matches	no matches
1056	738.505	3.682	X738.505_3.682	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1061	385.212	2.166	X385.212_2.166	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1062	166.042	2.246	X166.042_2.246	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1064	818.588	3.431	X818.588_3.431	PC O-40:7	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.948007	7	84.0183	3.42	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1073	240.079	2.202	X240.079_2.202	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1075	796.546	3.457	X796.546_3.457	PC 36:5;O	Spectral Match to 1-(9Z-Octadecenyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.91888	9	63.991	3.39	Yes	[M+H] ⁺	no matches	no matches
1078	816.573	3.372	X816.573_3.372	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1083	434.748	2.917	X434.748_2.917	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1084	276.101	0.432	X276.101_0.432	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1095	646.407	3.044	X646.407_3.044	PC 25:3;O	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.947236	5	80.0248	1.24	Yes	[M+H] ⁺	no matches	no matches
1098	552.33	2.601	X552.33_2.601	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1099	409.256	2.787	X409.256_2.787	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
1107	524.459	5.116	X524.459_5.116	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1116	403.357	3.548	X403.357_3.548	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1117	297.242	3.077	X297.242_3.077	FA 18:2;O (annotated via network annotation propagation)	2-hydroxypentadecanal oxime	NA	NA	NA	1.35	Yes	[M+H] ⁺	no matches	no matches

1124	798.562	3.497	X798.562_3.497	PC 36:4;O	Spectral Match to 1-(9Z-Octadecenyl)-2-tetradecenyl-sn-glycero-3-phosphocholine from NIST14	0.945794	9	66.0091	2.88	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1135	510.392	3.067	X510.392_3.067	LPC O-18:0	Spectral Match to Lyso-PAF C-18 from NIST14	0.939065	7	0.00061	0.39	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1136	454.389	3.079	X454.389_3.079	CAR 20:1	Palmitoylcarnitine	0.920822	6	54.0466	0.22	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1147	654.397	2.898	X654.397_2.898	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1159	189.16	0.256	X189.16_0.256	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1164	328.32	3.278	X328.32_3.278	NAE 18:0	Spectral Match to Palmitoyl ethanolamide from NIST14	0.988697	9	28.0314	3.05	Yes	[M+H] ⁺	Amines	Organic nitrogen compounds
1165	193.155	0.314	X193.155_0.314	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1186	852.536	3.244	X852.536_3.244	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1195	455.224	2.636	X455.224_2.636	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1204	188.176	0.265	X188.176_0.265	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1209	412.426	2.649	X412.426_2.649	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1214	616.358	2.994	X616.358_2.994	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1228	364.054	0.242	X364.054_0.242	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1232	462.297	2.913	X462.297_2.913	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1241	389.189	2.458	X389.189_2.458	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1254	169.036	0.407	X169.036_0.407	Urate	Urate	0.885339	5	0.000397	2.35	No	[M+H] ⁺	Purines and purine derivatives	Organoheterocyclic compounds
1260	708.443	2.998	X708.443_2.998	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1269	454.176	2.774	X454.176_2.774	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1272	636.386	2.982	X636.386_2.982	PC 23:2;O2	1,2-didecanoyl-sn-glycero-3-phosphocholine	0.949775	5	70.0041	1.73	Yes	[M+H] ⁺	no matches	no matches
1274	558.282	2.697	X558.282_2.697	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1275	353.298	3.807	X353.298_3.807	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1281	710.459	3.077	X710.459_3.077	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1285	600.197	2.523	X600.197_2.523	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1298	800.577	3.616	X800.577_3.616	PC 36:3;O	Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.949628	7	94.0385	3.75	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1299	606.376	3.012	X606.376_3.012	PC 22:2;O	1,2-didecanoyl-sn-glycero-3-phosphocholine	0.924675	4	39.9942	0.82	Yes	[M+H] ⁺	no matches	no matches
1306	854.551	3.264	X854.551_3.264	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1307	744.589	4.087	X744.589_4.087	PC O-34:2 or LPC 34:2 or LPC O-34:3;O	PAF (o-16:0/18:2)	0.760068	5	0.000183	1.61	No	[M+H] ⁺	no matches	no matches
1310	582.339	3.043	X582.339_3.043	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1313	554.307	2.94	X554.307_2.94	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1314	630.844	2.095	X630.844_2.095	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

1317	688.453	3.179	X688.453_3.179	PC 28:3;O	Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.941834	7	18.0859	2.61	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1320	694.427	2.931	X694.427_2.931	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1332	214.059	0.31	X214.059_0.31	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1333	386.29	2.692	X386.29_2.692	CAR 14:1;O	Lauroylcarnitine	0.904952	8	42.0095	0.26	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1341	566.308	2.55	X566.308_2.55	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1353	842.587	3.499	X842.587_3.499	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1356	674.437	3.083	X674.437_3.083	PC 27:3;O	Spectral Match to 1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.930096	5	0.004944	3.11	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1359	526.519	3.661	X526.519_3.661	NAE 31:0;O (annotated via network annotation propagation)	NA	NA	NA	NA	0.76	No	[M+H] ⁺	no matches	no matches
1373	666.397	2.95	X666.397_2.95	PC 24:2;O3	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.944588	10	68.1731	1.05	Yes	[M+H] ⁺	no matches	no matches
1376	433.23	2.157	X433.23_2.157	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1379	846.546	3.241	X846.546_3.241	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1380	786.526	3.267	X786.526_3.267	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1385	748.473	3.065	X748.473_3.065	PC 30:3;O3	Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.930123	7	41.9344	3.87	Yes	[M+H] ⁺	no matches	no matches
1386	150.977	0.291	X150.977_0.291	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1392	383.691	2.198	X383.691_2.198	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1412	774.525	3.277	X774.525_3.277	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1421	822.562	3.491	X822.562_3.491	PC 38:6;O	Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.941622	10	61.98	2.8	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1424	233.19	3.075	X233.19_3.075	NA	NA	NA	NA	NA	NA	NA	NA	Benzoic acids and derivatives	Benzenoids
1430	218.154	3.079	X218.154_3.079	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1432	524.277	2.717	X524.277_2.717	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1436	728.485	3.268	X728.485_3.268	PC 29:1;O	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.926575	12	54.0833	1.78	Yes	[M+Na] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1438	179.143	3.037	X179.143_3.037	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1448	139.112	2.644	X139.112_2.644	4-Hydroxynonenal	Spectral Match to 4-Hydroxynonenal from NIST14	0.73892	5	0.000702	5.05	No	[M+H-H ₂ O] ⁺	no matches	no matches
1459	361.708	2.318	X361.708_2.318	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1460	736.475	3.108	X736.475_3.108	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1462	318.192	2.287	X318.192_2.287	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1465	326.15	2.308	X326.15_2.308	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1473	518.288	2.772	X518.288_2.772	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1476	692.448	3.047	X692.448_3.047	PC 27:2;O2	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-	0.949057	8	40.1054	2.46	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules

					glycero-3-phosphocholine from NIST14								
1477	820.546	3.428	X820.546_3.428	PC 38:7;O	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.949924	9	85.9763	3.29	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1488	332.218	2.234	X332.218_2.234	Thr-Val-Leu	Spectral Match to Thr-Val-Leu from NIST14	0.834945	9	0	0	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
1496	329.247	3.041	X329.247_3.041	FA 22:6	Spectral Match to (+/-)-5-Hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid from NIST14	0.800826	13	28.0308	1.52	Yes	[M+H-H ₂ O] ⁺	no matches	no matches
1497	536.335	2.729	X536.335_2.729	PC 18:1 or LPC 18:2;O	Spectral Match to Lyso-PC(16:0) from NIST14	0.939852	8	40.0056	0.56	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1498	440.336	2.811	X440.336_2.811	CAR 18:2;O	Massbank:RP025103 Oleoyl-L-Carnitine[(3R)-3-[(Z)-octadec-9-enyl]oxy-4-(trimethylazaniumyl)butanoate	0.933578	6	13.9785	2.27	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1501	870.547	3.242	X870.547_3.242	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1513	351.216	2.562	X351.216_2.562	Prostaglandin I3	Spectral Match to Prostaglandin I3 from NIST14	0.853887	11	0.002197	6.26	No	[M+H] ⁺	Eicosanoids	Lipids and lipid-like molecules
1518	429.22	2.59	X429.22_2.59	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1519	412.202	2.199	X412.202_2.199	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1534	662.438	3.092	X662.438_3.092	PC 26:2;O	1,2-didecanoyl-sn-glycero-3-phosphocholine	0.937874	5	96.0556	1.66	Yes	[M+H] ⁺	no matches	no matches
1535	688.415	3.018	X688.415_3.018	PC 25:1;O2	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenyl)-sn-glycero-3-phosphocholine from NIST14	NA	NA	NA	1.45	Yes	[M+Na] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1549	387.876	2.056	X387.876_2.056	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1551	716.448	3.124	X716.448_3.124	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1553	770.495	3.29	X770.495_3.29	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1558	410.41	2.603	X410.41_2.603	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
1589	308.139	2.484	X308.139_2.484	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1595	584.334	2.733	X584.334_2.733	LPC 22:6;O or PC 22:5	Spectral Match to Lyso-PC(16:0) from NIST14	0.939956	9	88.0052	1.2	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1596	342.242	2.966	X342.242_2.966	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1600	240.115	2.336	X240.115_2.336	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1602	240.078	2.502	X240.078_2.502	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1619	445.303	2.339	X445.303_2.339	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1626	163.075	2.662	X163.075_2.662	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1632	239.128	2.665	X239.128_2.665	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1637	163.031	2.245	X163.031_2.245	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1654	381.263	2.596	X381.263_2.596	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1657	251.121	2.383	X251.121_2.383	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1670	359.22	2.793	X359.22_2.793	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1678	705.797	2.188	X705.797_2.188	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

1697	346.198	2.21	X346.198_2.21	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1708	295.932	0.279	X295.932_0.279	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1716	189.047	2.245	X189.047_2.245	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1726	348.311	2.749	X348.311_2.749	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1745	347.221	2.609	X347.221_2.609	Corticosterone	Corticosterone	0.885048	13	0.000793	2.29	No	[M+H] ⁺	Hydroxysteroids	Lipids and lipid-like molecules
1752	481.262	2.266	X481.262_2.266	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
1757	302.214	2.71	X302.214_2.71	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1770	242.075	2.501	X242.075_2.501	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1785	309.127	2.302	X309.127_2.302	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1789	530.348	3.198	X530.348_3.198	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1797	333.205	2.685	X333.205_2.685	ST 20:3;O4	Corticosterone	0.807135	11	14.0167	3	Yes	[M+H] ⁺	Hydroxysteroids	Lipids and lipid-like molecules
1806	424.165	2.532	X424.165_2.532	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1808	516.299	2.565	X516.299_2.565	Taurocholic acid	Taurocholic acid	0.85167	18	0.001831	3.55	No	[M+H] ⁺	Bile acids, alcohols and derivatives	Lipids and lipid-like molecules
1812	247.042	0.319	X247.042_0.319	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1816	236.11	2.907	X236.11_2.907	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1817	277.032	0.298	X277.032_0.298	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1820	222.068	2.501	X222.068_2.501	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1821	480.277	2.51	X480.277_2.51	Taurocholic acid	Taurocholic acid	0.918758	19	0.001892	3.94	No	[M-2H2O+H] ⁺	Bile acids, alcohols and derivatives	Lipids and lipid-like molecules
1822	533.325	2.566	X533.325_2.566	Taurocholic acid	Taurocholic acid	0.885235	22	0.00061	1.14	No	[M+NH4] ⁺	Bile acids, alcohols and derivatives	Lipids and lipid-like molecules
1824	269.226	3.258	X269.226_3.258	Retinol isomer	Spectral Match to Retinol from NIST14	0.962401	10	0	0	No	[M+H-H2O] ⁺	no matches	no matches
1831	267.113	2.356	X267.113_2.356	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1832	299.2	2.636	X299.2_2.636	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1833	260.053	0.281	X260.053_0.281	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1834	384.274	2.644	X384.274_2.644	CAR 14:2;O	Massbank:RP025103 Oleoyl-L-Carnitine[(3R)-3-[(2Z)-octadec-9-enoyloxy-4-(trimethylazaniumyl)]butanoate	0.848795	5	42.084	1.04	Yes	[M+H] ⁺	no matches	no matches
1835	462.267	2.555	X462.267_2.555	Taurocholic acid	Taurocholic acid	0.904983	20	0.000824	1.78	No	[M-3H2O+H] ⁺	Bile acids, alcohols and derivatives	Lipids and lipid-like molecules
1837	614.924	2.093	X614.924_2.093	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1838	370.258	2.803	X370.258_2.803	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1841	310.126	2.392	X310.126_2.392	NA	NA	NA	NA	NA	NA	NA	NA	Benzenesulfonamides	Benzenoids
1847	593.707	2.185	X593.707_2.185	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1850	538.281	2.558	X538.281_2.558	Taurocholic acid	Spectral Match to Taurocholic acid from NIST14	0.902686	17	0.000183	0.34	No	[M+Na] ⁺	no matches	no matches

1853	163.075	3.045	X163.075_3.045	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1860	202.144	2.436	X202.144_2.436	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1864	348.07	0.429	X348.07_0.429	Adenosine 5'-monophosphate	Adenosine 5'-monophosphate	0.969597	4	0.001129	3.24	No	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
1870	230.99	0.417	X230.99_0.417	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1874	628.731	2.439	X628.731_2.439	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1879	332.095	0.35	X332.095_0.35	N-Acetylneuraminic Acid Dimer	Spectral Match to N-Acetylneuraminic Acid Dimer from NIST14	0.829662	9	9.16E-05	0.28	No	[M+H] ⁺	no matches	no matches
1881	557.287	3.087	X557.287_3.087	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1888	358.258	2.585	X358.258_2.585	CAR 12:1:0	Decanoylcarnitine	0.871043	8	42.0103	2.23	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1889	370.295	2.802	X370.295_2.802	CAR 14:1	Lauroylcarnitine	0.96244	8	26.0146	0.54	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1895	703.363	2.883	X703.363_2.883	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1897	344.279	2.746	X344.279_2.746	CAR 12:0	Lauroylcarnitine	0.972066	8	0.00119	1.45	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1899	268.658	2.147	X268.658_2.147	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1905	330.245	3.044	X330.245_3.044	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1906	539.276	3.087	X539.276_3.087	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1914	151.031	2.247	X151.031_2.247	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1918	254.094	2.561	X254.094_2.561	Desmethyleketamine, methylated and hydroxylated	Spectral Match to Desmethyleketamine from NIST14	0.948289	7	30.0099	133923	Yes	[M+H] ⁺	Halobenzenes	Benzenoids
1919	238.045	0.31	X238.045_0.31	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1927	301.216	2.778	X301.216_2.778	FA 20:6	Spectral Match to 11(12)-EET from NIST14	0.827148	13	2.0152	0.66	Yes	[M+H- H ₂ O] ⁺	no matches	no matches
1953	818.603	3.941	X818.603_3.941	PC O-40:7	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.949798	7	84.0333	3.42	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1989	345.677	2.313	X345.677_2.313	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2000	289.121	2.54	X289.121_2.54	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2003	452.373	3.008	X452.373_3.008	CAR 20:2	Massbank:RP025103 Oleoyl-L-Carnitine[(3R)-3-[(Z)-octadec-9-enoyloxy-4-(trimethylazaniumyl)]butanoate	0.913719	6	26.0152	0.88	Yes	[M+H] ⁺	no matches	no matches
2015	381.079	0.257	X381.079_0.257	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2024	297.123	2.444	X297.123_2.444	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2030	325.118	2.485	X325.118_2.485	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2042	181.134	2.533	X181.134_2.533	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2052	222.636	2.547	X222.636_2.547	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2053	241.191	2.446	X241.191_2.446	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
2058	237.11	2.442	X237.11_2.442	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

2060	293.193	2.546	X293.193_2.546	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2063	594.307	2.242	X594.307_2.242	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2065	820.01	2.376	X820.01_2.376	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2067	822.759	2.317	X822.759_2.317	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2072	447.99	2.099	X447.99_2.099	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2076	294.073	2.401	X294.073_2.401	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2110	511.282	3.265	X511.282_3.265	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2121	256.073	2.563	X256.073_2.563	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2124	796.51	3.314	X796.51_3.314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2125	419.209	2.218	X419.209_2.218	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2128	700.35	2.257	X700.35_2.257	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2138	447.721	2.211	X447.721_2.211	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2143	690.346	2.315	X690.346_2.315	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2148	550.519	3.516	X550.519_3.516	NAE 33:2;O (annotated via network annotation propagation)	NA	NA	NA	NA	0.73	No	[M+H] ⁺	no matches	no matches
2165	385.273	3.138	X385.273_3.138	NA	NA	NA	NA	NA	NA	NA	NA	Fatty amides	Lipids and lipid-like molecules
2166	524.504	3.483	X524.504_3.483	NAE 31:1;O (annotated via network annotation propagation)	NA	NA	NA	NA	0.57	No	[M+H] ⁺	no matches	no matches
2183	548.467	3.135	X548.467_3.135	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2191	400.157	0.457	X400.157_0.457	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2192	446.147	2.538	X446.147_2.538	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2193	204.102	2.446	X204.102_2.446	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2194	305.166	2.198	X305.166_2.198	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
2197	1464.088	3.707	X1464.088_3.707	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2201	826.534	3.633	X826.534_3.633	PC 38:7 or PC O-38:8;O	Spectral Match to 1-Octadecanoyl-2-octadecenoyl-sn-glycero-3-phosphocholine from NIST14	0.856654	10	15.9428	2.06	Yes	[M+Na] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
2205	269.226	5.109	X269.226_5.109	Retinol isomer	Spectral Match to Retinol from NIST14	0.941753	10	9.16E-05	0.34	No	[M+H-H ₂ O] ⁺	no matches	no matches
2220	246.065	2.241	X246.065_2.241	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2224	692.521	3.735	X692.521_3.735	PC 29:0 or LPC 29:1;O or PC O-29:1;O	Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.952015	7	14.0184	2.17	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
2226	726.504	3.674	X726.504_3.674	LPC 30:2;O or PC 30:1 or PC O-30:2;O	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.92535	7	56.0649	0.55	Yes	[M+Na] ⁺	no matches	no matches
2243	350.139	2.796	X350.139_2.796	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2244	657.95	2.082	X657.95_2.082	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

2246	601.421	3.324	X601.421 3.324	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2250	572.4	3.271	X572.4 3.271	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2262	455.189	2.751	X455.189 2.751	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2263	799.334	2.873	X799.334 2.873	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2269	241.154	2.583	X241.154 2.583	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2272	454.243	2.829	X454.243 2.829	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2283	213.208	0.274	X213.208 0.274	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
2294	448.764	3.005	X448.764 3.005	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2299	588.365	3.077	X588.365 3.077	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2302	536.371	3.168	X536.371 3.168	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2333	566.55	4.37	X566.55 4.37	NA	NA	NA	NA	NA	NA	NA	NA	Phosphosphingolipids	Lipids and lipid-like molecules
2337	833.649	3.305	X833.649 3.305	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
2349	1412.063	3.834	X1412.063 3.834	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2369	770.53	3.374	X770.53 3.374	PC 32:1;O	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.920258	8	12.0392	0.78	Yes	[M+Na] ⁺	no matches	no matches
2370	407.24	2.744	X407.24 2.744	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2372	347.107	2.304	X347.107 2.304	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2387	462.762	2.872	X462.762 2.872	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2399	538.483	3.479	X538.483 3.479	CAR 26:1 (annotated via network annotation propagation)	NA	NA	NA	NA	0	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
2412	476.795	3.052	X476.795 3.052	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2413	501.351	3.136	X501.351 3.136	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2440	933.63	2.948	X933.63 2.948	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2478	420.247	2.581	X420.247 2.581	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2480	391.193	2.828	X391.193 2.828	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2526	212.119	2.291	X212.119 2.291	1,3-Diphenylguanidine	Massbank:LU084503 1,3-Diphenylguanidine [1,2-diphenylguanidine]	0.988144	8	0.00061	2.88	No	[M+H] ⁺	NA	Benzenoids
2534	403.204	2.679	X403.204 2.679	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2589	576.293	2.626	X576.293 2.626	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2600	649.341	2.154	X649.341 2.154	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2615	963.638	3.025	X963.638 3.025	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2640	629.313	2.244	X629.313 2.244	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2648	714.469	3.234	X714.469 3.234	PC 30:4;O	Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.947782	7	7.92969	1.96	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules

2674	716.558	4.038	X716.558_4.038	PC O-32:2 or LPC 32:2 or LPC O-32:3;O	Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.828169	4	44.0244	1.26	Yes	[M+H] ⁺	no matches	no matches
2689	257.147	0.284	X257.147_0.284	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
2750	367.247	2.84	X367.247_2.84	FA 21:4;O3	Spectral Match to trans-EKODE-(E)-lb from NIST14	0.715915	9	56.0262	2.45	Yes	[M+H] ⁺	no matches	no matches
2765	284.056	2.477	X284.056_2.477	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2780	423.573	2.3	X423.573_2.3	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2808	466.267	2.338	X466.267_2.338	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
2861	318.119	0.429	X318.119_0.429	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2868	352.247	2.919	X352.247_2.919	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2893	302.196	2.265	X302.196_2.265	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2898	216.063	0.345	X216.063_0.345	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2905	596.355	3.051	X596.355_3.051	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
2928	520.248	2.245	X520.248_2.245	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
2939	475.194	2.404	X475.194_2.404	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2956	278.069	2.295	X278.069_2.295	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2964	300.051	2.295	X300.051_2.295	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2982	727.333	2.221	X727.333_2.221	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2983	466.293	2.753	X466.293_2.753	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3021	169.134	2.427	X169.134_2.427	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3039	776.578	3.539	X776.578_3.539	PC 34:1;O	Spectral Match to 1-Hexadecyl-2-(9Z-octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.885556	10	30.0089	2.58	Yes	[M+H] ⁺	no matches	no matches
3049	764.544	3.404	X764.544_3.404	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3051	468.346	3.053	X468.346_3.053	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3070	261.217	0.273	X261.217_0.273	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3073	330.662	2.32	X330.662_2.32	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3075	902.537	3.122	X902.537_3.122	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3091	742.573	4.065	X742.573_4.065	PC O-34:3 or LPC 34:3 or PC O-34:3	Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine from NIST14	0.904542	6	44.0308	2.02	Yes	[M+H] ⁺	no matches	no matches
3099	426.261	2.805	X426.261_2.805	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3100	466.33	2.972	X466.33_2.972	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3107	1459.119	3.733	X1459.119_3.733	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3120	669.418	2.979	X669.418_2.979	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3157	355.263	2.696	X355.263_2.696	Cholic acid	Spectral Match to Cholic acid from NIST14	0.776603	13	0.000397	1.12	No	[M+H-H ₂ O] ⁺	Bile acids, alcohols and derivatives	Lipids and lipid-like molecules

3171	706.464	3.127	X706.464_3.127	PC 28:2;O2	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.956677	9	26.0878	1.98	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
3176	462.191	2.402	X462.191_2.402	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3182	123.044	2.179	X123.044_2.179	NA	NA	NA	NA	NA	NA	NA	NA	Pyridinecarboxylic acids and derivatives	Organoheterocyclic compounds
3185	375.214	2.643	X375.214_2.643	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3194	315.109	2.391	X315.109_2.391	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3210	449.265	3.153	X449.265_3.153	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3222	460.27	2.627	X460.27_2.627	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3225	552.329	3.039	X552.329_3.039	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3239	789.4	2.899	X789.4_2.899	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3253	197.118	2.443	X197.118_2.443	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3269	158.118	0.319	X158.118_0.319	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
3273	338.051	0.279	X338.051_0.279	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3277	350.679	2.259	X350.679_2.259	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3278	652.347	2.245	X652.347_2.245	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3280	655.953	2.376	X655.953_2.376	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3286	483.239	2.22	X483.239_2.22	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3297	354.263	2.859	X354.263_2.859	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3303	410.201	2.12	X410.201_2.12	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3310	224.066	2.174	X224.066_2.174	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3311	166.084	0.281	X166.084_0.281	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
3312	660.345	2.436	X660.345_2.436	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3316	254.058	2.376	X254.058_2.376	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3351	315.161	2.247	X315.161_2.247	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3352	228.042	2.469	X228.042_2.469	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3359	229.032	0.295	X229.032_0.295	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3362	490.425	3.263	X490.425_3.263	Î±-Tocopheryl acetate	alpha-TOCOPHERYL ACETATE	0.762717	5	0.000122	0.25	No	[M+NH4] ⁺	Amines	Organic nitrogen compounds
3366	917.597	2.95	X917.597_2.95	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3378	534.318	3.024	X534.318_3.024	LPE 21:3;O or PE 21:2 or PE O-21:3;O (annotated via network annotation propagation)	NCGC00380283-0114-[5-[4-[5-[acetyl(hydroxy)amino]pentylamino]-4-oxobutanoyl]-hydroxyamino]pentylamino]-4-oxobutanoic acid	NA	NA	NA	1.87	Yes	[M+NH4] ⁺	Fatty amides	Lipids and lipid-like molecules
3389	287.018	2.397	X287.018_2.397	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

3527	160.064	2.547	X160.064_2.547	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3533	398.194	2.43	X398.194_2.43	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3568	593.808	2.282	X593.808_2.282	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3587	267.12	2.512	X267.12_2.512	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3593	507.269	5.592	X507.269_5.592	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3628	515.258	2.444	X515.258_2.444	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3661	361.215	2.243	X361.215_2.243	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3694	552.268	2.184	X552.268_2.184	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3766	364.172	2.763	X364.172_2.763	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3776	587.115	2.318	X587.115_2.318	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3781	485.287	5.806	X485.287_5.806	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3803	553.235	2.782	X553.235_2.782	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3807	311.04	0.311	X311.04_0.311	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3813	580.566	3.857	X580.566_3.857	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3827	492.262	2.098	X492.262_2.098	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3828	537.766	2.188	X537.766_2.188	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3832	538.252	2.238	X538.252_2.238	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3833	357.177	2.08	X357.177_2.08	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3837	433.547	2.097	X433.547_2.097	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3938	356.542	2.187	X356.542_2.187	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3948	554.371	3.239	X554.371_3.239	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3957	835.665	3.274	X835.665_3.274	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
4013	403.284	3.147	X403.284_3.147	NA	NA	NA	NA	NA	NA	NA	NA	Fatty amides	Lipids and lipid-like molecules
4081	364.065	0.422	X364.065_0.422	Guanosine 5_- monophosphate	Guanosine 5_-monophosphate	0.946635	5	0.005005	1	No	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
4090	283.163	0.275	X283.163_0.275	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
4091	180.1	0.278	X180.1_0.278	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
4162	409.198	2.509	X409.198_2.509	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
4210	421.723	2.143	X421.723_2.143	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S2: Infection-perturbed metabolites in the plasma. NA, not applicable.

Cluster Index	M/Z	RT	features	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
722	400.341	2.951	X400.341 2.951	CAR 16:0	Palmitoylcarnitine	0.969065	8	0.00061	1.52	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
753	150.058	0.323	X150.058 0.323	Methionine	Methionine	0.999057	7	0.000397	2.64	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
760	426.357	2.982	X426.357 2.982	CAR 18:1	Massbank:RP025103 Oleoyl-L-Carnitine [(3R)-3-[(Z)-octadec-9-enoyl]oxy-4-(trimethylazaniumyl)butanoate	0.941071	5	0.000702	1.65	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
951	424.342	2.918	X424.342 2.918	CAR 18:2	Massbank:RP025103 Oleoyl-L-Carnitine [(3R)-3-[(Z)-octadec-9-enoyl]oxy-4-(trimethylazaniumyl)butanoate	0.94911	7	2.01639	0.24	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
980	247.202	0.268	X247.202 0.268	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1093	398.326	2.887	X398.326 2.887	CAR 16:1	Palmitoylcarnitine	0.958329	10	2.0162	1.26	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
4090	283.163	0.275	X283.163 0.275	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
4375	190.086	2.56	X190.086 2.56	Indolepropionic acid	Indolepropionic acid	0.984352	4	0.000107	0.56	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
4386	196.074	0.275	X196.074 0.275	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S3: Infection-perturbed metabolites in the lung at position 1. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected/uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
91	162.113	0.297	X162.113 0.297	2.790602682	0.407892858	3.77E-07	7.53E-05	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
152	349.036	2.524	X349.036 2.524	1.001001503	0.479357958	0.001866845	0.035264257	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
475	507.27	3.2	X507.27 3.2	1.727020141	0.455667281	0.000327577	0.010765154	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
564	438.297	2.951	X438.297 2.951	1.657607478	0.442623374	0.000101285	0.004189364	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
575	467.277	3.202	X467.277 3.202	1.87421423	0.383785888	0.002297484	0.041131823	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
592	332.646	5.656	X332.646 5.656	1.266939792	0.469559729	0.00111058	0.024436603	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
709	313.131	5.657	X313.131 5.657	1.34406228	0.235330266	0.001170409	0.024629916	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
710	240.096	2.268	X240.096 2.268	1.665105604	4.407194783	0.000427732	0.013501704	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
730	221.186	0.271	X221.186 0.271	1.804781035	0.299518628	1.51E-06	0.000207489	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
746	147.076	0.32	X147.076 0.32	2.522058142	2.089605933	3.77E-07	7.53E-05	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
772	393.224	2.51	X393.224 2.51	1.411207742	0.48597765	0.000247753	0.008740592	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
773	169.058	0.306	X169.058 0.306	2.715630079	2.12711785	3.77E-07	7.53E-05	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds

779	218.139	0.499	X218.139 0.499	1.841880226	0.417854341	2.52E-05	0.001551794	CAR 3:0	Propionylcarnitine	0.985552	5	0.000305176	1.4	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
782	253.101	2.236	X253.101 2.236	2.570209343	0.338152571	1.13E-05	0.000903283	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
797	704.522	3.673	X704.522 3.673	2.55582722	0.390711518	3.77E-07	7.53E-05	LPC 30:2;O or PC 30:1 or PC O-30:2;O	Spectral Match to 1-(9Z-Octadecenyl)-2-tetradecenyl-sn-glycero-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
810	134.045	0.322	X134.045 0.322	1.639548499	2.165918479	0.000551282	0.016437521	Aspartate	Aspartate - 50.00 eV	0.970598	5	9.16E-05	0.68	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
835	508.376	2.982	X508.376 2.982	1.001001503	4.410806724	4.52E-06	0.0004169	LPC O-18:1	Spectral Match to 1-(12-Octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.813677	5	0.00088501	0.2	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
840	191.041	0.284	X191.041 0.284	3.058661092	2.081459816	3.77E-07	7.53E-05	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
854	185.033	0.306	X185.033 0.306	1.825942797	2.482657804	1.51E-06	0.000207489	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
860	349.209	2.688	X349.209 2.688	1.001001503	0.39610393	0.001418733	0.028942693	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
863	266.075	0.278	X266.075 0.278	2.235869682	2.075943062	3.77E-07	7.53E-05	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
874	700.571	3.709	X700.571 3.709	2.089632982	31.82201705	5.94E-05	0.002851839	HexCer 34:1;O2 (annotated via network annotation propagation)	NA	NA	NA	NA	NA	Yes	NA	Phosphosphingolipids	Lipids and lipid-like molecules
886	220.118	0.659	X220.118 0.659	2.536120264	3.850610132	2.78E-05	0.00162695	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
888	184.095	0.281	X184.095 0.281	1.001001503	0.370332517	0.000532174	0.016437521	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
895	441.738	2.143	X441.738 2.143	1.632984128	3.076058723	4.40E-05	0.002295664	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
905	766.572	3.984	X766.572 3.984	1.713510275	0.43810164	4.52E-06	0.0004169	PC O-36:5	Spectral Match to Eicosapentaenyl PAF C-16 from NIST14	0.90891	9	0	3.26	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
910	146.165	0.287	X146.165 0.287	1.621802403	2.597529612	7.15E-06	0.000612942	Spermidine	Massbank-PB000484 Spermidine[N-(3-aminopropyl)butane-1,4-diamine	0.907869	6	0.000793457	5.43	No	[M+H] ⁺	Amines	Organic nitrogen compounds
915	248.149	0.435	X248.149 0.435	1.60010765	3.269810285	0.000673785	0.01796012	CAR 4:0;O	Propionylcarnitine	0.923178	5	30.0101	0.81	Yes	[M+H] ⁺	no matches	no matches
933	244.093	0.307	X244.093 0.307	1.812157901	2.236115871	8.60E-05	0.003749097	Cytidine	3'-CMP - 60.0 eV	0.92616	4	79.9663	0.82	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
948	678.505	3.633	X678.505 3.633	2.333300267	0.244920039	3.77E-07	7.53E-05	PC 28:0 or LPC 28:1;O or PC O-28:1;O	Spectral Match to 1,2-Ditetradecenyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102 0.311	1.909584589	0	1.73E-06	0.000207489	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
976	220.089	2.267	X220.089 2.267	1.417050503	10.1421504	0.000344855	0.011179824	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
979	207.014	0.281	X207.014 0.281	2.236932473	2.353401215	7.53E-07	0.00012904	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202 0.268	3.322029404	0	1.73E-06	0.000207489	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
981	262.165	0.512	X262.165 0.512	1.302069277	0.050232176	0.000104757	0.004259539	CAR 5:0;O	Acetyl-carnitine	0.939864	5	58.0417	0.38	Yes	[M+H] ⁺	no matches	no matches
996	728.52	3.641	X728.52 3.641	1.818386716	0.355753658	0.000810937	0.020056054	LPC 30:1;O or PC 30:0 or PC O-30:1;O	NA	NA	NA	NA	3.43	Yes	[M+Na] ⁺	no matches	no matches
1004	154.059	0.285	X154.059 0.285	3.562542717	2.222431386	3.77E-07	7.53E-05	NA	NA	NA	NA	NA	NA	NA	NA	NA	Nucleosides, nucleotides, and analogues
1009	209.092	0.631	X209.092 0.631	2.209979305	18.50383621	2.12E-05	0.001376728	Kynurenine	Kynurenine	0.940758	8	0.000106812	0.51	No	[M+H] ⁺	Carbonyl compounds	Organic oxygen compounds
1015	176.103	0.321	X176.103 0.321	2.431325525	15.24231563	9.47E-05	0.004054912	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

1019	265.212	0.265	X265.212_0.265	2.731367072	0.249693236	2.78E-05	0.00162695	NA	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1020	660.422	3.07	X660.422_3.07	1.001001503	16.04829071	0.003444002	0.049772055	PC 26:3;O	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.949795	5	94.0404	2.65	Yes	[M+H] ⁺	no matches	no matches	
1135	510.392	3.067	X510.392_3.067	1.001001503	3.219315529	0.002197847	0.040249119	LPC O-18:0	Spectral Match to Lyso-PAF C-18 from NIST14	0.939065	7	0.000610352	0.39	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules	
1159	189.16	0.256	X189.16_0.256	1.9888412	2.138505681	3.77E-07	7.53E-05	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
1165	193.155	0.314	X193.155_0.314	1.001001503	0	0.000698424	0.018016331	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives	
1199	136.048	0.284	X136.048_0.284	2.16799744	2.137783894	3.77E-07	7.53E-05	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
1253	135.003	0.293	X135.003_0.293	2.37390311	2.360438678	3.77E-07	7.53E-05	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
1254	169.036	0.407	X169.036_0.407	1.389425036	3.544192109	0.001082164	0.024436603	Urate	Urate	0.885339	5	0.000396729	2.35	No	[M+H] ⁺	Purines and purine derivatives	Organoheterocyclic compounds	
1275	353.298	3.807	X353.298_3.807	1.001001503	5.778113115	0.002817974	0.043247524	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
1304	648.422	3.036	X648.422_3.036	1.001001503	2.089435557	0.000673785	0.01796012	PC 25:2;O	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.955571	5	82.0401	2.31	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules	
1307	744.589	4.087	X744.589_4.087	1.779663169	10.76169237	0.000119016	0.004758664	LPC 34:2 or LPC O-34:3;O or PC O-34:2	PAF (o-16:0/18:2)	0.760068	5	0.000183105	1.61	No	[M+H] ⁺	no matches	no matches	
1341	566.308	2.55	X566.308_2.55	1.001001503	2.606832702	0.002807687	0.043247524	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
1356	674.437	3.083	X674.437_3.083	1.907308841	3.946129426	8.52E-05	0.003749097	PC 27:3;O	Spectral Match to 1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.930096	5	0.00494385	3.11	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules	
1359	526.519	3.661	X526.519_3.661	1.366929044	0.365133965	0.001187403	0.024770267	NAE 31:0;O (annotated via network annotation propagation)	NA	NA	NA	NA	0.76	No	[M+H] ⁺	no matches	no matches	
1386	150.977	0.291	X150.977_0.291	1.322255749	2.935809265	4.40E-05	0.002295664	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
1488	332.218	2.234	X332.218_2.234	2.156055093	0.046263936	3.59E-05	0.002037638	Thr-Val-Leu	Spectral Match to Thr-Val-Leu from NIST14	0.834945	9	0	0	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives	
1600	240.115	2.336	X240.115_2.336	1.663615346	5.162971391	0.00111058	0.024436603	Ketamine + H2	Massbank:EA282604 Ketamine 2-(2-chlorophenyl)-2-(methylamino)-1-cyclohexanone	0.980975	9	2.0159	2.08	Yes	[M+H] ⁺	Halobenzenes	Benzenoids	
1619	445.303	2.339	X445.303_2.339	2.531730038	0.01893036	3.17E-06	0.00033021	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives	
1629	166.123	2.513	X166.123_2.513	1.001001503	0.23942441	0.0029971	0.044382978	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
1836	388.269	2.51	X388.269_2.51	1.374578563	0.264464286	0.000643802	0.01755093	NA	NA	NA	NA	NA	NA	NA	NA	Eicosanoids	Lipids and lipid-like molecules	
1874	628.731	2.439	X628.731_2.439	1.417050503	0	0.002482983	0.041948417	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
1919	238.045	0.31	X238.045_0.31	1.954645477	0.286522206	6.08E-05	0.002860951	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
2053	241.191	2.446	X241.191_2.446	1.001001503	0.082333721	0.001721621	0.033578604	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives	
2058	237.11	2.442	X237.11_2.442	1.001001503	0	0.002482983	0.041948417	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
2121	256.073	2.563	X256.073_2.563	1.8197482	0.010619777	1.94E-05	0.001294937	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
2349	1412.063	3.834	X1412.063_3.834	1.878790713	0.147532332	0.000128869	0.005068153	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
2450	703.297	2.519	X703.297_2.519	1.001001503	0	0.000698424	0.018016331	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	
2898	216.063	0.345	X216.063_0.345	1.324604203	0	0.000698424	0.018016331	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches	

3070	261.217	0.273	X261.217 0.273	1.793696593	0	1.73E-06	0.000207489	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3094	428.205	2.244	X428.205 2.244	1.571121578	0	0.002482983	0.041948417	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3099	426.261	2.805	X426.261 2.805	2.644457897	0	1.73E-06	0.000207489	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3176	462.191	2.402	X462.191 2.402	1.001001503	0.068894051	0.002679571	0.043247524	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3269	158.118	0.319	X158.118 0.319	1.001001503	0	0.000180805	0.006673086	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds

Table S4: Infection-perturbed metabolites in the lung at position 2. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected/uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
91	162.113	0.297	X162.113 0.297	2.411688553	0.354960116	2.64E-06	0.000949219	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
619	115.054	2.251	X115.054 2.251	2.054530478	17.04661885	0.000472843	0.023840744	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
710	240.096	2.268	X240.096 2.268	1.604730814	4.105904559	0.001151036	0.046059724	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
721	204.123	0.316	X204.123 0.316	1.266939792	0.481233337	5.23E-05	0.006944287	CAR 2:0	Acetyl-carnitine	0.985822	5	9.16E-05	0	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
746	147.076	0.32	X147.076 0.32	2.960698594	2.01551009	3.77E-07	0.000316406	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
747	160.133	0.303	X160.133 0.303	1.240529378	0.392568934	0.000554998	0.026906709	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
760	426.357	2.982	X426.357 2.982	1.687887867	2.000105928	0.000101285	0.010001327	CAR 18:1	Massbank:RP025103 Oleoyl-L-Carnitine [(3R)-3-[(Z)-octadec-9-enyloxy-4-(trimethylazaniumyl)butanoate	0.941071	5	0.000701904	1.88	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
773	169.058	0.306	X169.058 0.306	3.331411821	2.135009195	3.77E-07	0.000316406	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds
782	253.101	2.236	X253.101 2.236	1.229330712	0.363982143	0.000137808	0.011206909	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
793	258.11	0.35	X258.11 0.35	1.261597993	0.284184638	0.000455824	0.023451685	Glycerophosphocholine	Sn-glycero-3-phosphocholine	0.957974	7	9.16E-05	0.39	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
797	704.522	3.673	X704.522 3.673	2.473060056	0.438926365	0.000247753	0.015614653	LPC 30:2:O or PC 30:1 or PC O-30:2:O	Spectral Match to 1-(9Z-Octadecenyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
835	508.376	2.982	X508.376 2.982	2.806044404	4.67554363	3.65E-05	0.006138283	LPC O-18:1	Spectral Match to 1-(12-Octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.813677	5	0.00088501	0.2	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
854	185.033	0.306	X185.033 0.306	2.002051595	2.919145742	1.51E-06	0.000726804	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
863	266.075	0.278	X266.075 0.278	3.15492984	2.355048318	8.52E-05	0.00934252	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
878	112.051	0.31	X112.051 0.31	3.550027324	3.019011597	2.70E-05	0.005677224	Cytosine	Spectral Match to Cytosine from NIST14	0.829279	4	0.000205994	1.84	No	[M+H] ⁺	no matches	no matches
886	220.118	0.659	X220.118 0.659	1.516811185	3.16271584	0.000200632	0.01445123	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
910	146.165	0.287	X146.165 0.287	2.134661192	2.425512988	0.000712761	0.030980546	Spermidine	Massbank:PB000484 Spermidine [N-(3-aminopropyl)butane-1,4-diamine	0.907869	6	0.000793457	5.43	No	[M+H] ⁺	Amines	Organic nitrogen compounds
933	244.093	0.307	X244.093 0.307	2.585063284	3.311786984	6.53E-05	0.008159471	Cytidine	3'-CMP - 60.0 eV	0.92616	4	79.9663	0.82	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
948	678.505	3.633	X678.505 3.633	1.689850829	0.282087008	0.000669497	0.030687292	LPC 28:1:O or PC 28:0 or PC O-28:1:O	Spectral Match to 1,2-Ditetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102 0.311	2.143772526	0	1.73E-06	0.000726804	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives

976	220.089	2.267	X220.089_2.267	1.967326614	29.18961243	0.000173889	0.013406141	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
979	207.014	0.281	X207.014_0.281	1.698914472	2.509221567	3.77E-07	0.000316406	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	1.902220727	0.006526106	1.94E-05	0.004898844	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
981	262.165	0.512	X262.165_0.512	1.955372368	0.127737684	0.001042911	0.043101283	CAR 5:0:O	Acetyl-carnitine	0.939864	5	58.0417	0.38	Yes	[M+H] ⁺	no matches	no matches
1004	154.059	0.285	X154.059_0.285	2.30503374	2.426348096	0.000224294	0.014880132	NA	NA	NA	NA	NA	NA	NA	NA	NA	Nucleosides, nucleotides, and analogues
1009	209.092	0.631	X209.092_0.631	2.103820546	18.06435845	7.63E-05	0.008741453	Kynurenine	Kynurenine	0.940758	8	0.000106812	0.51	No	[M+H] ⁺	Carbonyl compounds	Organic oxygen compounds
1136	454.389	3.079	X454.389_3.079	1.13210885	7.686443528	0.000532174	0.026306112	CAR 20:1	Palmitoylcarnitine	0.920822	6	54.0466	0.22	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1199	136.048	0.284	X136.048_0.284	2.166109502	2.324195326	0.000224294	0.014880132	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1307	744.589	4.087	X744.589_4.087	1.001001503	8.309489508	0.000119016	0.010001327	LPC 34:2 or LPC O-34:3:O or PC O-34:2	PAF (o-16:0/18:2)	0.760068	5	0.000183105	1.61	No	[M+H] ⁺	no matches	no matches
1333	386.29	2.692	X386.29_2.692	1.998798261	3.701858108	0.000436615	0.022931385	CAR 14:1:O	LAUROYL-CARNITINE	0.904952	8	42.0095	0.26	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1365	414.321	2.782	X414.321_2.782	2.278794096	2.821572581	6.80E-05	0.008159471	CAR 16:1:O	Massbank:RP025103 Oleoyl-L-Carnitine [(3R)-3-[(2Z)-octadec-9-enoyloxy-4-(trimethylazaniumyl)]butanoate	0.937557	6	12.0372	0.97	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1386	150.977	0.291	X150.977_0.291	1.940838091	3.491814774	0.000219328	0.014880132	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1600	240.115	2.336	X240.115_2.336	1.64786381	7.355665341	0.000598121	0.028450243	Ketamine + H2	Massbank:EA282604 ketamine [2-(2-chlorophenyl)-2-(methylamino)-1-cyclohexanone	0.980975	9	2.0159	2.08	Yes	[M+H] ⁺	Halobenzenes	Benzenoids
1745	347.221	2.609	X347.221_2.609	1.823715025	19.99708333	9.36E-05	0.009835716	Corticosterone	Corticosterone	0.885048	13	0.000793457	2.29	No	[M+H] ⁺	Hydroxysteroids	Lipids and lipid-like molecules
1752	481.262	2.266	X481.262_2.266	2.480121398	4.267741062	0.000103987	0.010001327	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
1919	238.045	0.31	X238.045_0.31	2.258413542	0.28955222	0.000277732	0.016378551	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2041	339.133	2.641	X339.133_2.641	1.34406228	0	0.000180051	0.013406141	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2349	1412.063	3.834	X1412.063_3.834	2.15314702	0.22850008	0.000339627	0.018613037	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2706	292.198	0.293	X292.198_0.293	1.001001503	0	0.000698424	0.030980546	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3070	261.217	0.273	X261.217_0.273	2.643406194	0	9.08E-06	0.002862359	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3099	426.261	2.805	X426.261_2.805	1.001001503	0.061811295	0.000273015	0.016378551	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3269	158.118	0.319	X158.118_0.319	1.402752094	0	0.000180805	0.013406141	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
3273	338.051	0.279	X338.051_0.279	2.226247001	0.049008633	3.31E-05	0.006138283	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
4140	375.21	2.67	X375.21_2.67	1.001001503	8.948554023	0.001151036	0.046059724	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S5: Infection-perturbed metabolites in the lung at position 3. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected/uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
91	162.113	0.297	X162.113_0.297	1.383175667	0.452236414	0.000137808	0.006923478	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
168	790.558	3.323	X790.558_3.323	1.001001503	3.366480242	0.000712761	0.018650592	PC O-38:7	Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine from NIST14	0.807308	13	3.95612	4.43	Yes	[M+H] ⁺	no matches	no matches
238	526.314	5.678	X526.314_5.678	2.192201247	0.44996914	4.52E-06	0.00094583	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
291	788.542	3.343	X788.542_3.343	1.001001503	4.535108905	0.002765951	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
311	840.572	3.374	X840.572_3.374	1.359252073	3.645756616	0.00274648	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
404	454.292	2.911	X454.292_2.911	1.674598038	0.48572477	2.64E-06	0.000662081	LPE 16:0 or LPE O-16:1:O	Spectral Match to 1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine from NIST14	0.853875	10	0.000701904	1.76	No	[M+H] ⁺	Glycerophosphoethanolamines	Lipids and lipid-like molecules
424	485.287	3.195	X485.287_3.195	2.004555308	0.458170888	1.69E-05	0.002660148	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
564	438.297	2.951	X438.297_2.951	2.555689196	0.356914044	2.64E-06	0.000662081	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
575	467.277	3.202	X467.277_3.202	2.091501868	0.238841685	0.000104272	0.005835741	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
592	332.646	5.656	X332.646_5.656	1.894715848	0.226952333	4.29E-05	0.00341981	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
619	115.054	2.251	X115.054_2.251	1.395403415	3.327935223	0.004592045	0.044537522	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
642	814.558	3.367	X814.558_3.367	1.001001503	4.273836152	0.004048366	0.040705549	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
662	313.273	3.211	X313.273_3.211	1.910618032	0.357243309	0.000105995	0.005835741	MG O-16:2	Spectral Match to Monolaurin from NIST14	0.93717	8	56.0621	2.23	Yes	[M+H- ⁺ H ₂ O]	no matches	no matches
710	240.096	2.268	X240.096_2.268	1.414595408	5.042526671	3.65E-05	0.00341981	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
730	221.186	0.271	X221.186_0.271	1.366929044	0.353493955	7.30E-05	0.004475392	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
746	147.076	0.32	X147.076_0.32	1.710987118	2.383118044	5.23E-05	0.003756298	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
761	464.313	2.984	X464.313_2.984	2.888211221	0.314201413	1.51E-06	0.000543157	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
764	466.329	3.067	X466.329_3.067	1.89375435	0.37548061	1.51E-06	0.000543157	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
773	169.058	0.306	X169.058_0.306	1.218040668	2.117528602	0.000909684	0.021557793	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds
783	140.068	0.286	X140.068_0.286	1.001001503	0.444540164	0.000673785	0.018650592	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
797	704.522	3.673	X704.522_3.673	3.047245309	0.35239615	3.77E-07	0.000543157	LPC 30:2;O or PC 30:1 or PC O-30:2;O	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecenoyl-sn-glycero-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
827	790.571	3.867	X790.571_3.867	1.671315676	0.376672697	0.000741724	0.019012365	PC O-38:7	Spectral Match to 1-Octadecenoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine from NIST14	0.90469	8	20.0279	4.43	Yes	[M+H] ⁺	no matches	no matches
829	434.147	2.645	X434.147_2.645	1.001001503	0.356721767	0.000800912	0.020118903	NA	NA	NA	NA	NA	NA	NA	NA	Arylsulfates	Organic acids and derivatives
845	319.147	2.41	X319.147_2.41	1.001001503	19.98771279	0.002843725	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
854	185.033	0.306	X185.033_0.306	1.408348959	2.636856957	0.000909684	0.021557793	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
878	112.051	0.31	X112.051_0.31	1.64257642	2.144076311	0.000224294	0.009253474	Cytosine	Spectral Match to Cytosine from NIST14	0.829279	4	0.000205994	1.84	No	[M+H] ⁺	no matches	no matches
886	220.118	0.659	X220.118_0.659	1.414595408	3.928648872	0.000200632	0.008689437	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds

888	184.095	0.281	X184.095_0.281	2.404761985	0.308952206	2.70E-05	0.003008154	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
889	949.623	2.916	X949.623_2.916	1.001001503	0.371486377	0.00098561	0.023138808	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
901	596.355	2.942	X596.355_2.942	1.406064428	7.692564117	0.000455824	0.015066186	PC 20:1,O2	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.949106	5	29.9728	1.34	Yes	[M+H] ⁺	no matches	no matches
905	766.572	3.984	X766.572_3.984	2.093962393	0.335393637	1.51E-06	0.000543157	PC O-36:5	Spectral Match to Eicosapentaenoyl PAF C-16 from NIST14	0.90891	9	0	3.26	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
922	143.808	3.867	X1438.08_3.867	2.488100751	0.492080844	5.47E-05	0.003815488	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
926	148.809	3.842	X1488.096_3.842	1.637330668	0.474169702	0.000192665	0.008642401	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
933	244.093	0.307	X244.093_0.307	1.309934291	2.33084318	0.000200632	0.008689437	Cytidine (annotated via network annotation propagation)	3'-CMP-60.0 eV	0.92616	4	79.9663	1	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
948	678.505	3.633	X678.505_3.633	2.929724438	0.212557264	2.75E-05	0.003008154	LPC 28:1,O or PC 28:0 or PC O-28:1,O	Spectral Match to 1,2-Ditradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
976	220.089	2.267	X220.089_2.267	1.710868304	11.54543968	0.000750132	0.019033662	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
979	207.014	0.281	X207.014_0.281	1.83406535	2.302612358	0.001709999	0.028322725	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	1.983879452	0	1.73E-06	0.000543157	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
996	728.52	3.641	X728.52_3.641	1.001001503	0.399744139	6.72E-05	0.004475392	LPC 30:1,O or PC 30:0 or PC O-30:1,O	Spectral Match to 1,2-Dihexadecanoyl-sn-glycero-3-phosphocholine from NIST14	0.775339	10	28.0325	0.14	Yes	[M+Na] ⁺	no matches	no matches
101	722.511	3.776	X722.511_3.776	1.045475303	0.40576099	0.002297484	0.032548314	PE O-36:6	PE(P-16:0/20:5); [M+H] ⁺ C41H73N107P1	0.726445	13	1.00562	1.25	Yes	[M+H] ⁺	no matches	no matches
101	265.212	0.265	X265.212_0.265	3.097146765	0.226852052	2.75E-05	0.003008154	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
103	427.741	2.93	X427.741_2.93	1.001001503	16.80173267	0.004134717	0.041063242	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
104	552.402	3.155	X552.402_3.155	1.001001503	0.40606463	0.000726292	0.018808729	LPC 20:0 or LPC O-20:1,O or PC O-20:0	Spectral Match to 1-Arachidoyl-2-hydroxy-sn-glycero-3-phosphocholine from NIST14	0.92906	11	0.0012207	0.72	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
105	738.505	3.682	X738.505_3.682	1.477088144	0.3110933	0.000401801	0.01410164	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
106	385.212	2.166	X385.212_2.166	1.963764364	41.08322737	0.000173889	0.008495276	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
107	816.573	3.372	X816.573_3.372	1.001001503	16.38741833	0.00111037	0.024322477	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
108	434.748	2.917	X434.748_2.917	1.001001503	12.29720247	0.001289747	0.025533228	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
109	552.33	2.601	X552.33_2.601	1.001001503	16.7268176	0.002073249	0.031000002	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
110	772.524	3.719	X772.524_3.719	1.34406228	0.245923006	0.00010472	0.005835741	PE O-40:9 (annotated via network annotation propagation)	NA	NA	NA	NA	4.66	No	NA	no matches	no matches
112	624.386	3.046	X624.386_3.046	1.001001503	10.59247145	0.004268777	0.04221272	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
115	348.08	0.244	X348.08_0.244	1.495891938	0.466992917	0.000106865	0.005835741	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
115	189.16	0.256	X189.16_0.256	1.707779792	2.089321733	0.000137808	0.006923478	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
121	534.283	2.67	X534.283_2.67	1.001001503	35.47402277	0.001200632	0.024322477	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
123	462.297	2.913	X462.297_2.913	2.404607988	0.265668297	4.36E-05	0.00341981	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
124	279.113	2.589	X279.113_2.589	1.001001503	0.400924741	0.004135748	0.041063242	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
125	682.427	2.967	X682.427_2.967	1.001001503	18.9659375	0.002494179	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
126	181.08	2.368	X181.08_2.368	1.001001503	0.47580359	0.001151036	0.024322477	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
127	636.386	2.982	X636.386_2.982	1.001001503	8.021727935	0.000381548	0.013692113	PC 23:2,O2	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.949775	5	70.0041	1.73	Yes	[M+H] ⁺	no matches	no matches

129	606.376	3.012	X606.376_3.012	1.603331289	7.895039726	0.000181498	0.008495276	PC 22:2;O	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.924675	4	39.9942	0.82	Yes	[M+H] ⁺	no matches	no matches
130	648.422	3.036	X648.422_3.036	2.311670799	2.145139474	7.30E-05	0.004475392	PC 25:2;O	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.955571	5	82.0401	2.31	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
130	744.589	4.087	X744.589_4.087	1.001001503	9.07597379	0.001888192	0.030211067	LPC 34:2 or LPC O-34:3;O or PC O-34:2	PAF (o-16:0/18:2)	0.760068	5	0.000183105	1.61	No	[M+H] ⁺	no matches	no matches
135	674.437	3.083	X674.437_3.083	1.389425036	3.39124951	0.000186003	0.008495276	PC 27:3;O	Spectral Match to 1,2-Oi-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.930096	5	0.00494385	3.11	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
137	650.402	3.025	X650.402_3.025	1.001001503	5.052875	0.00106482	0.024322477	PC 24:2;O2	Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.922597	7	56.1374	1.23	Yes	[M+H] ⁺	no matches	no matches
137	326.15	2.391	X326.15_2.391	1.001001503	3.67270318	0.00441218	0.043294515	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
141	618.375	3.071	X618.375_3.071	1.001001503	37.903538	0.002859938	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
143	524.277	2.717	X524.277_2.717	1.001001503	11.8621481	0.00206919	0.031000002	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
147	820.546	3.428	X820.546_3.428	1.001001503	5.987931483	0.002352207	0.032548314	PC 38:7;O	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.949924	9	85.9763	3.29	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
148	458.171	2.552	X458.171_2.552	1.001001503	0.405217698	0.00333377	0.035749311	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
150	644.39	3.056	X644.39_3.056	1.001001503	2.487340751	0.003392489	0.035749311	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
151	226.105	0.283	X226.105_0.283	1.001001503	0.491534137	0.000372583	0.013564163	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
156	772.582	4.051	X772.582_4.051	1.001001503	5.108466749	0.001983721	0.030932515	PC 35:2 or PC O-35:3;O	Spectral Match to 1-(1Z-Octadecenyl)-2-(9Z-octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.942699	8	0.00408936	4.01	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
164	679.417	3.27	X679.417_3.27	1.001001503	0.035275277	0.002142331	0.031843411	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
165	381.263	2.596	X381.263_2.596	1.001001503	10.2568869	0.000570362	0.017909363	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
168	472.186	2.587	X472.186_2.587	1.001001503	0.30668068	0.00336656	0.035749311	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
171	189.047	2.245	X189.047_2.245	1.73727046	2.175192449	0.003388313	0.035749311	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
183	614.924	2.093	X614.924_2.093	1.001001503	0.203732913	0.002445903	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
191	238.045	0.31	X238.045_0.31	2.424185024	0.335871298	2.33E-05	0.003008154	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
212	256.073	2.563	X256.073_2.563	1.326497378	0.271808852	0.002574302	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
218	548.467	3.135	X548.467_3.135	2.375884608	0.043054248	9.59E-06	0.001720542	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
222	692.521	3.735	X692.521_3.735	1.745634931	0.281619139	0.000330941	0.012595825	LPC 29:1;O or PC 29:0 or PC O-29:1;O	Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.952015	7	14.0184	2.17	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
267	716.558	4.038	X716.558_4.038	1.001001503	0.220202121	0.001457447	0.025657148	LPC 32:2 or LPC O-32:3;O or PC O-32:2	Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.828169	4	44.0244	1.26	Yes	[M+H] ⁺	no matches	no matches
305	468.346	3.053	X468.346_3.053	1.001001503	0	0.002482983	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
307	261.217	0.273	X261.217_0.273	2.017366779	0	1.73E-06	0.000543157	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
309	426.261	2.805	X426.261_2.805	2.41657726	0.020481245	3.17E-06	0.000722961	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
310	466.33	2.972	X466.33_2.972	1.001001503	0	4.27E-05	0.00341981	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
326	158.118	0.319	X158.118_0.319	1.659583913	0	4.27E-05	0.00341981	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
327	338.051	0.279	X338.051_0.279	3.021907708	0	1.72E-06	0.000543157	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
327	652.347	2.245	X652.347_2.245	1.360646731	0	0.002482983	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

332	799.667	3.361	X799.667_3.361	1.001001503	0	0.000698424	0.018650592	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
335	428.037	0.441	X428.037_0.441	1.322255749	0	0.002482983	0.032548314	Adenosine diphosphate	Adenosine 3',5'-diphosphate - 40.0 eV	0.940462	5	0.000396729	0.93	No	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
335	575.778	2.229	X575.778_2.229	1.001001503	0	0.002482983	0.032548314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S6: Infection-perturbed metabolites in the lung at position 4. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected /uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
91	162.113	0.297	X162.113_0.297	1.001001503	0.335296453	1.51E-06	0.000419822	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
152	349.036	2.524	X349.036_2.524	1.289591936	0.429750662	0.000327577	0.009464623	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
168	790.558	3.323	X790.558_3.323	1.001001503	2.640453626	0.003392489	0.032288508	PC O-38:7	Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine from NIST14	0.807308	13	3.95612	4.43	Yes	[M+H] ⁺	no matches	no matches
223	643.135	2.525	X643.135_2.525	1.001001503	0.206732252	0.000267048	0.008291283	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
291	788.542	3.343	X788.542_3.343	1.001001503	5.708431882	0.000163886	0.006857795	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
311	840.572	3.374	X840.572_3.374	1.530344023	6.313557352	0.000276355	0.008291283	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
397	580.36	2.98	X580.36_2.98	1.001001503	2.222724211	5.23E-05	0.003098092	PC 20:1:O	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.751682	4	13.9783	1.55	Yes	[M+H] ⁺	no matches	no matches
642	814.558	3.367	X814.558_3.367	2.036021367	7.357437013	6.72E-05	0.003706203	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
710	240.096	2.268	X240.096_2.268	1.637365307	6.358542633	5.23E-05	0.003098092	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
713	132.077	0.31	X132.077_0.31	1.598049631	2.042068075	3.77E-07	0.000182765	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
721	204.123	0.316	X204.123_0.316	2.072571137	0.468546942	7.15E-06	0.001157513	CAR 2:0	Acetyl-carnitine	0.985822	5	9.16E-05	0	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
730	221.186	0.271	X221.186_0.271	2.044658525	0.348141272	3.65E-05	0.002770034	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
746	147.076	0.32	X147.076_0.32	3.035065373	2.155617652	3.77E-07	0.000182765	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
747	160.133	0.303	X160.133_0.303	1.412859872	0.353233077	2.52E-05	0.002354859	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
772	393.224	2.51	X393.224_2.51	1.409701052	0.493517897	0.000712761	0.012626803	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
773	169.058	0.306	X169.058_0.306	2.397023542	2.020924428	7.53E-07	0.000304609	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds
779	218.139	0.499	X218.139_0.499	1.417050503	0.447602521	0.000554998	0.012626803	CAR 3:0	Propionylcarnitine	0.985552	5	0.000305176	1.38	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
782	253.101	2.236	X253.101_2.236	1.001001503	0.411376246	0.000186003	0.007165554	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
783	140.068	0.286	X140.068_0.286	1.87040211	0.411497701	1.69E-05	0.001869189	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
829	434.147	2.645	X434.147_2.645	1.825927327	0.300074806	5.23E-05	0.003098092	NA	NA	NA	NA	NA	NA	NA	NA	Arylsulfates	Organic acids and derivatives
835	508.376	2.982	X508.376_2.982	2.699011387	4.587735538	3.77E-07	0.000182765	LPC O-18:1	Spectral Match to 1-(12-Octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.813677	5	0.00088501	0.2	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
845	319.147	2.41	X319.147_2.41	1.001001503	24.74410292	0.001641212	0.021187352	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
854	185.033	0.306	X185.033_0.306	1.001001503	2.939035101	2.64E-06	0.000581525	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

874	700.571	3.709	X700.571_3.709	2.605358342	23.94293175	5.94E-05	0.003354795	HexCer 34:1;O2 (annotated via network annotation propagation)	GalCer(d18:1/16:1); [M+H] ⁺ C40H76N1O8	NA	NA	NA	1.71	Yes	[M+H] ⁺	Phosphosphingolipids	Lipids and lipid-like molecules
878	112.051	0.31	X112.051_0.31	1.001001503	2.565945039	5.47E-05	0.003159755	Cytosine	Spectral Match to Cytosine from NIST14	0.829279	4	0.000205994	1.84	No	[M+H] ⁺	no matches	no matches
886	220.118	0.659	X220.118_0.659	1.808548203	6.085831752	5.22E-05	0.003098092	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
888	184.095	0.281	X184.095_0.281	2.026105035	0.359790481	0.000669497	0.012626803	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
901	596.355	2.942	X596.355_2.942	1.001001503	6.887277426	0.000673785	0.012626803	PC 20:1;O2	1,2-didecanoyl-sn-glycero-3-phosphocholine	0.949106	5	29.9728	1.34	Yes	[M+H] ⁺	no matches	no matches
911	431.236	2.63	X431.236_2.63	1.322255749	88.79873301	0.001368693	0.019320847	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
912	192.701	2.574	X192.701_2.574	1.001001503	20.58573789	0.001625206	0.021187352	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
915	248.149	0.435	X248.149_0.435	1.001001503	3.413309283	0.000810937	0.013860165	CAR 4:0;O	Propionylcarnitine	0.923178	5	30.0101	0.81	Yes	[M+H] ⁺	no matches	no matches
923	772.547	3.474	X772.547_3.474	1.753567198	25.17550444	0.000175595	0.007102802	PC 34:3;O	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.934941	6	37.9772	2.2	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
931	610.37	2.946	X610.37_2.946	1.001001503	4.142407572	0.000247753	0.008291283	PC 21:1;O2	Spectral Match to 1-Palmitoyl-2-glutaryl phosphatidylcholine from NIST14	0.927889	8	0.000793457	2.46	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
933	244.093	0.307	X244.093_0.307	1.302069277	3.021260168	8.38E-05	0.004196214	Cytidine	3'-CMP - 60.0 eV	NA	NA	NA	1	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
960	144.102	0.311	X144.102_0.311	2.946176493	0	1.73E-06	0.000419822	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
976	220.089	2.267	X220.089_2.267	1.001001503	40.27198761	7.47E-05	0.003929086	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
979	207.014	0.281	X207.014_0.281	2.86523729	2.725131118	3.50E-05	0.002770034	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	2.137340107	0.005977221	3.17E-06	0.00064029	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
981	262.165	0.512	X262.165_0.512	1.73125673	0.127072912	0.001042911	0.016874298	CAR 5:0;O	Acetyl-Carnitine	0.939864	5	58.0417	0.38	Yes	[M+H] ⁺	no matches	no matches
1004	154.059	0.285	X154.059_0.285	2.480047302	2.3100813	3.77E-07	0.000182765	NA	NA	NA	NA	NA	NA	NA	NA	NA	Nucleosides, nucleotides, and analogues
1015	176.103	0.321	X176.103_0.321	1.753958728	25.47363589	0.001200632	0.018679061	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1019	265.212	0.265	X265.212_0.265	2.314054224	0.191321285	2.70E-05	0.002410124	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1036	319.226	2.93	X319.226_2.93	1.001001503	7.045756589	0.000880918	0.014950965	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1062	166.042	2.246	X166.042_2.246	1.413899397	2.660047474	0.00098561	0.016272623	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1120	624.386	3.046	X624.386_3.046	1.413899397	5.42709262	0.005575756	0.045855602	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1124	798.562	3.497	X798.562_3.497	1.001001503	42.38206776	0.000472843	0.012473804	PC 36:4;O	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.945794	9	66.0091	2.88	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1136	454.389	3.079	X454.389_3.079	1.409701052	3.302677708	0.003252941	0.032224029	CAR 20:1	Palmitoylcarnitine	0.920822	6	54.0466	0.22	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1191	252.078	2.624	X252.078_2.624	1.374578563	19.9241342	0.000173889	0.007102802	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1199	136.048	0.284	X136.048_0.284	1.667612621	2.028152891	1.51E-06	0.000419822	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1204	188.176	0.265	X188.176_0.265	2.1472671	31.74370117	1.88E-05	0.001983635	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1245	279.113	2.589	X279.113_2.589	1.001001503	0.294266573	0.000327577	0.009464623	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1251	682.427	2.967	X682.427_2.967	1.001001503	22.68642105	0.001132207	0.018078069	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1253	135.003	0.293	X135.003_0.293	1.546865125	2.114865298	7.15E-06	0.001157513	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

1254	169.036	0.407	X169.036_0.407	1.001001503	3.496282216	0.004048366	0.037676989	Urate	Urate	0.885339	5	0.000396729	2.35	No	[M+H] ⁺	Purines and purine derivatives	Organoheterocyclic compounds
1272	636.386	2.982	X636.386_2.982	2.035051334	12.70895428	9.18E-05	0.004453907	PC 23:2;O2	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.949775	5	70.0041	1.73	Yes	[M+H] ⁺	no matches	no matches
1299	606.376	3.012	X606.376_3.012	1.67952992	21.82345506	0.000249903	0.008291283	PC 22:2;O	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.924675	4	39.9942	0.82	Yes	[M+H] ⁺	no matches	no matches
1302	700.452	3.2	X700.452_3.2	1.001001503	2.913329767	0.000200632	0.007608338	PC 29:4;O	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecenoyl-sn-glycero-3-phosphocholine from NIST14	0.934001	8	32.1008	4	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1304	648.422	3.036	X648.422_3.036	1.71058925	2.048036818	1.69E-05	0.001869189	PC 25:2;O	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.955571	5	82.0401	2.31	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1307	744.589	4.087	X744.589_4.087	2.754859398	32.87557782	2.12E-05	0.002061339	PC O-34:2 or LPC 34:2 or LPC O-34:3;O	PAF (o-16:0/18:2)	0.760068	5	0.000183105	1.61	No	[M+H] ⁺	no matches	no matches
1333	386.29	2.692	X386.29_2.692	1.001001503	3.792534258	0.001543477	0.020582519	CAR 14:1;O	Lauroylcarnitine	0.904952	8	42.0095	0.26	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1341	566.308	2.55	X566.308_2.55	1.324604203	4.05430865	0.001005784	0.016469707	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1356	674.437	3.083	X674.437_3.083	2.512433799	4.38533138	2.78E-05	0.002410124	PC 27:3;O	Spectral Match to 1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.930096	5	0.00494385	3.11	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1374	650.402	3.025	X650.402_3.025	1.34406228	7.395515062	0.000276355	0.008291283	PC 24:2;O2	Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.922597	7	56.1374	1.23	Yes	[M+H] ⁺	no matches	no matches
1386	150.977	0.291	X150.977_0.291	2.421397715	4.057540256	0.000134733	0.005945398	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1391	193.983	0.293	X193.983_0.293	1.001001503	2.121666983	0.00333377	0.032288508	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1421	822.562	3.491	X822.562_3.491	1.380621367	25.7586789	0.000472843	0.012473804	PC 38:6;O	Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.941622	10	61.98	2.8	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1450	229.98	0.284	X229.98_0.284	1.001001503	2.07533026	0.004663722	0.042234531	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1510	226.105	0.283	X226.105_0.283	1.746131415	0.442668443	7.15E-06	0.001157513	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1534	662.438	3.092	X662.438_3.092	1.374578563	6.540641002	8.47E-05	0.004196214	PC 26:2;O	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.937874	5	96.0556	1.66	Yes	[M+H] ⁺	no matches	no matches
1535	688.415	3.018	X688.415_3.018	1.001001503	2.90354023	0.002504284	0.028805197	PC 25:1;O2	Spectral Match to 1-Hexadecenoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14	NA	NA	NA	1.45	Yes	[M+Na] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1581	840.363	2.762	X840.363_2.762	1.001001503	0.423249731	0.004010624	0.037582179	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1582	616.175	2.776	X616.175_2.776	1.181411561	0.34452361	0.001214615	0.018776239	Hemin	Spectral Match to Hemin cation from NIST14	0.841594	5	0.00457764	7.43	No	Cat	no matches	no matches
1585	990.367	2.866	X990.367_2.866	1.001001503	0.244013255	0.000961754	0.015987522	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1600	240.115	2.336	X240.115_2.336	1.659583913	11.74786765	0.000344855	0.009707438	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1619	445.303	2.339	X445.303_2.339	1.001001503	0.113930706	0.00034798	0.009707438	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1649	440.16	2.639	X440.16_2.639	1.674141442	0.122228787	4.84E-05	0.003098092	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1684	472.186	2.587	X472.186_2.587	1.001001503	0.192650621	0.000501076	0.012626803	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1732	233.19	2.927	X233.19_2.927	1.417050503	0.182708965	0.001946939	0.024231906	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1752	481.262	2.266	X481.262_2.266	1.644248117	6.489690722	0.000570834	0.012626803	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
1770	242.075	2.501	X242.075_2.501	1.001001503	0.104359678	7.27E-05	0.003920044	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1805	208.891	0.269	X208.891_0.269	1.001001503	0.094424037	0.001588111	0.021062	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1820	222.068	2.501	X222.068_2.501	1.637607655	0.067142899	7.61E-05	0.003929086	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1919	238.045	0.31	X238.045_0.31	1.710313899	0.27951935	2.12E-05	0.002061339	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

2004	506.187	2.316	X506.187_2.316	1.001001503	7.977134146	0.004268777	0.038948582	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2024	297.123	2.444	X297.123_2.444	1.648993202	0.02643343	3.59E-05	0.002770034	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2121	256.073	2.563	X256.073_2.563	1.778132136	0.141111822	0.00022484	0.008291283	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2349	1412.063	3.834	X1412.063_3.834	1.409989919	0.222550685	0.002352207	0.027984351	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3070	261.217	0.273	X261.217_0.273	2.885747758	0	1.73E-06	0.000419822	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3099	426.261	2.805	X426.261_2.805	1.001001503	0.029228667	0.000104757	0.004889346	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3269	158.118	0.319	X158.118_0.319	1.001001503	0	0.000180805	0.007165554	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
3273	338.051	0.279	X338.051_0.279	1.001001503	0.095392476	4.63E-05	0.003098092	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S7: Infection-perturbed metabolites in the lung at position 5. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected /uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
91	162.113	0.297	X162.113_0.297	3.913393203	0.313499764	3.77E-07	0.000226574	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
283	156.042	0.281	X156.042_0.281	1.528837901	0.487932598	7.30E-05	0.005023465	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
710	240.096	2.268	X240.096_2.268	2.657975007	4.906642954	5.23E-05	0.004343967	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
713	132.077	0.31	X132.077_0.31	1.853985248	2.062616672	7.53E-07	0.000302098	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
730	221.186	0.271	X221.186_0.271	2.680896341	0.293694086	2.64E-06	0.000576733	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
746	147.076	0.32	X147.076_0.32	3.084860843	2.188840569	7.53E-07	0.000302098	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
747	160.133	0.303	X160.133_0.303	1.666907786	0.38569893	7.30E-05	0.005023465	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
773	169.058	0.306	X169.058_0.306	2.336847127	2.157490487	3.77E-07	0.000226574	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds
783	140.068	0.286	X140.068_0.286	2.604370687	0.394038969	7.15E-06	0.001229972	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
797	704.522	3.673	X704.522_3.673	1.673652378	0.416426573	0.000101285	0.006589011	PC 30:1 or LPC 30:2;O or PC O-30:2;O	Spectral Match to 1-(9Z-Octadecenyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
835	508.376	2.982	X508.376_2.982	3.252250432	4.498860846	3.77E-07	0.000226574	LPC O-18:1	Spectral Match to 1-(1Z-Octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.813677	5	0.00088501	0.2	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
854	185.033	0.306	X185.033_0.306	2.756115338	2.778596842	7.15E-06	0.001229972	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
863	266.075	0.278	X266.075_0.278	2.160483554	2.315806175	3.65E-05	0.003662943	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
878	112.051	0.31	X112.051_0.31	2.046237967	2.822105021	4.36E-05	0.003962558	Cytosine	Spectral Match to Cytosine from NIST14	0.829279	4	0.000205994	1.84	No	[M+H] ⁺	no matches	no matches
886	220.118	0.659	X220.118_0.659	2.335684728	8.189291953	2.49E-05	0.003159901	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
905	766.572	3.984	X766.572_3.984	1.33847911	0.424330842	0.000673785	0.030600021	PC O-36:5	Spectral Match to Eicosapentaenoyl PAF C-16 from NIST14	0.90891	9	0	3.26	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
910	146.165	0.287	X146.165_0.287	1.276282649	2.246829268	3.65E-05	0.003662943	Spermidine	Massbank:PB000484 Spermidine [N-(3-aminopropyl)butane-1,4-diamine	0.907869	6	0.000793457	5.43	No	[M+H] ⁺	Amines	Organic nitrogen compounds
915	248.149	0.435	X248.149_0.435	1.001001503	4.836252347	0.000365721	0.018339363	CAR 4:0;O	Propionylcarnitine	0.923178	5	30.0101	0.81	Yes	[M+H] ⁺	no matches	no matches

933	244.093	0.307	X244.093_0.307	2.272881803	3.620196599	8.15E-05	0.005448225	Cytidine	3'-CMP - 60.0 eV	NA	NA	NA	1	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
948	678.505	3.633	X678.505_3.633	2.098214268	0.219934735	6.72E-05	0.005023465	PC 28:0 or LPC 28:1:O or PC O-28:1:O	Spectral Match to 1,2-Ditetradececanoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102_0.311	2.212122465	0	1.73E-06	0.000462625	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
976	220.089	2.267	X220.089_2.267	1.822357424	19.97465062	0.00026781	0.014324834	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
979	207.014	0.281	X207.014_0.281	2.615012097	2.585328212	5.52E-05	0.004426046	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	2.914612814	0	1.73E-06	0.000462625	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1004	154.059	0.285	X154.059_0.285	2.468017519	2.377538304	3.77E-07	0.000226574	NA	NA	NA	NA	NA	NA	NA	NA	NA	Nucleosides, nucleotides, and analogues
1009	209.092	0.631	X209.092_0.631	1.3932675	20.93769591	0.000472843	0.022316336	Kynurenine	Kynurenine	0.940758	8	0.000106812	0.51	No	[M+H] ⁺	Carbonyl compounds	Organic oxygen compounds
1019	265.212	0.265	X265.212_0.265	1.679693459	0.14364334	3.30E-05	0.003662943	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1048	198.085	0.309	X198.085_0.309	2.265398499	50.98298767	1.88E-05	0.002661625	Citrulline	Spectral Match to L-Citrulline from NIST14	0.876088	5	9.16E-05	0.46	No	M+Na	no matches	no matches
1159	189.16	0.256	X189.16_0.256	2.750766196	2.32306517	2.64E-06	0.000576733	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1199	136.048	0.284	X136.048_0.284	3.078873036	2.553674048	2.78E-05	0.003346369	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1253	135.003	0.293	X135.003_0.293	2.153469837	2.021281338	1.13E-05	0.00181259	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1254	169.036	0.407	X169.036_0.407	2.500534353	9.086211005	5.22E-05	0.004343967	Urate	Urate	0.885339	5	0.000396729	2.35	No	[M+H] ⁺	Purines and purine derivatives	Organoheterocyclic compounds
1323	260.186	2.341	X260.186_2.341	1.892593971	0.494404174	0.000427732	0.020591027	CAR 6:0	Massbank:RP025502 Hexanoyl-L-Carnitine [L-Hexanoylcarnitine] (3R)-3-hexanoyloxy-4-(trimethylazaniumyl)butanoate	0.928943	5	0.000213623	1.54	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1510	226.105	0.283	X226.105_0.283	1.412136112	0.460668181	0.000327577	0.016776103	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1600	240.115	2.336	X240.115_2.336	1.415707668	7.295424403	0.001129453	0.046872314	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1919	238.045	0.31	X238.045_0.31	2.255252029	0.25191848	2.12E-05	0.002839379	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2259	694.519	3.493	X694.519_3.493	1.001001503	0.027628305	0.000500788	0.023180689	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2898	216.063	0.345	X216.063_0.345	1.380621367	0	0.000180805	0.010614562	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3070	261.217	0.273	X261.217_0.273	3.280239407	0	1.73E-06	0.000462625	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3269	158.118	0.319	X158.118_0.319	1.285248776	0	0.000180805	0.010614562	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
3273	338.051	0.279	X338.051_0.279	2.579836295	0.134736016	5.95E-05	0.004620077	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S8: Infection-perturbed metabolites in the lung at position 6. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected /uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
91	162.113	0.297	X162.113_0.297	2.100259118	0.347921059	2.64E-06	0.000554929	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
387	594.376	2.991	X594.376_2.991	1.001001503	2.13399031	0.001806943	0.041434445	PC 21:1;O	Spectral Match to 2-[5-Oxovaleryl]phosphatidylcholine from NIST14	0.797125	7	0.00195312	0.84	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
397	580.36	2.98	X580.36_2.98	1.001001503	2.078611001	0.000327577	0.014049396	PC 20:1;O	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.751682	4	13.9783	1.55	Yes	[M+H] ⁺	no matches	no matches
564	438.297	2.951	X438.297_2.951	1.734690778	0.388075661	2.64E-06	0.000554929	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
699	566.345	3.085	X566.345_3.085	1.229736691	4.003016092	0.002358484	0.047673421	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
710	240.096	2.268	X240.096_2.268	1.837591572	3.353173887	0.000186003	0.009790535	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
730	221.186	0.271	X221.186_0.271	1.22526648	0.413947746	0.000101285	0.006702185	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
746	147.076	0.32	X147.076_0.32	2.181524285	2.404852226	7.53E-07	0.000348813	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
747	160.133	0.303	X160.133_0.303	1.652924919	0.444762142	0.001151036	0.033501968	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
761	464.313	2.984	X464.313_2.984	2.722039535	0.340460172	2.64E-06	0.000554929	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
773	169.058	0.306	X169.058_0.306	2.848861998	2.312275711	7.53E-07	0.000348813	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds
779	218.139	0.499	X218.139_0.499	1.001001503	0.468221217	0.000427732	0.017379434	CAR 3:0	Propionylcarnitine	0.985552	5	0.000305176	1.38	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
783	140.068	0.286	X140.068_0.286	2.015227026	0.453047483	3.65E-05	0.003844866	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
797	704.522	3.673	X704.522_3.673	1.69221678	0.432093599	0.000909684	0.028470649	PC 30:1 or LPC 30:2;O or PC O-30:2;O	Spectral Match to 1-(9Z-Octadecenyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
835	508.376	2.982	X508.376_2.982	2.035495354	2.725253608	1.69E-05	0.002308319	LPC O-18:1	Spectral Match to 1-(1Z-Octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.813677	5	0.00088501	0.2	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
854	185.033	0.306	X185.033_0.306	2.137956751	3.080792887	3.77E-07	0.000348813	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
863	266.075	0.278	X266.075_0.278	1.402752094	2.180939979	0.000275574	0.012975807	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
878	112.051	0.31	X112.051_0.31	2.299574204	3.057542706	6.72E-05	0.004990266	Cytosine	Spectral Match to Cytosine from NIST14	0.829279	4	0.000205994	1.84	No	[M+H] ⁺	no matches	no matches
886	220.118	0.659	X220.118_0.659	2.109058369	6.208086205	0.000134733	0.008211619	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
905	766.572	3.984	X766.572_3.984	3.182092236	0.379836808	5.23E-05	0.004179737	PC O-36:5	Spectral Match to Eicosapentaenoyl PAF C-16 from NIST14	0.90891	9	0	3.26	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
915	248.149	0.435	X248.149_0.435	1.739690664	3.94464483	0.001144054	0.033501968	CAR 4:0;O	Propionylcarnitine	0.923178	5	30.0101	0.81	Yes	[M+H] ⁺	no matches	no matches
918	295.226	2.849	X295.226_2.849	2.108921391	3.23206975	0.000810937	0.026997317	FA 18:3;O	Spectral Match to 13-Keto-9Z,11E-octadecadienoic acid from NIST14	0.784467	9	0.000701904	2.71	No	[M+H] ⁺	Fatty amides	Lipids and lipid-like molecules
922	1438.08	3.867	X1438.08_3.867	1.001001503	0.480220039	0.002618398	0.047673421	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
933	244.093	0.307	X244.093_0.307	1.952599782	4.699954695	4.99E-05	0.004179737	Cytidine	3'-CMP - 60.0 eV	NA	NA	NA	1	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
948	678.505	3.633	X678.505_3.633	1.101994866	0.208749996	0.000357271	0.015044342	PC 28:0 or LPC 28:1;O or PC O-28:1;O	Spectral Match to 1,2-Ditradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102_0.311	3.693206002	0	1.73E-06	0.000554929	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
964	828.537	3.257	X828.537_3.257	1.001001503	162.3982639	0.002859938	0.047673421	PC 36:5;O3	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.937119	9	93.9668	1.81	Yes	[M+H] ⁺	no matches	no matches

976	220.089	2.267	X220.089_2.267	1.233032172	25.43790486	0.00026781	0.01292181	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
979	207.014	0.281	X207.014_0.281	2.423502253	2.701043929	3.77E-07	0.000348813	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	2.2422249	0	1.73E-06	0.000554929	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
981	262.165	0.512	X262.165_0.512	1.001001503	0.124043091	0.000676312	0.024638142	CAR 5:0;O	Acetyl-Carnitine	0.939864	5	58.0417	0.38	Yes	[M+H] ⁺	no matches	no matches
1004	154.059	0.285	X154.059_0.285	2.200184415	2.0316718	7.53E-07	0.000348813	NA	NA	NA	NA	NA	NA	NA	NA	Nucleosides, nucleotides, and analogues	Organic acids and derivatives
1009	209.092	0.631	X209.092_0.631	2.108081007	23.36268046	0.000173889	0.009790535	Kynurenine	Kynurenine	0.940758	8	0.000106812	0.51	No	[M+H] ⁺	Carbonyl compounds	Organic oxygen compounds
1015	176.103	0.321	X176.103_0.321	2.117685043	44.026353	0.001200632	0.033501968	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1019	265.212	0.265	X265.212_0.265	1.893441995	0.242921502	6.90E-05	0.004990266	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1036	319.226	2.93	X319.226_2.93	1.951921802	7.982360849	0.000155992	0.009263543	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1062	166.042	2.246	X166.042_2.246	1.40050759	2.331786059	0.002354387	0.047673421	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1107	524.459	5.116	X524.459_5.116	1.001001503	0.17910854	0.001193214	0.033501968	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1142	156.991	2.403	X156.991_2.403	1.75522707	3.156395017	0.00192393	0.043684533	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1154	348.08	0.244	X348.08_0.244	1.519761115	0.483192339	0.000101285	0.006702185	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1159	189.16	0.256	X189.16_0.256	1.888051427	2.113956154	3.65E-05	0.003844866	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1162	554.175	3.479	X554.175_3.479	2.377601907	0.081316188	0.000215767	0.011104792	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1164	328.32	3.278	X328.32_3.278	1.417050503	0.280801673	0.002640716	0.047673421	NAE 18:0	Spectral Match to Palmitoyl ethanolamide from NIST14	0.988697	9	28.0314	3.05	Yes	[M+H] ⁺	Amines	Organic nitrogen compounds
1232	462.297	2.913	X462.297_2.913	2.170737158	0.327782742	2.64E-06	0.000554929	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1299	606.376	3.012	X606.376_3.012	1.389425036	22.37675121	0.001200632	0.033501968	PC 22:2;O	1,2-didecanoyl-sn-glycero-3-phosphocholine	0.924675	4	39.9942	0.82	Yes	[M+H] ⁺	no matches	no matches
1304	648.422	3.036	X648.422_3.036	1.001001503	2.250552229	0.00130203	0.035669797	PC 25:2;O	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.955571	5	82.0401	2.31	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1332	214.059	0.31	X214.059_0.31	1.628248385	52.24967508	5.94E-05	0.004588619	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1356	674.437	3.083	X674.437_3.083	1.414595408	3.863412081	0.000741724	0.025262262	PC 27:3;O	Spectral Match to 1,2-Di-(9Z-tetradecenyl)-sn-glycero-3-phosphocholine from NIST14	0.930096	5	0.00494385	3.11	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1386	150.977	0.291	X150.977_0.291	2.6963377	2.846066827	3.50E-05	0.003844866	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1450	229.98	0.284	X229.98_0.284	1.669110119	2.572498855	0.001465809	0.035734884	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1608	674.461	3.269	X674.461_3.269	1.802271584	0.153925164	0.00022484	0.011320216	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1619	445.303	2.339	X445.303_2.339	1.977423529	0.183645447	0.001562787	0.037702245	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1654	381.263	2.596	X381.263_2.596	1.612528977	6.146694215	0.000618881	0.023497201	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1874	628.731	2.439	X628.731_2.439	1.233032172	0	0.000698424	0.024638142	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1919	238.045	0.31	X238.045_0.31	1.586286185	0.359216368	4.14E-05	0.004171929	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1925	333.278	3.361	X333.278_3.361	1.001001503	0.076571891	0.002679571	0.047673421	FA 22:3;O or FA 22:4	Spectral Match to cis-7,10,13,16-Docosatetraenoic acid from NIST14	0.89298	13	0.00119019	2.4	No	[M+H- H2O] ⁺	Fatty amides	Lipids and lipid-like molecules
2024	297.123	2.444	X297.123_2.444	1.001001503	0.114907885	0.00214236	0.04725435	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2128	700.35	2.257	X700.35_2.257	1.001001503	0	0.002474025	0.047673421	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

2226	726.504	3.674	X726.504_3.674	1.001001503	0.043731093	0.002142331	0.04725435	LPC 30:2;O or PC 30:1 or PC O-30:2;O	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.92535	7	56.0649	0.55	Yes	[M+Na] ⁺	no matches	no matches
2440	933.63	2.948	X933.63_2.948	1.001001503	0.061450225	0.002142331	0.04725435	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2480	391.193	2.828	X391.193_2.828	1.001001503	0	0.002482983	0.047673421	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2898	216.063	0.345	X216.063_0.345	1.901060501	0.042801277	0.000104757	0.006739387	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3070	261.217	0.273	X261.217_0.273	1.694234874	0.023497208	3.17E-06	0.000611006	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3099	426.261	2.805	X426.261_2.805	1.001001503	0.031872103	0.000500788	0.019996968	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3100	466.33	2.972	X466.33_2.972	1.001001503	0	0.000180805	0.009790535	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3269	158.118	0.319	X158.118_0.319	1.001001503	0	0.000180805	0.009790535	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
3273	338.051	0.279	X338.051_0.279	1.952530172	0.032156955	4.20E-06	0.000747456	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S9: Infection-perturbed metabolites in the lung at position 7. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected /uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
18	333.062	2.524	X333.062_2.524	1.001001503	0.468668919	0.000427732	0.012102472	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
91	162.113	0.297	X162.113_0.297	2.898914578	0.318782135	3.77E-07	0.000179753	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
223	643.135	2.525	X643.135_2.525	1.374578563	0.291673284	0.000499874	0.013583864	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
283	156.042	0.281	X156.042_0.281	1.001001503	0.497306275	0.000101285	0.005209494	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
311	840.572	3.374	X840.572_3.374	1.001001503	7.812667803	0.000423127	0.012102472	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
599	309	2.395	X309_2.395	1.912861219	0.375959256	0.000373447	0.011283762	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
642	814.558	3.367	X814.558_3.367	1.36232155	3.907697138	0.000815981	0.019095557	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
649	172.591	2.548	X172.591_2.548	1.934612352	0.31544685	0.000430964	0.012102472	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
658	161.084	2.547	X161.084_2.547	1.001001503	0.379905314	0.003663248	0.040205333	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
709	313.131	5.657	X313.131_5.657	1.374578563	0.2796048	0.002352207	0.032643716	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
710	240.096	2.268	X240.096_2.268	2.142428679	5.701323576	3.65E-05	0.002724381	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
721	204.123	0.316	X204.123_0.316	1.713510275	0.484746247	1.69E-05	0.002128653	CAR 2:0	Acetyl-carnitine	0.985822	5	9.16E-05	0	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
730	221.186	0.271	X221.186_0.271	2.638260579	0.324307786	7.15E-06	0.001219752	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
746	147.076	0.32	X147.076_0.32	3.443932121	2.174833011	3.77E-07	0.000179753	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
747	160.133	0.303	X160.133_0.303	1.001001503	0.35990126	2.52E-05	0.002370403	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
773	169.058	0.306	X169.058_0.306	2.314657555	2.118866118	3.77E-07	0.000179753	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds
779	218.139	0.499	X218.139_0.499	1.399641645	0.412264385	0.000101285	0.005209494	CAR 3:0	Propionylcarnitine	0.985552	5	0.000305176	1.38	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules

783	140.068	0.286	X140.068_0.286	1.001001503	0.42670142	3.65E-05	0.002724381	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
797	704.522	3.673	X704.522_3.673	2.206213492	0.486422016	0.000909684	0.019921244	PC 30:1 or LPC 30:2,O or PC O-30:2,O	Spectral Match to 1-(9Z-Octadecenyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
805	420.899	2.095	X420.899_2.095	1.001001503	0.32447403	0.000427732	0.012102472	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
835	508.376	2.982	X508.376_2.982	2.067003334	4.890198196	7.53E-07	0.00025679	LPC O-18:1	Spectral Match to 1-(12-Octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.813677	5	0.00088501	0.2	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
845	319.147	2.41	X319.147_2.41	1.001001503	9.432233876	0.00403222	0.043161028	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
854	185.033	0.306	X185.033_0.306	2.013806954	2.546378482	7.53E-07	0.00025679	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
863	266.075	0.278	X266.075_0.278	1.955012912	2.550021342	2.78E-05	0.002370403	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
874	700.571	3.709	X700.571_3.709	2.691499572	38.59619444	6.65E-05	0.004067538	HexCer 34:1;O2 (annotated via network annotation propagation)	GalCer(d18:1/16:1); [M+H] ⁺ C40H76N108	NA	NA	NA	1.71	Yes	[M+H] ⁺	Phosphosphingolipids	Lipids and lipid-like molecules
878	112.051	0.31	X112.051_0.31	2.557205985	2.93853367	2.75E-05	0.002370403	Cytosine	Spectral Match to Cytosine from NIST14	0.829279	4	0.000205994	1.84	No	[M+H] ⁺	no matches	no matches
886	220.118	0.659	X220.118_0.659	2.075714326	4.852987076	0.000177937	0.007278521	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
910	146.165	0.287	X146.165_0.287	1.298588192	2.115304623	0.000327577	0.010259179	Spermidine	Massbank:PB000484 Spermidine[N-(3-aminopropyl)butane-1,4-diamine	0.907869	6	0.000793457	5.43	No	[M+H] ⁺	Amines	Organic nitrogen compounds
911	431.236	2.63	X431.236_2.63	1.001001503	27.70706316	0.003268419	0.037032311	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
915	248.149	0.435	X248.149_0.435	1.559474881	2.731995667	0.002618398	0.033468708	CAR 4:0,O	Propionylcarnitine	0.923178	5	30.0101	0.81	Yes	[M+H] ⁺	no matches	no matches
923	772.547	3.474	X772.547_3.474	1.001001503	5.252603472	0.002197847	0.031414733	PC 34:3,O	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.934941	6	37.9772	2.2	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
933	244.093	0.307	X244.093_0.307	2.575532277	2.674584284	2.78E-05	0.002370403	Cytidine	3'-CMP - 60.0 eV	NA	NA	NA	1	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
948	678.505	3.633	X678.505_3.633	1.603068556	0.298821969	0.001082164	0.022859518	PC 28:0 or LPC 28:1,O or PC O-28:1,O	Spectral Match to 1,2-Ditetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102_0.311	2.774268479	0	1.73E-06	0.000375366	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
966	838.556	3.34	X838.556_3.34	1.408348959	3.321497369	0.001418733	0.023435573	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
976	220.089	2.267	X220.089_2.267	1.001001503	34.52390491	0.000173889	0.007278521	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
979	207.014	0.281	X207.014_0.281	2.888958979	2.207985936	3.77E-07	0.000179753	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	2.858973064	0	1.73E-06	0.000375366	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
981	262.165	0.512	X262.165_0.512	1.565161932	0.093713132	9.71E-05	0.005209494	CAR 5:0,O	Acetyl-Carnitine	0.939864	5	58.0417	0.38	Yes	[M+H] ⁺	no matches	no matches
1019	265.212	0.265	X265.212_0.265	2.562559746	0.177331948	2.75E-05	0.002370403	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1025	449.266	3.234	X449.266_3.234	1.001001503	0.030005391	0.000104757	0.005209494	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1036	319.226	2.93	X319.226_2.93	1.001001503	6.860685983	0.0012837	0.023414321	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1038	444.141	2.464	X444.141_2.464	1.001001503	16.48157837	0.001771543	0.027298556	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1062	166.042	2.246	X166.042_2.246	2.584968973	2.216509326	0.000337689	0.01033415	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1083	434.748	2.917	X434.748_2.917	1.001001503	43.72163212	0.001460578	0.023435573	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1124	798.562	3.497	X798.562_3.497	1.001001503	24.859797	0.000330941	0.010259179	PC 36:4,O	Spectral Match to 1-(9Z-Octadecenyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.945794	9	66.0091	2.88	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules

1135	510.392	3.067	X510.392_3.067	1.001001503	3.558426455	0.00441218	0.046395917	LPC O-18:0	Spectral Match to Lyso-PAF C-18 from NIST14	0.939065	7	0.000610352	0.39	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1154	348.08	0.244	X348.08_0.244	1.782269816	0.399089041	1.13E-05	0.001797529	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1199	136.048	0.284	X136.048_0.284	1.965939247	2.117206732	3.65E-05	0.002724381	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1253	135.003	0.293	X135.003_0.293	1.34406228	2.093210408	1.69E-05	0.002128653	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1281	710.459	3.077	X710.459_3.077	1.001001503	6.321677618	0.001888192	0.02816946	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1302	700.452	3.2	X700.452_3.2	1.636339721	2.906174617	0.001536515	0.024451073	PC 29:4;O	Spectral Match to 1-(9Z-Octadecenyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.934001	8	32.1008	4	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1333	386.29	2.692	X386.29_2.692	2.061257593	4.259956728	0.000155992	0.006895438	CAR 14:1;O	Lauroylcarnitine	0.904952	8	42.0095	0.26	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1341	566.308	2.55	X566.308_2.55	1.001001503	3.478613342	0.003872977	0.041643227	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1356	674.437	3.083	X674.437_3.083	1.88888671	2.601720896	0.000554998	0.014719769	PC 27:3;O	Spectral Match to 1,2-Di-(9Z-tetradecenyl)-sn-glycero-3-phosphocholine from NIST14	0.930096	5	0.00494385	3.11	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1365	414.321	2.782	X414.321_2.782	1.882681318	2.101918169	0.003410276	0.038038925	CAR 16:1;O	Massbank:RP025103 Oleoyl-L-Carnitine [(3R)-3-[(Z)-octadec-9-enyloxy-4-(trimethylazaniumyl)butanoate	0.937557	6	12.0372	0.97	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1386	150.977	0.291	X150.977_0.291	2.158430055	3.12347959	1.51E-06	0.000375366	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1418	331.263	3.102	X331.263_3.102	1.001001503	2.922853029	0.001187403	0.023414321	FA 22:4;O	Spectral Match to 8S-Hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid from NIST14	0.910257	13	28.0316	0.6	Yes	[M+H-2O] ⁺	Fatty amides	Lipids and lipid-like molecules
1488	332.218	2.234	X332.218_2.234	2.027698331	0.059680206	0.000161217	0.00696822	Thr-Val-Leu	Spectral Match to Thr-Val-Leu from NIST14	0.834945	9	0	0	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
1498	440.336	2.811	X440.336_2.811	1.001001503	3.446340848	0.000493118	0.013583864	CAR 18:2;O	Massbank:RP025103 Oleoyl-L-Carnitine [(3R)-3-[(Z)-octadec-9-enyloxy-4-(trimethylazaniumyl)butanoate	0.933578	6	13.9785	2.27	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1499	664.417	3.087	X664.417_3.087	1.41662751	4.611711907	0.004719803	0.048771295	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1510	226.105	0.283	X226.105_0.283	1.951431169	0.448895693	0.000224294	0.008498248	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1534	662.438	3.092	X662.438_3.092	1.001001503	3.176585369	0.002741737	0.033468708	PC 26:2;O	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.937874	5	96.0556	1.66	Yes	[M+H] ⁺	no matches	no matches
1571	471.267	2.732	X471.267_2.732	1.001001503	25.9968388	0.003444002	0.038059412	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1600	240.115	2.336	X240.115_2.336	2.422479325	9.000751488	0.000267048	0.00928722	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1619	445.303	2.339	X445.303_2.339	2.367988505	0.088294776	7.08E-05	0.004227595	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1637	163.031	2.245	X163.031_2.245	1.604730814	2.364382759	0.002232728	0.031535627	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1654	381.263	2.596	X381.263_2.596	1.677706217	9.428118698	0.00084744	0.019639223	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1707	422.129	2.383	X422.129_2.383	1.001001503	0.304407438	0.003722404	0.040205333	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1708	295.932	0.279	X295.932_0.279	1.001001503	0.05068364	0.000134957	0.006325921	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1716	189.047	2.245	X189.047_2.245	1.331422542	2.673591814	0.001139552	0.023414321	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1770	242.075	2.501	X242.075_2.501	1.546955129	0.112648609	0.000714687	0.01689067	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1836	388.269	2.51	X388.269_2.51	1.001001503	0.171875	0.001713795	0.027091588	NA	NA	NA	NA	NA	NA	NA	NA	Eicosanoids	Lipids and lipid-like molecules
1837	614.924	2.093	X614.924_2.093	1.190983812	0.034749801	0.000104362	0.005209494	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1899	268.658	2.147	X268.658_2.147	1.001001503	0.073613463	0.001390848	0.023414321	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives

1919	238.045	0.31	X238.045_0.31	1.669110119	0.308878274	0.000149963	0.006753983	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2014	389.25	2.536	X389.25_2.536	1.001001503	0.319843914	0.003273489	0.037032311	NA	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
2021	302.305	2.854	X302.305_2.854	1.001001503	2.184032197	0.000247753	0.009098264	SPB 18:0;O2	Sphinganine	0.960601	13	0.000183105	0.99	No	[M+H] ⁺	Amines	Organic nitrogen compounds	
2052	222.636	2.547	X222.636_2.547	1.247109264	0.303605212	0.000330941	0.010259179	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2065	820.01	2.376	X820.01_2.376	1.001001503	0.063002964	5.95E-05	0.003737693	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2101	464.207	2.32	X464.207_2.32	1.001001503	0.179668305	0.001946939	0.028865491	NA	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
2349	1412.063	3.834	X1412.063_3.834	1.697324654	0.192847847	0.002601549	0.033468708	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2674	716.558	4.038	X716.558_4.038	1.364369486	0.172767399	0.003715492	0.040205333	PC O-32:2 or LPC 32:2 or LPC O-32:3;O	Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.828169	4	44.0244	1.26	Yes	[M+H] ⁺	no matches	no matches	
2898	216.063	0.345	X216.063_0.345	1.793423771	0.03850925	1.94E-05	0.002208787	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3070	261.217	0.273	X261.217_0.273	1.671315676	0.013951763	1.94E-05	0.002208787	NA	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3099	426.261	2.805	X426.261_2.805	1.296180731	0.029820261	0.000134957	0.006325921	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3120	669.417	2.981	X669.417_2.981	1.67802512	0.046956886	0.002142331	0.030806111	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
4162	409.198	2.509	X409.198_2.509	1.722696699	0.034975669	0.000500788	0.013583864	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S10: Infection-perturbed metabolites in the lung at position 8. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected /uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
18	333.062	2.524	X333.062_2.524	1.416205397	0.457137655	7.30E-05	0.004009403	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
91	162.113	0.297	X162.113_0.297	1.599256291	0.369091856	4.52E-06	0.000797157	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
152	349.036	2.524	X349.036_2.524	1.652924919	0.377522115	0.000248697	0.009352441	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
223	643.135	2.525	X643.135_2.525	2.348851425	0.123193431	9.16E-05	0.004918281	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
238	526.314	5.678	X526.314_5.678	1.001001503	0.488899687	0.000101285	0.005211966	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
283	156.042	0.281	X156.042_0.281	1.214465419	0.4993022	3.65E-05	0.002653282	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
592	332.646	5.656	X332.646_5.656	1.36232155	0.33426009	0.000344859	0.011516214	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
649	172.591	2.548	X172.591_2.548	1.322255749	0.217479375	0.001884433	0.032778526	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
658	161.084	2.547	X161.084_2.547	1.328055689	0.147023233	0.000579063	0.016440071	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
709	313.131	5.657	X313.131_5.657	1.001001503	0.227191782	0.001562787	0.029243067	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
710	240.096	2.268	X240.096_2.268	1.001001503	4.743529299	0.000247753	0.009352441	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
730	221.186	0.271	X221.186_0.271	2.188401755	0.319281029	7.15E-06	0.001104394	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
746	147.076	0.32	X147.076_0.32	1.857533645	2.124086432	1.51E-06	0.000610372	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
747	160.133	0.303	X160.133_0.303	1.066753424	0.430226108	0.000327577	0.011237697	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
772	393.224	2.51	X393.224_2.51	1.242382268	0.437906689	1.69E-05	0.001902306	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
773	169.058	0.306	X169.058_0.306	2.296213356	2.089289985	4.52E-06	0.000797157	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds
779	218.139	0.499	X218.139_0.499	1.417050503	0.431867075	0.000101285	0.005211966	CAR 3:0	Propionylcarnitine	0.985552	5	0.000305176	1.38	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
782	253.101	2.236	X253.101_2.236	1.812756014	0.378072827	5.23E-05	0.003314673	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
783	140.068	0.286	X140.068_0.286	1.943183363	0.427112048	4.52E-06	0.000797157	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
805	420.899	2.095	X420.899_2.095	1.709763268	0.359297424	0.000137808	0.006188836	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
827	790.571	3.867	X790.571_3.867	1.745147588	0.496000621	0.00130203	0.025728108	PC O-38:7	Spectral Match to 1-Octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine from NIST14	0.90469	8	20.0279	4.43	Yes	[M+H] ⁺	no matches	no matches
835	508.376	2.982	X508.376_2.982	1.377947886	3.173198539	2.52E-05	0.00225396	LPC O-18:1	Spectral Match to 1-(12-Octadecenyl)-sn-glycero-3-phosphocholine from NIST14	0.813677	5	0.00088501	0.2	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
854	185.033	0.306	X185.033_0.306	2.085331889	2.385142608	4.52E-06	0.000797157	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
863	266.075	0.278	X266.075_0.278	1.901342366	2.022452795	1.51E-06	0.000610372	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
874	700.571	3.709	X700.571_3.709	2.267646278	26.94399116	5.94E-05	0.003495823	HexCer 34:1;O2 (annotated via network annotation propagation)	GalCer(d18:1/16:1); [M+H] ⁺ C40H76N1O8	NA	NA	NA	1.71	Yes	[M+H] ⁺	Phosphosphingolipids	Lipids and lipid-like molecules
877	295.066	0.357	X295.066_0.357	2.344392757	0.088192224	5.94E-05	0.003495823	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

878	112.051	0.31	X112.051_0.31	3.429423966	2.290252338	2.78E-05	0.002289304	Cytosine	Spectral Match to Cytosine from NIST14	0.829279	4	0.000205994	1.84	No	[M+H] ⁺	no matches	no matches
883	170.042	0.332	X170.042_0.332	1.001001503	2.133814486	0.002011665	0.034505641	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
886	220.118	0.659	X220.118_0.659	2.458354317	3.478153309	2.78E-05	0.002289304	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
888	184.095	0.281	X184.095_0.281	1.833449244	0.357245554	0.001651233	0.030436906	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
895	441.738	2.143	X441.738_2.143	1.001001503	3.769870458	0.000305155	0.011084299	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
905	766.572	3.984	X766.572_3.984	1.399641645	0.409097535	7.15E-06	0.001104394	PC O-36:5	Spectral Match to Eicosapentaenoyl PAF C-16 from NIST14	0.90891	9	0	3.26	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
910	146.165	0.287	X146.165_0.287	2.407687049	2.374432105	3.65E-05	0.002653282	Spermidine	Massbank:PB000484 Spermidine [N-(3-aminopropyl)butane-1,4-diamine	0.907869	6	0.000793457	5.43	No	[M+H] ⁺	Amines	Organic nitrogen compounds
917	200.069	0.278	X200.069_0.278	1.622947256	0.237277055	0.003984575	0.040209499	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
933	244.093	0.307	X244.093_0.307	3.073353766	2.44970218	3.50E-05	0.002653282	Cytidine	3'-CMP - 60.0 eV	NA	NA	NA	1	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
948	678.505	3.633	X678.505_3.633	1.725567994	0.37958411	0.000137808	0.006188836	PC 28:0 or LPC 28:1;O or PC O-28:1;O	Spectral Match to 1,2-Ditetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102_0.311	2.5287535	0	1.73E-06	0.000610372	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
979	207.014	0.281	X207.014_0.281	2.284921105	2.162012426	1.51E-06	0.000610372	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	2.197603173	0.008414129	3.17E-06	0.000781961	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
981	262.165	0.512	X262.165_0.512	1.717371624	0.105206078	5.94E-05	0.003495823	CAR 5:0;O	Acetyl-Carnitine	0.939864	5	58.0417	0.38	Yes	[M+H] ⁺	no matches	no matches
992	351.282	3.539	X351.282_3.539	1.001001503	2.095660097	0.000669497	0.017430899	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1009	209.092	0.631	X209.092_0.631	1.88826484	19.47424371	2.12E-05	0.002225396	Kynurenine	Kynurenine	0.940758	8	0.000106812	0.51	No	[M+H] ⁺	Carbonyl compounds	Organic oxygen compounds
1011	462.78	2.966	X462.78_2.966	1.34406228	4.486432981	0.00106482	0.022289018	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1015	176.103	0.321	X176.103_0.321	1.532407553	21.21605422	0.000249903	0.009352441	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1019	265.212	0.265	X265.212_0.265	3.806887345	0.217189678	3.77E-07	0.000465008	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1031	427.741	2.93	X427.741_2.93	1.220774308	24.96666667	0.001771543	0.031879629	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1078	816.573	3.372	X816.573_3.372	1.001001503	10.5848906	0.002817974	0.039607302	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1136	454.389	3.079	X454.389_3.079	1.305609134	4.412168888	0.001840059	0.032233658	CAR 20:1	Palmitoylcarnitine	0.920822	6	54.0466	0.22	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1159	189.16	0.256	X189.16_0.256	2.361399259	2.12267506	5.23E-05	0.003314673	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1165	193.155	0.314	X193.155_0.314	1.001001503	0	0.002482983	0.039063484	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1191	252.078	2.624	X252.078_2.624	1.001001503	10.21408811	0.000696195	0.017430899	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1232	462.297	2.913	X462.297_2.913	1.001001503	0.369761975	0.000327577	0.011237697	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1275	353.298	3.807	X353.298_3.807	1.001001503	5.243598116	0.002359184	0.038590633	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1307	744.589	4.087	X744.589_4.087	1.001001503	8.789711045	0.000515714	0.01612422	PC O-34:2 or LPC 34:2 or LPC O-34:3;O	PAF (o-16:0/18:2)	0.760068	5	0.000183105	1.61	No	[M+H] ⁺	no matches	no matches
1332	214.059	0.31	X214.059_0.31	2.165100371	11.29432942	2.49E-05	0.002225396	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1333	386.29	2.692	X386.29_2.692	1.001001503	2.787082102	0.000895262	0.02006178	CAR 14:1;O	Lauroylcarnitine	0.904952	8	42.0095	0.26	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules

1356	674.437	3.083	X674.437_3.083	1.389425036	3.28156828	0.000186003	0.007786916	PC 27:3-O	Spectral Match to 1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.930096	5	0.00494385	3.11	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1386	150.977	0.291	X150.977_0.291	2.269578825	2.17868618	2.64E-06	0.000781961	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1477	820.546	3.428	X820.546_3.428	1.001001503	21.14956638	0.003444002	0.043401455	PC 38:7-O	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.949924	9	85.9763	3.29	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1488	332.218	2.234	X332.218_2.234	1.266939792	0.127913533	0.000119016	0.005879399	Thr-Val-Leu	Spectral Match to Thr-Val-Leu from NIST14	0.834945	9	0	0	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
1600	240.115	2.336	X240.115_2.336	1.684985243	5.237904072	0.000291574	0.010749065	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1602	240.078	2.502	X240.078_2.502	1.730034102	0.23574483	0.001005784	0.021562889	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1619	445.303	2.339	X445.303_2.339	1.383175667	0.216359559	0.000213368	0.008639657	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1716	189.047	2.245	X189.047_2.245	2.001679951	2.297599548	0.001700632	0.031115273	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1770	242.075	2.501	X242.075_2.501	1.999588299	0.200533953	0.000613952	0.017092396	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1820	222.068	2.501	X222.068_2.501	1.468291755	0.197169942	0.000430964	0.013824417	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1836	388.269	2.51	X388.269_2.51	1.628495084	0.302839117	0.003698916	0.046143042	NA	NA	NA	NA	NA	NA	NA	NA	Ecosanoids	Lipids and lipid-like molecules
1914	151.031	2.247	X151.031_2.247	1.409498539	11.52667984	0.002854301	0.039607302	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1919	238.045	0.31	X238.045_0.31	2.006830332	0.380208108	0.000192665	0.007931376	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2021	302.305	2.854	X302.305_2.854	1.001001503	2.116063604	0.003392489	0.043401455	SP8 18:0;O2	Sphinganine	0.960601	13	0.000183105	0.99	No	[M+H] ⁺	Amines	Organic nitrogen compounds
2030	325.118	2.485	X325.118_2.485	1.372519052	0.236200479	0.003578915	0.044872691	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2101	464.207	2.32	X464.207_2.32	1.36232155	0.080283149	0.001119585	0.023238453	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
2121	256.073	2.563	X256.073_2.563	1.408348959	0.34328965	0.000880918	0.02006178	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2124	796.51	3.314	X796.51_3.314	1.001001503	27.77972794	0.003444002	0.043401455	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2205	269.226	5.109	X269.226_5.109	1.001001503	0.205669347	0.000532141	0.016127576	Retinol isomer	Spectral Match to Retinol from NIST14	0.941753	10	9.16E-05	0.34	No	[M+H- H2O] ⁺	no matches	no matches
3070	261.217	0.273	X261.217_0.273	2.442683307	0.009074716	3.17E-06	0.000781961	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3099	426.261	2.805	X426.261_2.805	2.143059599	0.099470855	0.000128869	0.006188836	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3159	576.498	3.241	X576.498_3.241	1.001001503	0	0.000698424	0.017430899	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3176	462.191	2.402	X462.191_2.402	1.001001503	0	0.000698424	0.017430899	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3269	158.118	0.319	X158.118_0.319	1.411207742	0	0.000180805	0.007786916	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
3278	652.347	2.245	X652.347_2.245	1.001001503	0	0.002482983	0.039063484	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S11: Infection-perturbed metabolites in the lung at position 9. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected /uninfected)	pval	padj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
291	788.542	3.343	X788.542_3.343	2.144414639	4.216150532	0.000137808	0.005988812	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
387	594.376	2.991	X594.376_2.991	1.001001503	2.108963061	0.002243335	0.026629215	PC 21:1;O	Spectral Match to 2-(5-Oxovaleryl)phosphatidylcholine from NIST14	0.797125	7	0.00195312	0.84	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
475	507.27	3.2	X507.27_3.2	1.001001503	0.451653506	0.000712761	0.015357314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
564	438.297	2.951	X438.297_2.951	1.799518742	0.417532063	2.64E-06	0.000337893	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
619	115.054	2.251	X115.054_2.251	1.397694154	4.399423848	0.001003717	0.018414795	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
642	814.558	3.367	X814.558_3.367	1.001001503	3.807171606	0.000909684	0.017752483	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
699	566.345	3.085	X566.345_3.085	1.001001503	2.733997245	0.002243335	0.026629215	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
709	313.131	5.657	X313.131_5.657	1.001001503	0.364989183	0.00403222	0.035975796	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
710	240.096	2.268	X240.096_2.268	1.309934291	5.172871238	0.000137808	0.005988812	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
713	132.077	0.31	X132.077_0.31	3.120157316	2.297500998	3.77E-07	9.65E-05	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
730	221.186	0.271	X221.186_0.271	2.309845353	0.397255461	3.65E-05	0.002754257	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
746	147.076	0.32	X147.076_0.32	2.850535104	2.720426684	3.77E-07	9.65E-05	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
761	464.313	2.984	X464.313_2.984	2.396062244	0.364608244	4.52E-06	0.000526587	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
764	466.329	3.067	X466.329_3.067	1.919030515	0.428429447	2.64E-06	0.000337893	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
773	169.058	0.306	X169.058_0.306	2.322950838	2.743827083	3.77E-07	9.65E-05	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds
779	218.139	0.499	X218.139_0.499	1.233032172	0.498786395	0.000327577	0.010606667	CAR 3:0	Propionylcarnitine	0.985552	5	0.000305176	1.38	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
797	704.522	3.673	X704.522_3.673	2.742073901	0.456477416	7.53E-07	0.000160902	PC 30:1 or LPC 30:2;O or PC O-30:2;O	Spectral Match to 1-[9Z-Octadecenyl]-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
810	134.045	0.322	X134.045_0.322	1.001001503	2.377304327	0.00333377	0.031862053	Aspartate	Aspartate - 50.00 eV	0.970598	5	9.16E-05	0.68	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
827	790.571	3.867	X790.571_3.867	1.400782	0.419180932	0.001151036	0.019164008	PC O-38:7	Spectral Match to 1-Octadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenyl)-sn-glycero-3-phosphocholine from NIST14	0.90469	8	20.0279	4.43	Yes	[M+H] ⁺	no matches	no matches
835	508.376	2.982	X508.376_2.982	1.983481384	2.690649909	0.000186003	0.007337113	LPC O-18:1	Spectral Match to 1-[1Z-Octadecenyl]-sn-glycero-3-phosphocholine from NIST14	0.813677	5	0.00088501	0.2	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
840	191.041	0.284	X191.041_0.284	1.001001503	2.409369262	2.64E-06	0.000337893	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
845	319.147	2.41	X319.147_2.41	1.001001503	17.03182607	0.002359184	0.027223004	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
854	185.033	0.306	X185.033_0.306	2.241643206	3.373034817	3.77E-07	9.65E-05	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
857	295.226	3.017	X295.226_3.017	1.220356984	2.601198716	0.000909684	0.017752483	FA 18:3;O	Spectral Match to 13-Keto-9Z,11E-octadecadienoic acid from NIST14	0.763788	8	0.000793457	2.71	No	[M+H] ⁺	Fatty amides	Lipids and lipid-like molecules
863	266.075	0.278	X266.075_0.278	1.860618156	2.307977638	7.15E-06	0.000733711	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
874	700.571	3.709	X700.571_3.709	1.681596258	11.28517599	7.63E-05	0.004252004	HexCer 34:1;O2 (annotated via network annotation propagation)	GalCer(d18:1/16:1); [M+H] ⁺ C40H76N1O8	NA	NA	NA	1.71	Yes	[M+H] ⁺	Phosphosphingolipids	Lipids and lipid-like molecules
878	112.051	0.31	X112.051_0.31	2.971211193	2.265162237	3.77E-07	9.65E-05	Cytosine	Spectral Match to Cytosine from NIST14	0.829279	4	0.000205994	1.84	No	[M+H] ⁺	no matches	no matches

886	220.118	0.659	X220.118 0.659	1.913113742	3.891445344	6.84E-05	0.004252004	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
895	441.738	2.143	X441.738 2.143	1.3845828	4.951259399	0.000669497	0.015357314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
904	810.525	3.34	X810.525 3.34	1.001001503	9.09950714	0.00111058	0.019164008	PC 36:6;O2 (annotated via network annotation propagation)	Spectral Match to N-Oleoyl-D-erythro-sphingomyelinophosphorylcholine from NIST14	NA	NA	NA	3.7	Yes	[M+Na] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
905	766.572	3.984	X766.572 3.984	2.070302309	0.338923671	7.15E-06	0.000733711	PC D-36:5	Spectral Match to Eicosapentaenoyl PAF C-16 from NIST14	0.90891	9	0	3.26	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
911	431.236	2.63	X431.236 2.63	1.001001503	136.6545421	0.001641212	0.022640435	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
915	248.149	0.435	X248.149 0.435	1.001001503	2.498324588	0.000673785	0.015357314	CAR 4:0;O	Propionylcarnitine	0.923178	5	30.0101	0.81	Yes	[M+H] ⁺	no matches	no matches
918	295.226	2.849	X295.226 2.849	1.417050503	5.094565372	0.001144054	0.019164008	FA 18:3;O	Spectral Match to 13-Keto-92,11-octadecadienoic acid from NIST14	0.784467	9	0.000701904	2.71	No	[M+H] ⁺	Fatty amides	Lipids and lipid-like molecules
923	772.547	3.474	X772.547 3.474	1.001001503	6.410951061	0.001279707	0.020539816	PC 34:3;O	Spectral Match to 1-Myristoyl-2-stearoyl-sn-glycero-3-phosphocholine from NIST14	0.934941	6	37.9772	2.2	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
931	610.37	2.946	X610.37 2.946	1.263285679	5.092925057	0.001447738	0.020690178	PC 21:1;O2	Spectral Match to 1-Palmitoyl-2-glutaryl phosphatidylcholine from NIST14	0.927889	8	0.000793457	2.46	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
933	244.093	0.307	X244.093 0.307	1.716932483	2.490192987	1.51E-06	0.00029568	Cytidine	3'-CMP - 60.0 eV	NA	NA	NA	1	Yes	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
948	678.505	3.633	X678.505 3.633	2.521876111	0.269764052	3.77E-07	9.65E-05	PC 28:0 or LPC 28:1;O or PC D-28:1;O	Spectral Match to 1,2-Ditetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102 0.311	2.781615841	0	1.73E-06	0.00029568	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
976	220.089	2.267	X220.089 2.267	1.306403737	12.41791813	0.000517747	0.01427424	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
979	207.014	0.281	X207.014 0.281	1.953822665	2.949017199	3.77E-07	9.65E-05	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202 0.268	2.708646198	0.004784534	3.17E-06	0.000386533	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
981	262.165	0.512	X262.165 0.512	1.415084048	0.129094244	0.000487576	0.013737844	CAR 5:0;O	Acetyl-Carnitine	0.939864	5	58.0417	0.38	Yes	[M+H] ⁺	no matches	no matches
996	728.52	3.641	X728.52 3.641	1.728421406	0.449571613	5.23E-05	0.003626809	LPC 30:1;O or PC 30:0 or PC D-30:1;O	Spectral Match to 1,2-Dihexadecanoyl-sn-glycero-3-phosphocholine from NIST14	0.775339	10	28.0325	0.14	Yes	[M+Na] ⁺	no matches	no matches
1004	154.059	0.285	X154.059 0.285	2.204441839	2.653148712	3.77E-07	9.65E-05	NA	NA	NA	NA	NA	NA	NA	NA	NA	Nucleosides, nucleotides, and analogues
1007	381.351	3.783	X381.351 3.783	1.001001503	0.485779884	0.00098561	0.018414795	NA	NA	NA	NA	NA	NA	NA	NA	Bile acids, alcohols and derivatives	Lipids and lipid-like molecules
1009	209.092	0.631	X209.092 0.631	2.100152407	27.86738412	5.94E-05	0.003809981	Kynurenine	Kynurenine	0.940758	8	0.000106812	0.51	No	[M+H] ⁺	Carbonyl compounds	Organic oxygen compounds
1018	722.511	3.776	X722.511 3.776	1.001001503	0.424422163	0.003855565	0.035041022	PE O-36:6	PE(16:0/20:5); [M+H] ⁺ C41H73N1O7P1	0.726445	13	1.00562	1.25	Yes	[M+H] ⁺	no matches	no matches
1019	265.212	0.265	X265.212 0.265	1.963513269	0.270378352	2.64E-06	0.000337893	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1020	660.422	3.07	X660.422 3.07	1.001001503	22.22193848	0.000712528	0.015357314	PC 26:3;O	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.949795	5	94.0404	2.27	Yes	[M+H] ⁺	no matches	no matches
1031	427.741	2.93	X427.741 2.93	1.001001503	23.21598006	0.001132207	0.019164008	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1048	198.085	0.309	X198.085 0.309	2.089718943	47.65690657	5.94E-05	0.003809981	Citrulline	Spectral Match to L-Citrulline from NIST14	0.876088	5	9.16E-05	0.46	No	M+Na	no matches	no matches
1056	738.505	3.682	X738.505 3.682	1.19683263	0.412360205	0.000357271	0.011171243	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1062	166.042	2.246	X166.042 2.246	1.283646399	2.127694852	0.000247753	0.00920637	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1075	796.546	3.457	X796.546 3.457	1.415084048	5.634845462	0.0012837	0.020539816	PC 36:5;O	Spectral Match to 1-[9Z-Octadecenoyl]-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.91888	9	63.991	3.39	Yes	[M+H] ⁺	no matches	no matches
1083	434.748	2.917	X434.748 2.917	1.001001503	12.88756101	0.0006318	0.015357314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1095	646.407	3.044	X646.407 3.044	1.001001503	28.95019425	0.000712528	0.015357314	PC 25:3;O	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.947236	5	80.0248	1.24	Yes	[M+H] ⁺	no matches	no matches

1105	772.524	3.719	X772.524_3.719	1.544770634	0.324151461	0.000267048	0.009706265	PE O-40:9 (annotated via network annotation propagation)	NA	NA	NA	NA	4.66	No	NA	no matches	no matches
1107	524.459	5.116	X524.459_5.116	1.686376577	0.289392289	0.000246272	0.00920637	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1120	624.386	3.046	X624.386_3.046	1.001001503	7.535395833	0.001011119	0.018414795	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1124	798.562	3.497	X798.562_3.497	1.001001503	4.138629809	0.005830113	0.04502533	PC 36:4:O	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecenoyl-sn-glycero-3-phosphocholine from NIST14	0.945794	9	66.0091	2.88	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1159	189.16	0.256	X189.16_0.256	2.356633902	2.645159129	3.77E-07	9.65E-05	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1164	328.32	3.278	X328.32_3.278	1.34406228	0.293022798	1.69E-05	0.001448114	NAE 18:0	Spectral Match to Palmitoyl ethanamide from NIST14	0.988697	9	28.0314	3.05	Yes	[M+H] ⁺	Amines	Organic nitrogen compounds
1199	136.048	0.284	X136.048_0.284	1.637616205	2.419331443	3.77E-07	9.65E-05	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1213	534.283	2.67	X534.283_2.67	1.001001503	19.7684689	0.001771543	0.023398993	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1241	389.189	2.458	X389.189_2.458	1.001001503	94.96185897	0.003444002	0.031878778	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1251	682.427	2.967	X682.427_2.967	1.001001503	23.53717973	0.001970379	0.025055473	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1253	135.003	0.293	X135.003_0.293	1.324604203	2.369793534	7.30E-05	0.004252004	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1260	708.443	2.998	X708.443_2.998	1.001001503	51.07339065	0.001132207	0.019164008	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1274	558.282	2.697	X558.282_2.697	1.001001503	18.25200382	0.000870878	0.017752483	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1299	606.376	3.012	X606.376_3.012	1.001001503	3.763630174	0.002297484	0.027021785	PC 22:2:O	1,2,-didecanoyl-sn-glycero-3-phosphocholine	0.924675	4	39.9942	0.82	Yes	[M+H] ⁺	no matches	no matches
1307	744.589	4.087	X744.589_4.087	2.705469467	23.98763294	0.000114226	0.005567808	PC O-34:2 or LPC 34:2 or LPC O-34:3:O	PAF (o-16:0/18:2)	0.760068	5	0.000183105	1.61	No	[M+H] ⁺	no matches	no matches
1332	214.059	0.31	X214.059_0.31	1.637330668	40.61653761	5.94E-05	0.003809981	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1333	386.29	2.692	X386.29_2.692	1.241911033	3.257195505	0.000551282	0.014670245	CAR 14:1:O	Lauroylcarnitine	0.904952	8	42.0095	0.26	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1353	842.587	3.499	X842.587_3.499	1.001001503	13.1396415	0.00111037	0.019164008	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1356	674.437	3.083	X674.437_3.083	1.546800618	3.632146688	0.000554998	0.014670245	PC 27:3:O	Spectral Match to 1,2-Di-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.930096	5	0.00494385	3.11	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1386	150.977	0.291	X150.977_0.291	1.584404616	3.084343121	7.53E-07	0.000160902	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1438	179.143	3.037	X179.143_3.037	1.001001503	23.38021552	0.003444002	0.031878778	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1468	385.292	3.132	X385.292_3.132	1.001001503	0.477504944	0.001334593	0.020646091	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
1497	536.335	2.729	X536.335_2.729	1.001001503	15.3985	0.004268777	0.036976842	PC 18:1 or LPC 18:2:O	Spectral Match to Lyso-PC([16:0]) from NIST14	0.939852	8	40.0056	0.56	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1506	617.321	2.316	X617.321_2.316	1.001001503	0.304963826	0.001757192	0.023398993	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1518	429.22	2.59	X429.22_2.59	1.001001503	177.8061636	0.000472843	0.013470772	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1539	798.526	3.319	X798.526_3.319	1.001001503	11.82911872	0.001061347	0.019030033	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1553	770.495	3.29	X770.495_3.29	1.001001503	11.92916714	0.001562787	0.021896103	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1599	699.27	2.871	X699.27_2.871	1.001001503	0.342524902	0.003444002	0.031878778	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1600	240.115	2.336	X240.115_2.336	1.001001503	6.252989611	0.000436615	0.012867597	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1602	240.078	2.502	X240.078_2.502	1.001001503	0.298287429	0.00111037	0.019164008	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids

1608	674.461	3.269	X674.461_3.269	1.001001503	0.097865876	0.000193001	0.007497798	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1637	163.031	2.245	X163.031_2.245	1.37733732	2.323346496	0.00098561	0.018414795	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1654	381.263	2.596	X381.263_2.596	1.574456599	3.083438552	0.000727826	0.015422687	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1707	422.129	2.383	X422.129_2.383	1.001001503	0.134609124	0.003881299	0.035041022	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1716	189.047	2.245	X189.047_2.245	1.001001503	2.124121772	0.003410276	0.031878778	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1745	347.221	2.609	X347.221_2.609	1.001001503	19.48905109	0.000581285	0.014904139	Corticosterone	Corticosterone	0.885048	13	0.000793457	2.29	No	[M+H] ⁺	Hydroxysteroids	Lipids and lipid-like molecules
1820	222.068	2.501	X222.068_2.501	1.411207742	0.259949178	0.0014011	0.020646091	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1824	269.226	3.258	X269.226_3.258	1.001001503	0.075072645	0.000302327	0.010335557	Retinol isomer	Spectral Match to Retinol from NIST14	0.962401	10	0	0	No	[M+H- H2O] ⁺	no matches	no matches
1836	388.269	2.51	X388.269_2.51	1.001001503	0.200507137	0.00313961	0.031466676	NA	NA	NA	NA	NA	NA	NA	NA	Eicosanoids	Lipids and lipid-like molecules
1889	370.295	2.802	X370.295_2.802	1.001001503	5.035939323	0.000933153	0.017989515	CAR 14:1	Lauroylcarmitine	0.96244	8	26.0146	0.54	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1925	333.278	3.361	X333.278_3.361	1.001001503	0.190591324	0.000472843	0.013470772	FA 22:3;O or FA 22:4	Spectral Match to cis-7,10,13,16-Docosatetraenoic acid from NIST14	0.89298	13	0.00119019	2.4	No	[M+H- H2O] ⁺	Fatty amides	Lipids and lipid-like molecules
1946	280.263	2.769	X280.263_2.769	1.40691652	0.442178693	0.000712761	0.015357314	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2015	381.079	0.257	X381.079_0.257	1.001001503	9.486384196	0.003722404	0.034086586	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2058	237.11	2.442	X237.11_2.442	3.221776089	0.039829802	8.79E-06	0.000867032	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2065	820.01	2.376	X820.01_2.376	1.001001503	0.156601349	0.001977233	0.025055473	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2124	796.51	3.314	X796.51_3.314	1.389425036	12.03446027	0.001289747	0.020539816	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2160	279.171	2.306	X279.171_2.306	1.409989919	0.025626588	0.002679571	0.02941402	Leu-Phe	Spectral Match to Leu-Phe from NIST14	0.926877	4	0.00167847	6.01237	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
2179	740.555	5.56	X740.555_5.56	1.706844389	0.357054949	0.000357271	0.011171243	PE O-37:4	NA	NA	NA	NA	5.266292796	No	NA	no matches	no matches
2183	548.467	3.135	X548.467_3.135	1.983481384	0.111919331	0.000791116	0.016626405	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2205	269.226	5.109	X269.226_5.109	1.001001503	0.195055032	0.000148046	0.00632652	Retinol isomer	Spectral Match to Retinol from NIST14	0.941753	10	9.16E-05	0.34	No	[M+H- H2O] ⁺	no matches	no matches
2224	692.521	3.735	X692.521_3.735	1.54407561	0.468024999	0.000880918	0.017752483	PC 29:0 or LPC 29:1;O or PC O-29:1;O	Spectral Match to 1-Myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.952015	7	14.0184	2.17	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
2226	726.504	3.674	X726.504_3.674	1.34406228	0.09144491	9.05E-05	0.004836325	LPC 30:2;O or PC 30:1 or PC O-30:2;O	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.92535	7	56.0649	0.55	Yes	[M+Na] ⁺	no matches	no matches
2227	429.282	2.824	X429.282_2.824	1.001001503	0.166339636	0.001586548	0.022108202	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2301	854.472	2.928	X854.472_2.928	1.326497378	31.91528465	0.001200632	0.019483671	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2349	1412.063	3.834	X1412.063_3.834	1.793107252	0.28944385	2.70E-05	0.002099658	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2381	868.489	2.917	X868.489_2.917	1.001001503	23.85289948	0.001771543	0.023398993	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2386	782.53	3.391	X782.53_3.391	1.001001503	8.81271319	0.005905392	0.045333604	PC 33:2;O	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.874262	12	0.0379639	0.766743179	Yes	[M+Na] ⁺	no matches	no matches
2409	294.127	2.621	X294.127_2.621	1.001001503	15.26154891	0.006420066	0.047575289	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2445	437.266	3.275	X437.266_3.275	1.001001503	0.180209507	0.002089566	0.026262974	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2492	706.501	3.293	X706.501_3.293	1.376098011	0.265160634	0.000913934	0.017752483	PC 29:1;O	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.965433	9	26.052	0.990797333	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules

2579	762.527	3.24	X762.527_3.24	1.001001503	12.87731265	0.001460578	0.020690178	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2615	963.638	3.025	X963.638_3.025	1.686704824	0.190833668	0.000183652	0.007337113	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2636	606.449	3.324	X606.449_3.324	1.001001503	0.260893387	0.001957944	0.025055473	LPC 24:1 or LPC O-24:2;O or PC O-24:1	Spectral Match to 1-[9Z-Octadecenoyl]-sn-glycero-3-phosphocholine from NIST14	0.956062	11	84.0888	0.494682738	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
2644	494.36	3.193	X494.36_3.193	1.001001503	0.233634259	7.35E-05	0.004252004	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2674	716.558	4.038	X716.558_4.038	1.001001503	0.357147053	0.002817106	0.02941402	PC O-32:2 or LPC 32:2 or LPC O-32:3;O	Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.828169	4	44.0244	1.26	Yes	[M+H] ⁺	no matches	no matches
2898	216.063	0.345	X216.063_0.345	1.001001503	0.126131722	8.54E-05	0.004658326	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3051	468.346	3.053	X468.346_3.053	1.60451006	0.073771201	7.61E-05	0.004252004	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3070	261.217	0.273	X261.217_0.273	1.848743008	0	1.73E-06	0.00079568	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3099	426.261	2.805	X426.261_2.805	1.868185111	0.137533908	2.05E-05	0.001696962	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3273	338.051	0.279	X338.051_0.279	1.677706217	0.065731708	0.000221542	0.008478118	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3356	575.778	2.229	X575.778_2.229	1.001001503	0	0.002482983	0.028045671	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3366	917.597	2.95	X917.597_2.95	1.001001503	0.127650368	0.002609579	0.02909113	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S12: Infection-perturbed metabolites in the lung at position 10. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected/uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
91	162.113	0.297	X162.113_0.297	1.968583019	0.366239897	1.69E-05	0.00302613	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
397	580.36	2.98	X580.36_2.98	1.001001503	2.39125372	0.001151036	0.02007244	PC 20:1;O	1,2-Didecanoyl-sn-glycero-3-phosphocholine	0.751682	4	13.9783	1.55	Yes	[M+H] ⁺	no matches	no matches
404	454.292	2.911	X454.292_2.911	1.898670231	0.484217047	1.51E-06	0.001158533	LPE 16:0 or LPE O-16:1;O	Spectral Match to 1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine from NIST14	0.853875	10	0.000701904	1.76	No	[M+H] ⁺	Glycerophosphoethanolamines	Lipids and lipid-like molecules
564	438.297	2.951	X438.297_2.951	2.044659115	0.336294047	1.51E-06	0.001158533	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
575	467.277	3.202	X467.277_3.202	2.160326913	0.353494204	0.000106865	0.006657919	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
592	332.646	5.656	X332.646_5.656	1.001001503	0.442285424	0.000965985	0.02007244	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
619	115.054	2.251	X115.054_2.251	1.276282649	2.815217391	0.002992009	0.027831917	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
662	313.273	3.211	X313.273_3.211	1.001001503	0.486413247	5.23E-05	0.005007524	MG O-16:2	Spectral Match to Monolaurin from NIST14	0.93717	8	56.0621	2.23	Yes	[M+H-H ₂ O] ⁺	no matches	no matches
709	313.131	5.657	X313.131_5.657	1.001001503	0.273469733	0.000696195	0.0181856	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
710	240.096	2.268	X240.096_2.268	1.001001503	5.02221245	5.23E-05	0.005007524	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
747	160.133	0.303	X160.133_0.303	1.066785738	0.449928412	0.001151036	0.02007244	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
761	464.313	2.984	X464.313_2.984	1.857880685	0.305783118	2.64E-06	0.001176828	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
764	466.329	3.067	X466.329_3.067	1.724970896	0.359666878	1.51E-06	0.001158533	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
772	393.224	2.51	X393.224_2.51	2.461702929	0.372288878	7.30E-05	0.005435826	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

779	218.139	0.499	X218.139_0.499	1.001001503	0.366619119	0.000132514	0.007532314	CAR 3:0	Propionylcarnitine	0.985552	5	0.000305176	1.38	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
782	253.101	2.236	X253.101_2.236	1.677366338	0.268470459	1.69E-05	0.00302613	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
797	704.522	3.673	X704.522_3.673	2.080031669	0.465632575	0.000132514	0.007532314	PC 30:1 or LPC 30:2:0 or PC O-30:2:0	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecenoyl-sn-glycero-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
804	175.002	0.301	X175.002_0.301	1.001001503	0.491811376	0.000455824	0.014892107	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
827	790.571	3.867	X790.571_3.867	1.001001503	0.408768732	0.001700632	0.02277997	PC O-38:7	Spectral Match to 1-Octadecenoyl-2-(5Z,8Z,11Z,14Z-icosatetraenoyl)-sn-glycero-3-phosphocholine from NIST14	0.90469	8	20.0279	4.43	Yes	[M+H] ⁺	no matches	no matches
840	191.041	0.284	X191.041_0.284	1.904413263	2.252025714	5.23E-05	0.005007524	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
874	700.571	3.709	X700.571_3.709	1.001001503	14.48659591	0.002073249	0.026076211	HexCer 34:1:0:2 (annotated via network annotation propagation)	GalCer(d18:1/16:1); [M+H] ⁺ C40H76N1O8	NA	NA	NA	1.71	Yes	[M+H] ⁺	Phosphosphingolipids	Lipids and lipid-like molecules
877	295.066	0.357	X295.066_0.357	1.001001503	0.136132799	0.000241933	0.010056533	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
886	220.118	0.659	X220.118_0.659	2.371434726	2.661647962	0.000275574	0.011018845	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
889	949.623	2.916	X949.623_2.916	2.112486808	0.455350108	0.000247753	0.010056533	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
892	298.274	2.766	X298.274_2.766	1.615848402	0.471831283	2.64E-06	0.001176828	SPB 18:2:0:2	Spectral Match to D-erythro-C18-Sphingosine from NIST14	0.905047	8	2.0155	0	Yes	[M+H] ⁺	no matches	no matches
901	596.355	2.942	X596.355_2.942	1.001001503	12.71665577	0.001562498	0.021576968	PC 20:1:0:2	1,2-didecanoyl-sn-glycero-3-phosphocholine	0.949106	5	29.9728	1.34	Yes	[M+H] ⁺	no matches	no matches
905	766.572	3.984	X766.572_3.984	1.401529663	0.366566157	0.000106865	0.006657919	PC O-36:5	Spectral Match to Eicosapentaenoyl PAF C-16 from NIST14	0.90891	9	0	3.26	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
925	385.346	3.401	X385.346_3.401	1.001001503	0.054425928	0.000635753	0.017592825	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
948	678.505	3.633	X678.505_3.633	3.408545898	0.262839631	2.78E-05	0.003547163	PC 28:0 or LPC 28:1:0 or PC O-28:1:0	Spectral Match to 1,2-Ditetradecenoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102_0.311	3.155637731	0	1.73E-06	0.001158533	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
976	220.089	2.267	X220.089_2.267	1.001001503	10.05880814	0.000933153	0.02007244	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
980	247.202	0.268	X247.202_0.268	2.33463455	0.008813393	5.12E-06	0.001715965	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1011	462.78	2.966	X462.78_2.966	1.001001503	6.974289307	0.003354871	0.030151901	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1018	722.511	3.776	X722.511_3.776	1.360681572	0.290338425	0.000344855	0.012318218	PE O-36:6	PE(P-16:0/20:5); [M+H] ⁺ C41H73N1O7P1	0.726445	13	1.00562	1.25	Yes	[M+H] ⁺	no matches	no matches
1019	265.212	0.265	X265.212_0.265	1.397198711	0.239895605	7.15E-06	0.001742317	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1025	449.266	3.234	X449.266_3.234	1.001001503	0.131376473	0.000487576	0.015655724	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1036	319.226	2.93	X319.226_2.93	1.001001503	10.11782831	0.004764954	0.039521088	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1048	198.085	0.309	X198.085_0.309	1.324604203	26.88303846	0.000750132	0.018781351	Citrulline	Spectral Match to L-Citrulline from NIST14	0.876088	5	9.16E-05	0.46	No	M+Na	no matches	no matches
1056	738.505	3.682	X738.505_3.682	1.001001503	0.466836399	0.000243264	0.010056533	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1058	792.587	3.933	X792.587_3.933	1.528902977	0.472428192	6.90E-05	0.005435826	PC O-38:6	Spectral Match to Docosahexaenoyl PAF C-16 from NIST14	0.883538	9	0.00262451	4.04	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1098	552.33	2.601	X552.33_2.601	1.001001503	66.42459416	0.002859938	0.027092413	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1105	772.524	3.719	X772.524_3.719	1.643918786	0.300299693	0.000175595	0.008168897	PE O-40:9 (annotated via network annotation propagation)	NA	NA	NA	NA	4.66	No	NA	no matches	no matches
1107	524.459	5.116	X524.459_5.116	1.656903263	0.284676105	0.000401801	0.014163483	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

1117	297.242	3.077	X297.242_3.077	1.001001503	3.038986807	0.001447738	0.020274035	FA 18:2;O (annotated via network annotation propagation)	2-hydroxypentadecanal oxime	NA	NA	NA	1.35	Yes	[M+H] ⁺	no matches	no matches
1164	328.32	3.278	X328.32_3.278	1.391005192	0.311955822	8.60E-05	0.006059659	NAE 18:0	Spectral Match to Palmitoyl ethanolamide from NIST14	0.988697	9	28.0314	3.05	Yes	[M+H] ⁺	Amines	Organic nitrogen compounds
1181	448.342	2.92	X448.342_2.92	1.001001503	0.463093728	0.000554998	0.016338884	CAR 20:4	Massbank:RPQ25103 Oleoyl-L- Carnitine [(3R)-3-[(2-octadec-9- enyl)oxy-4-(trimethylazanium)]butanoate	0.861432	5	21.9836	0.22	Yes	[M+H] ⁺	no matches	no matches
1232	462.297	2.913	X462.297_2.913	1.386087321	0.270911275	6.90E-05	0.005435826	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1245	279.113	2.589	X279.113_2.589	1.001001503	0.382710775	0.001806943	0.023499025	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1253	135.003	0.293	X135.003_0.293	1.399641645	2.302228137	0.000186003	0.008168897	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1267	181.08	2.368	X181.08_2.368	1.364369486	0.340648563	0.000186003	0.008168897	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1269	454.176	2.774	X454.176_2.774	1.266939792	0.411408531	0.002399702	0.027092413	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1304	648.422	3.036	X648.422_3.036	1.416435531	2.204592766	0.002243335	0.027092413	PC 25:2;O	1,2-Didecanoyl-sn-glycero-3- phosphocholine	0.955571	5	82.0401	2.31	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1307	744.589	4.087	X744.589_4.087	1.247109264	32.85324178	0.002073249	0.026076211	PC O-34:2 or LPC O- 34:3;O	PAF (o-16:0/18:2)	0.760068	5	0.000183105	1.61	No	[M+H] ⁺	no matches	no matches
1332	214.059	0.31	X214.059_0.31	1.001001503	4.111311649	0.001923254	0.024779851	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1356	674.437	3.083	X674.437_3.083	1.001001503	5.23340465	0.000186003	0.008168897	PC 27:3;O	Spectral Match to 1,2-Di-(9Z- tetradecenyl)-sn-glycero-3- phosphocholine from NIST14	0.930096	5	0.00494385	3.11	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1359	526.519	3.661	X526.519_3.661	1.001001503	0.345546028	0.000554917	0.016338884	NAE 31:0;O (annotated via network annotation propagation)	NA	NA	NA	NA	0.76	No	[M+H] ⁺	no matches	no matches
1374	650.402	3.025	X650.402_3.025	1.376098011	8.286042706	0.005202116	0.04236009	PC 24:2;O2	Spectral Match to 1-Myristoyl-2- palmitoyl-sn-glycero-3- phosphocholine from NIST14	0.922597	7	56.1374	1.23	Yes	[M+H] ⁺	no matches	no matches
1387	263.139	2.23	X263.139_2.23	1.471468578	0.427427703	0.001368693	0.02007244	Phe-Pro	Spectral Match to Phe-Pro from NIST14	0.989461	6	0.000183105	0.7	No	M+H	Amino acids, peptides, and analogues	Organic acids and derivatives
1535	688.415	3.018	X688.415_3.018	1.644210242	8.656232394	0.005202116	0.04236009	PC 25:1;O2	Spectral Match to 1-Hexadecanoyl-2- (9Z-octadecenyl)-sn-glycero-3- phosphocholine from NIST14	NA	NA	NA	1.45	Yes	[M+Na] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
1547	554.55	3.841	X554.55_3.841	1.266939792	0.392729182	0.003728057	0.032321896	NAE 33:0;O (annotated via network annotation propagation)	NA	NA	NA	NA	0	No	NA	no matches	no matches
1554	456.155	2.616	X456.155_2.616	1.001001503	0.453869085	0.006457157	0.046253274	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1582	616.175	2.776	X616.175_2.776	1.331422542	0.275931505	0.001170409	0.02007244	Hemin	Spectral Match to Hemin cation from NIST14	0.841594	5	0.00457764	7.43	No	Cat	no matches	no matches
1589	308.139	2.484	X308.139_2.484	1.326497378	0.35741836	0.003578915	0.031230988	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1602	240.078	2.502	X240.078_2.502	1.129139782	0.311749729	0.001345236	0.02007244	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1620	262.074	2.476	X262.074_2.476	1.001001503	0.3596536	0.002883899	0.027092413	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1649	440.16	2.639	X440.16_2.639	1.40691652	0.258986807	0.00064356	0.017592825	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1697	346.198	2.21	X346.198_2.21	1.40691652	0.11769875	0.000974278	0.02007244	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1707	422.129	2.383	X422.129_2.383	1.001001503	0.168789365	0.000879351	0.02007244	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1714	331.263	3.282	X331.263_3.282	1.734480783	0.441053079	0.000305155	0.011847966	FA 22:5	Spectral Match to Eicosapentaenoic acid ethyl ester from NIST14	0.755169	10	0.000396729	0.6	No	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1715	328.51	2.097	X328.51_2.097	1.001001503	0.065700134	0.002154032	0.026715985	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1757	302.214	2.71	X302.214_2.71	1.001001503	9.196428571	0.00451709	0.037816514	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1770	242.075	2.501	X242.075_2.501	1.36232155	0.30829012	0.000804095	0.019583358	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids

1820	222.068	2.501	X222.068_2.501	1.387070967	0.311038296	0.000984022	0.02007244	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1824	269.226	3.258	X269.226_3.258	1.415440167	0.489860086	0.001460578	0.020274035	Retinol isomer	Spectral Match to Retinol from NIST14	0.962401	10	0	0	No	[M+H-H2O] ⁺	no matches	no matches
1832	299.2	2.636	X299.2_2.636	1.001001503	0.233537566	0.006298969	0.045855807	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1836	388.269	2.51	X388.269_2.51	1.001001503	0.15625	0.000115091	0.006851763	NA	NA	NA	NA	NA	NA	NA	NA	Eicosanoids	Lipids and lipid-like molecules
1837	614.924	2.093	X614.924_2.093	1.322255749	0.100775879	4.43E-05	0.004751503	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1842	219.095	2.325	X219.095_2.325	1.001001503	0.218579158	0.000501076	0.015792736	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1864	348.07	0.429	X348.07_0.429	1.001001503	0.364278909	0.00192393	0.024779851	Adenosine 5'-monophosphate	Adenosine 5'-monophosphate	0.969597	4	0.00112915	3.24	No	[M+H] ⁺	NA	Nucleosides, nucleotides, and analogues
1874	628.731	2.439	X628.731_2.439	1.397694154	0.050530467	0.001721621	0.022832783	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1899	268.658	2.147	X268.658_2.147	1.001001503	0.159400949	0.001713795	0.022832783	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1919	238.045	0.31	X238.045_0.31	1.732435983	0.45035688	0.000436615	0.014806223	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1925	333.278	3.361	X333.278_3.361	1.001001503	0.154266027	0.0014011	0.02007244	FA 22:3,O or FA 22:4	Spectral Match to cis-7,10,13,16-Docosatetraenoic acid from NIST14	0.89298	13	0.00119019	2.4	No	[M+H-H2O] ⁺	Fatty amides	Lipids and lipid-like molecules
2052	222.636	2.547	X222.636_2.547	1.408348959	0.309364619	0.002601549	0.027092413	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2058	237.11	2.442	X237.11_2.442	1.34406228	0	0.000698424	0.0181856	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2101	464.207	2.32	X464.207_2.32	1.417050503	0	0.000180805	0.008168897	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
2121	256.073	2.563	X256.073_2.563	1.001001503	0.174833741	0.000148046	0.00793233	NAE 33:1,O (annotated via network annotation propagation)	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2145	552.535	3.682	X552.535_3.682	2.151786048	0.250812159	0.000490885	0.015655724	NAE 33:2,O (annotated via network annotation propagation)	NA	NA	NA	NA	0	No	NA	no matches	no matches
2148	550.519	3.516	X550.519_3.516	1.389425036	0.17152421	0.000630364	0.017592825	NAE 31:1,O (annotated via network annotation propagation)	NA	NA	NA	NA	0.73	No	[M+H] ⁺	no matches	no matches
2166	524.504	3.483	X524.504_3.483	1.904234223	0.043881906	0.000215767	0.009323209	NA	NA	NA	NA	NA	0.57	No	[M+H] ⁺	no matches	no matches
2205	269.226	5.109	X269.226_5.109	2.118007875	0.236611111	0.000183652	0.008168897	Retinol isomer	Spectral Match to Retinol from NIST14	0.941753	10	9.16E-05	0.34	No	[M+H-H2O] ⁺	no matches	no matches
2226	726.504	3.674	X726.504_3.674	1.796819804	0.185474844	0.000712528	0.0181856	LPC 30:2,O or PC 30:1 or PC O-30:2,O	Spectral Match to 1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine from NIST14	0.92535	7	56.0649	0.55	Yes	[M+Na] ⁺	no matches	no matches
2349	1412.063	3.834	X1412.063_3.834	2.447993777	0.301449123	2.75E-05	0.003547163	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2440	933.63	2.948	X933.63_2.948	1.001001503	0.215498264	0.001957944	0.025097278	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2445	437.266	3.275	X437.266_3.275	1.001001503	0.209261847	0.003076678	0.028520483	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2492	706.501	3.293	X706.501_3.293	1.001001503	0.191476409	0.001200632	0.02007244	PC 29:1,O	Spectral Match to 1-(9Z-Octadecenoyl)-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.965433	9	26.052	0.990797333	Yes	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
2615	963.638	3.025	X963.638_3.025	1.001001503	0.159290647	0.000310162	0.01870351	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2644	494.36	3.193	X494.36_3.193	1.001001503	0.217488978	5.87E-05	0.005425657	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2674	716.558	4.038	X716.558_4.038	2.123470245	0.334512626	8.47E-05	0.006059659	PC O-32:2 or LPC 32:2 or LPC O-32:3,O	Spectral Match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine from NIST14	0.828169	4	44.0244	1.26	Yes	[M+H] ⁺	no matches	no matches
2706	292.198	0.293	X292.198_0.293	1.001001503	0.063188705	0.000635753	0.017592825	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
2765	284.056	2.477	X284.056_2.477	1.001001503	0.090785055	0.001390848	0.02007244	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

2861	318.119	0.429	X318.119_0.429	1.001001503	0.154997066	0.00320033	0.029462828	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2898	216.063	0.345	X216.063_0.345	1.001001503	0.140151209	9.05E-05	0.006219376	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3051	468.346	3.053	X468.346_3.053	1.415084048	0.033151434	2.54E-05	0.003547163	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3070	261.217	0.273	X261.217_0.273	2.213599535	0.013678212	3.17E-06	0.00121161	NA	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3091	742.573	4.065	X742.573_4.065	1.878236397	0.134908232	2.19E-05	0.003547163	PC O-34:3 or LPC 34:3 or PC O-34:3	Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycerol-3-phosphocholine from NIST14	0.904542	6	44.0308	2.02	Yes	[M+H] ⁺	no matches	no matches	
3099	426.261	2.805	X426.261_2.805	2.518084375	0.079470861	1.32E-05	0.002716326	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3211	421.271	3.498	X421.271_3.498	1.402752094	0.084026468	0.000344273	0.012318218	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3250	123.041	2.395	X123.041_2.395	1.001001503	0.11268056	0.004139181	0.034870646	NA	NA	NA	NA	NA	NA	NA	NA	NA	Pyridinecarboxylic acids and derivatives	Organoheterocyclic compounds
3273	338.051	0.279	X338.051_0.279	1.001001503	0.059629901	0.000803944	0.019583358	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3352	228.042	2.469	X228.042_2.469	1.001001503	0	0.000698424	0.0181856	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3366	917.597	2.95	X917.597_2.95	1.34406228	0.037925007	3.31E-05	0.003968402	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3593	507.269	5.592	X507.269_5.592	1.001001503	0.101755066	0.001977233	0.025223845	NA	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S13: Infection-perturbed metabolites in the lung at position 11. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected /uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
18	333.062	2.524	X333.062_2.524	1.001001503	0.417538402	0.001151036	0.030815245	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
32	205.131	2.547	X205.131_2.547	1.507035299	0.436401307	0.000247753	0.011094844	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
91	162.113	0.297	X162.113_0.297	1.6632134	0.384555699	0.000247753	0.011094844	Carnitine	Spectral Match to L-Carnitine from NIST14	0.843951	5	0.00050354	3.11	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
152	349.036	2.524	X349.036_2.524	1.319266327	0.33340771	0.000673785	0.021355845	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
223	643.135	2.525	X643.135_2.525	1.313391379	0.171048872	0.000366573	0.01480115	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
356	224.084	2.243	X224.084_2.243	1.001001503	3.404732975	0.0029971	0.047018196	Desmethyleketamine	Spectral Match to Desmethyleketamine from NIST14	0.897255	5	0.000396729	1.77	No	[M+H] ⁺	Halobenzenes	Benzenoids
564	438.297	2.951	X438.297_2.951	2.389974454	0.435059379	0.000327577	0.013447019	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
649	172.591	2.548	X172.591_2.548	2.158936983	0.085182089	3.21E-05	0.003591737	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
658	161.084	2.547	X161.084_2.547	1.324604203	0.077394799	9.71E-05	0.005801521	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
710	240.096	2.268	X240.096_2.268	2.46592647	4.43421265	5.23E-05	0.004158255	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
730	221.186	0.271	X221.186_0.271	1.968382489	0.392448073	0.000186003	0.008982863	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
740	125.016	2.245	X125.016_2.245	1.001001503	2.146585741	7.30E-05	0.00507804	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
746	147.076	0.32	X147.076_0.32	2.122394679	2.042374539	7.30E-05	0.00507804	Glutamine	Glutamine	0.972569	6	0.00050354	3.42	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
761	464.313	2.984	X464.313_2.984	1.939012126	0.412399601	0.002243335	0.04582439	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
766	179.062	2.244	X179.062_2.244	1.956298763	2.037211584	0.000137808	0.007542696	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
773	169.058	0.306	X169.058_0.306	1.732312402	2.157004532	7.53E-07	0.00046369	NA	NA	NA	NA	NA	NA	NA	NA	Purines and purine derivatives	Organoheterocyclic compounds
797	704.522	3.673	X704.522_3.673	2.229073974	0.492802954	0.000427732	0.016461006	PC 30:1 or LPC 30:2;O or PC O-30:2;O	Spectral Match to 1-[9Z-Octadecenoyl]-2-tetradecanoyl-sn-glycero-3-phosphocholine from NIST14	0.958277	9	28.0308	0.71	Yes	[M+H] ⁺	no matches	no matches
840	191.041	0.284	X191.041_0.284	1.654786801	2.116640218	4.52E-06	0.001011688	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
854	185.033	0.306	X185.033_0.306	1.611626782	2.40074752	0.000101285	0.005801521	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
874	700.571	3.709	X700.571_3.709	1.178329475	20.88561765	0.000750132	0.022284549	HexCer 34:1;O2 (annotated via network annotation propagation)	GalCer(d18:1/16:1); [M+H] ⁺ C40H76N10O8	NA	NA	NA	1.71	Yes	[M+H] ⁺	Phosphosphingolipids	Lipids and lipid-like molecules
877	295.066	0.357	X295.066_0.357	1.413899397	0.159820003	0.00262999	0.04582439	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
886	220.118	0.659	X220.118_0.659	3.268644145	3.379099149	2.78E-05	0.003591737	Pantothenic acid	Pantothenic acid	0.978773	11	9.16E-05	0.42	No	[M+H] ⁺	Alcohols and polyols	Organic oxygen compounds
888	184.095	0.281	X184.095_0.281	1.642215464	0.412044807	0.000101285	0.005801521	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
895	441.738	2.143	X441.738_2.143	1.520460011	4.748729967	0.000237329	0.011094844	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
905	766.572	3.984	X766.572_3.984	1.637365307	0.448123962	0.002765951	0.04582439	PC O-36:5	Spectral Match to Eicosapentaenoyl PAF C-16 from NIST14	0.90891	9	0	3.26	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules

910	146.165	0.287	X146.165_0.287	1.731415749	2.550366539	1.69E-05	0.002980866	Spermidine	Massbank:P8000484 Spermidine N-(3-aminopropyl)butane-1,4-diamine	0.907869	6	0.000793457	5.43	No	[M+H] ⁺	Amines	Organic nitrogen compounds
917	200.069	0.278	X200.069_0.278	1.001001503	0.362024979	0.002640716	0.04582439	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
948	678.505	3.633	X678.505_3.633	1.601010777	0.287904332	0.000101285	0.005801521	PC 28:0 or LPC 28:1:0 or PC O-28:1:0	Spectral Match to 1,2-Ditetradeconoyl-sn-glycero-3-phosphocholine from NIST14	0.946793	8	0.00268555	2.65	No	[M+H] ⁺	Glycerophosphocholines	Lipids and lipid-like molecules
960	144.102	0.311	X144.102_0.311	2.71808211	0	1.73E-06	0.000608642	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
976	220.089	2.267	X220.089_2.267	2.409688445	16.44030214	7.61E-05	0.00507804	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
979	207.014	0.281	X207.014_0.281	1.417050503	2.362715195	3.65E-05	0.003598237	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	1.417050503	0	1.73E-06	0.000608642	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1004	154.059	0.285	X154.059_0.285	2.338728522	2.118442946	3.77E-07	0.00046369	NA	NA	NA	NA	NA	NA	NA	NA	NA	Nucleosides, nucleotides, and analogues
1015	176.103	0.321	X176.103_0.321	1.001001503	12.87487889	0.002494179	0.04582439	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1019	265.212	0.265	X265.212_0.265	1.001001503	0.27341335	0.000741724	0.022284549	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1025	449.266	3.234	X449.266_3.234	1.229736691	0.053286681	0.000500788	0.017886234	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1048	198.085	0.309	X198.085_0.309	1.989790697	22.9238909	7.63E-05	0.00507804	Citrulline	Spectral Match to L-Citrulline from NIST14	0.876088	5	9.16E-05	0.46	No	M+Na	no matches	no matches
1062	166.042	2.246	X166.042_2.246	1.71058925	2.227476787	0.000101285	0.005801521	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1098	552.33	2.601	X552.33_2.601	1.001001503	22.03313119	0.001460578	0.03561786	NA	NA	NA	NA	NA	NA	NA	NA	Glycerophosphocholines	Lipids and lipid-like molecules
1199	136.048	0.284	X136.048_0.284	2.459543682	2.068890323	2.78E-05	0.003591737	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1232	462.297	2.913	X462.297_2.913	1.414595408	0.385357209	0.000909684	0.025460813	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1253	135.003	0.293	X135.003_0.293	2.241623575	2.167036388	2.52E-05	0.003591737	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1254	169.036	0.407	X169.036_0.407	1.817772194	4.137876354	0.000427732	0.016461006	Urate	Urate	0.885339	5	0.000396729	2.35	No	[M+H] ⁺	Purines and purine derivatives	Organoheterocyclic compounds
1307	744.589	4.087	X744.589_4.087	1.3932675	77.71931928	0.000472843	0.017645641	PC O-34:2 or LPC 34:2 or LPC O-34:3:0	PAF (o-16:0/18:2)	0.760068	5	0.000183105	1.61	No	[M+H] ⁺	no matches	no matches
1332	214.059	0.31	X214.059_0.31	2.416336126	9.783126437	3.15E-05	0.003591737	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1333	386.29	2.692	X386.29_2.692	1.488042882	2.91975243	0.000163886	0.008588341	CAR 14:1:0	Lauroylcarnitine	0.904952	8	42.0095	0.26	Yes	[M+H] ⁺	Fatty acid esters	Lipids and lipid-like molecules
1341	566.308	2.55	X566.308_2.55	1.001001503	6.620285888	0.001368693	0.035485165	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1386	150.977	0.291	X150.977_0.291	2.691547816	2.534339196	3.12E-05	0.003591737	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1407	291.231	2.99	X291.231_2.99	1.001001503	2.003302395	0.002044107	0.04582439	FA 19:4	Spectral Match to cis-7,10,13,16-Docosatetraenoic acid from NIST14	0.700905	11	42.0467	3.09	Yes	[M+H] ⁺	no matches	no matches
1488	332.218	2.234	X332.218_2.234	1.645838978	0.125167307	0.003106322	0.048423234	Thr-Val-Leu	Spectral Match to Thr-Val-Leu from NIST14	0.834945	9	0	0	No	[M+H] ⁺	Amino acids, peptides, and analogues	Organic acids and derivatives
1600	240.115	2.336	X240.115_2.336	1.322255749	7.78407754	0.000175595	0.008982863	NA	NA	NA	NA	NA	NA	NA	NA	Halobenzenes	Benzenoids
1637	163.031	2.245	X163.031_2.245	2.008636961	2.978933873	0.000163886	0.008588341	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1654	381.263	2.596	X381.263_2.596	2.099500378	26.52351763	2.39E-05	0.003591737	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1716	189.047	2.245	X189.047_2.245	1.634507626	3.250821168	0.000436615	0.016544351	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
1745	347.221	2.609	X347.221_2.609	1.001001503	18.05	0.001200632	0.031797379	Corticosterone	Corticosterone	0.885048	13	0.000793457	2.29	No	[M+H] ⁺	Hydroxysteroids	Lipids and lipid-like molecules

1752	481.262	2.266	X481.262_2.266	1.001001503	2.579896907	0.001651233	0.03987242	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
1834	384.274	2.644	X384.274_2.644	1.417050503	15.61473214	0.000501076	0.017886234	CAR 14:2;O	Massbank:RP025103 Oleoyl-L-Carnitine[(3R)-3-[(Z)-octadec-9-enyl]oxy-4-(trimethylazaniumyl)]butanoate	0.848795	5	42.084	1.04	Yes	[M+H] ⁺	no matches	no matches
1919	238.045	0.31	X238.045_0.31	1.001001503	0.346956184	9.73E-05	0.005801521	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2052	222.636	2.547	X222.636_2.547	1.691107181	0.106225855	7.08E-05	0.00507804	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2060	293.193	2.546	X293.193_2.546	1.481939135	0.067163003	0.000750961	0.022284549	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2099	217.105	0.302	X217.105_0.302	1.001001503	0	0.002482983	0.04582439	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2166	524.504	3.483	X524.504_3.483	1.001001503	0.037055444	0.002142331	0.04582439	NAE 31:1;O (annotated via network annotation propagation)	NA	NA	NA	NA	0.57	No	[M+H] ⁺	no matches	no matches
2349	1412.063	3.834	X1412.063_3.834	1.402752094	0.228945254	0.002601549	0.04582439	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2898	216.063	0.345	X216.063_0.345	1.408348959	0.064901774	5.56E-05	0.004280673	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
2928	520.248	2.245	X520.248_2.245	1.383593455	0	0.000180805	0.008982863	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3070	261.217	0.273	X261.217_0.273	2.263181181	0.015523025	1.94E-05	0.003190758	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3091	742.573	4.065	X742.573_4.065	1.001001503	0.045794761	0.00111654	0.03022019	PC O-34:3 or LPC 34:3 or PC O-34:3	Spectral Match to 1-Stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine from NIST14	0.904542	6	44.0308	2.02	Yes	[M+H] ⁺	no matches	no matches
3099	426.261	2.805	X426.261_2.805	1.526092483	0.070382531	0.000390972	0.015531664	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3212	407.255	3.426	X407.255_3.426	1.001001503	0	0.002482983	0.04582439	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3215	439.281	3.488	X439.281_3.488	1.001001503	0	0.002482983	0.04582439	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches

Table S14: Infection-perturbed metabolites in the lung at position 12. NA, not applicable.

Cluster Index	M/Z	RT min	features	Mean Decrease Accuracy	fold change (infected /uninfected)	pval	pAdj	Annotation	GNPS Library Match	Cosine Score	No of Shared Peaks	Mass Difference to Library Reference	ppm error	Analog GNPS Library Match	Adduct	Compound subclass	Compound superclass
960	144.102	0.311	X144.102_0.311	3.066327457	0.024857774	7.54E-06	0.003050827	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
980	247.202	0.268	X247.202_0.268	3.19181902	0	1.73E-06	0.00122513	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1165	193.155	0.314	X193.155_0.314	2.814326442	0.193445024	0.000175595	0.049745957	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
1516	138.053	0.299	X138.053_0.299	2.909051506	0.442597517	2.52E-05	0.008933569	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
3070	261.217	0.273	X261.217_0.273	3.579908887	0.022610127	3.17E-06	0.001793761	NA	NA	NA	NA	NA	NA	NA	NA	Amino acids, peptides, and analogues	Organic acids and derivatives
3269	158.118	0.319	X158.118_0.319	3.48692415	0.030190893	5.12E-06	0.002419474	NA	NA	NA	NA	NA	NA	NA	NA	Amines	Organic nitrogen compounds
3311	166.084	0.281	X166.084_0.281	3.366271079	0	1.73E-06	0.00122513	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives
4090	283.163	0.275	X283.163_0.275	3.498923653	0	1.73E-06	0.00122513	NA	NA	NA	NA	NA	NA	NA	NA	no matches	no matches
4091	180.1	0.278	X180.1_0.278	3.902745058	0	1.73E-06	0.00122513	NA	NA	NA	NA	NA	NA	NA	NA	Ureas	Organic acids and derivatives

Table S15: R² of metabolic perturbation in lung tissue

Position Number	R²
1	0.171834
2	0.106314
3	0.112465
4	0.175063
5	0.124246
6	0.144456
7	0.163849
8	0.190141
9	0.16932
10	0.16542
11	0.172299
12	0.053892

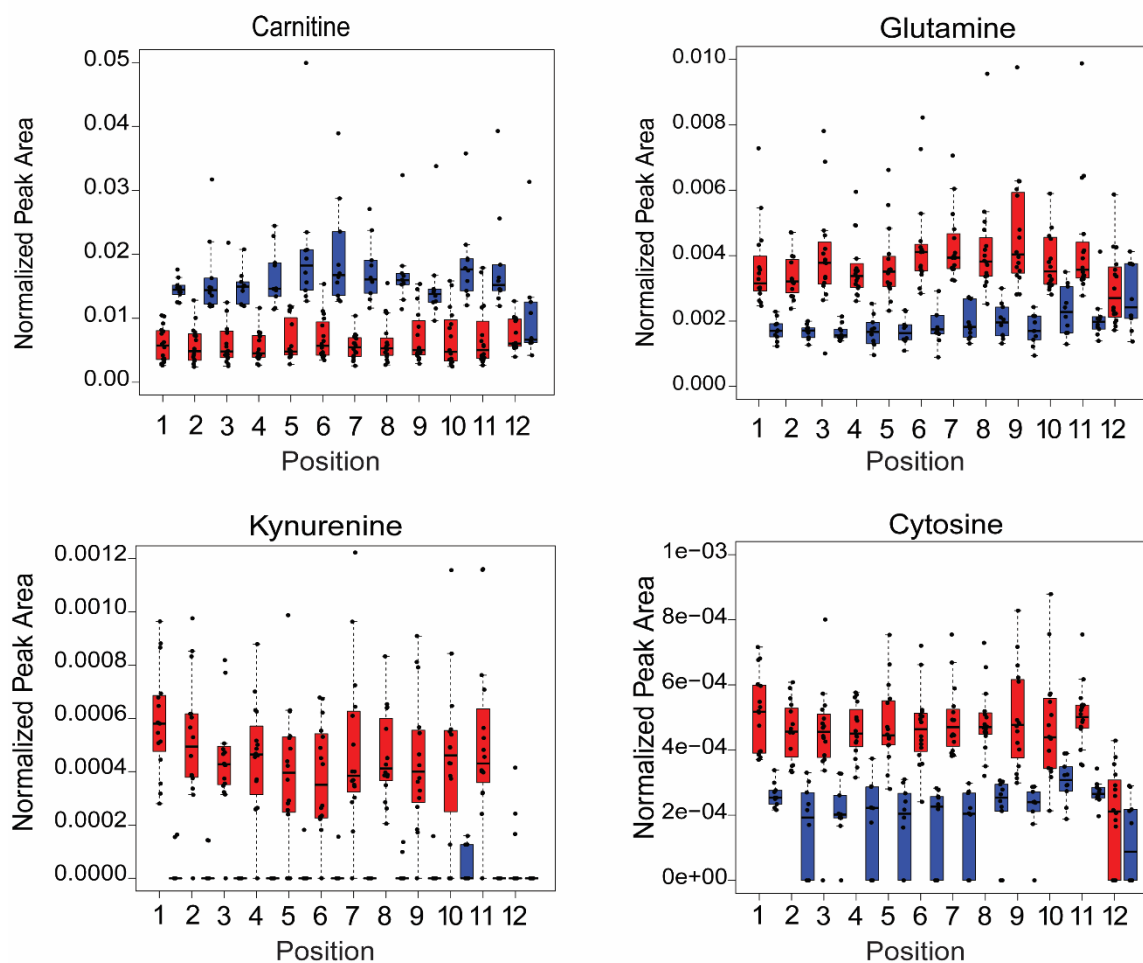


Fig S1: Boxplots of metabolites perturbed by IAV infection in lung tissue (Red - infected, Blue - Uninfected). Carnitine statistically different at all positions (p-value <0.05). Glutamine statistically different at all positions (p-value <0.05). Kynurenine statistically different at all positions (p-value <0.05). Cytosine statistically different at all positions (p-value <0.05).

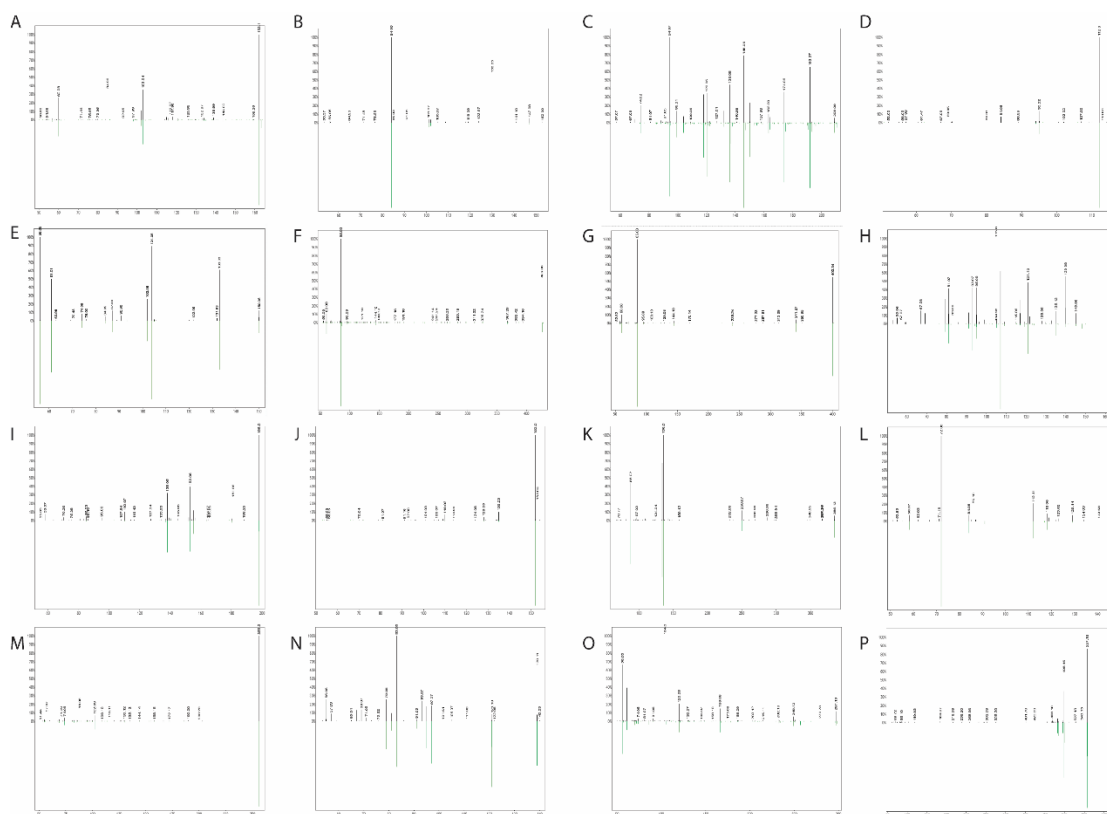


Fig S2: Representative mirror plots of metabolites perturbed by IAV infection in the lung tissue and plasma. **A:** Mirror plot of m/z 162.113, RT 0.297 min (top, black) to reference library spectrum (L-carnitine, bottom, green). **B:** Mirror plot of m/z 147.076, RT 0.32 min (top, black) to reference library spectrum (Glutamine, bottom, green). **C:** Mirror plot of m/z 209.092, RT 0.631 min (top, black) to library spectrum (Kynurenine, bottom, green). **D:** Mirror plot of m/z 112.0508, RT 0.31 min (top, black) to reference library spectrum (Cytosine, bottom, green). **E:** Mirror plot of m/z 159.058, RT 0.323 min (top, black) to reference library spectrum (Methionine, bottom, green). **F:** Mirror plot of m/z 426.357, RT 2.982 min (top, black) to reference library spectrum (Oleyl L-carnitine, bottom, green). **G:** Mirror plot of m/z 400.341, RT 2.952 min (top, black) to reference library spectrum (Palmitoylcarnitine, bottom, green). **H:** Mirror plot of m/z 166.042, RT 2.246 min (top, black) to reference library spectrum (Phenylalanine, bottom, green). **I:** Mirror plot of m/z 198.085, RT 0.309 min (top, black) to reference library spectrum (L-

citrulline, bottom, green). **J:** Mirror plot of m/z 150.977, RT 0.291 min (top, black) to reference library spectrum (Guanine, bottom, green). **K:** Mirror plot of m/z 385.212, RT 2.166 min (top, black) to reference library spectrum (S-Adenosyl-L-homocysteine, bottom, green). **L:** Mirror plot of m/z 146.165, RT 0.287 min (top, black) to reference library spectrum (Spermidine, bottom, green). **M:** Mirror plot of m/z 256.073, RT 2.563 min (top, black) to reference library spectrum (Palmitamide, bottom, green). **N:** Mirror plot of m/z 139.112, RT 2.644 min (top, black) to reference library spectrum (4-Hydroxynonenal, bottom, green). **O:** Mirror plot of m/z 297.123, RT 2.444 min (top, black) to reference library spectrum (Met-Phe, bottom, green). **P:** Mirror plot of m/z 616.358, 2.994 min (top, black) to reference library spectrum (Hemin cation, bottom, green).

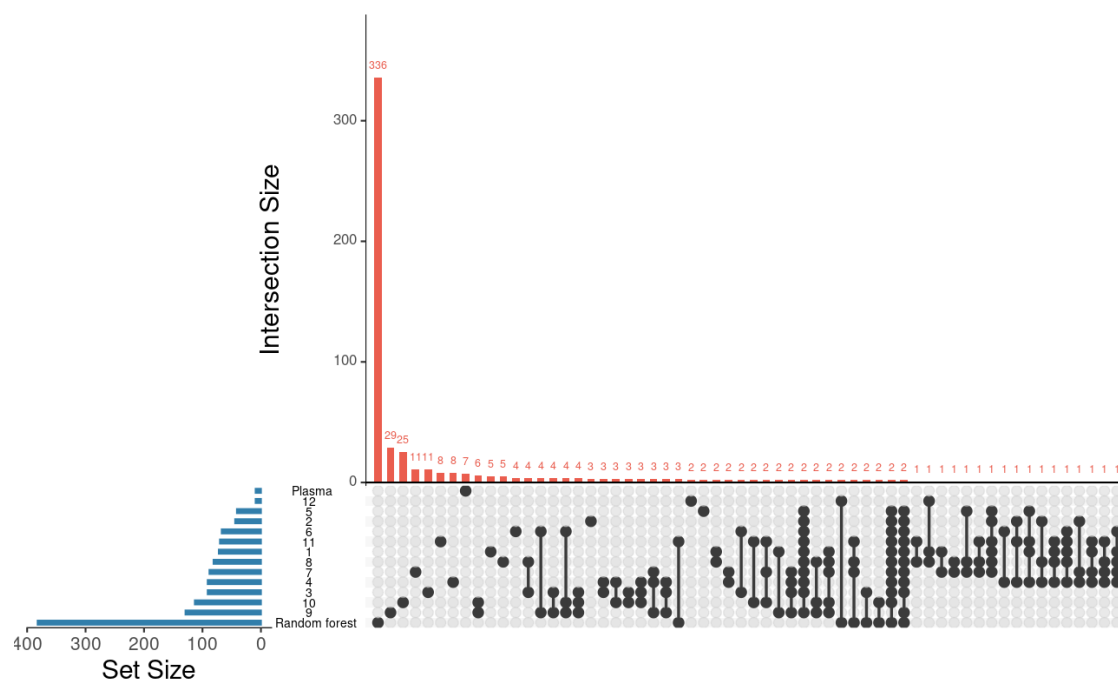


Fig S3: Limited overlap of metabolites differing in abundance between plasma and lung tissue and metabolites impacted by infection in plasma and at each lung position.

Metabolites differing between plasma and lung tissue were identified using random forest, with a cutoff of >1 (“Random forest” set). Likewise, metabolites differing between infected and uninfected samples were identified by random forest at each tissue site and in plasma individually, as in Fig. 2A (sets 1-12, representing lung segments 1-12, and plasma).