MEASURE OF X-INACTIVATION ESCAPE IN CIRCULATING CD11B MYELOID CELLS WITH AGE

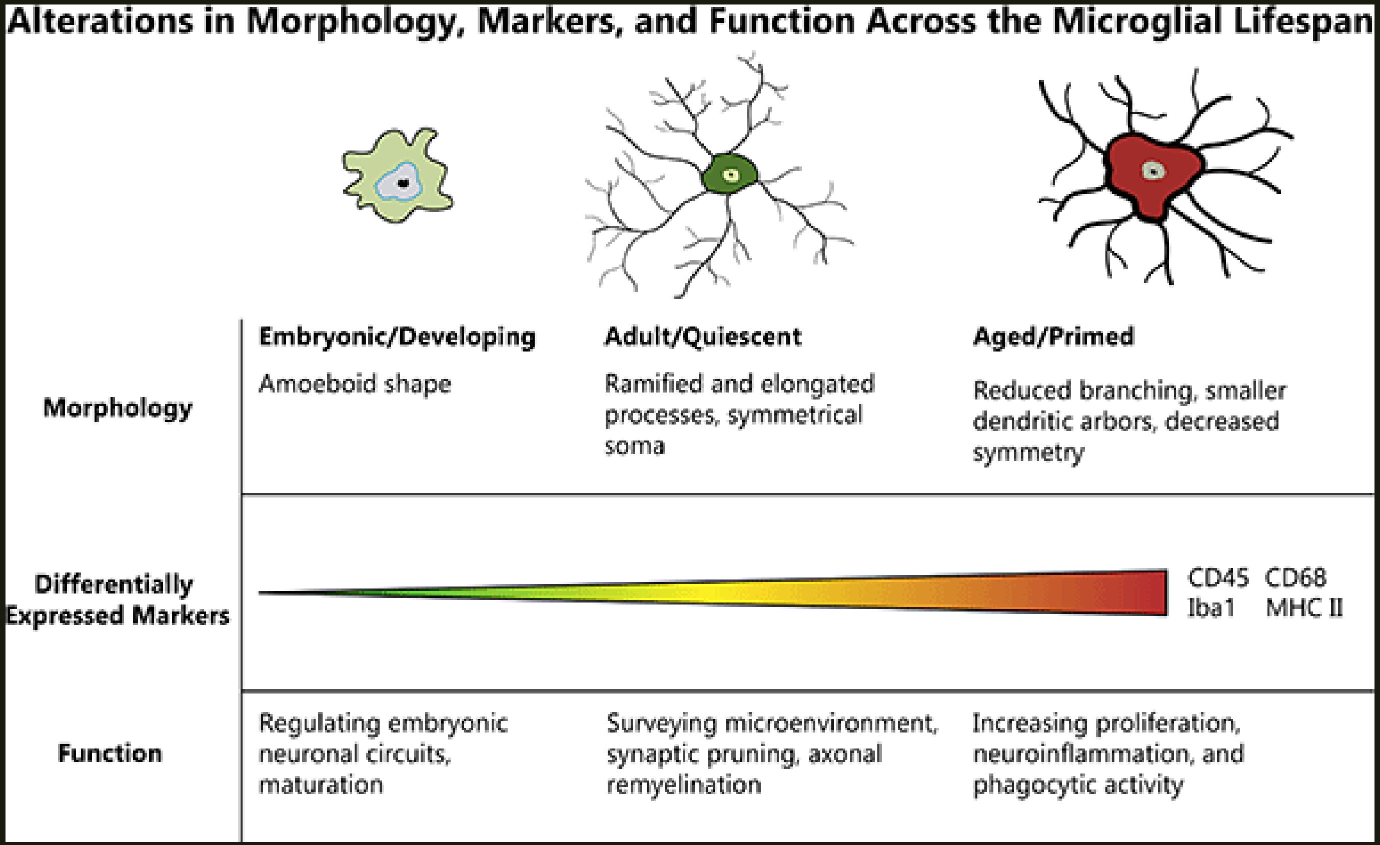
Jihra James

Gene and Human Disease

Key Words

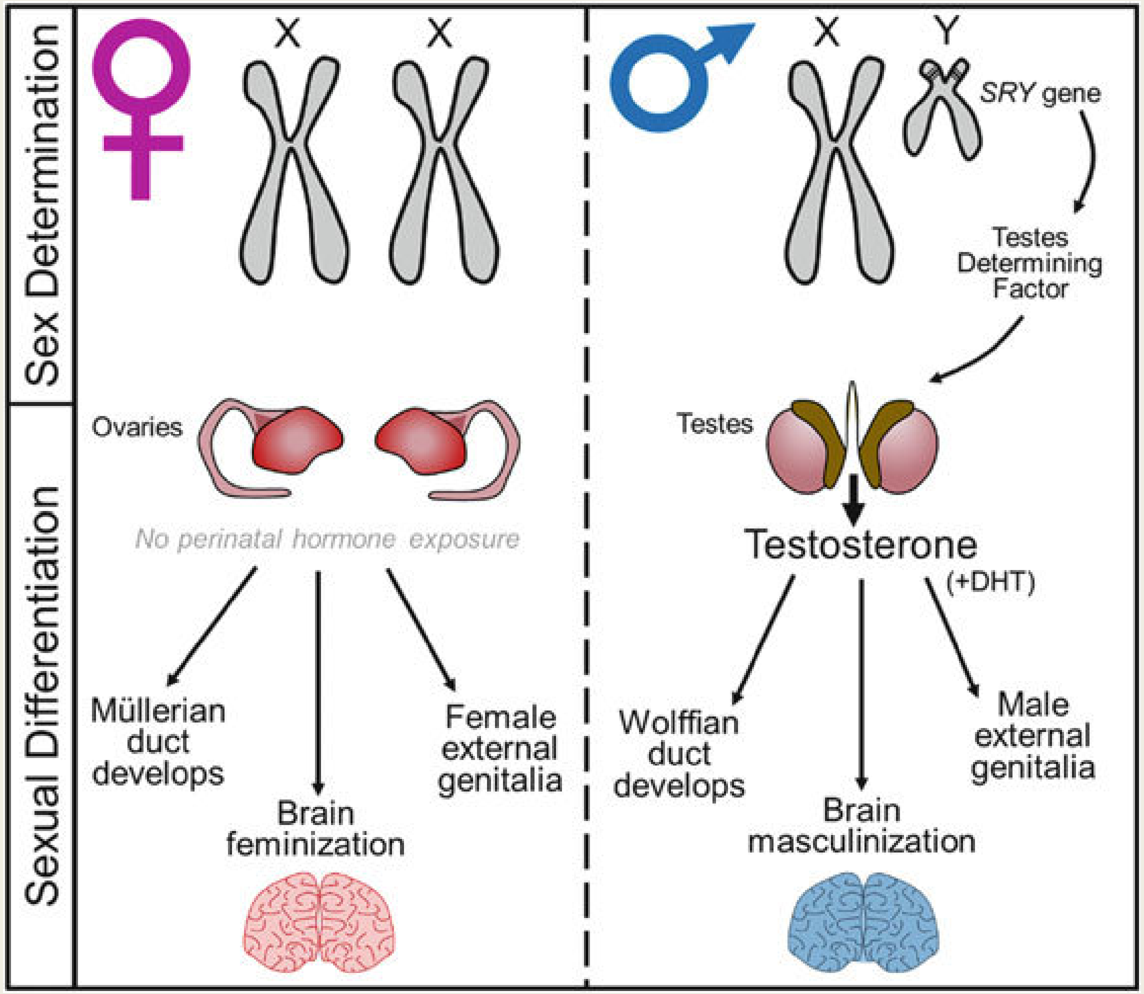
- Neuroinflammation with aging
- Sex differences
- Sex differences in microglia
- X-inactivation
- DNA Methylation

Neuroinflammation with aging

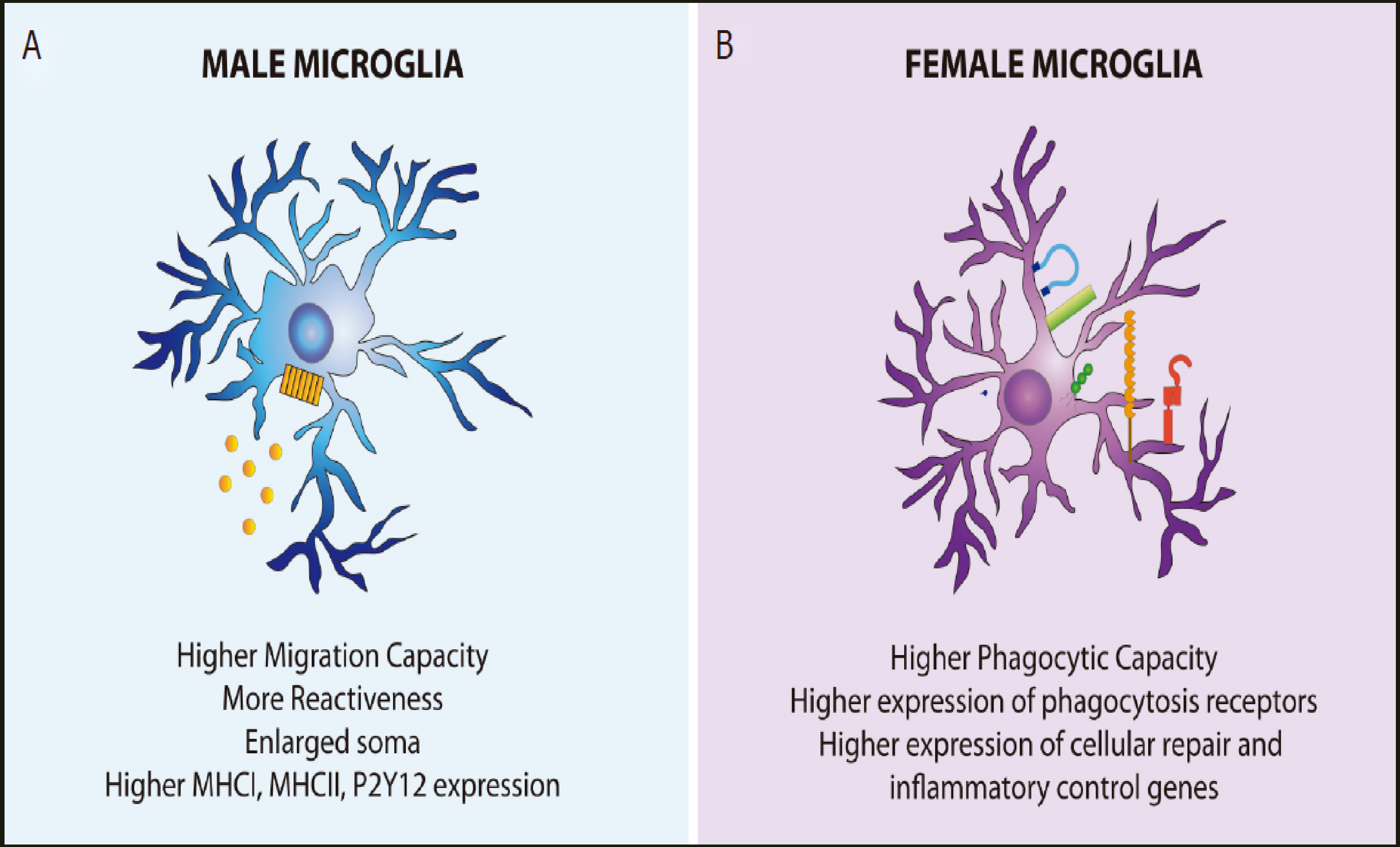


- Sterile inflammation
  - *Not in response to a "brain infection"*
- Microglia activate with aging
- Mice, rats, monkeys, humans
- Related to the development of Alzheimer's Disease
- Happens more in females than males

SEX DIFFERENCES



Turano et al, 2018; *Current Topics in Behavioral Neurosciences*



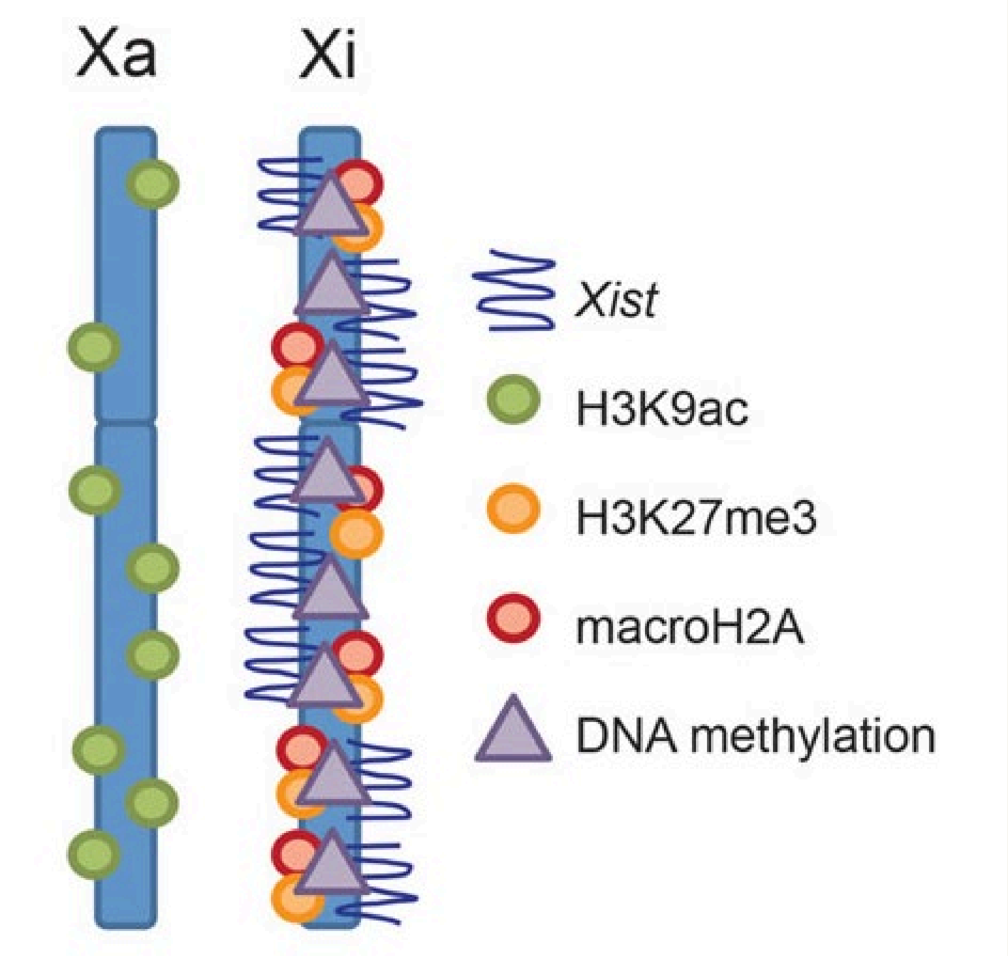
Sex Differences in Microglia

- What can cause the differences?
  - **Estrogen**
  - **Testosterone**
  - **Sex Chromosomes**

Yanguas-Casás, 2019; *Neuroimmunol Neuroinflammation*

X-Inactivation

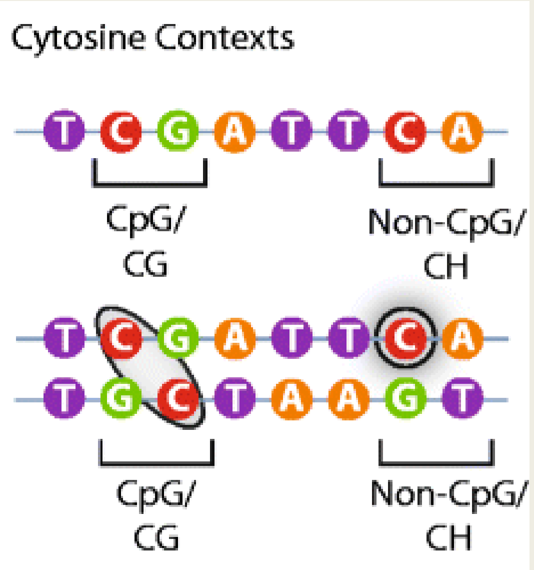
- Females have two X chromosomes
- Epigenetic mechanisms turn off one X chromosome
    - *Both active = problems*
- Disorders where there is incorrect x-inactivation
    - *Rett syndrome*
- X-inactivation has been proposed to fail with aging

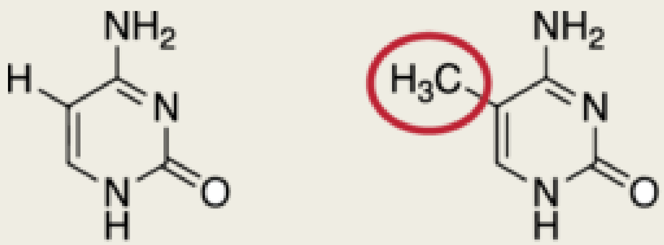


Geens et al., 2017; *Hum Reprod Update*

DNA METHYLATION

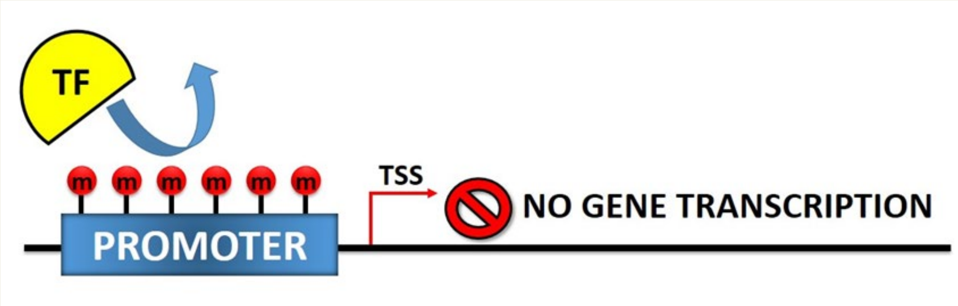
- Methyl groups added to the DNA molecule
- Added to cytosine
- Relation to gene expression

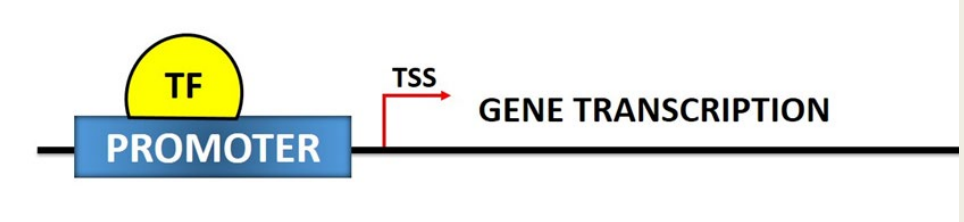




Cytosine **methylated Cytosine**

Masser et al., 2018; *GeroScience*





Background

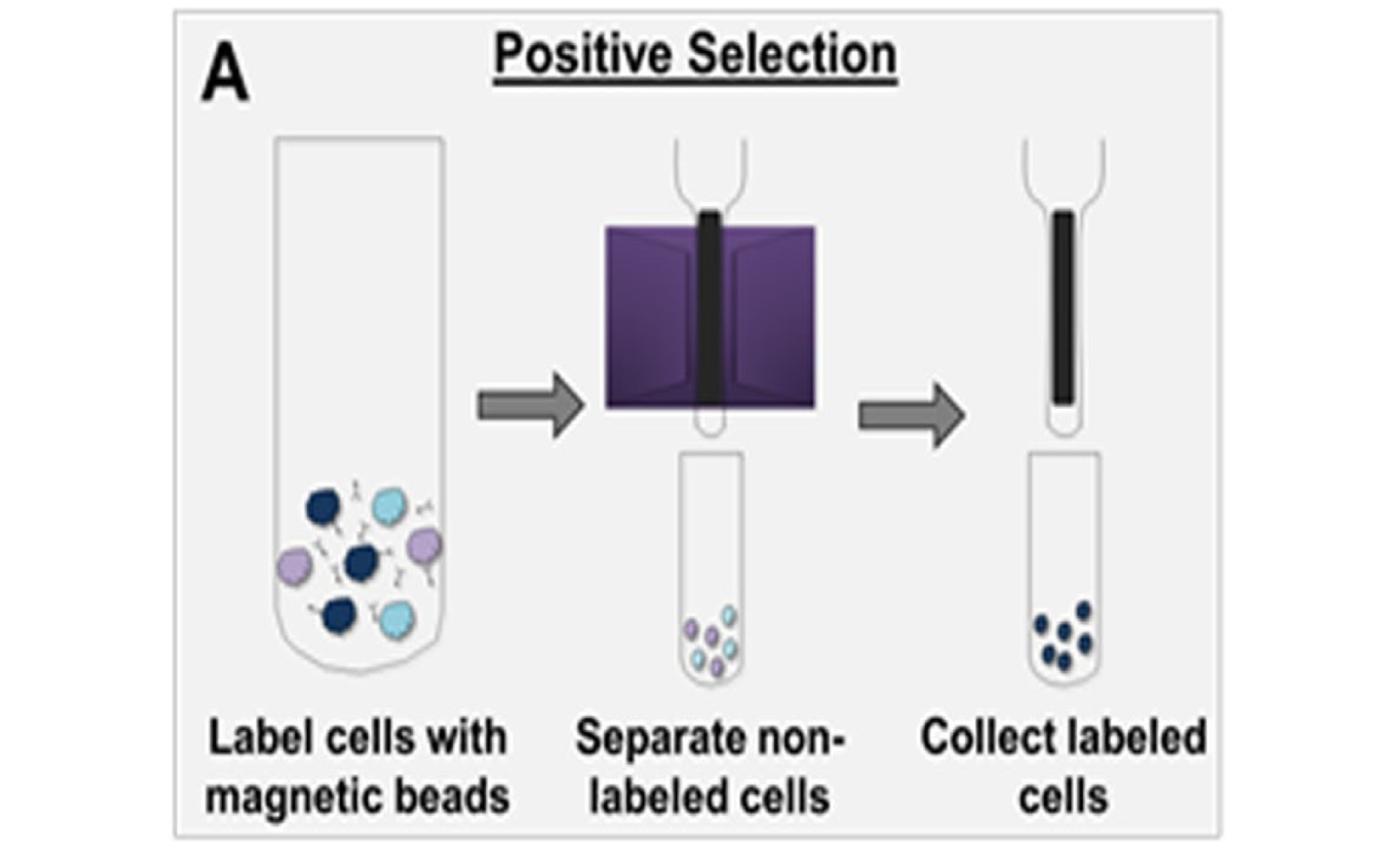
- Sex- chromosomally driven differences in gene expression
  - Age related disease
    - Neuroinflammation
- X-inactivation silences one of two X chromosomes in females
- Blood and brains collected from C57Bl6 mice
  - *Male*
  - *Female*
  - *Young (6 mo)*
  - *Old (25 mo)*
- First analyzed the blood

Hypothesis

X-chromosome DNA methylation will decrease with age in females in microglia and potentially in circulating macrophages as well, indicating escape from X-inactivation

MACS

Magnetic-Activated Cell Sorting

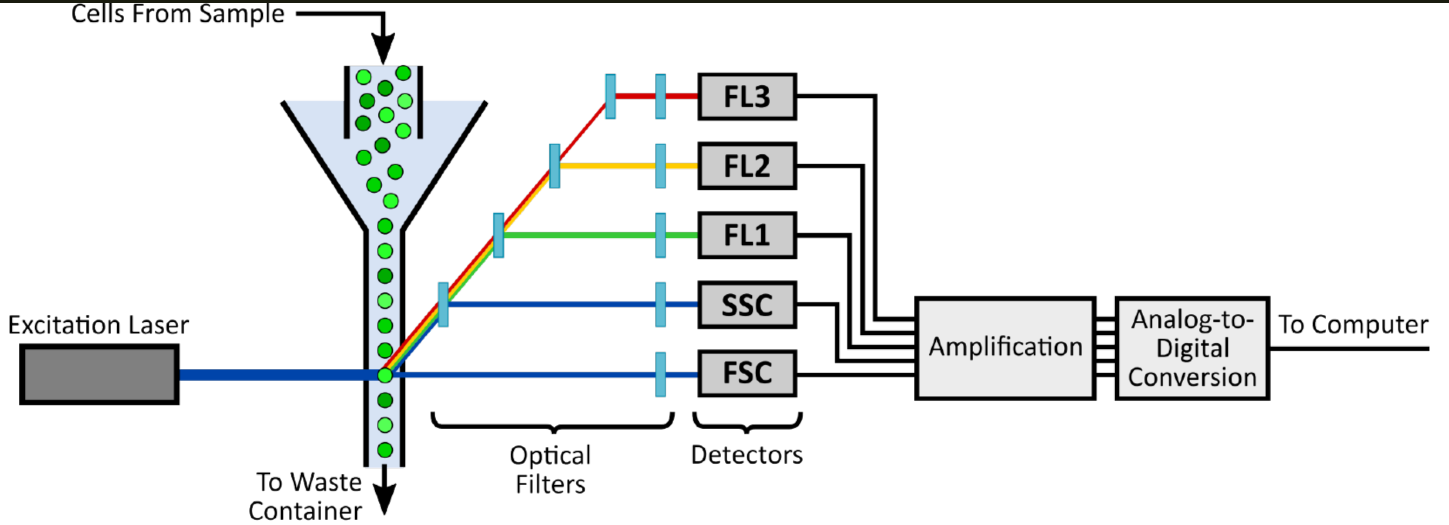


Cd11b+

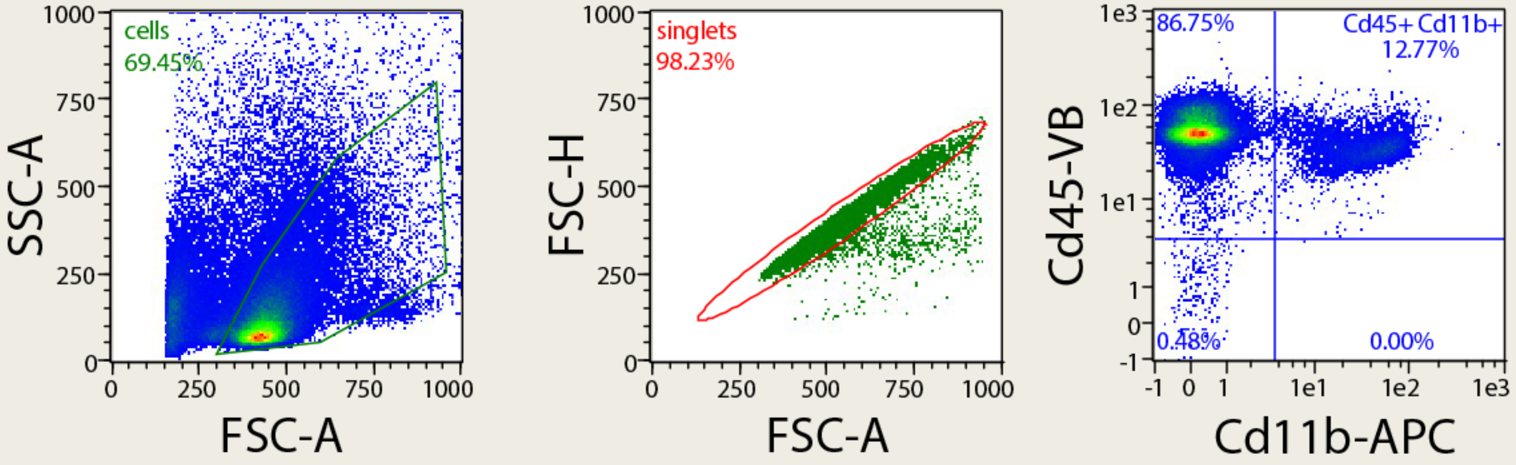
Holt & Olsen, 2016: *Novel Applications of Magnetic Cell Sorting to Analyze Cell-Type Specific Gene and Protein Expression in the Central Nervous System*

FLOW CYTOMETRY

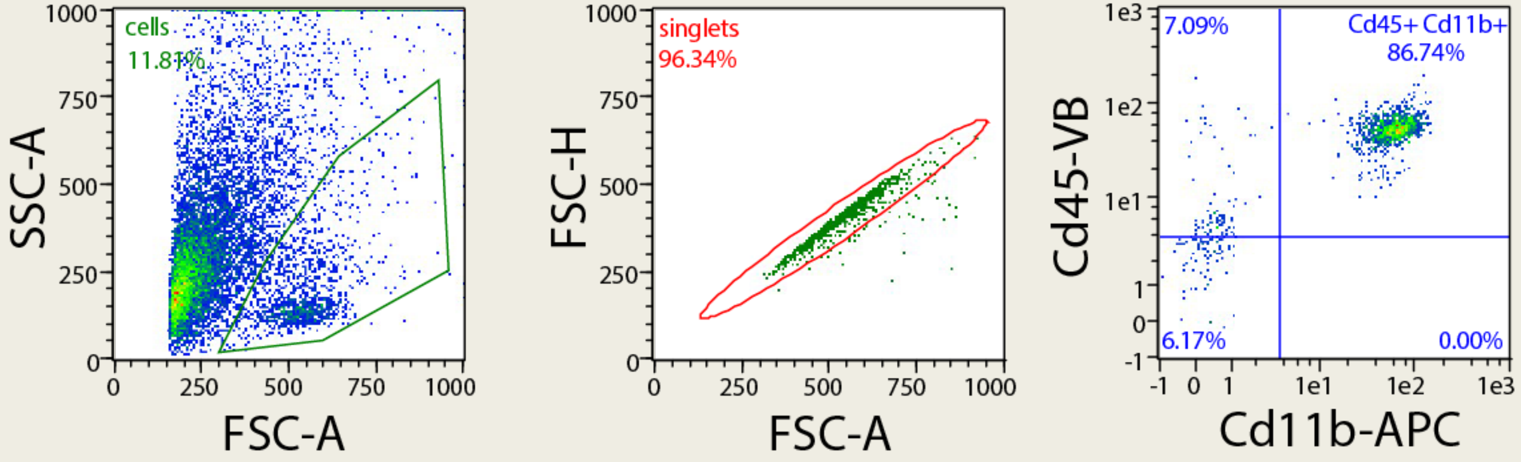
Tigh et al., 2012: Flow Cytometry

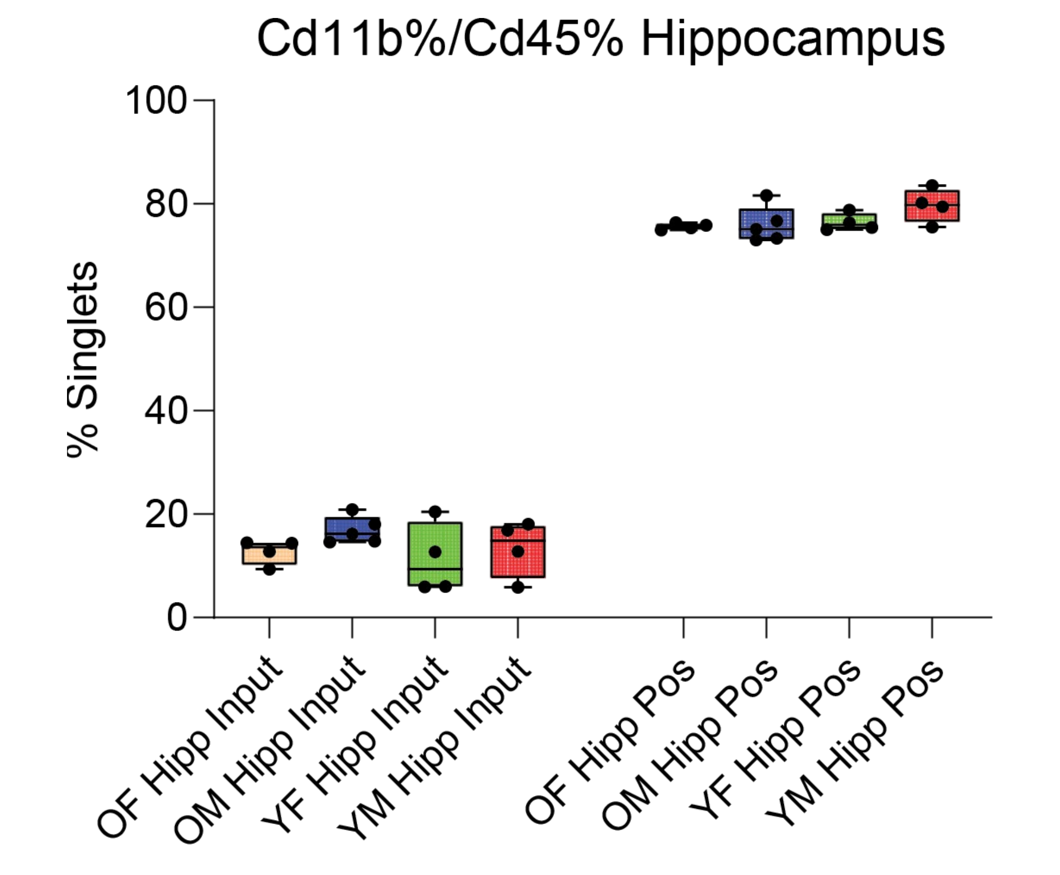


Blood Input

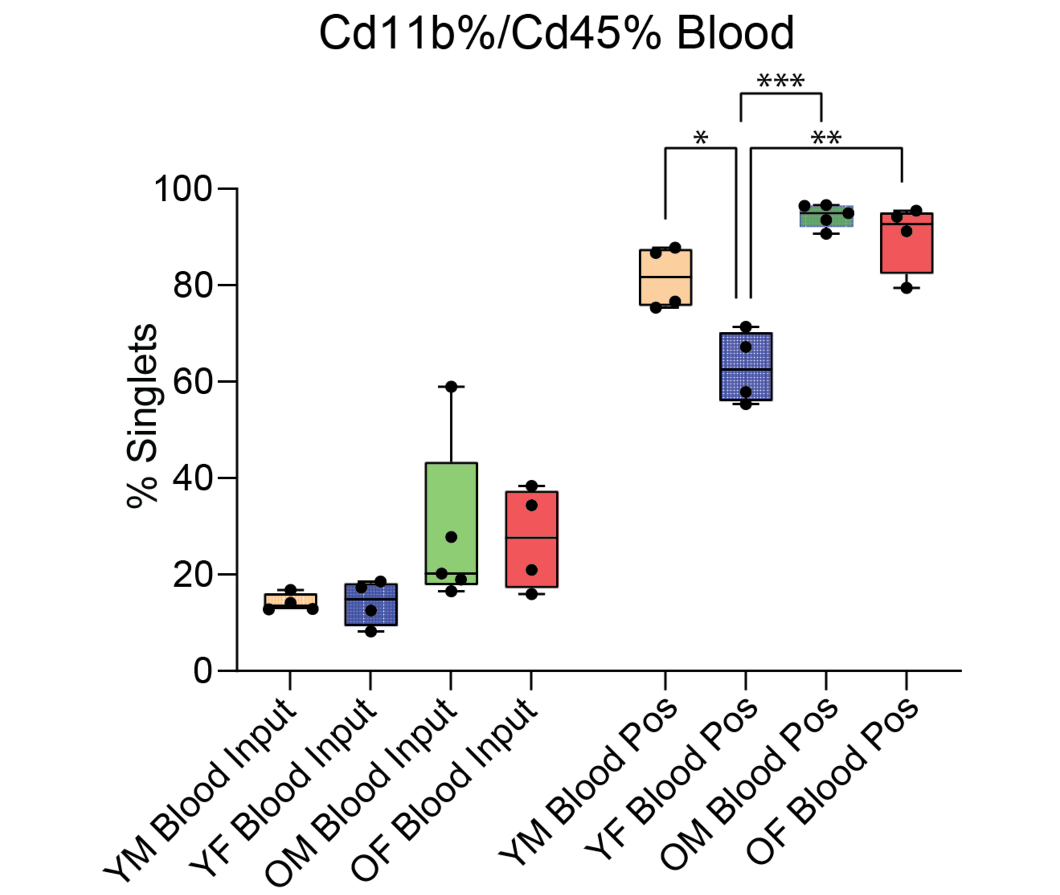


Blood Cd11b Positive





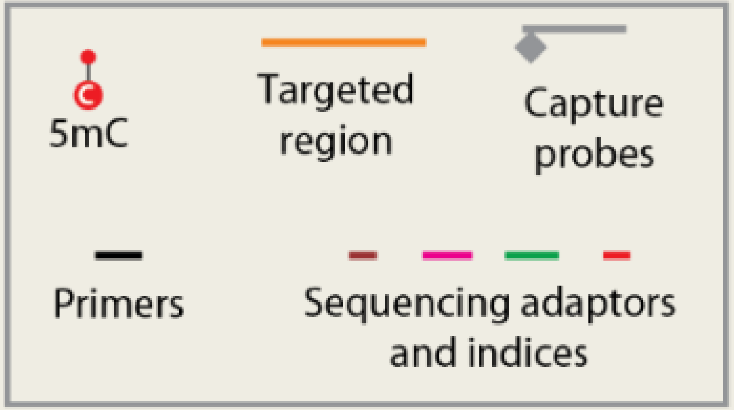
Cd11b%/Cd45% Hippocampus
% Singlets
100
80
60
40
20
0
OF Hipp Input
OM Hipp Input
YF Hipp Input
YM Hipp Input
OF Hipp Pos
OM Hipp Pos
YF Hipp Pos
YM Hipp Pos

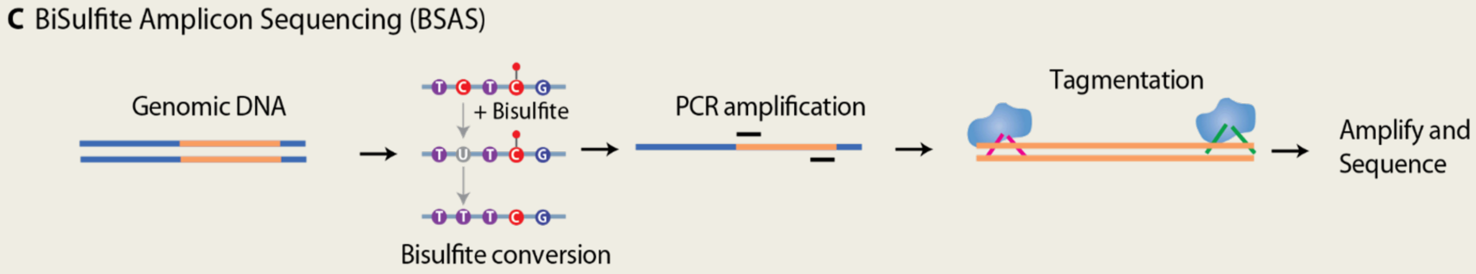


Cd11b%/Cd45% Blood
***
*
**
% Singlets
100
80
60
40
20
0
YM Blood Input
YF Blood Input
OM Blood Input
OF Blood Input
YM Blood Pos
YF Blood Pos
OM Blood Pos
OF Blood Pos

BSAS

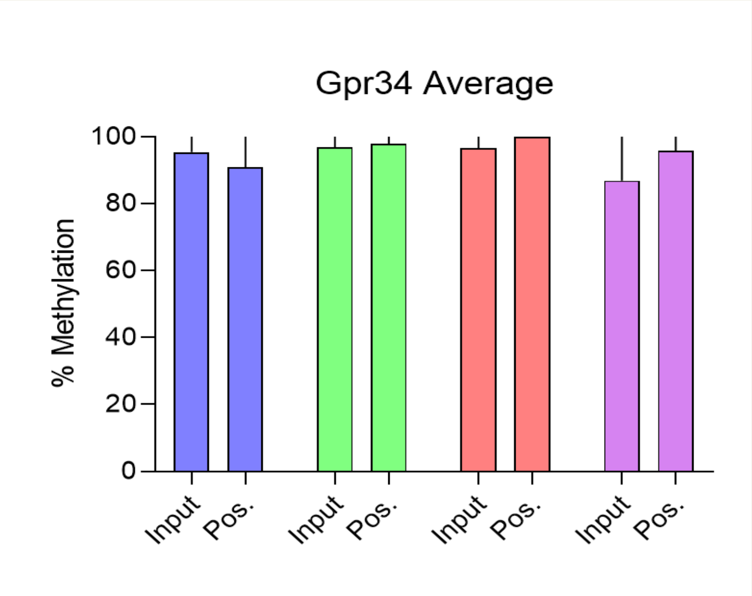
Bisulfite Amplicon Sequencing

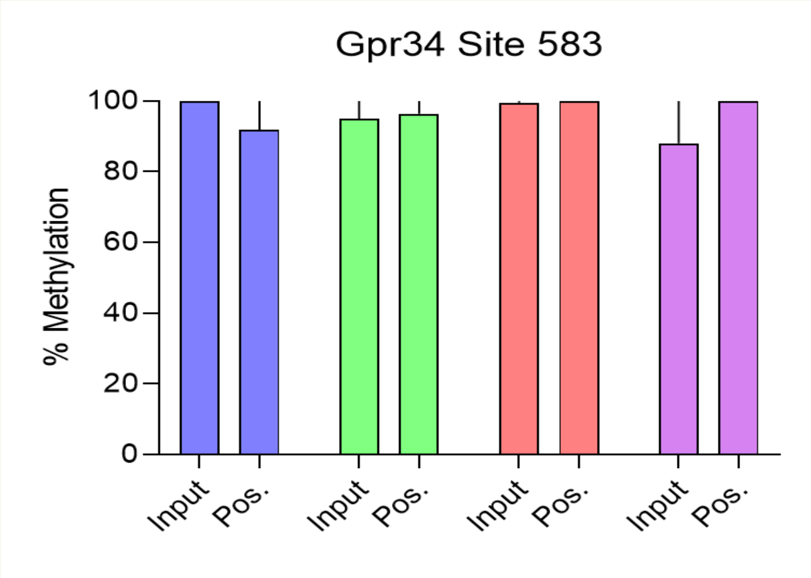




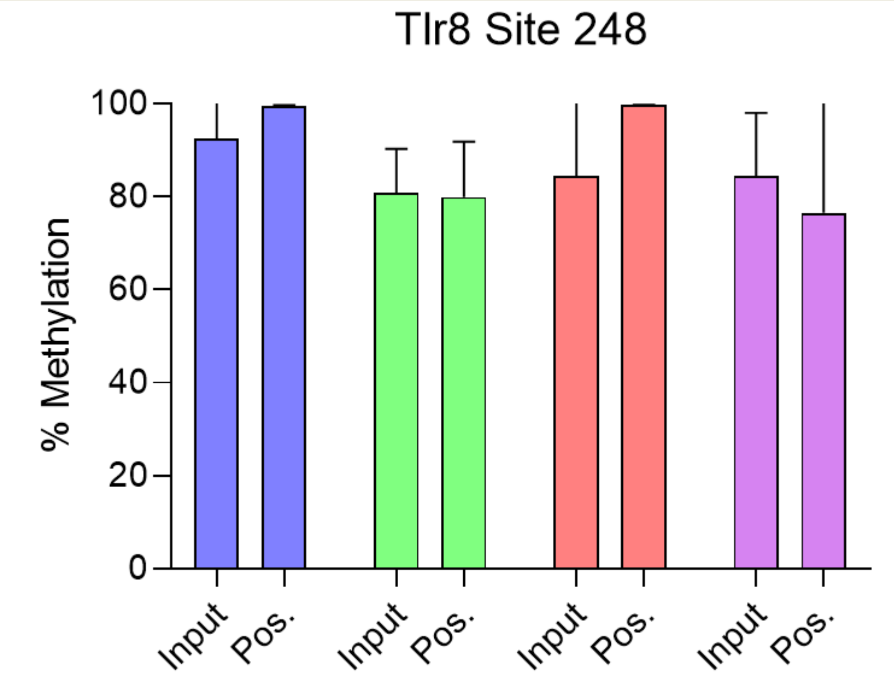
Masser et al., 2013: *Focused, high accuracy 5-methylcytosine quantitation with base resolution by benchtop next-generation sequencing*


Old Male
Old Female
Young Male
Young Female

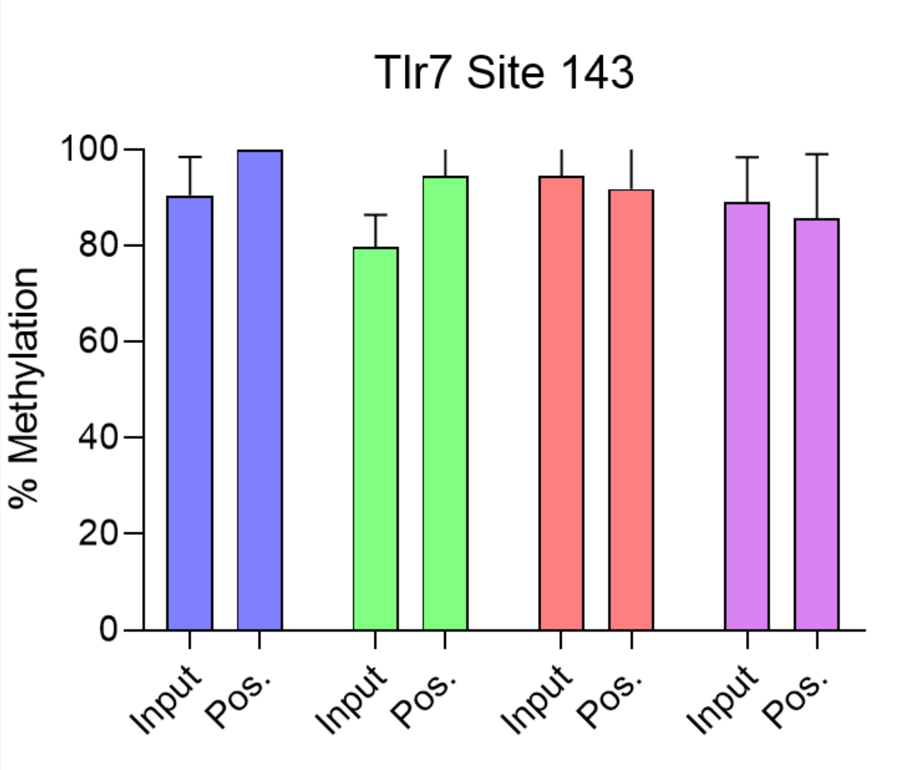

Gpr34 Average
% Methylation
0
20
40
60
80
100
Input
Pos.
Input
Pos.
Input
Pos.
Input
Pos.


Gpr34 Site 583
% Methylation
0
20
40
60
80
100
Input
Pos.
Input
Pos.
Input
Pos.
Input
Pos.


Gpr34 Site 757
% Methylation
0
20
40
60
80
100
Input
Pos.
Input
Pos.
Input
Pos.
Input
Pos.


Tlr8 Site 248
% Methylation
100
80
60
40
20
0
Input
Pos.
Input
Pos.
Input
Pos.
Input
Pos.


Old Male
Old Female
Young Male
Young Female


Tlr7 Site 143
% Methylation
100
80
60
40
20
0
Input
Pos.
Input
Pos.
Input
Pos.
Input
Pos.

Conclusion

- BSAS did not reveal any significant difference
  - *By sex or age*
  - *Do not escape X-inactivation ?*
- Future studies will examine microglia
- Compare/contrast short-lived circulating monocytes and long-lived brain resident microglia
  - *Similar functions*
  - *Different areas of the body*
  - *Surface markers (Cd11b, Cd45, Cx3cr1)*

Acknowledgments

- Gene and Human Disease lab
  - *Dr. Freeman*
  - *Sarah Ocañas*
  - *Kevin Pham*
- OMRF
  - *Courtney Greenwood*
  - *Autumn Huffman*
  - *Dr. Lewis*
- Langston University
  - *Dr. Quinn*
  - *Dr. Peterson*
  - *Dr. Coleman*