



# TAGGING ENDOGENOUS MITOCHONDRIAL PROTEINS USING CRISPR/CAS9 TECHNOLOGY

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Program*

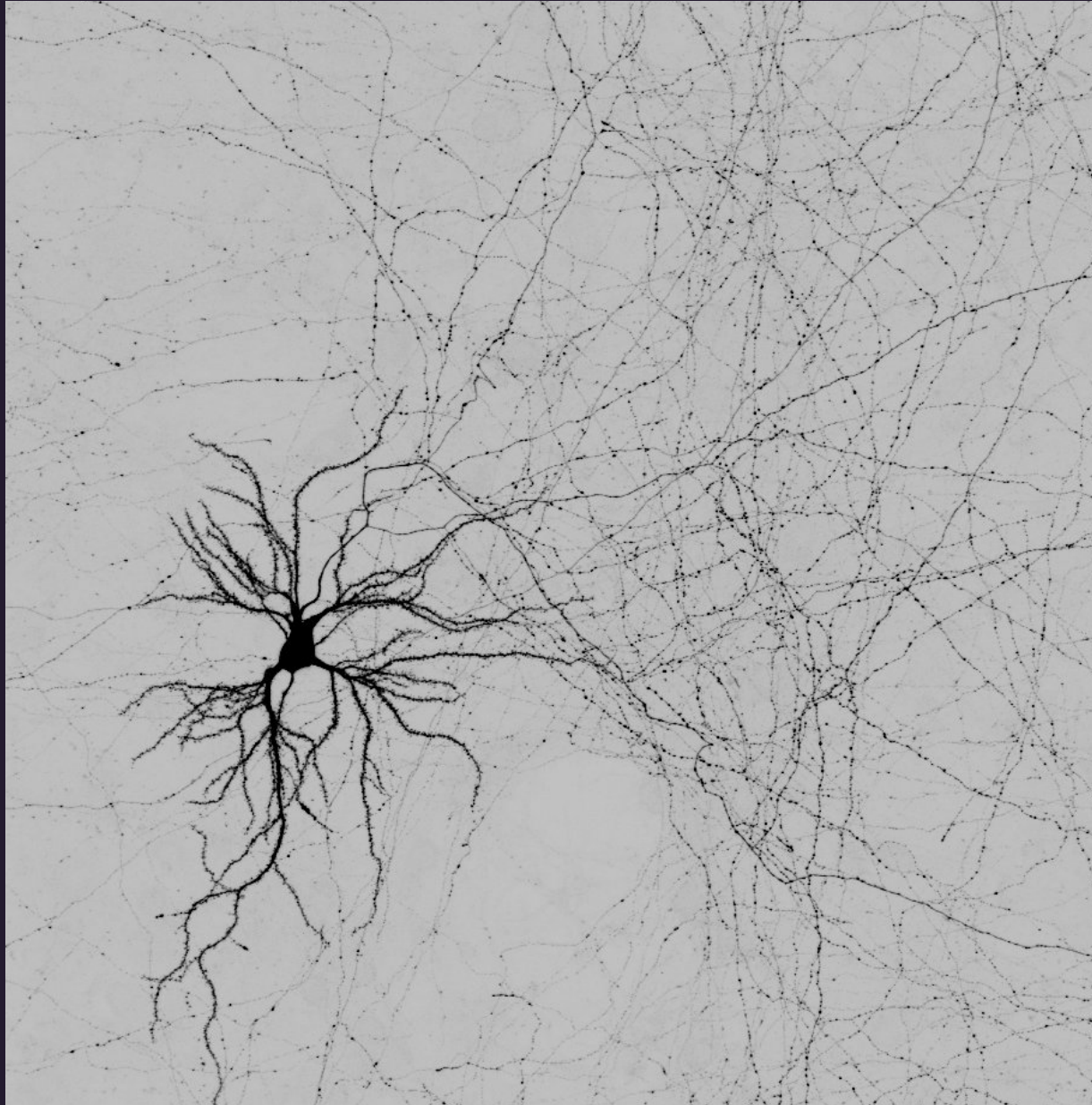
*Lewis Lab*

*Ageing and Metabolism*

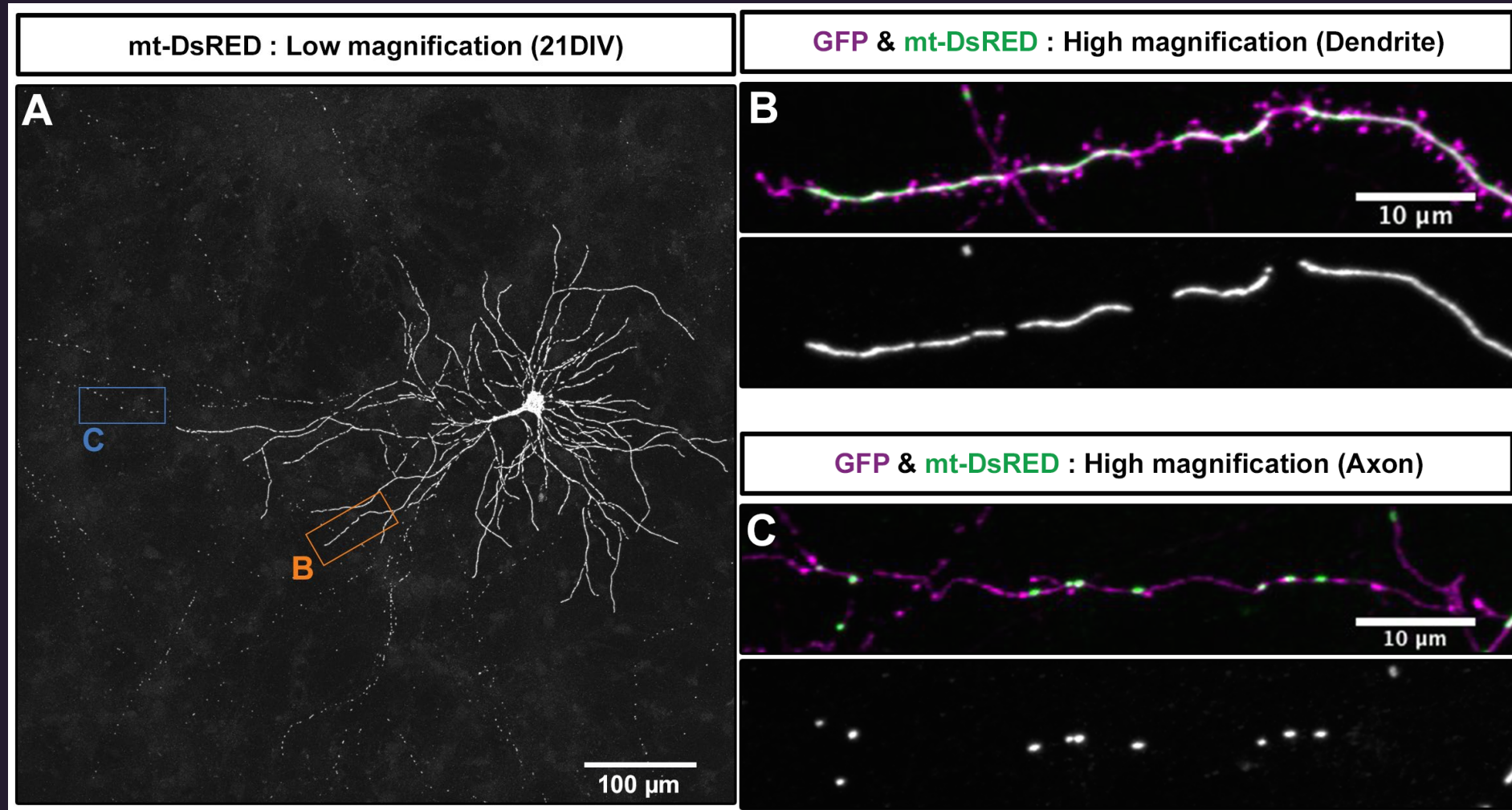
## WHY ARE MITOCHONDRIA IMPORTANT?

- **Mitochondria, known as the powerhouse of the cell, are believed to be an element to understanding the metabolism of the brain.**
- **The targeting and spatial organization of protein complexes underlie every aspect of neuronal functioning, which is why tagging mitochondrial proteins is important to understanding the brain.**

# NEURONAL MITOCHONDRIA

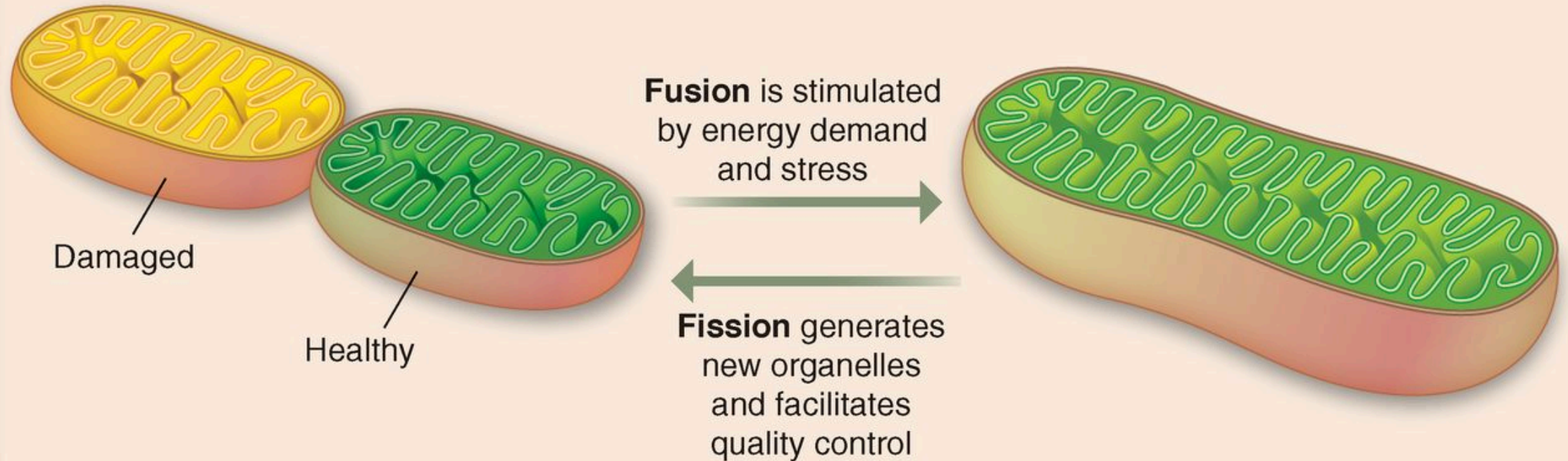


# DIFFERENTIATION OF MITOCHONDRIA



# MITOCHONDRIAL FISSION AND FUSION

## Complementation of mitochondrial function by fusion



# HYPOTHESIS

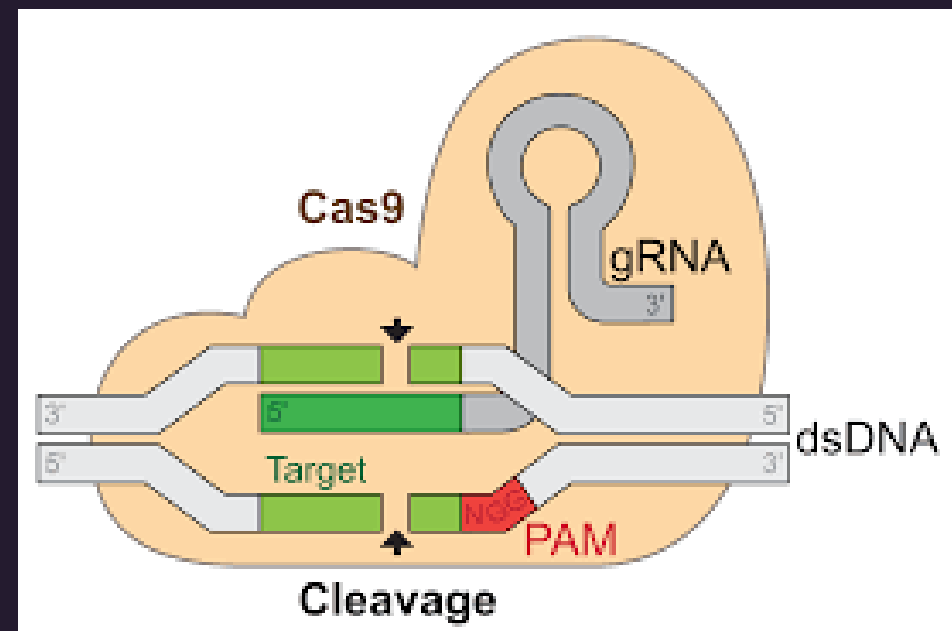
- **Mitochondrial proteins that regulate fission are either differently localized within the axon or dendrite or/and the abundance is different.**

## UNDERSTANDING

- **The whole premise of testing out this experiment is to see whether we can efficiently tag endogenous mitochondrial protein to be able to see its morphology and to overall help our understanding into what mitochondria really does and why.**

# WHAT IS CRISPR/CAS9 TECHNOLOGY?

- CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) /Cas9 technology is genome editing technology that can harness to modify, correct or delete precise regions of DNA.



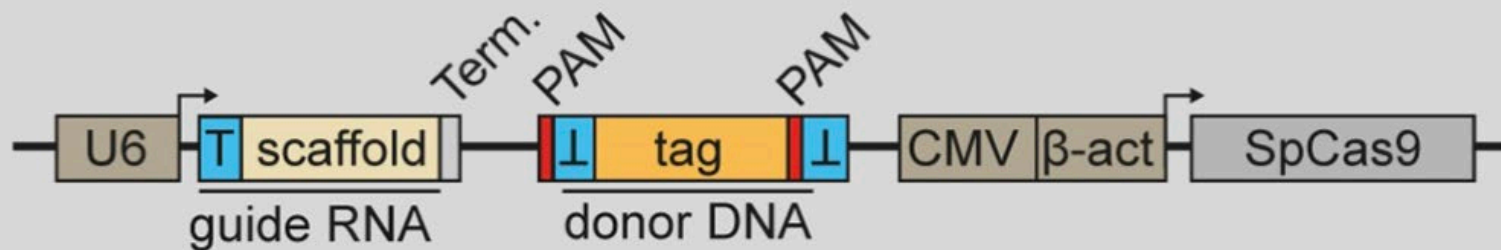


# ORANGE

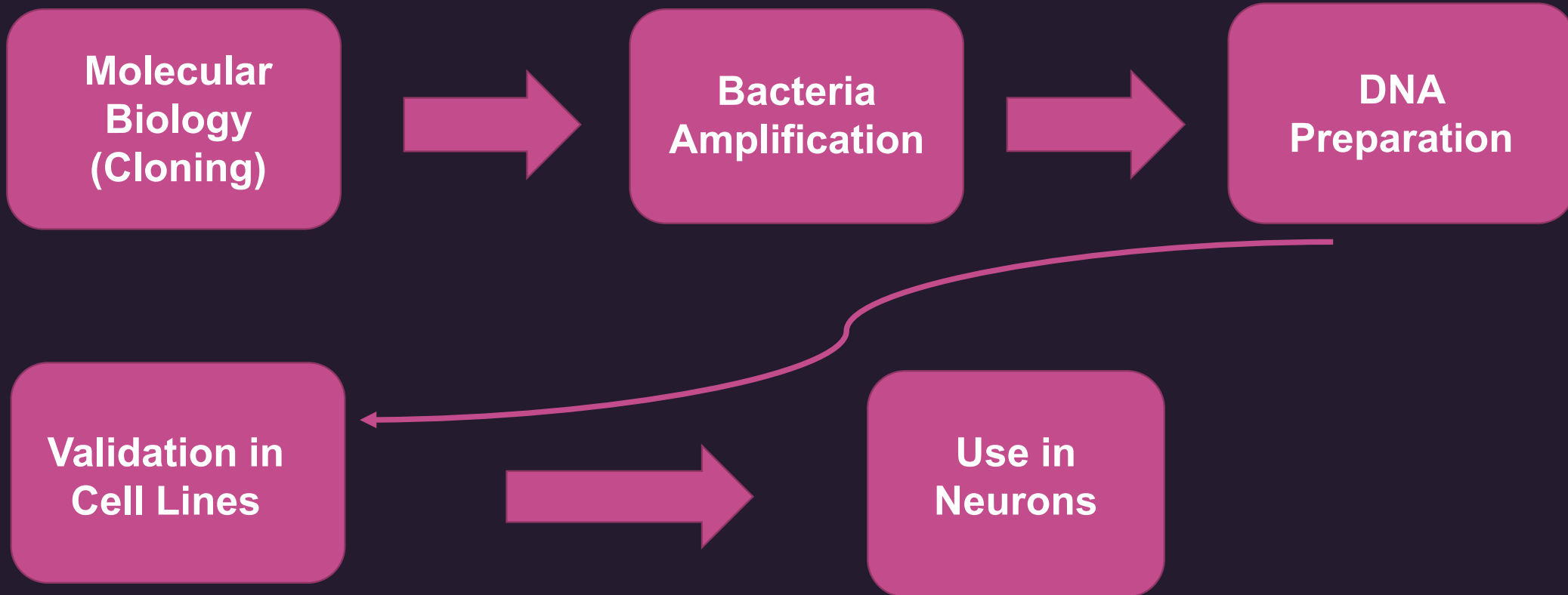
METHODS AND RESOURCES

## ORANGE: A CRISPR/Cas9-based genome editing toolbox for epitope tagging of endogenous proteins in neurons

Jelmer Willems<sup>1</sup> <sup>✉</sup>, Arthur P. H. de Jong<sup>1</sup> <sup>✉</sup>, Nicky Scheefhals<sup>1</sup> , Eline Mertens<sup>1</sup> , Lisa A. E. Catsburg<sup>1</sup> , Rogier B. Poorthuis<sup>2</sup> , Fred de Winter<sup>3</sup> , Joost Verhaagen<sup>3</sup> , Frank J. Meye<sup>2</sup> , Harold D. MacGillavry<sup>1\*</sup> 



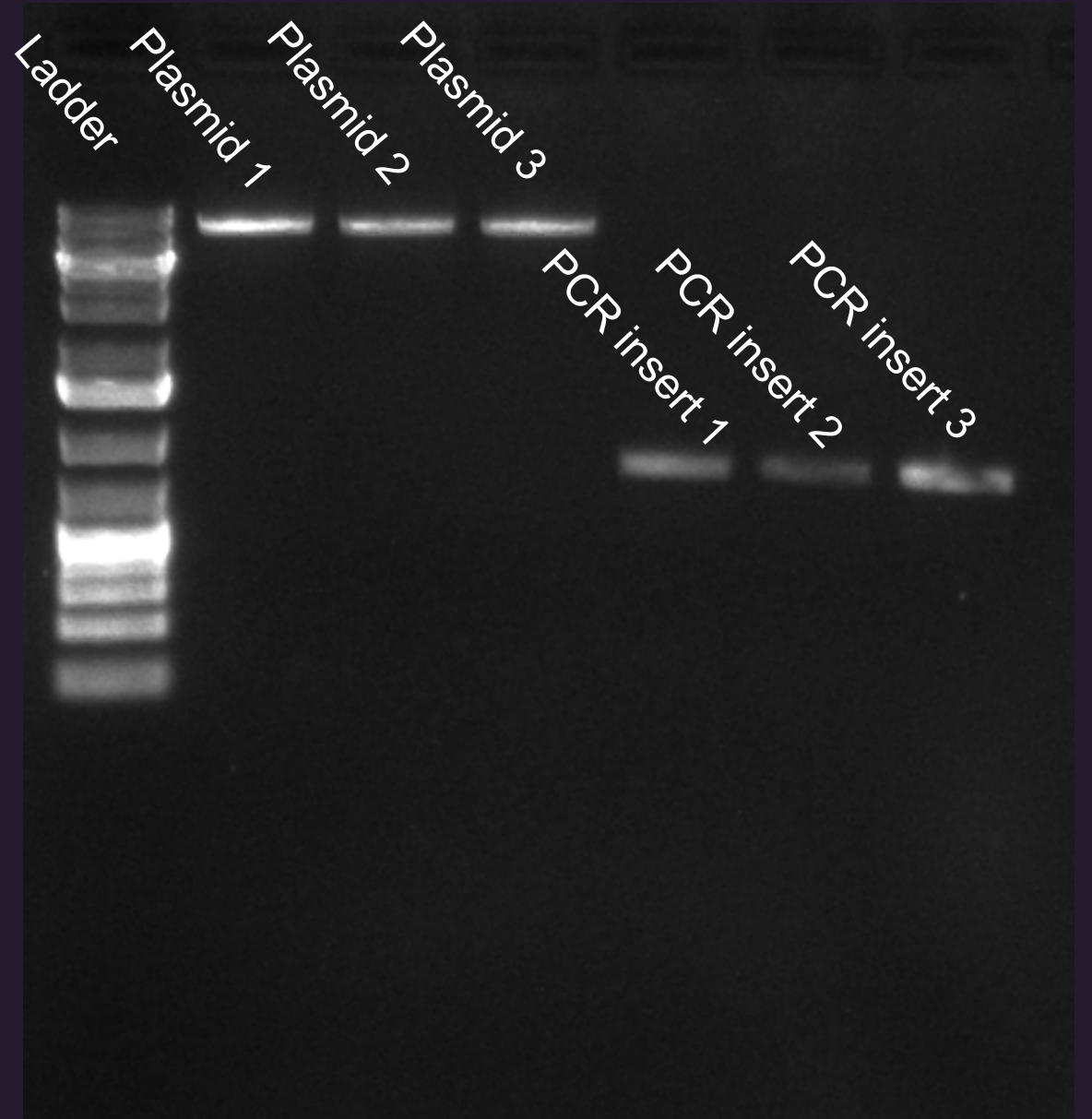
# Overview of the Editing/Tagging Process of MIEF 1



# MOLECULAR BIOLOGY

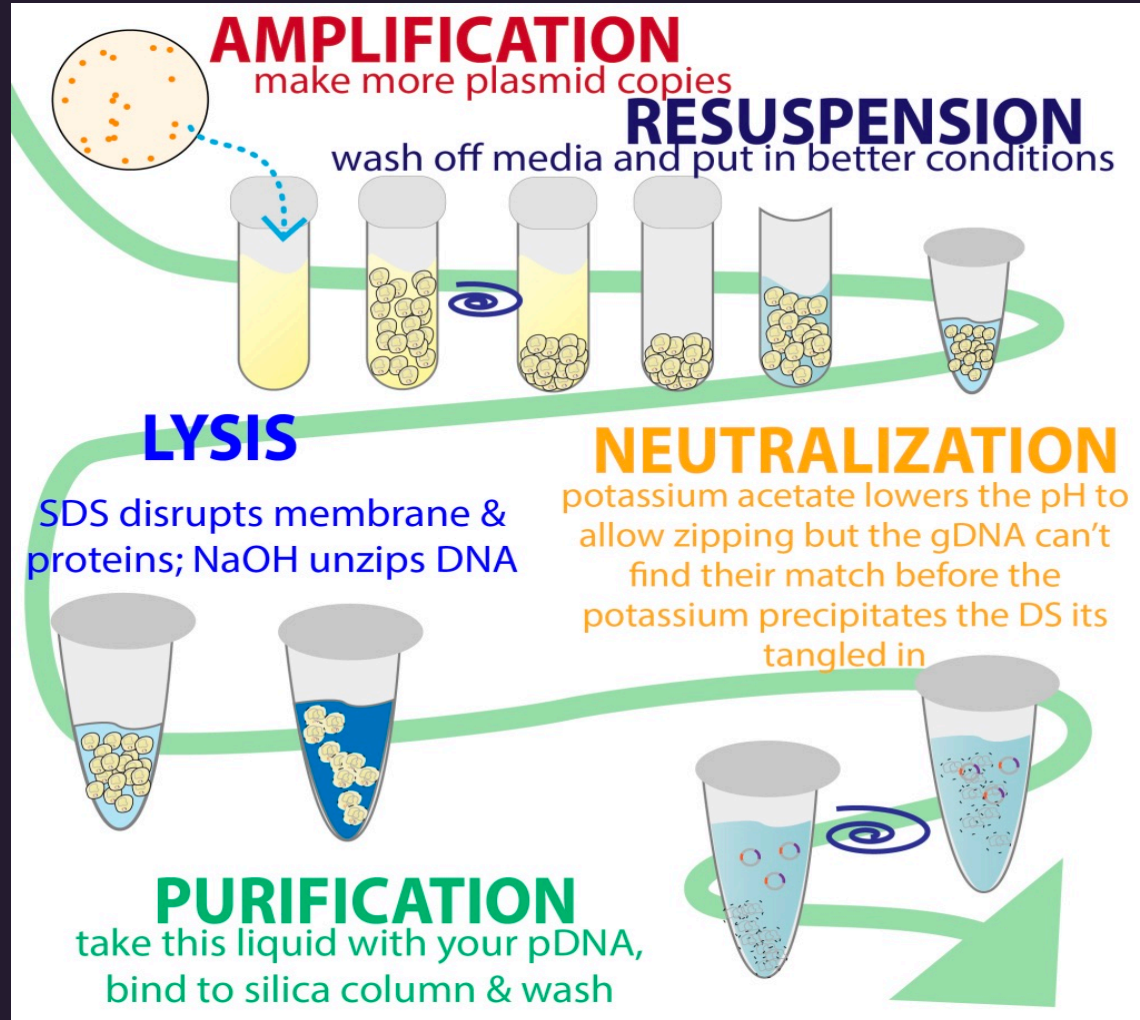
- **Constructed gRNA and donor DNA**
- **Inserted gRNA and donor DNA into the plasmid**
- **Sequenced to confirm the construct was correct**

# CONFIRMATION OF DNA

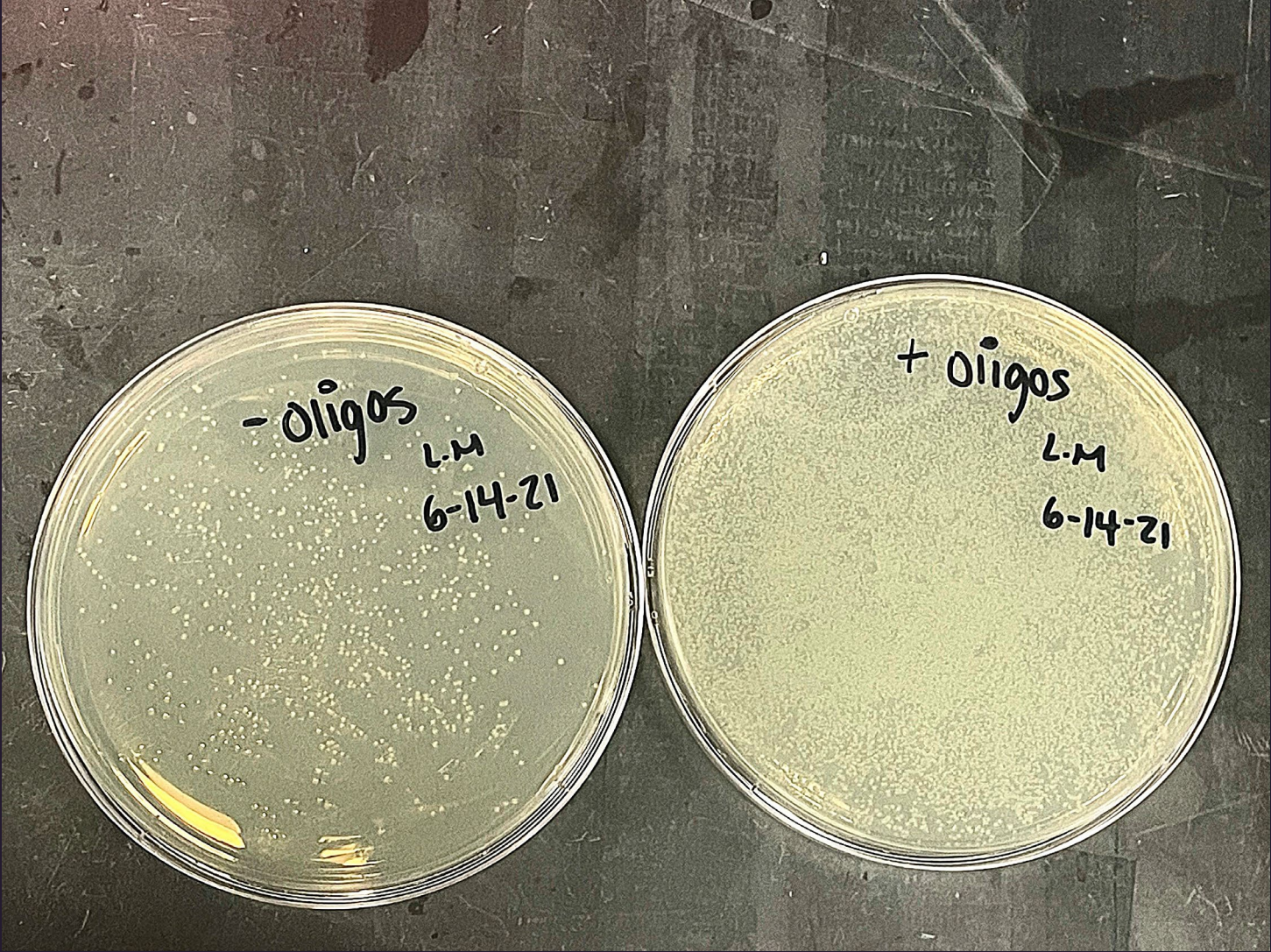


# BACTERIA AMPLIFICATION/DNA PREPARATION

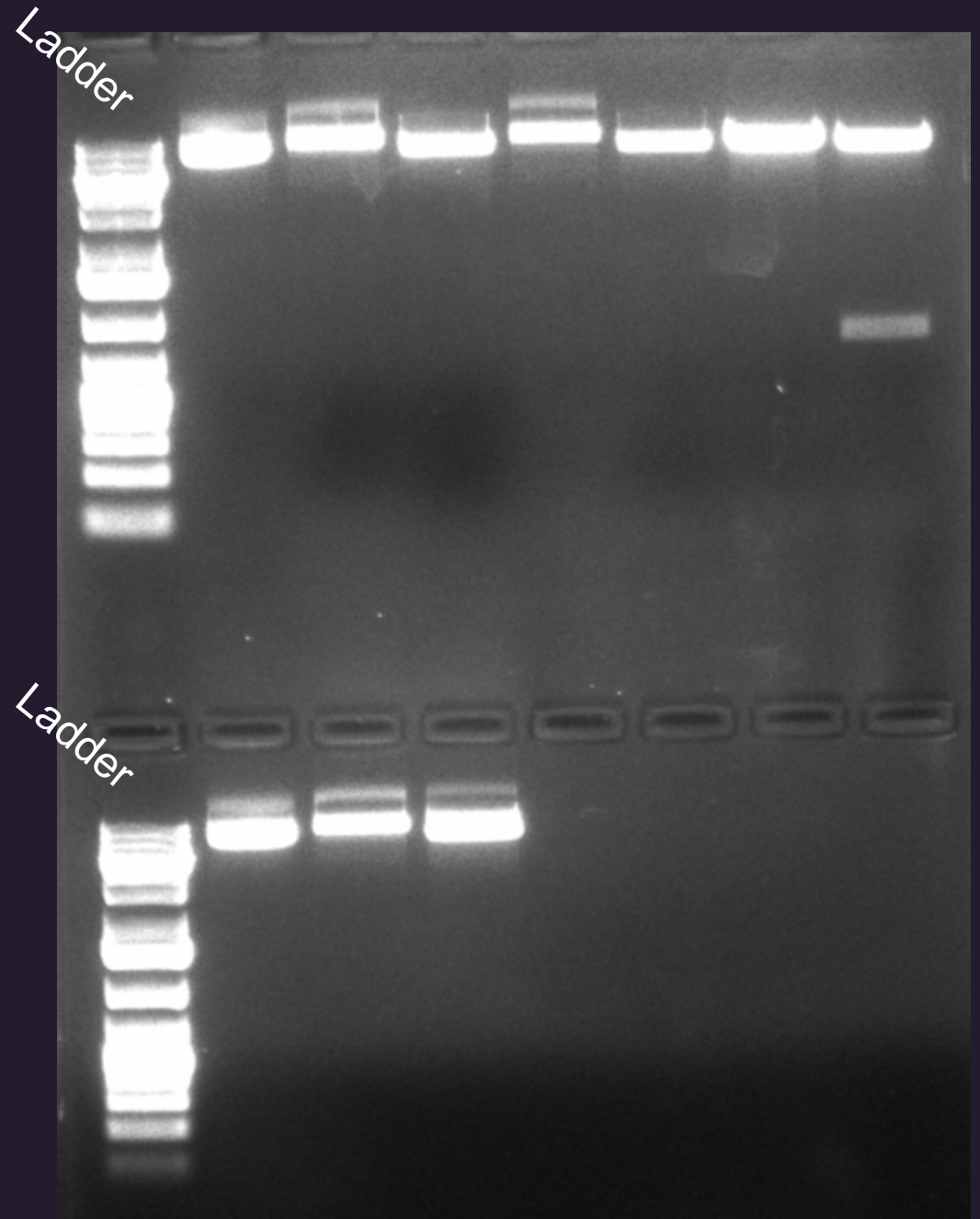
- Ligation & Transformation
- Mini Preparations



# TRANSFORMATION RESULTS



# DNA DONOR INSERTION



# VALIDATION IN CELL LINES

- **Cell Culture for NIH/3T3 cells**
- **Transfection into cells**
- **Fixing**
- **Staining**
- **Imaging**

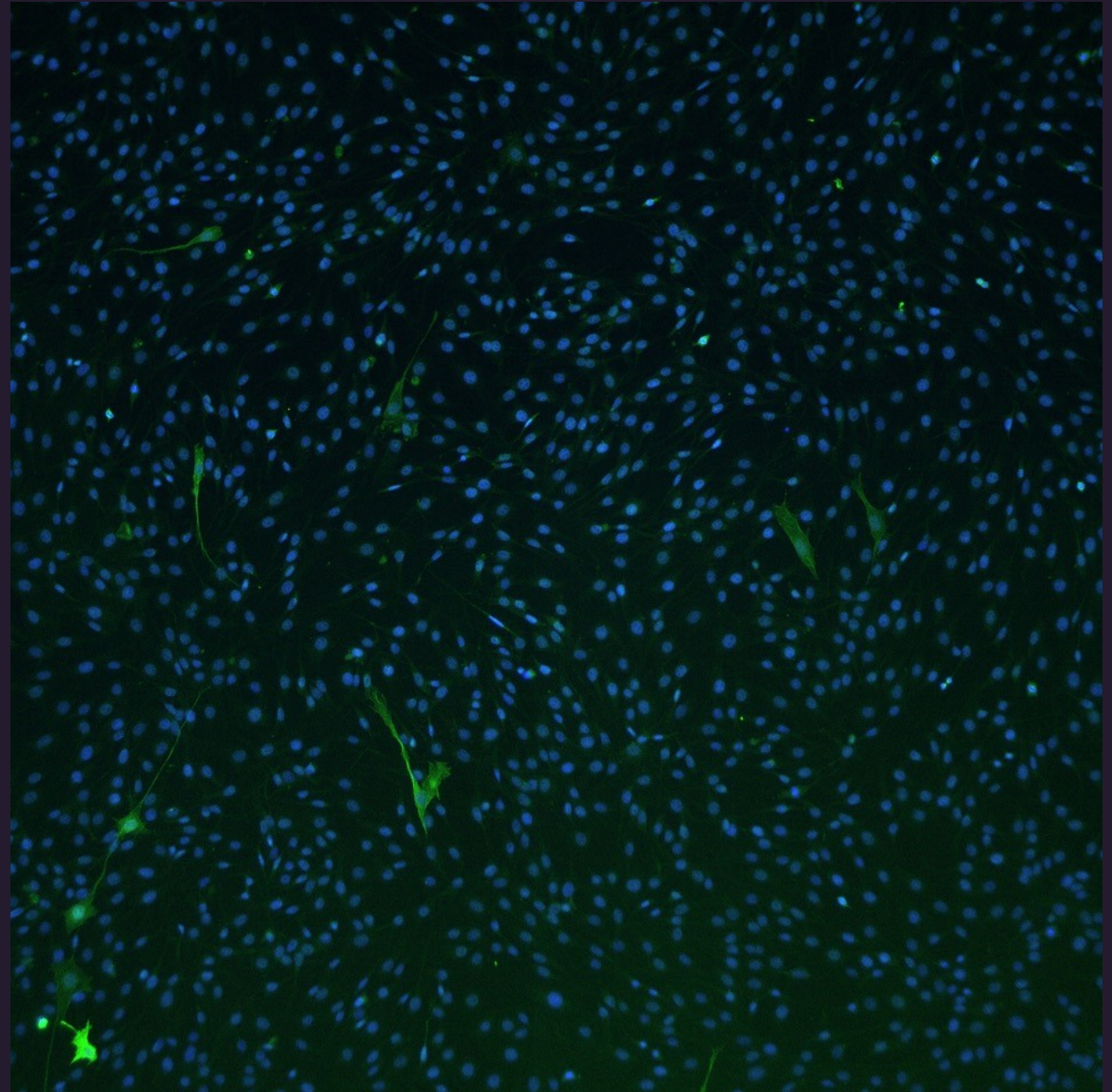


<https://www.cellsciences.com/resources/news/endotoxins-innovative-solutions-for-cell-culture-studies/>



# BETA ACTIN RESULTS

DAPI= NUCLEUS  
ACTIN-GFP



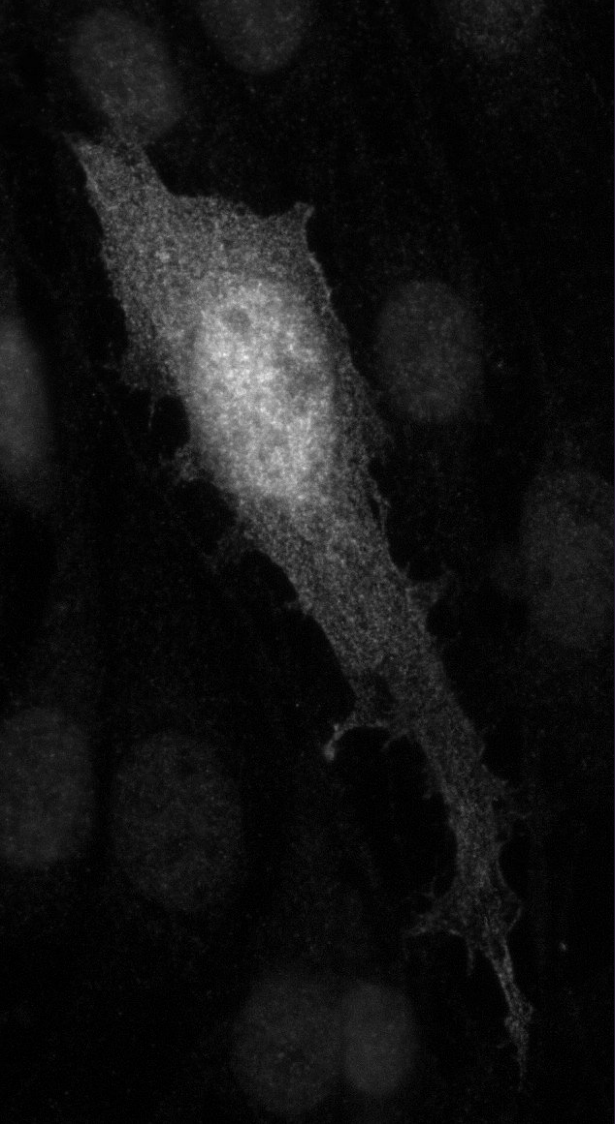
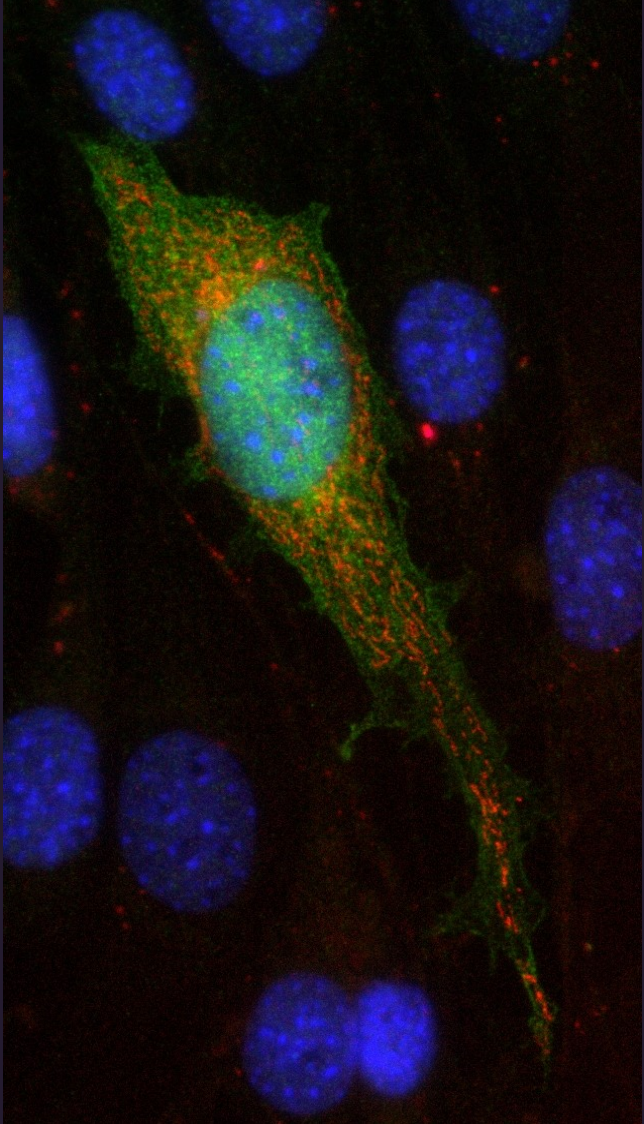
# MIEF1 RESULTS

DAPI= NUCLEUS

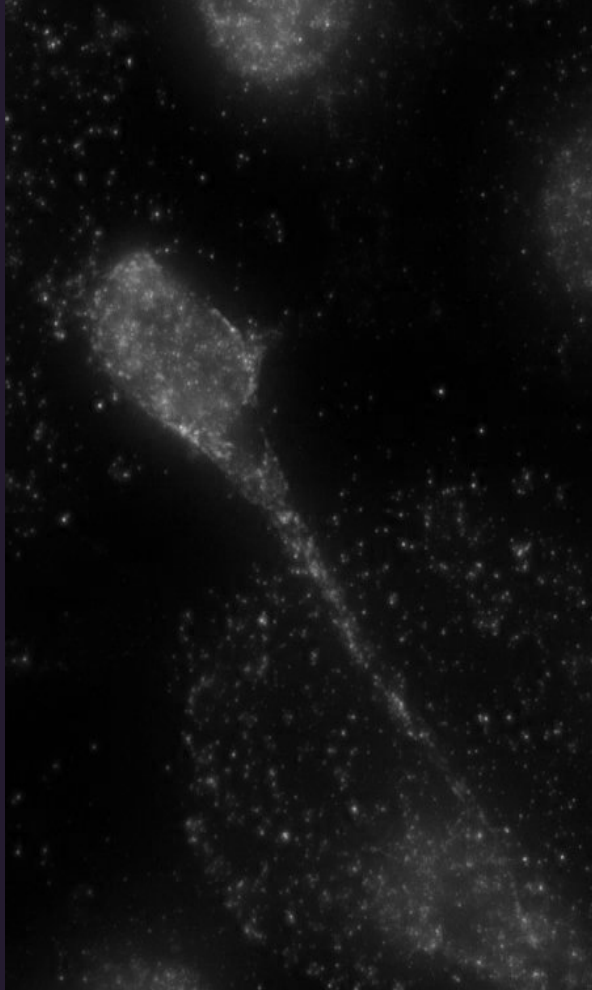
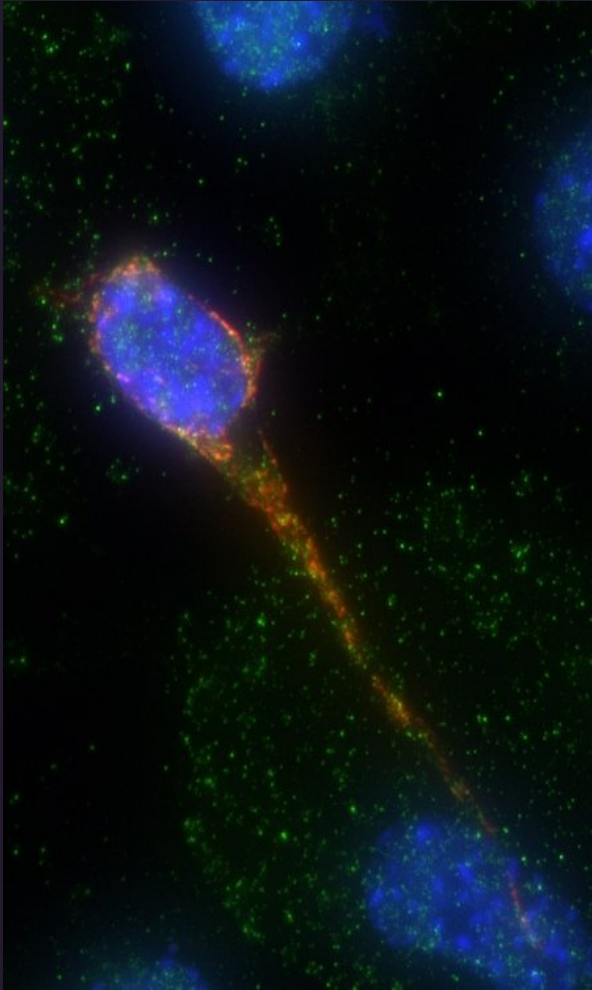
MITOCHONDRIA

MIEF1-GFP

Cell A



Cell B



Black & White= gfp channel

# FUTURE STUDIES

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- Validating more gRNA's for mitochondrial fission factors
- Use in neurons to visualize mitochondrial fission factors localization and abundance both in culture and *in vivo*.



<https://simplifaster.com/articles/monitor-athlete-mitochondria/>

# ACKNOWLEDGEMENTS

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