

Laboratory Research Update

Christina Nemeth Mertz, PhD

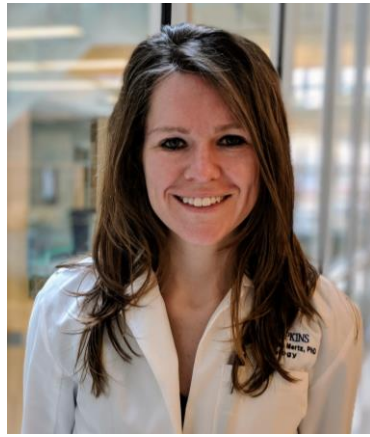
April 19, 2018



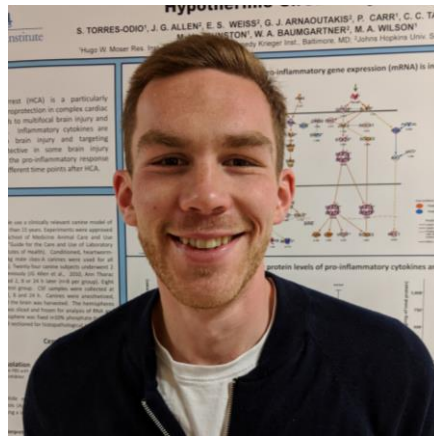
Meet the LBSL Team



Ali Fatemi, MD, MBA



Christina Nemeth Mertz, PhD
Postdoctoral Fellow
2016 - present



Philippe Hubo
Graduate Student
2017 - present



Sophia Tomlinson, BS
Research Technician
2017 - present



Melissa Rosen, BS
Research Technician
2018 - present
Lab Undergraduate
2015-2017



Oscar Larraza, JHU '19
Lab Undergraduate
2017 - present



Connor Murray, BA
Research Technician
2016-2017



Joel Marx, MS
Research Technician
2011-2016

Outline

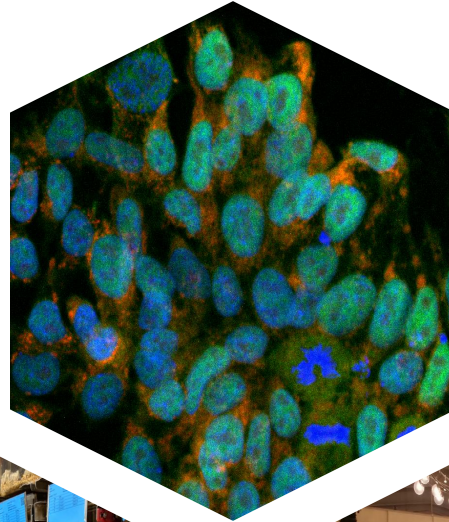
Mouse Models

- how we generate mice
- what we know about DARS2 in mice
- behavior and histology so far



Induced Pluripotent Stem Cells

- what they are and how they're made
- utility
- our data so far



Potential for Therapeutic Testing

- Understanding numbers
- Animals and cells as platforms



Collaborations

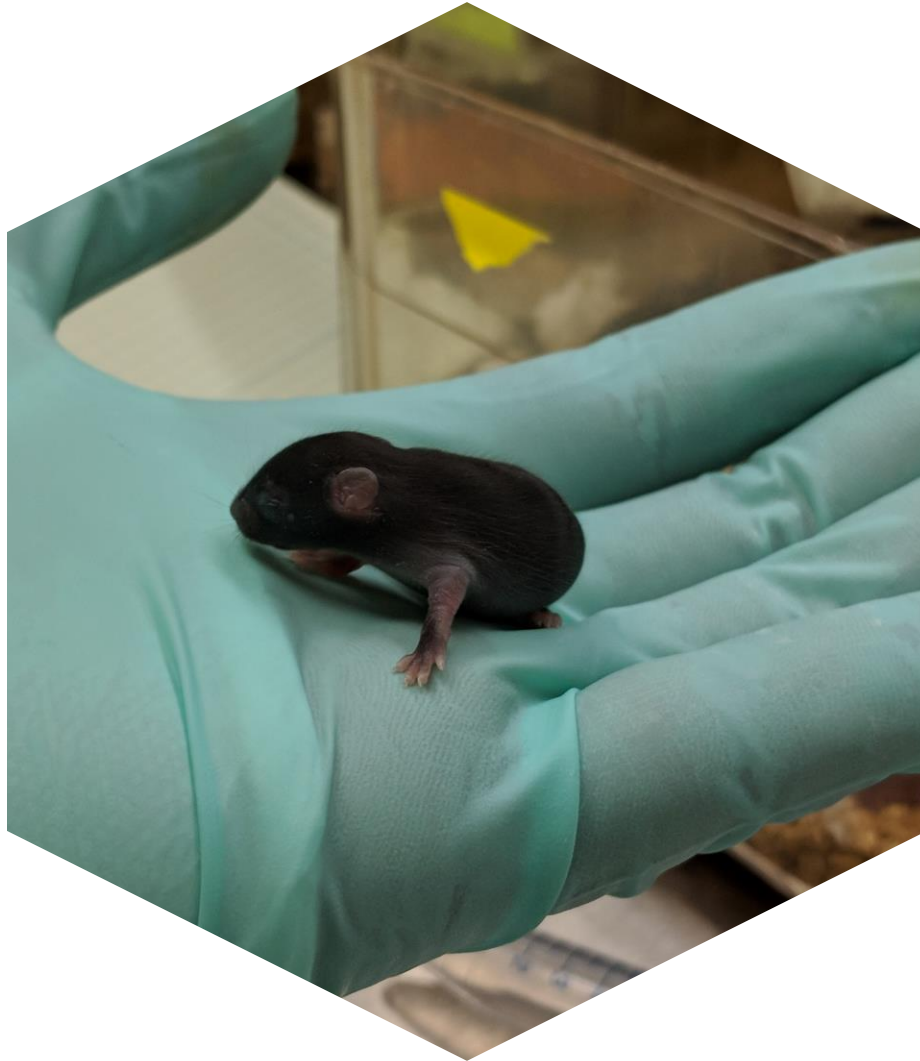
- Cerebral Organoids
- RNAseq



Outline

Mouse Models

- how we generate mice
- what we know about DARS2 in mice
- behavior and histology so far



Why mice?

Comparable genetic makeup to humans

Genetically easy to modify

Genetically identical!

Similar reproductive and nervous systems to humans

Relatively short life span (can study their *entire* life span)

Excellent model for studying changes in motor function, behavior

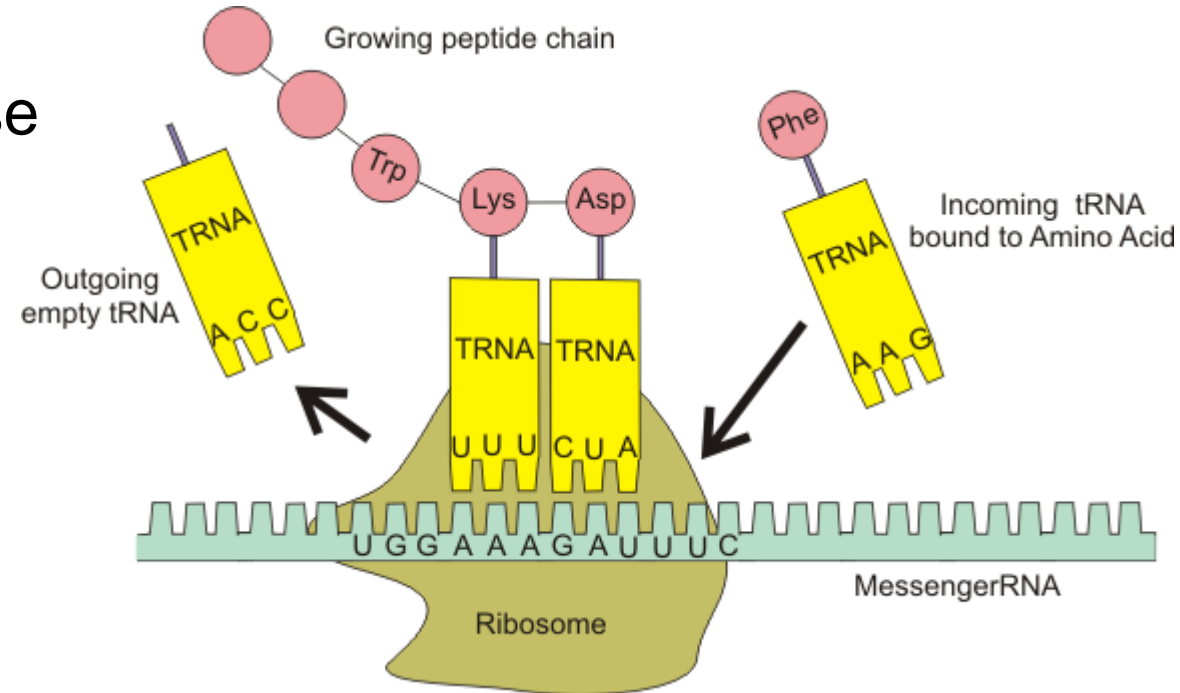
Useful for testing therapies

Not perfect!

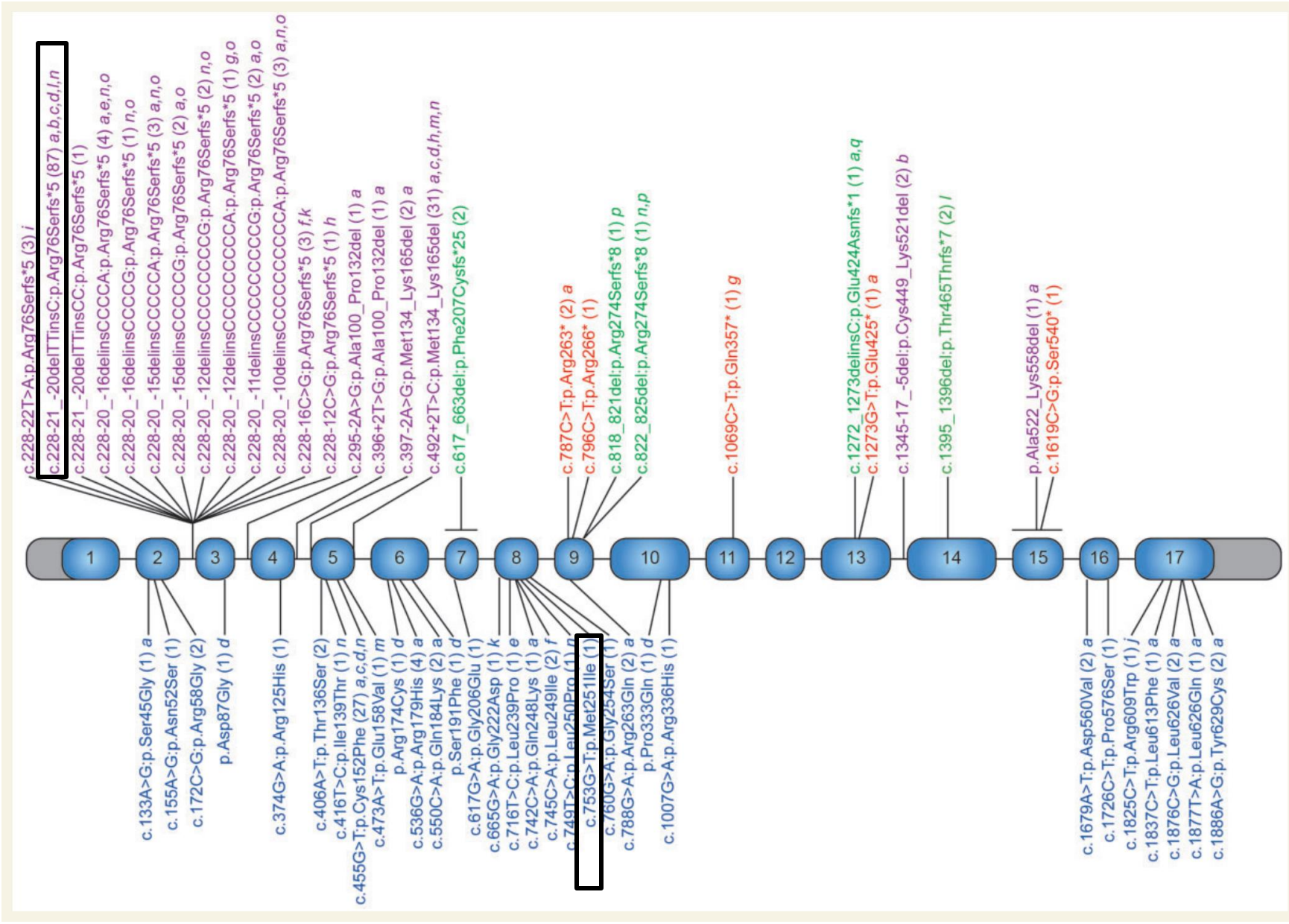


LBSL and *DARS2*

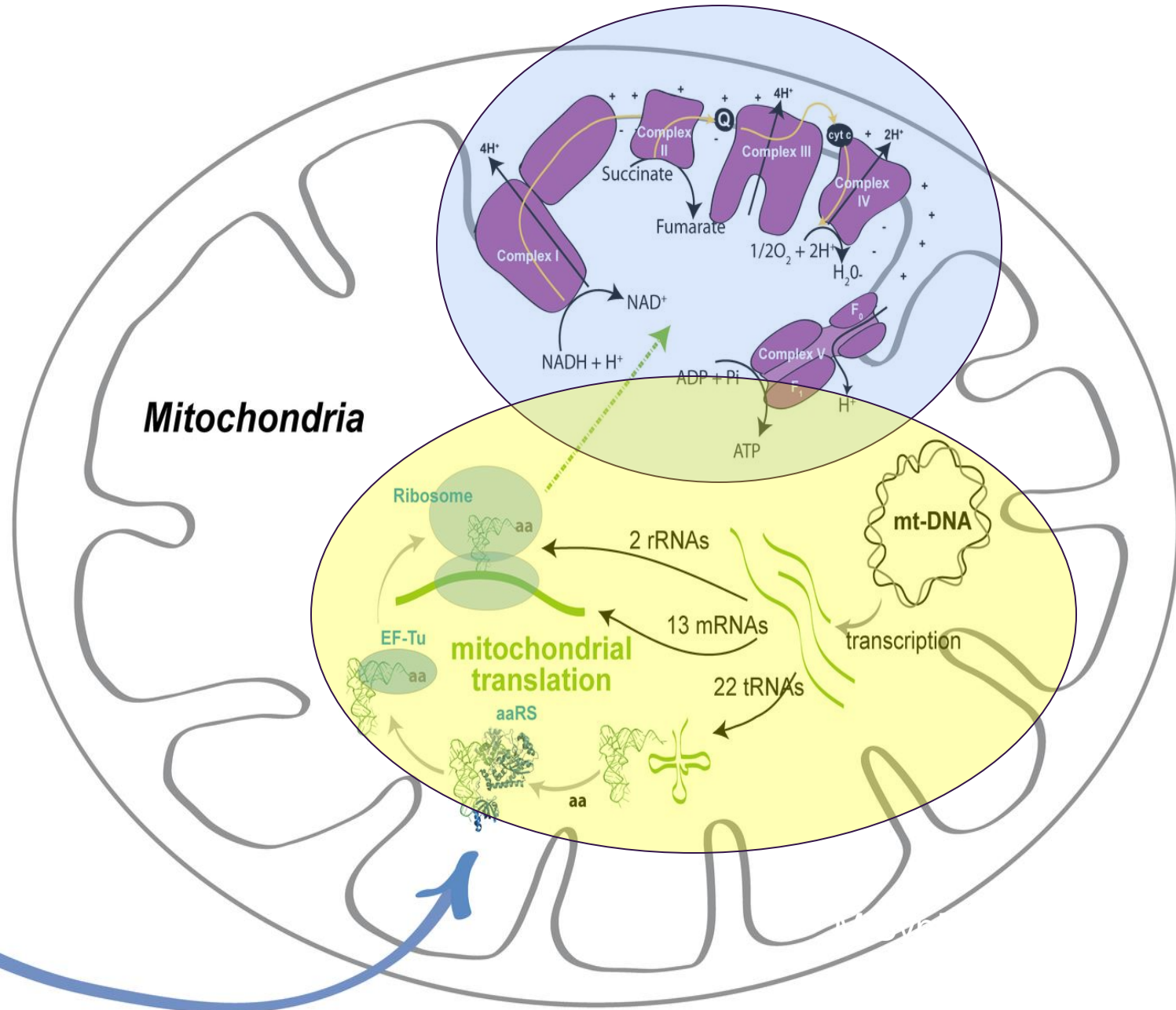
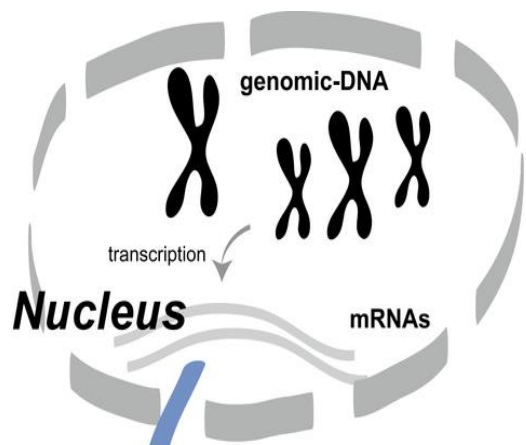
- Mitochondrial aspartyl-tRNA synthetase
 - Aspartic acid in mitochondrial protein translation (mtAspRS)
- Nuclear encoded
- Decreased activity; “leaky”



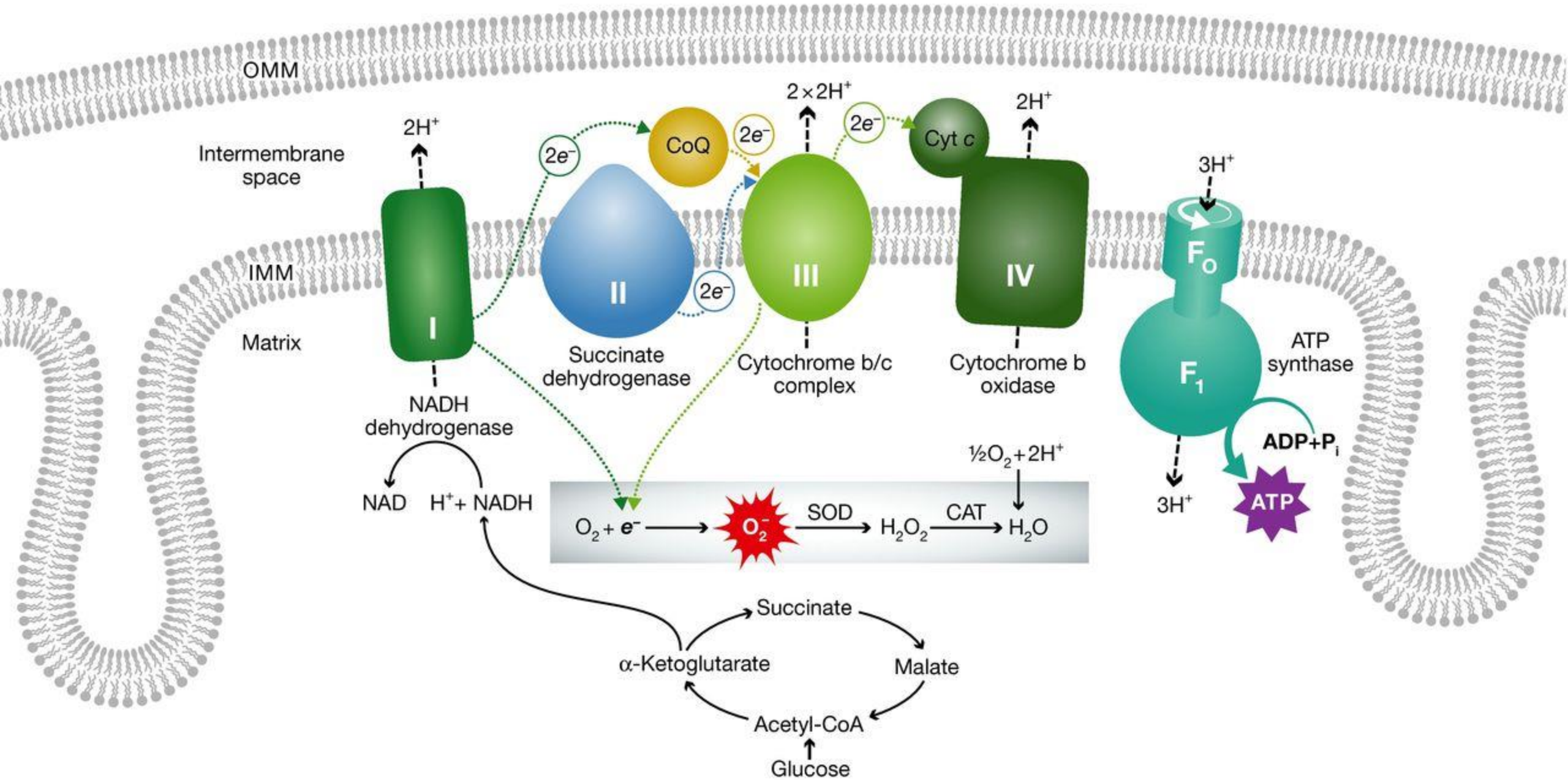
DARS2 gene



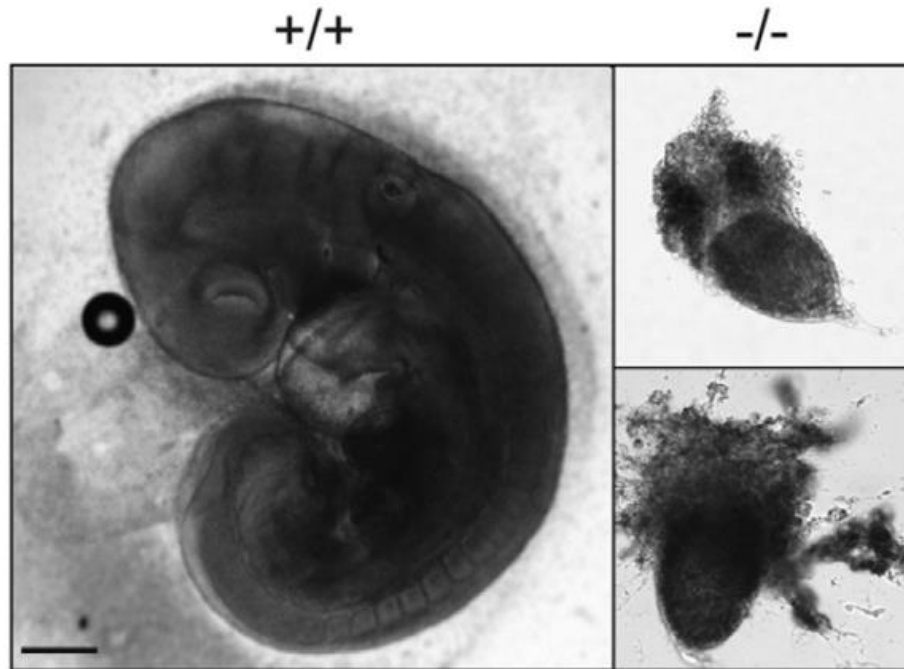
frameshift
missense
deletion
nonsense



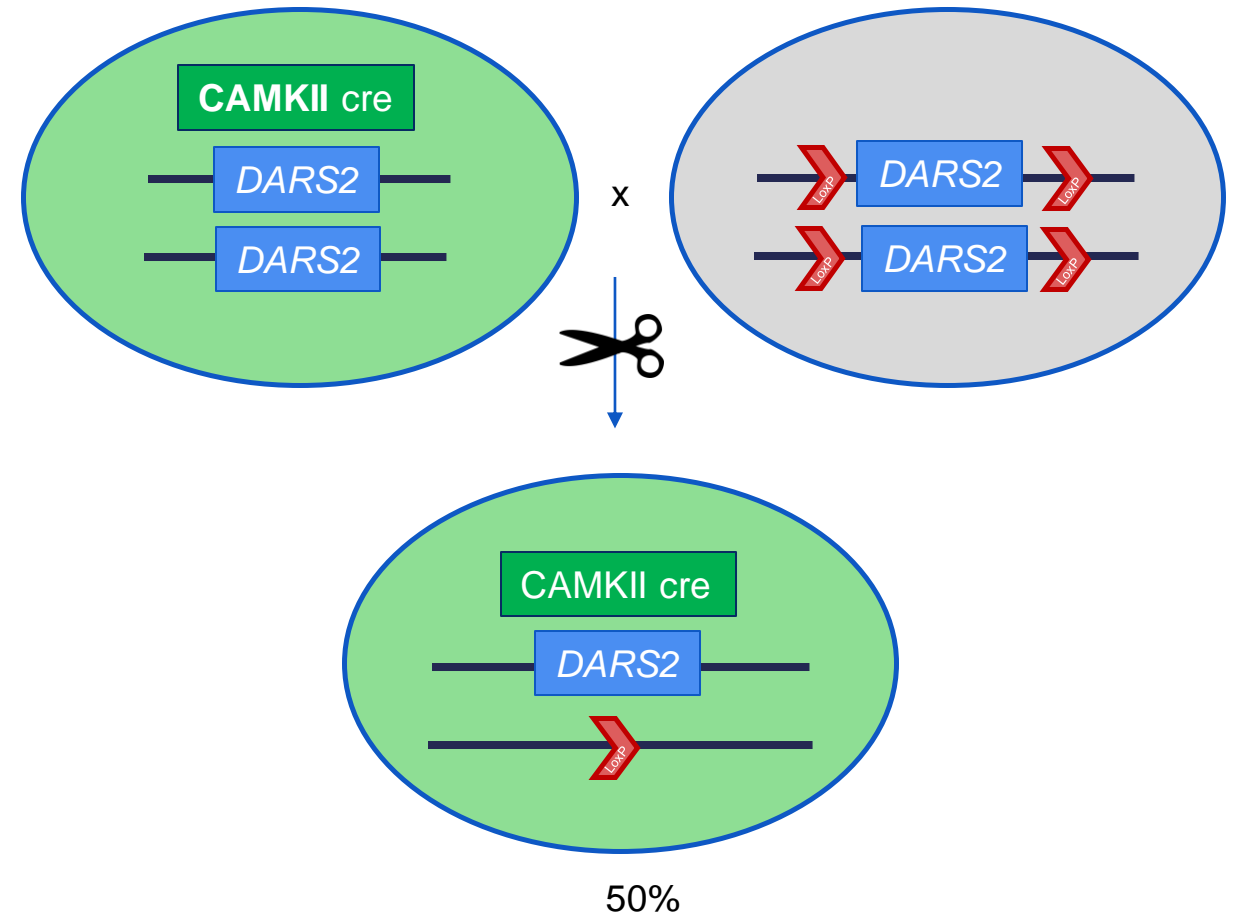
19 mt-aaRSs



Modeling LBSL

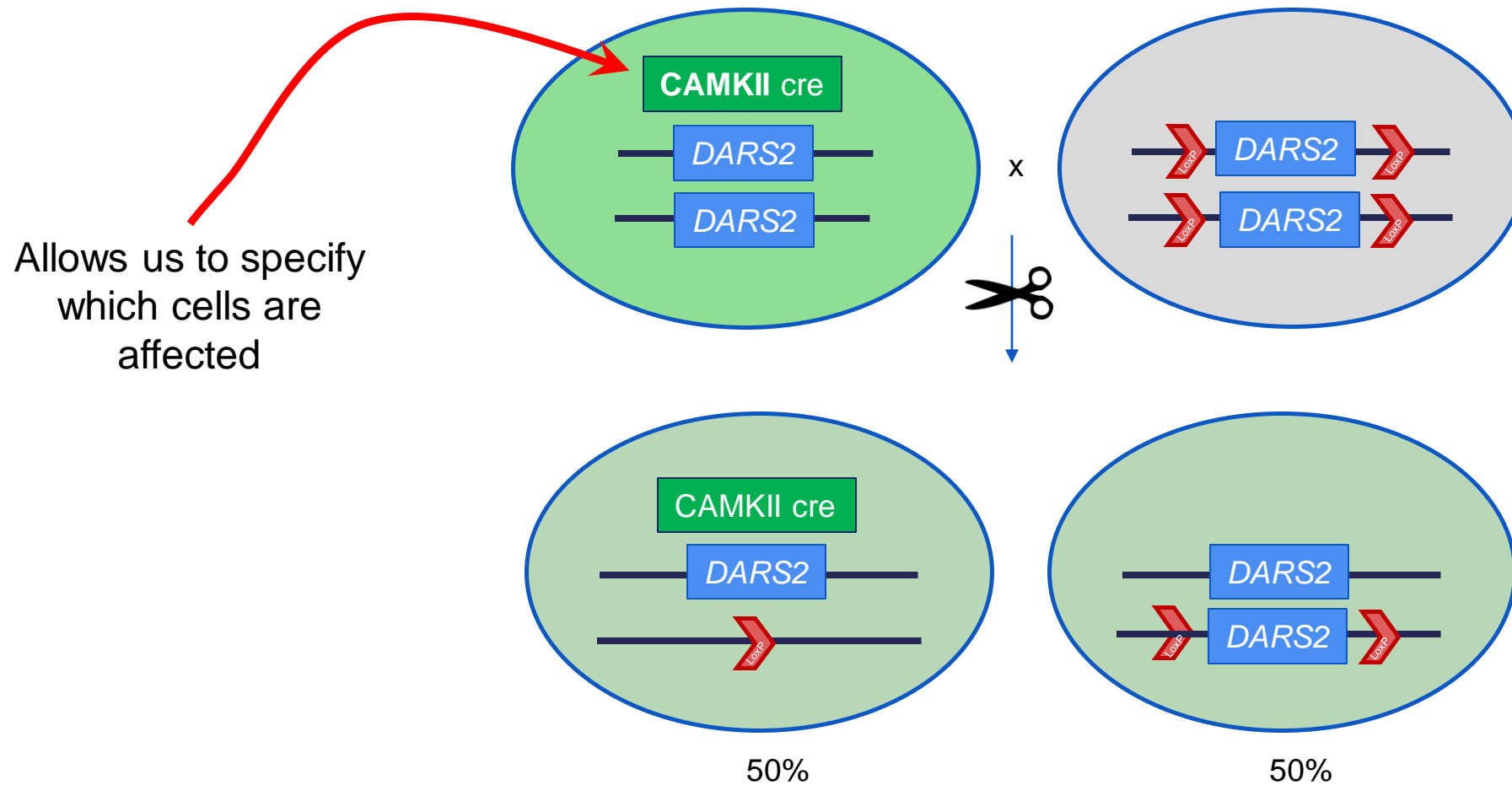


Cre-Lox Recombination

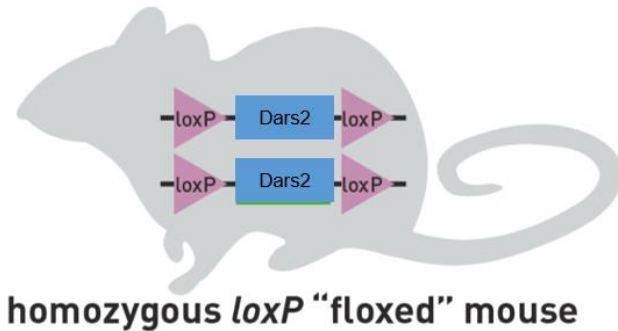


Full knockout of DARS2 is embryonic lethal and selective knockout results in mitochondrial dysfunction

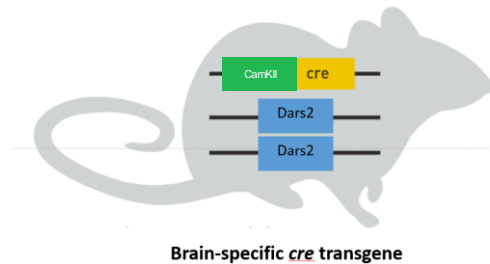
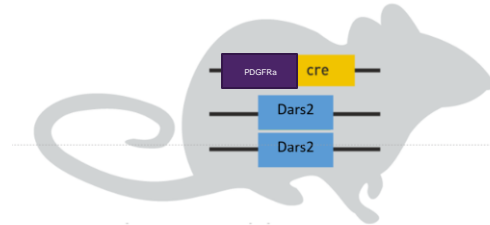
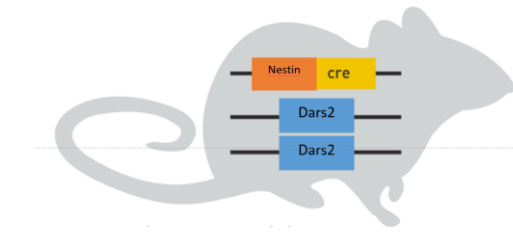
How does Cre-Lox work?



Our mice



Received from Dr. Aleksandra Trifunovic
at CECAD, Cologne, Germany



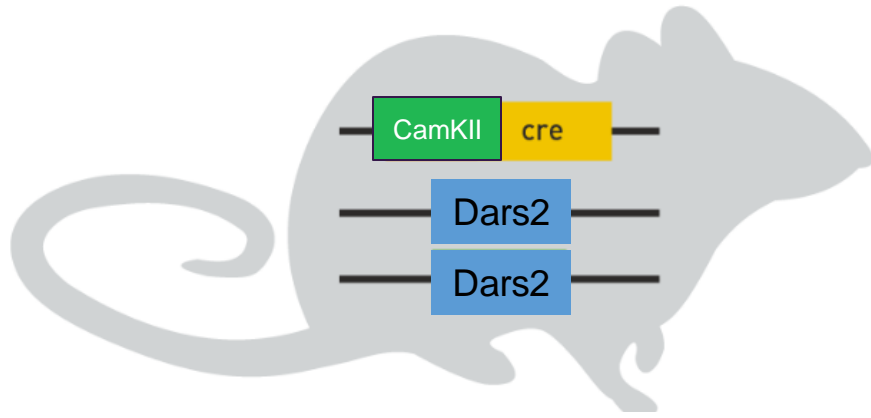
Purchased from Jackson Labs:

Nestin: neuronal precursor cells (all)

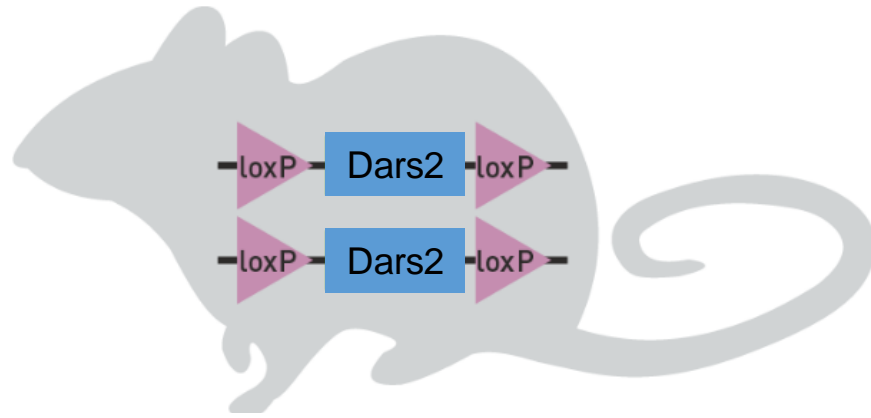
CamKII α : neurons in forebrain

PDGFR α : oligodendrocyte precursors (myelin)

FOUNDER



Brain-specific *cre* transgene

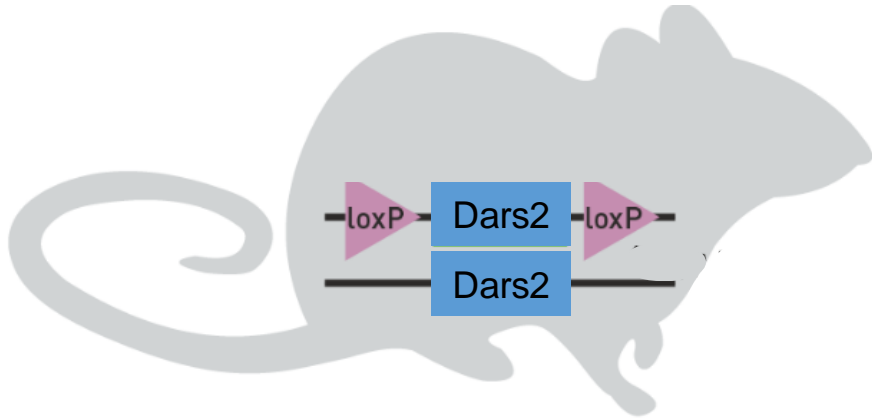


Homozygous loxP "floxed" mouse

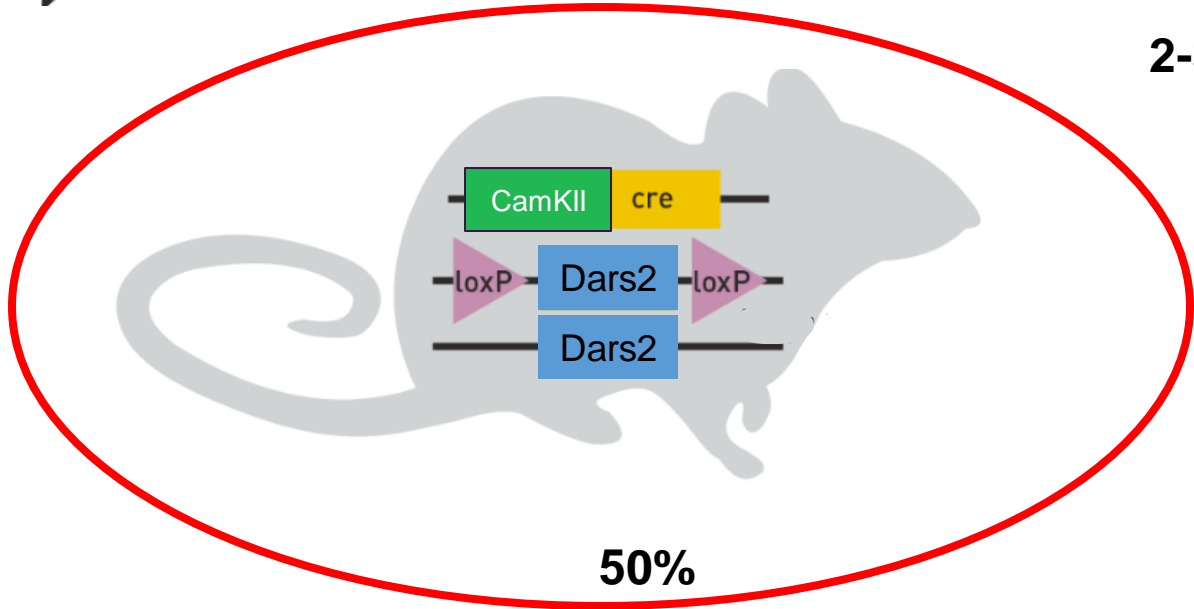
X



1st GENERATION



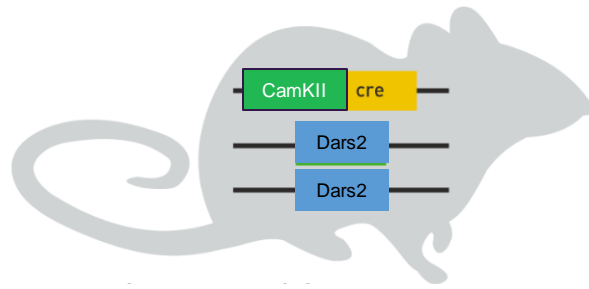
50%



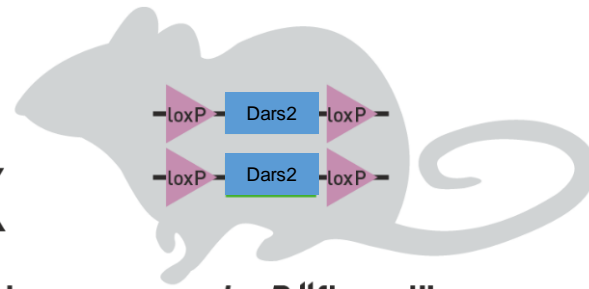
50%

2-3 months

FOUNDER



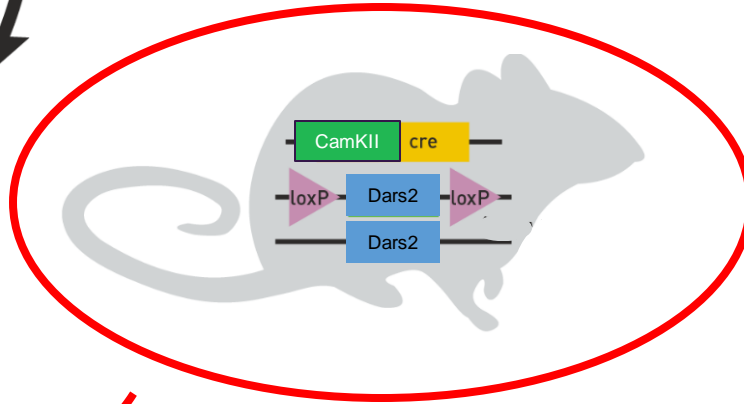
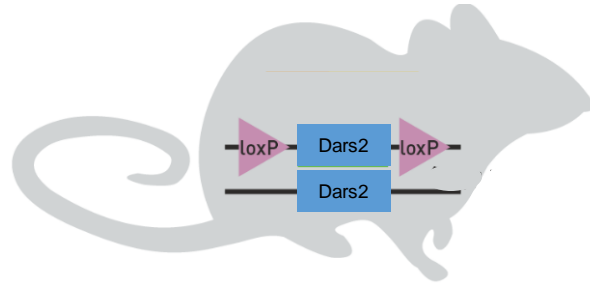
Brain-specific cre transgene



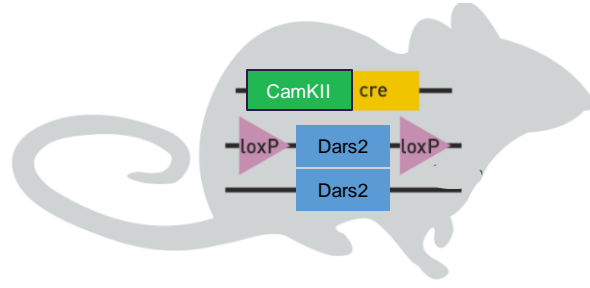
homozygous *loxP* "floxed" mouse

X

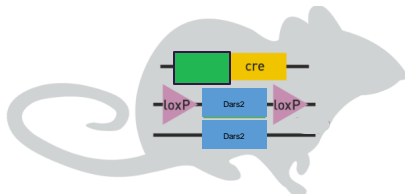
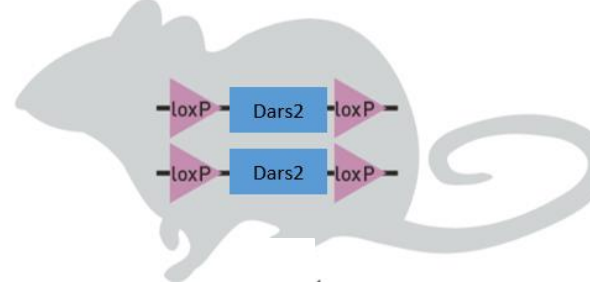
1st GENERATION



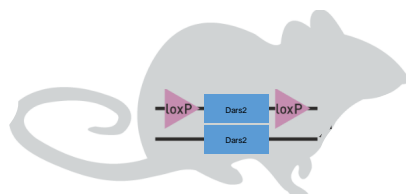
2nd GENERATION



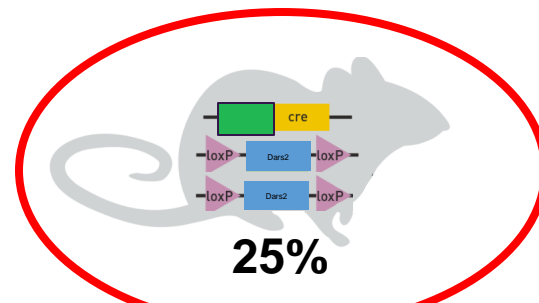
X



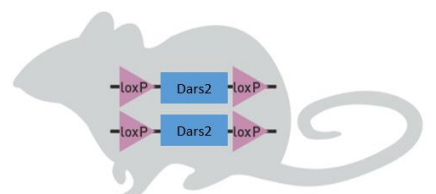
25%



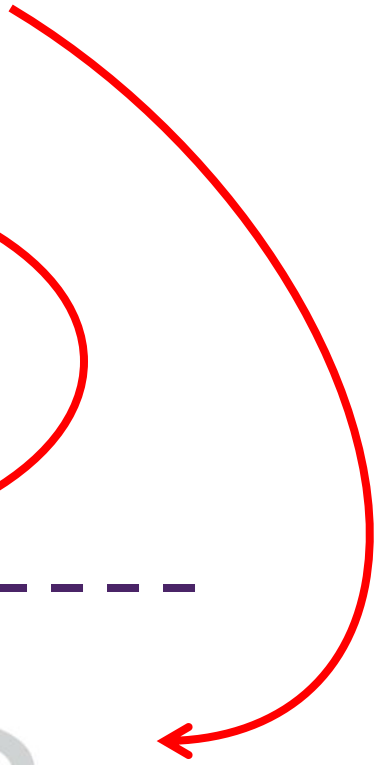
25%



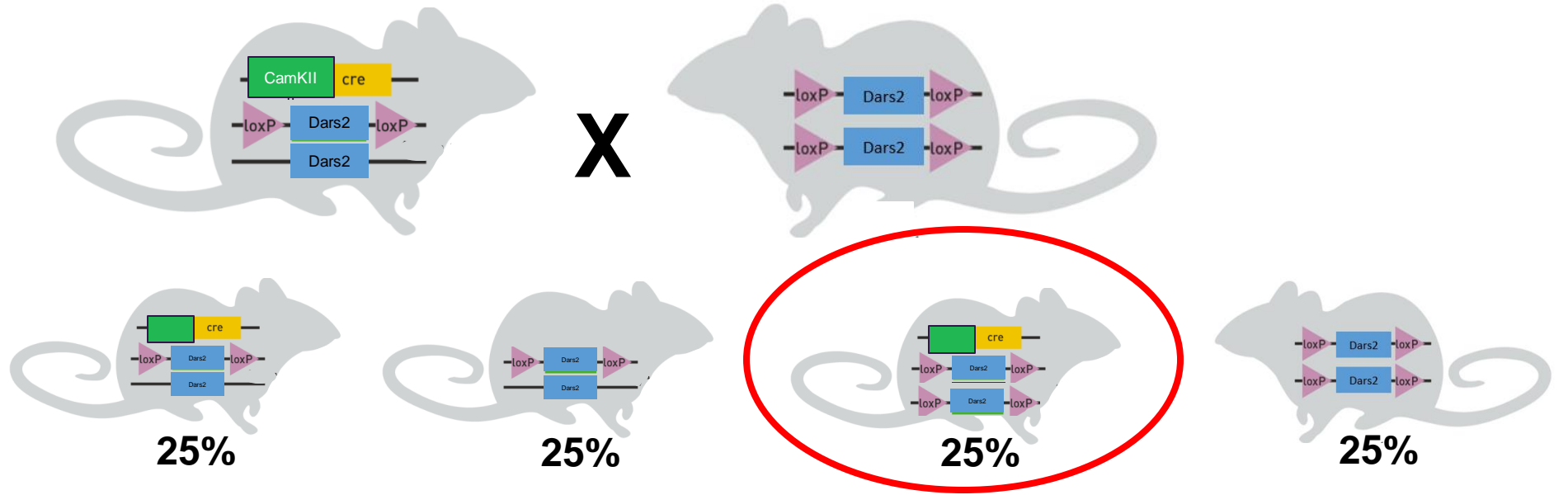
25%



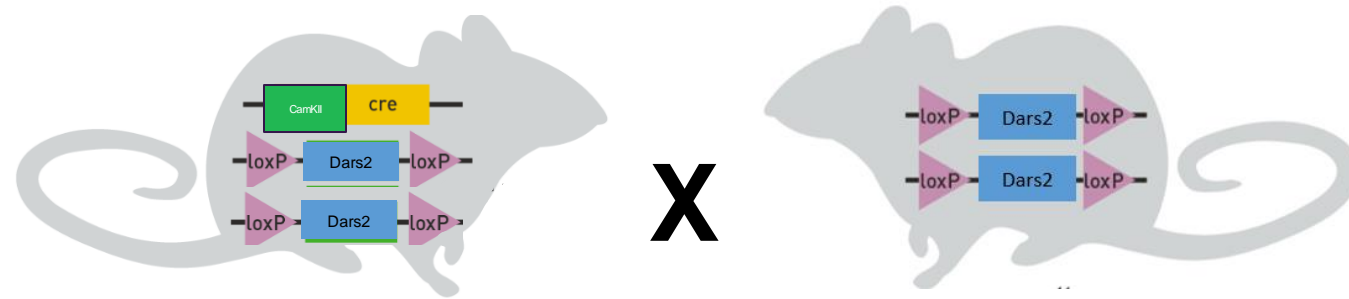
25%



2nd GENERATION



3rd GENERATION



50% Dars2 fl/fl Cre - (Control)
50% Dars2 fl/fl Cre + (Mutant)



- Activity
- Gait
- Cognition
- Reflexes
- Affect
- Weight



Oscar at work

Tissue-Specific Loss of DARS2 Activates Stress Responses Independently of Respiratory Chain Deficiency in the Heart

Sukru Anil Dogan,^{1,6} Claire Pujol,^{1,6} Priyanka Maiti,¹ Alexandra Kukat,¹ Shuaiyu Wang,² Steffen Hermans,¹ Katharina Senft,¹ Rolf Wibom,³ Elena I. Rugarli,^{1,2,4} and Aleksandra Trifunovic^{1,3,4,5,*}

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⁴Center for Molecular Medicine Cologne (CMMC), 50931 Cologne, Germany

⁵Institute for Mitochondrial Diseases and Aging, Medical Faculty, University of Cologne, 50931 Cologne, Germany

⁶These authors contributed equally to this work

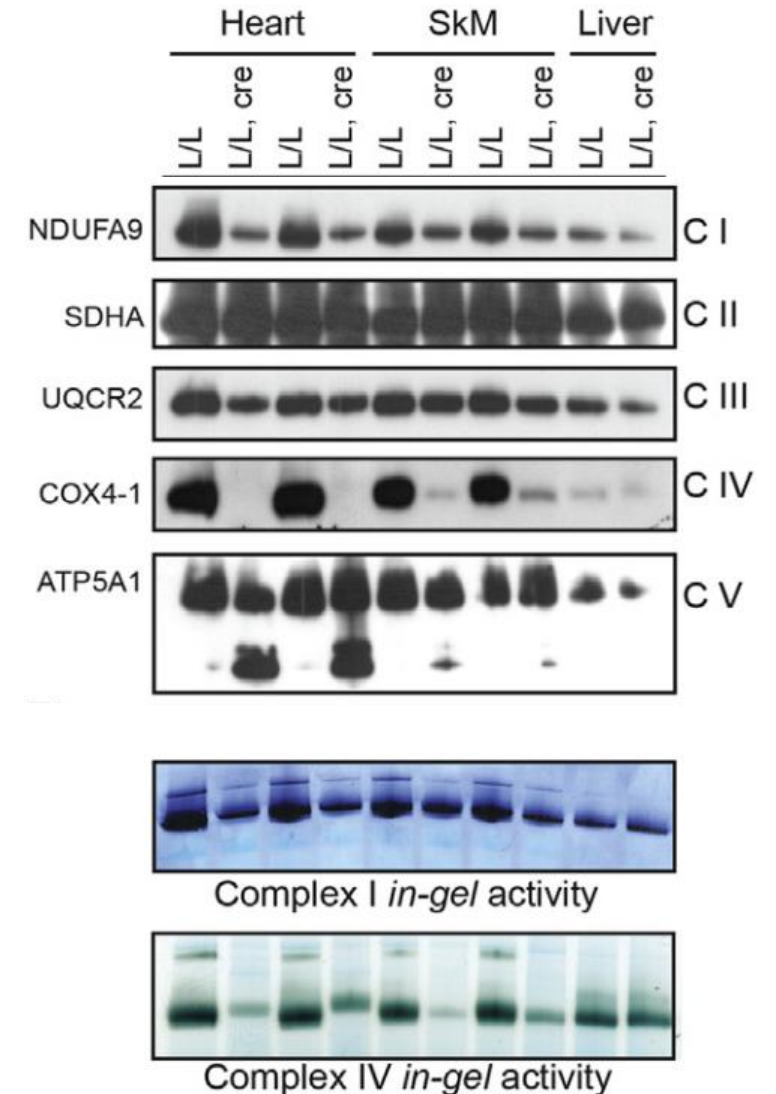
*Correspondence: aleksandra.trifunovic@uk-koeln.de

<http://dx.doi.org/10.1016/j.cmet.2014.02.004>

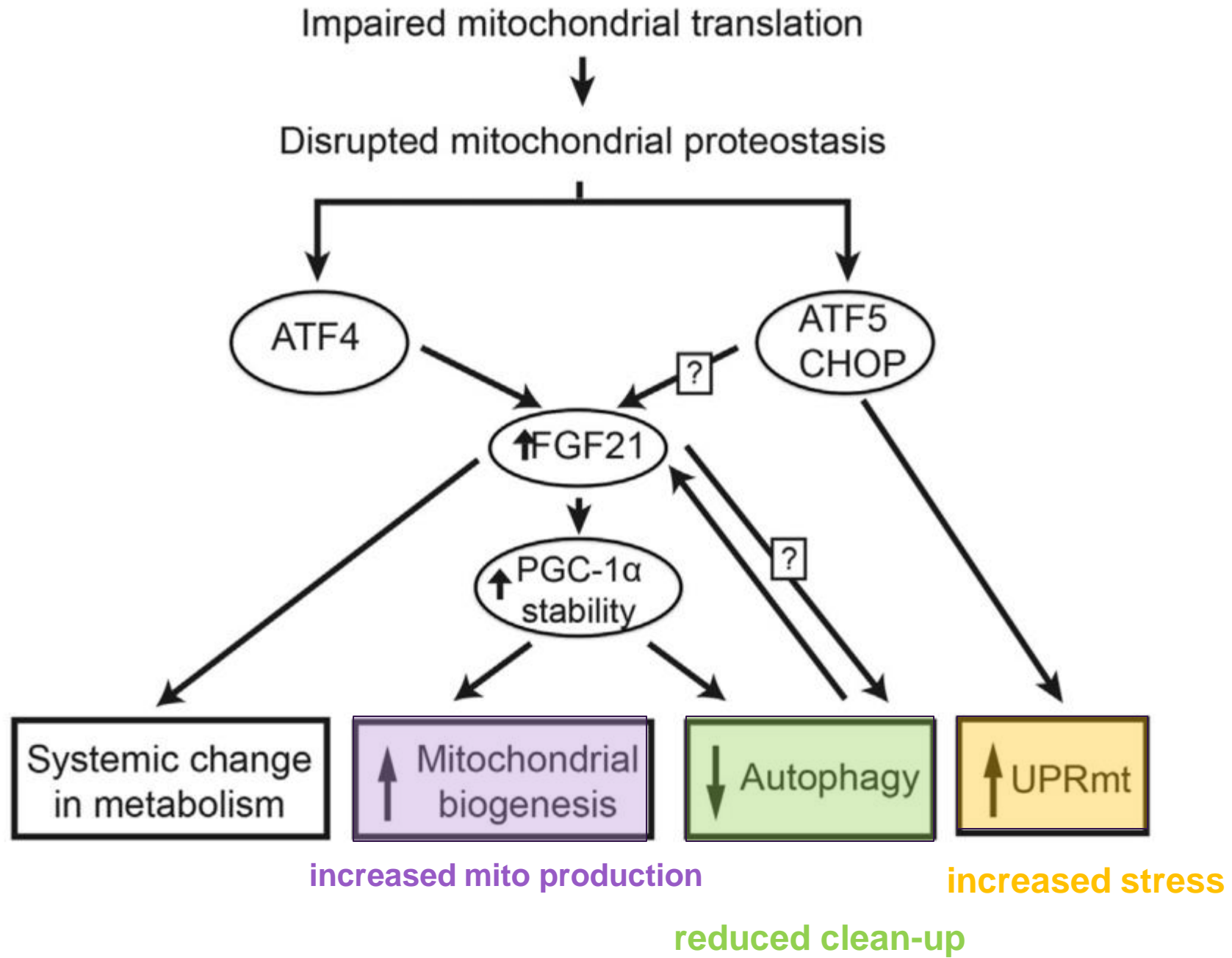
SUMMARY

Adaptive stress responses activated upon mitochondrial dysfunction are assumed to arise in order to counteract respiratory chain deficiency. Here, we demonstrate that loss of DARS2 (mitochondrial aspartyl-tRNA synthetase) leads to the activation of

presentations in diseases caused by mitochondrial DNA (mtDNA) mutations are often assigned to a plasmoly (disproportionately high) and different tissue) and different various



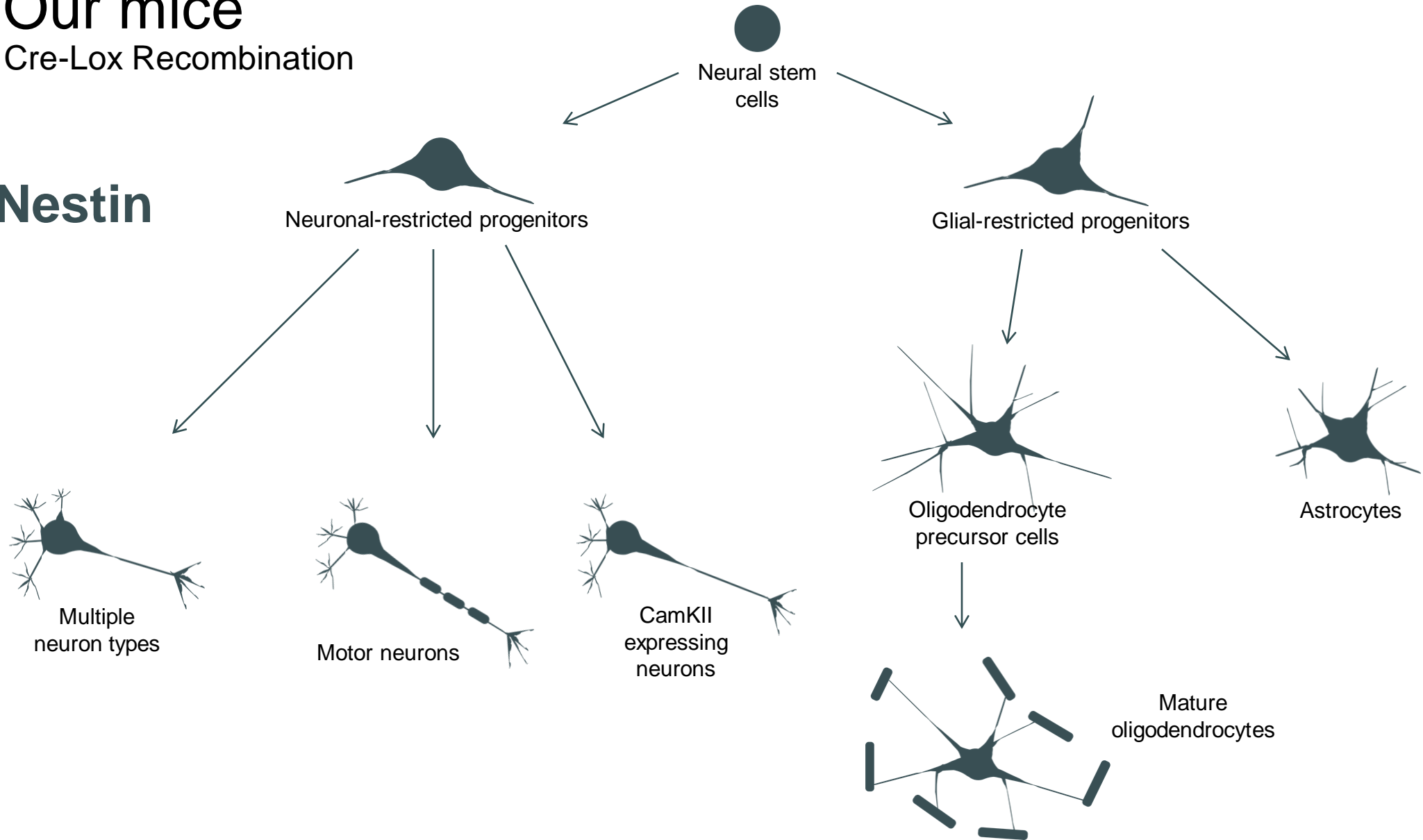
Full knockout of DARS2 is embryonic lethal and selective knockout results in mitochondrial dysfunction



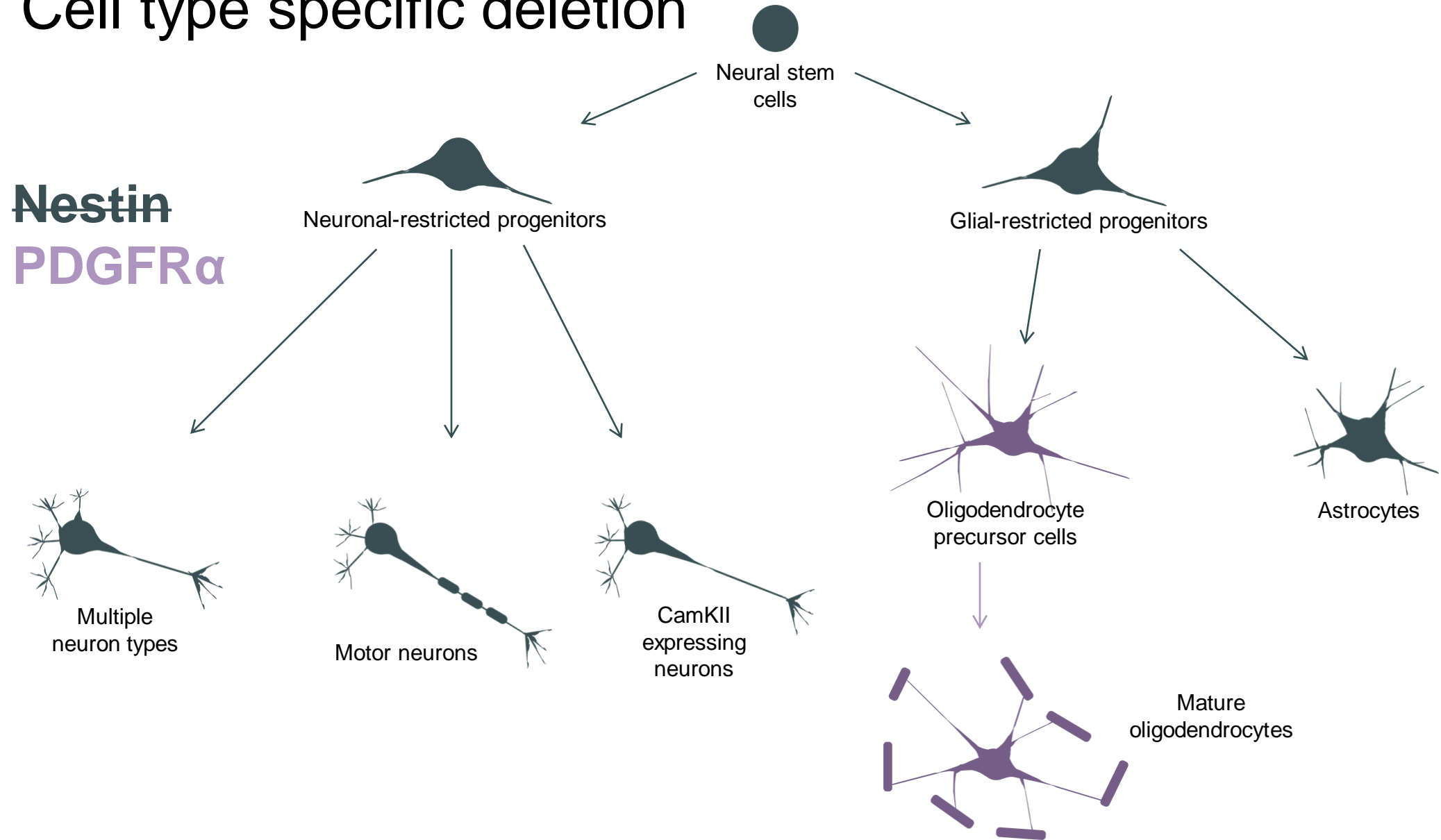
Our mice

Cre-Lox Recombination

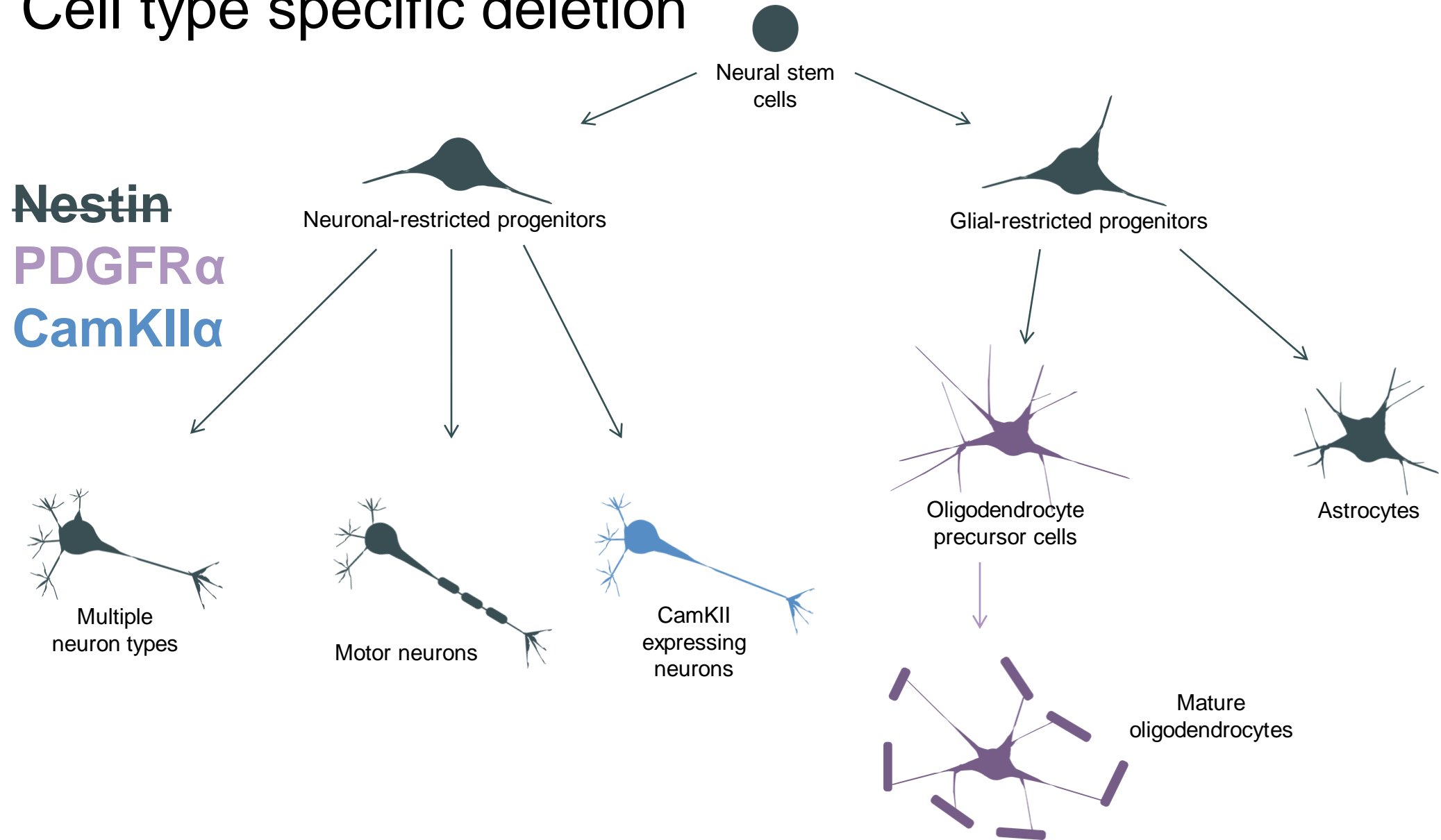
Nestin



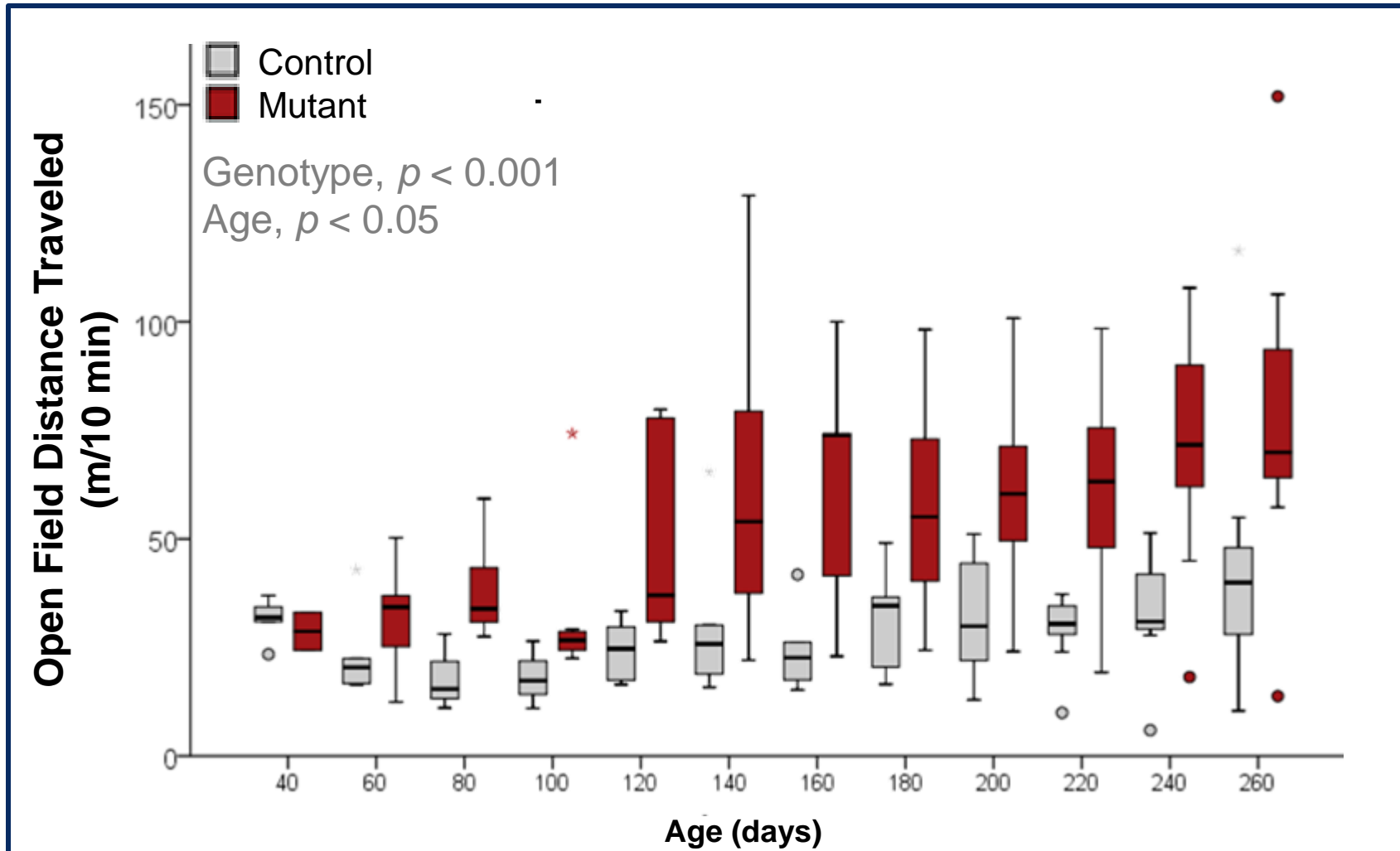
Cell type specific deletion



Cell type specific deletion

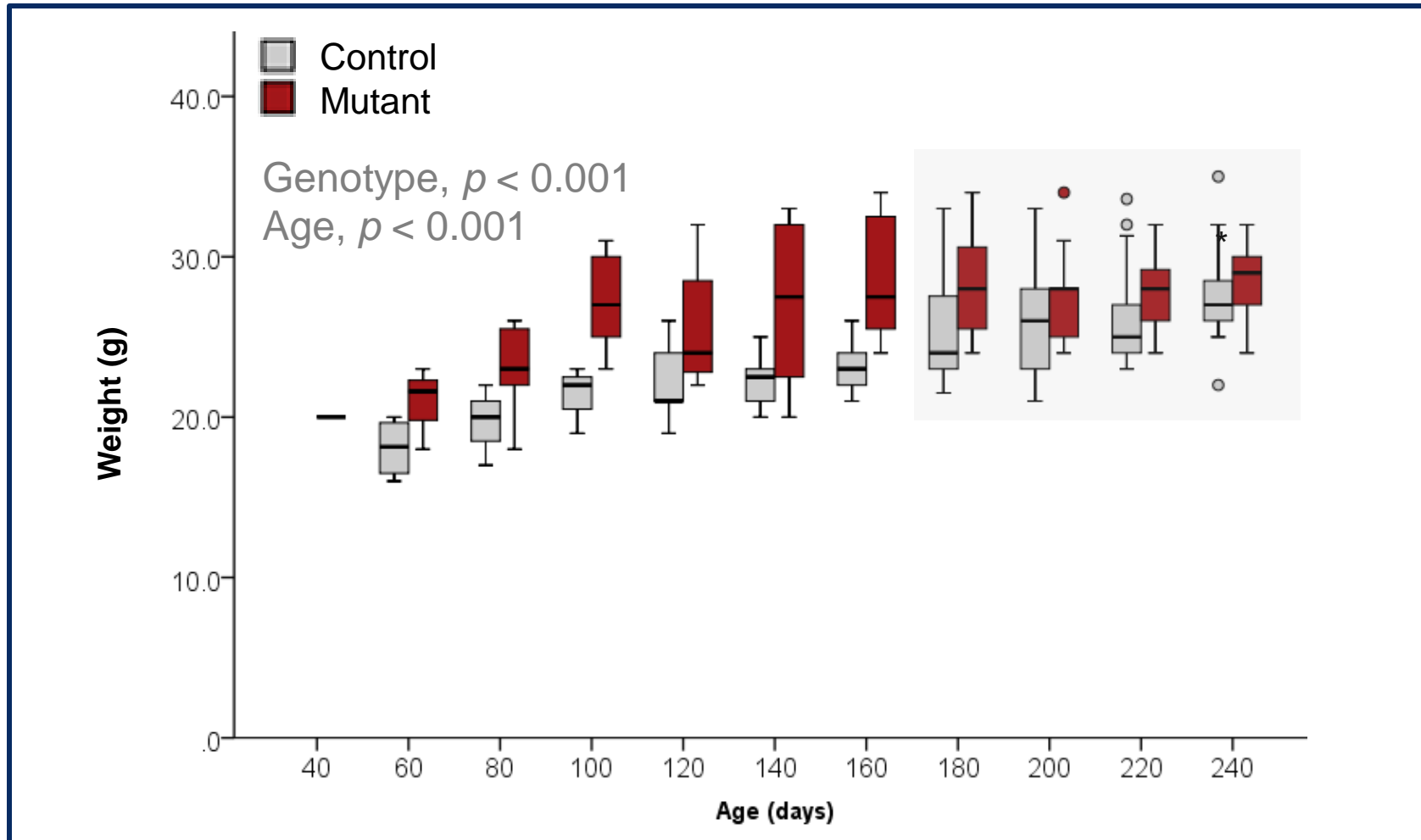


DARS2 deletion increases overall activity



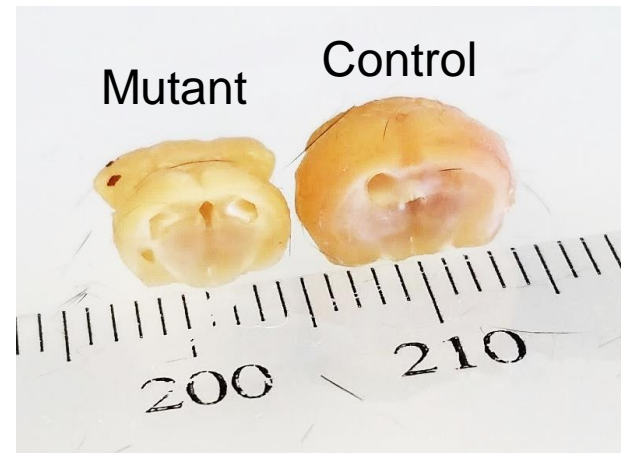
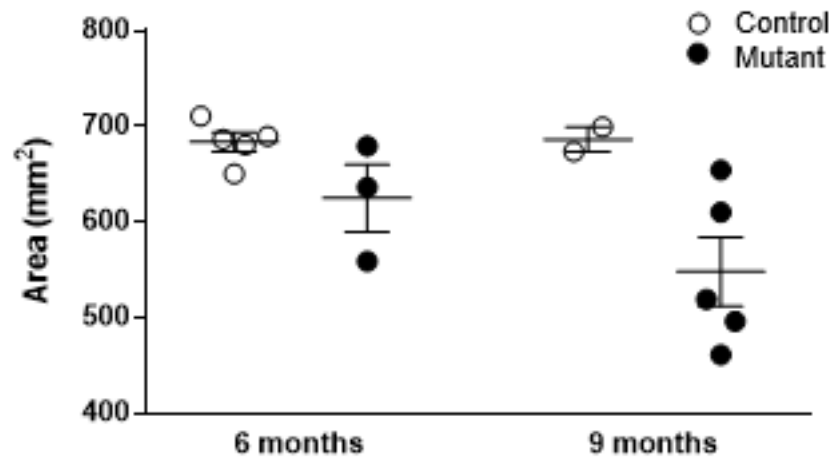
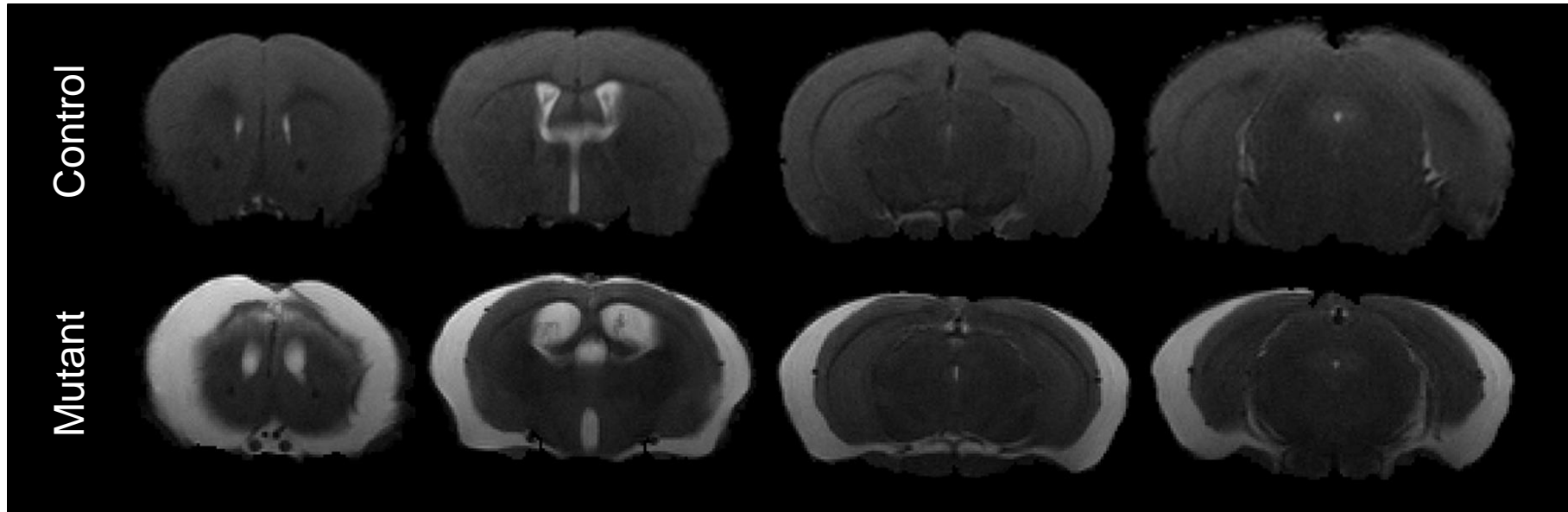
males & females
Cre- n = 13
Cre+ n = 12

Body mass of *DARS2* mutant mice decreases at ~6 months of age

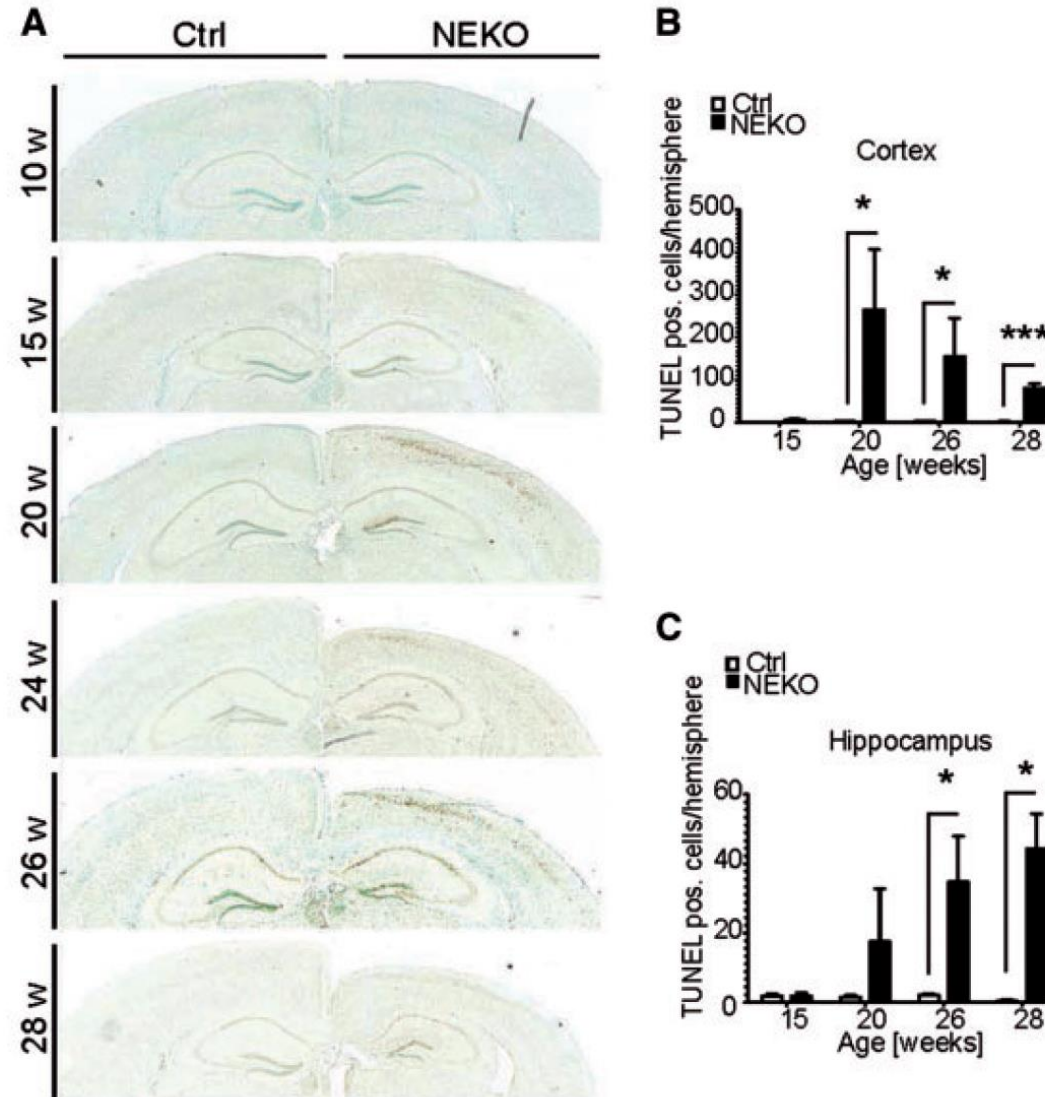


males & females
Cre- n = 13
Cre+ n = 12

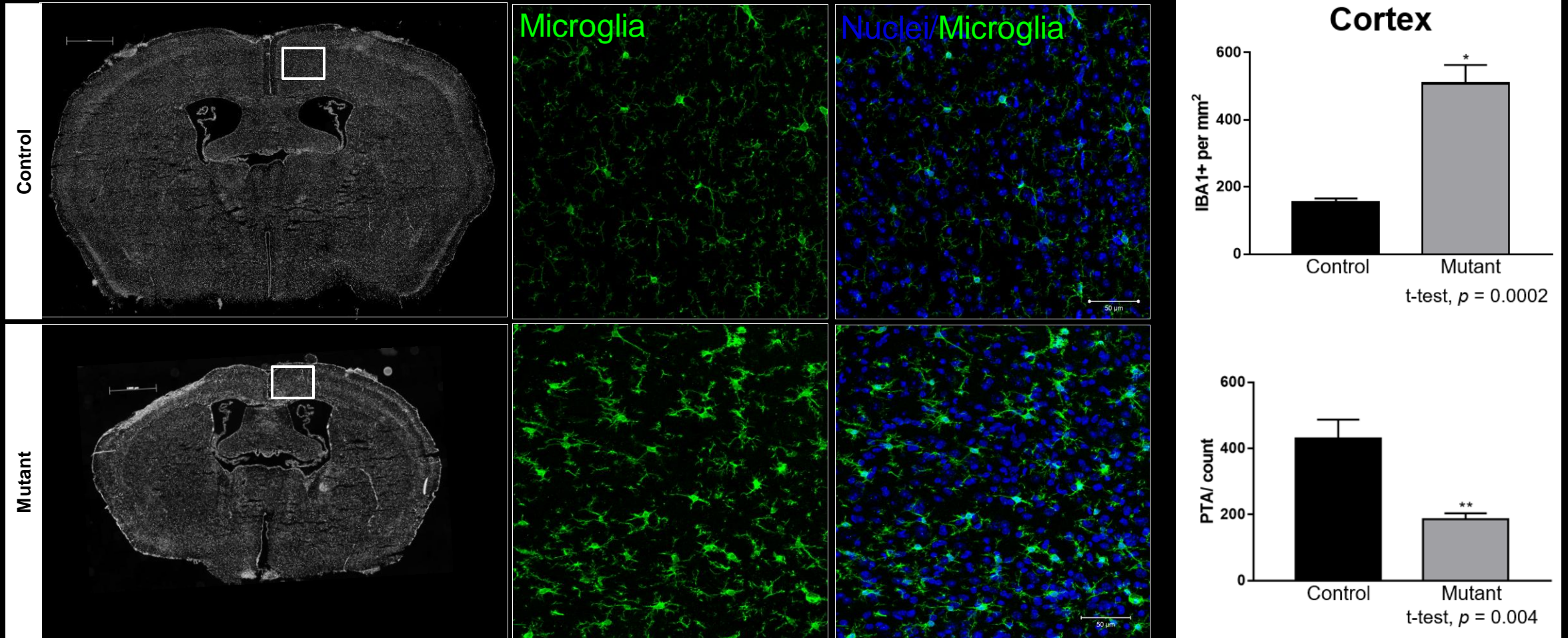
DARS2 deletion from neurons leads to severe brain atrophy



DARS2 deletion leads to neuronal cell loss

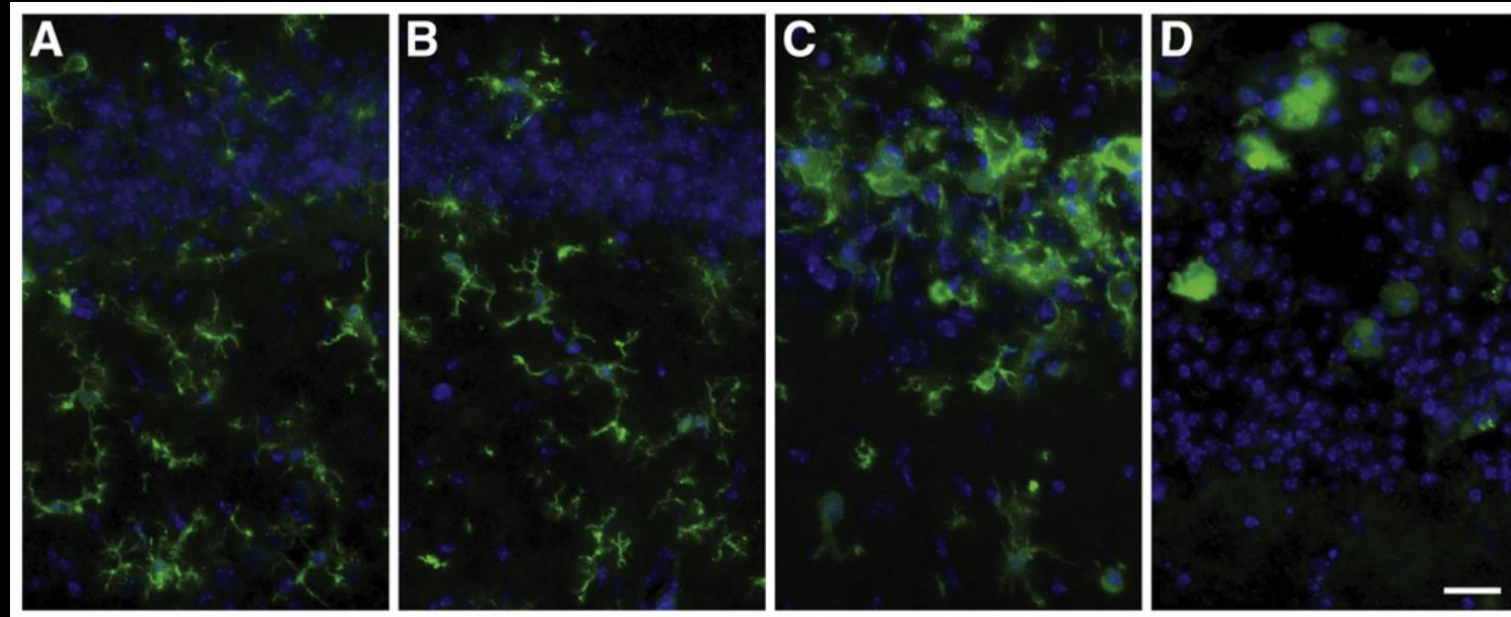


DARS2 deficiency increases brain inflammation

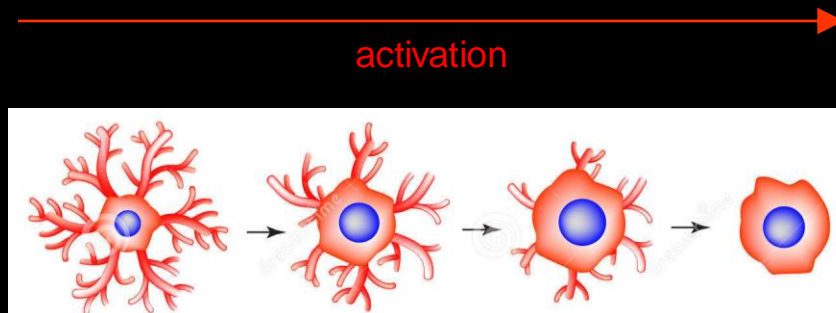


*Microglia are cells that produce inflammation and keep brain areas free from debris and harmful pathogens

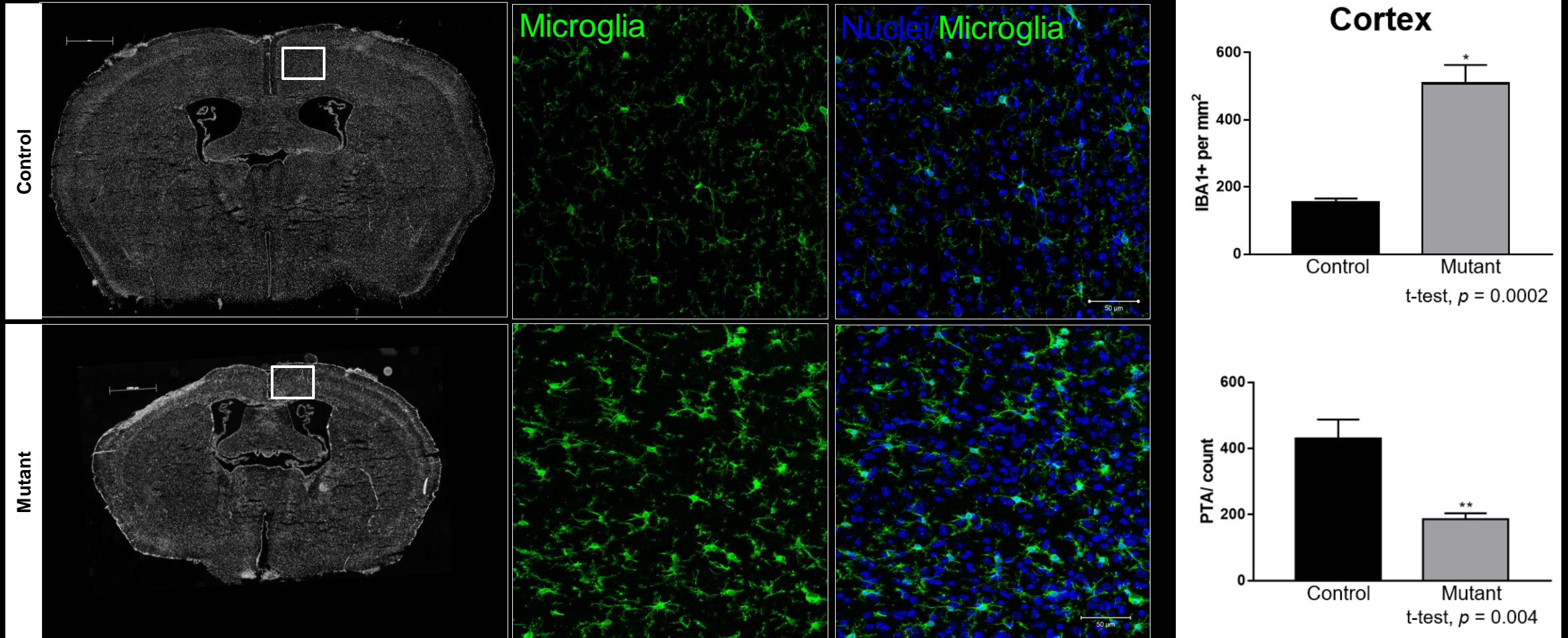
Microglial cells change shape upon activation



Nemeth et al., 2017

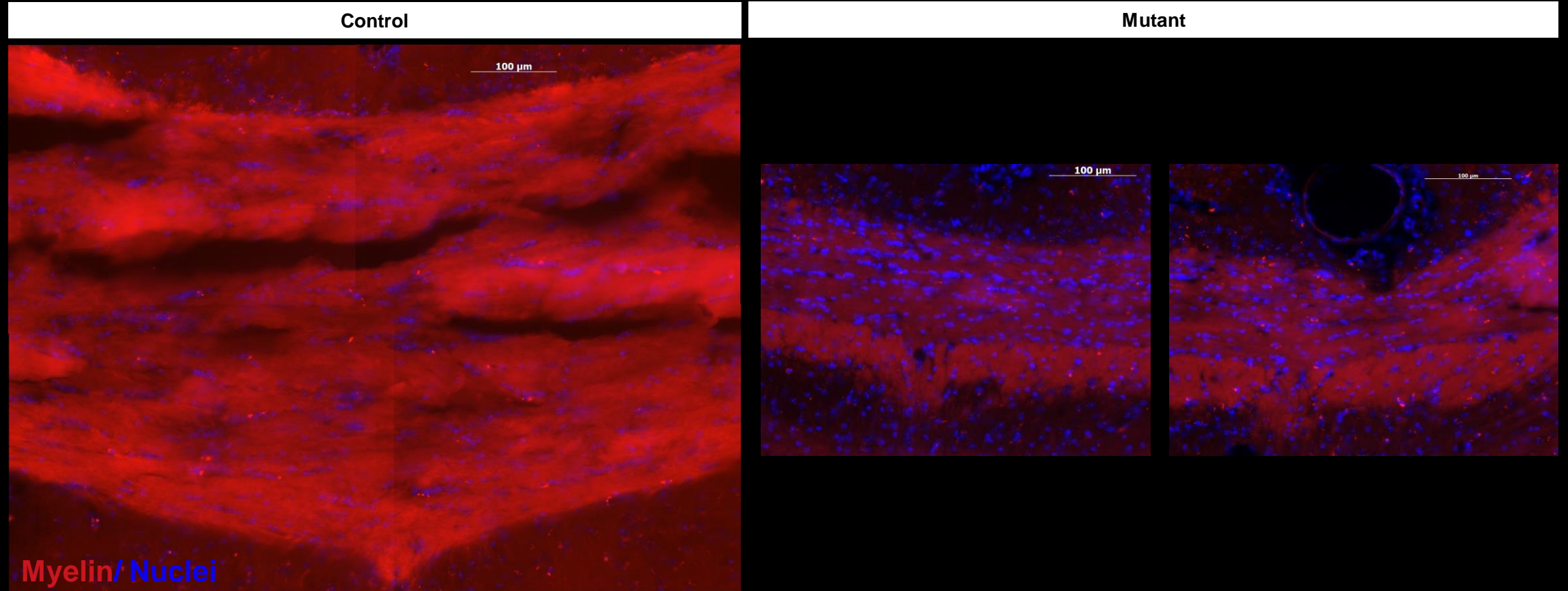


DARS2 deficiency increases brain inflammation



*Microglia are cells that produce inflammation and keep brain areas free from debris and harmful pathogens

DARS2 deficiency leads to reduced white matter area



Electron Microscopy

- Uses a beam of electrons as illumination source
- Allows for very high magnification and the visualization of tissue ultrastructure

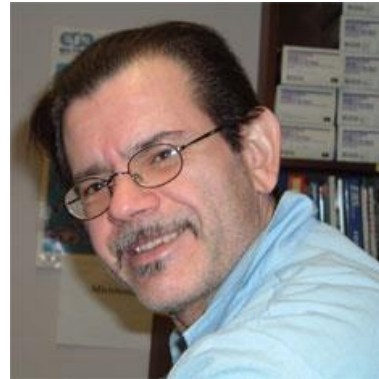


myelin

mitochondrion

axon

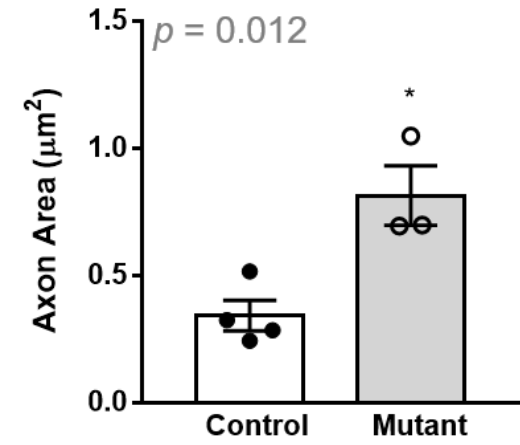
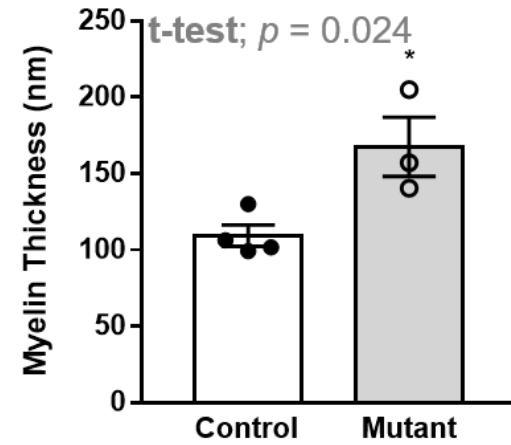
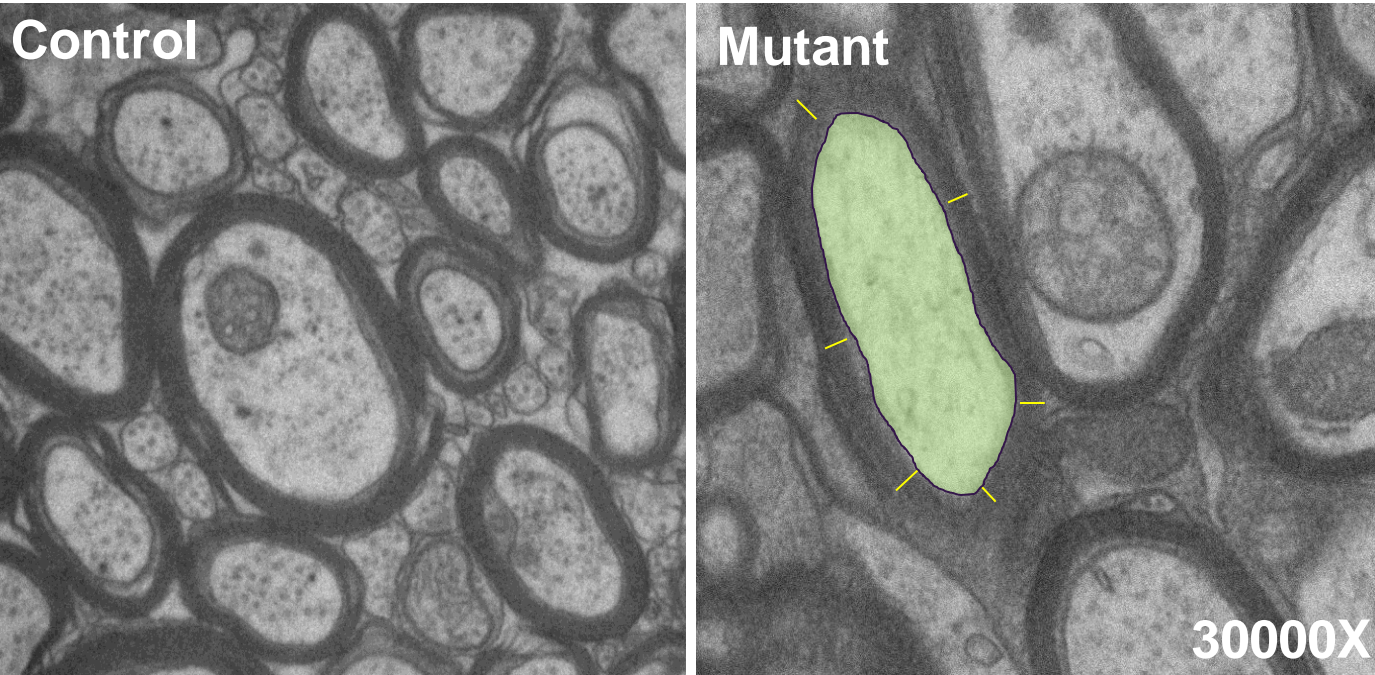
80,000x



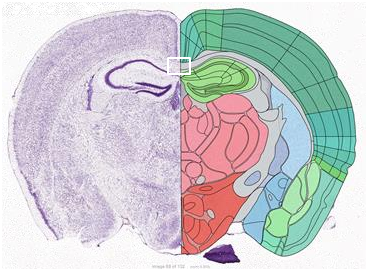
Michael Delannoy

JHU MicFac
Hitachi 7600 TEM

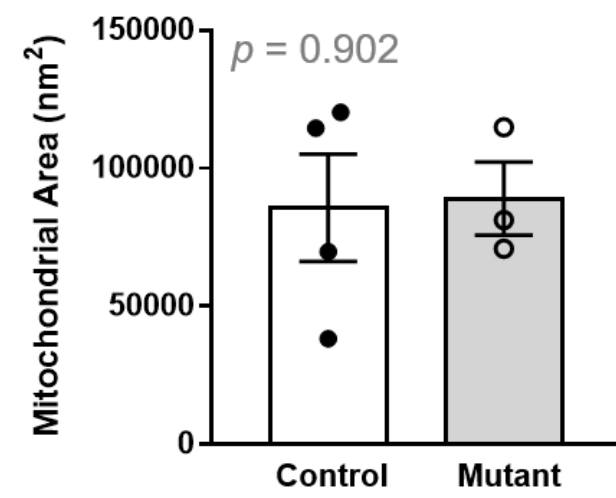
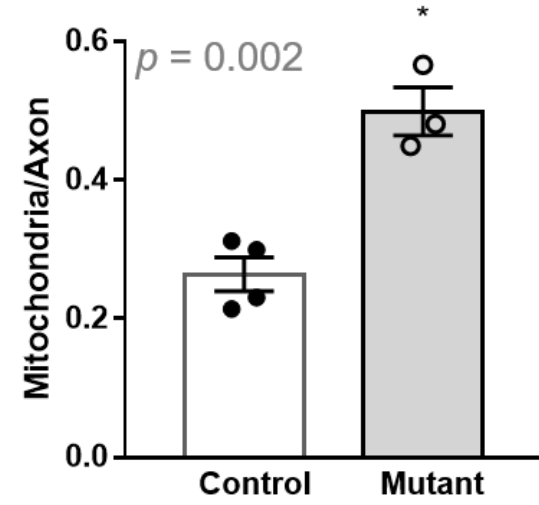
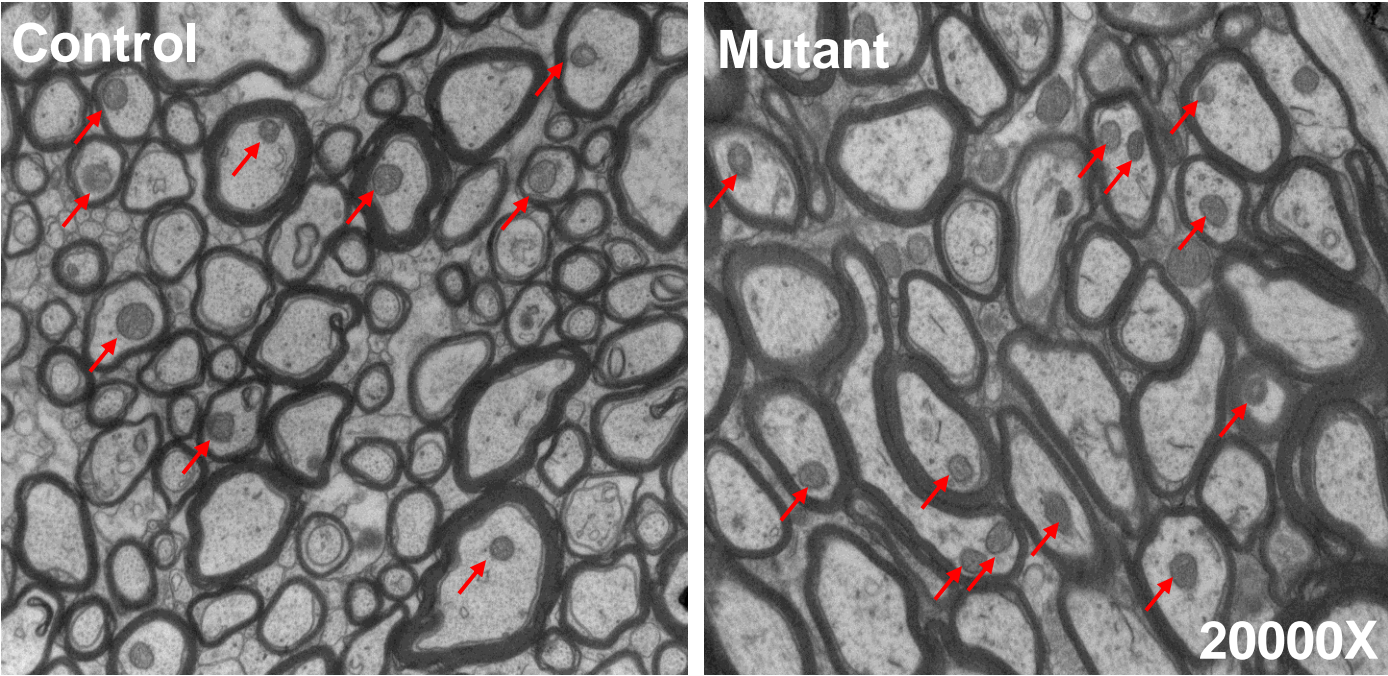
DARS2 deficiency alters brain axons

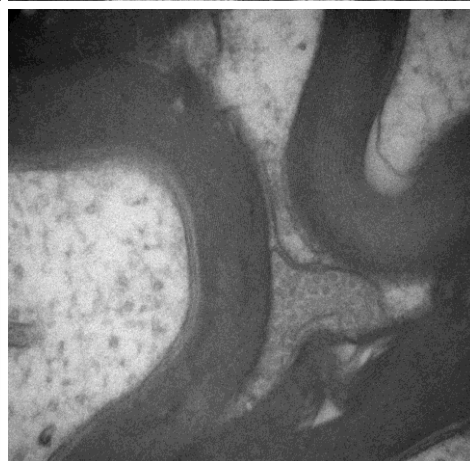
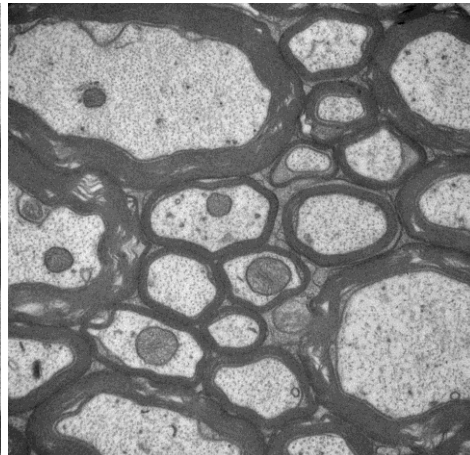
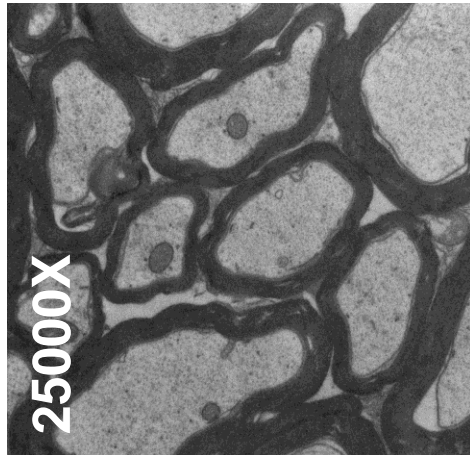
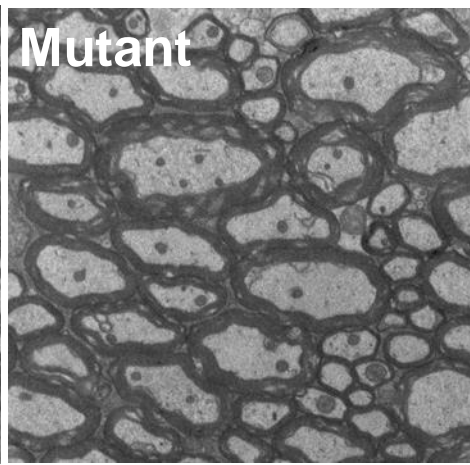
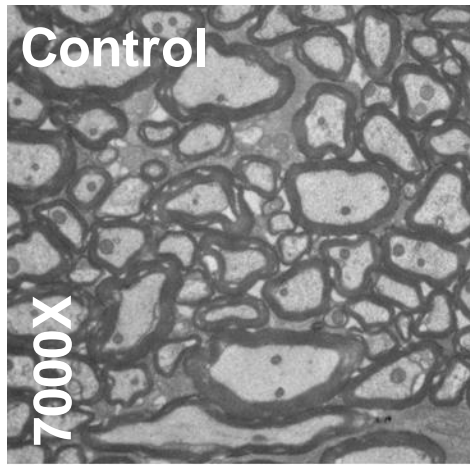


JHU MicFac; Hitachi 7600 TEM

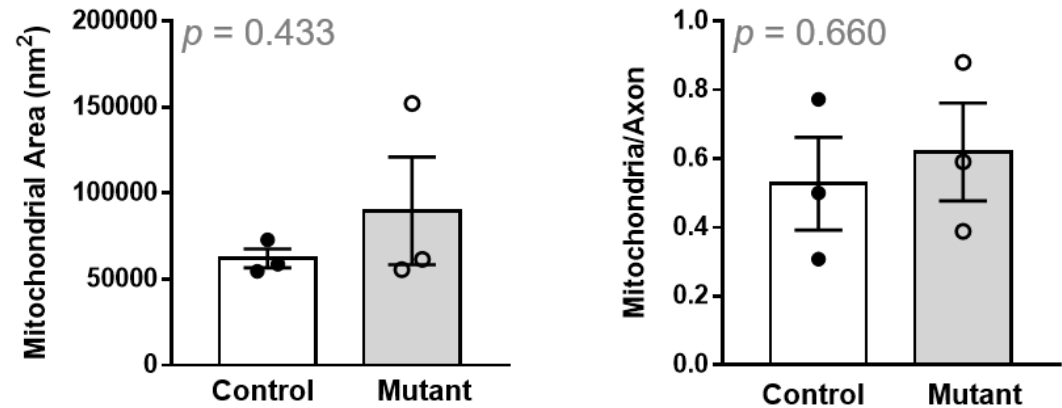
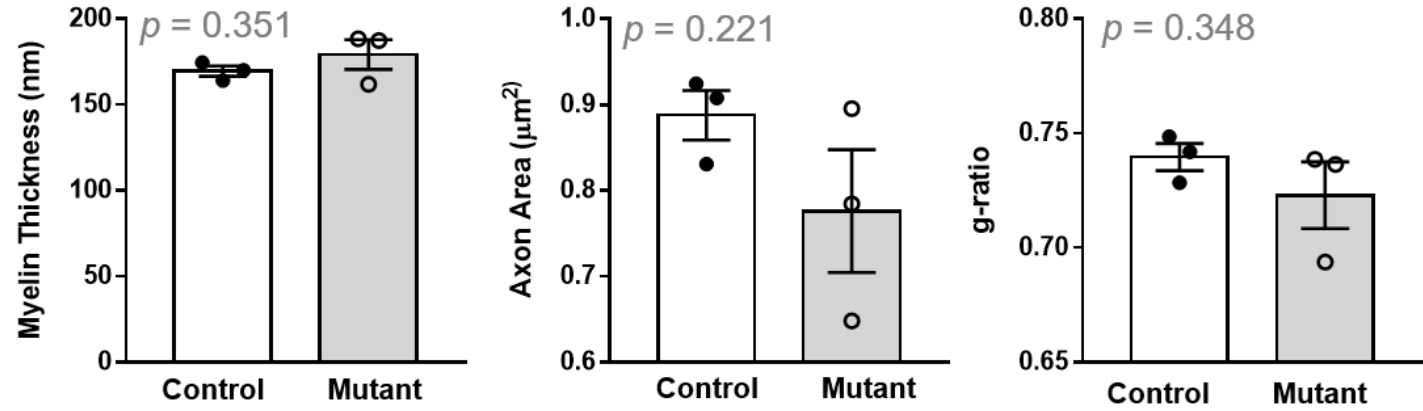


DARS2 deficiency increases mitochondrial count in brain axons

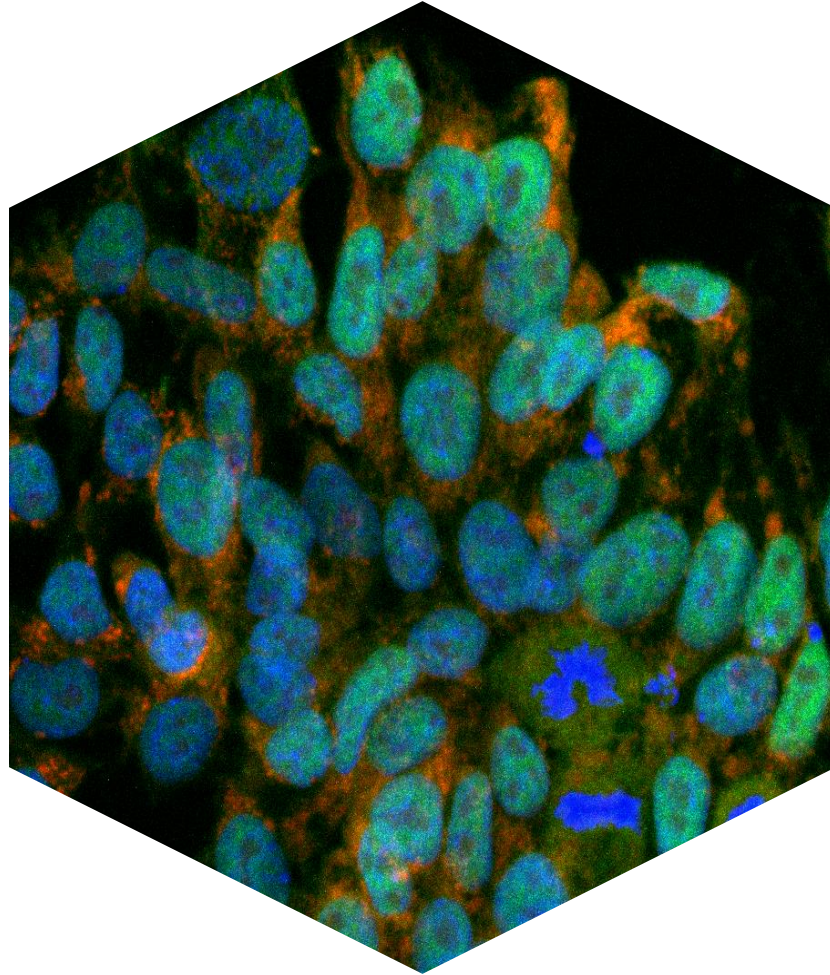




DARS2 deficiency in the brain does not alter nerves of the spinal cord



Outline



Induced Pluripotent Stem Cells

- What are they?
- How do we get them?
- Our data so far

Why iPSCs?

Non-invasive method with lots of flexibility

Allows us to assess the effect of specific mutations

Can be turned in to almost any cell type

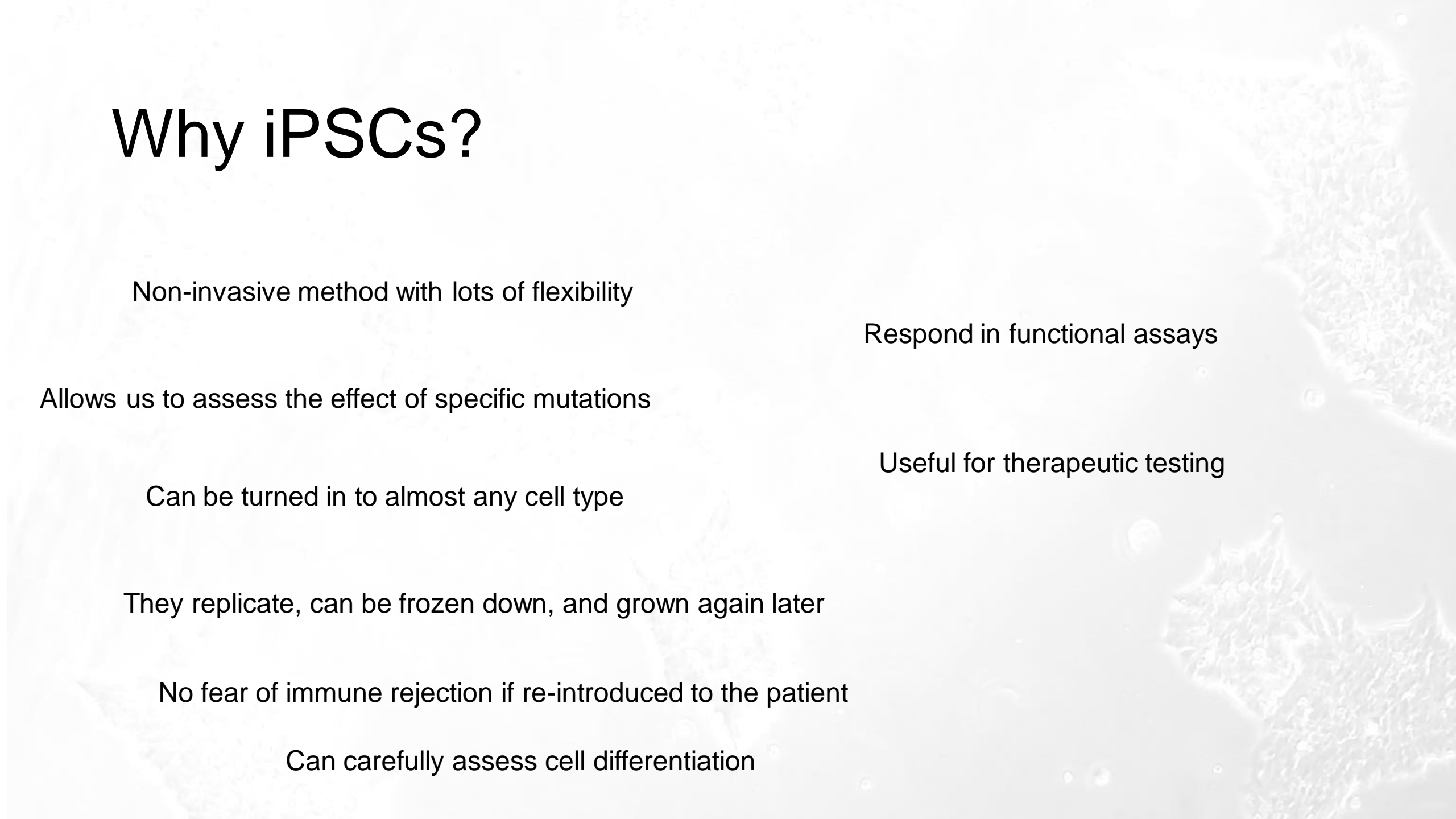
They replicate, can be frozen down, and grown again later

No fear of immune rejection if re-introduced to the patient

Can carefully assess cell differentiation

Respond in functional assays

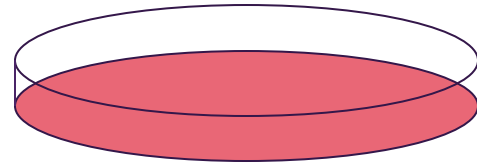
Useful for therapeutic testing



Induced Pluripotent Stem Cells (iPSCs)

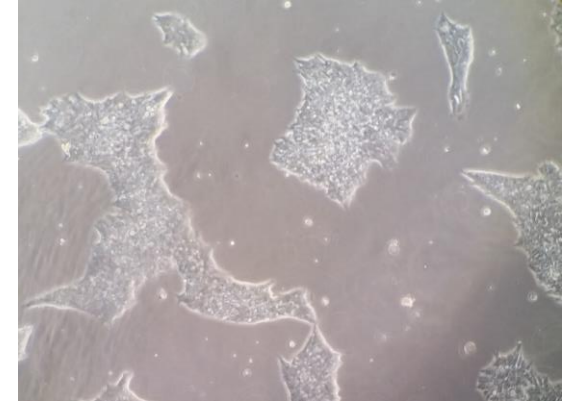


Isolate peripheral blood monocyte cells

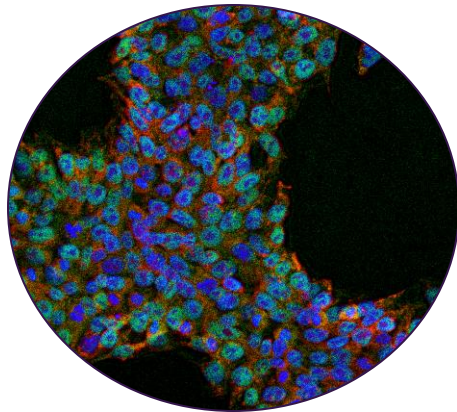


Cells are reprogrammed to induce pluripotency

Cells are monitored and tested to ensure reprogramming was successful



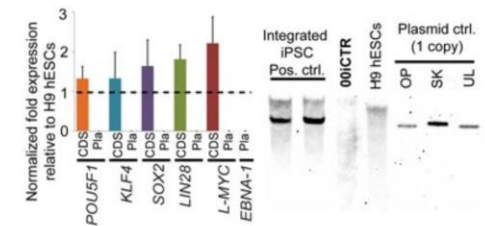
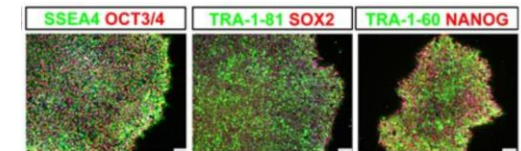
Cells are karyotyped, tested for pluripotency, and screened for bacterium



3 – 4 months



Frozen cells are mailed to us



From iPSCs to neurons

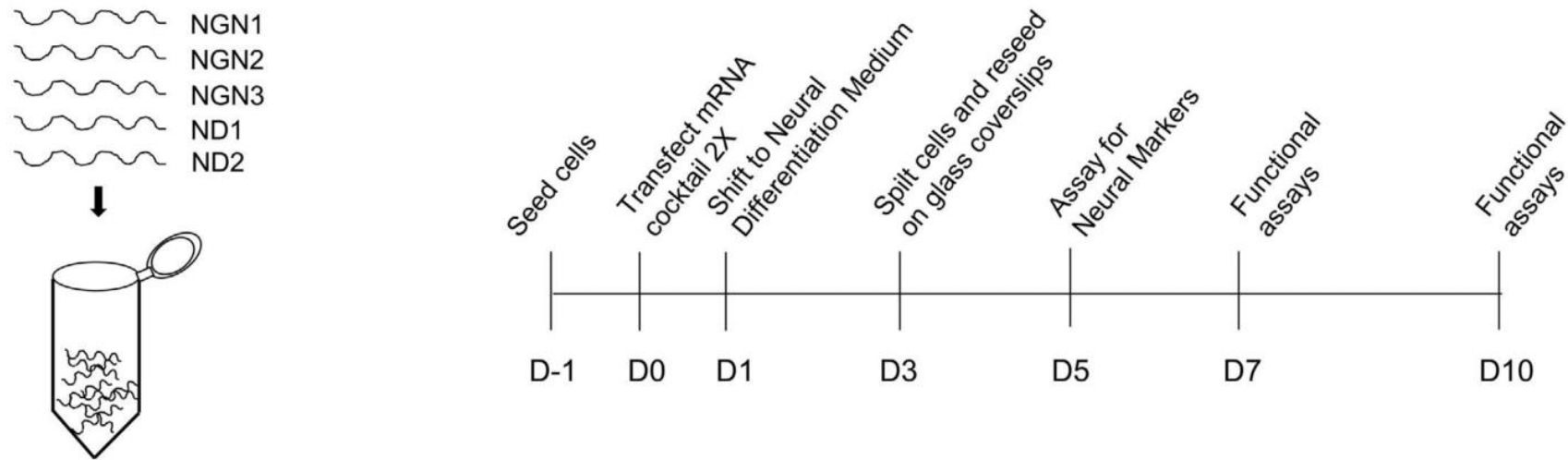
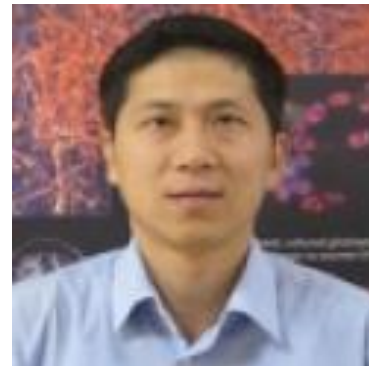
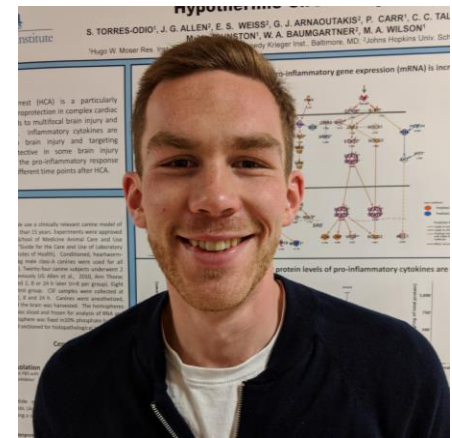


Figure 2. Induction of neurogenesis by syn-TFs mRNAs of Neurogenin and NeuroD families.

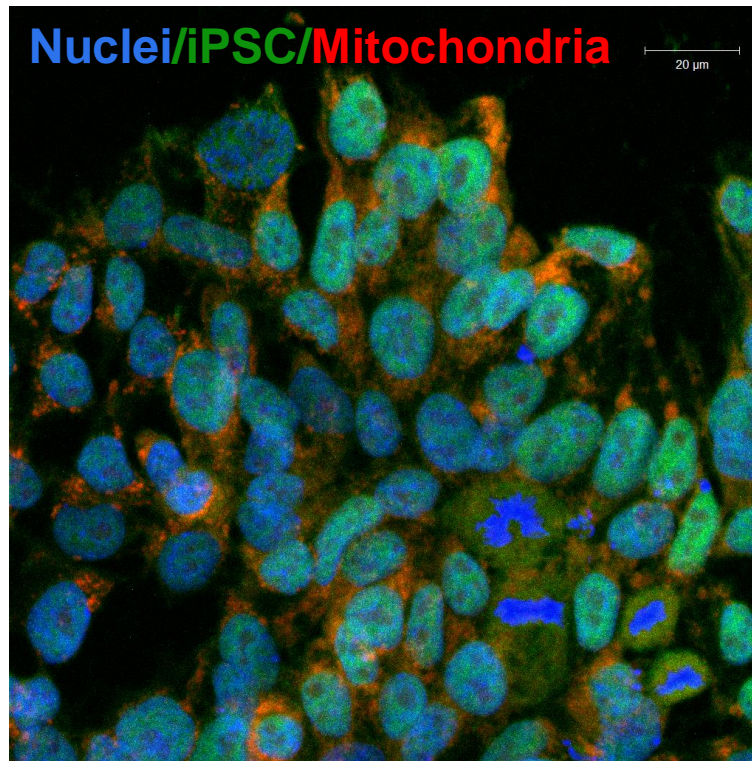
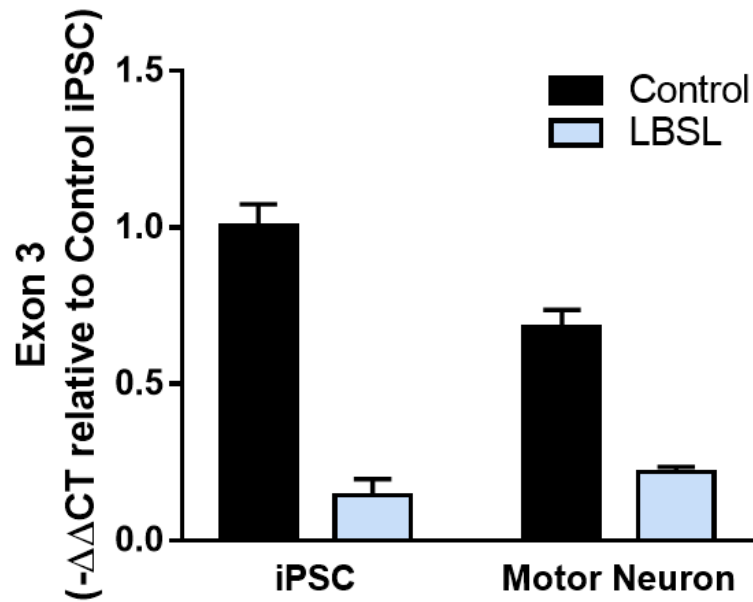


Mingyao Ying, PhD

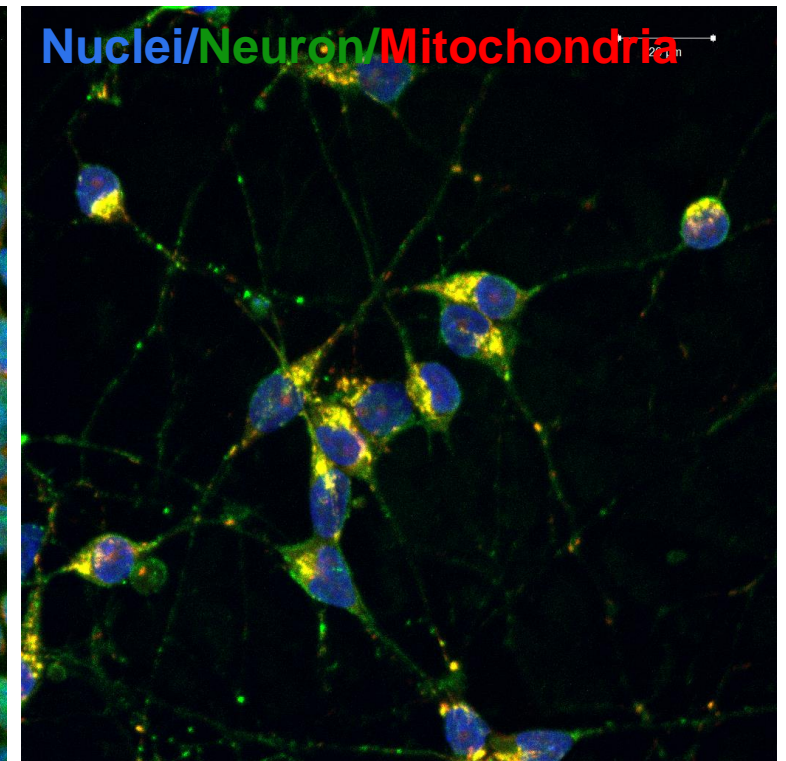
iPSC derived motor neurons



Philippe Hubo



iPSC



motor neuron

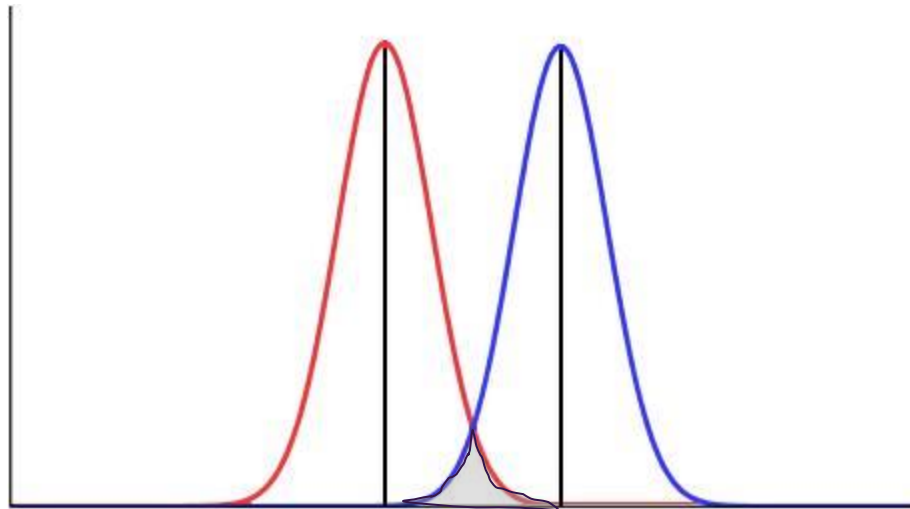
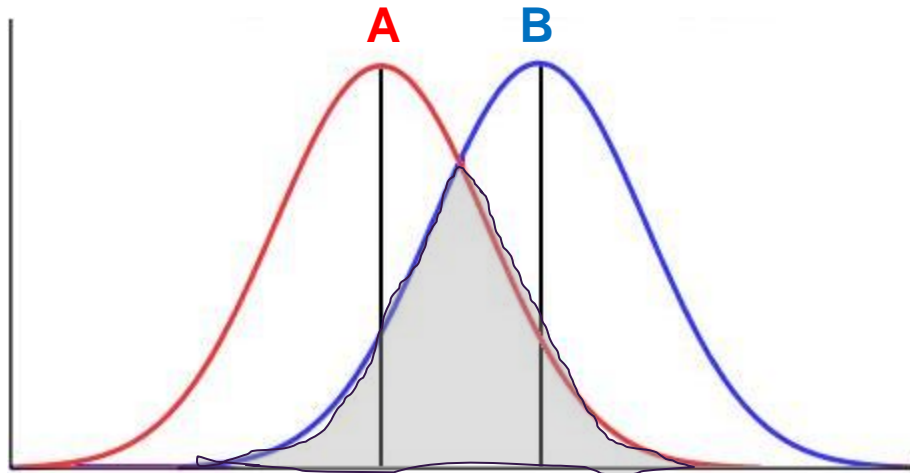
Outline

Potential for Therapeutic Testing

- Understanding numbers
- Animals and cells as platforms



The numbers



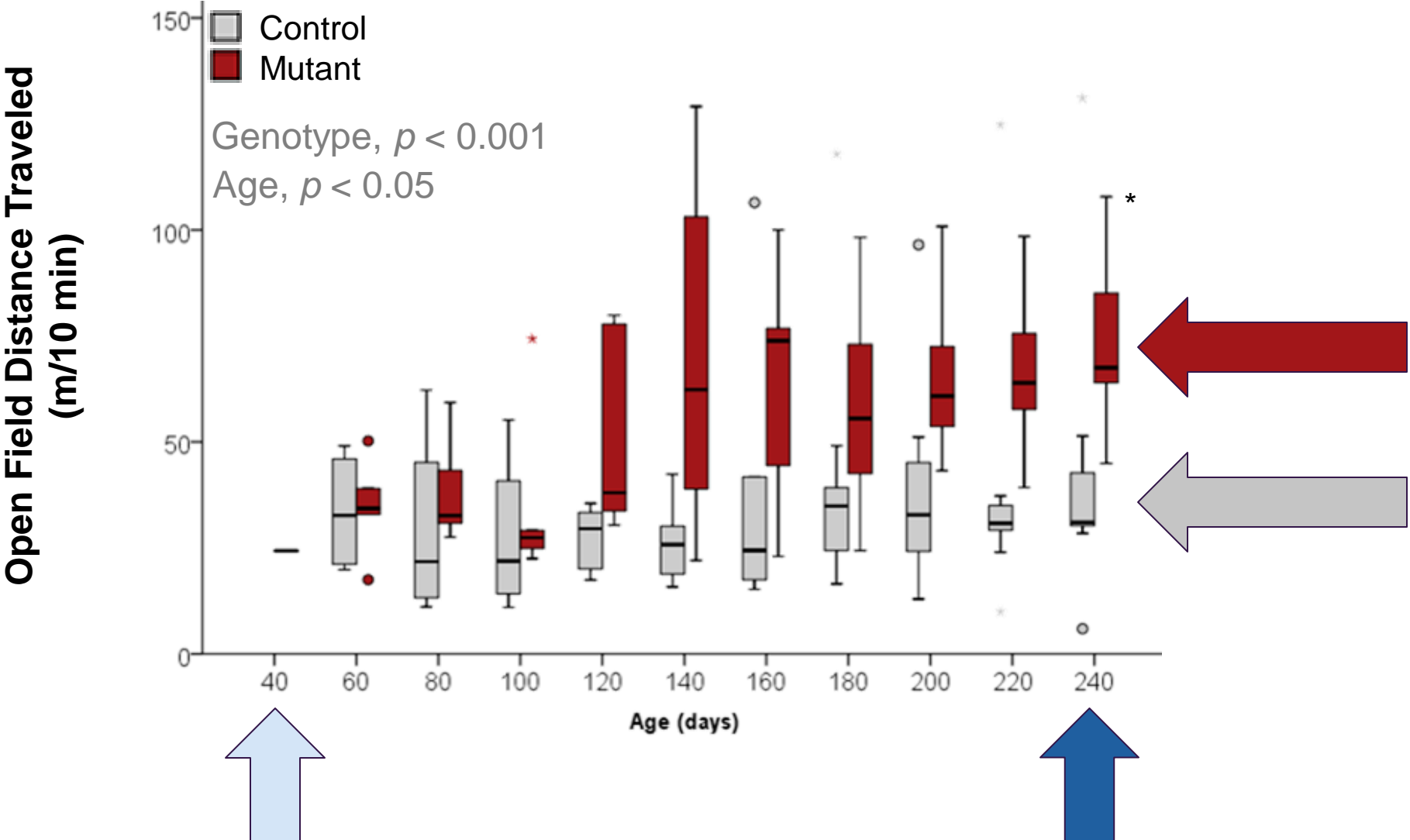
Sample sizes (# of animals) are calculated based on **statistical POWER**, or the confidence that your groups are truly different.

Animals are randomly assigned to groups and balanced for:

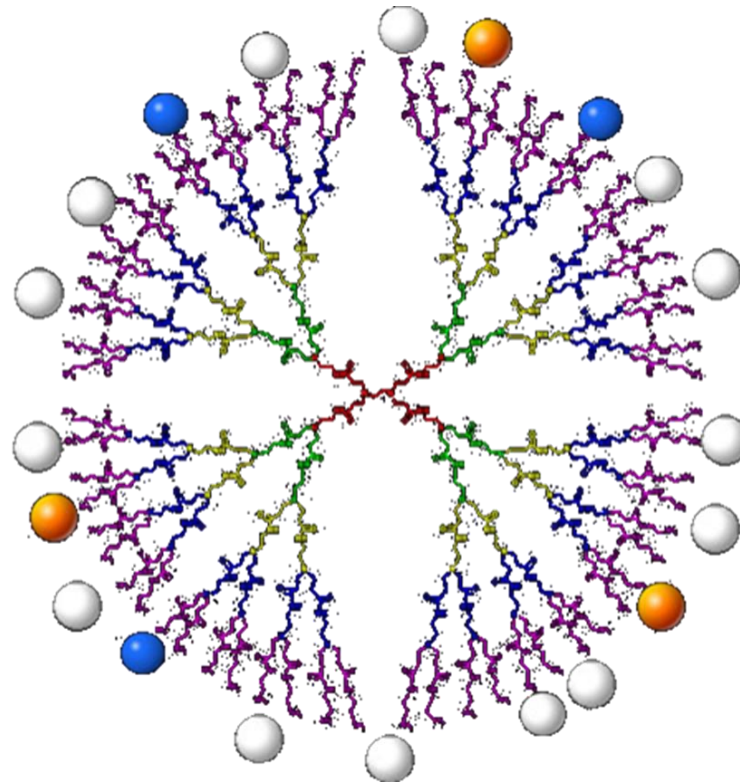
- Sex
- Litter
- Weight
- Housing

To achieve a less than < 5% chance of incorrect interpretation

The numbers



The Dendrimer Platform



4 – 10 nm in diameter

(a human hair is 50,000 nm thick!)



RM Kannan, PhD
Sujatha Kannan, MD



Outline



Collaborations

- Cerebral Organoids
- RNAseq

From iPSCs to “mini brains”

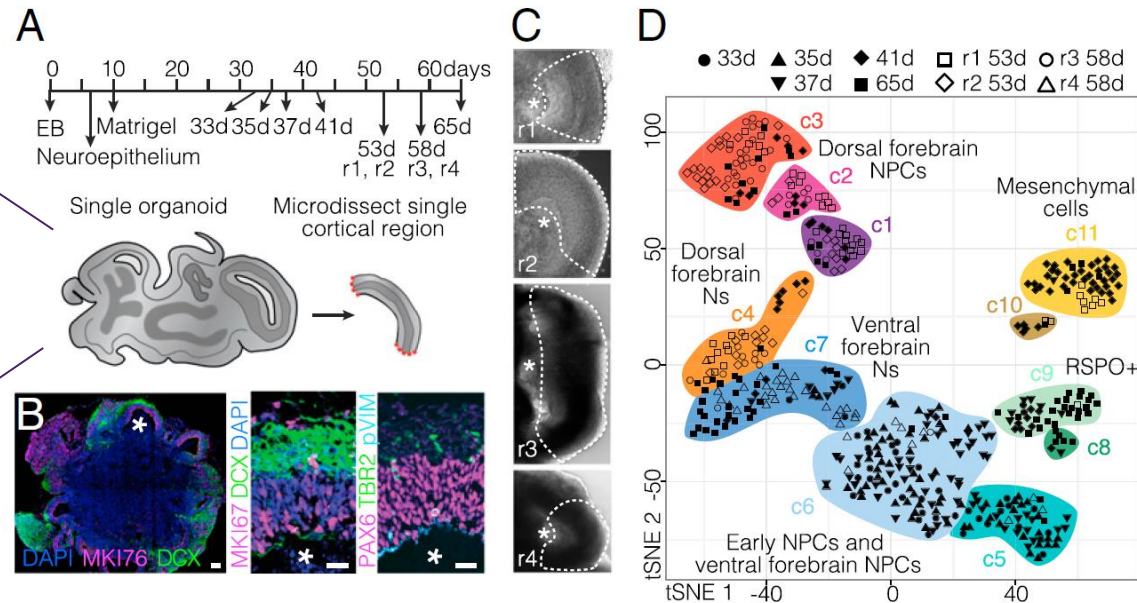
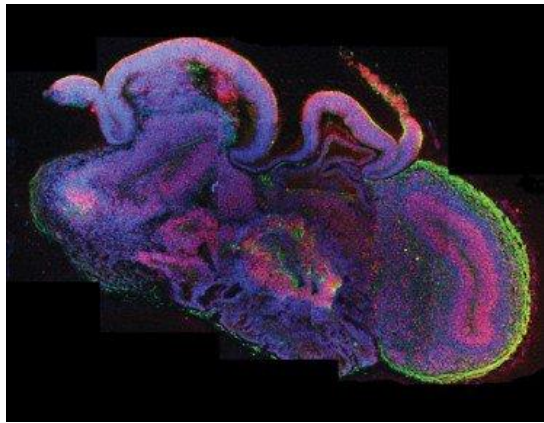
Human cerebral organoids recapitulate gene expression programs of fetal neocortex development

J. Gray Camp^{a,1}, Farhath Badsha^{b,1}, Marta Florio^b, Sabina Kanton^a, Tobias Gerber^a, Michaela Wilsch-Bräuninger^b, Eric Lewitus^c, Alex Sykes^b, Wulf Hevers^a, Madeline Lancaster^{d,e}, Juergen A. Knoblich^e, Robert Lachmann^f, Svante Pääbo^{a,2}, Wieland B. Huttner^{b,2}, and Barbara Treutlein^{a,b,2}

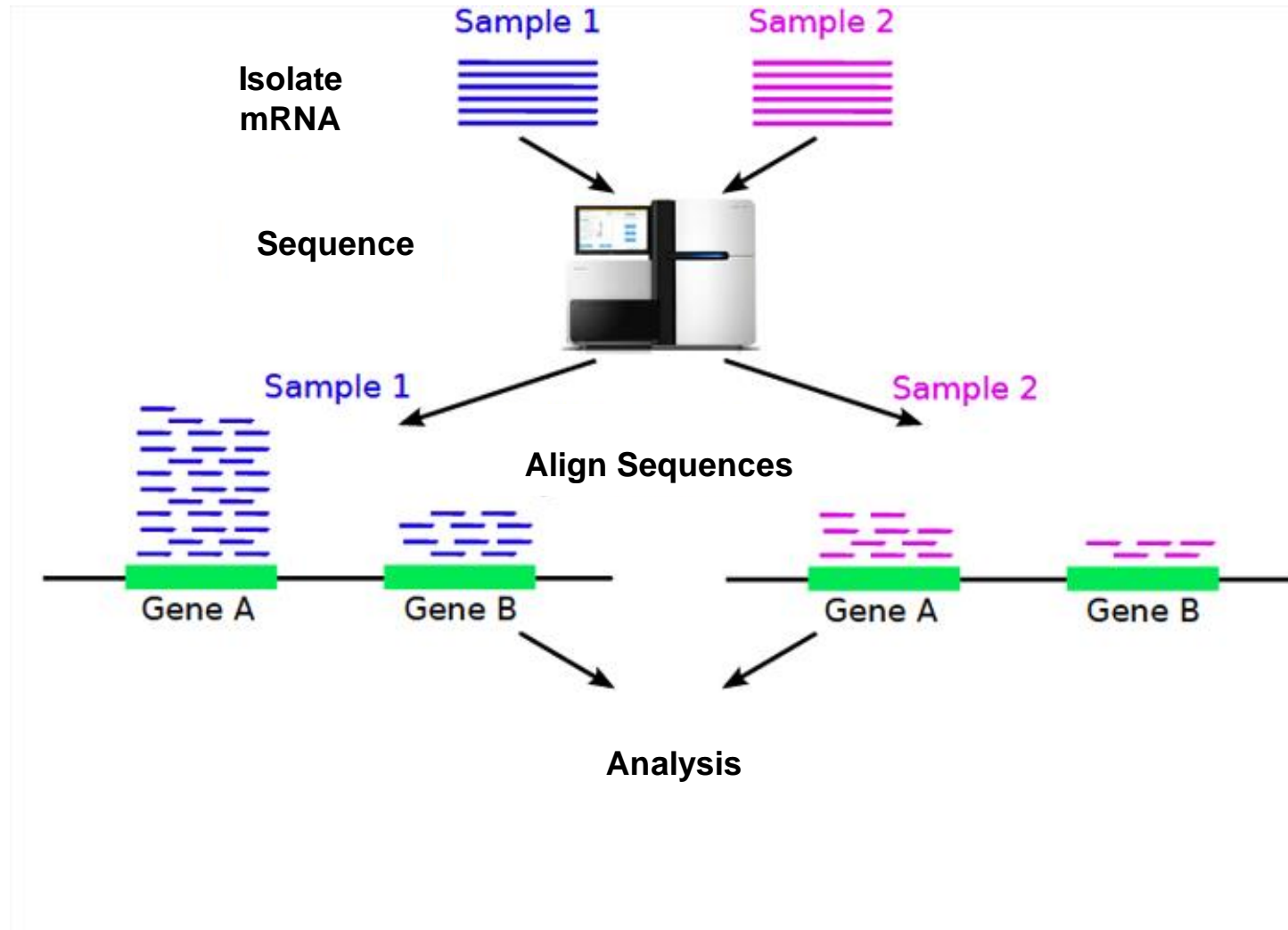
^aMax Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, 04103 Leipzig, Germany; ^bMax Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany; ^cInstitut de Biologie, Ecole Normale Supérieure, 75005 Paris, France; ^dMedical Research Council Laboratory of Molecular Biology, Cambridge CB2 0QH, United Kingdom; ^eInstitute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), 1030 Vienna, Austria; and ^fTechnische Universität Dresden, Universitätsklinikum Carl Gustav Carus, Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, 01307 Dresden, Germany



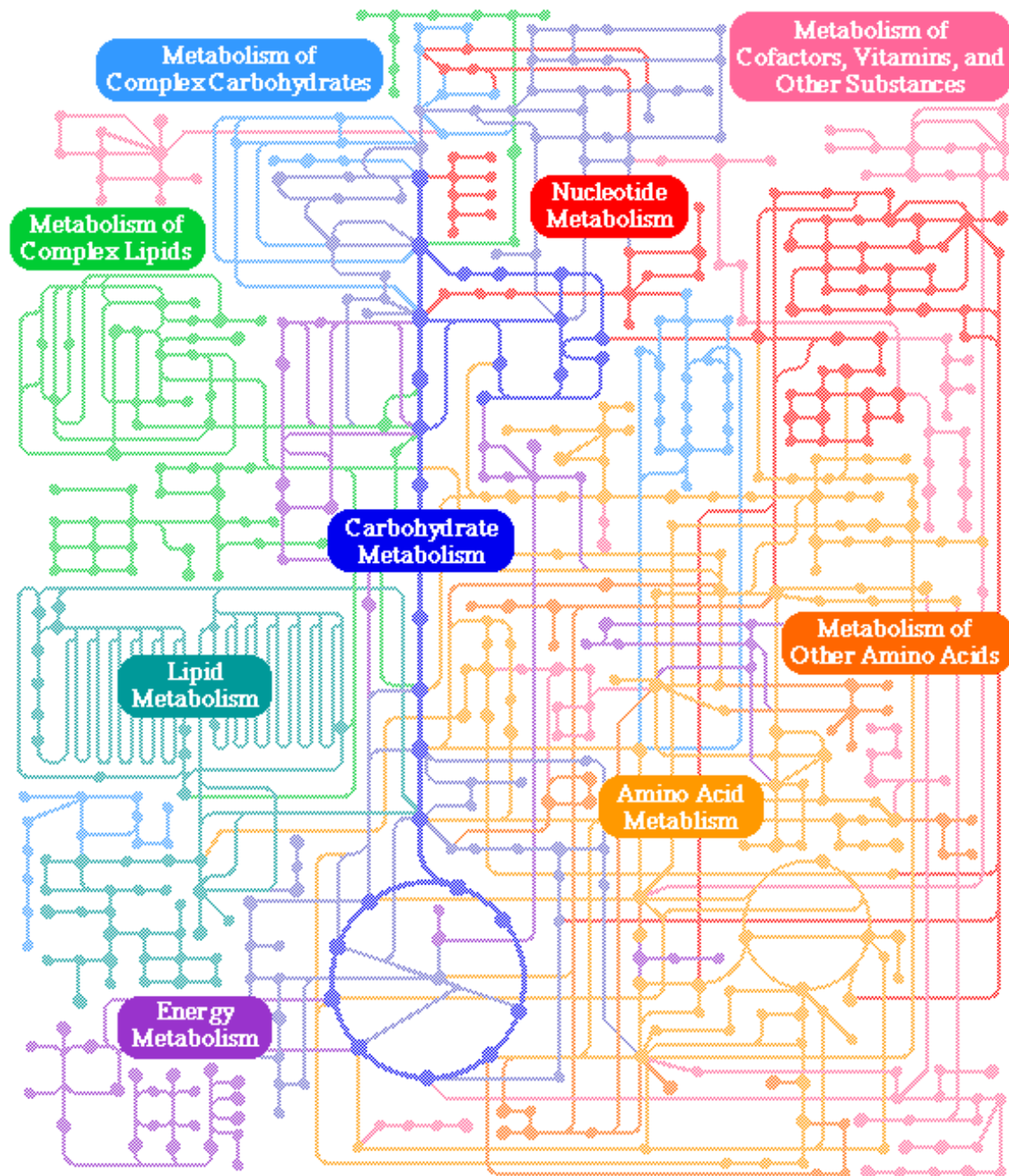
Paul Tesar, PhD



RNA-Seq



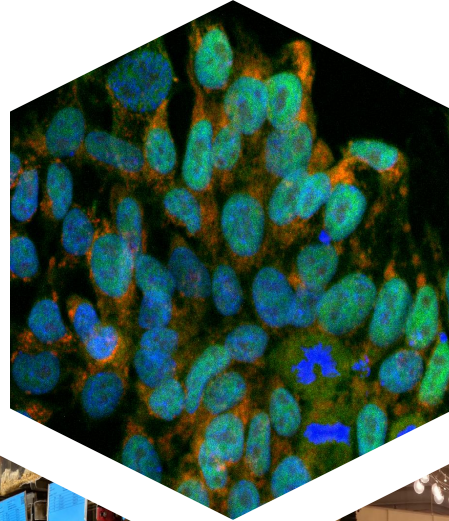
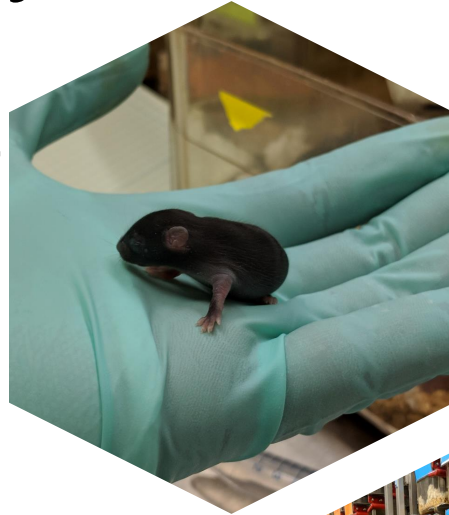
METABOLIC PATHWAYS



Summary

Mouse Models

- Mutant animals show loss of cells
- Increase in brain inflammation
- Increase in overall activity



Induced Pluripotent Stem Cells

- Can examine almost any cell type
- Mutation specific

Potential for Therapeutic Testing

- Dendrimer platform is in testing
- Cells and animals show deficits that we can attempt to remedy



Collaborations

- LBSL patient cells are being grown into cerebral organoids
- Will begin global gene expression studies on mice