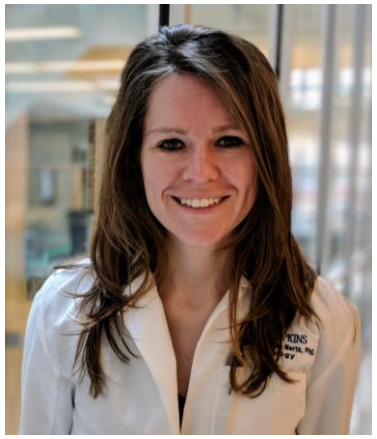
Laboratory Research Update

Christina Nemeth Mertz, PhD

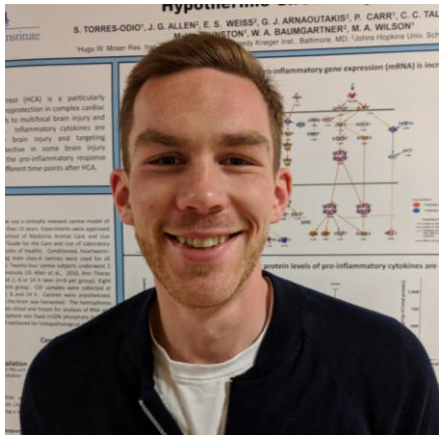
April 19. 2018

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

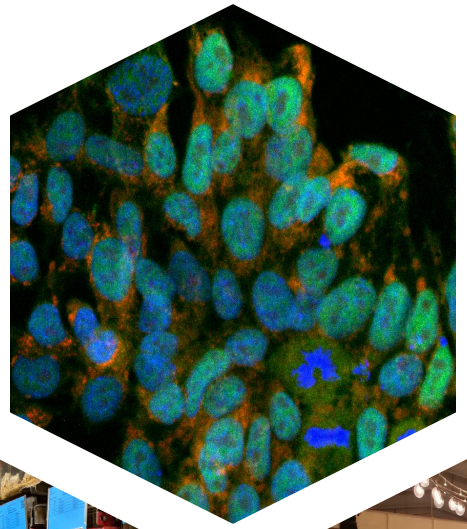
Research Technician
2016-2017

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

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-what we know about DARS2 in mice

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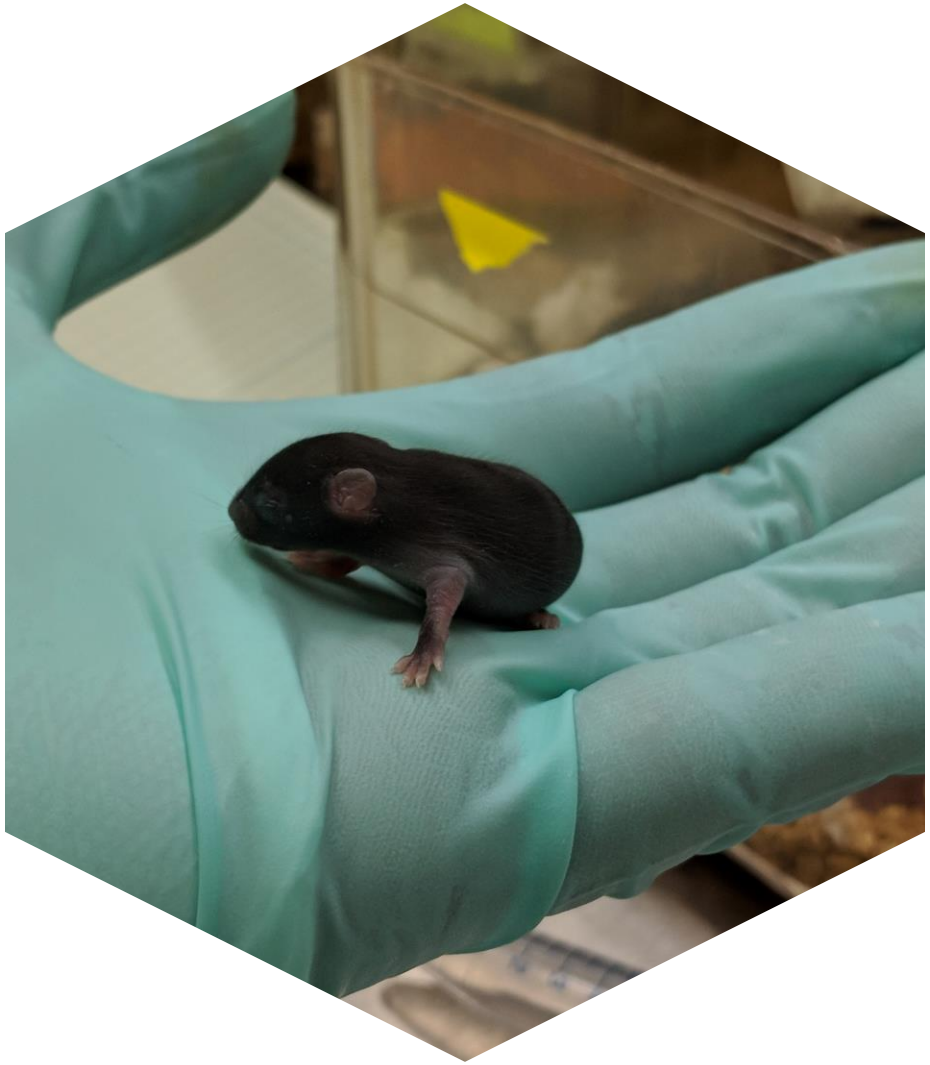


Why mice?

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)

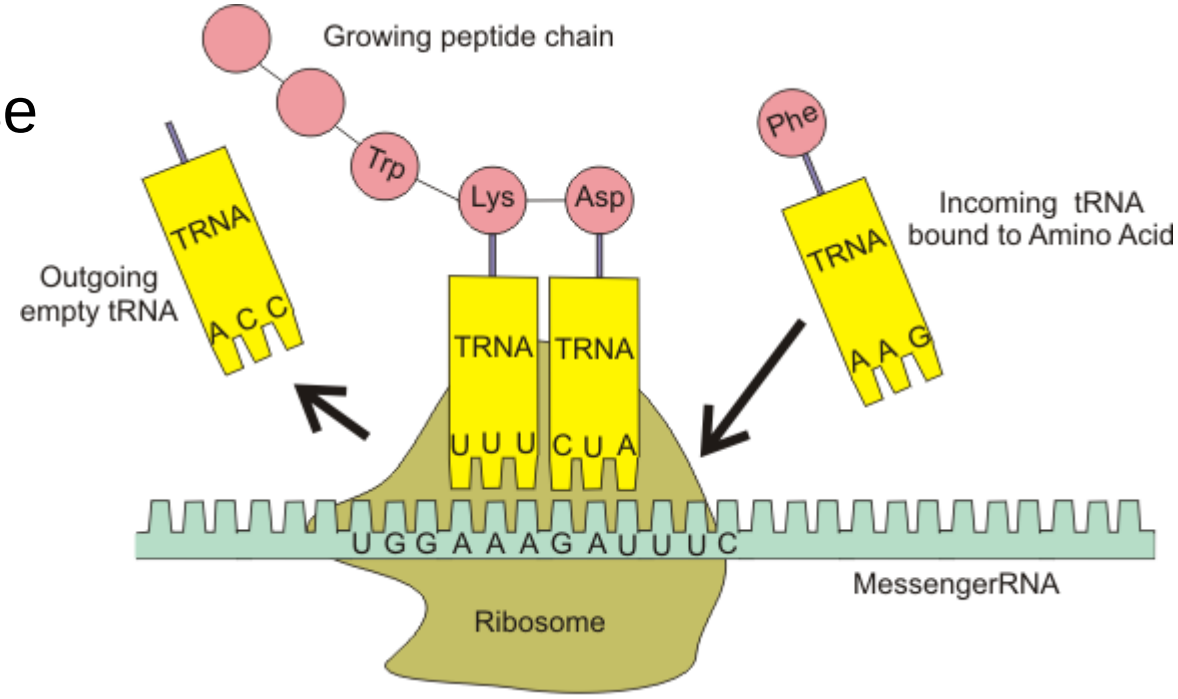
Excellent model for studying changes in motor function, behavior

Useful for testing therapies

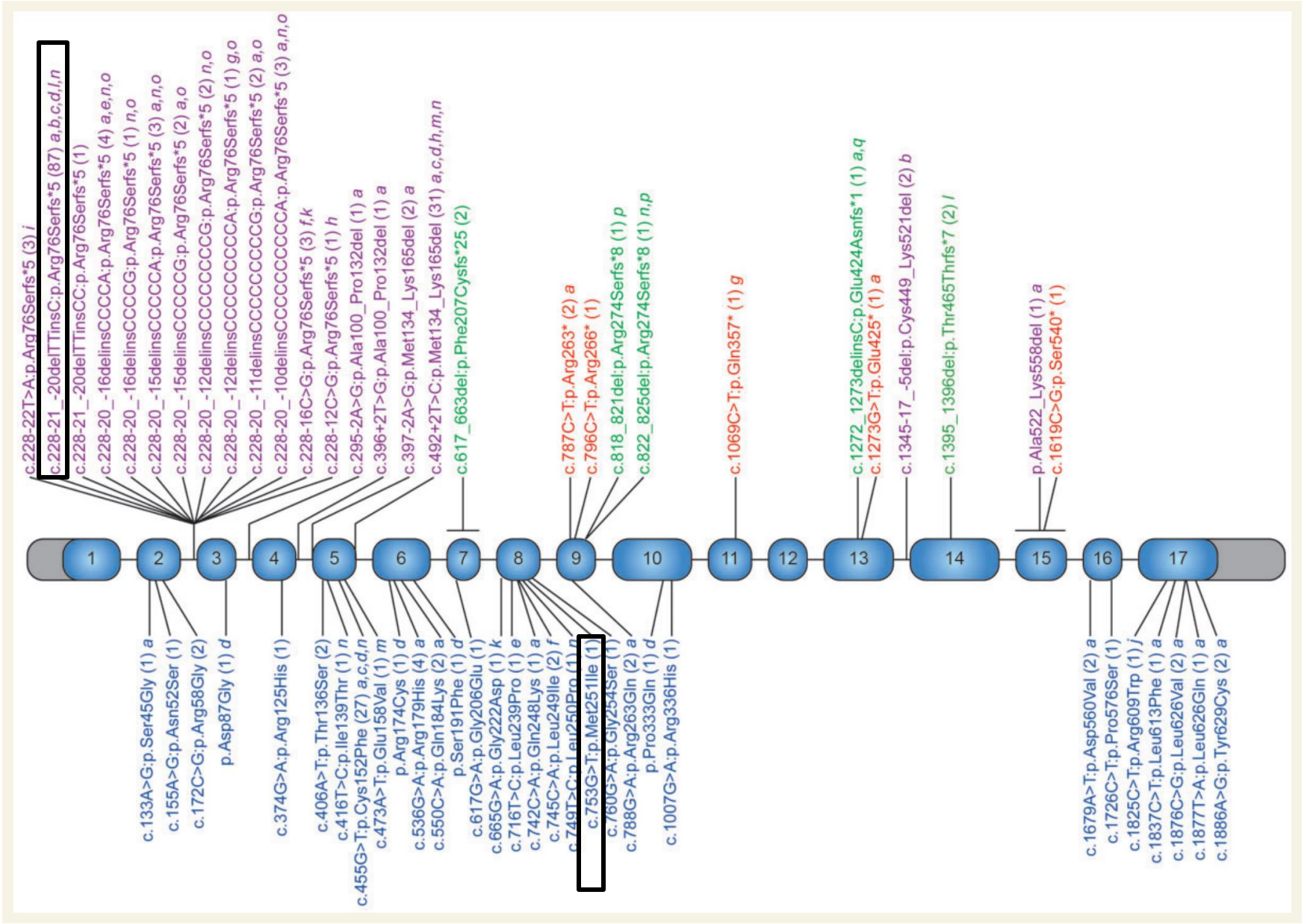
Not perfect!

LBSL and *DARS2*

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

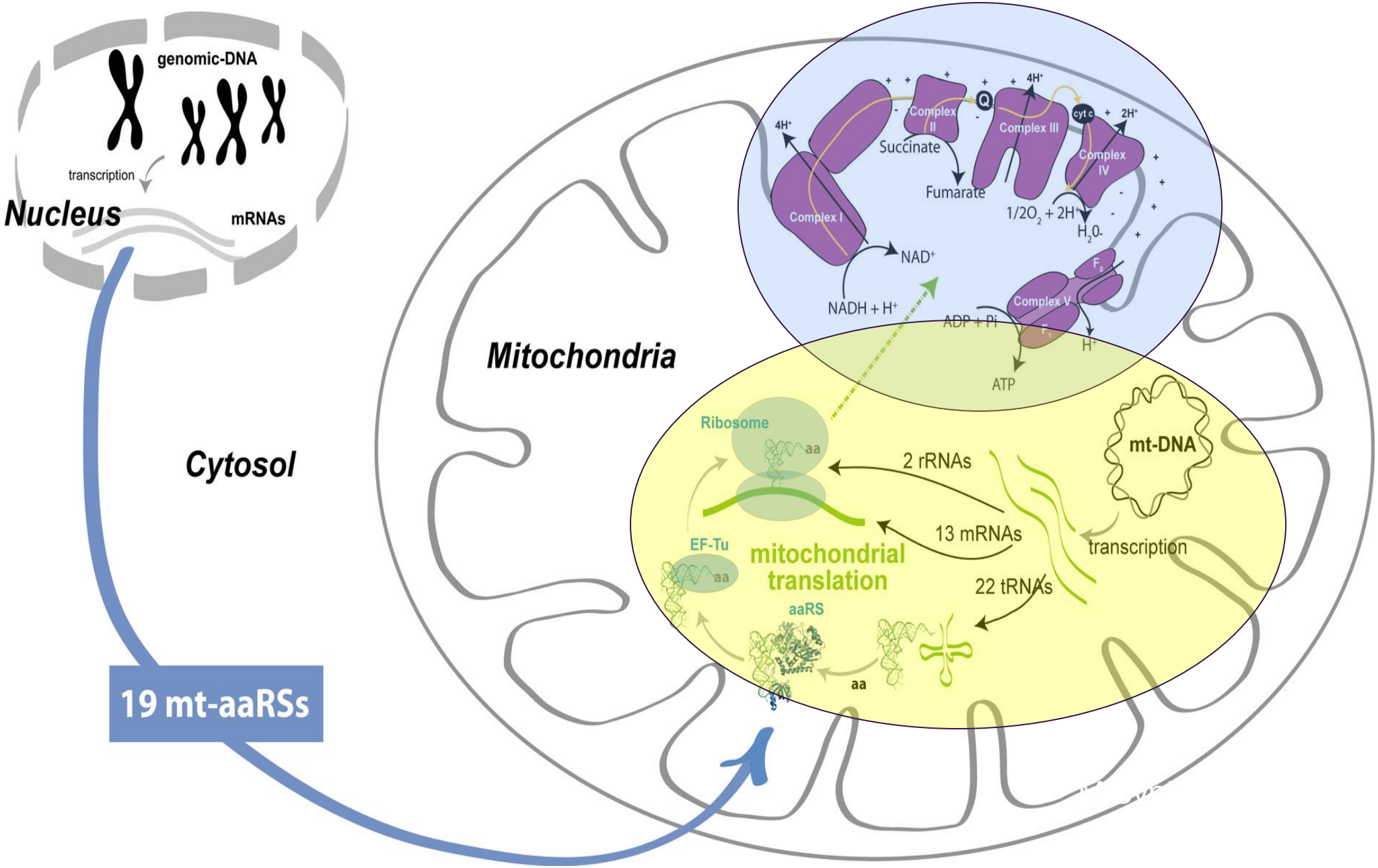


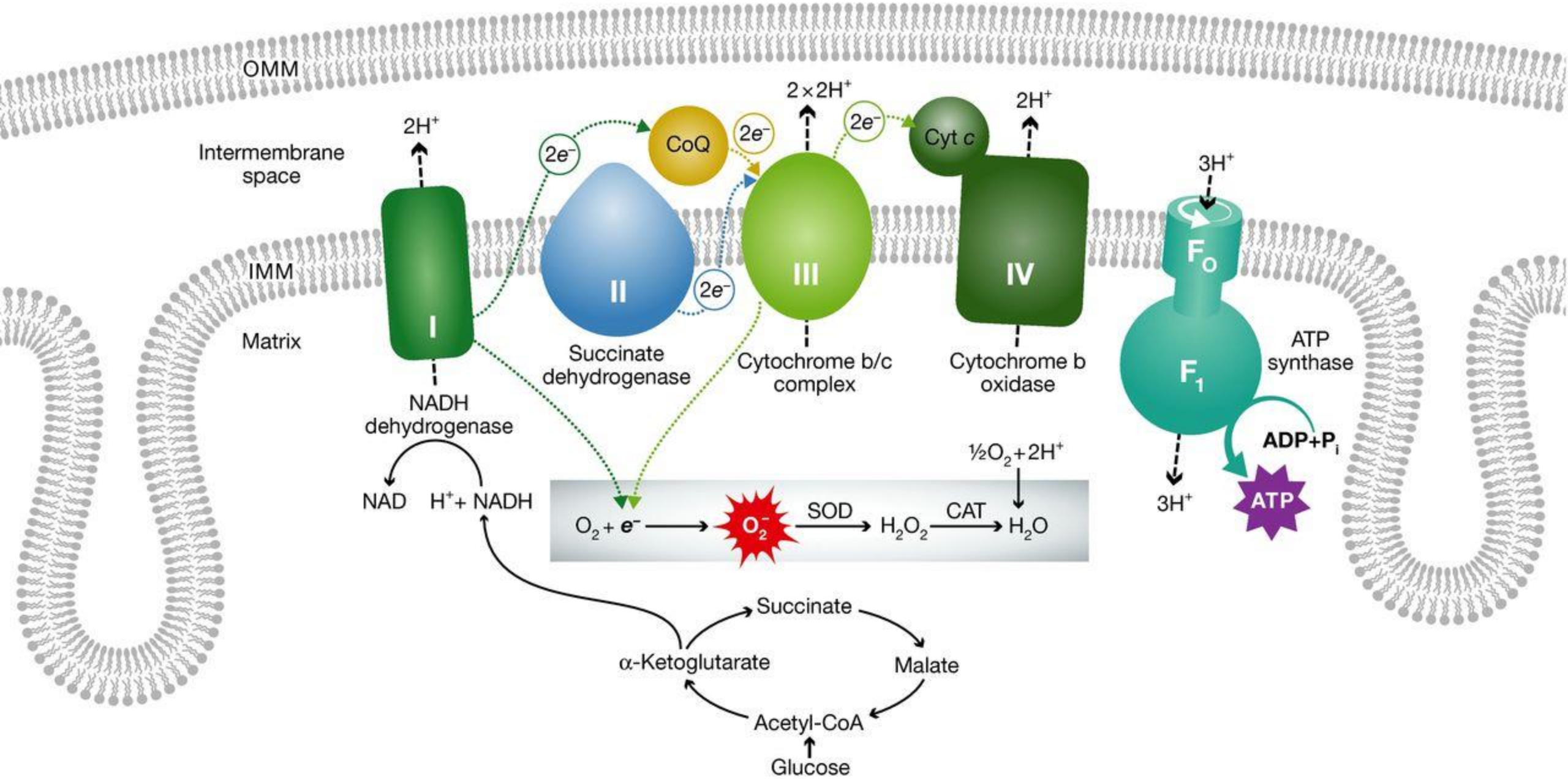
***DARS2* gene**



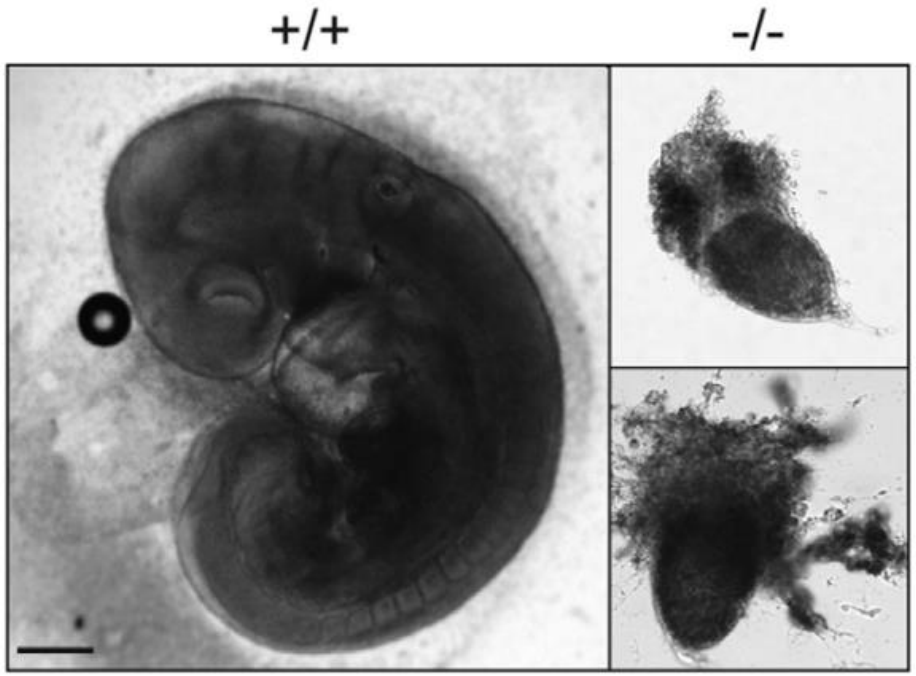
frameshift
missense
deletion
nonsense

van Berge et al., 2014; *Brain*

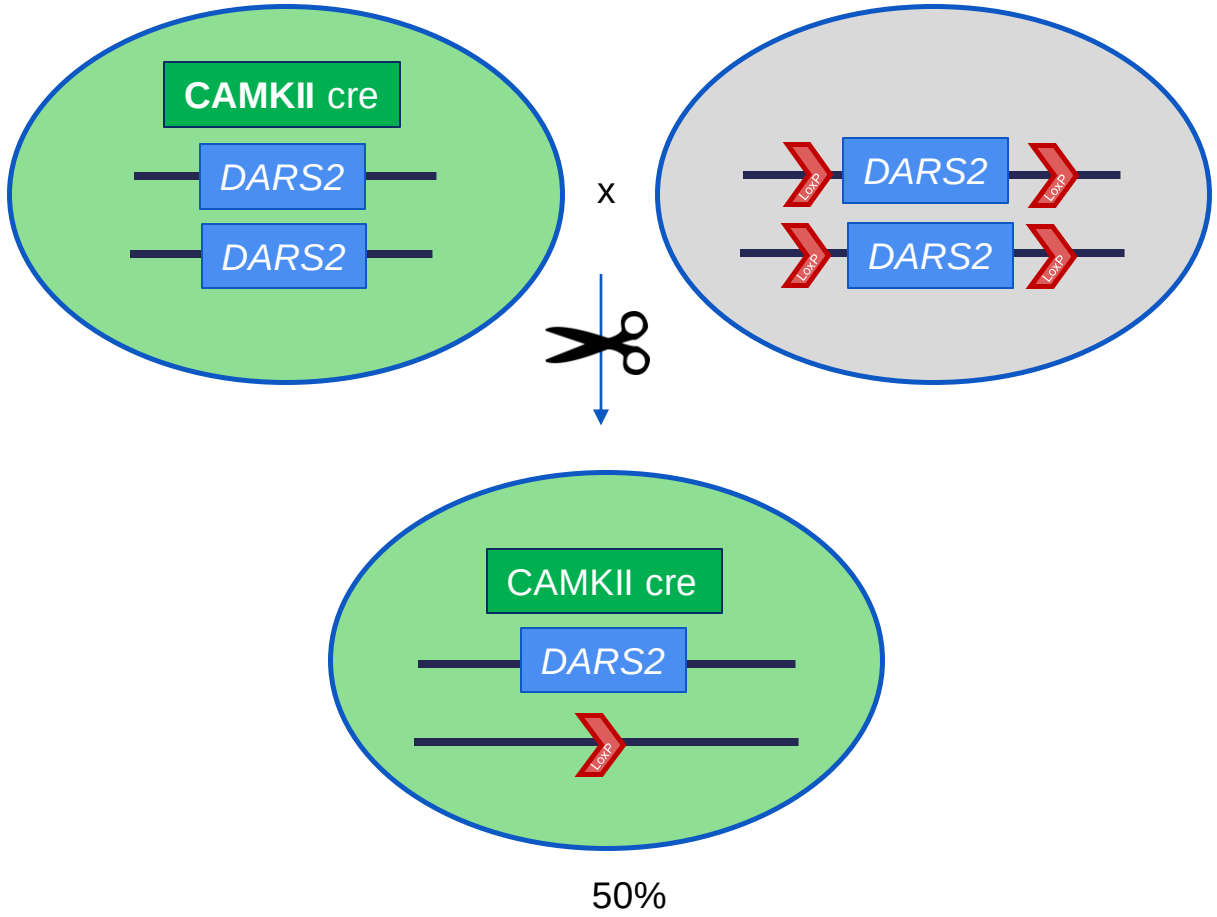



OMM
Intermembrane space
IMM
Matrix
2H+
2e−
CoQ
2e−
2×2H+
2e−
Cyt c
2H+
3H+
I
II
III
IV
Fo
F1
2e−
NADH dehydrogenase
Succinate dehydrogenase
Cytochrome b/c complex
Cytochrome b oxidase
ATP synthase
ADP+Pi
ATP
3H+
½O2+2H+
NAD
H+ + NADH
O2 + e−
O2−
SOD
H2O2
CAT
H2O
Succinate
α-Ketoglutarate
Malate
Acetyl-CoA
Glucose

Modeling LBSL



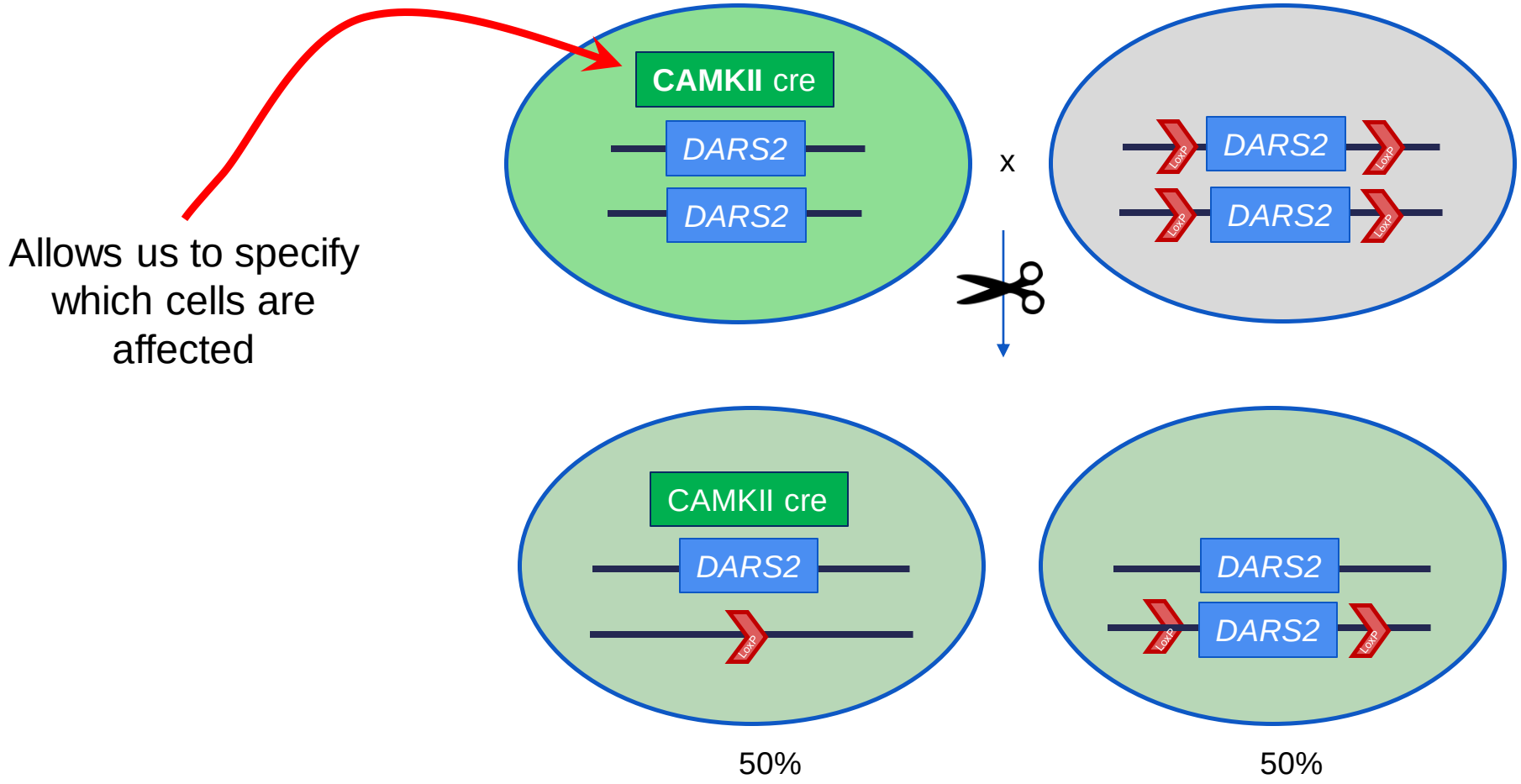
Cre-Lox Recombination



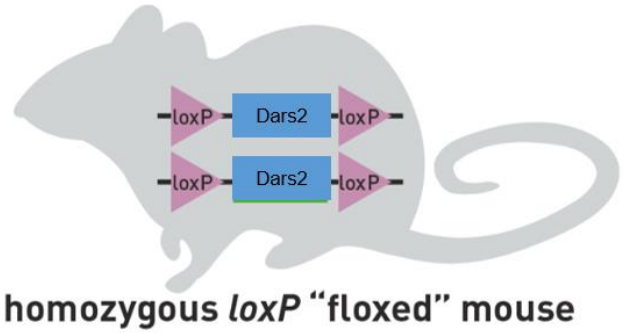
Full knockout of DARS2 is embryonic lethal and selective knockout results in mitochondrial dysfunction

Dogan et al, 2014; *Cell Metab*

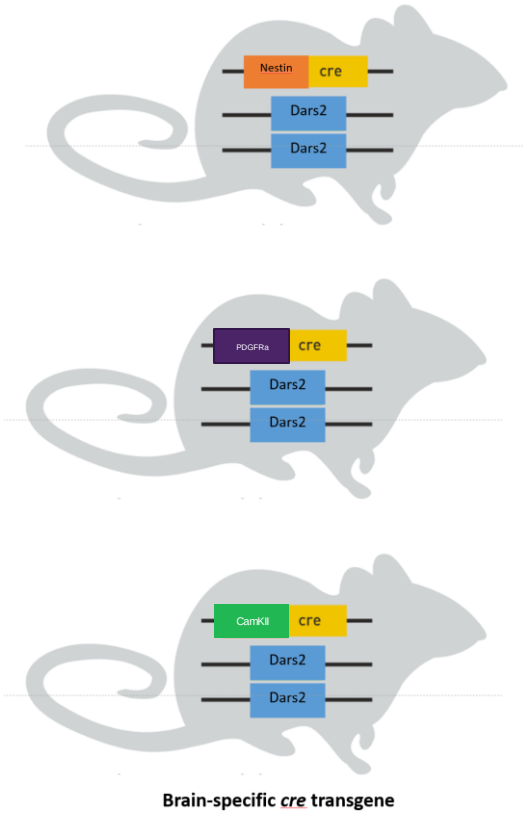
How does Cre-Lox work?



Our mice



homozygous *loxP* "floxed" mouse



Brain-specific *cre* transgene

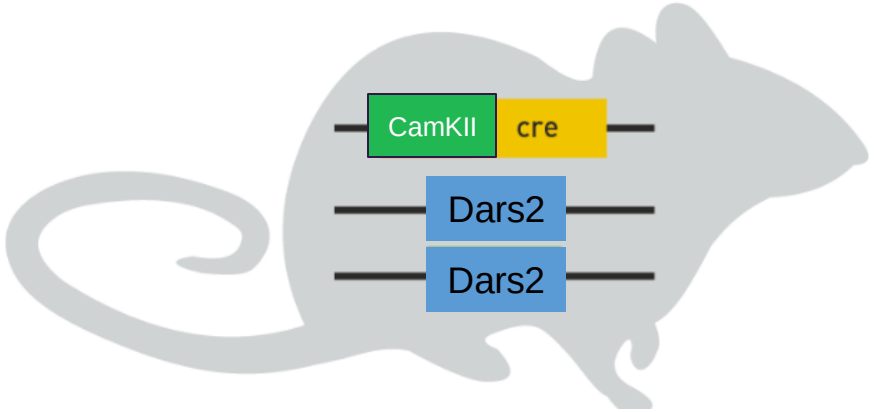
Received from Dr. Aleksandra Trifunovic
at CECAD, Cologne, Germany

Purchased from Jackson Labs:

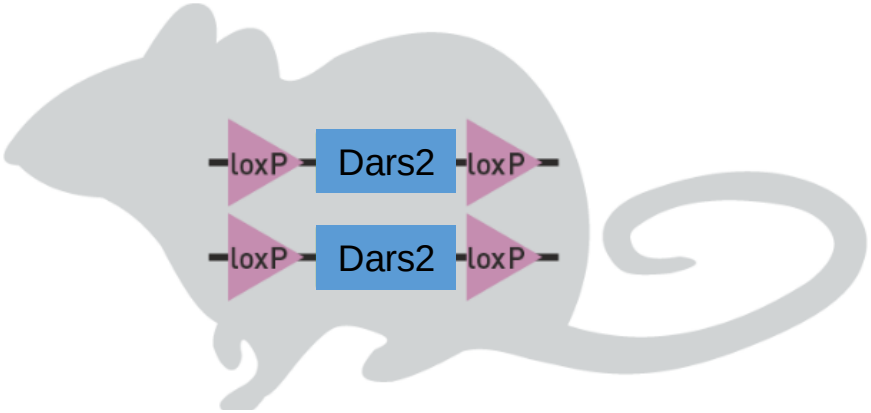
Nestin: neuronal precursor cells (all)
CamKIIα: neurons in forebrain
PDGFRα: oligodendrocyte precursors (myelin)

The Jackson Laboratory

FOUNDER



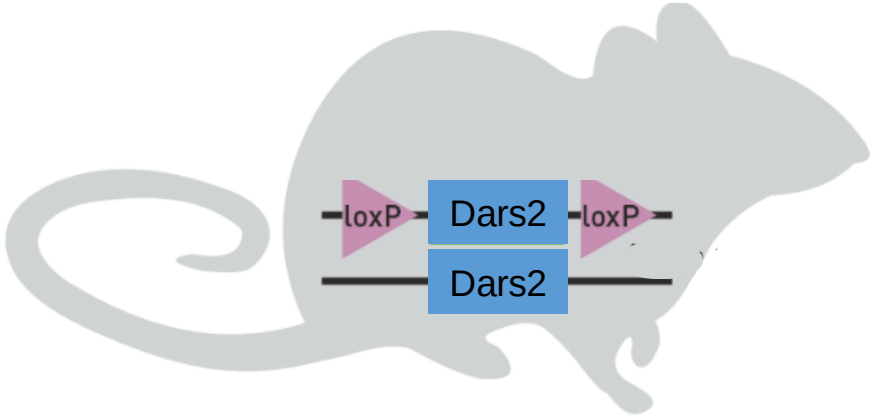
X



Brain-specific *cre* transgene

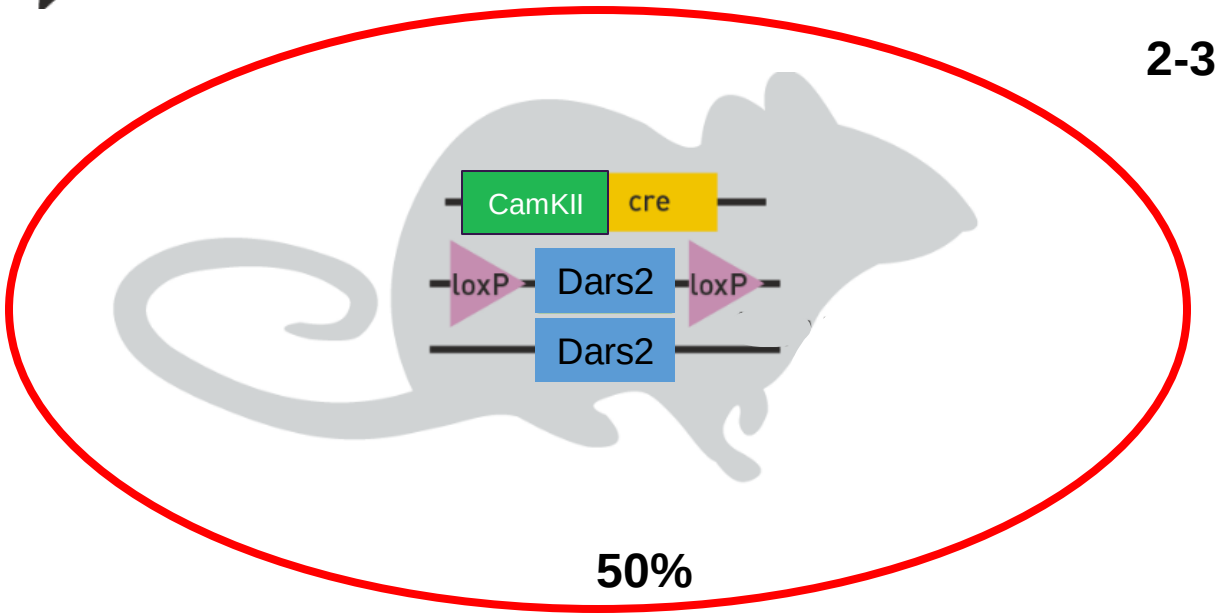
Homozygous loxP “floxed” mouse

1st GENERATION

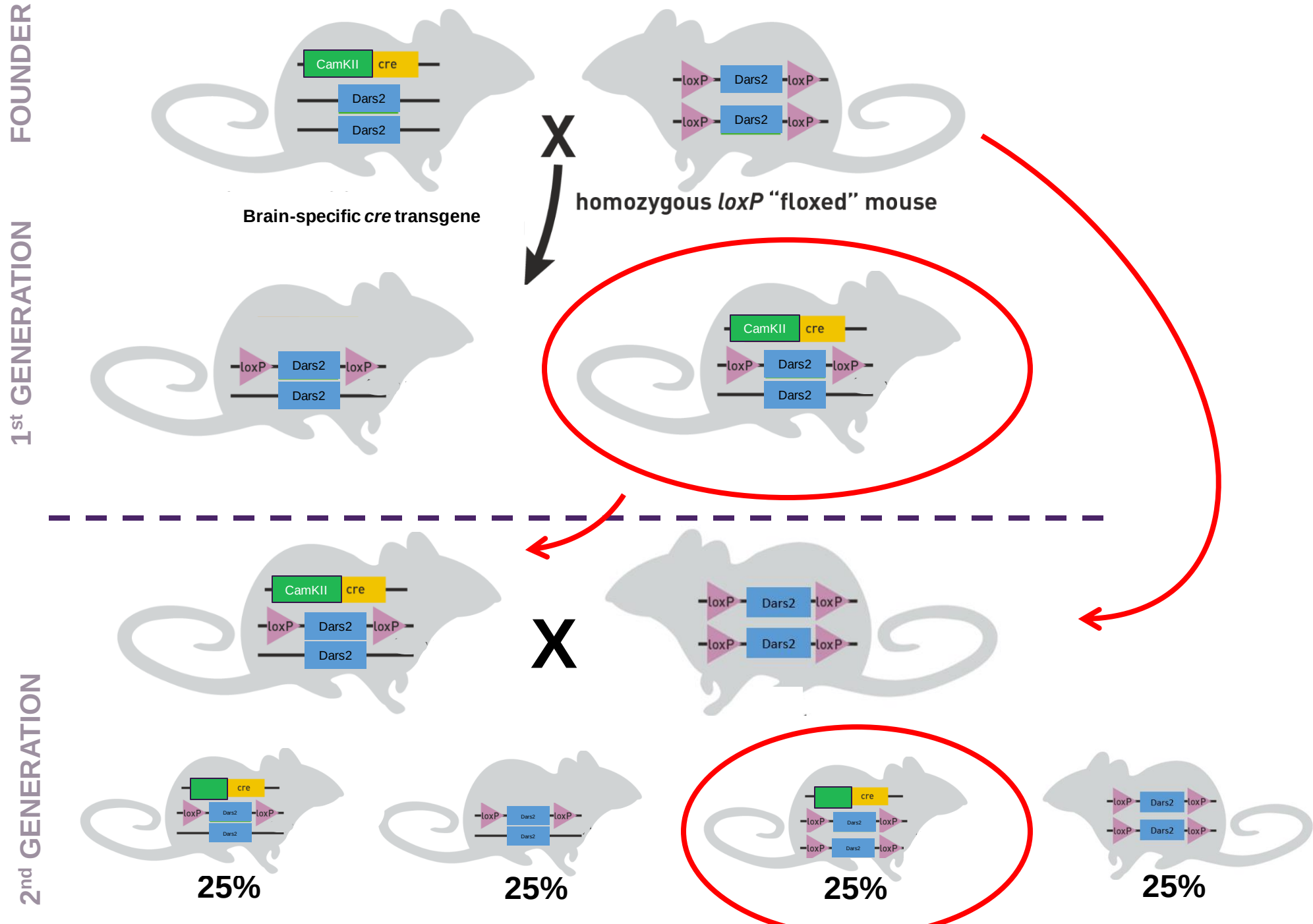


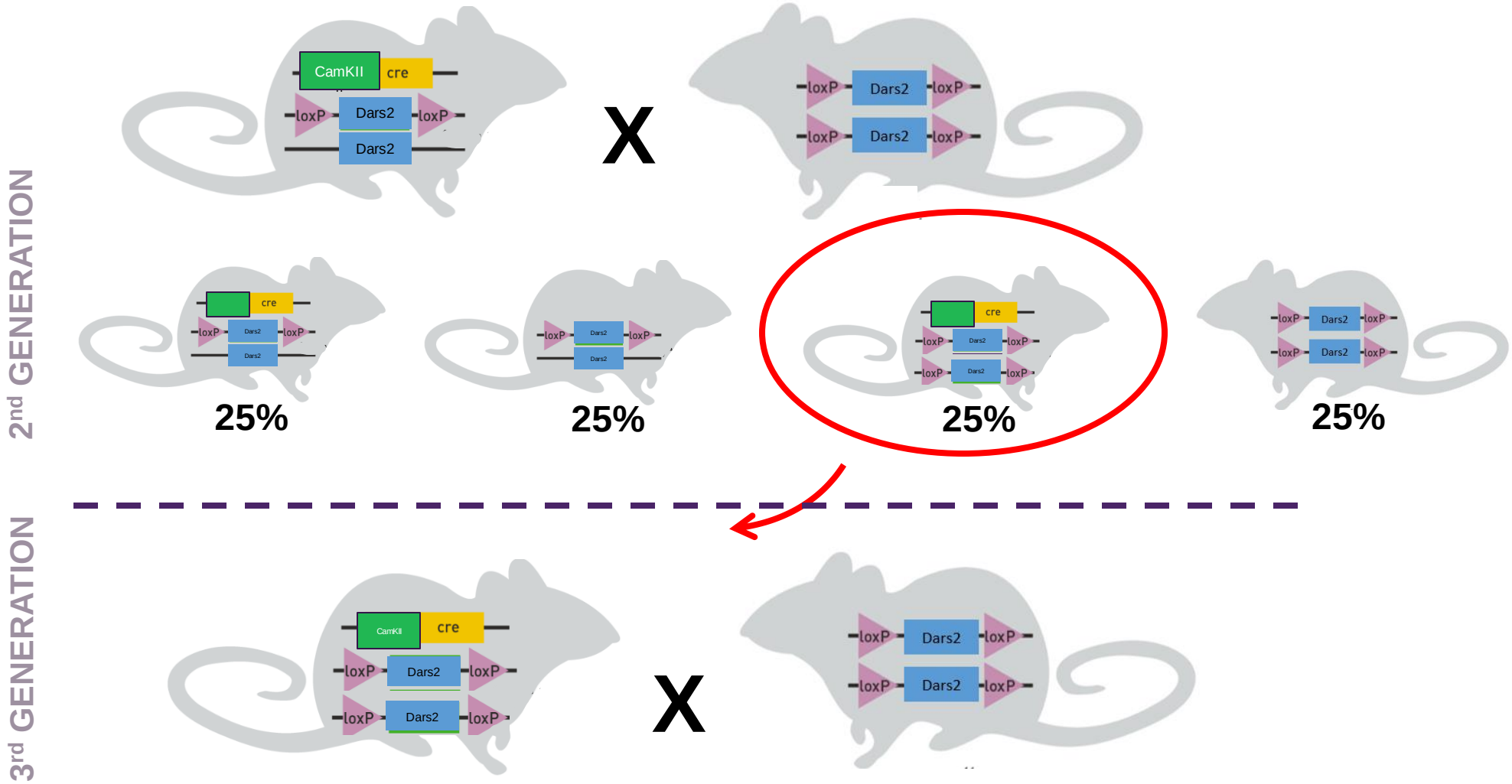
50%

2-3 months



50%


FOUNDER
1st GENERATION
2nd GENERATION
CamKII
cre
Dars2
Dars2
X
loxP
Dars2
loxP
loxP
Dars2
loxP
Brain-specific cre transgene
homozygous loxP "floxed" mouse
CamKII
cre
loxP
Dars2
loxP
Dars2
CamKII
cre
loxP
Dars2
loxP
Dars2
X
loxP
Dars2
loxP
loxP
Dars2
loxP
cre
loxP
Dars2
loxP
Dars2
loxP
Dars2
loxP
cre
loxP
Dars2
loxP
Dars2
loxP
Dars2
loxP
25%
25%
25%
25%



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

maintain colonies

Day 0

28

42-56

weaning

begin mating

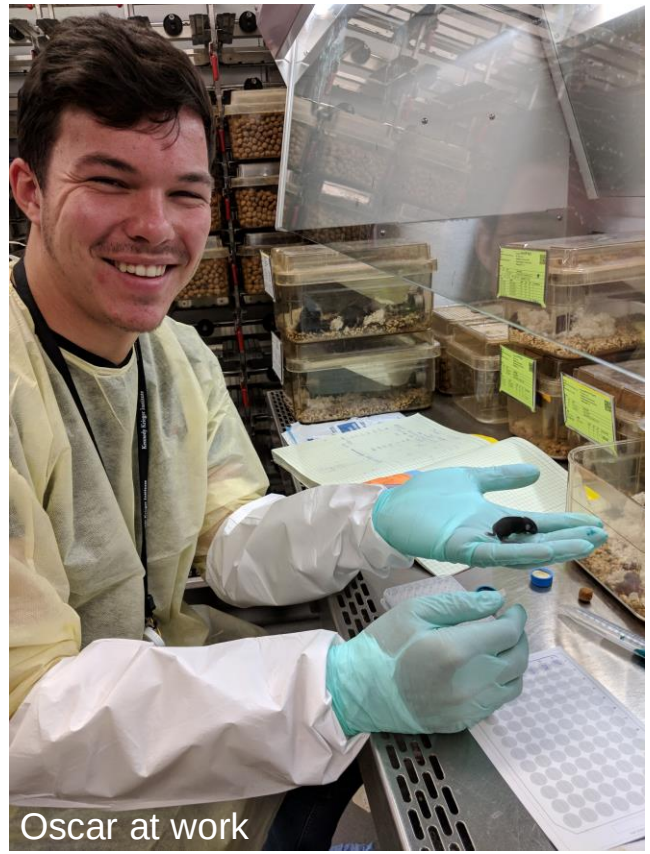
baseline phenotyping

Activity
Gait
Cognition
Reflexes
Affect
Weight

determine end date
based on behavioral
observations

-brain/spinal cord histology

Behavior, Imaging, Histology, Therapies



Oscar at work

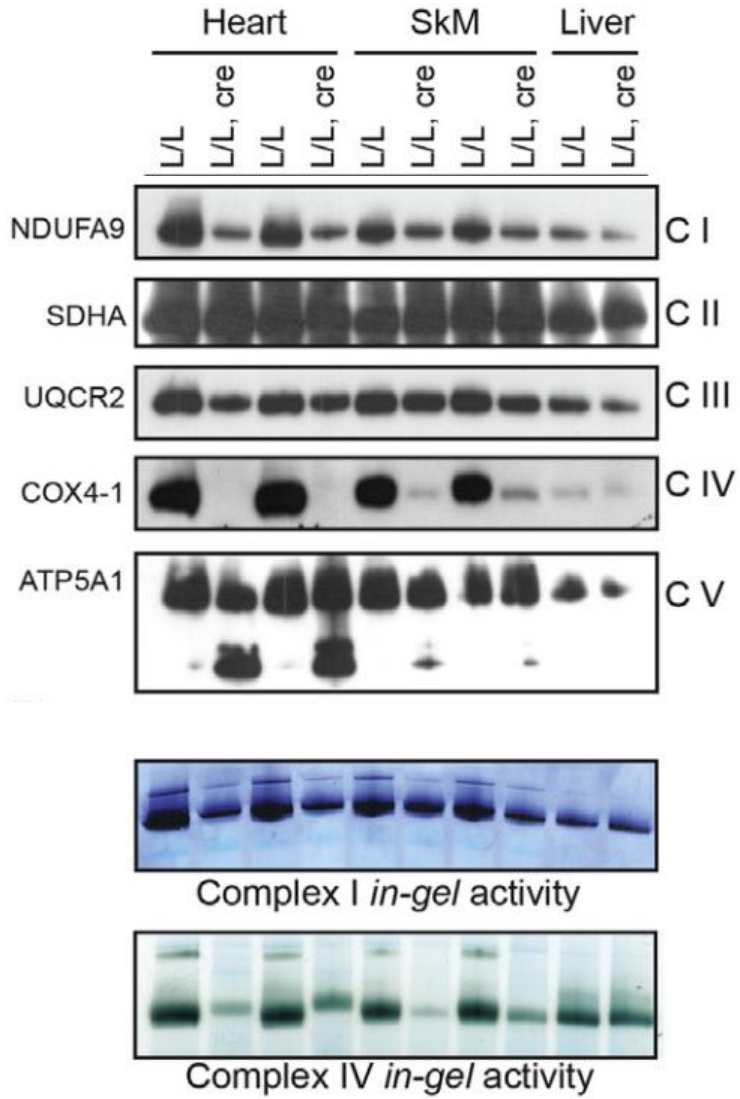
Cell Metabolism
Article

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

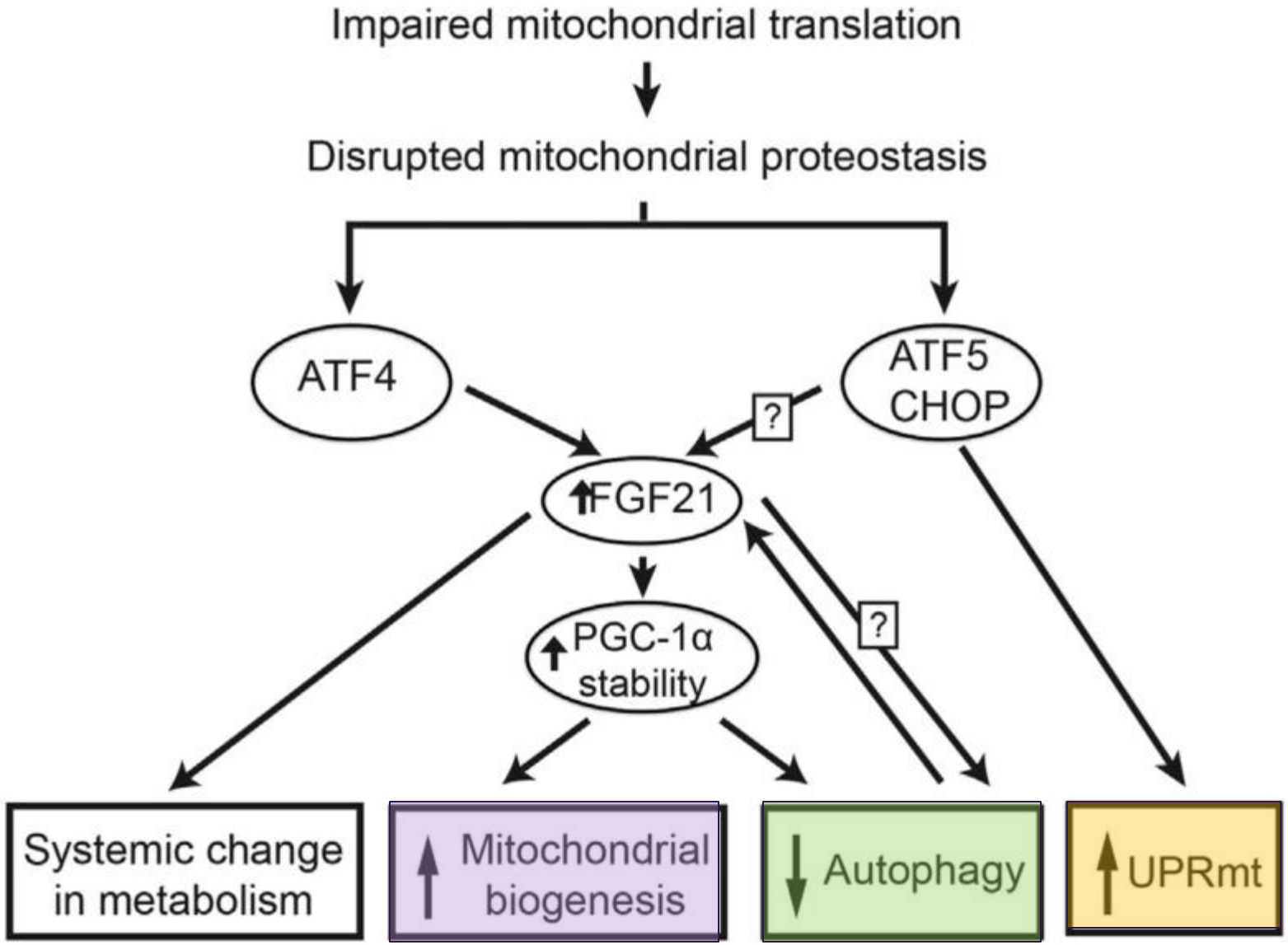
Sukru Anil Dogan,[1,6] Claire Pujol,[1,6] Priyanka Maiti,[1] Alexandra Kukat,[1] Shuaiyu Wang,[2] Steffen Hermans,[1] Katharina Senft,[1] Rolf Wibom,[3] Elena I. Rugarli,[1,2,4] and Aleksandra Trifunovic[1,3,4,5,*]
[1]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, 50674 Cologne, Germany
[2]Institute of Zoology, University of Cologne, 50674 Cologne, Germany
[3]Department of Laboratory Medicine, Karolinska Institutet, 17165 Stockholm, Sweden
[4]Center for Molecular Medicine Cologne (CMMC), 50931 Cologne, Germany
[5]Institute for Mitochondrial Diseases and Aging, Medical Faculty, University of Cologne, 50931 Cologne, Germany
[6]These 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 ... presentations in diseases caused by mitochondrial DNA (mtDNA) mutations are often assigned to a ... plasmy (disproportionately ... tissue) and ... various ...



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

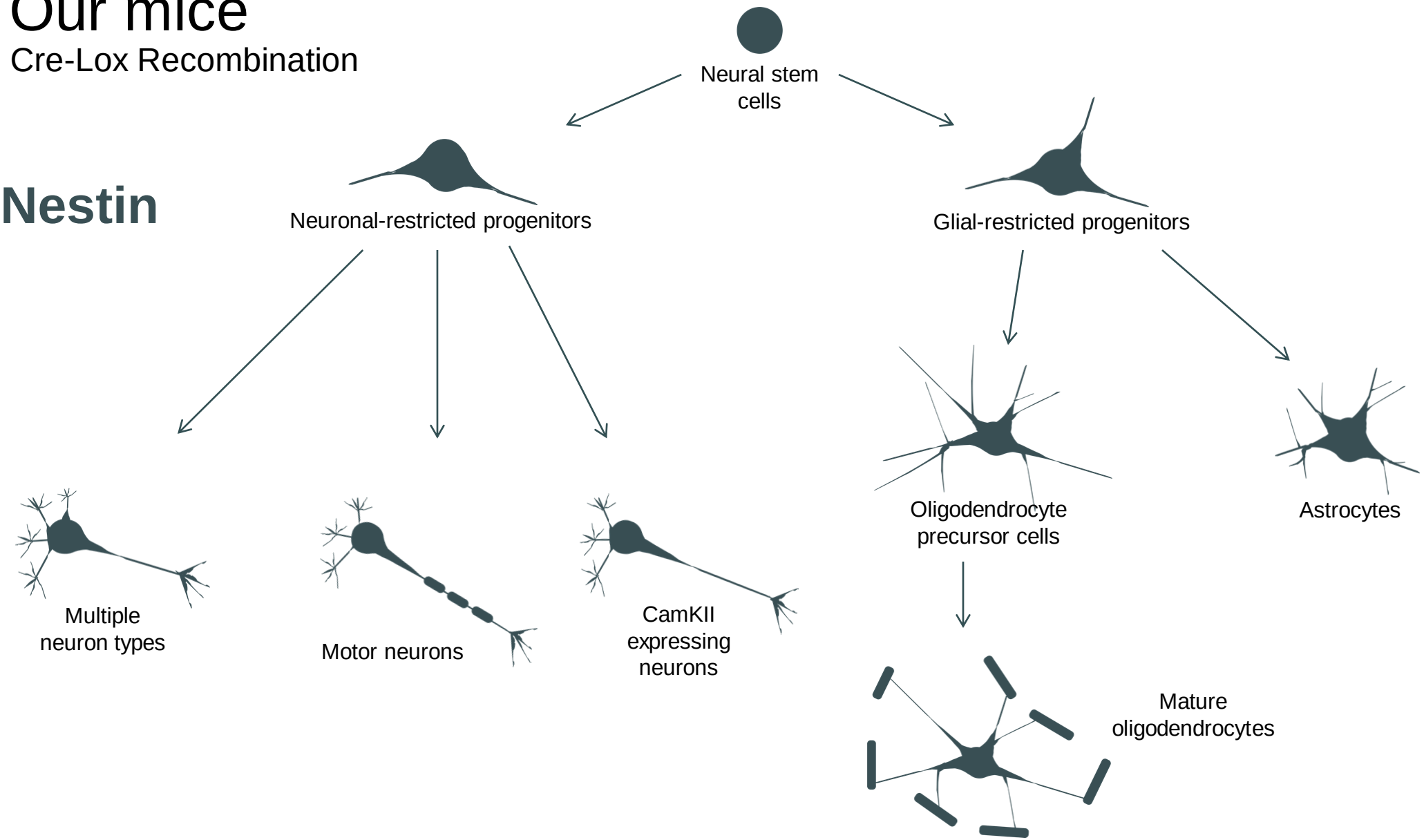


Dogan et al, 2014; *Cell Metab*

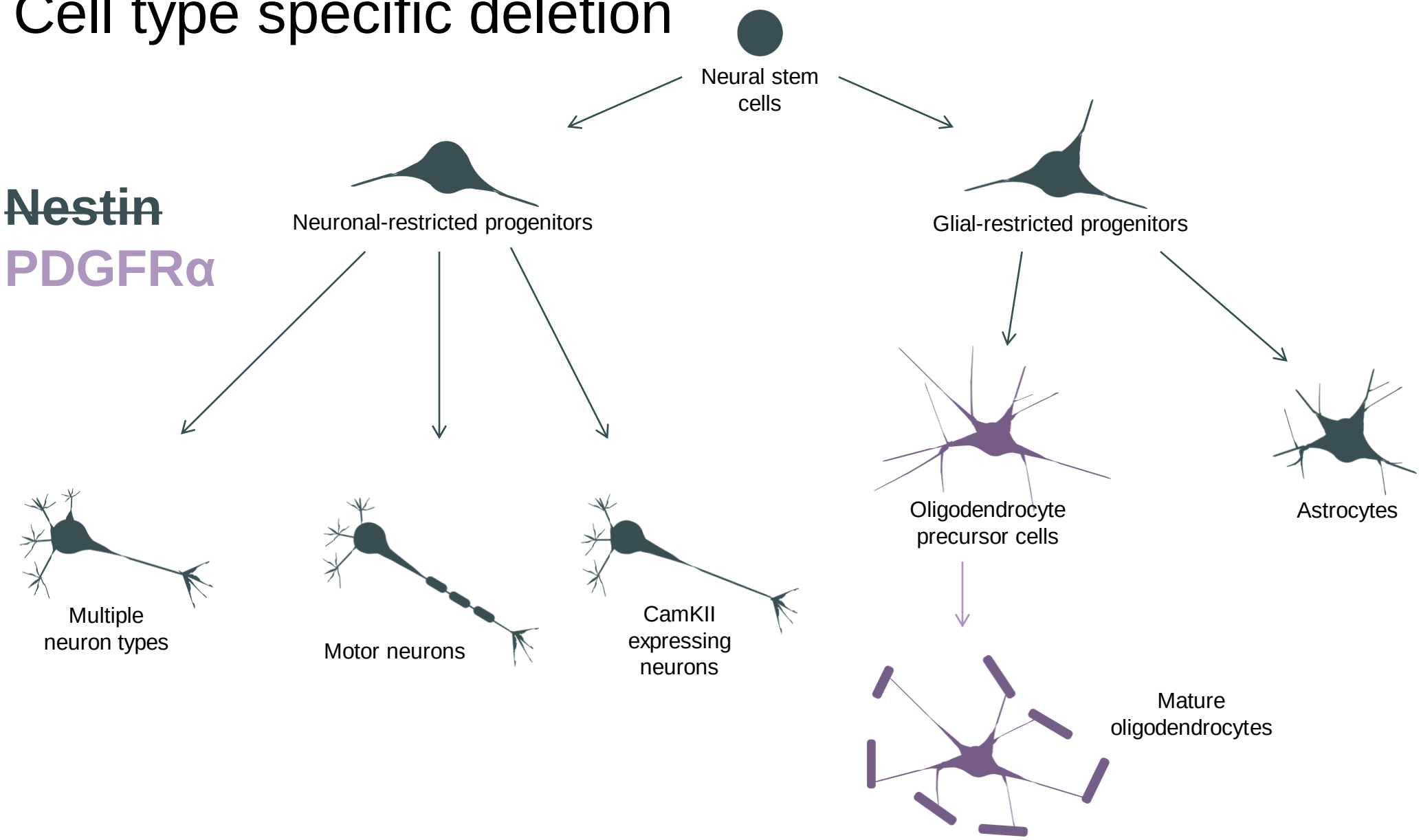
Our mice

Cre-Lox Recombination

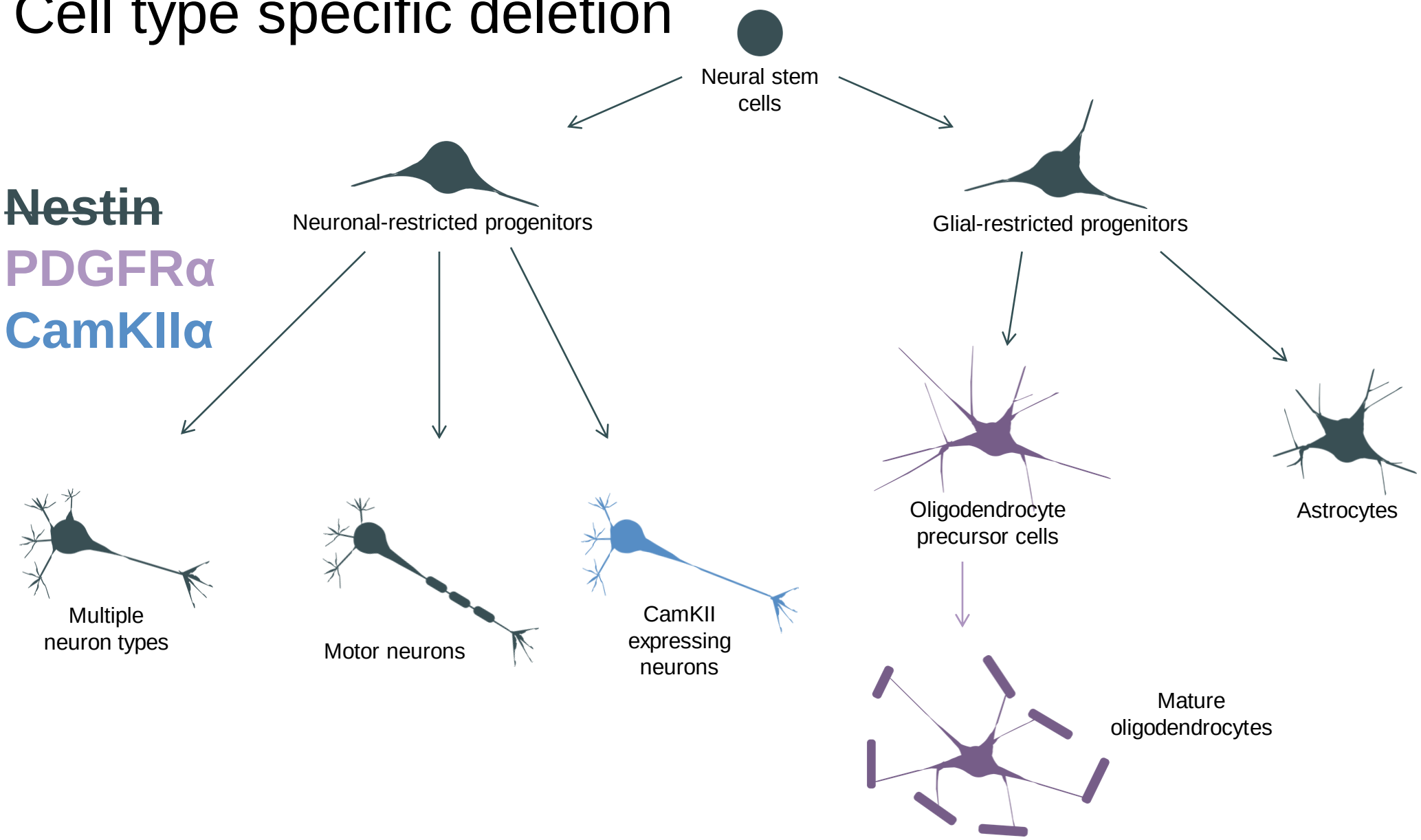
Nestin



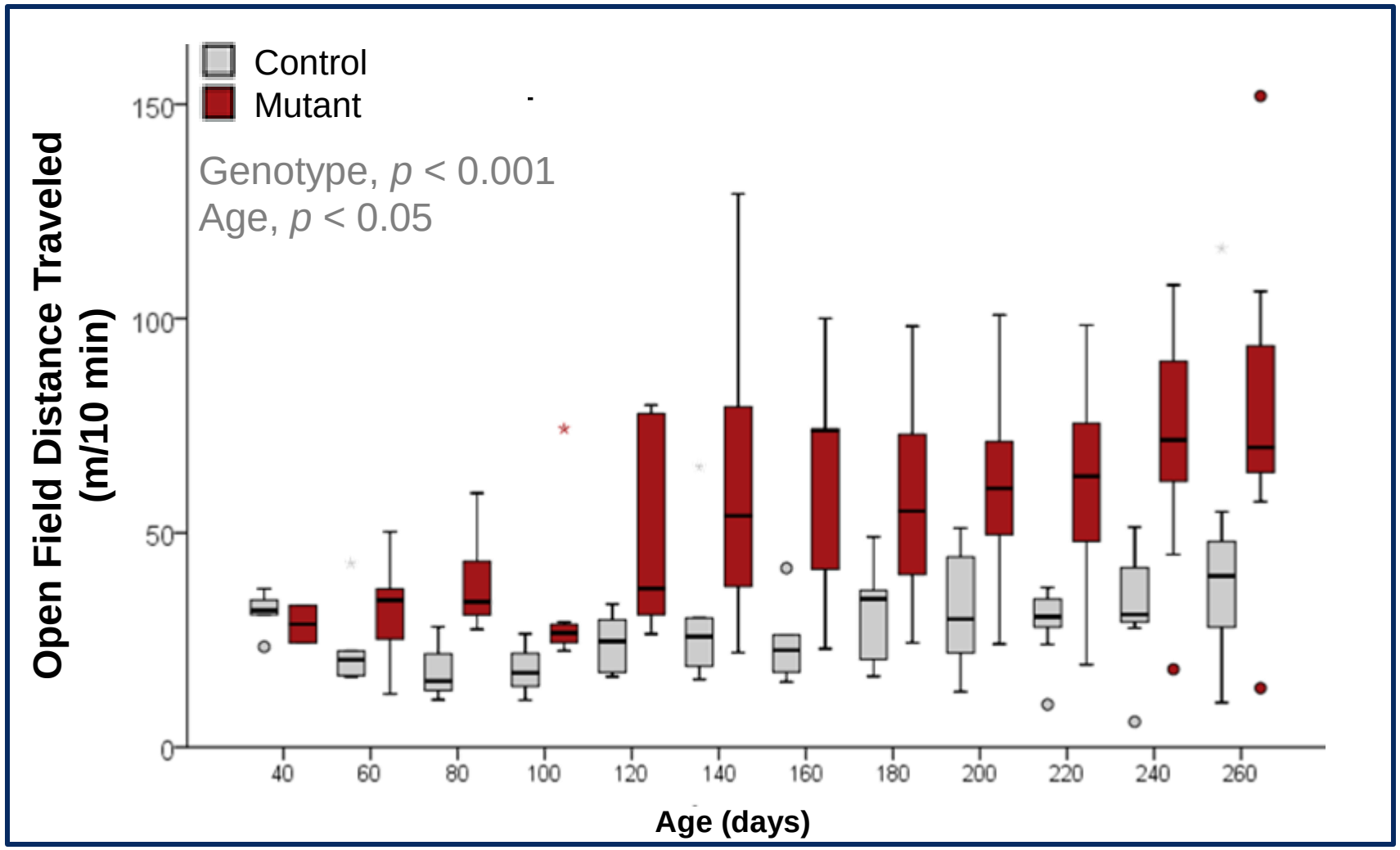
Cell type specific deletion



Cell type specific deletion

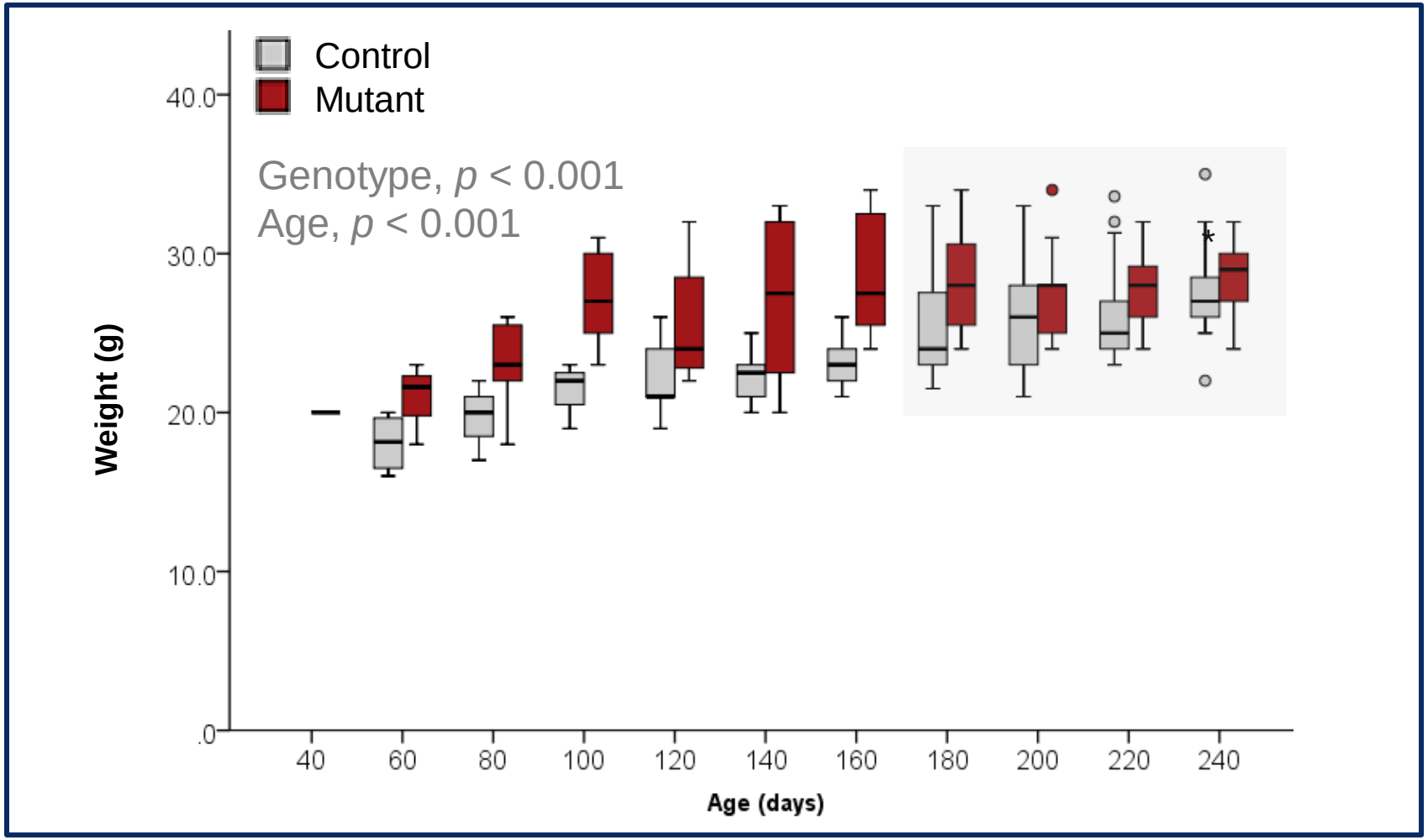


DARS2 deletion increases overall activity



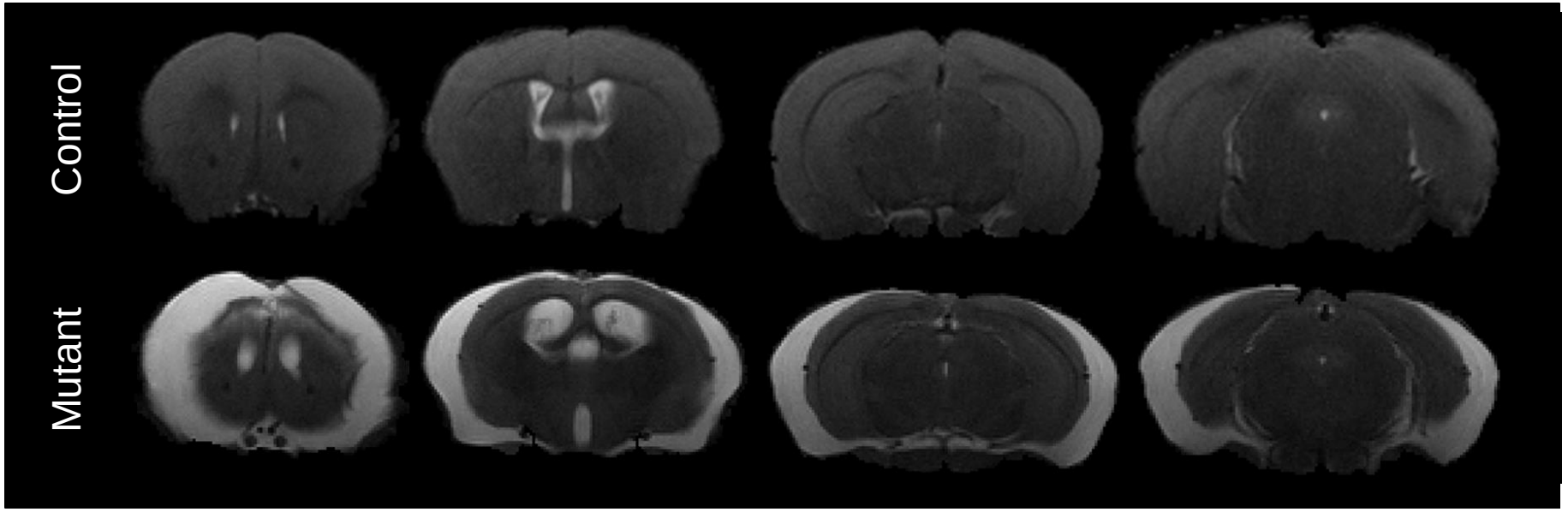
males & females
Cre- n = 13
Cre+ n = 12

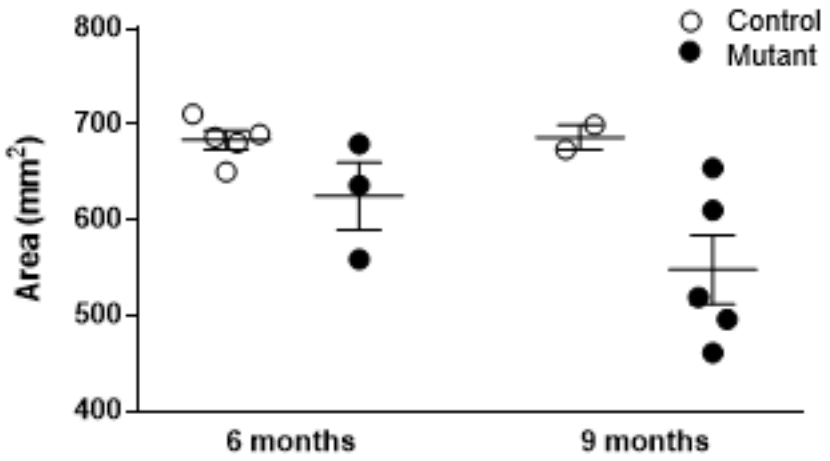
Body mass of *DARS2* mutant mice decreases at ~6 months of age

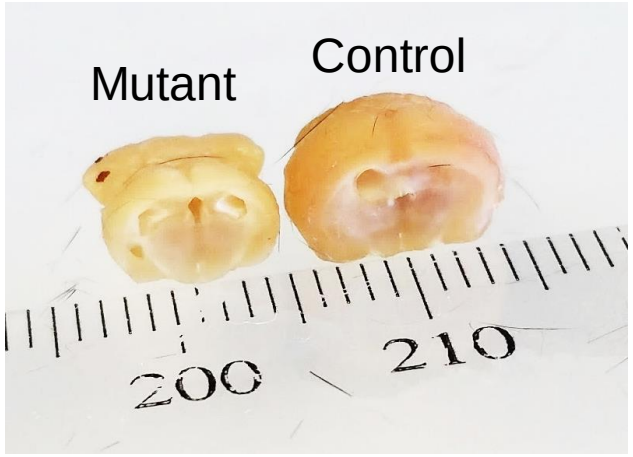


males & females
Cre- n = 13
Cre+ n = 12

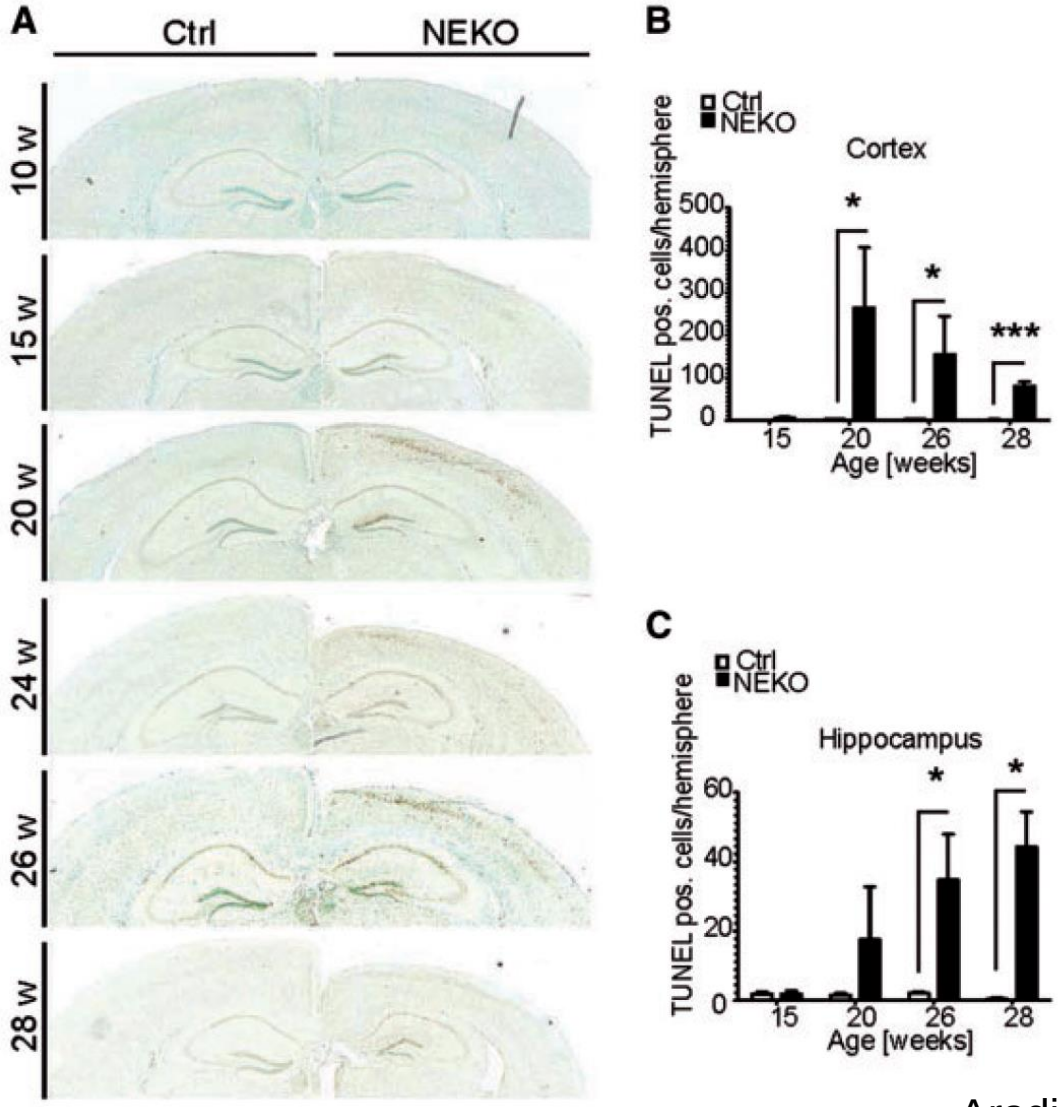
DARS2 deletion from neurons leads to severe brain atrophy





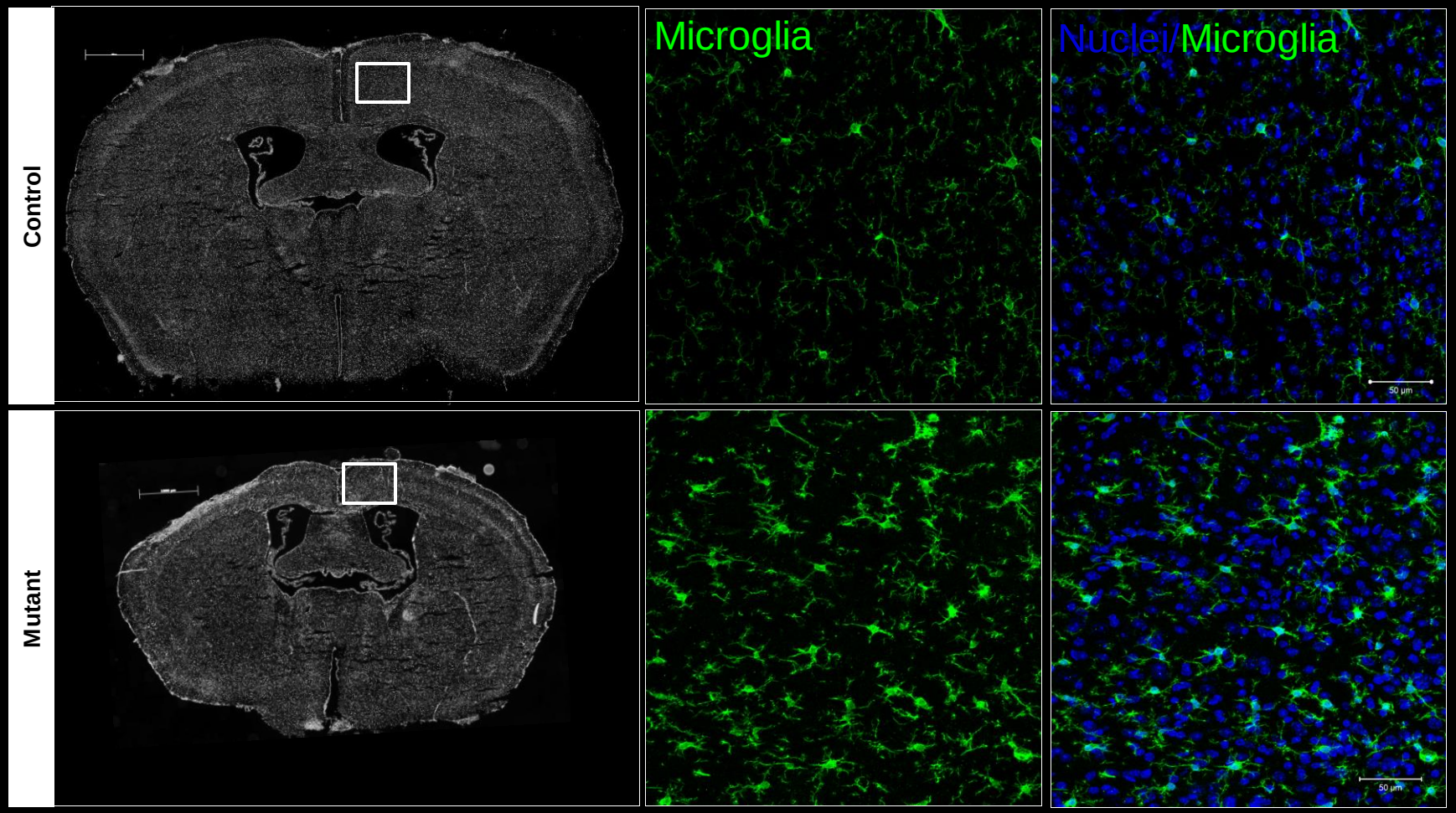


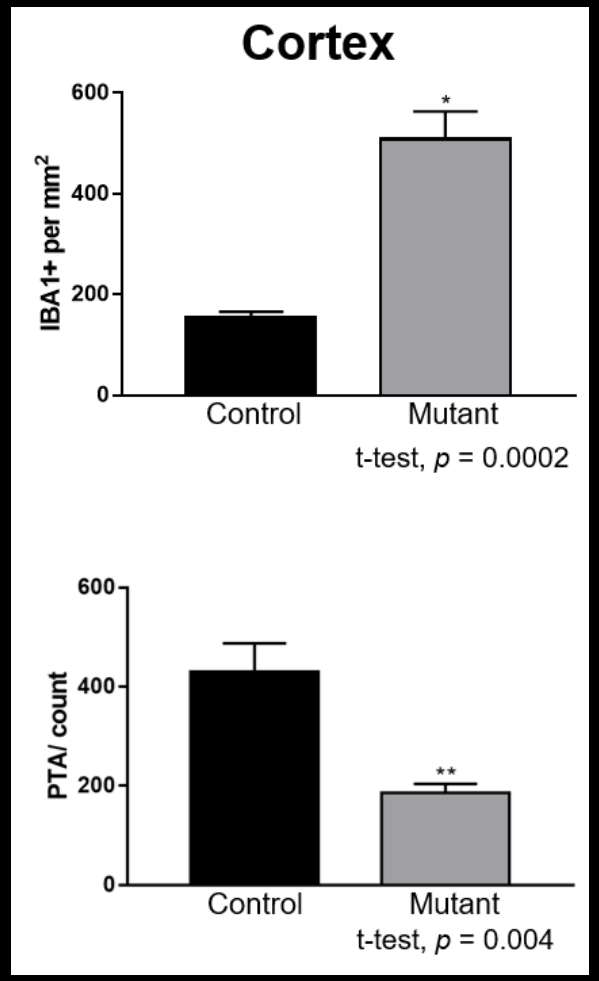
DARS2 deletion leads to neuronal cell loss



Aradjanski et al, 2017; *Hum Molec Genetics*

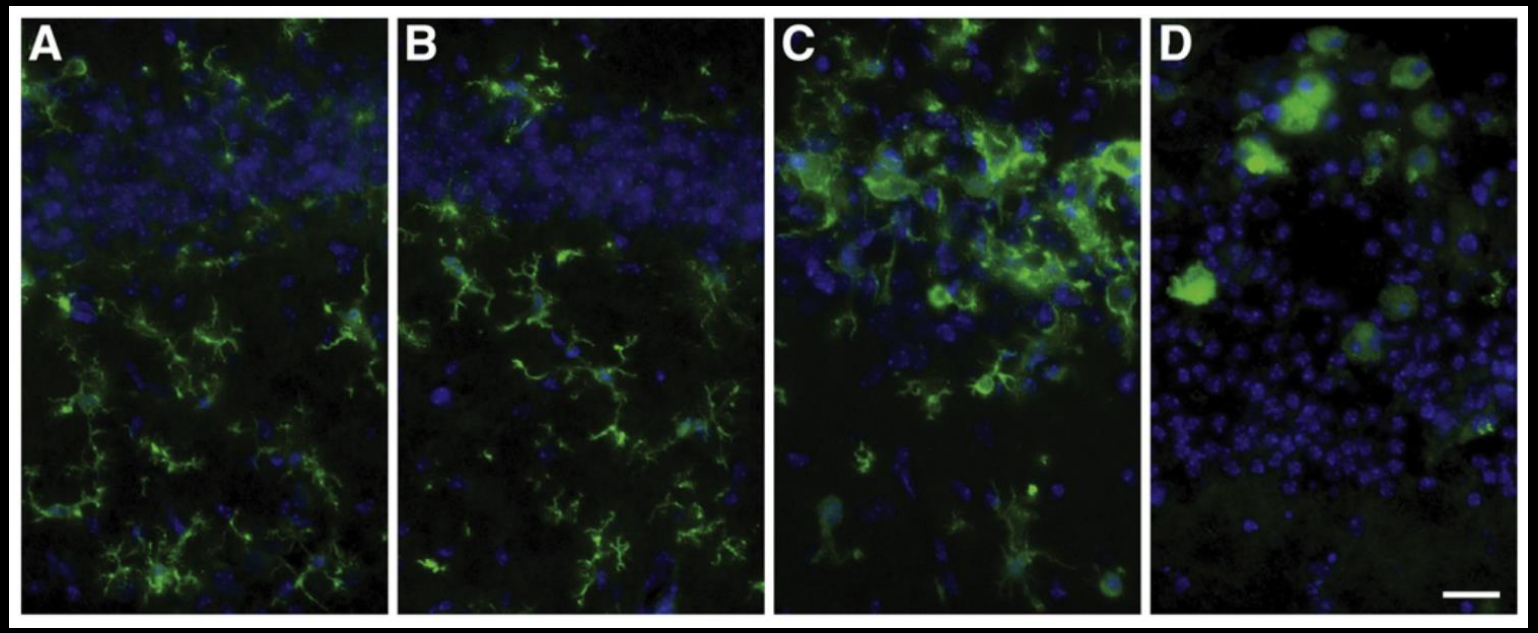
DARS2 deficiency increases brain inflammation





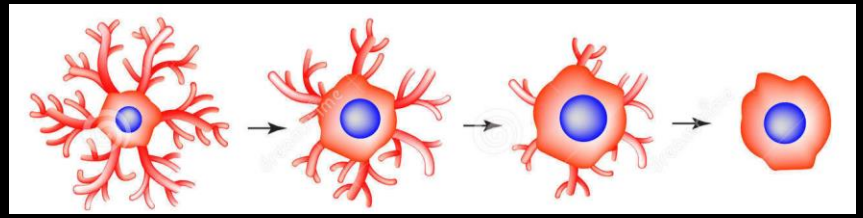
*Microglia are cells that produce inflammation and keep brain areas free from debris and harmful pathogens

Microglial cells change shape upon activation

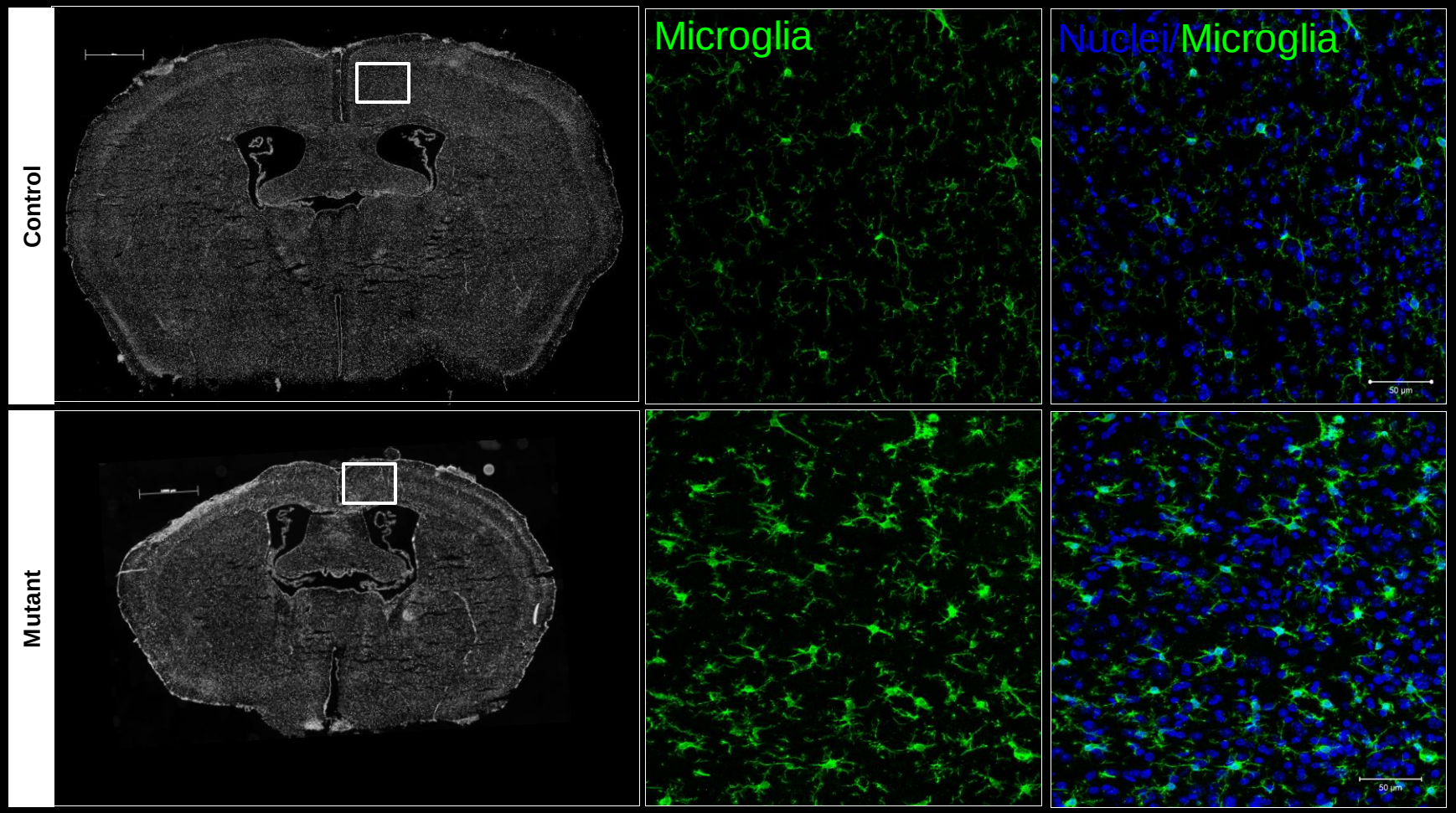


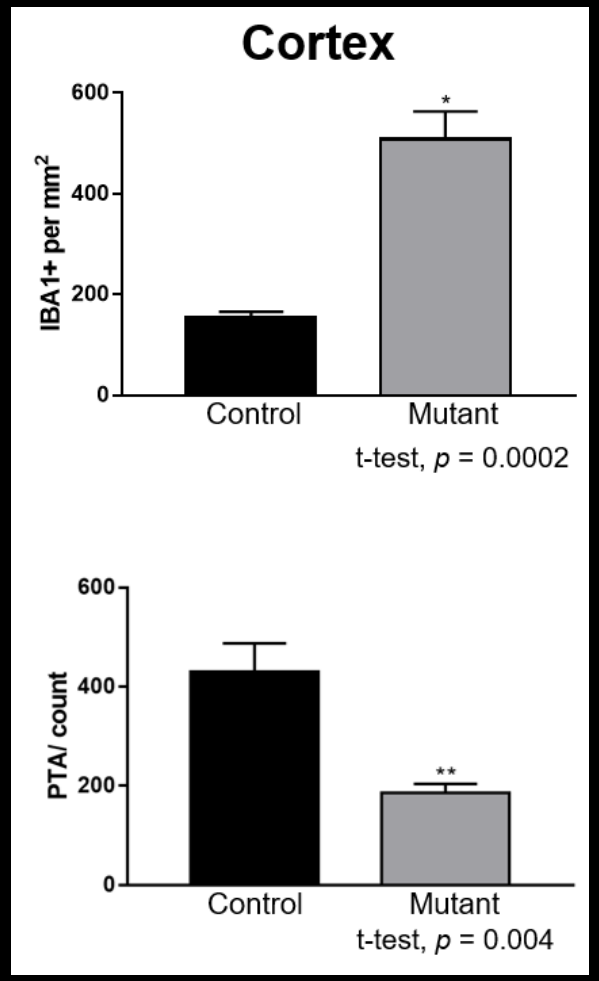
Nemeth et al., 2017

activation



DARS2 deficiency increases brain inflammation

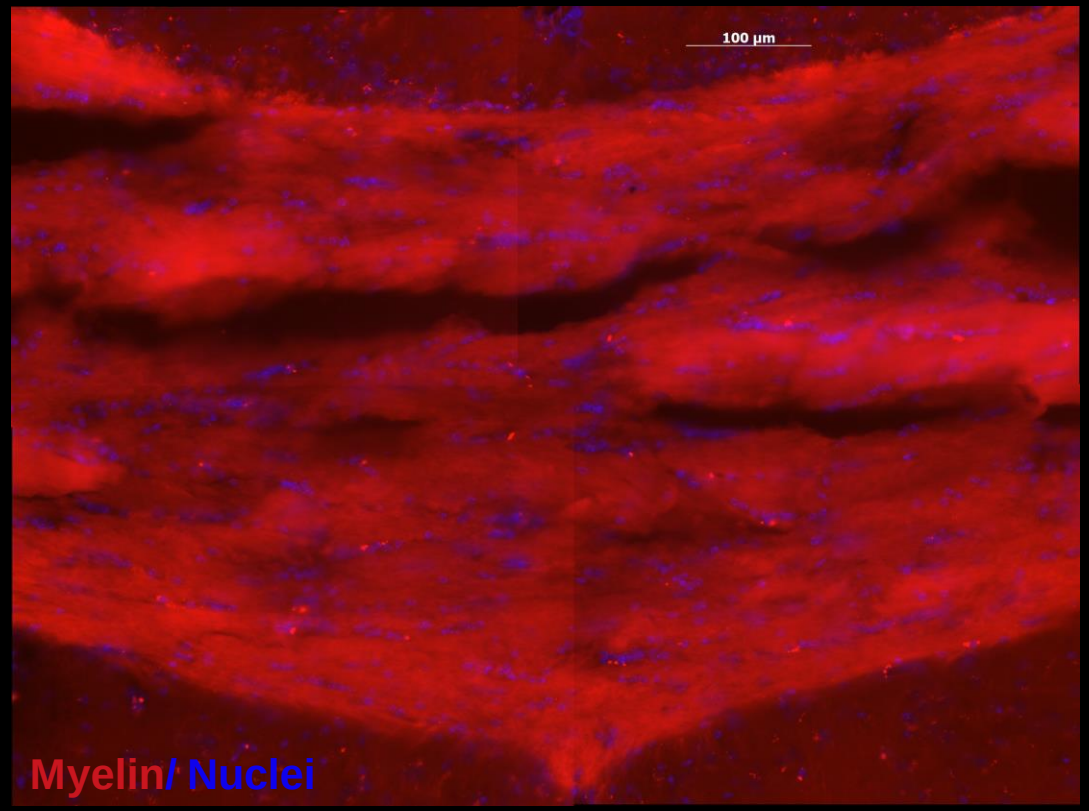




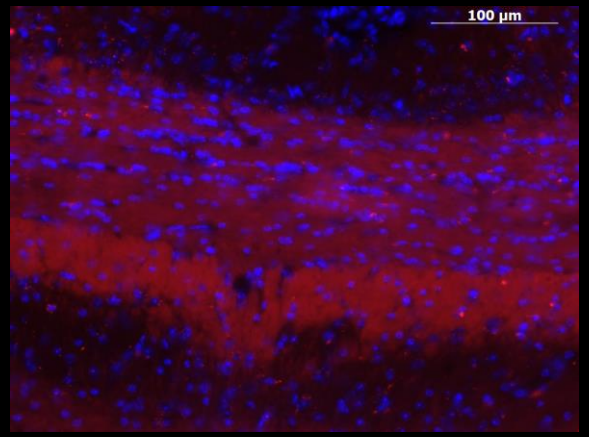
*Microglia are cells that produce inflammation and keep brain areas free from debris and harmful pathogens

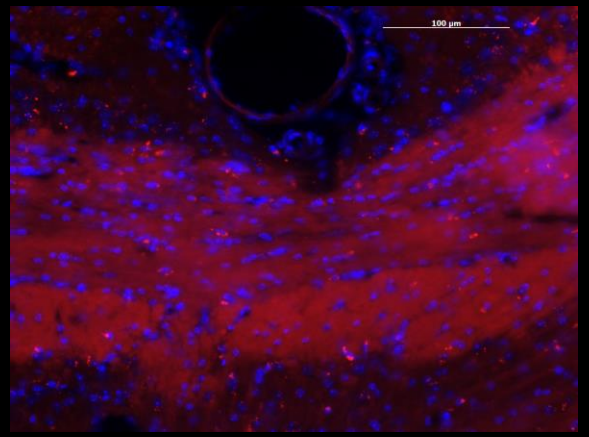
DARS2 deficiency leads to reduced white matter area

Control



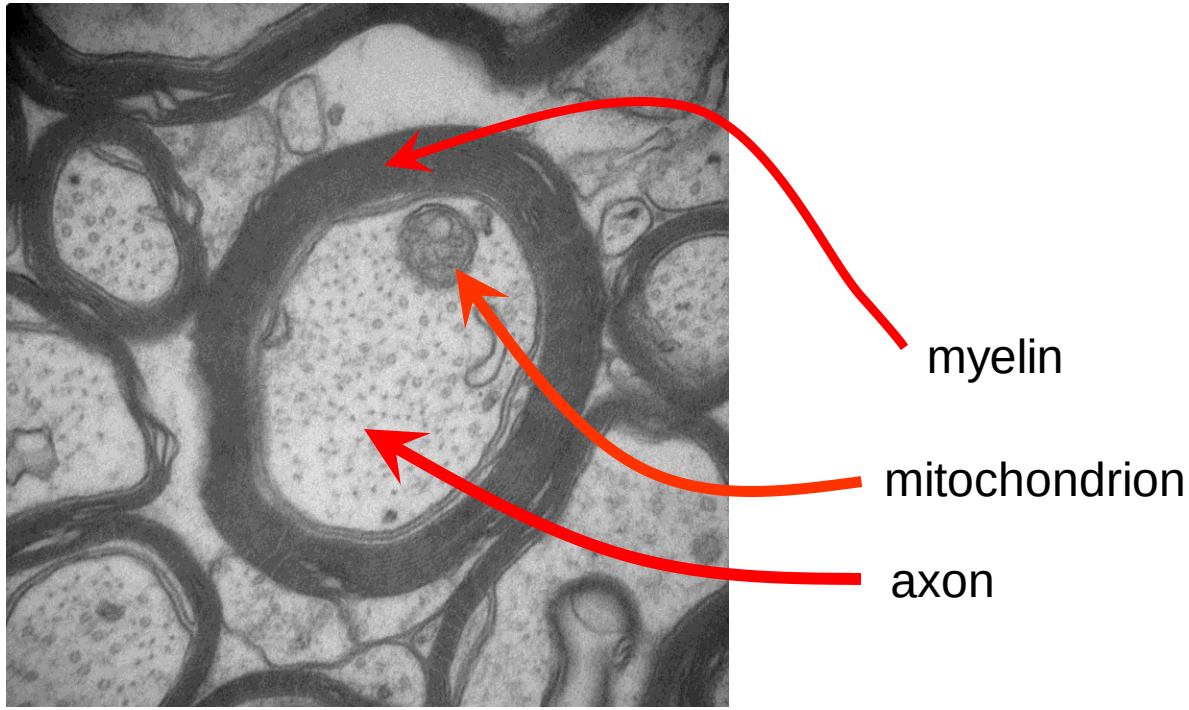
Mutant



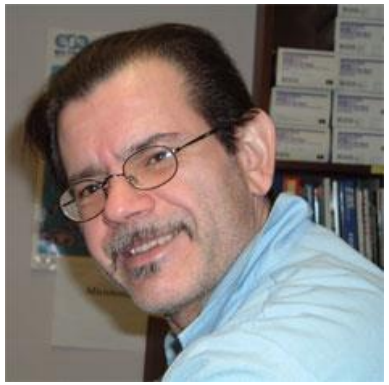


Electron Microscopy

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



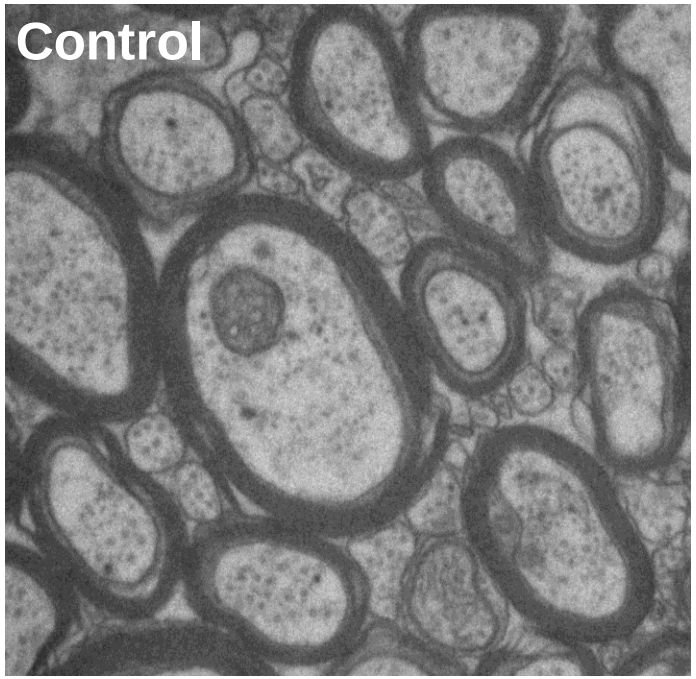
80,000x

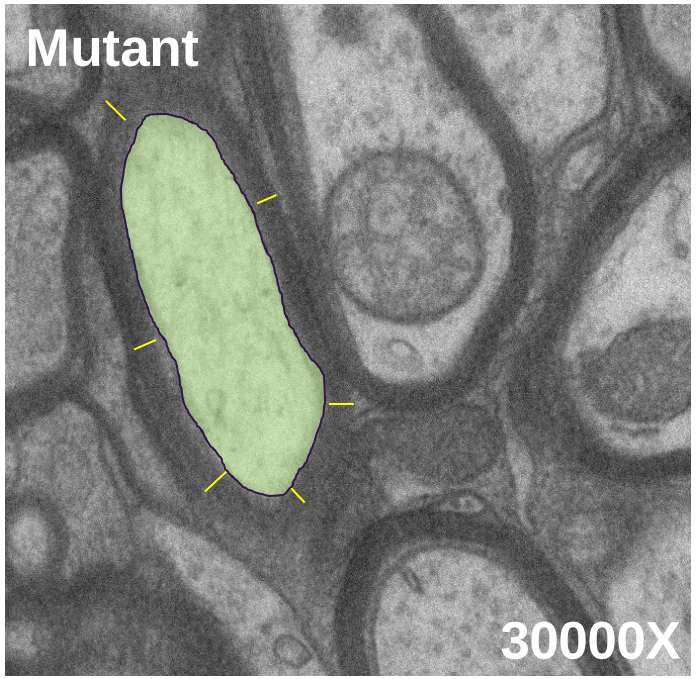


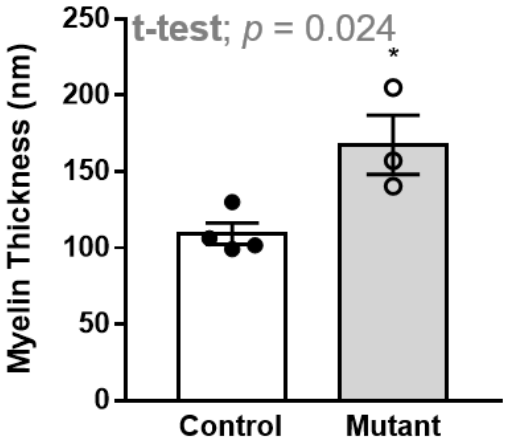
Michael Delannoy

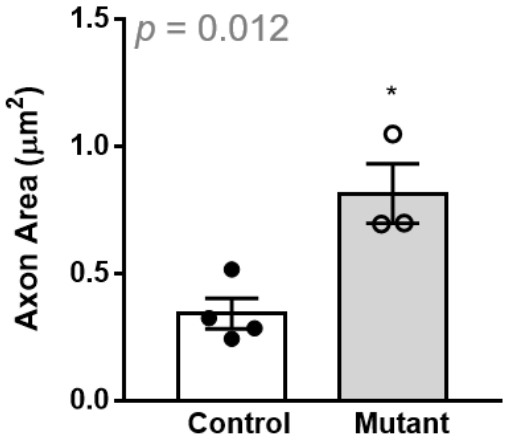
JHU MicFac
Hitachi 7600 TEM

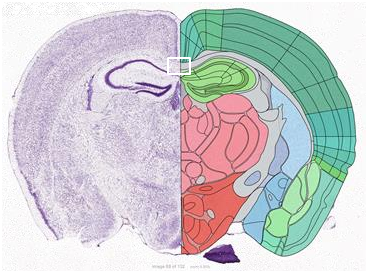
DARS2 deficiency alters brain axons





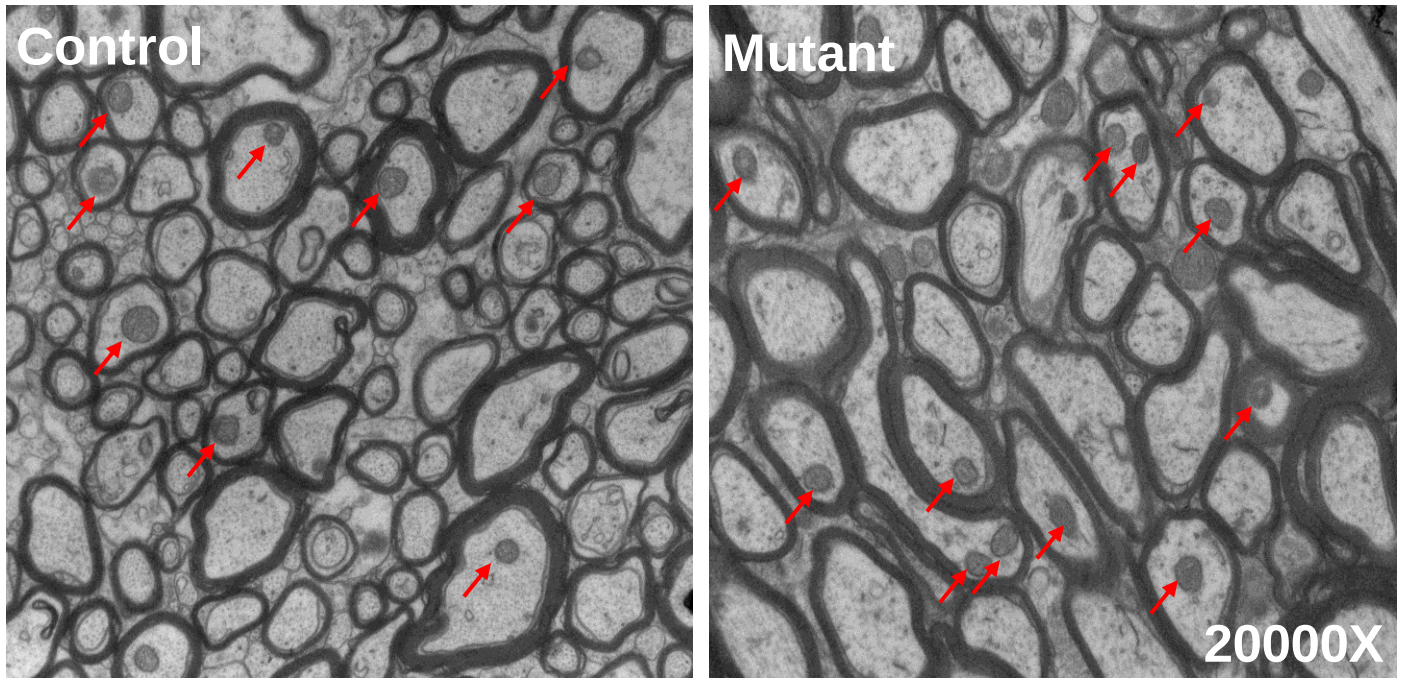


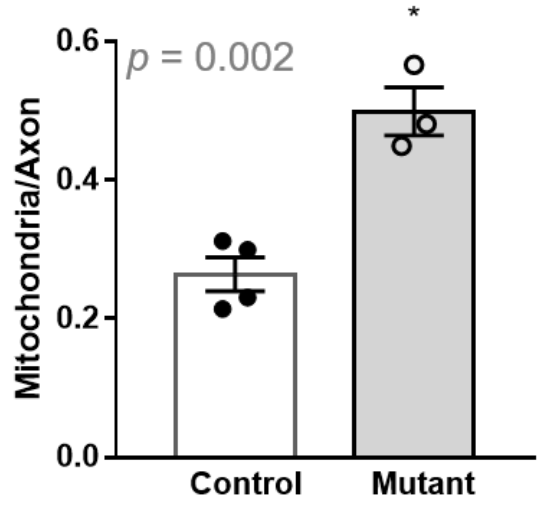


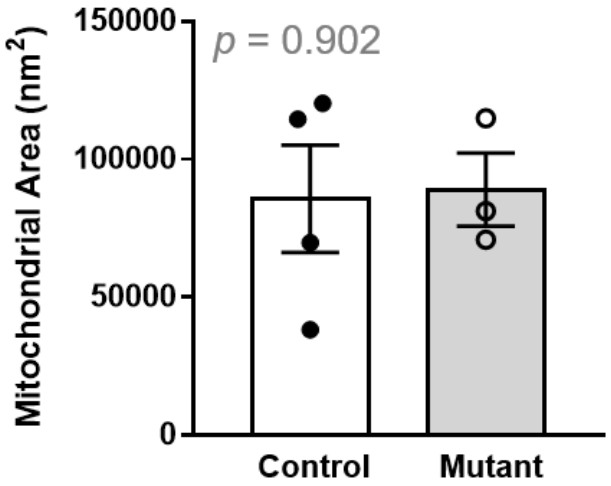


JHU MicFac; Hitachi 7600 TEM

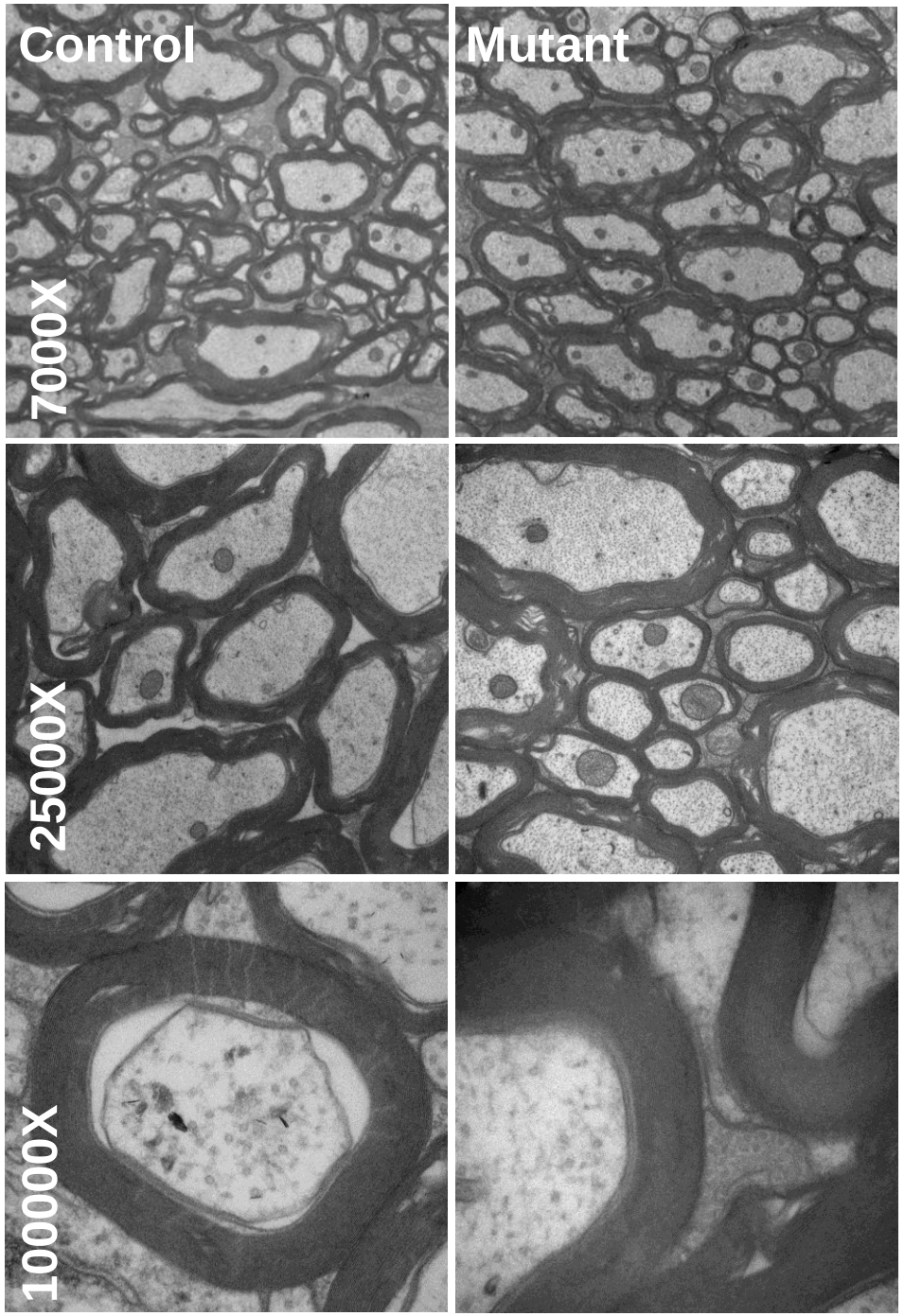
DARS2 deficiency increases mitochondrial count in brain axons



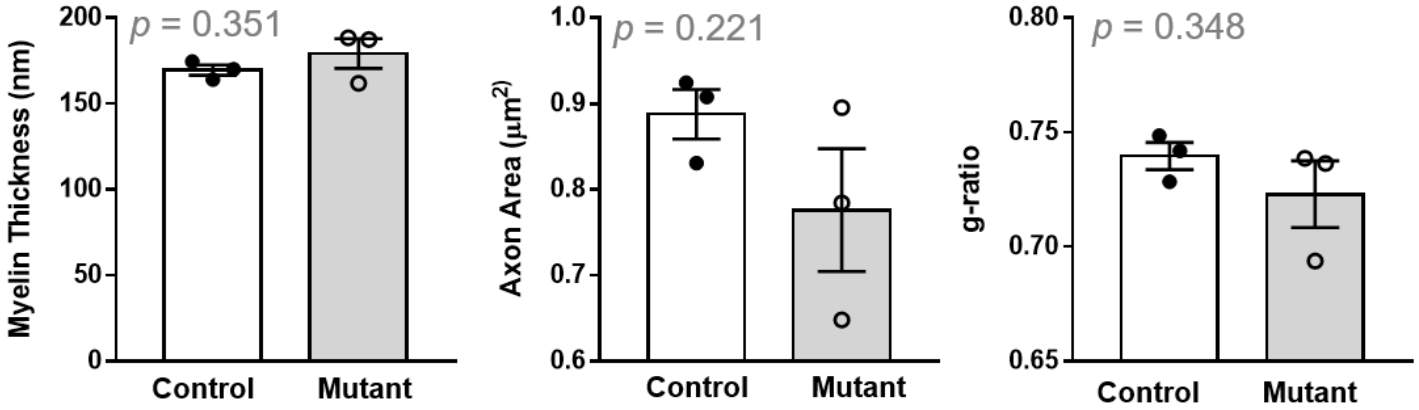


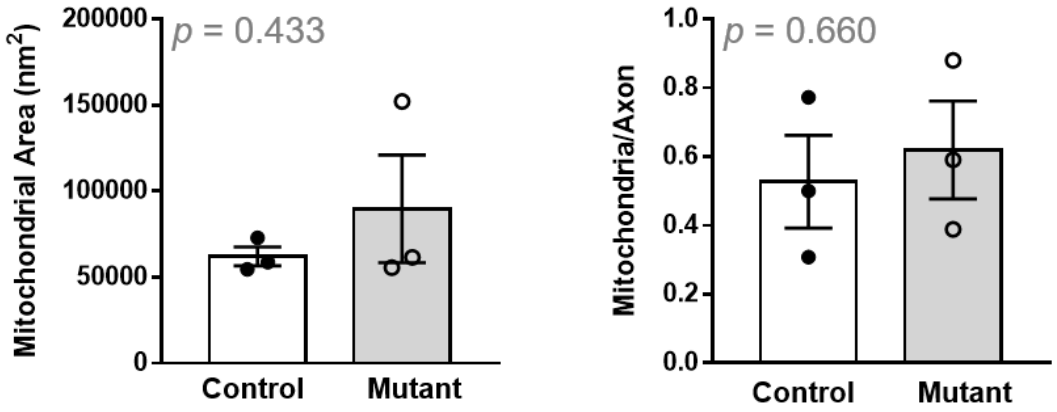


Cervical Spinal Cord
Dorsal Column

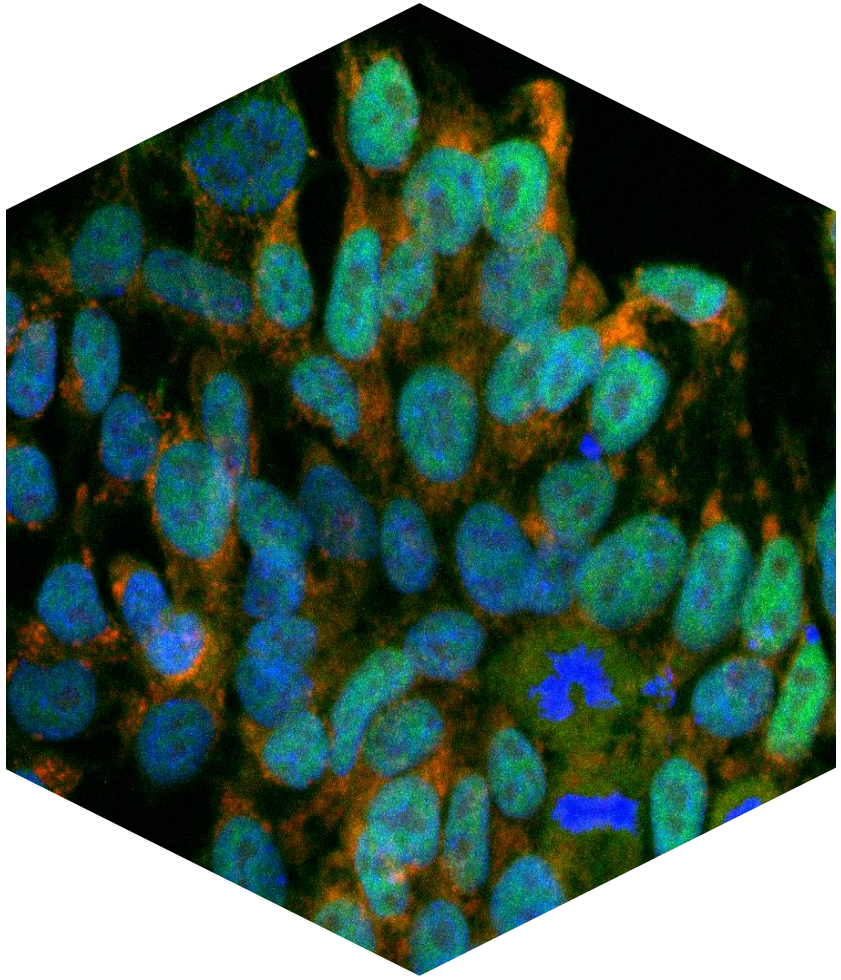


DARS2 deficiency in the brain does not alter nerves of the spinal cord





Outline



<u>Induced Pluripotent Stem Cells</u>
-What are they?
-How do we get them?
-Our data so far

Why iPSCs?

Non-invasive method with lots of flexibility

Respond in functional assays

Allows us to assess the effect of specific mutations

Useful for therapeutic testing

Can be turned in to almost any cell type

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

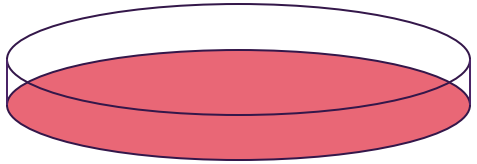
No fear of immune rejection if re-introduced to the patient

Can carefully assess cell differentiation

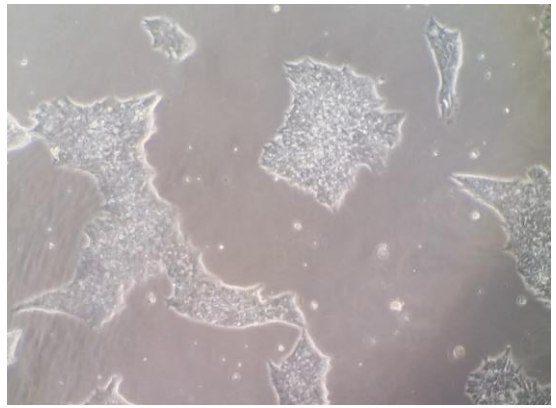
Induced Pluripotent Stem Cells (iPSCs)

Isolate peripheral blood monocyte cells

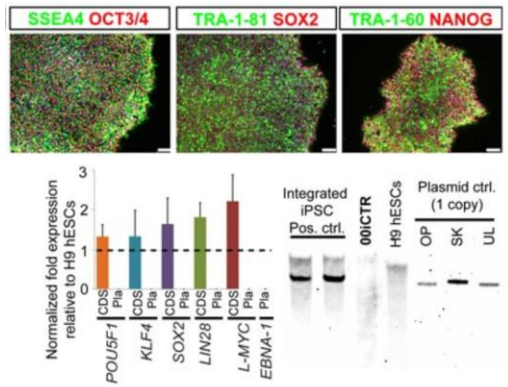
Cells are reprogrammed to induce pluripotency



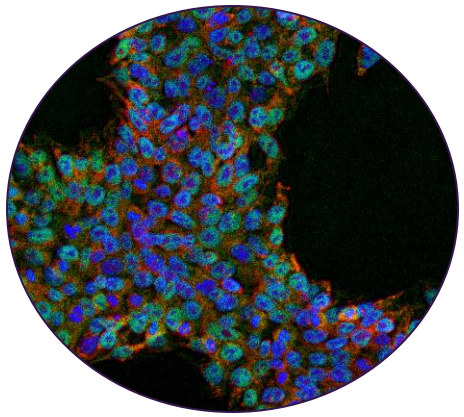
Cells are monitored and tested to ensure reprogramming was successful



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



Frozen cells are mailed to us



3 – 4 months

CEDARS-SINAI®

From iPSCs to neurons

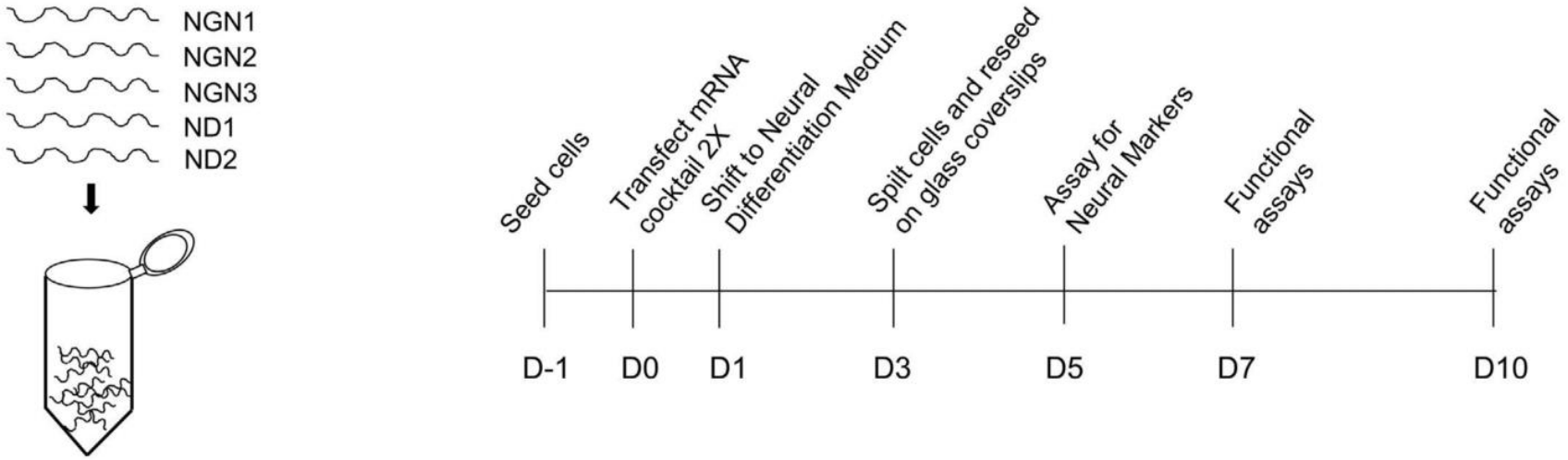
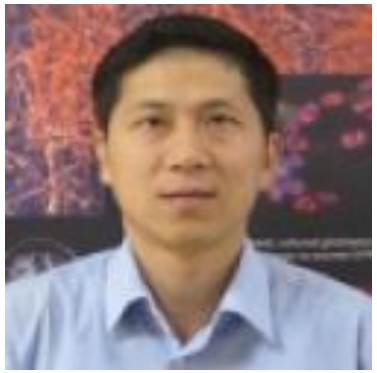
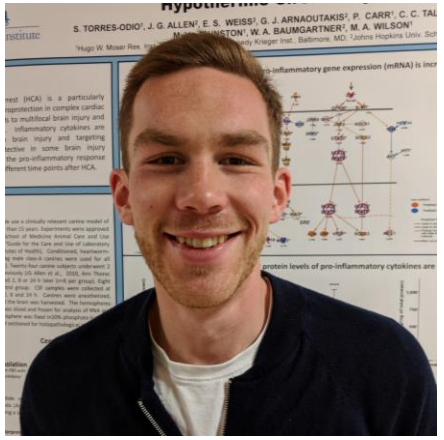


Figure 2. Induction of neurogenesis by syn-TFs mRNAs of Neurogenin and NeuroD families.

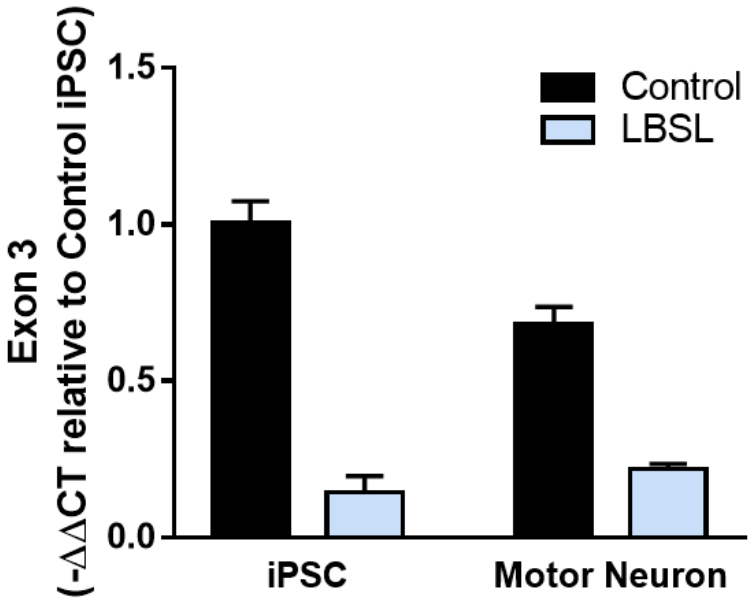


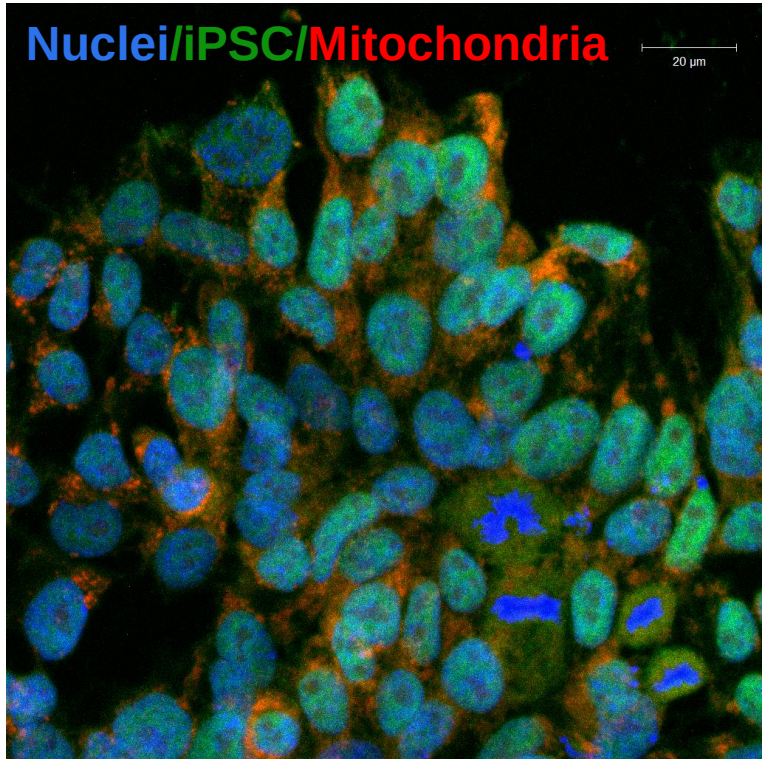
Mingyao Ying, PhD

iPSC derived motor neurons

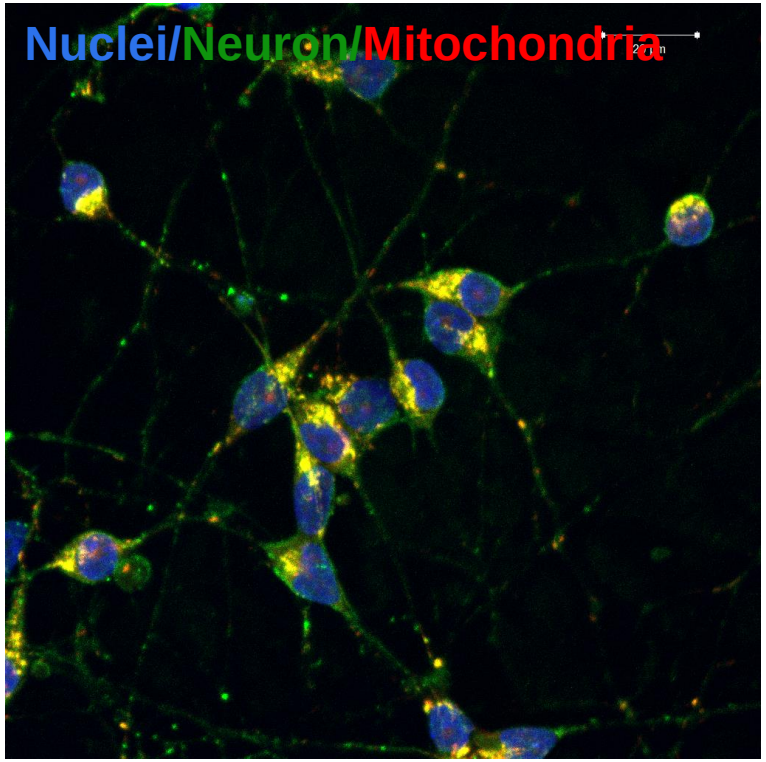


Philippe Hubo





iPSC



motor neuron

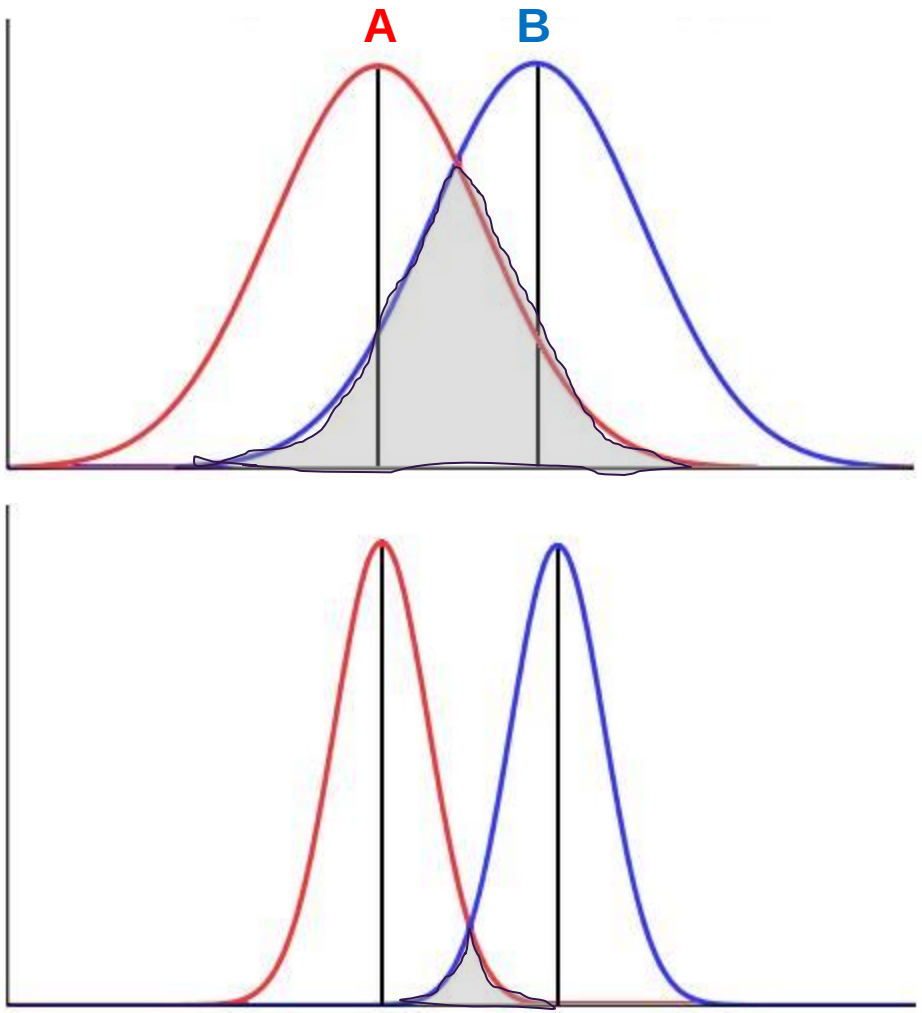
Outline

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

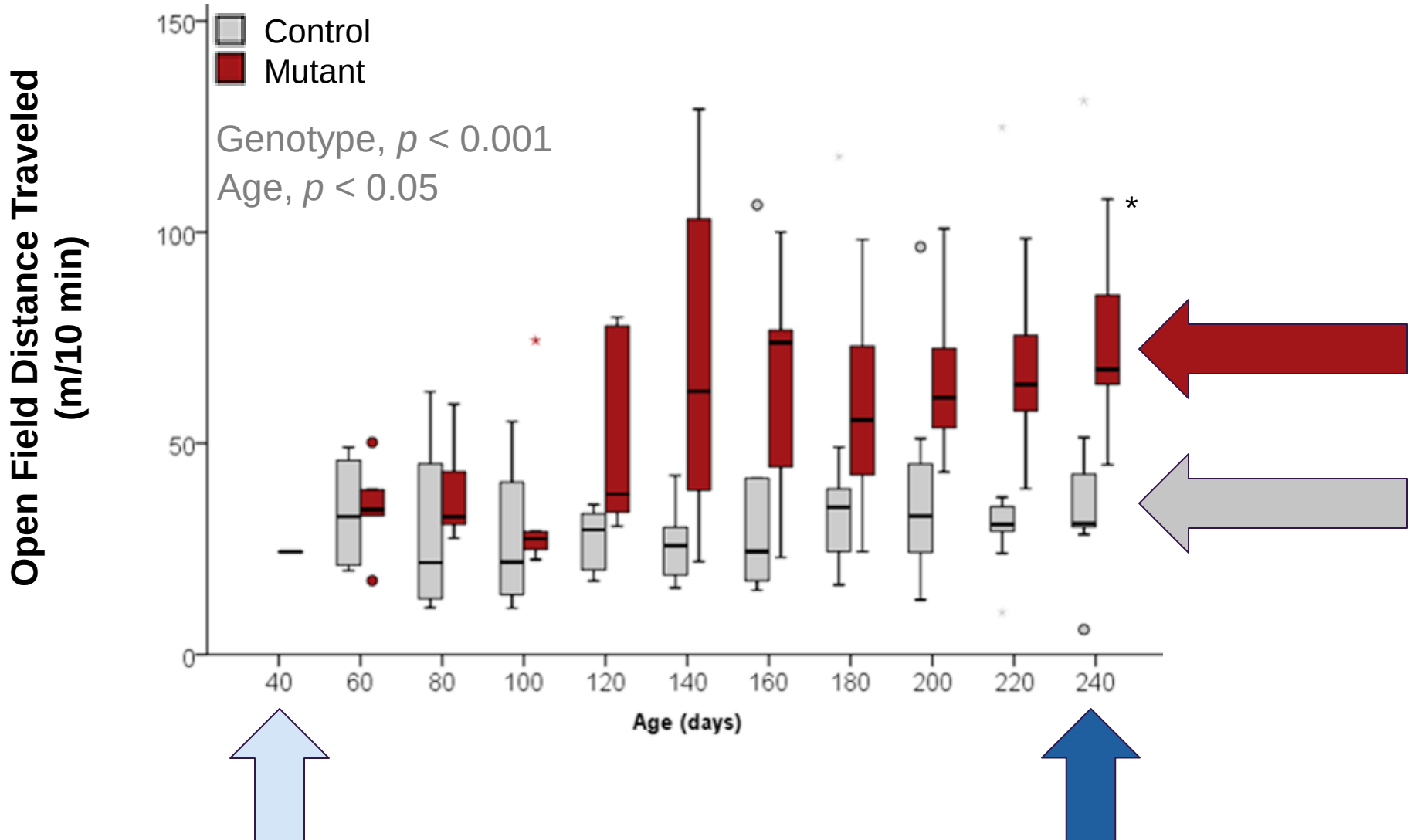
Litter

Weight

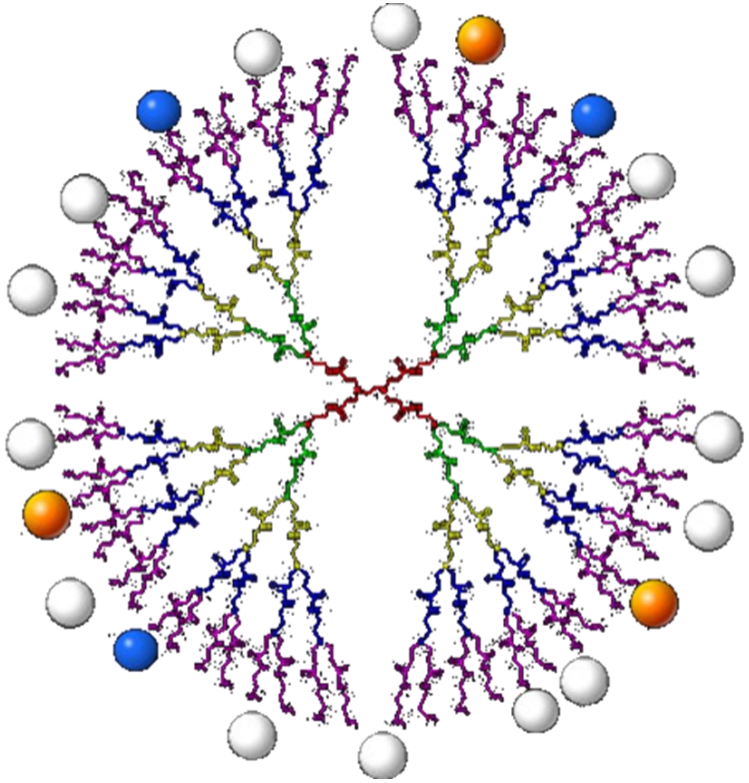
Housing

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

The numbers


Control
Mutant
Genotype, p < 0.001
Age, p < 0.05
Open Field Distance Traveled (m/10 min)
150
100
50
0
40
60
80
100
120
140
160
180
200
220
240
Age (days)
*

The Dendrimer Platform



4 – 10 nm in diameter

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

RM Kannan, PhD
Sujatha Kannan, MD

JOHNS HOPKINS
MEDICINE
THE CENTER FOR
NANOMEDICINE

Outline

Collaborations

-Cerebral Organoids

-RNAseq

From iPSCs to “mini brains”

Paul Tesar, PhD

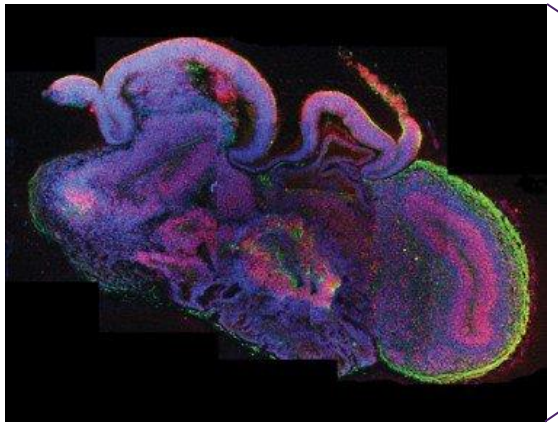
CASE WESTERN RESERVE UNIVERSITY
EST. 1826

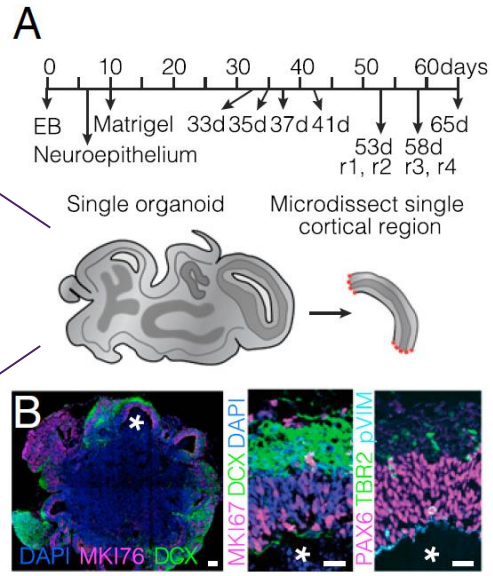
PNAS

Human cerebral organoids recapitulate gene expression programs of fetal neocortex development

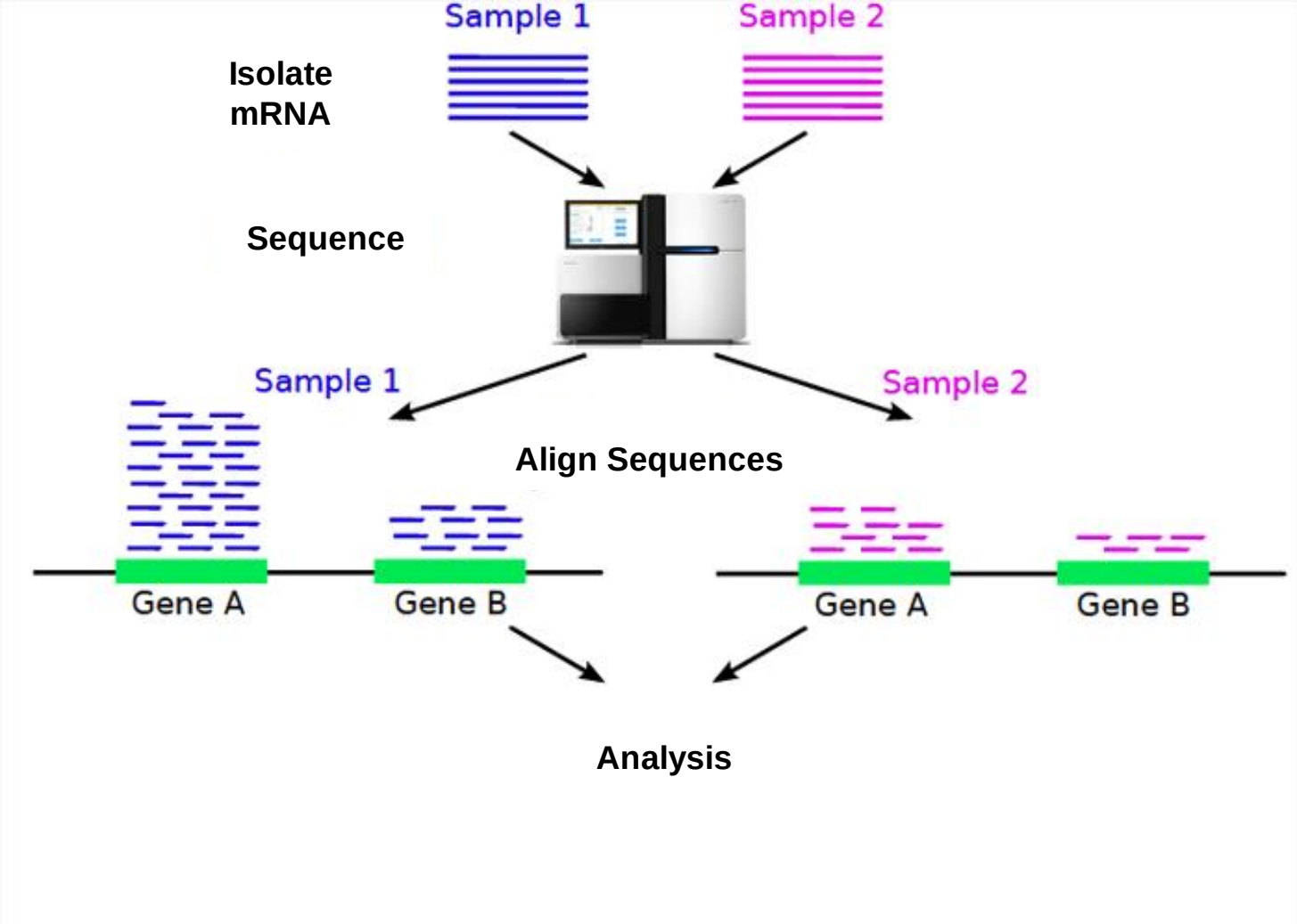
J. Gray Camp[a,1], Farhath Badsha[b,1], Marta Florio[b], Sabina Kanton[a], Tobias Gerber[a], Michaela Wilsch-Bräuninger[b], Eric Lewitus[c], Alex Sykes[b], Wulf Hevers[a], Madeline Lancaster[d,e], Juergen A. Knoblich[e], Robert Lachmann[f], Svante Pääbo[a,2], Wieland B. Huttner[b,2], and Barbara Treutlein[a,b,2]

[a]Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, 04103 Leipzig, Germany; [b]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany; [c]Institut de Biologie, Ecole Normale Superieure, 75005 Paris, France; [d]Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 0QH, United Kingdom; [e]Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), 1030 Vienna, Austria; and [f]Technische Universität Dresden, Universitätsklinikum Carl Gustav Carus, Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, 01307 Dresden, Germany

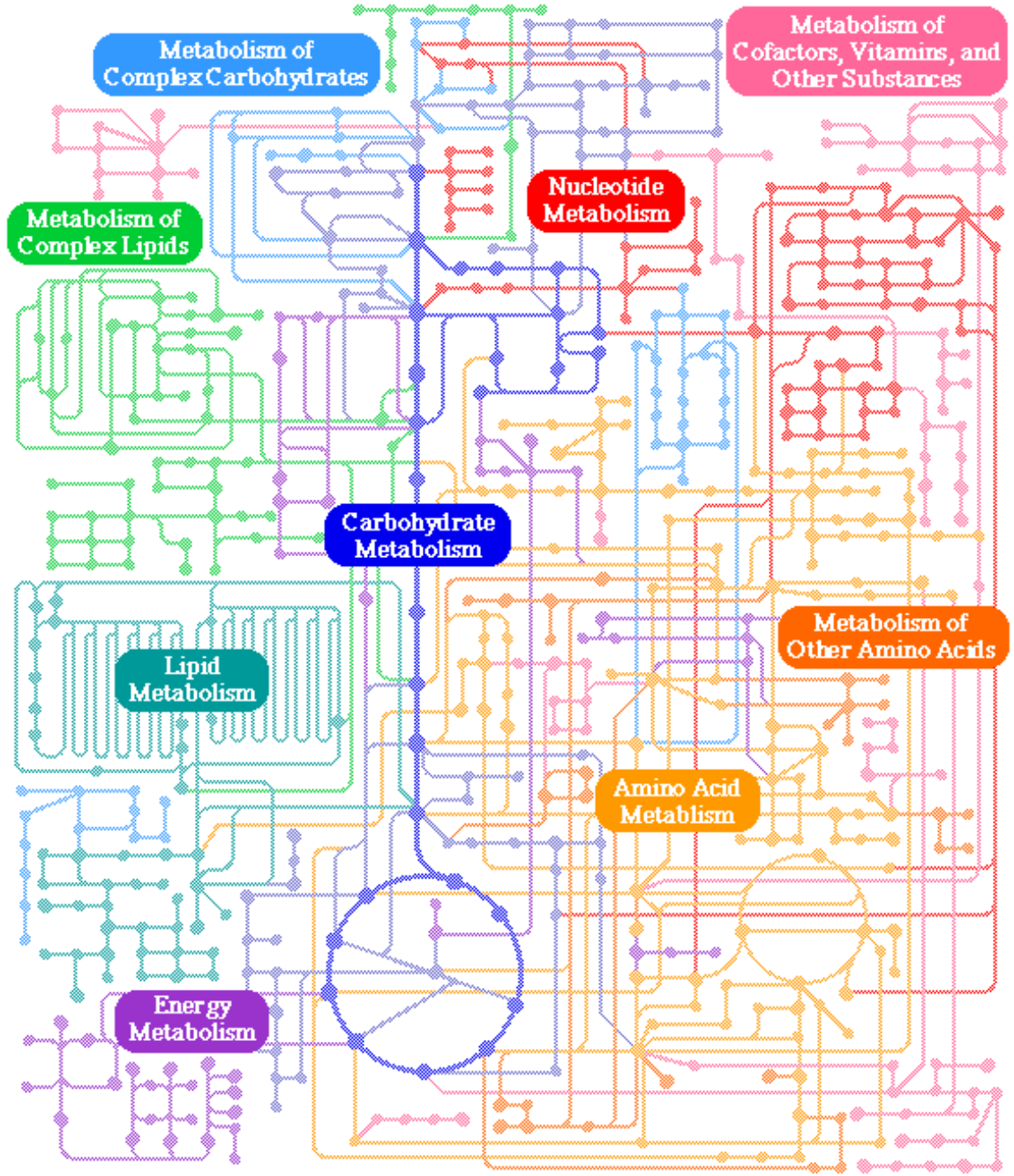




RNA-Seq

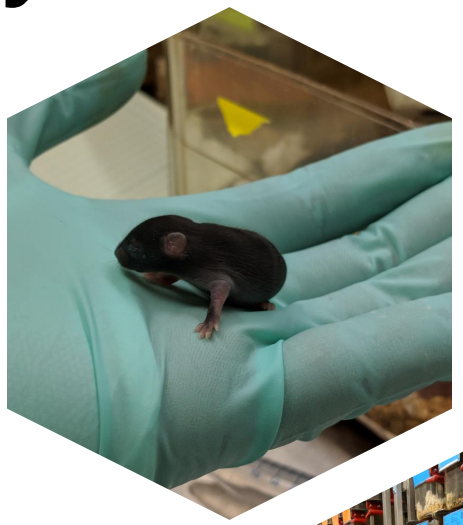

Sample 1
Sample 2
Isolate mRNA
Sequence
Sample 1
Sample 2
Align Sequences
Gene A
Gene B
Gene A
Gene B
Analysis

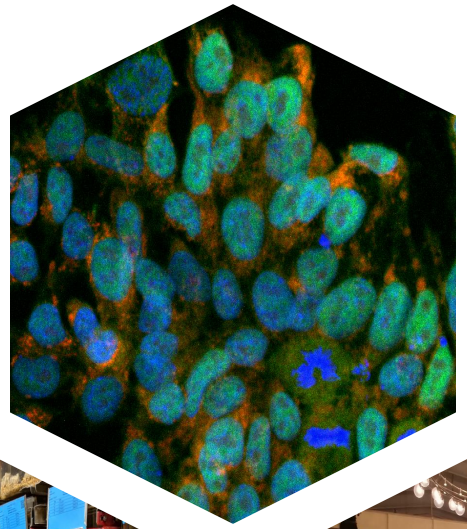
METABOLIC PATHWAYS



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