BOAZ BARAK
BRAIN BEHAVIOR LAB

New neurobiological insights in our understanding of Williams syndrome

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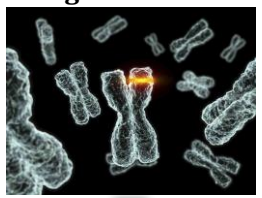
TEL AVIV UNIVERSITY

ASSOCIAZIONE ITALIANA
SINDROME DI WILLIAMS

Clinical indications and medical needs

Williams syndrome patients – 1:10,000

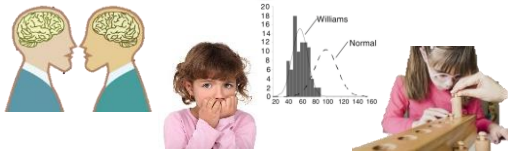
Deletion of 25 genes from chromosome 7

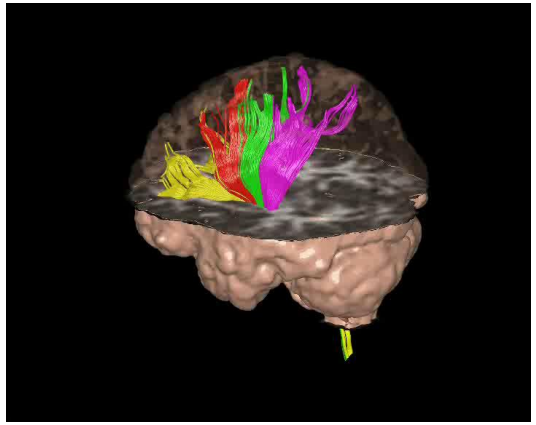


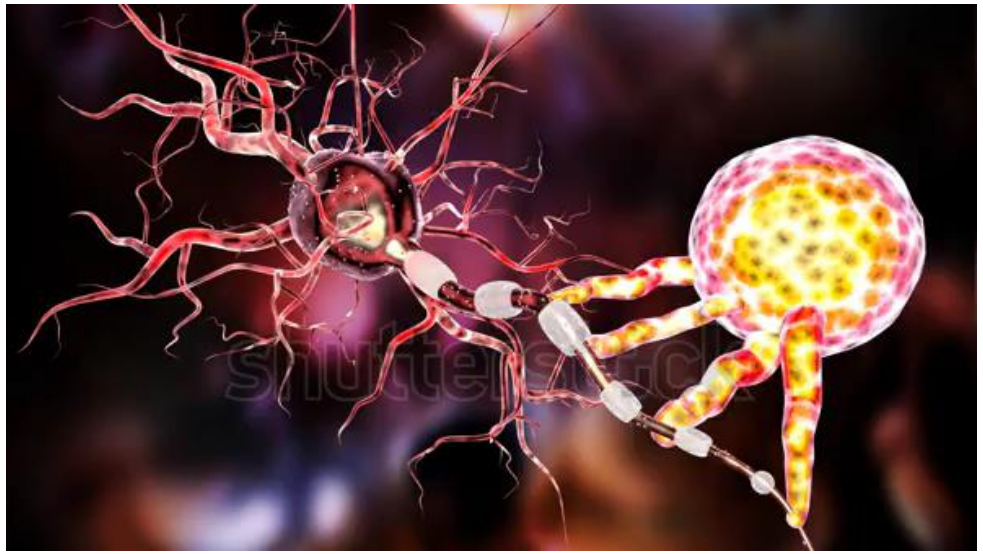
Why?

Brain function deficits

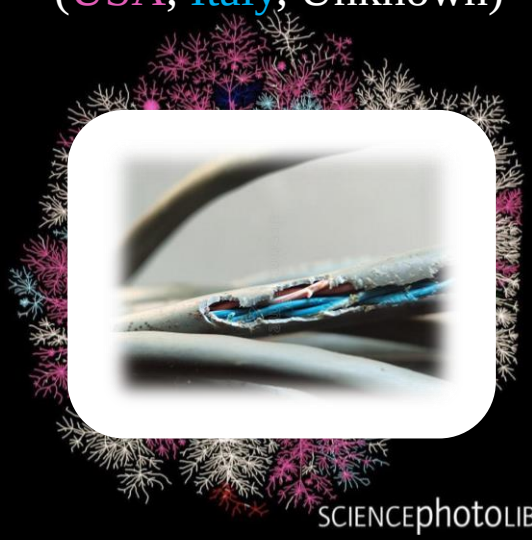
How to treat?



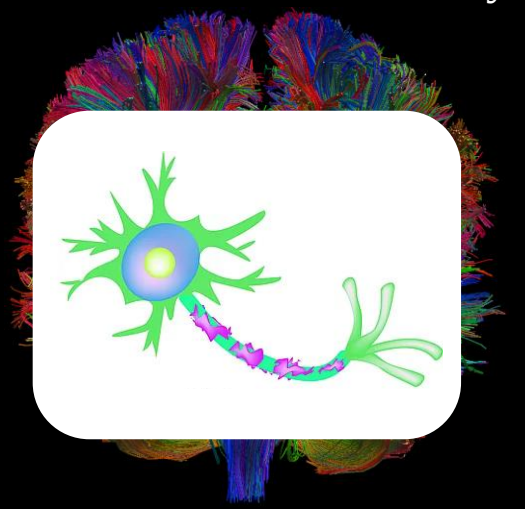




Global internet traffic
(USA, Italy, Unknown)

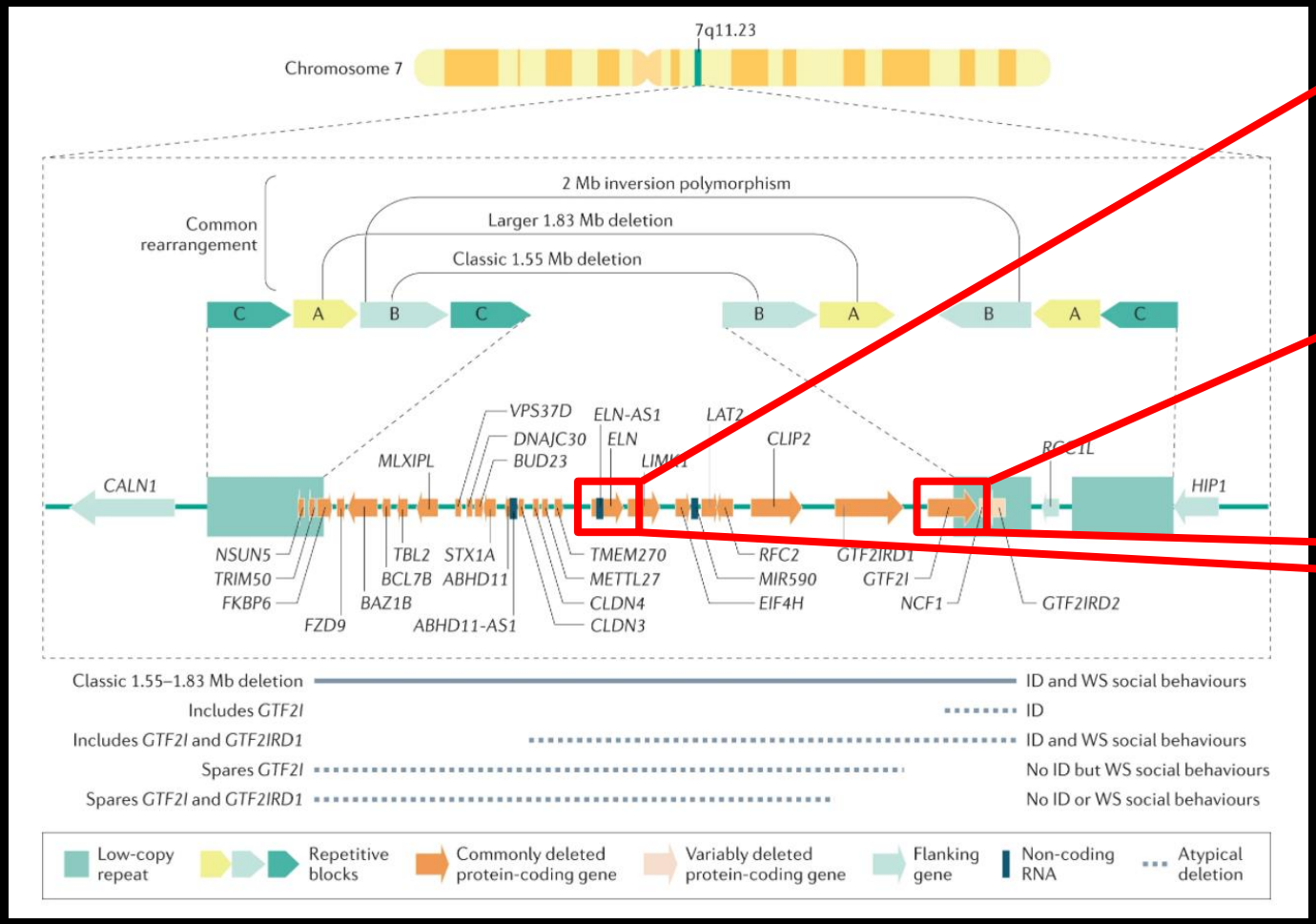


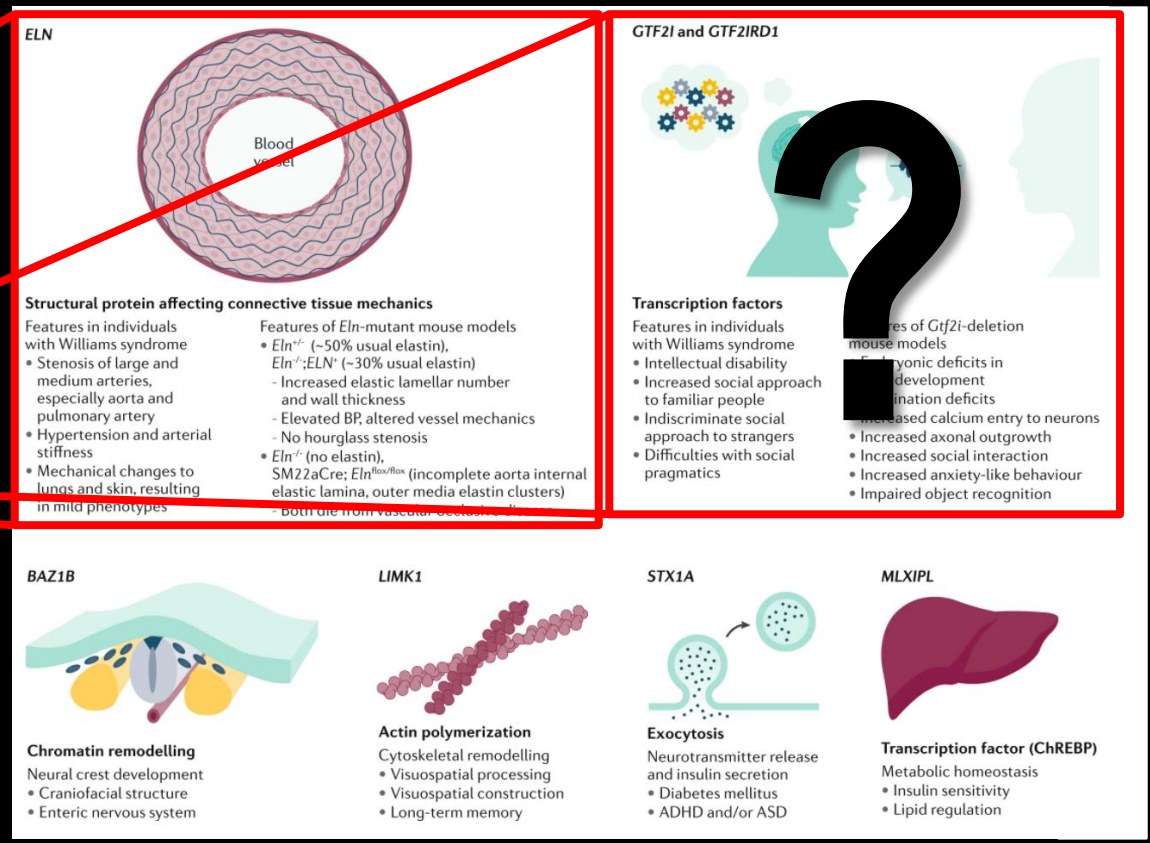
Human brain connectivity



Barak *et al.*, *Nature Neuroscience*, 2019

What is the genetics of WS?

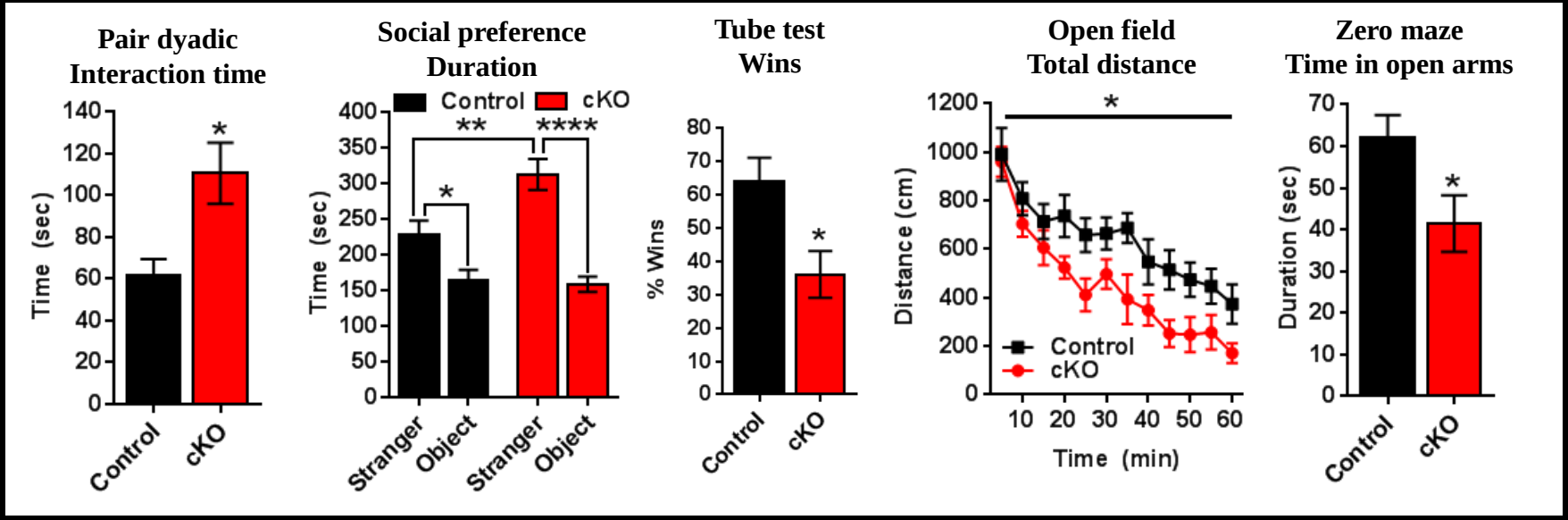




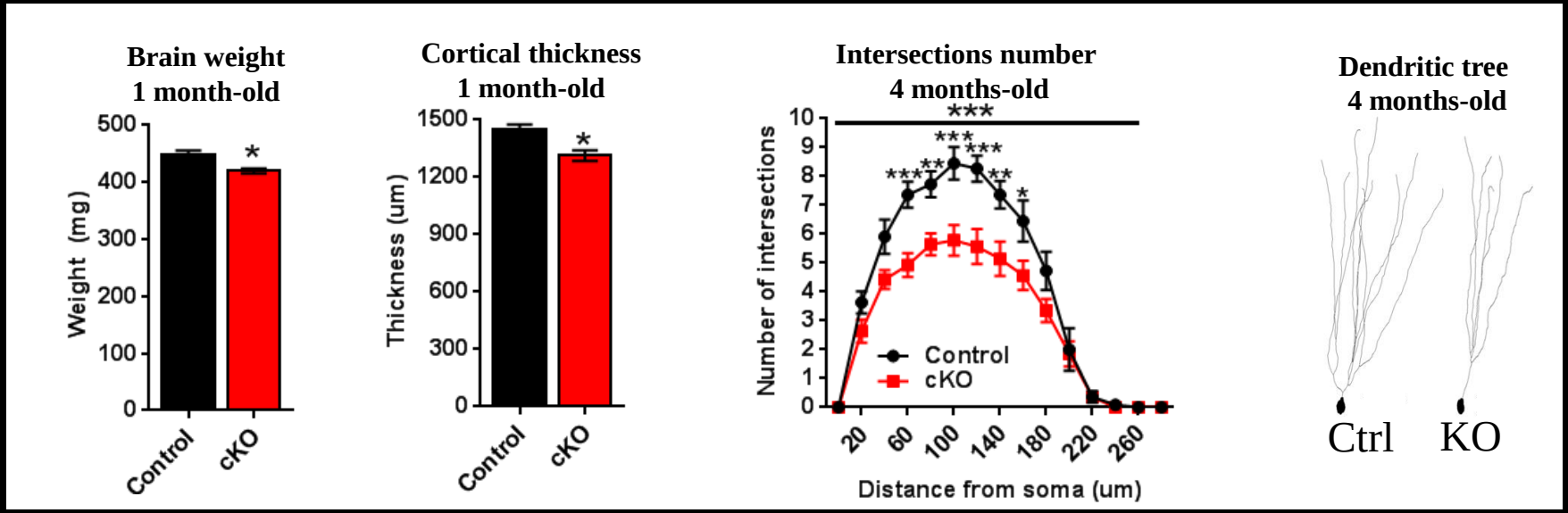
Studied the neuronal functions of *Gtf2i*
by deleting it from excitatory neurons in the mouse brain

Kozel *et al.*, *Nature Reviews Disease Primers*, 2021

What are the behavioral outcomes?

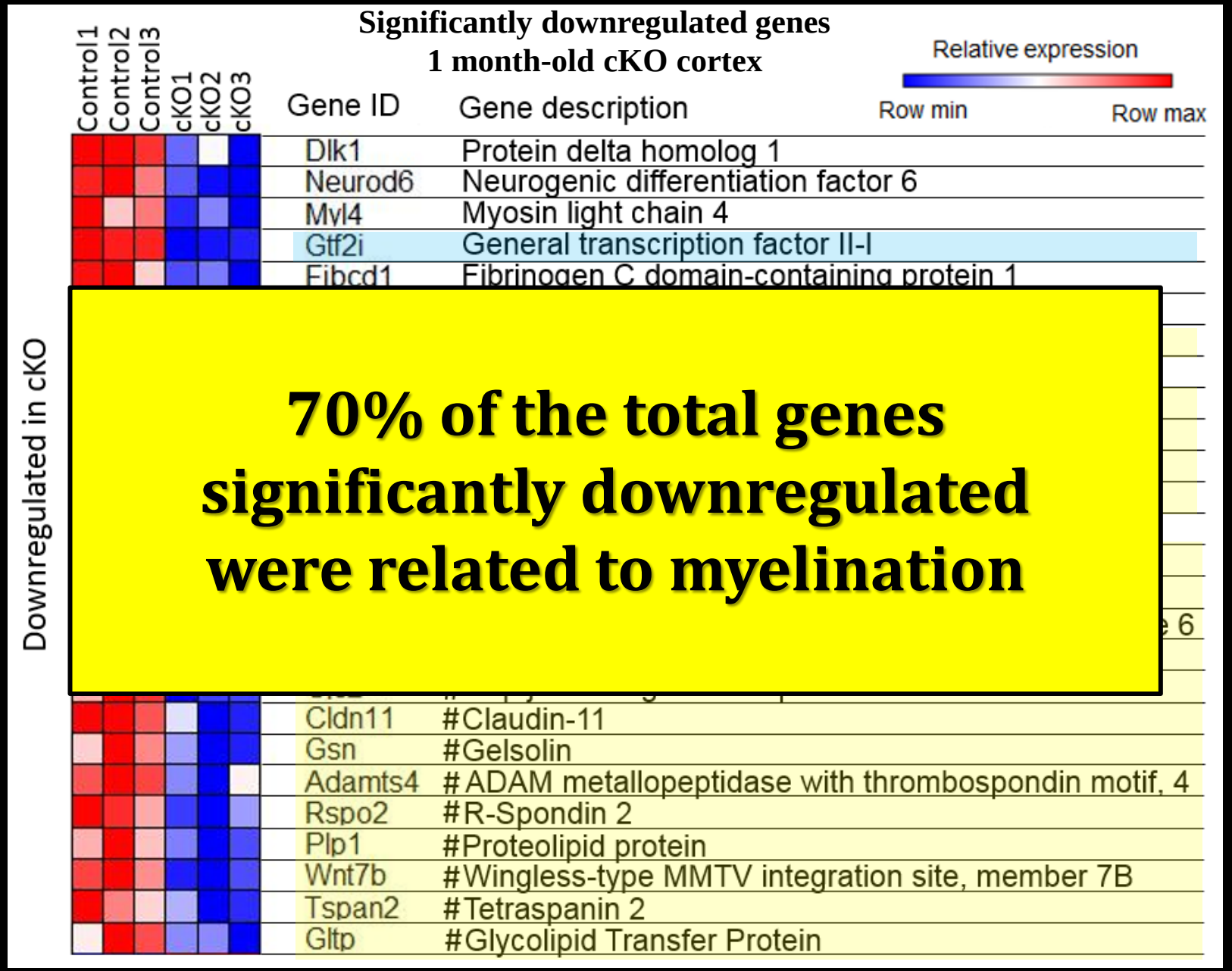


What are the neuroanatomical outcomes?



Barak *et al.*, *Nature Neuroscience*, 2019

What are the transcriptional outcomes?

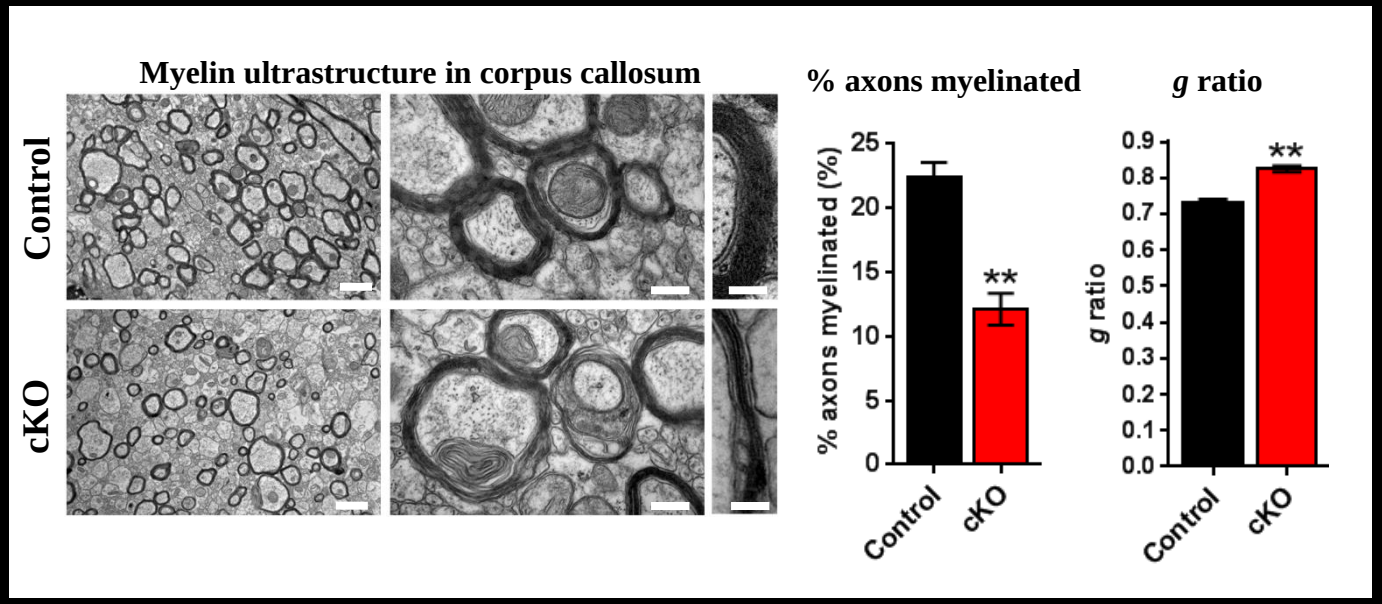


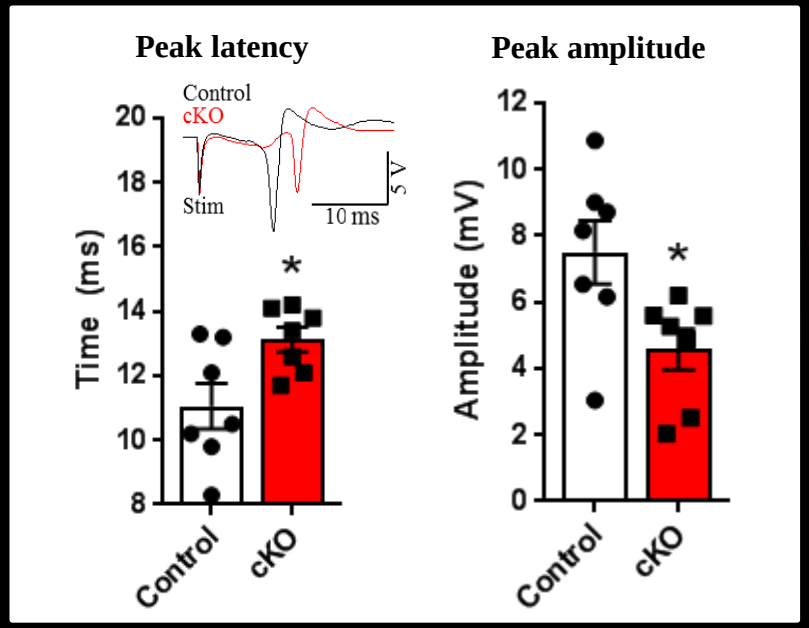
70% of the total genes significantly downregulated were related to myelination

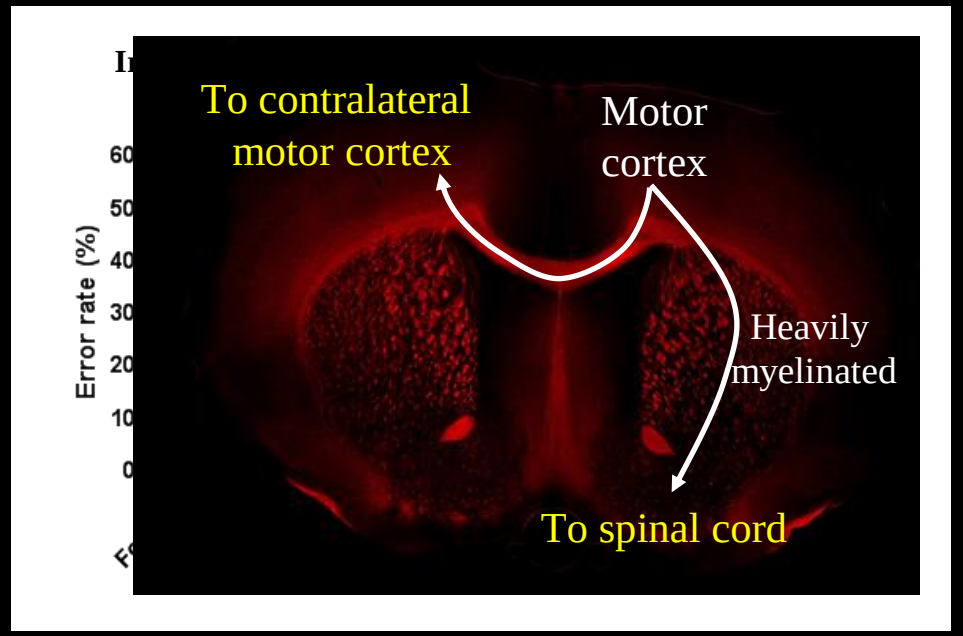
Barak et al., *Nature Neuroscience*, 2019

What is myelin and why is it important?

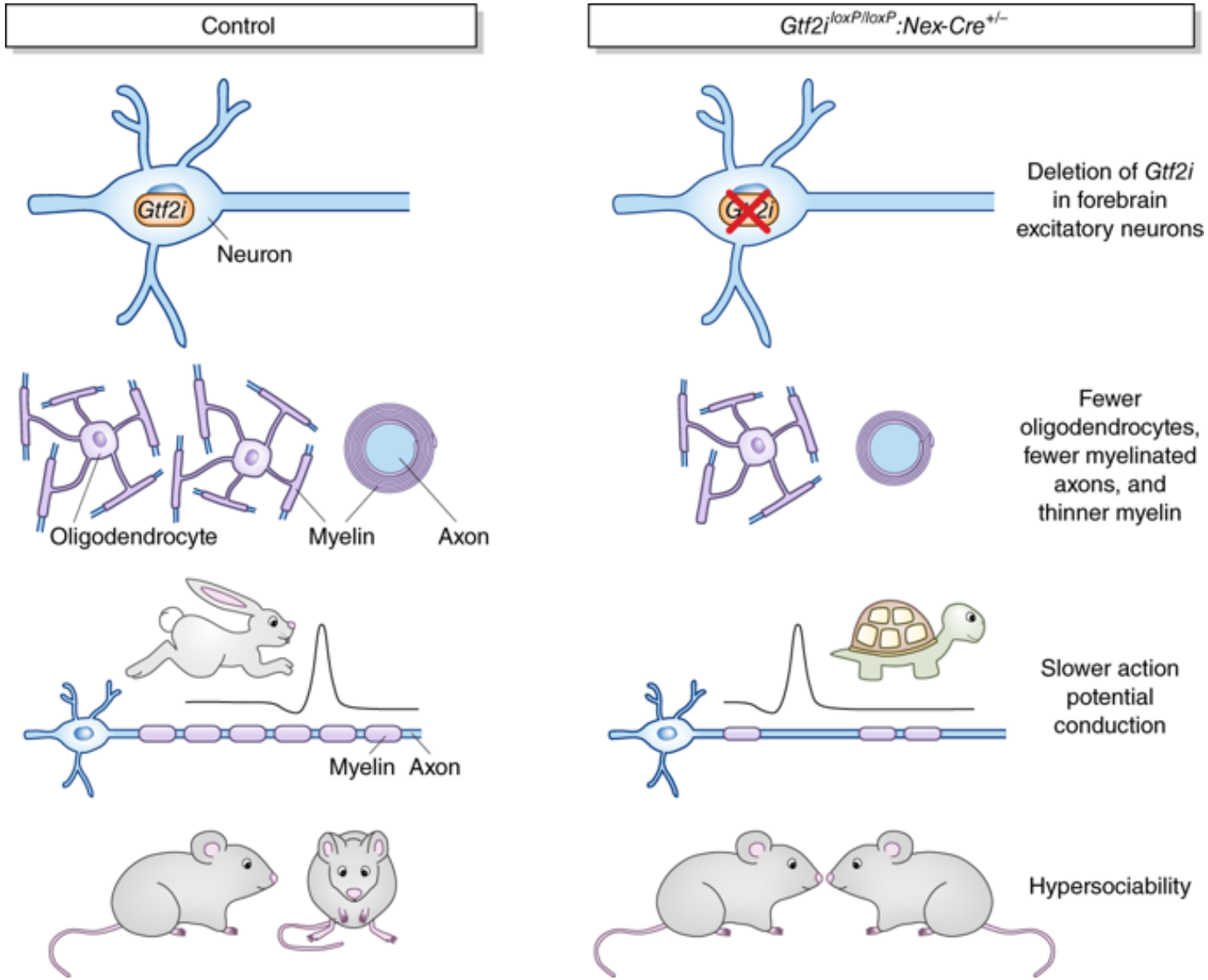
How does that affect myelin structure and function?



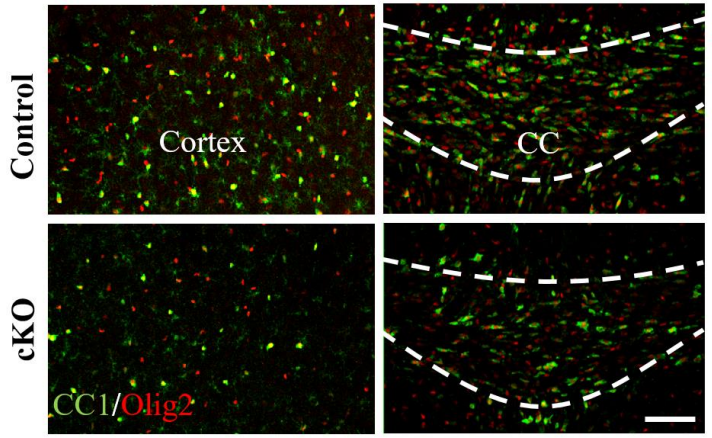


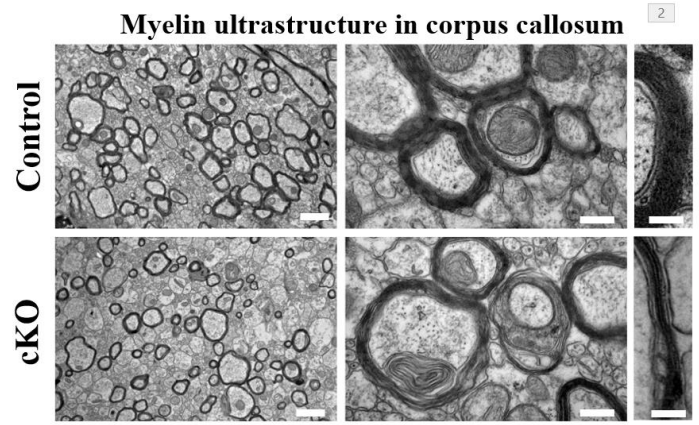


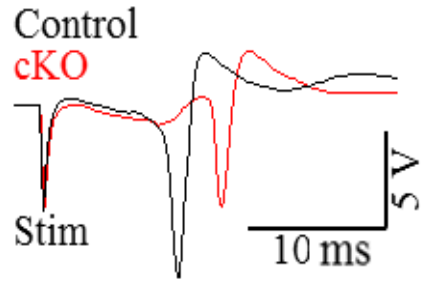
Barak *et al.*, *Nature Neuroscience*, 2019



Osso and Chan, *Nature Neuroscience* (commentary), 2019

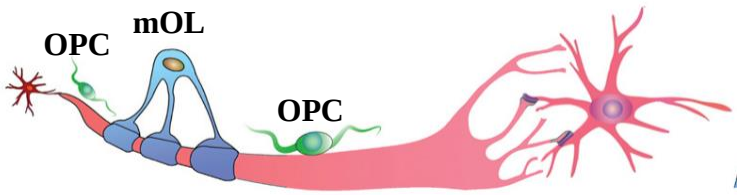






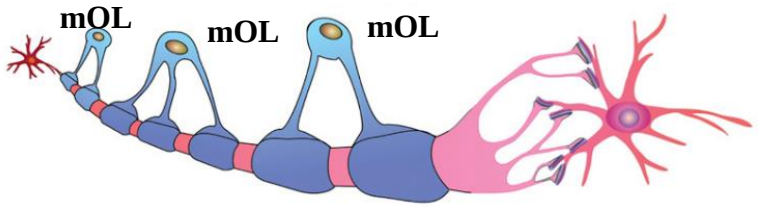
Barak *et al.*, *Nature Neuroscience*, 2019

Can we rescue myelination deficits?

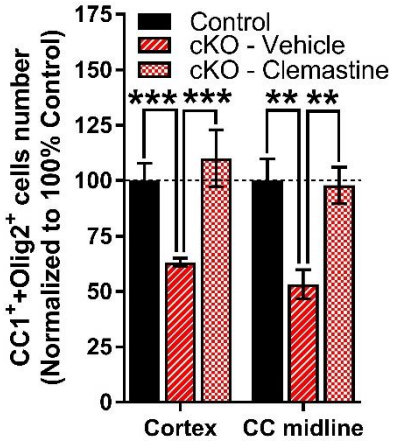


Clemastine

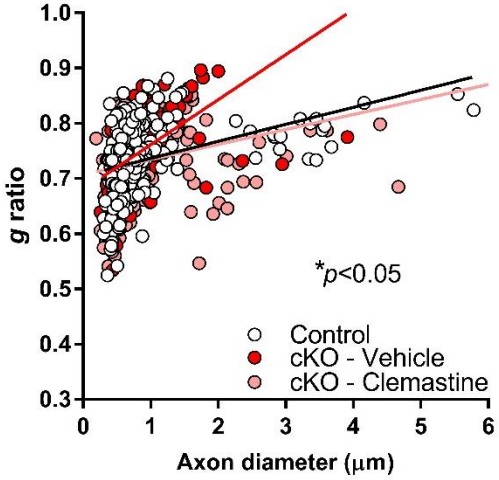
Increase OPC differentiation to mOL



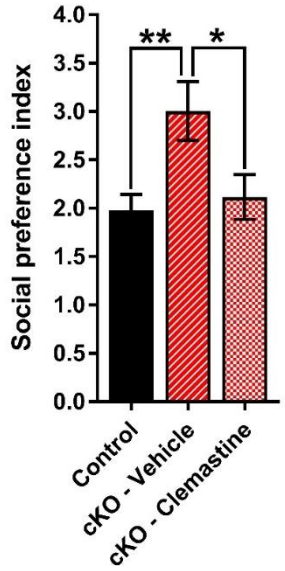
mOLs number Clemastine



***g* ratio Clemastine**

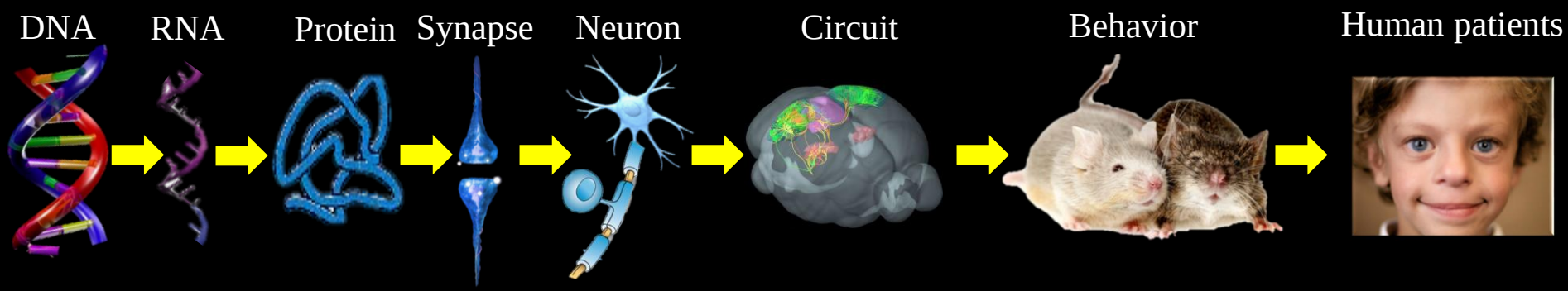


Social preference index Clemastine

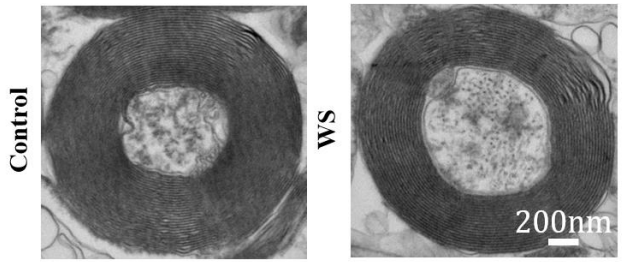


Barak *et al.*, *Nature Neuroscience*, 2019

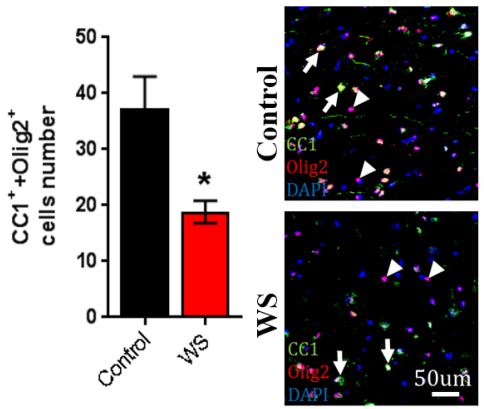
Barak *et al.*, *Nature Neuroscience*, 2019


DNA
RNA
Protein
Synapse
Neuron
Circuit
Behavior
Human patients

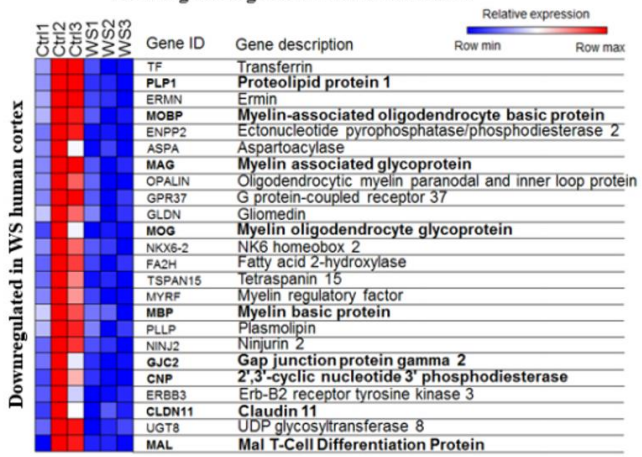
Myelin ultrastructure in human cortex

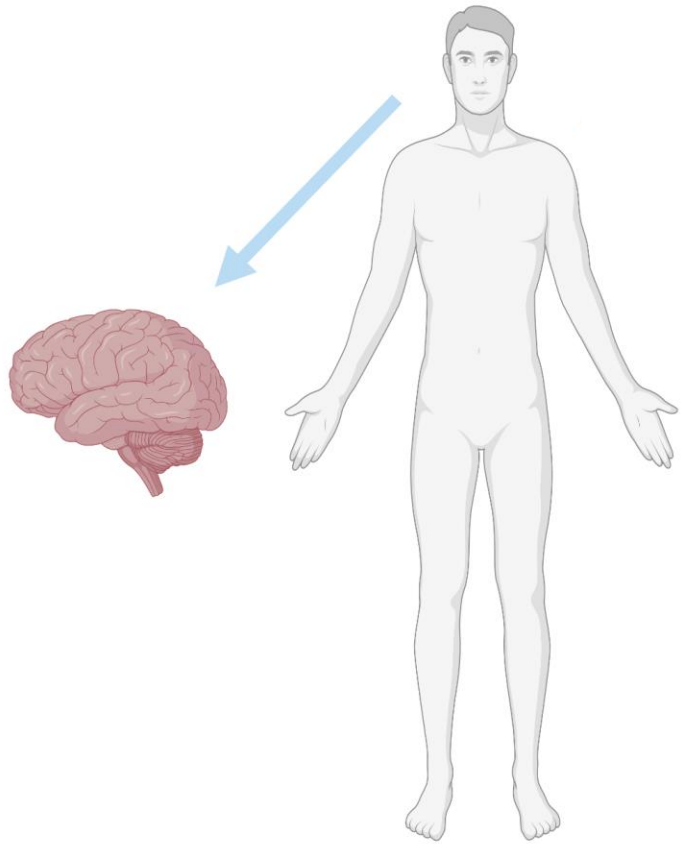


Myelinating oligodendrocytes in human cortex



Downregulated genes in WS human cortex

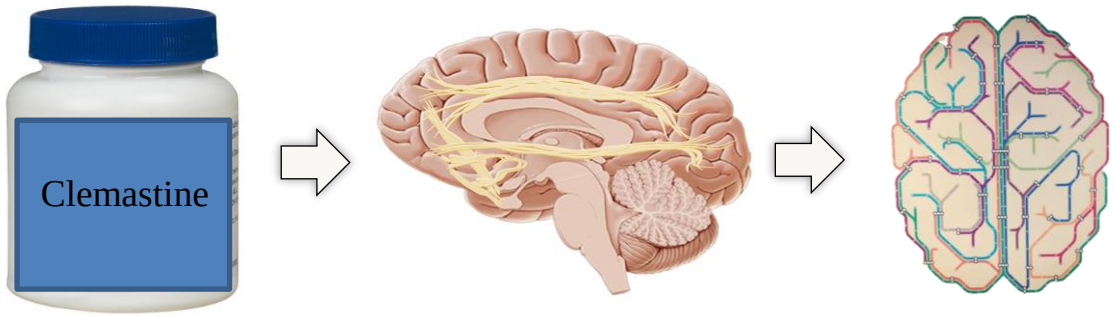




Individual with Williams syndrome

Barak *et al.*, *Nature Neuroscience*, 2019

Can we treat Williams syndrome in humans?



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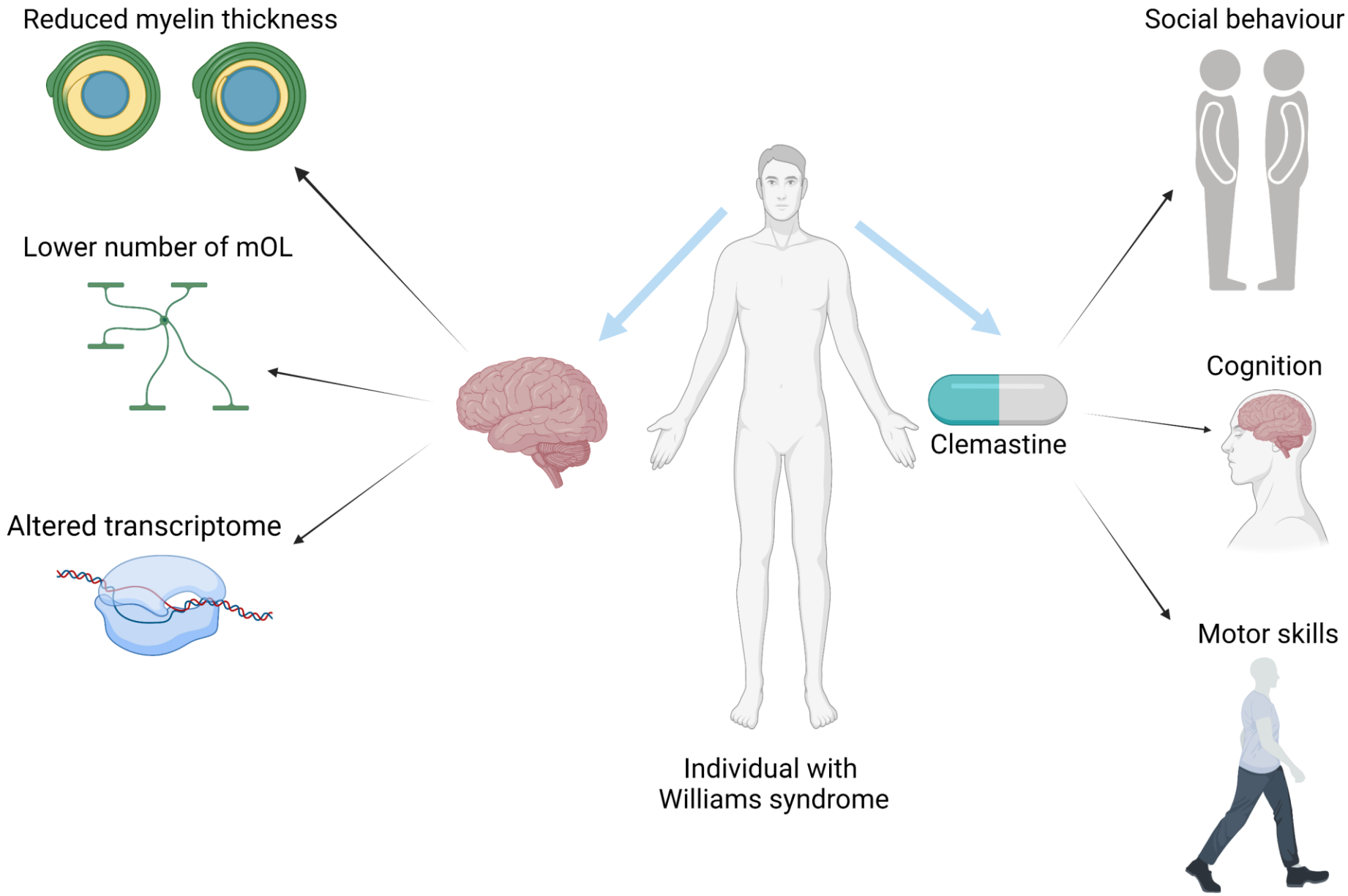
Barak *et al.*, *Nature Neuroscience*, 2019


Reduced myelin thickness
Lower number of mOL
Altered transcriptome
Individual with
Williams syndrome
Clemastine
Social behaviour
Cognition
Motor skills

Can we treat Williams syndrome in humans with clemastine?

Clinical trial in Sheba medical center, Israel

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Clinical trial project team

DR. BOAZ BARAK
Head of Neurogenetics Laboratory

PROF. DORON GOTHELF
Director of Child and Adolescent Psychiatry Unit

ARIEL NIR
Ph.D. Candidate, Barak Lab

DR. RONNIEWEINBERGER
Behavioral Neurogenetics Center Manager

DR. AMIR DORI
Head of Neuromuscular Department

DR. URI GIVON
The Walking and Gait Laboratory

DR. MEIR PLOTNIK
The Walking and Gait Laboratory

DR. URIEL KATZ
Director of the Pediatric Cardiology Unit

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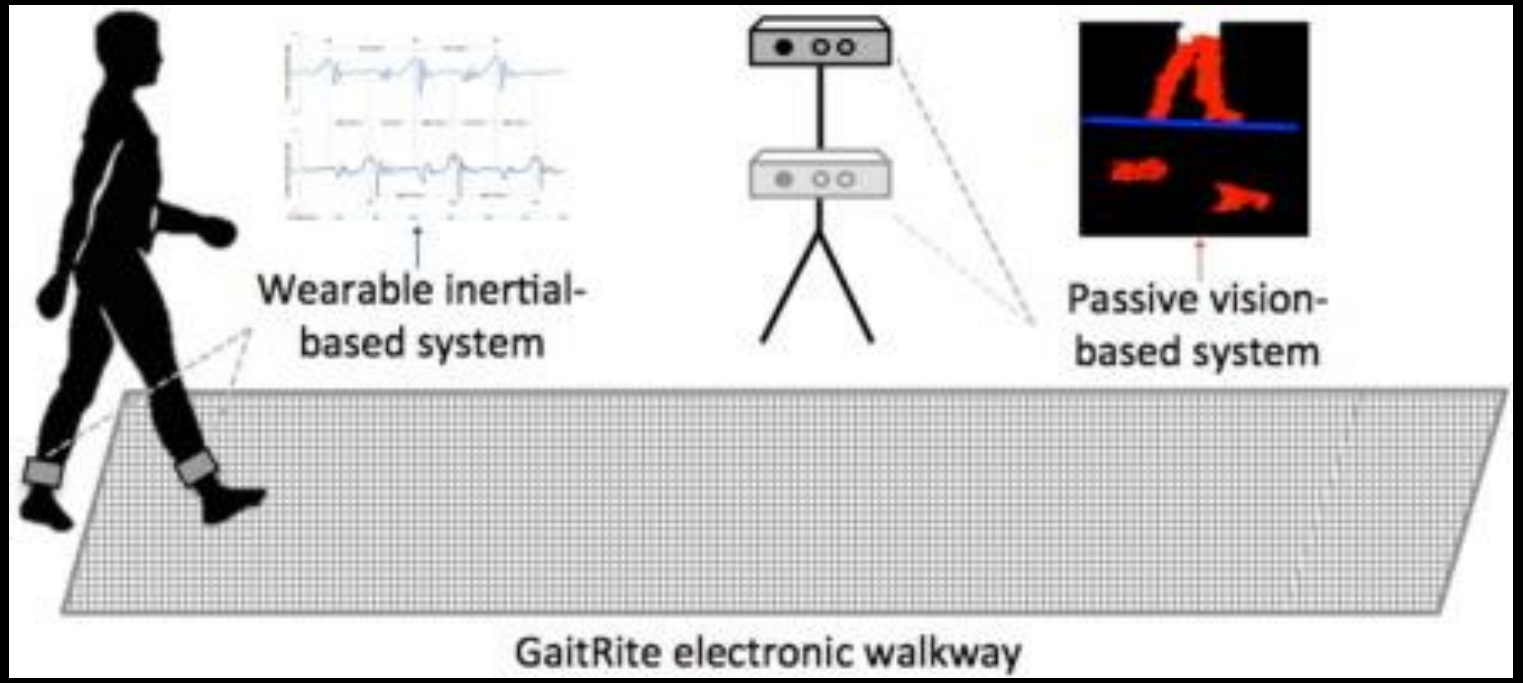
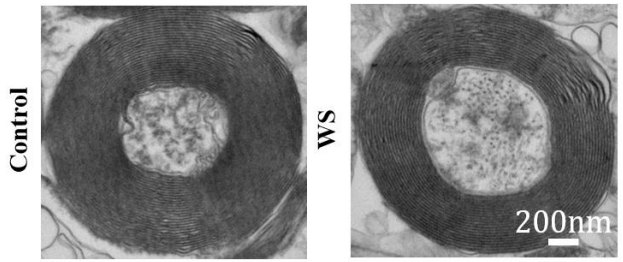
Are there detectable motor deficits in WS?



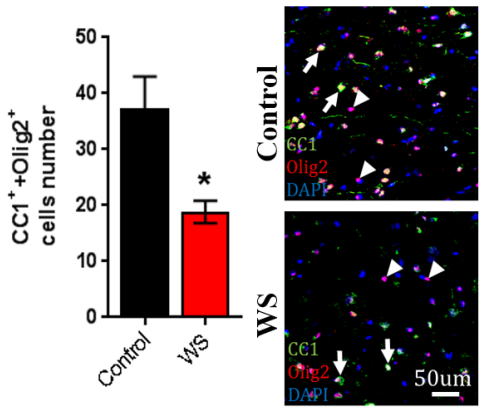
In collaboration with Prof. Doron Gothelf, Sheba Hospital

Ariel Nir *et al.*, Unpublished data

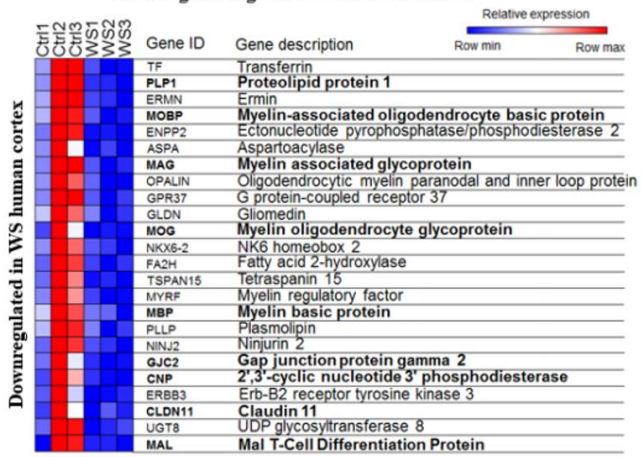
Myelin ultrastructure in human cortex

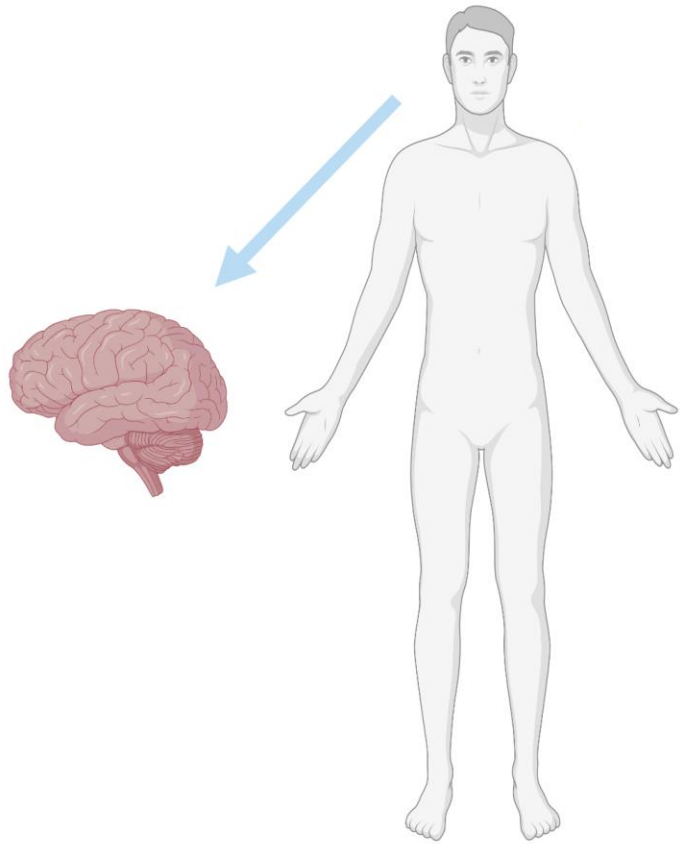


Myelinating oligodendrocytes in human cortex



Downregulated genes in WS human cortex

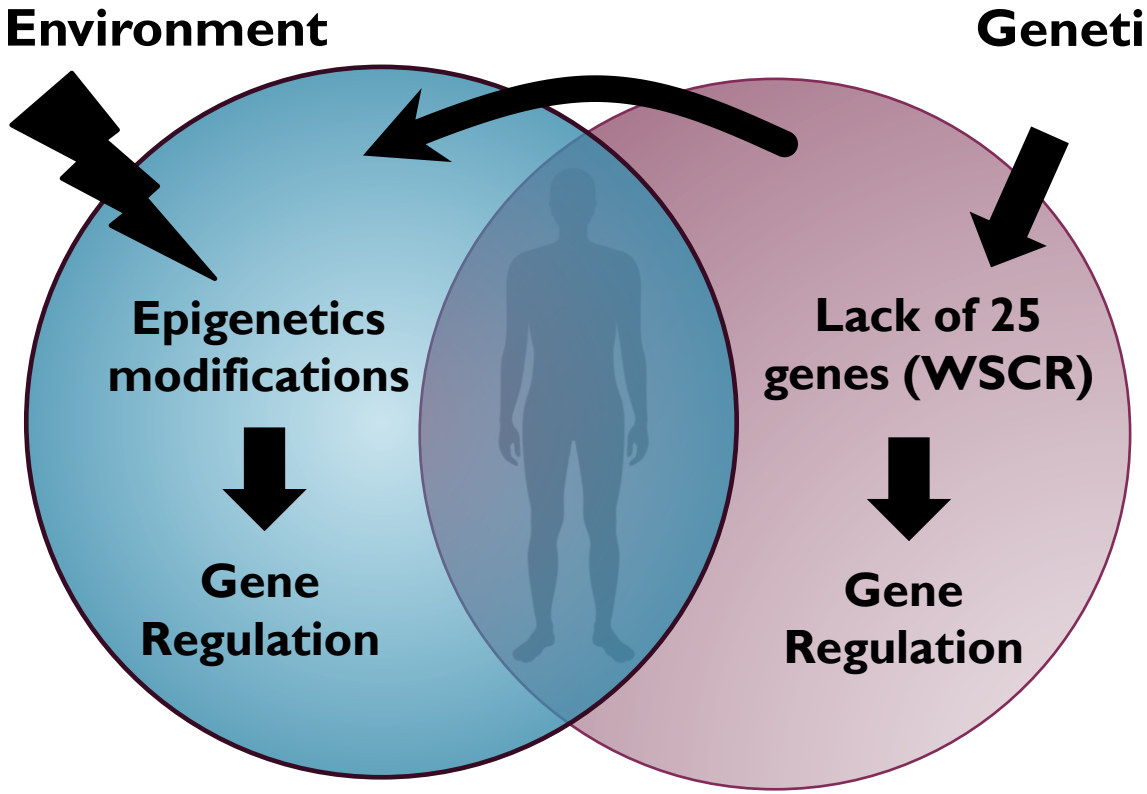


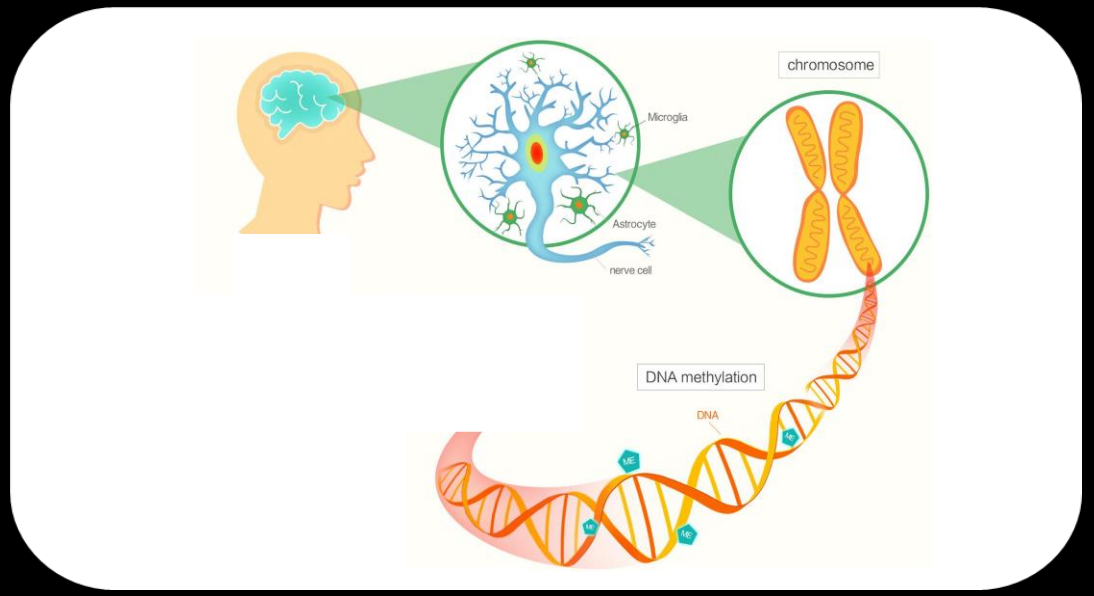


Individual with Williams syndrome

Barak *et al.*, *Nature Neuroscience*, 2019

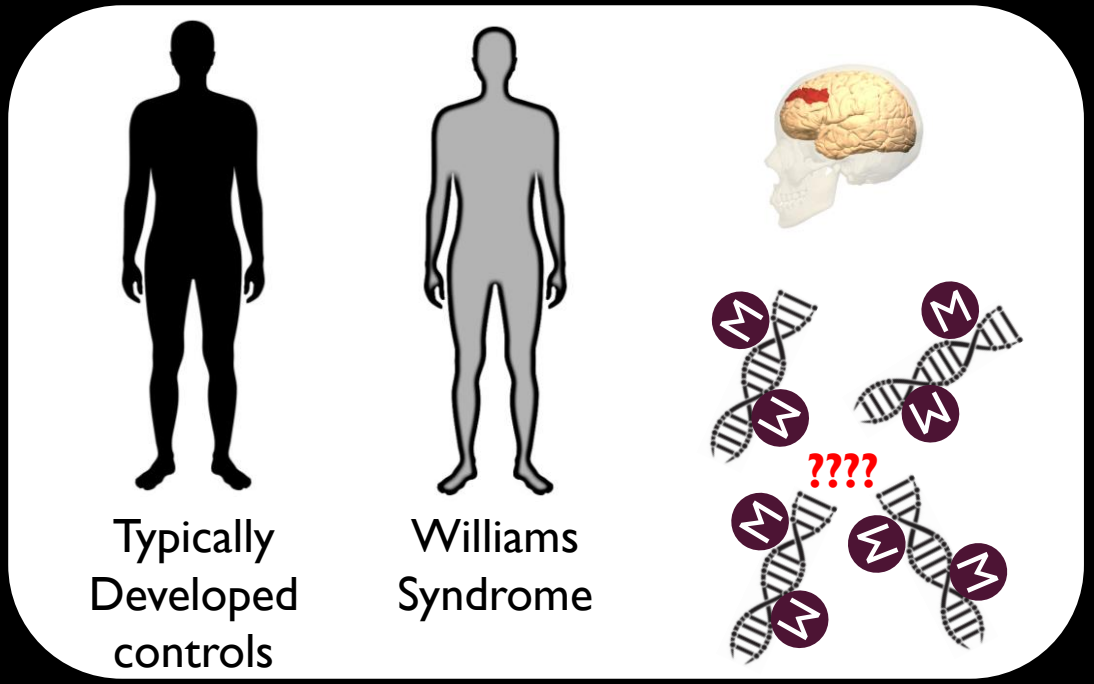
What is the role of epigenetic regulation in WS?





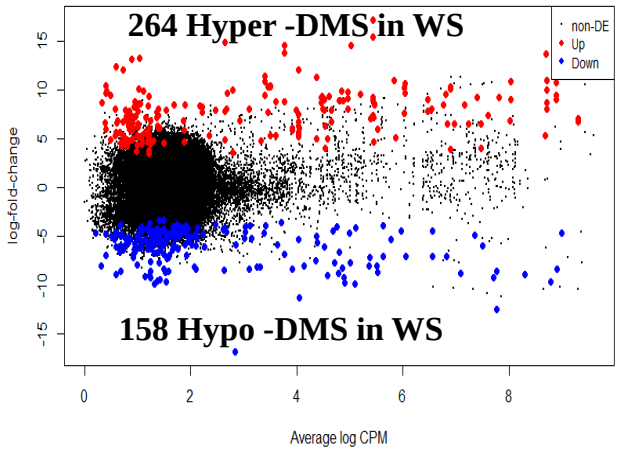
Hypothesis:

Some of the pathological outcomes in WS are the result of epigenetic changes propagating from the genetic variation in the WSCR

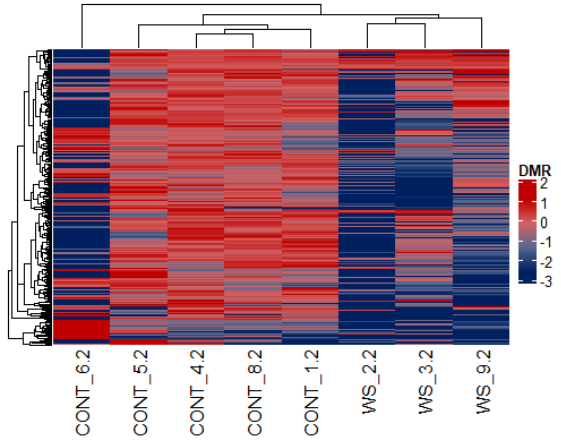


What is the role of epigenetic regulation in WS?

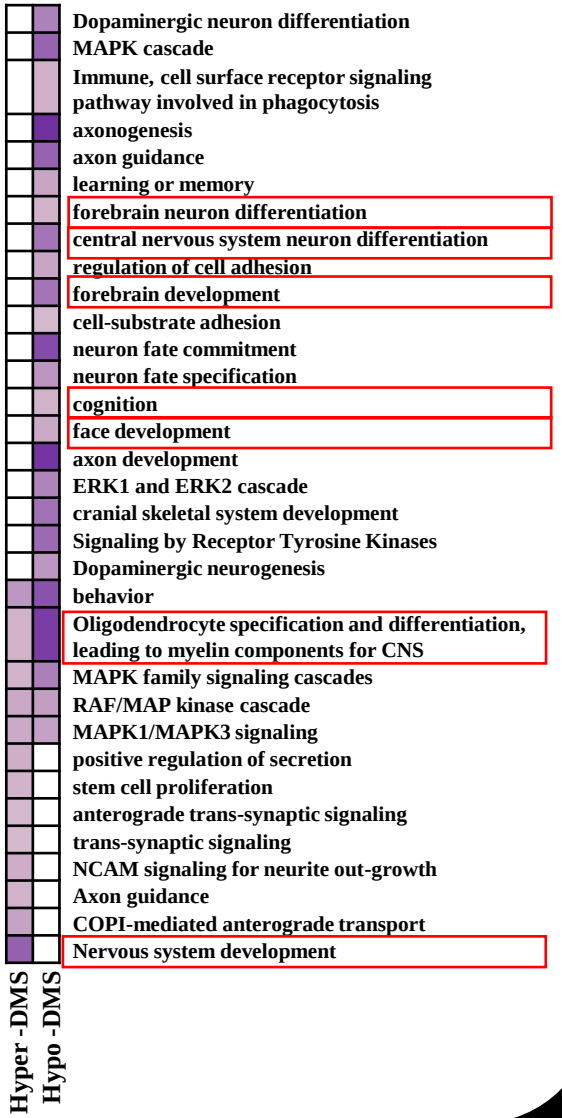
Differentially methylated sites (DMS)



Differentially methylated regions (DMR)

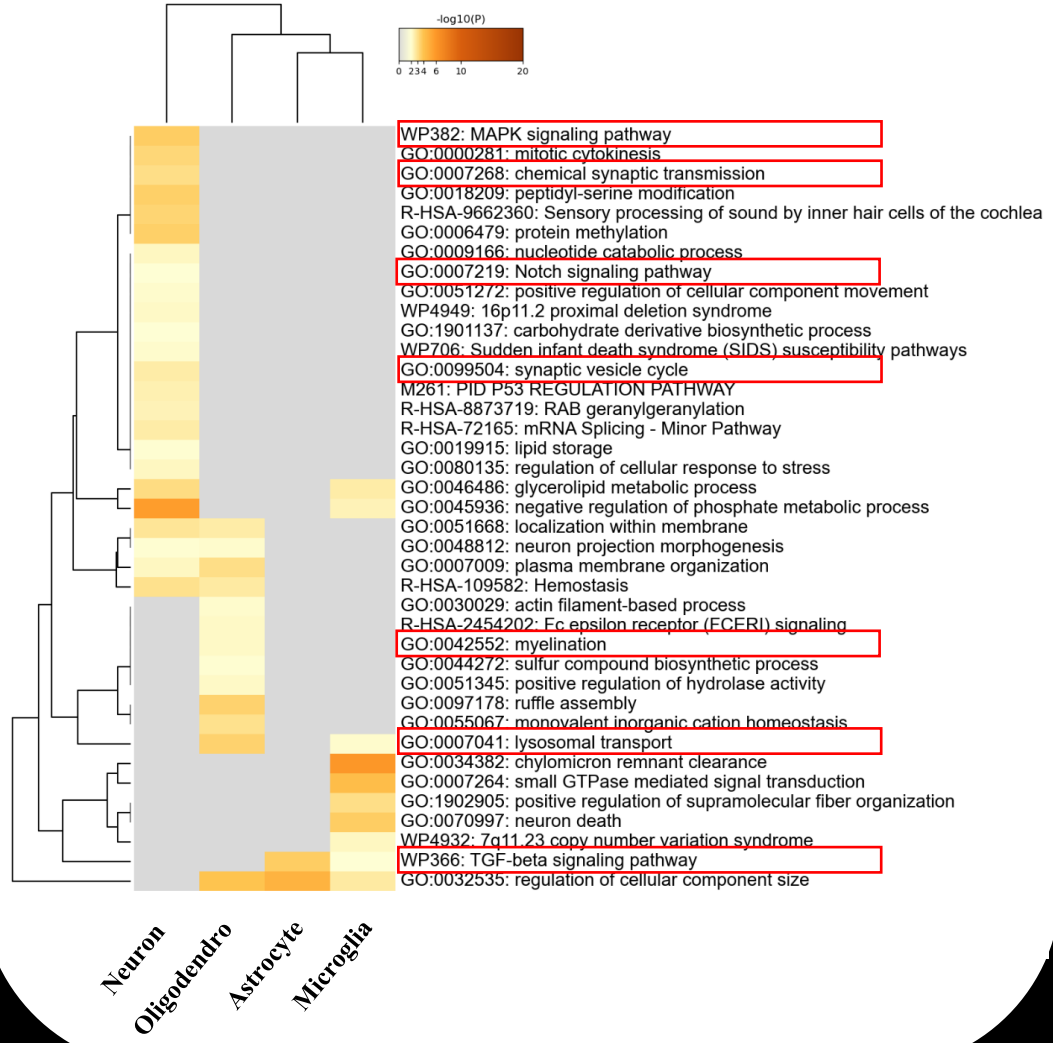


Pathway analysis



Cell-type specific methylation enrichment

Nott *et al.* Science 2019: Active promoters and enhancers in cell types of the human brain



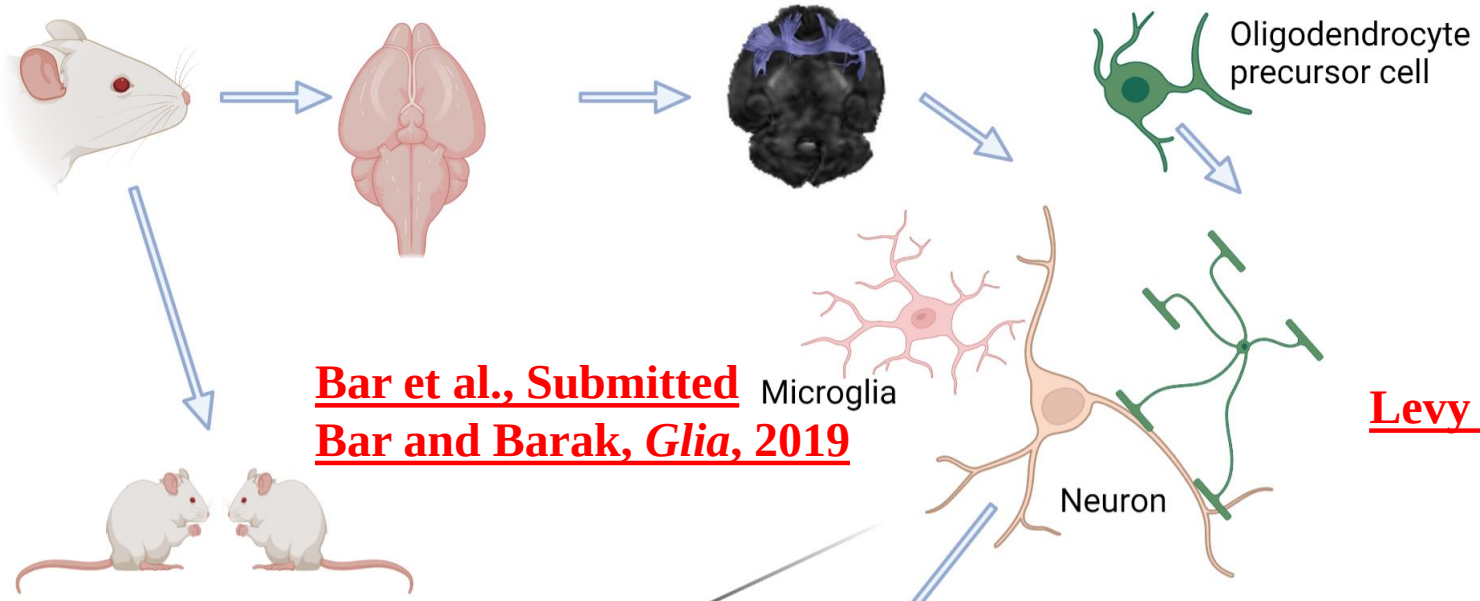
In collaboration with Dr. Asaf Marco, HUJI

Sari Trangle *et al.*, Under revision, *Molecular Psychiatry*

Barak et al., *Nature Neuroscience*, 2019

Grad et al., *Cells*, 2022

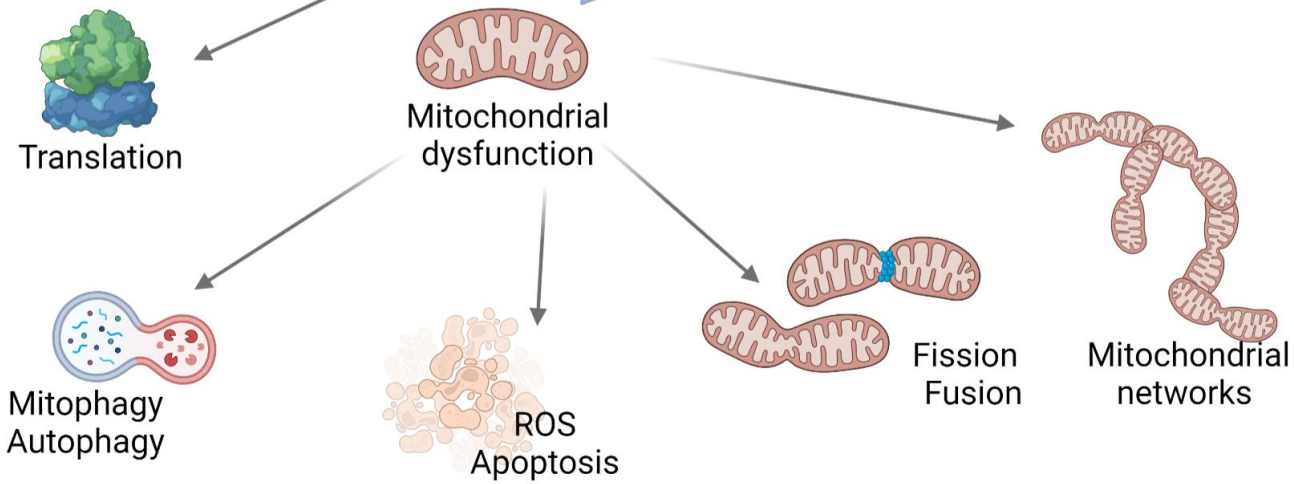
Nir et al., *Glia*, 2020



Bar et al., Submitted

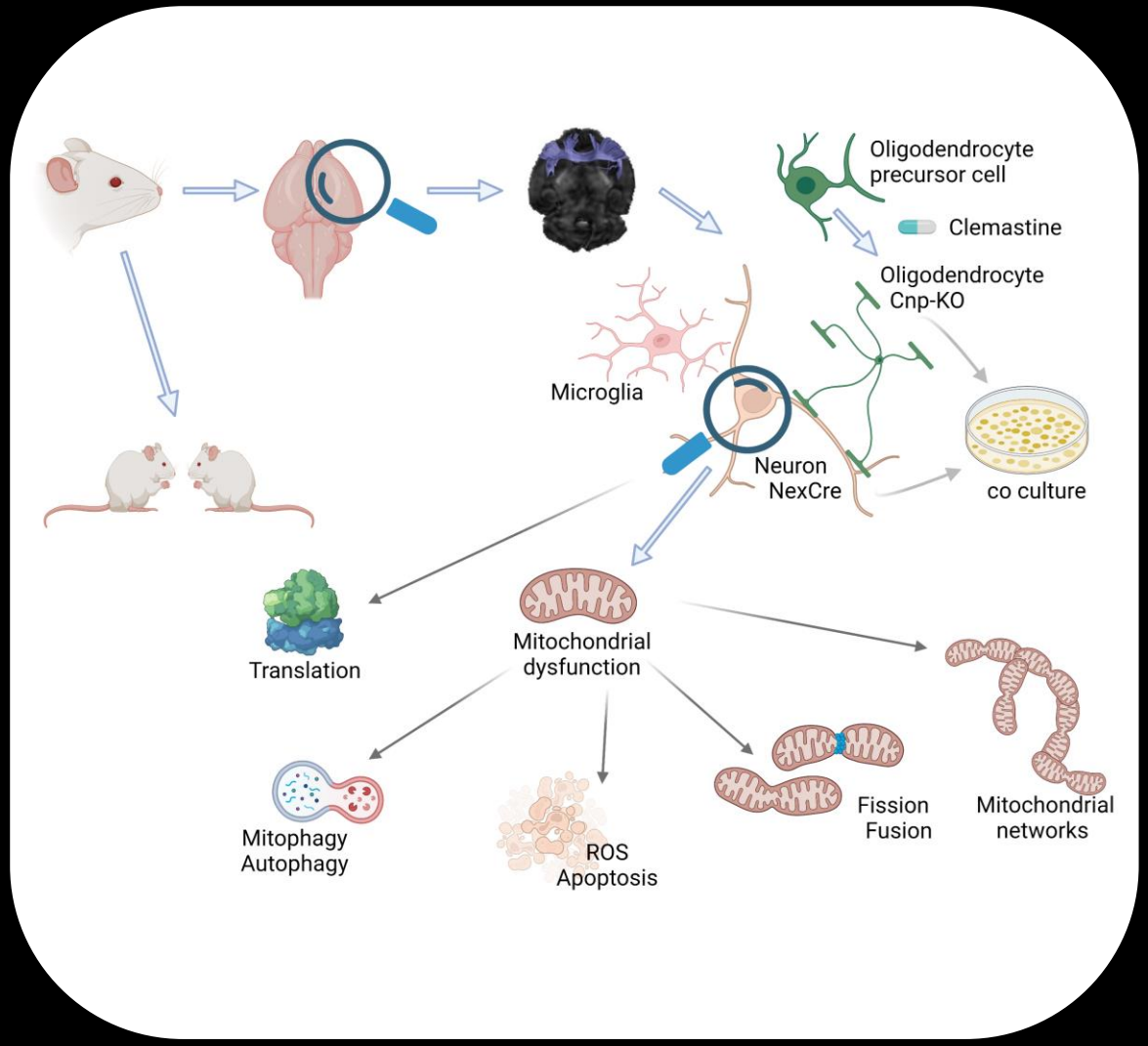
Bar and Barak, *Glia*, 2019

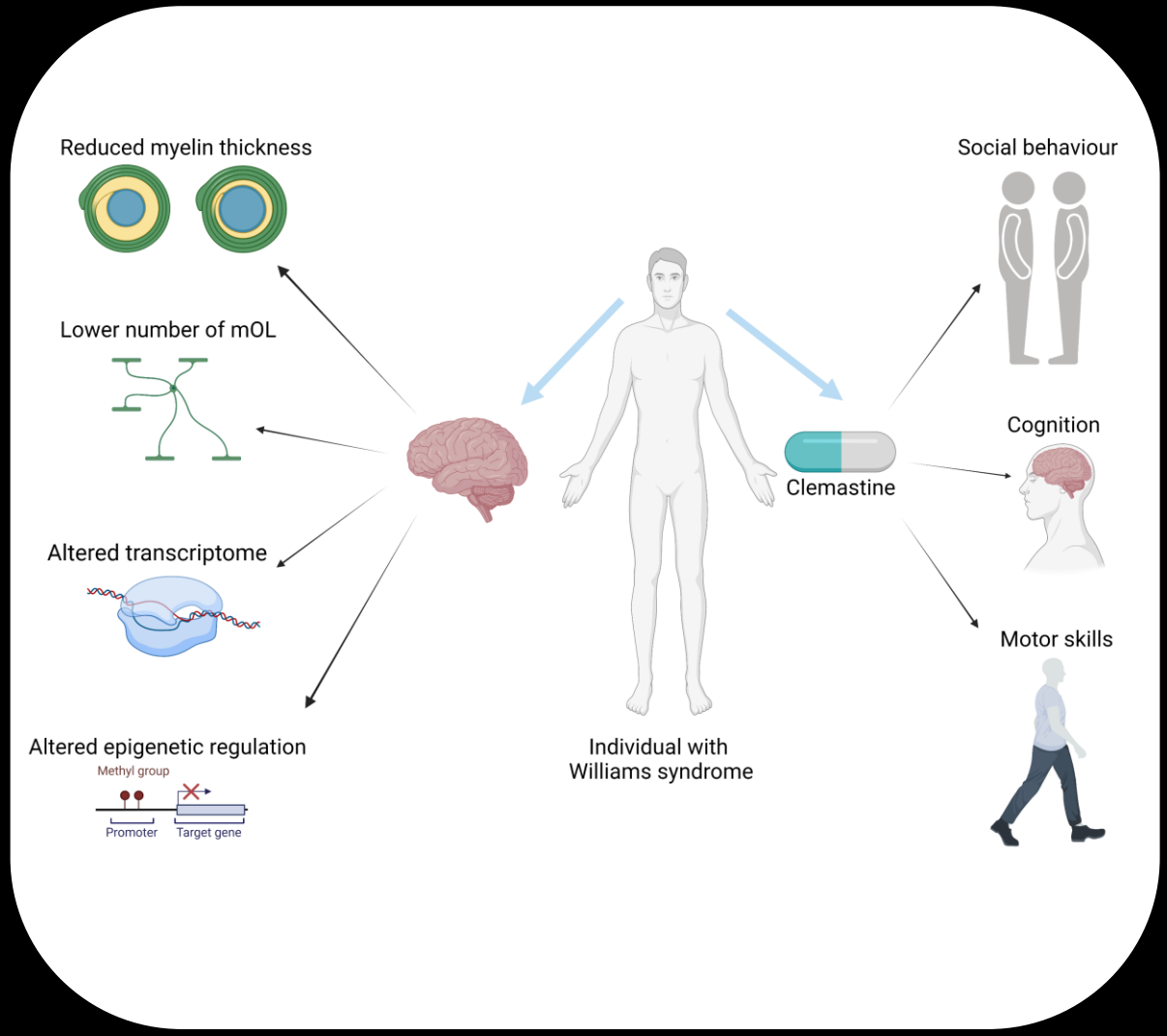
Levy et al., In preparation



Nir et al., In preparation

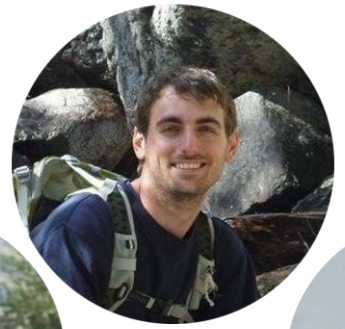
Summary


Oligodendrocyte precursor cell
Clemastine
Oligodendrocyte Cnp-KO
Microglia
Neuron NexCre
co culture
Translation
Mitochondrial dysfunction
Mitophagy Autophagy
ROS Apoptosis
Fission Fusion
Mitochondrial networks

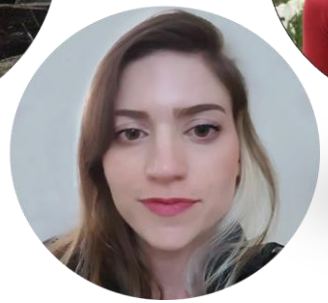

Reduced myelin thickness
Lower number of mOL
Altered transcriptome
Altered epigenetic regulation
Methyl group
Promoter
Target gene
Individual with Williams syndrome
Clemastine
Social behaviour
Cognition
Motor skills

Dr. Sari Trangle

Ariel Nir



Gilad Levy



Ela Bar

Inbar Fischer

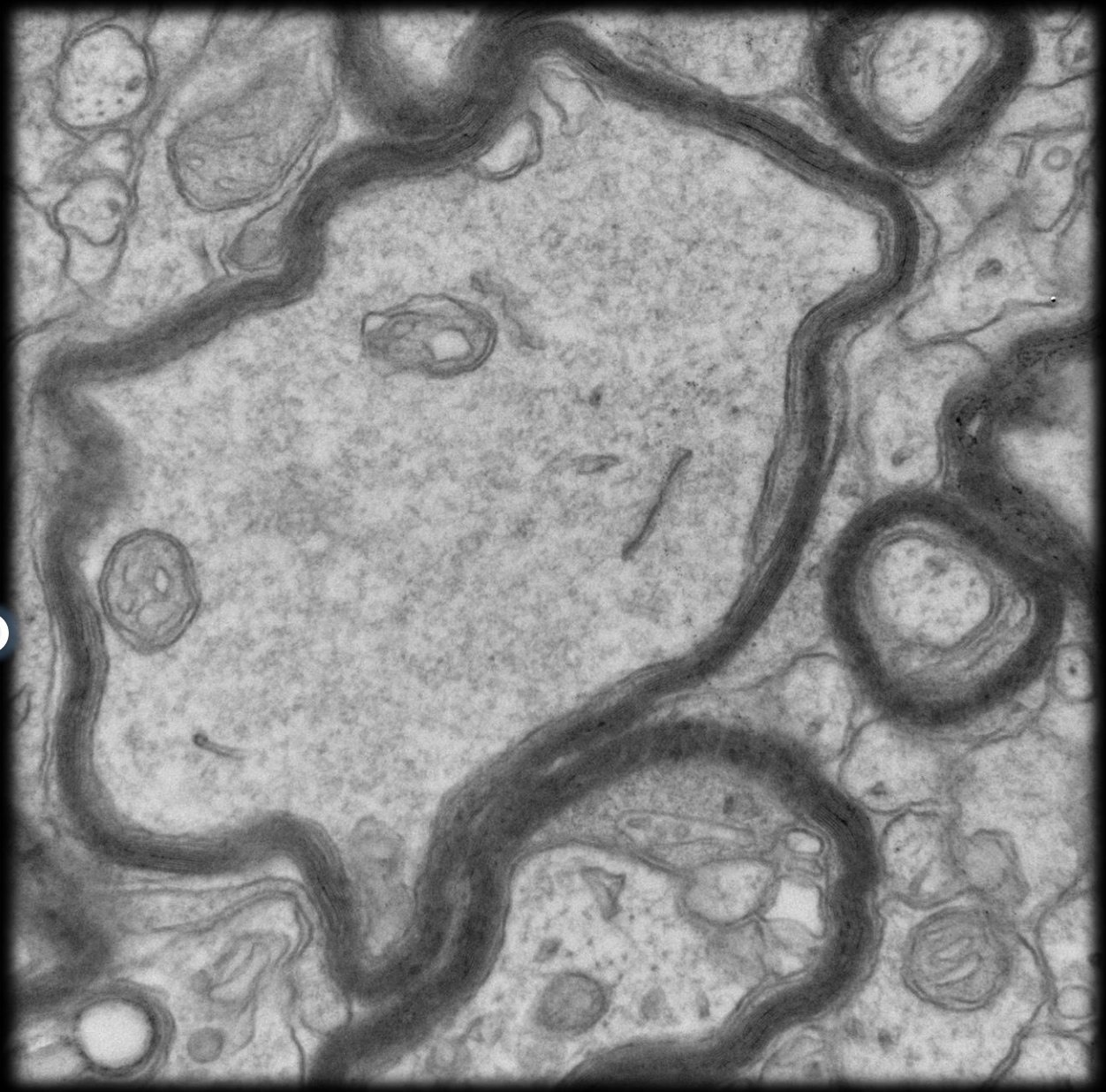
Omer Ophir

Omri Kimchi Feldhorn

May Rokach

Meitar Grad

Grazie mille!

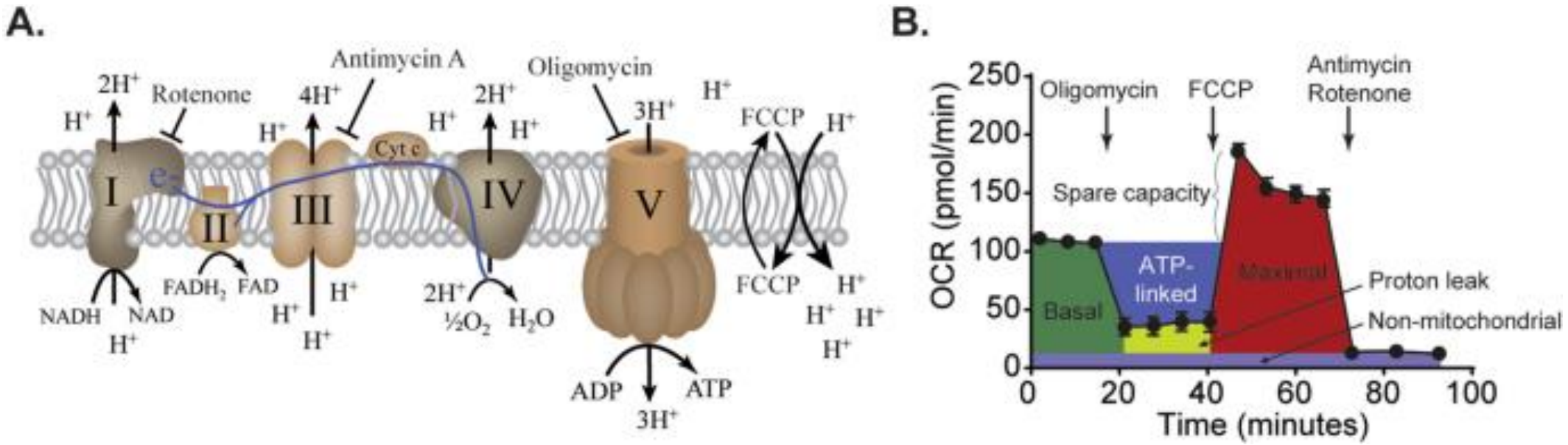


Lab website: https://people.socsci.tau.ac.il/mu/boazbarak/

E.mail: barakboaz@gmail.com

RESPIRATION

The electron transport chain



Oxygen consumption rate (OCR) measurements of purified mitochondria. (A) Simplified drawing of the electron transport chain located within the inner mitochondrial membrane. Electron transfer is coupled to the transfer of protons (H+) across the inner mitochondrial membrane into the inner membrane space, creating a proton gradient. This gradient is utilized by complex V for ATP synthesis. The protons react with oxygen to generate water. Thus, the OCR can be monitored by the Seahorse XF analyzers and used as a surrogate of mitochondrial respiration. The targets of the inhibitors (oligomycin, antimycin A, and rotenone) and uncoupler (FCCP) are indicated. (B) A representative OCR curve generated using isolated mitochondria showing the characteristic responses to mitochondrial inhibitors and the uncoupler FCCP.

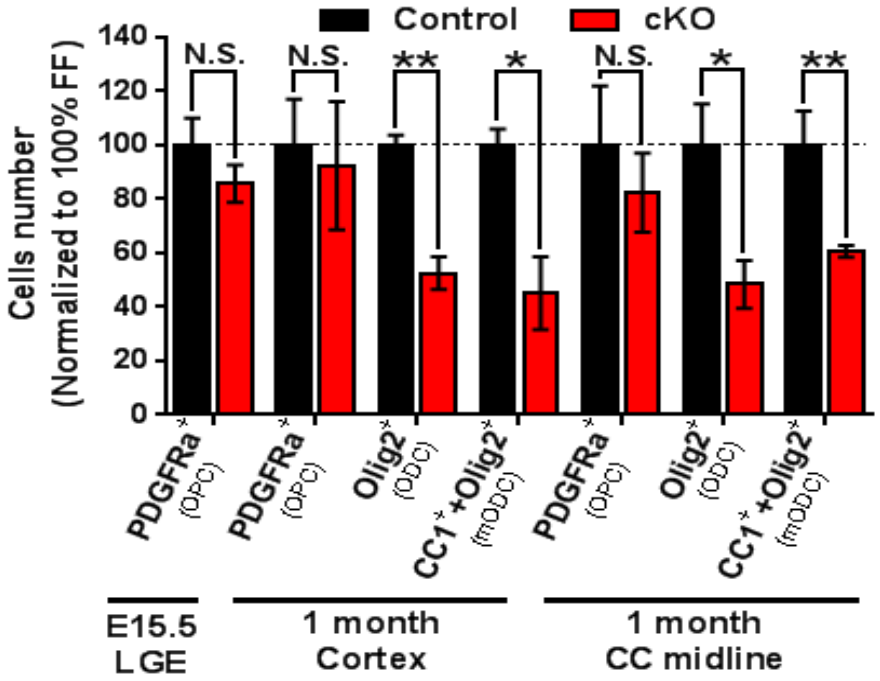
Taken from: “a method for assessing tissue respiration in anatomically defined brain regions”

What leads to the reduced genes expression?

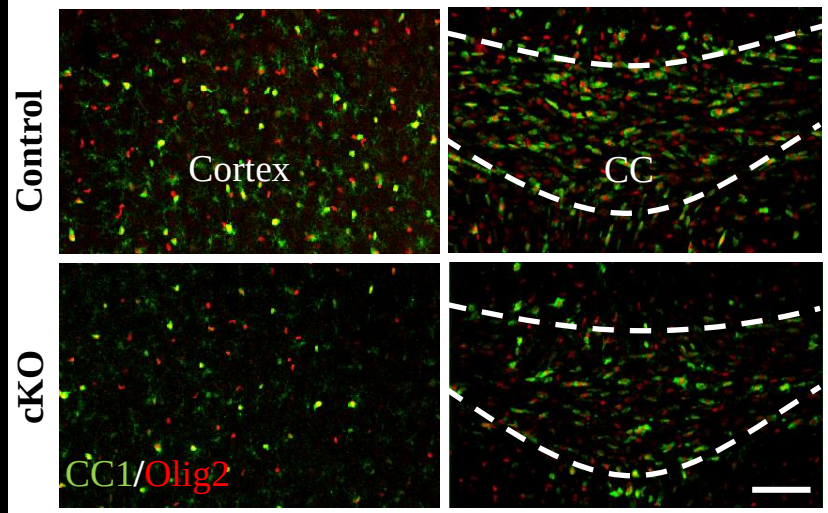
Oligodendrocytes precursor cells (PDGFRa) → Differentiation → Myelinating oligodendrocytes (CC1)

Olig2

Oligodendrocytes markers in development

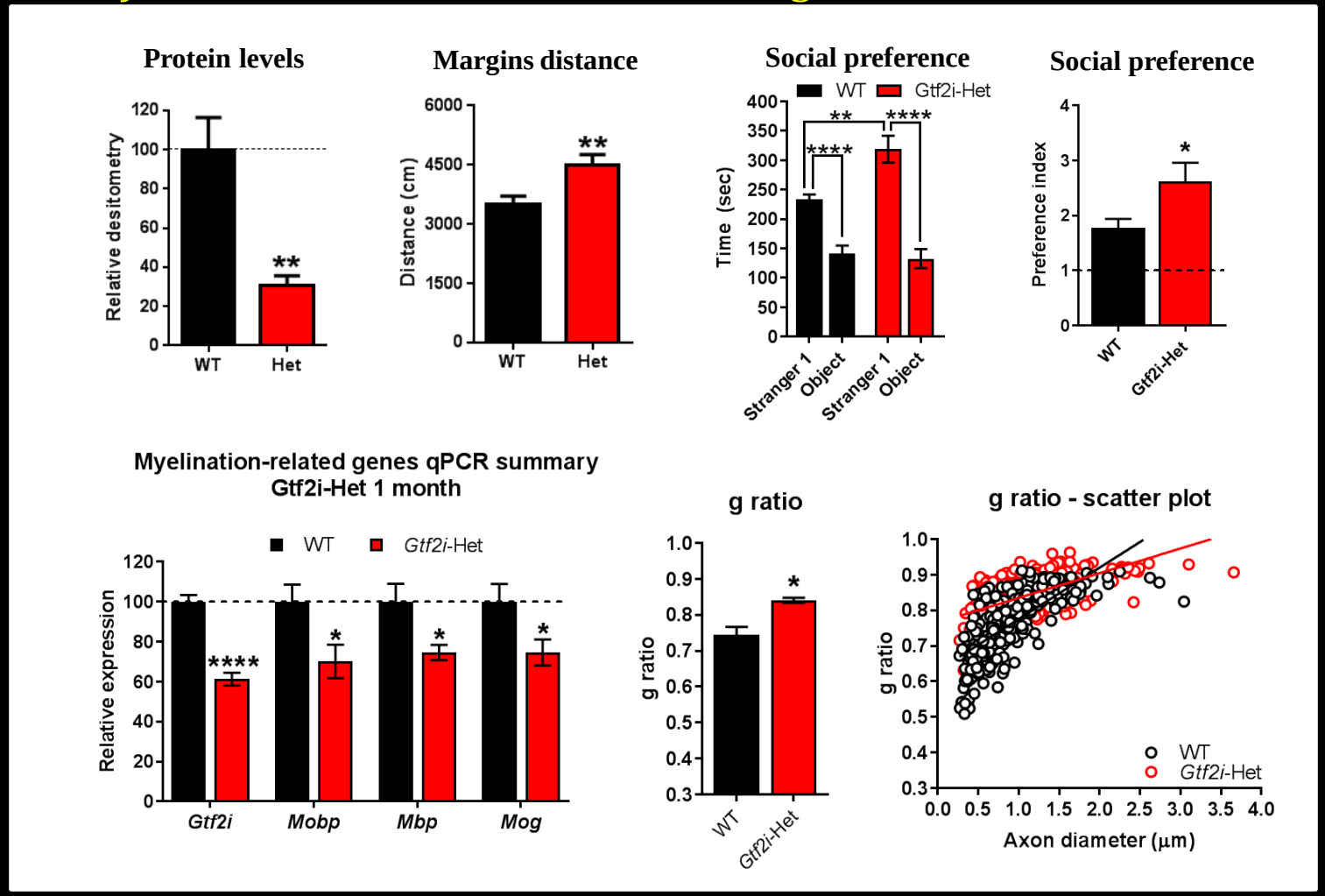


Mature oligodendrocytes in cortex and corpus callosum midline

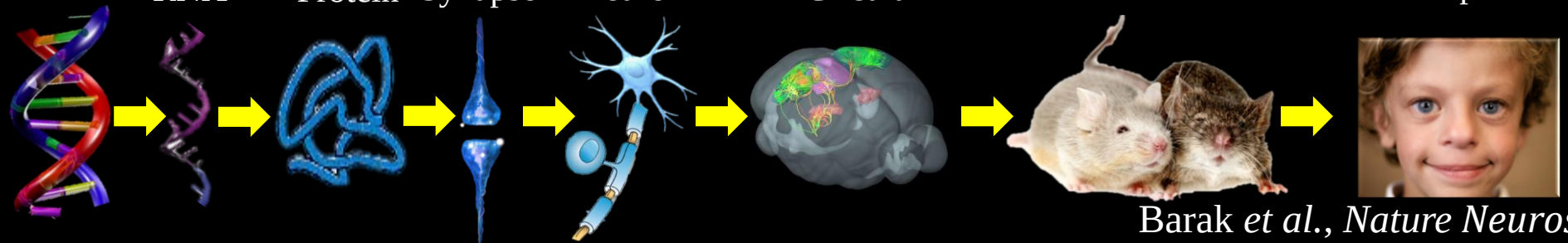


Barak *et al.*, *Nature Neuroscience*, 2019

Gtf2i-Hets as a model to the human genetic condition in WS

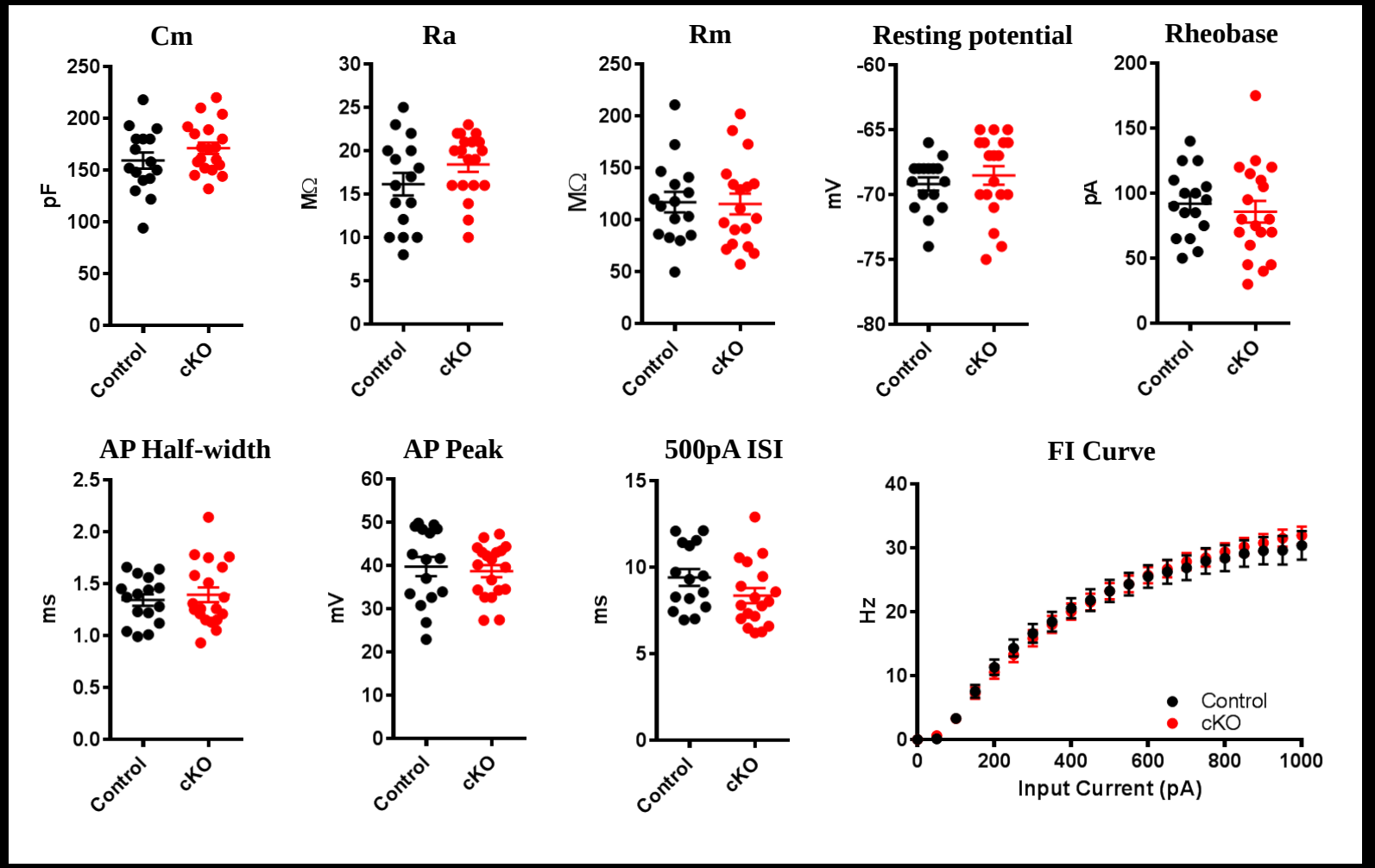


DNA RNA Protein Synapse Neuron Circuit Behavior Human patients



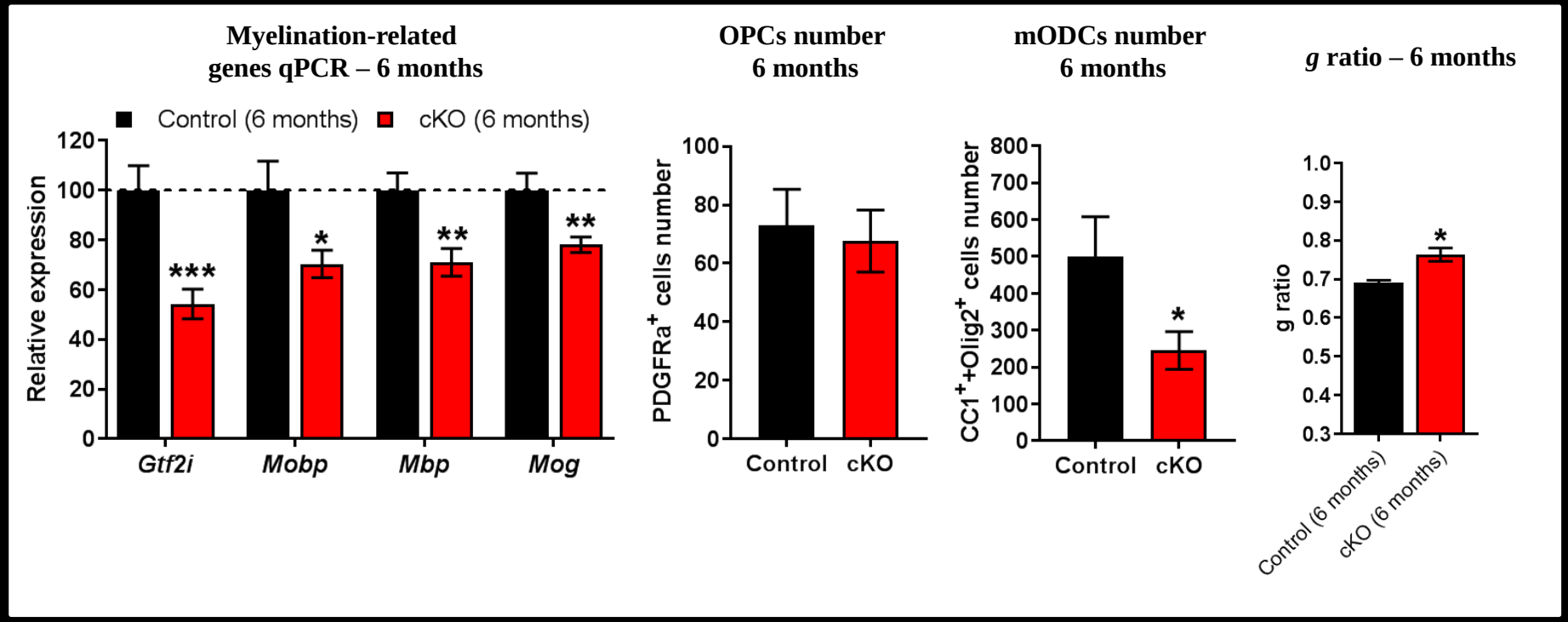
Barak *et al.*, *Nature Neuroscience*, In press

No effect on basal neuronal properties



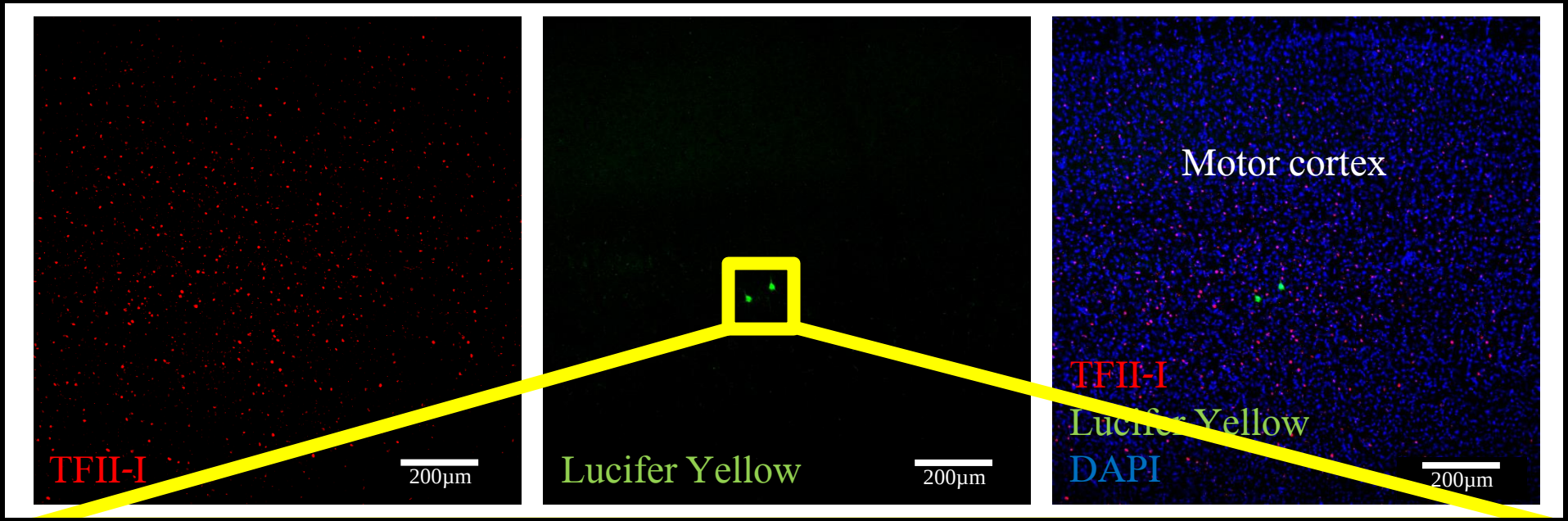
Barak *et al.*, *Nature Neuroscience*, In press

Is it simply a delayed myelination?



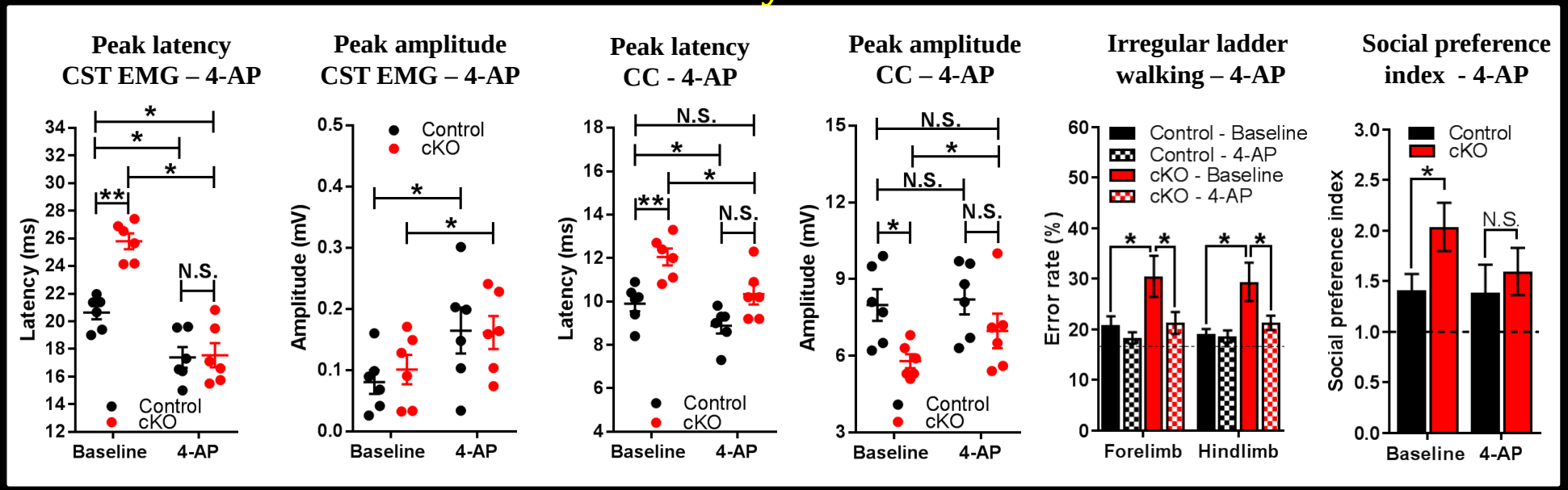
Barak *et al.*, *Nature Neuroscience*, In press

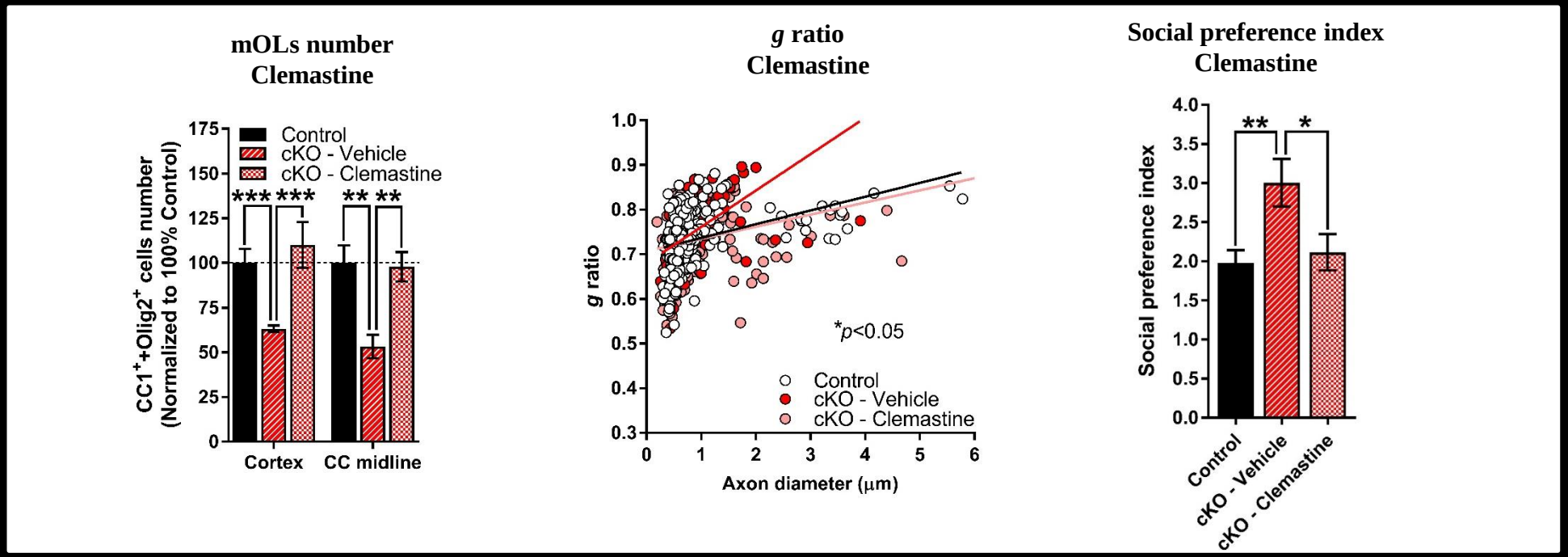
Does *Gtf2i*-KO affect electrophysiological properties?



Barak *et al.*, *Nature Neuroscience*, In press

Can we rescue myelination deficits?





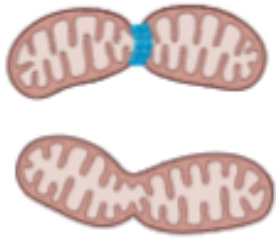
Barak *et al.*, *Nature Neuroscience*, 2019

Mitochondrial dysfunction

Mitochondrial ROS (MITOSOX) ?

Drp1 P1

Fission/Fusion



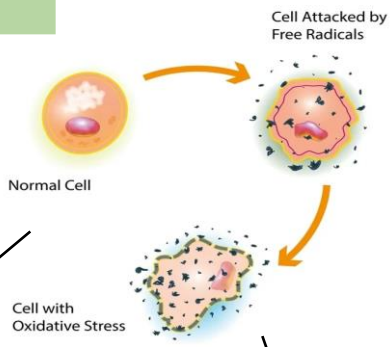
Count of individual mitochondria

Hypoxia/ROS

DCF

Hif1a

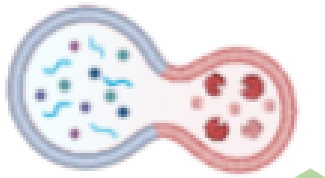
Pgc1a



Mfn2 P1

Protein level (WB) ?

Mitophagy



Pink/parkin pathway

Jun

Bax/Bak

Cyt c

Casp3

HSP60

? Apop tosis

ATP ?

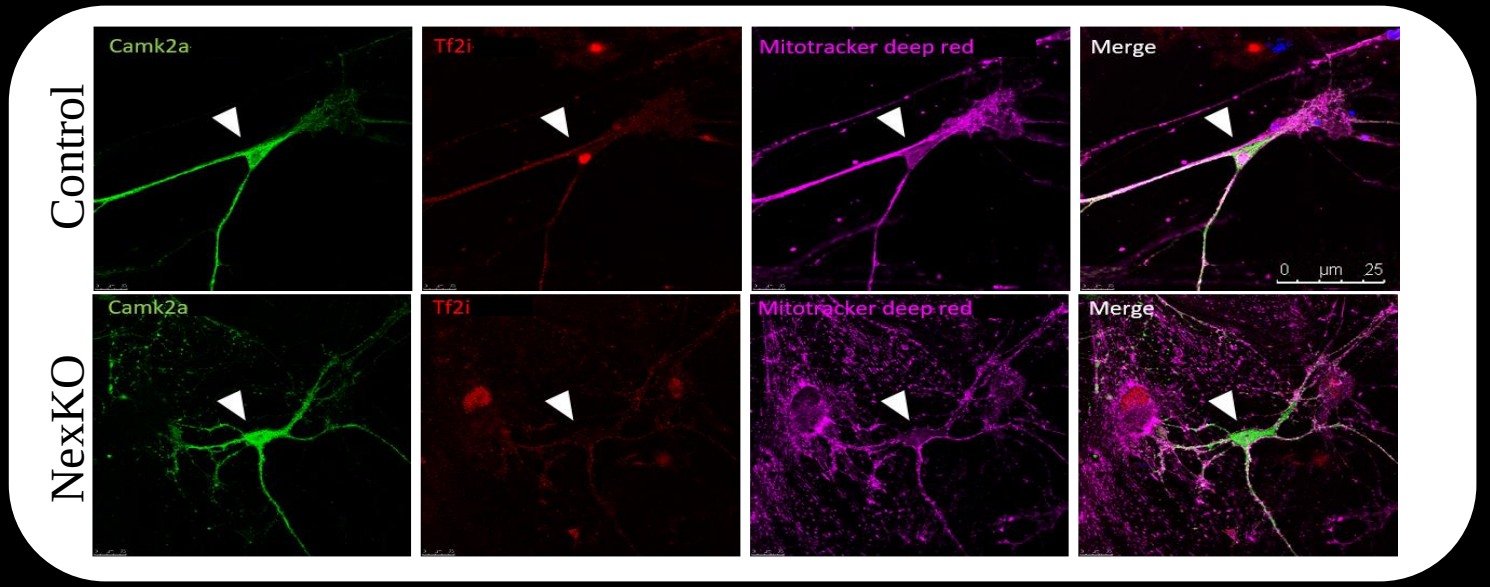
Pink1 P1,P30

Parkin P30

Tbk1 P1

Tbk1 P30

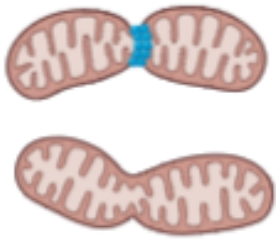
Mitochondrial network and morphology in primary neuronal cultures at DIV14



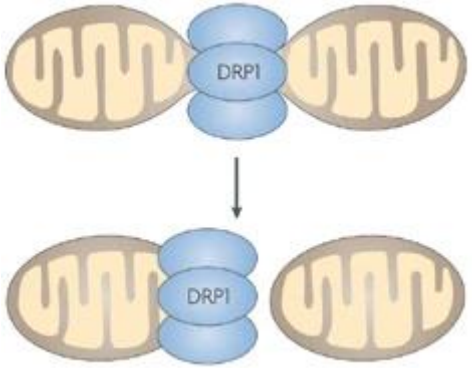
Ariel Nir and Dr. Sari Trangle, Unpublished data

Mitochondrial dysfunction

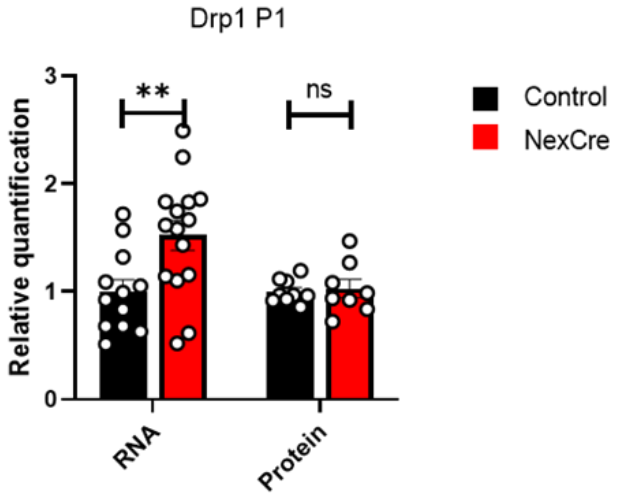
Fission/Fusion

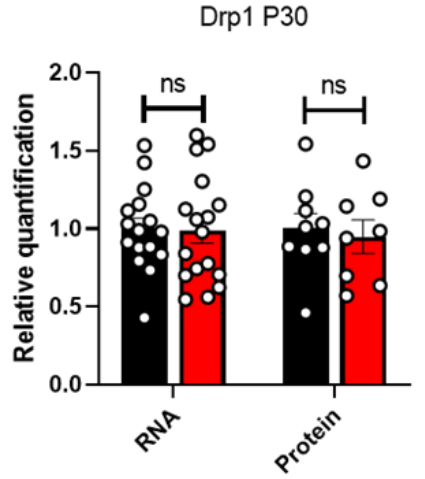


Fission

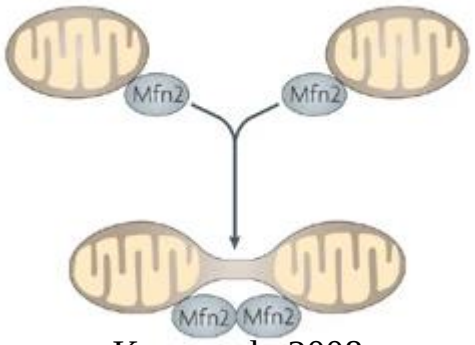


Knot et al., 2008

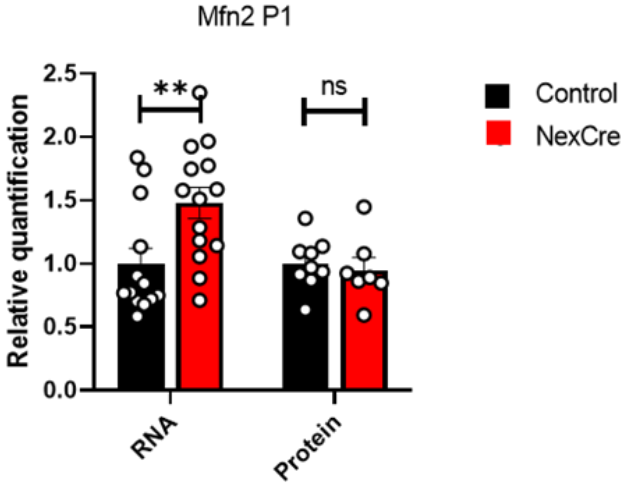


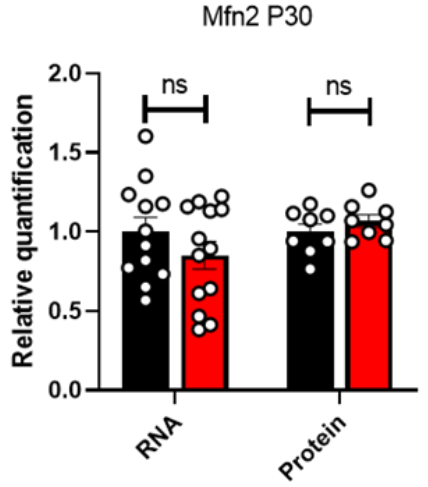


Fusion



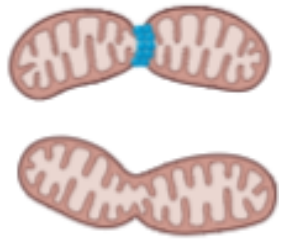
Knot et al., 2008





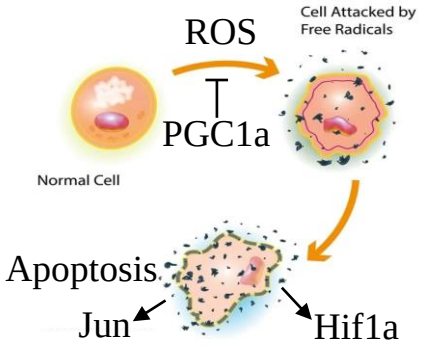
Ariel Nir and Dr. Sari Trangle, Unpublished data

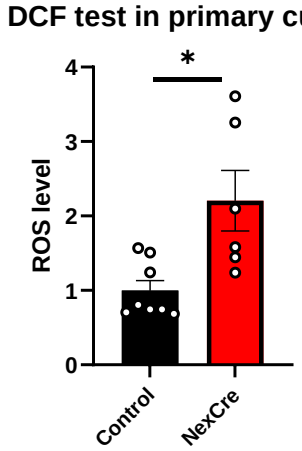
Fission/Fusion

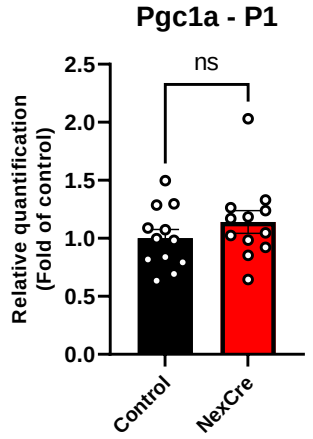


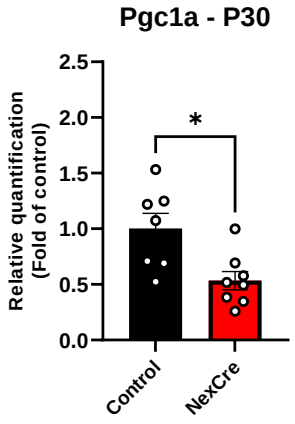
Mitochondrial dysfunction

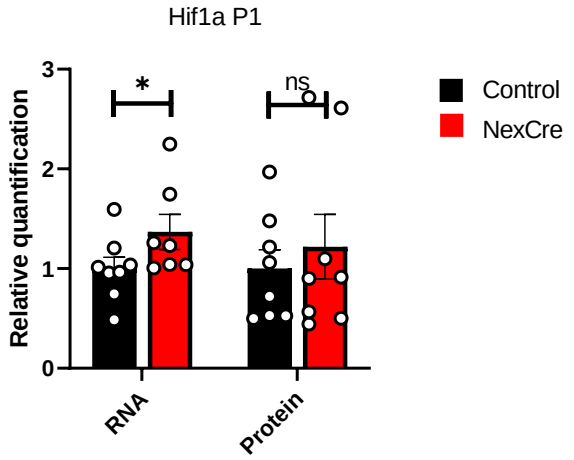
Hypoxia/ROS

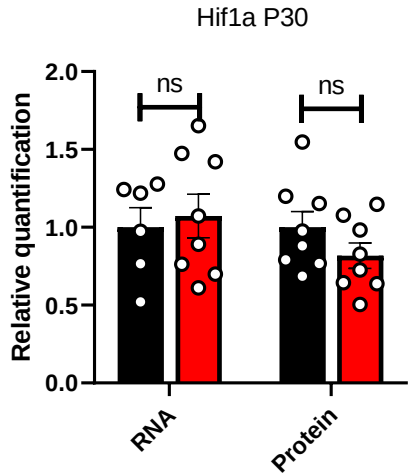


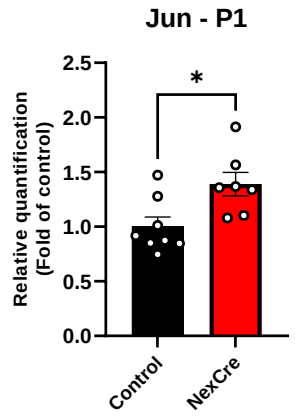


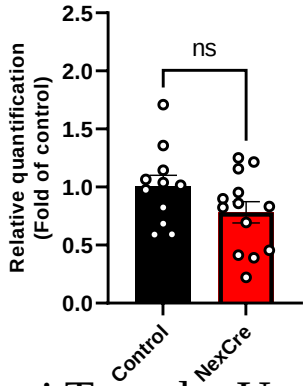




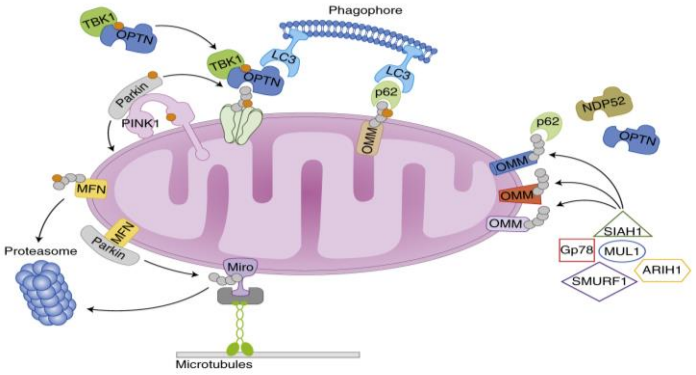






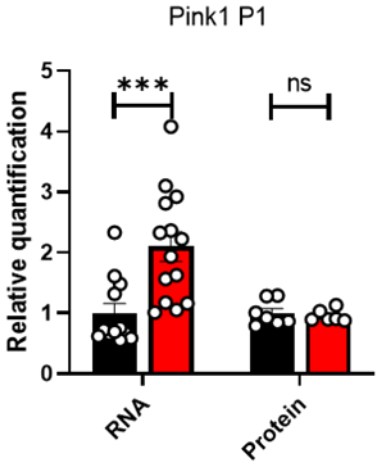


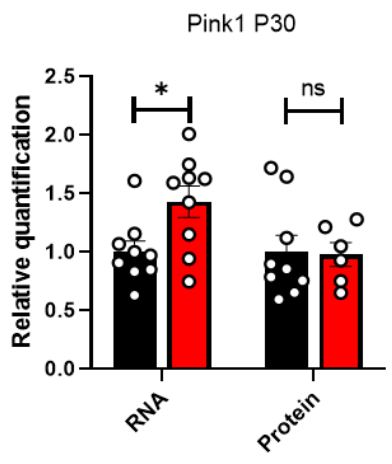
Ariel Nir and Dr. Sari Trangle, Unpublished data

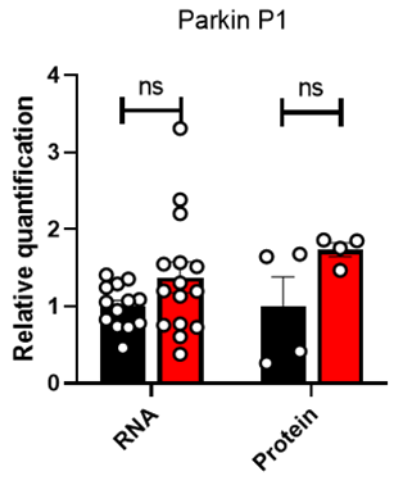


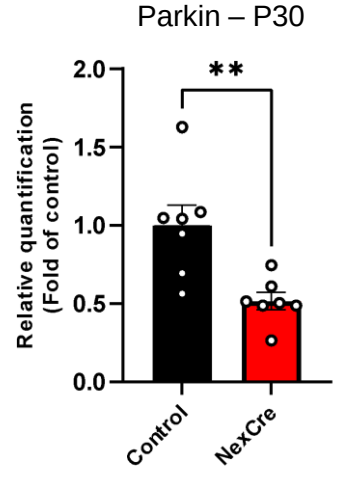
Mitochondrial dysfunction

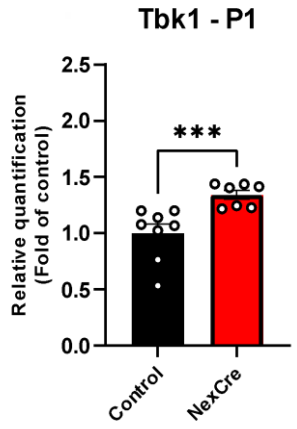
Mitophagy

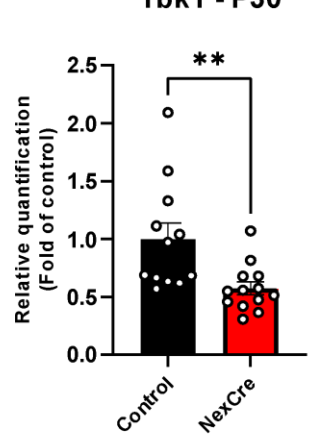












Mitochondrial stress
Glucose-deprivation by 2-DG
(2-deoxy-D-glucose)

⬇

Inhibits glycolysis

⬇

Reducing energy production

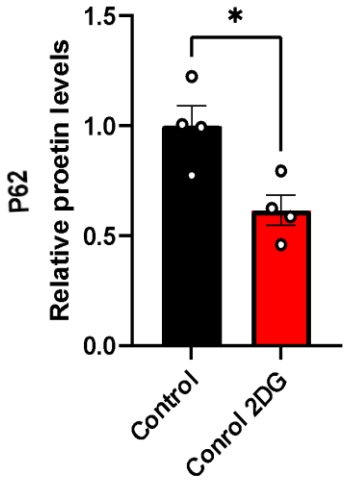
⬇

Mitochondrial activity increased

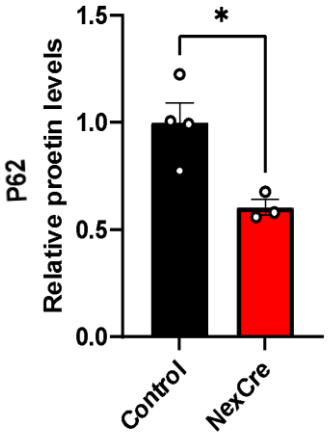
⬇

More autophagy – p62 degrades in lysosome

2DG (glucose-dep) in culture

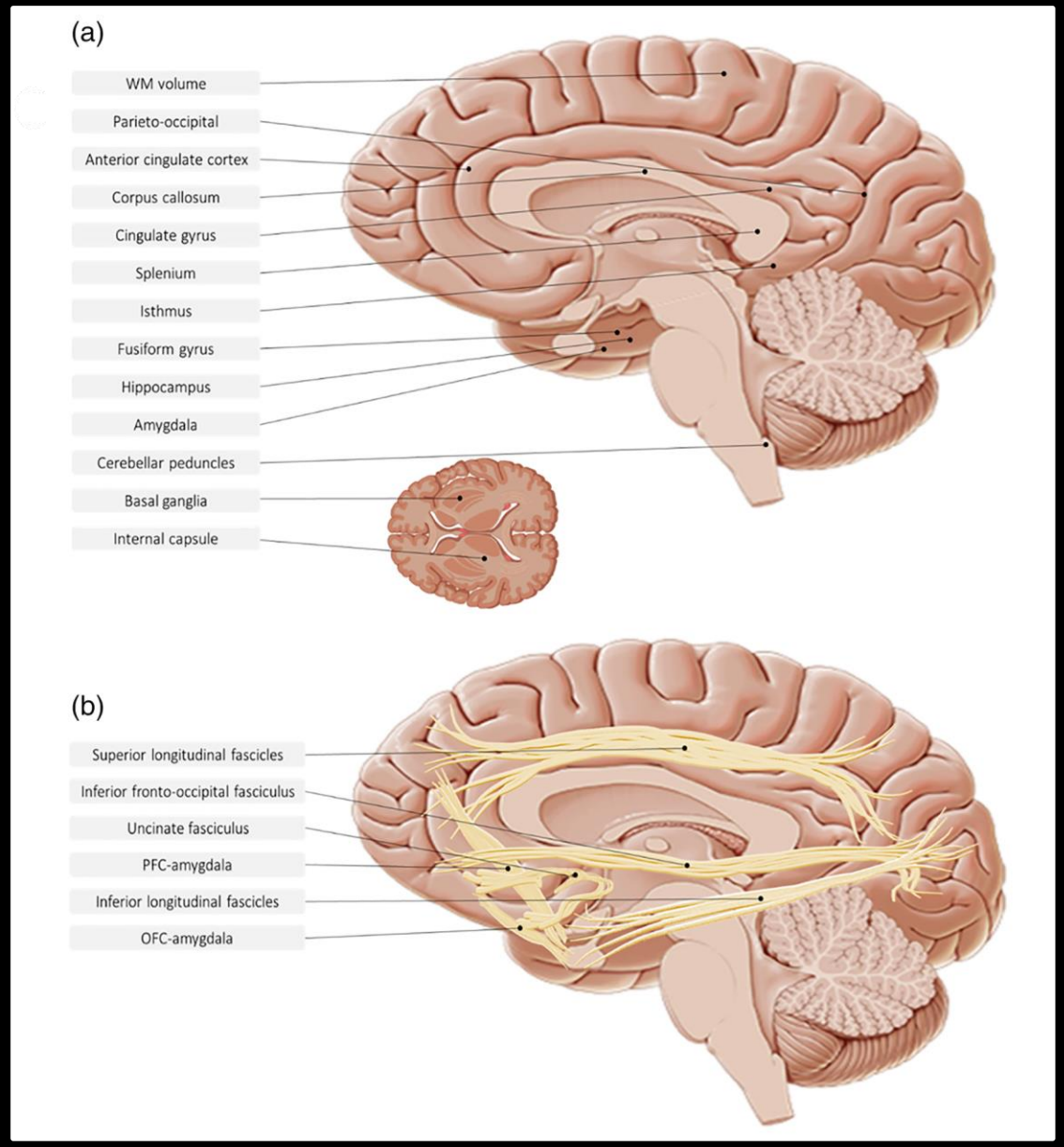


Normal medium in culture

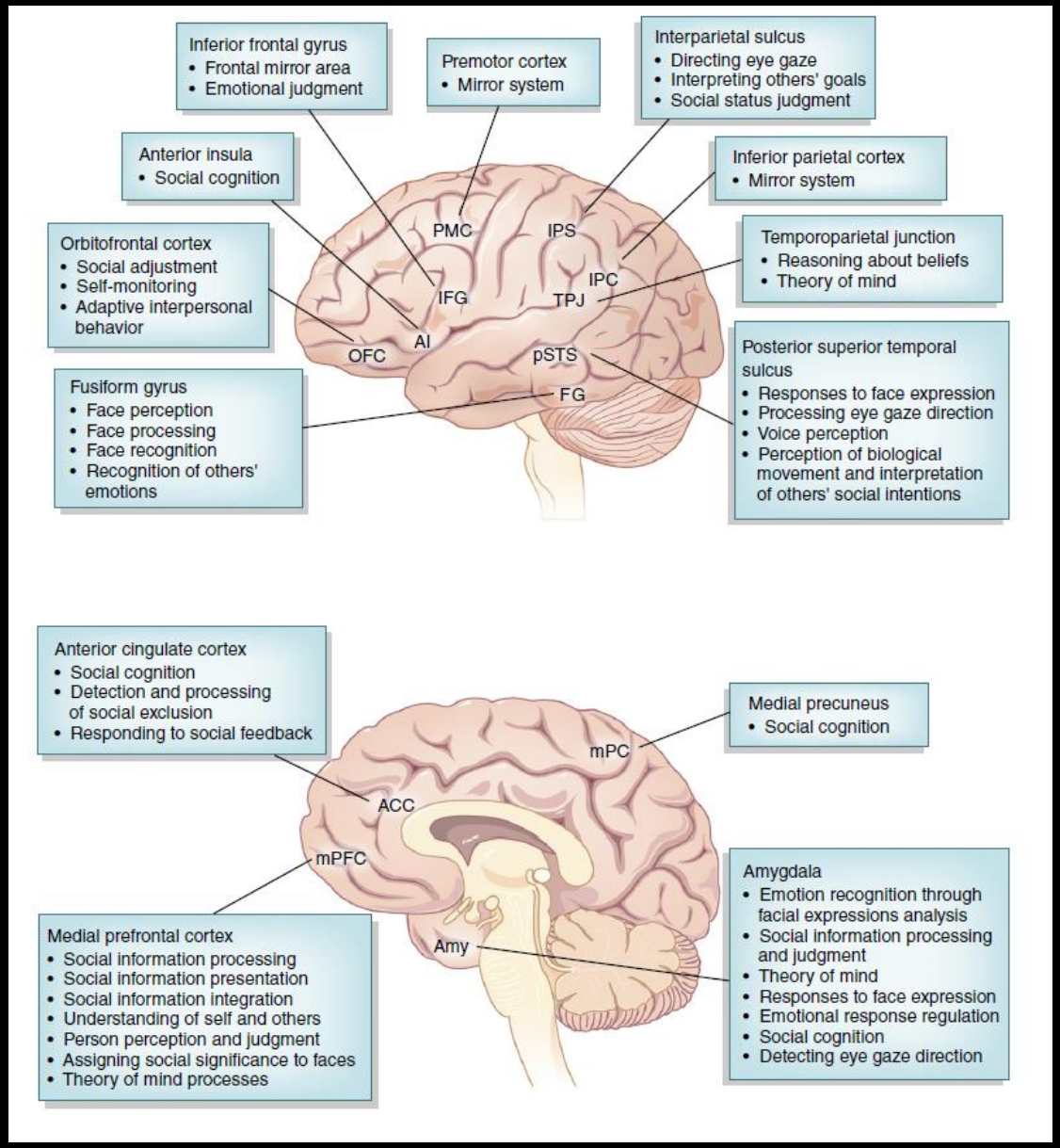


Ariel Nir and Dr. Sari Trangle, Unpublished data

How can we further study WS in humans?



Nir and Barak, *GLIA*, 2020

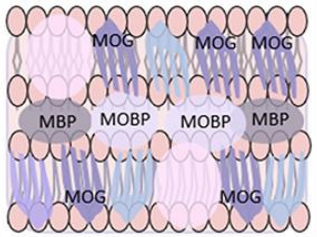


Barak and Feng, *Nature Neuroscience*, 2016

Interim summary

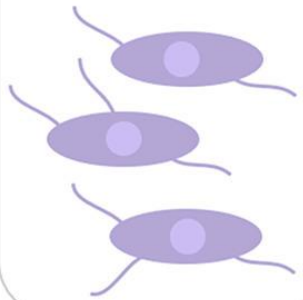
CONTROL

Myelin-Related Genes and Proteins Expression

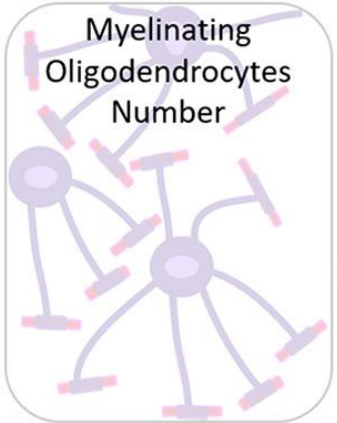


Myelin Thickness

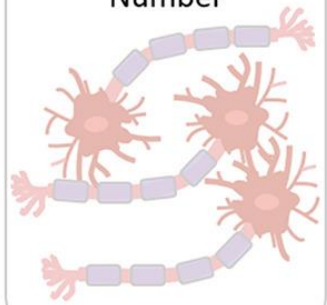
OPCs Number



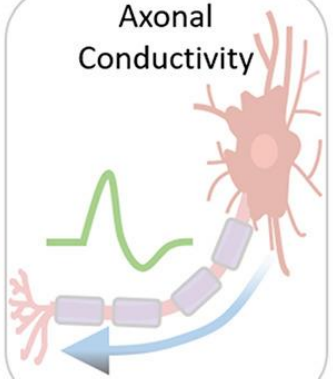
Myelinating Oligodendrocytes Number



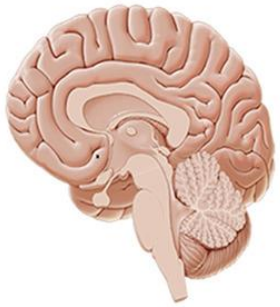
Myelinated Axons Number



Axonal Conductivity

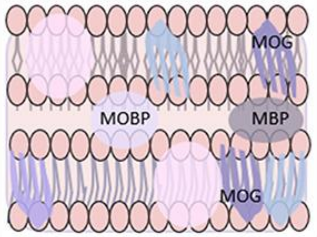


WM Properties



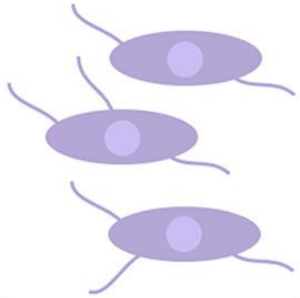
WILLIAMS SYNDROME

Reduced mRNA Expression of Myelin-Related Genes



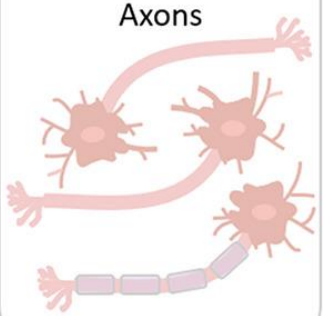
Decreased Myelin Thickness and Increased *g*–ratio

OPCs Normal Number

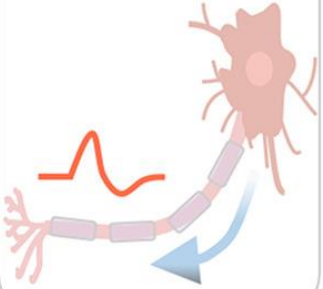


Fewer Myelinating Oligodendrocytes

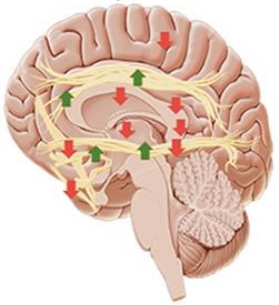
Fewer Myelinated Axons



Slower and Reduced Axonal Conductivity



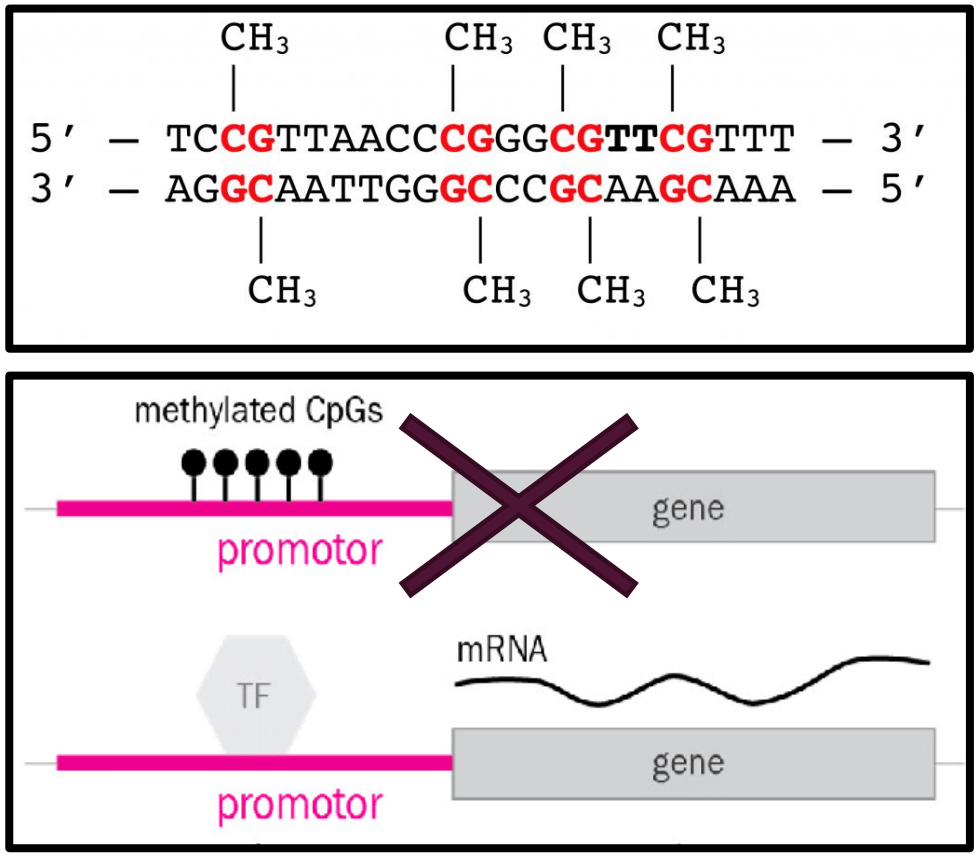
Altered WM Properties

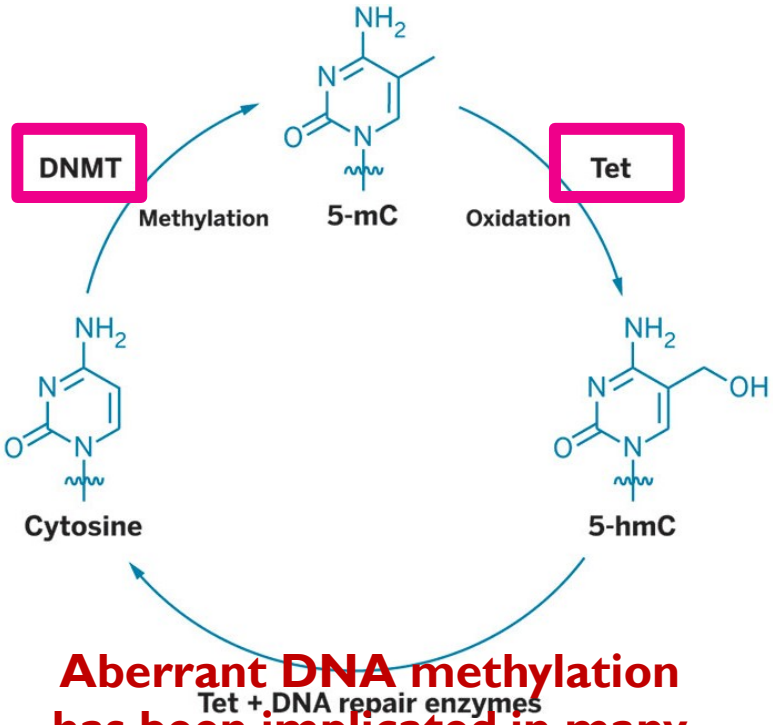


Nir and Barak, *GLIA*, 2020

DNA methylation

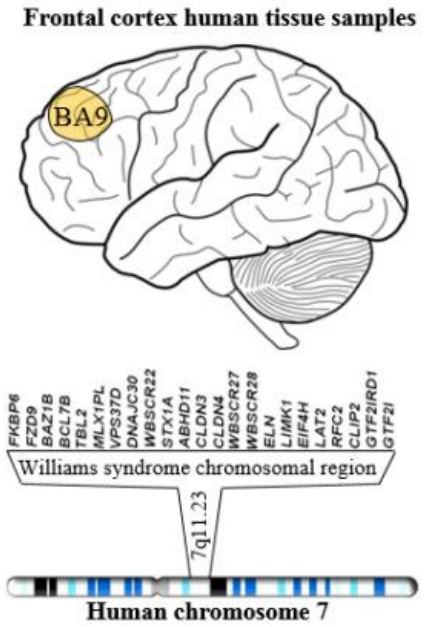
DNA methylation has been widely studied in the context of numerous biological and brain functions, including cell differentiation, neurodevelopment, myelination and neurogenesis.





Aberrant DNA methylation has been implicated in many neuropsychiatric disorders, including autism spectrum disorder (ASD) and schizophrenia.

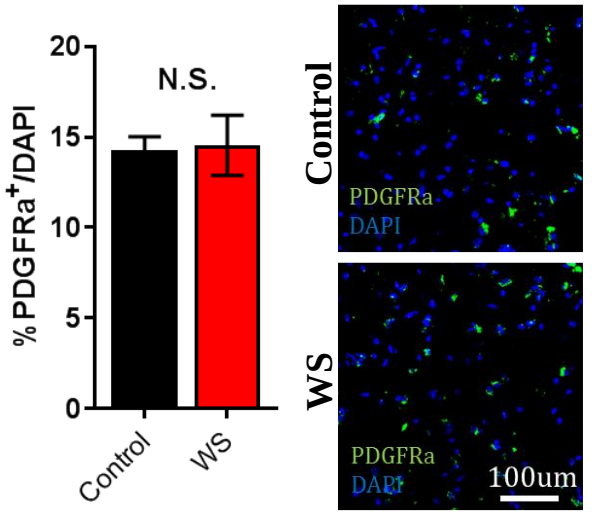
How translational are these findings?



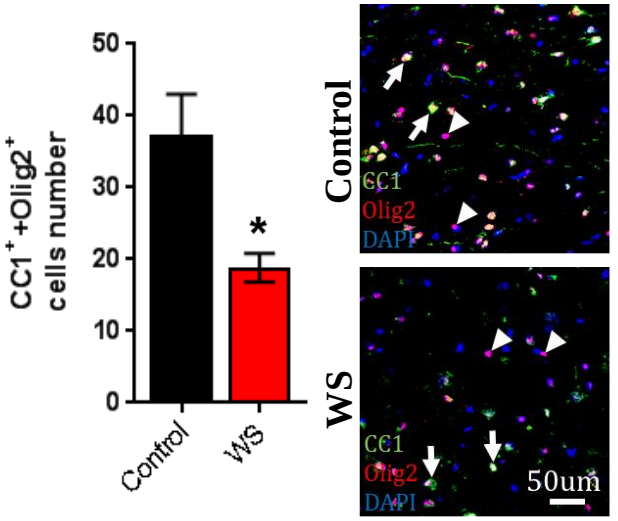
Barak *et al.*, *Nature Neuroscience*, 2019

Myelination-related abnormalities in WS subjects

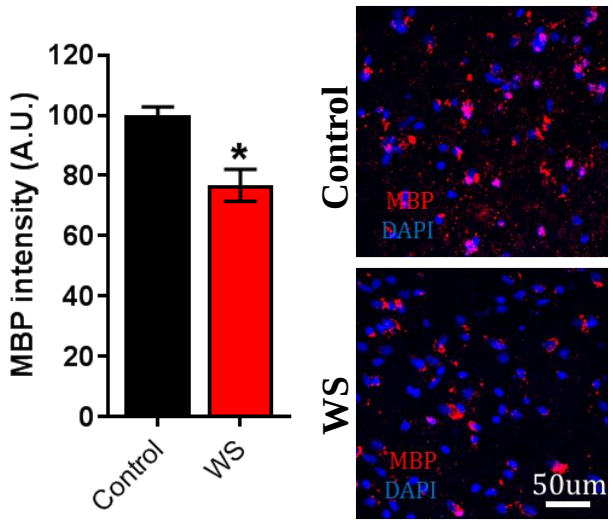
Oligodendrocyte precursor cells in human cortex



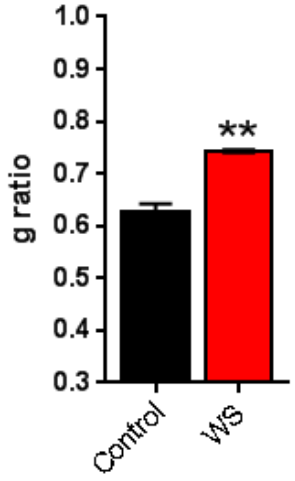
Myelinating oligodendrocytes in human cortex



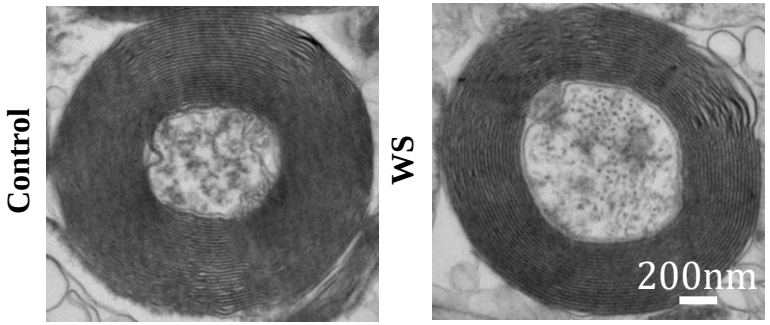
MBP intensity in human cortex



***g* ratio**



Myelin ultrastructure in human cortex



Barak *et al.*, *Nature Neuroscience*, 2019