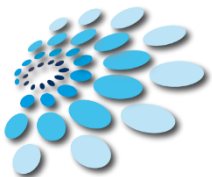


“ Techniques in Cellular  
and Molecular  
Neurobiology ”

International Master's Degree in Neuroscience

Lesson 8



DIPARTIMENTO DI  
SCIENZE DELLA VITA

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## **A model for neural development and treatment of Rett Syndrome using human induced pluripotent stem cells**

**Maria C. N. Marchetto<sup>1,†</sup>, Cassiano Carromeu<sup>2,†</sup>, Allan Acab<sup>2</sup>, Diana Yu<sup>1</sup>, Gene Yeo<sup>3</sup>,  
Yangling Mu<sup>1</sup>, Gong Chen<sup>4</sup>, Fred H. Gage<sup>1</sup>, and Alysson R. Muotri<sup>2,\*</sup>**

<sup>1</sup> The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

# Background



Autism spectrum disorders (ASD) are complex neurodevelopmental diseases in which different combinations of genetic mutations may contribute to the phenotype.

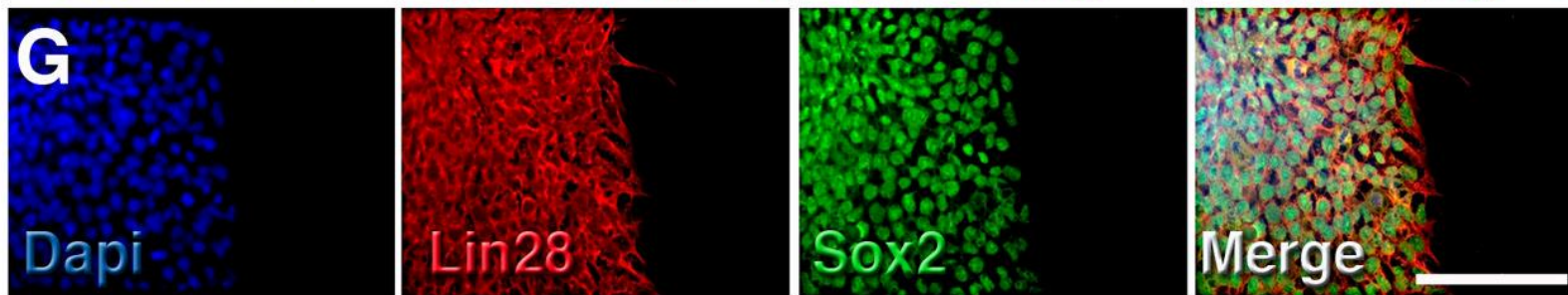
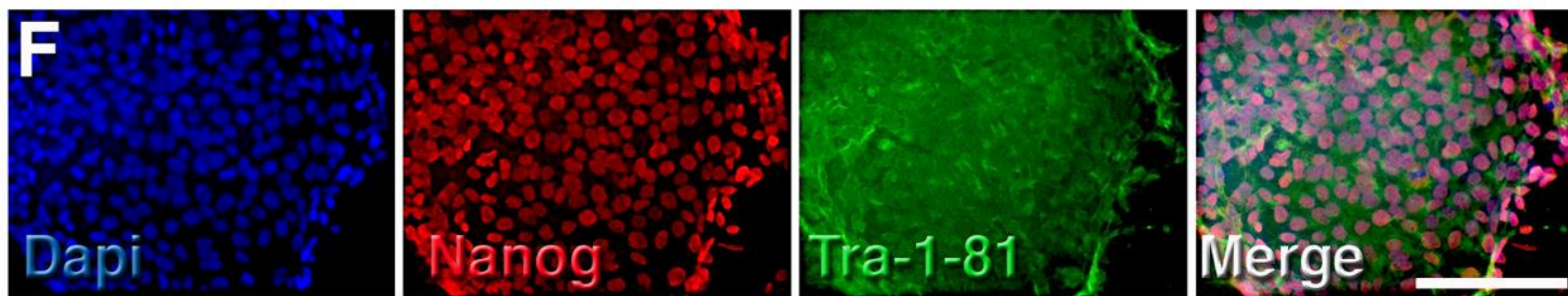
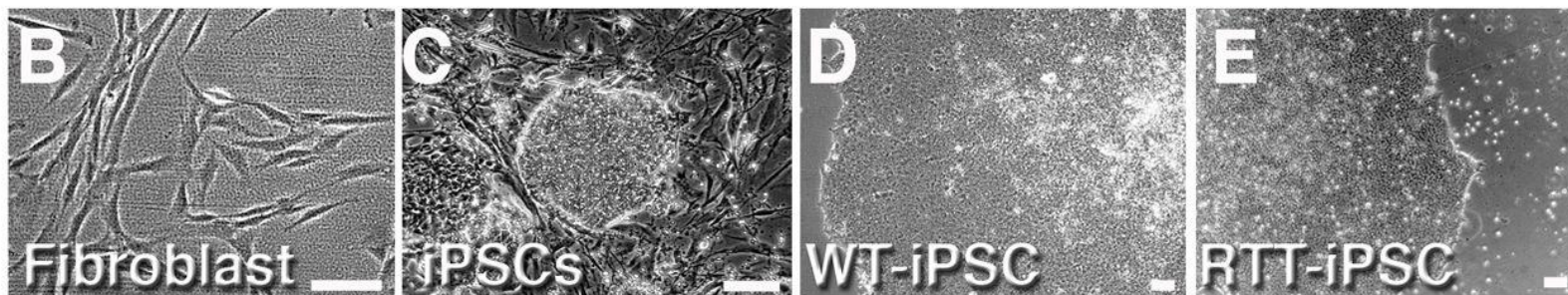
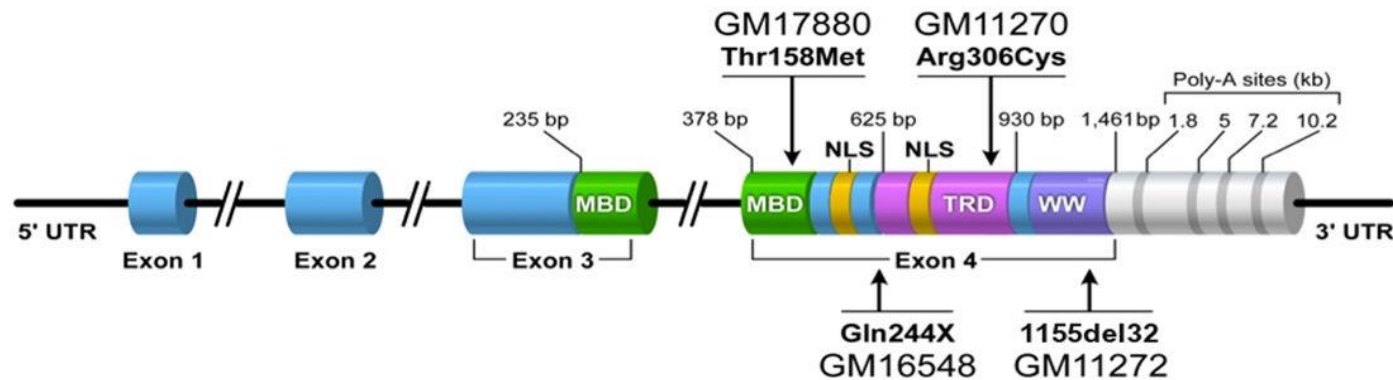
Using Rett syndrome (RTT) as an ASD genetic model, we developed a culture system using induced pluripotent stem cells (iPSCs) from RTT patients' fibroblasts.

RTT patients' iPSCs are able to undergo X-inactivation and generate functional neurons. Neurons derived from RTT-iPSCs had fewer synapses, reduced spine density, smaller soma size, altered calcium signaling and electrophysiological defects when compared to controls. Our data uncovered early alterations in developing human RTT neurons.

Finally, we used RTT neurons to test the effects of drugs in rescuing synaptic defects.



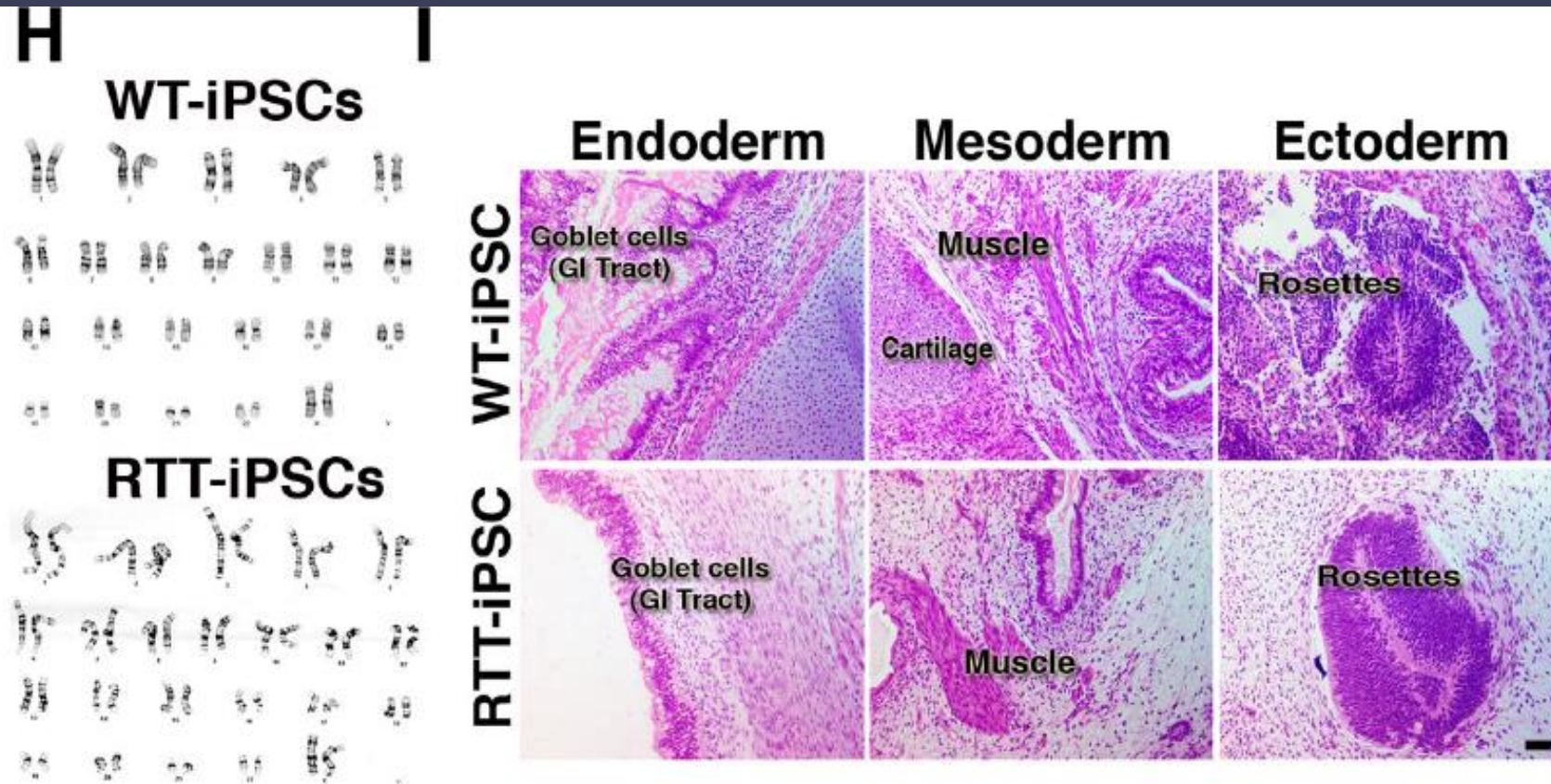
**A**



Generation of iPSCs. (A), Schematic representation of the MeCP2 gene structure and mutations used in this study



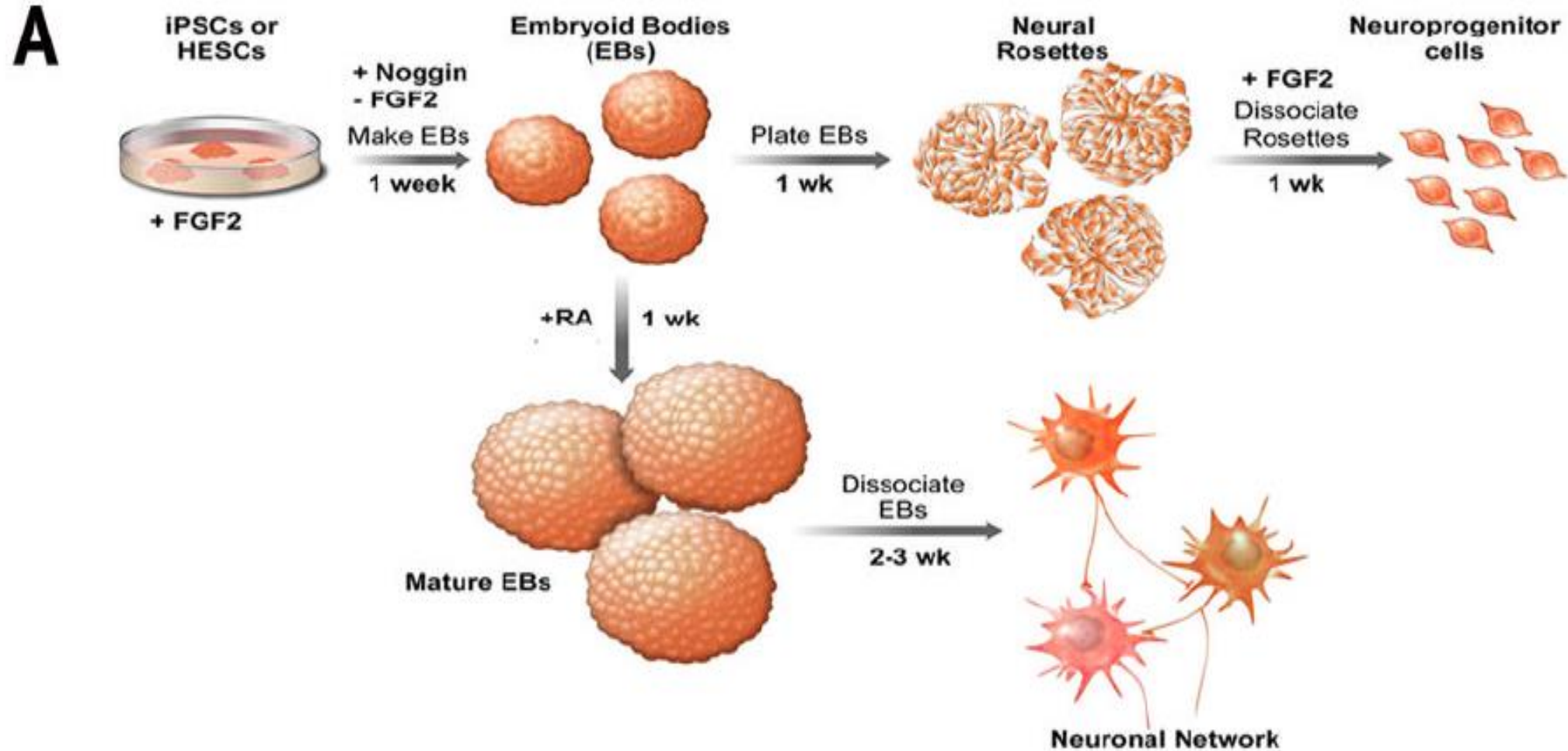
# Results



Representative images of teratoma sections.

NOTE : iPSCs are validated using animal models

# Results

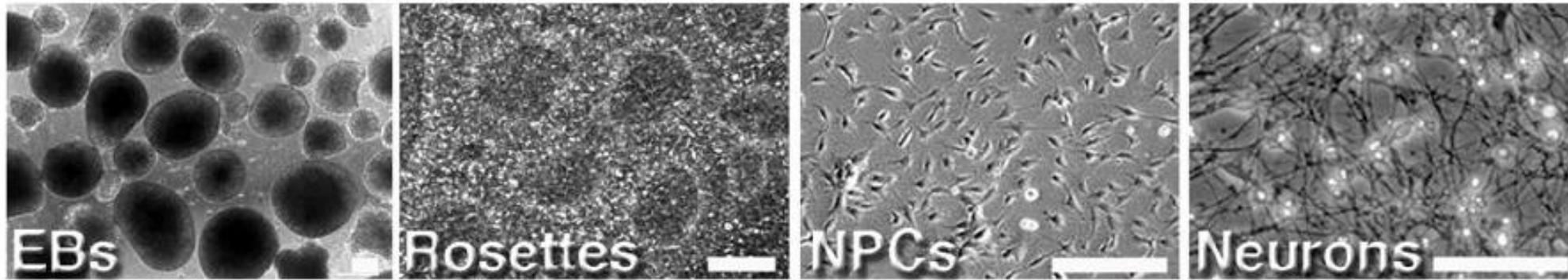




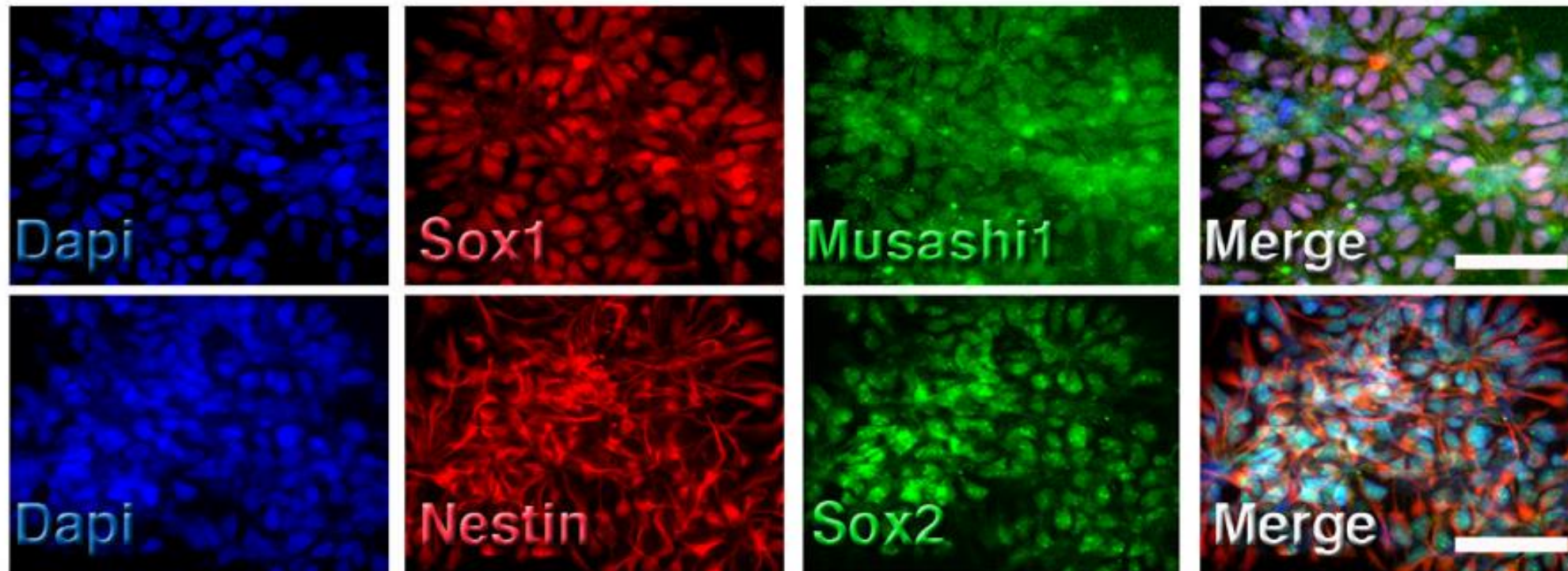
# Results



**B**

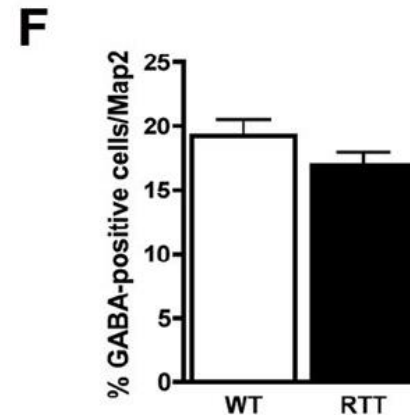
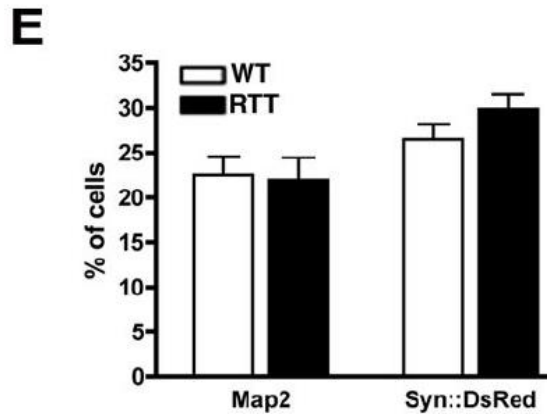
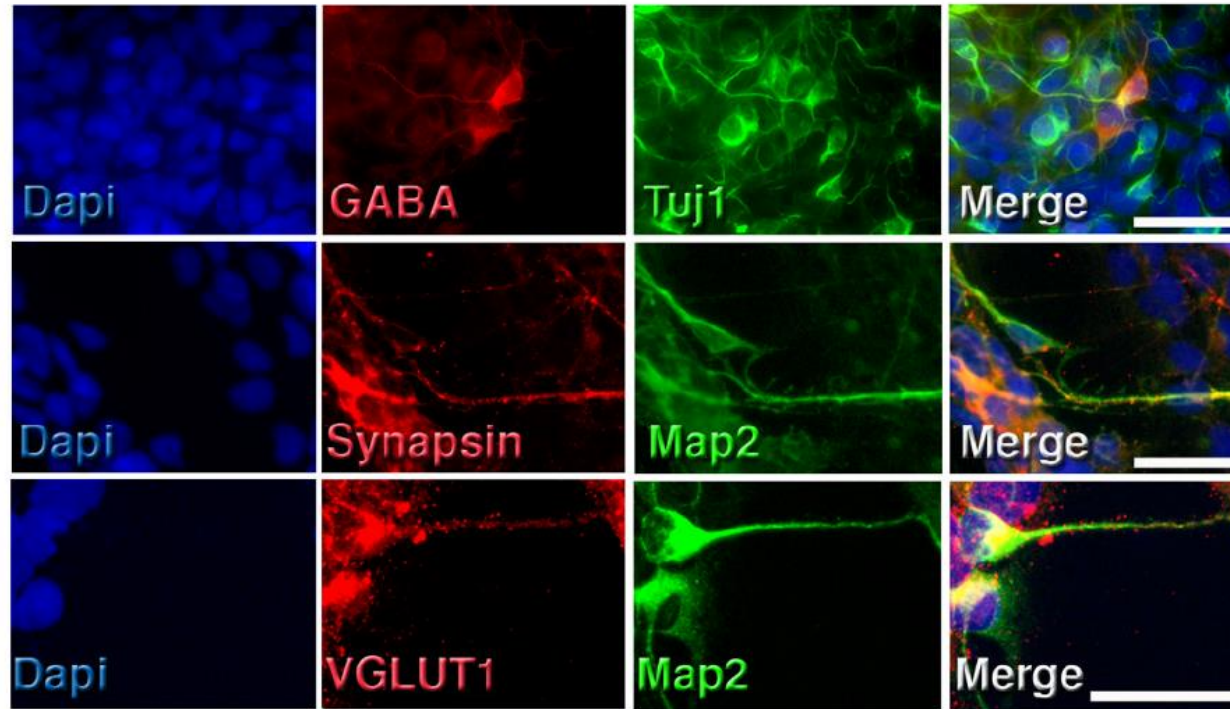


**C**



**D**

# Results

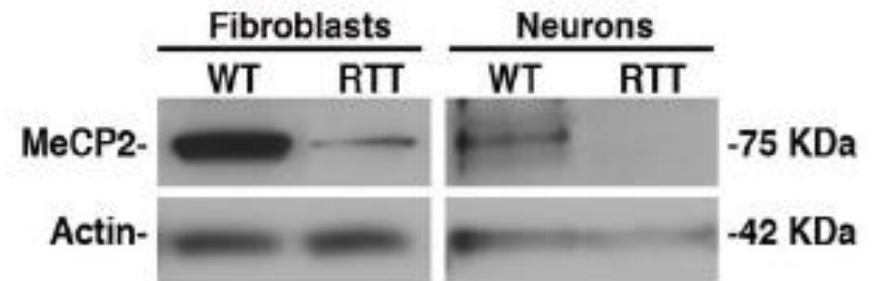
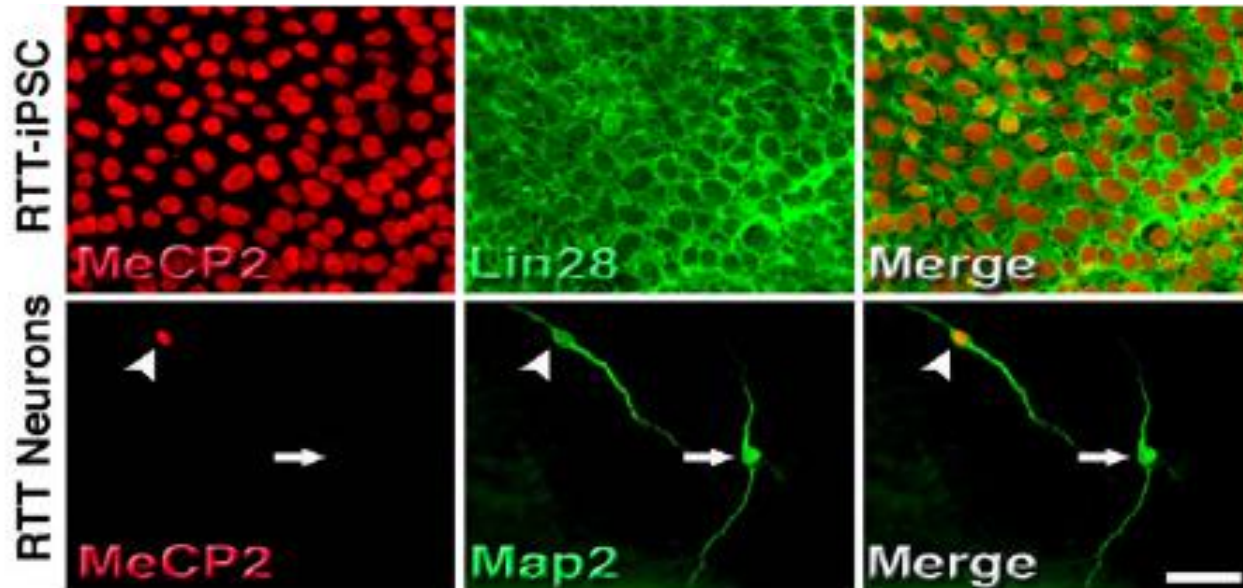
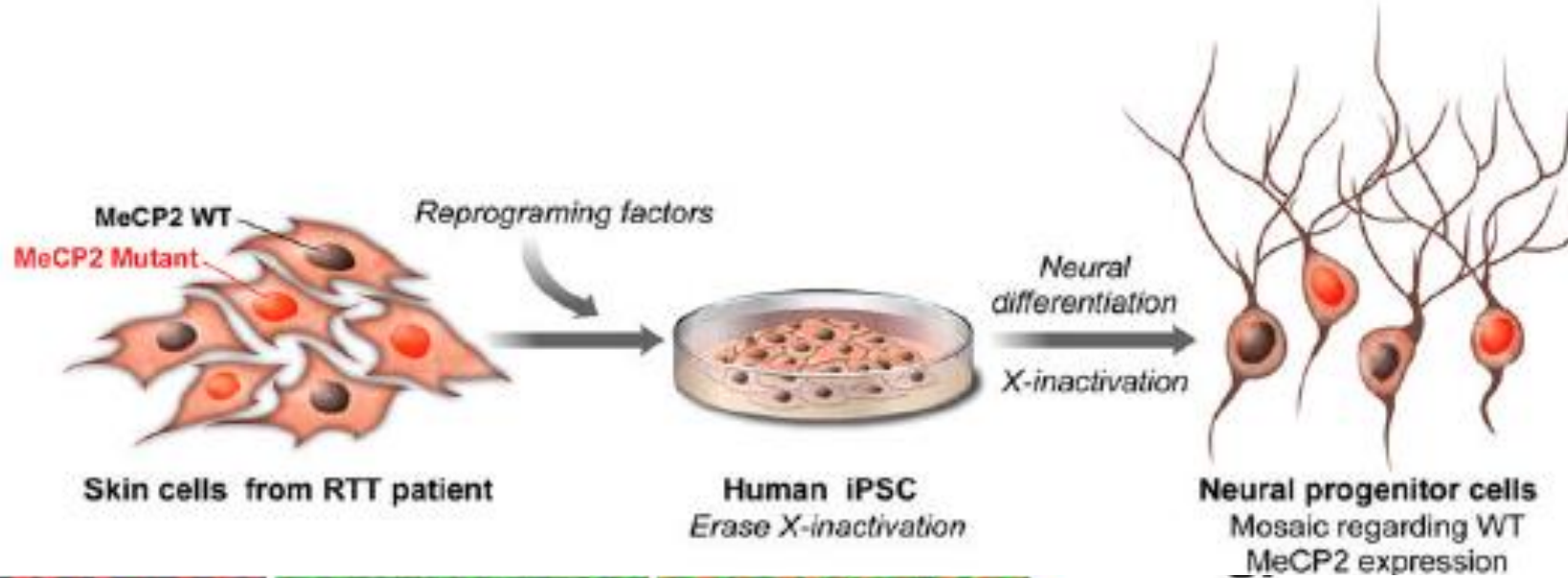




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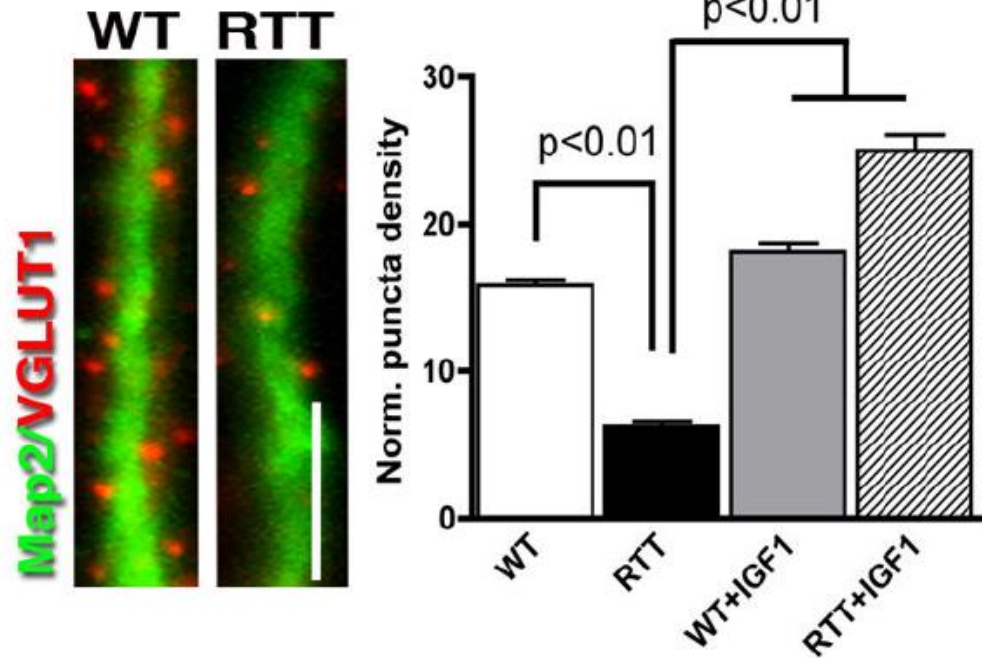
**A**



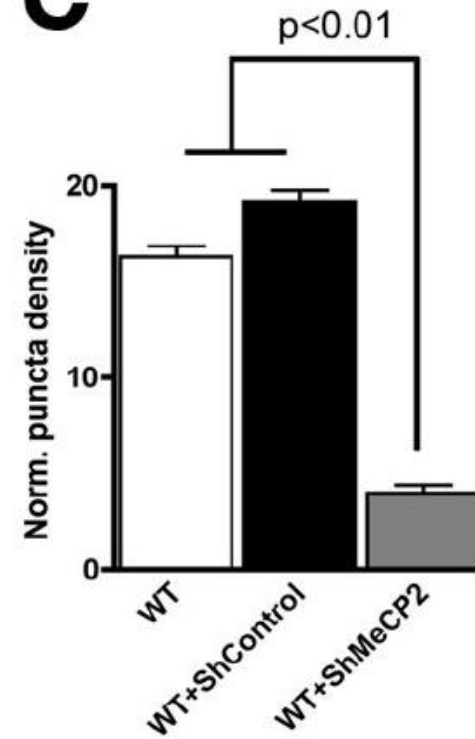
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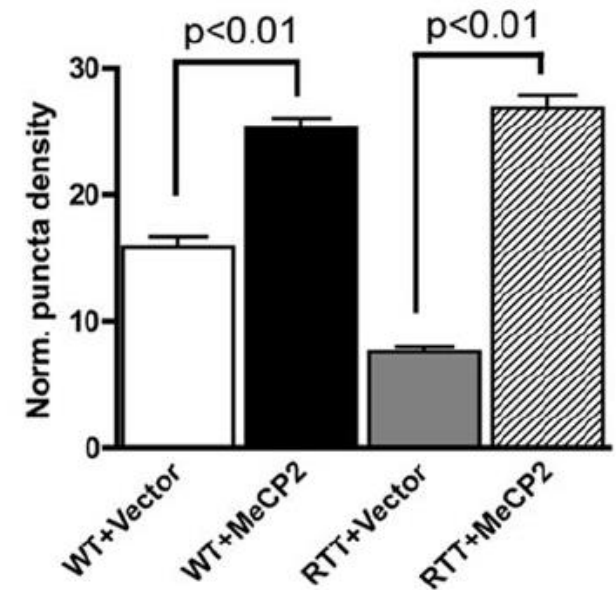
**B**



**C**



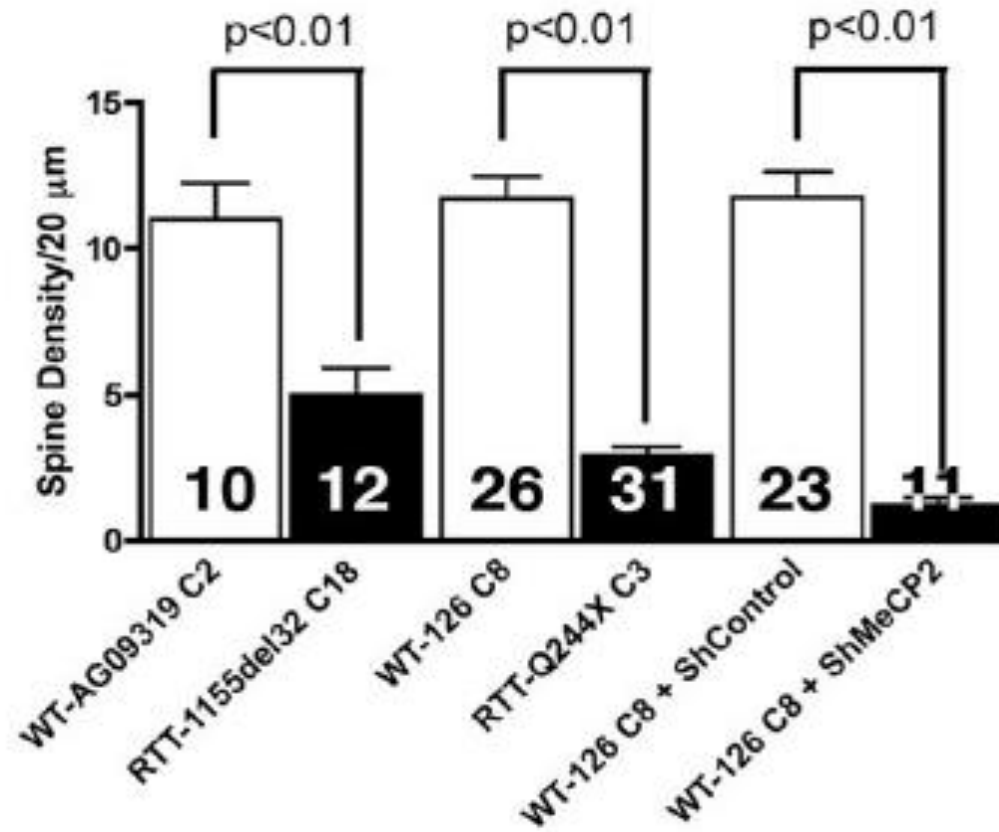
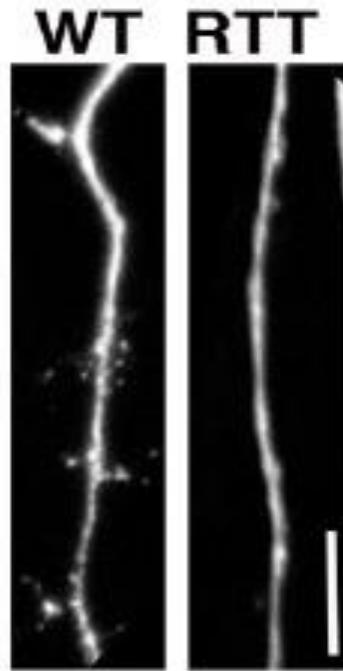
**D**



# Results



**E**

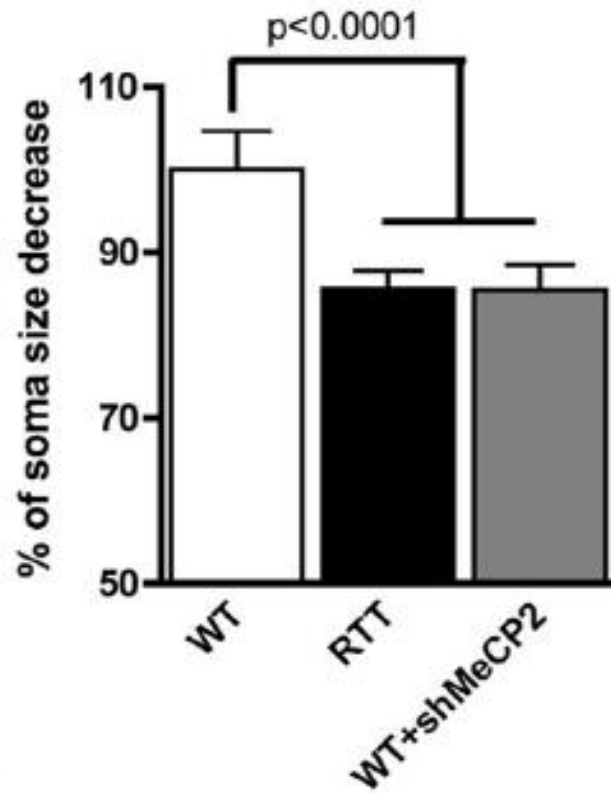
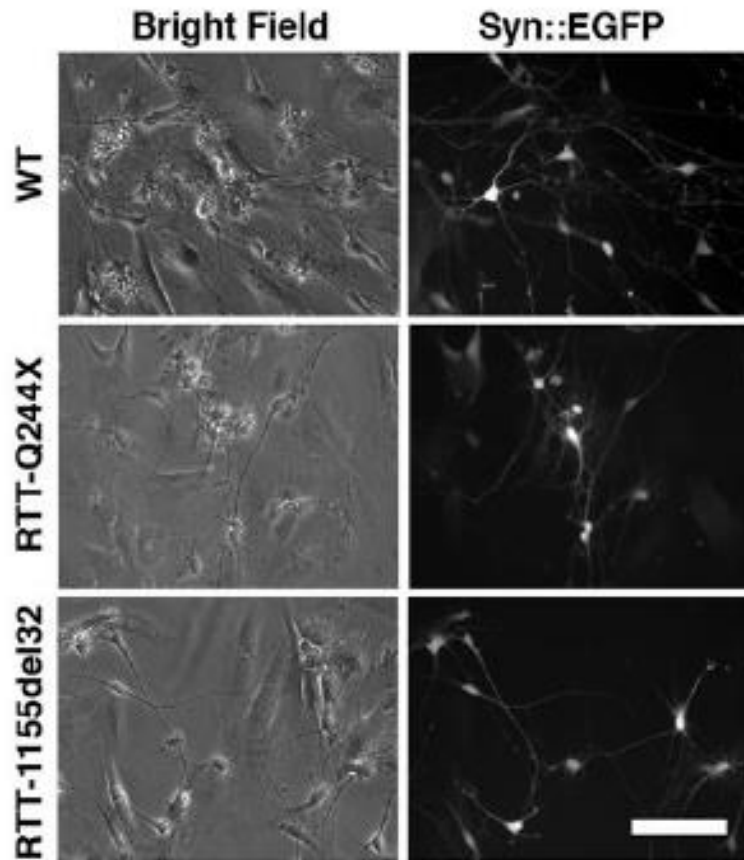




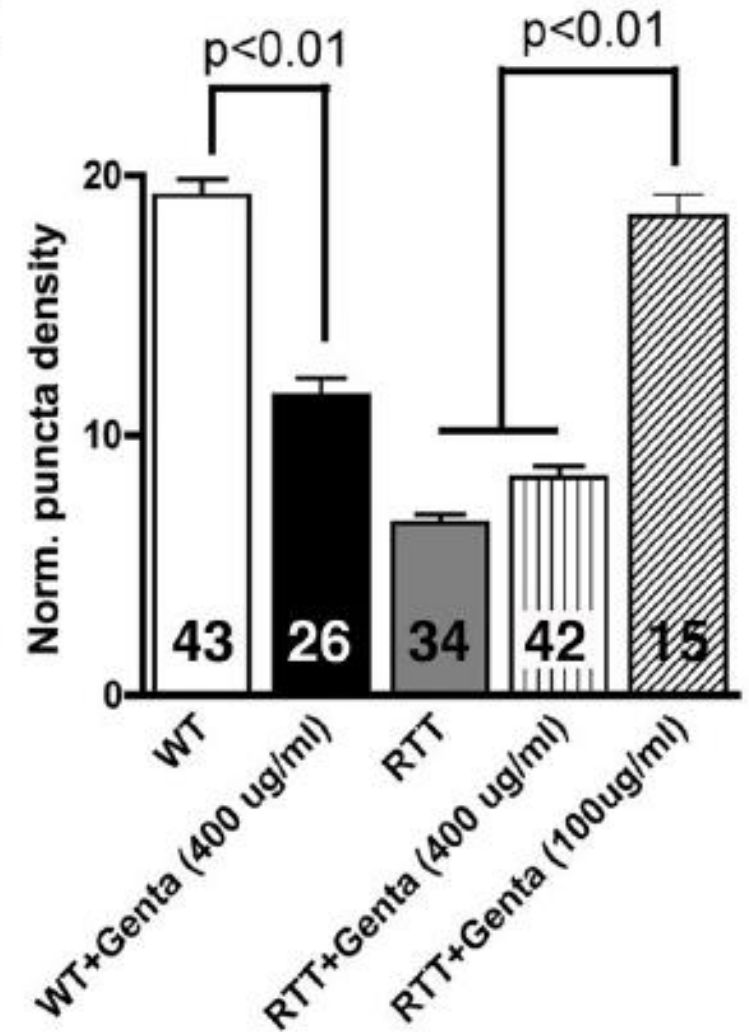
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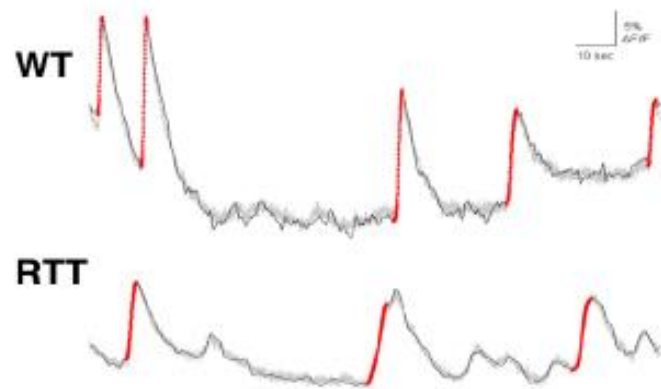
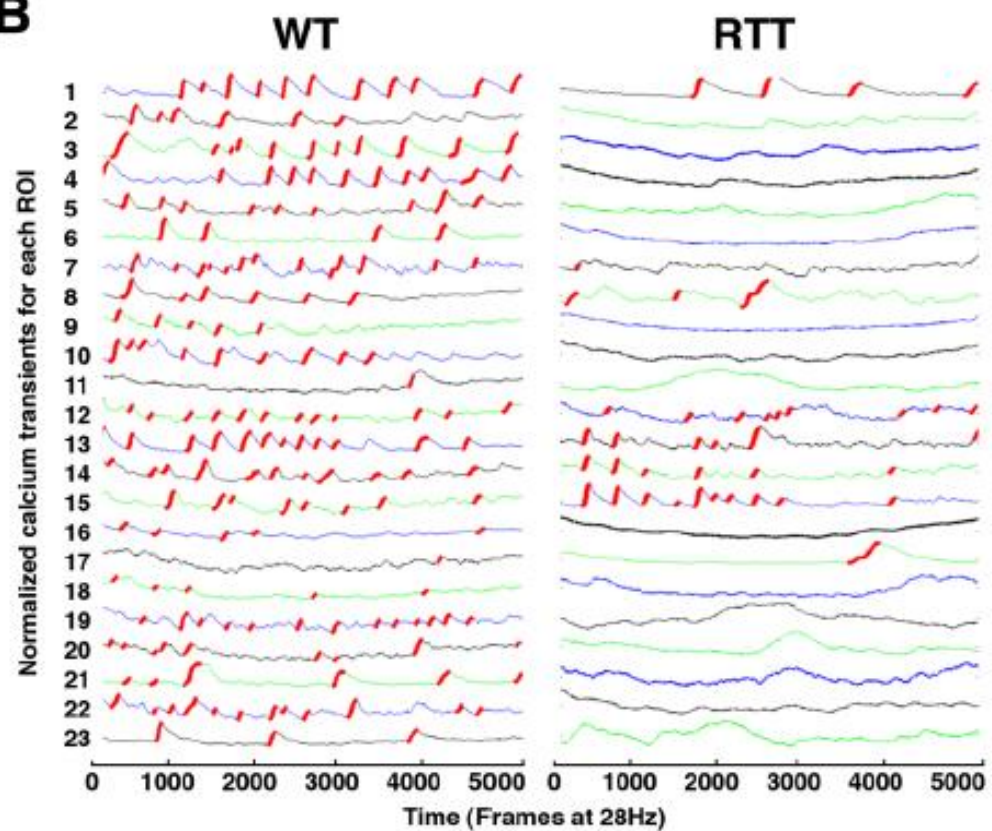
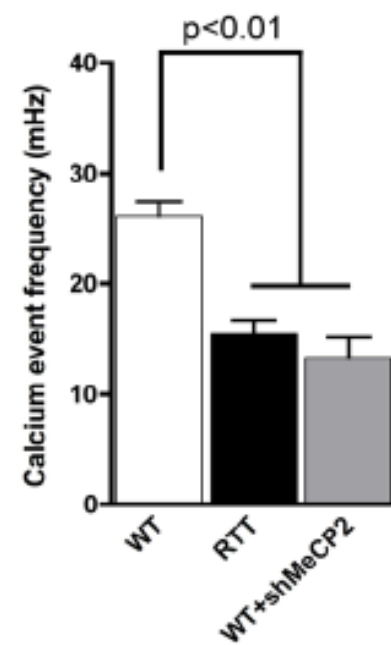
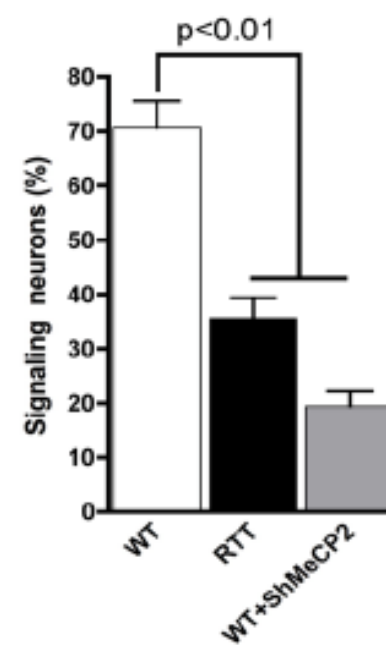


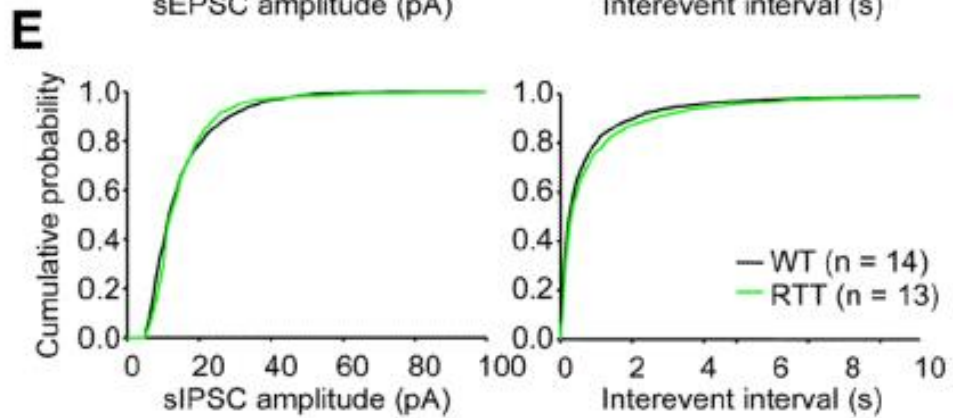
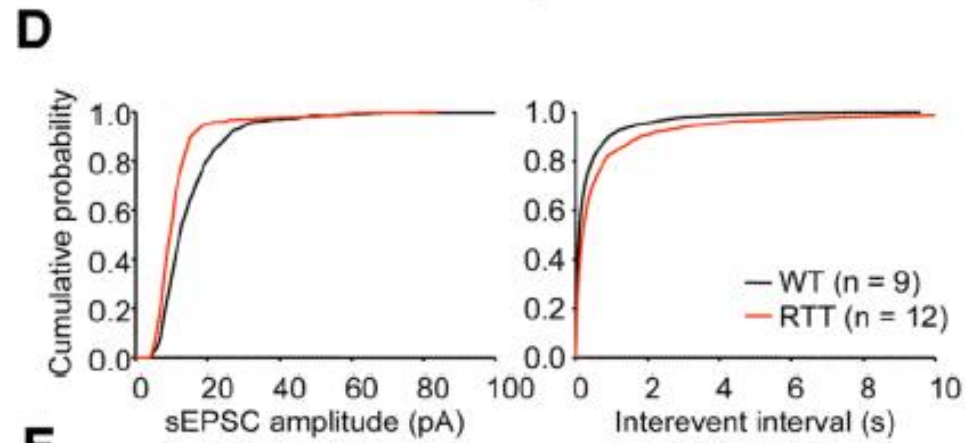
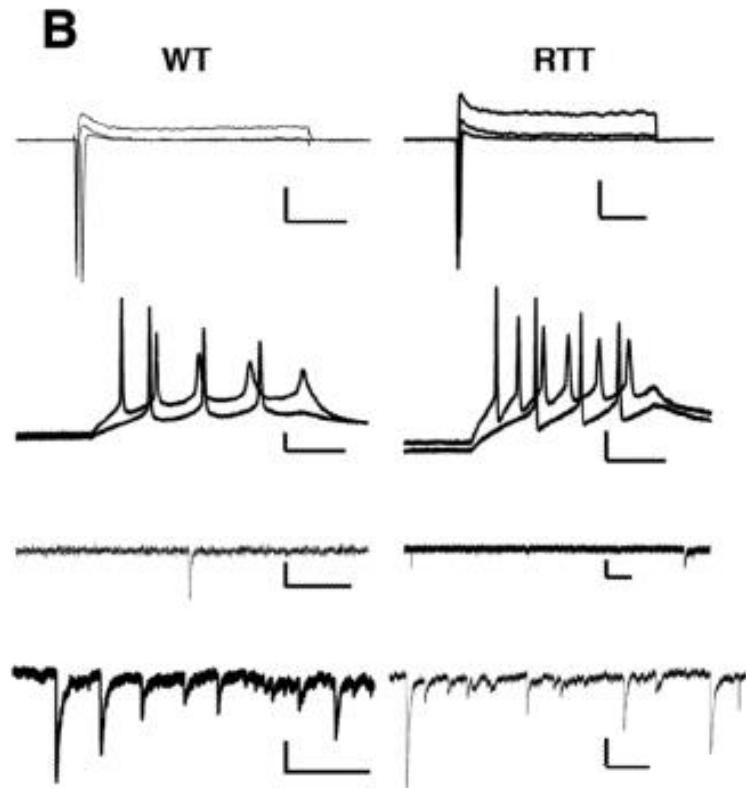
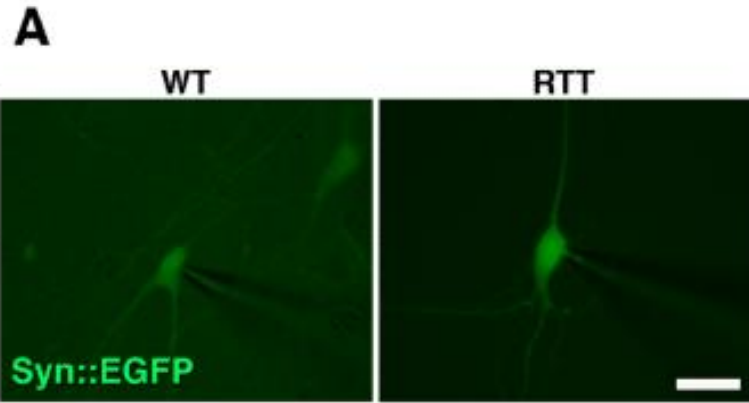
## F



## G



**A****B****C****D**







# Induced pluripotent stem cell models of the genomic imprinting disorders Angelman and Prader–Willi syndromes

Stormy J. Chamberlain<sup>a,b,2</sup>, Pin-Fang Chen<sup>b</sup>, Khong Y. Ng<sup>b</sup>, Fany Bourgois-Rocha<sup>b,1</sup>, Fouad Lemtiri-Chlieh<sup>c</sup>, Eric S. Levine<sup>c</sup>, and Marc Lalande<sup>a,b,2</sup>

<sup>a</sup>University of Connecticut Stem Cell Institute and Departments of <sup>b</sup>Genetics and Developmental Biology and <sup>c</sup>Neuroscience, University of Connecticut Health Center, Farmington, CT 06030

Edited by C. Thomas Caskey, University of Texas-Houston Health Science Center, Houston, TX, and approved August 27, 2010 (received for review April 3, 2010)

Angelman syndrome (AS) and Prader–Willi syndrome (PWS) are

# Background

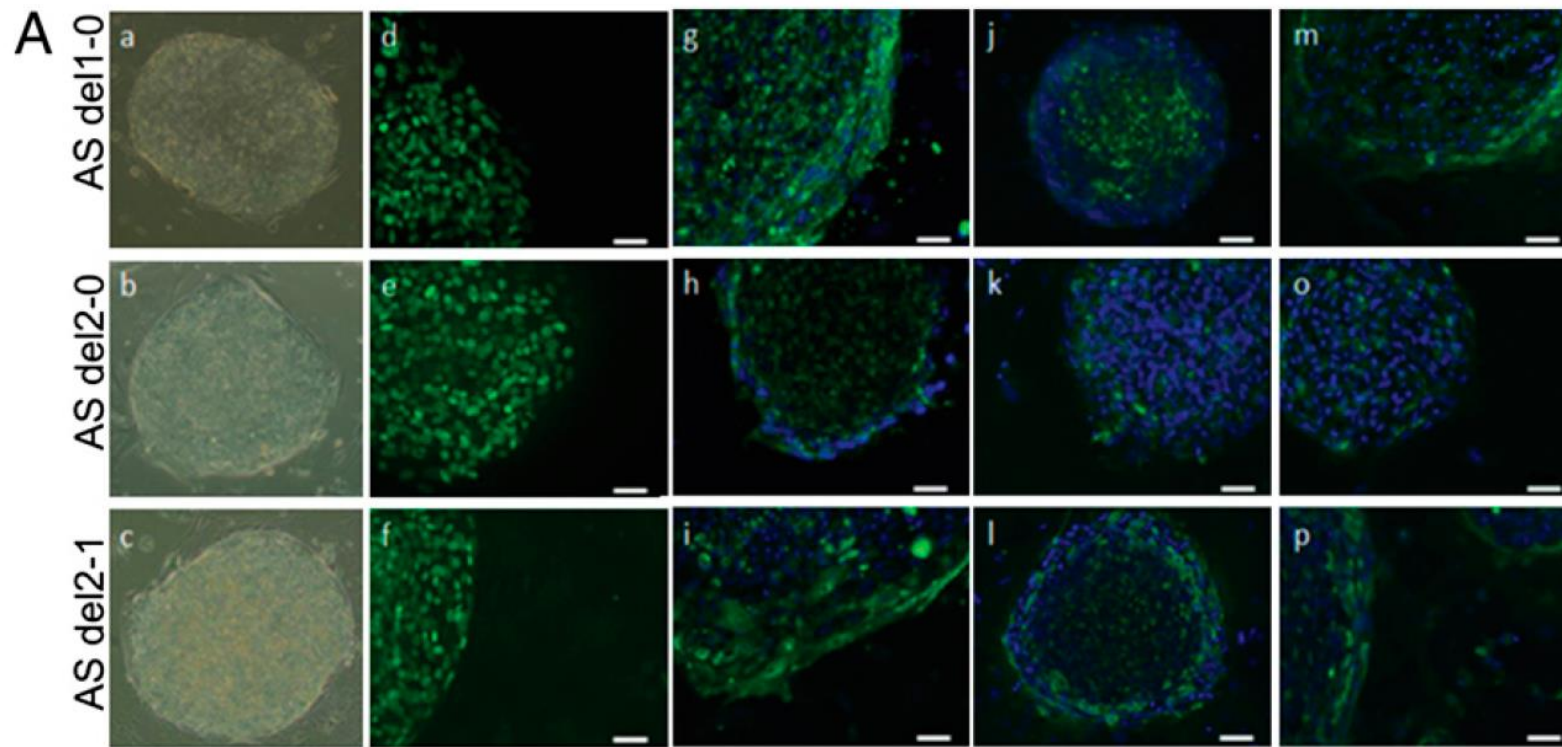


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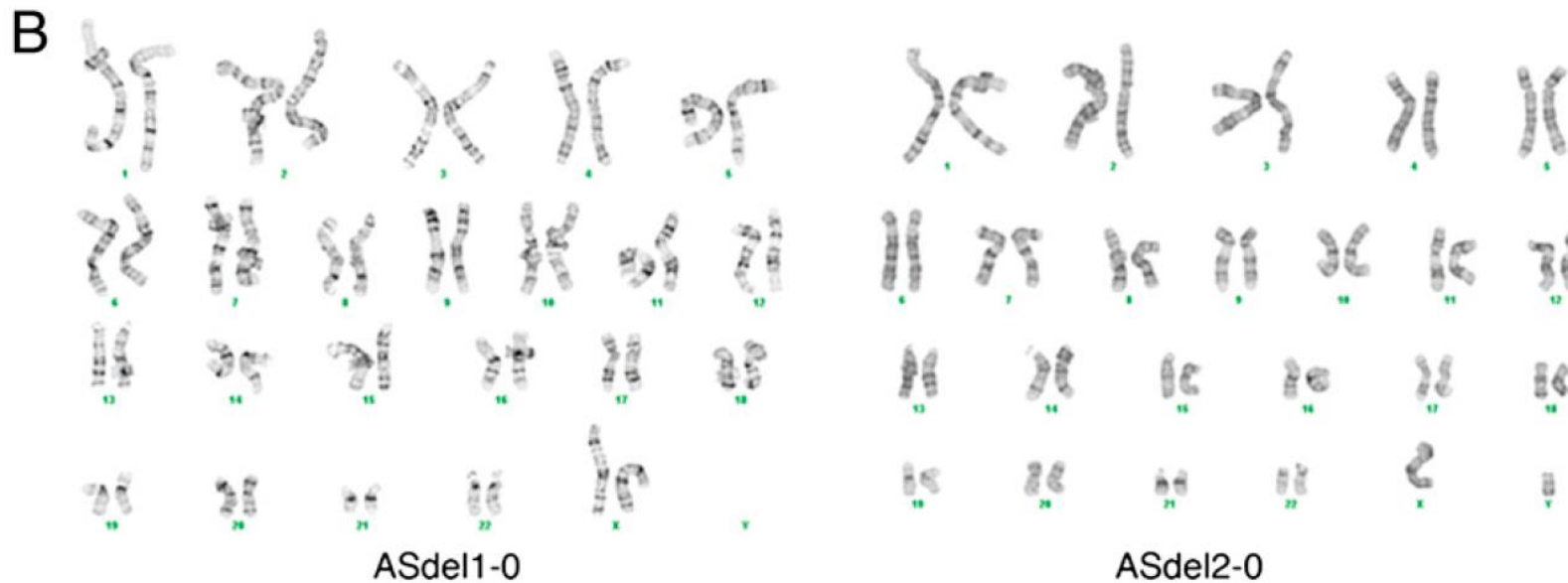
Angelman syndrome (AS) is a neurodevelopmental disorders of genomic imprinting. AS results from loss of function of the ubiquitin protein ligase E3A (UBE3A) gene.

## Biological question

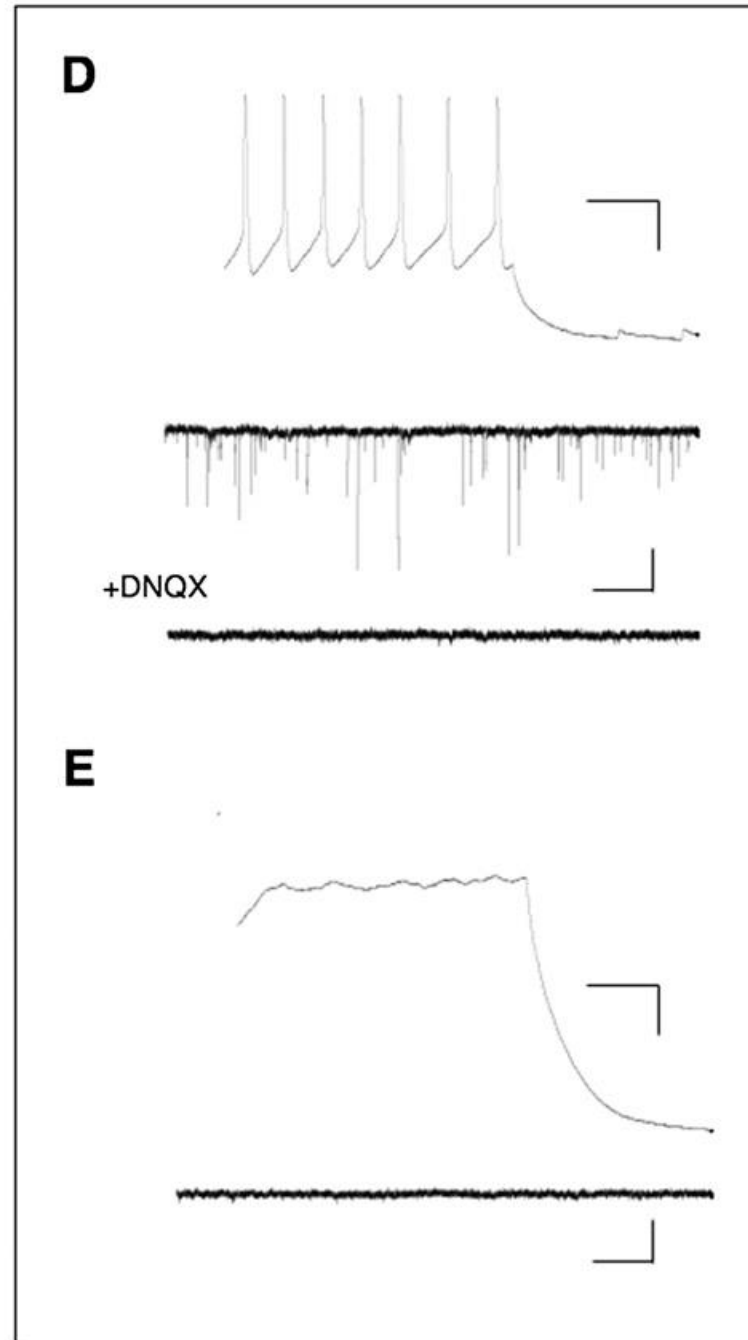
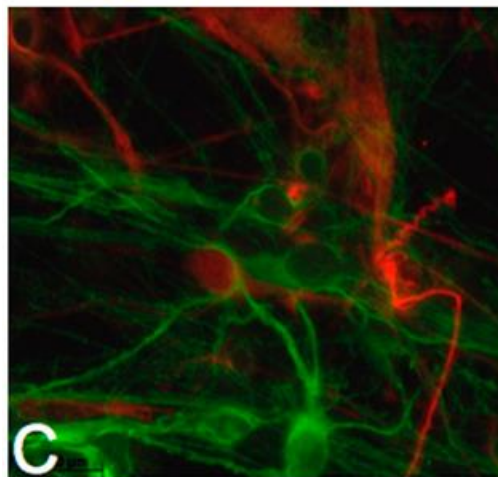
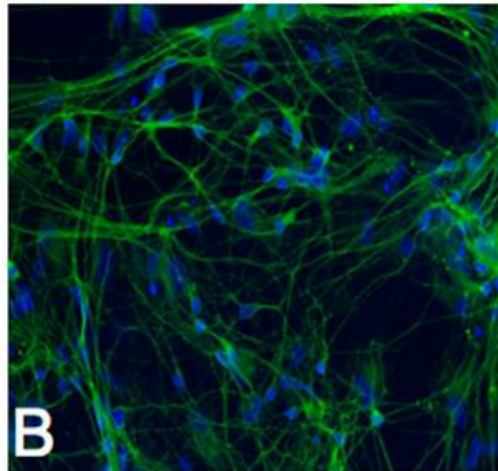
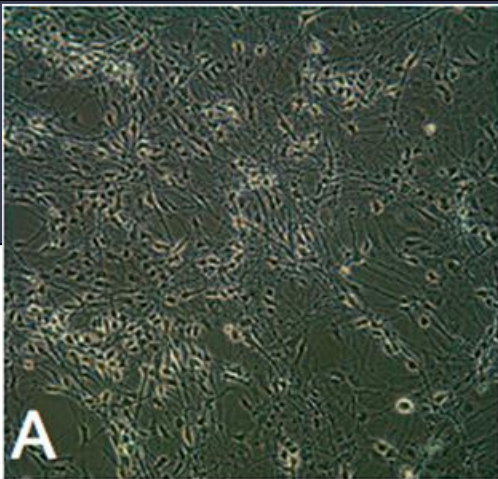
Can we generate a model of AS in vitro?



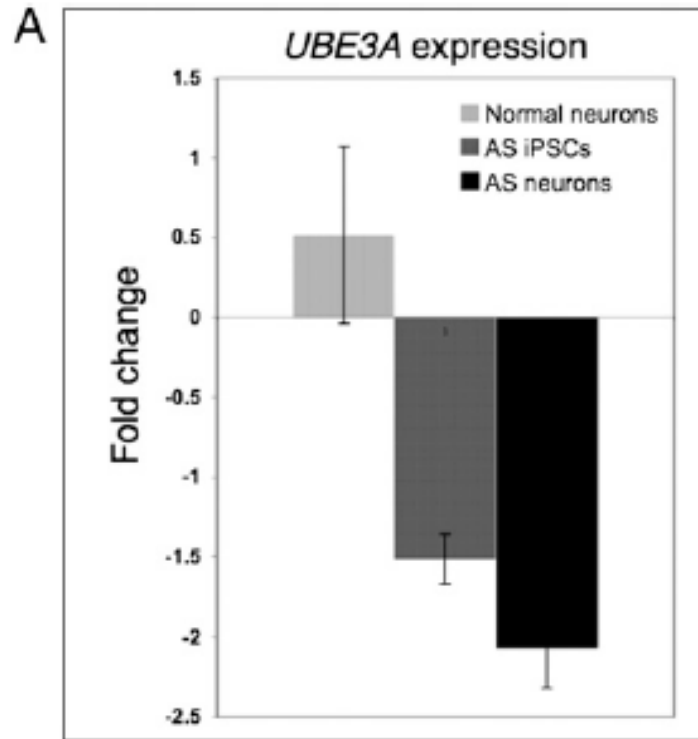
Immunocytochemistry for pluripotency markers on representative iPSC lines shows expression of NANOG (d–f), SSEA4 (g–i), TRA1-60 (j–l), and TRA1-81 (m–p).







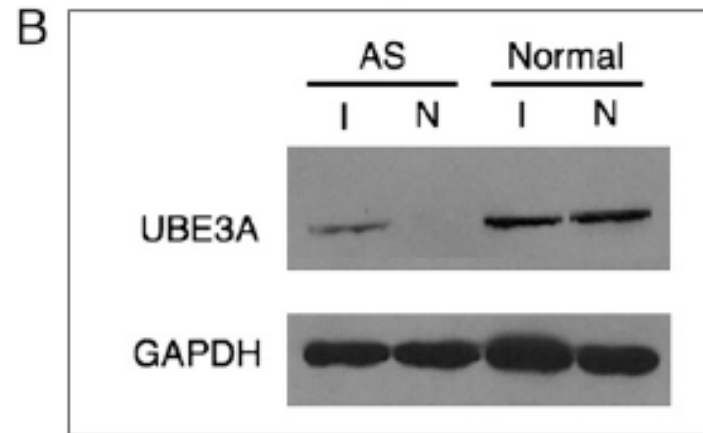
Functional neurons can be generated from AS iPSCs.  
(A) Phase-contrast image of 10-wk-old in vitro differentiated neurons generated from AS iPSCs.



Paternal UBE3A is repressed in AS iPSC-derived neurons.

(A) qRT-PCR analysis of UBE3A expression in AS and normal iPSCs and iPSC-derived neurons.

Gene expression is normalized to GAPDH and is presented as the fold change relative to UBE3A expression levels in normal iPSCs.



B) Western blot analysis of normal and AS iPSCs (I) and 10-wk-old iPSC-derived neurons (N).



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Research paper

## Human iPSC-derived astrocytes from ALS patients with mutated C9ORF72 show increased oxidative stress and neurotoxicity



Anastasya Birger<sup>a,b</sup>, Israel Ben-Dor<sup>a</sup>, Miri Ottolenghi<sup>a</sup>, Tikva Turetsky<sup>a</sup>, Yaniv Gil<sup>a</sup>, Sahar Sweetat<sup>b</sup>, Liat Perez<sup>b</sup>, Vitali Belzer<sup>b</sup>, Natania Casden<sup>b</sup>, Debora Steiner<sup>a</sup>, Michal Izrael<sup>c</sup>, Eithan Galun<sup>d</sup>, Eva Feldman<sup>e</sup>, Oded Behar<sup>b,\*</sup>, Benjamin Reubinoff<sup>a,\*</sup>



# Background



- Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motorneurons(MNs).
- It was shown that human astrocytes with mutations in genes associated with ALS, like C9orf72(C9) or SOD1, reduce survival of MNs.
- Astrocyte toxicity may be related to their dysfunction or the release of neurotoxic factors.

# Biological question

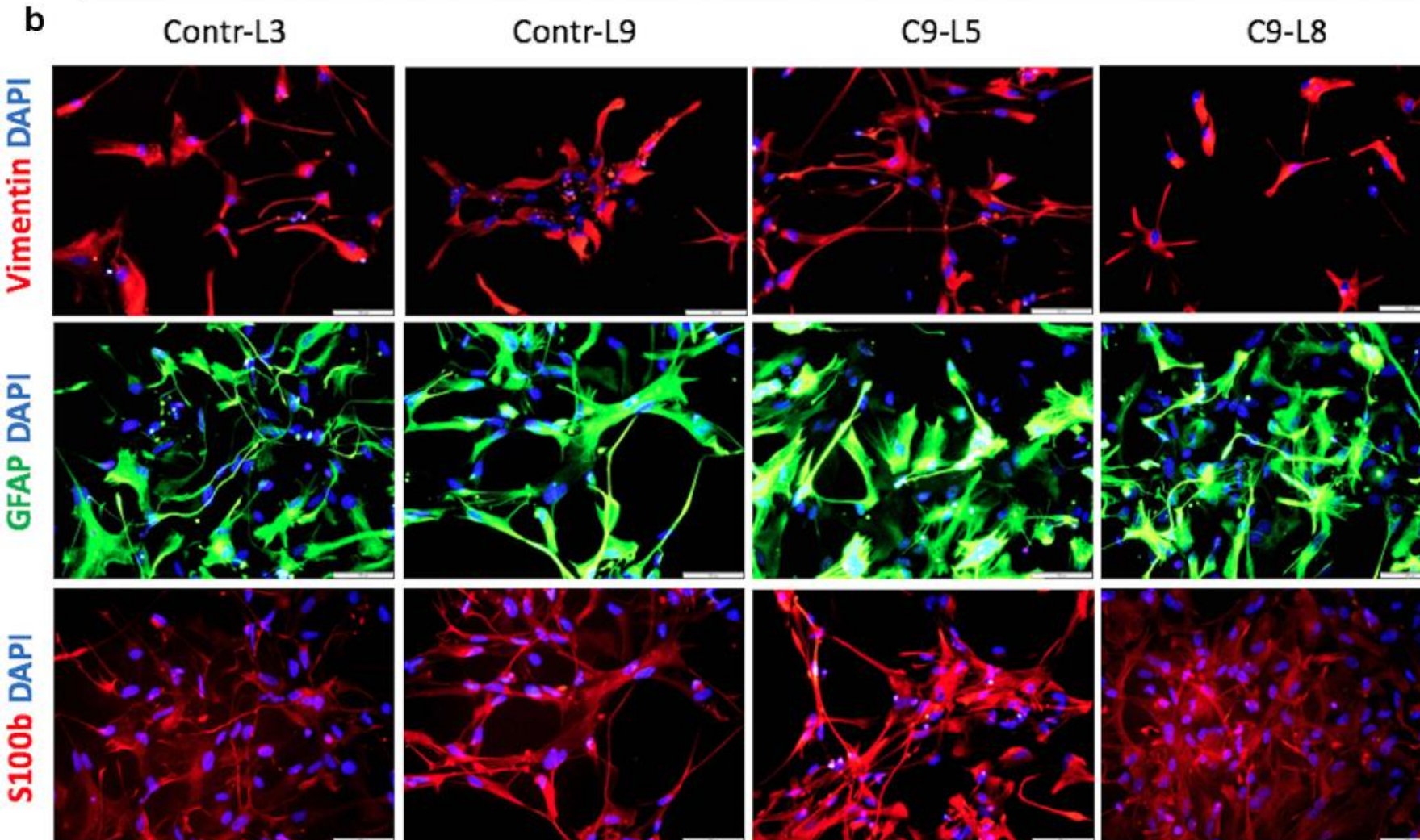
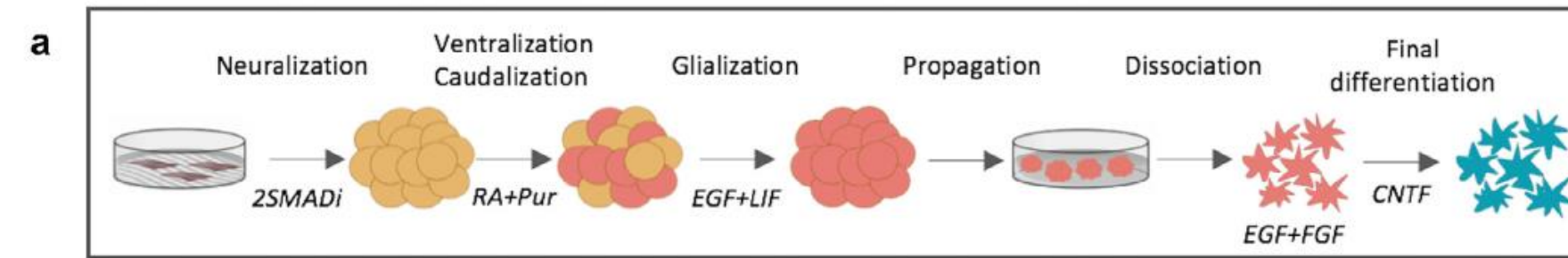


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The role of astrocytes in ALS caused by C9orf72 mutation and the mechanism of the possible induced toxicity

## Methods

- They used human induced pluripotent stem cell-derived astrocytes from ALS patients carrying C9orf72 mutations and non-affected donors.
- We utilized these cells to investigate astrocytic induced neuronal toxicity, changes in astrocyte transcription profile as well as changes in secretome profiles

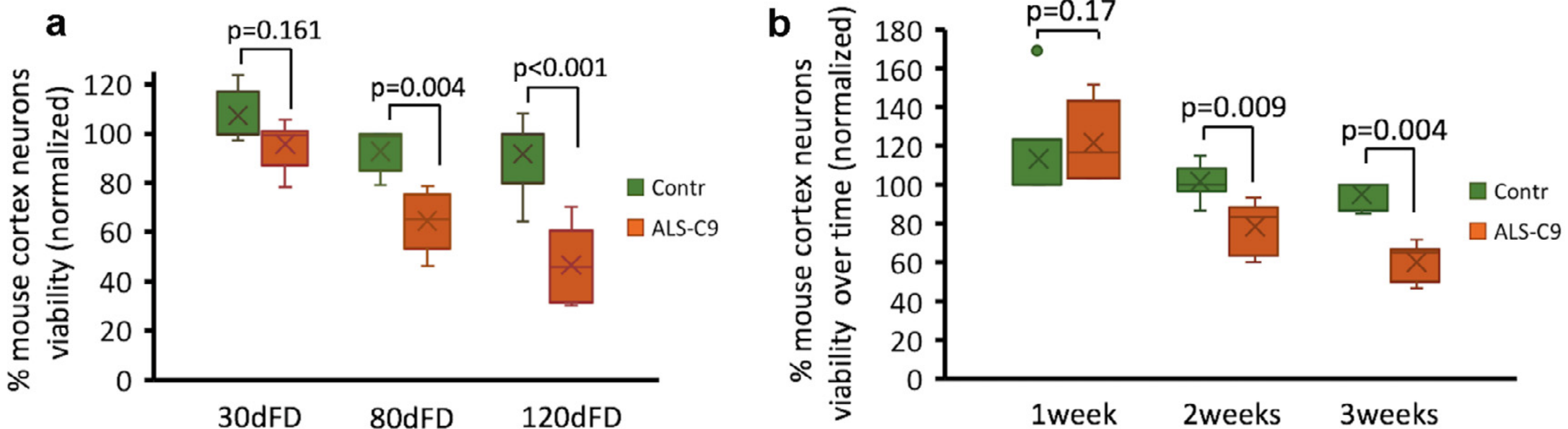


Generation of functional astrocytes from C9 mutated and control iPSClines.

(a) Schematic presentation of the protocol for induction of astrocyte differentiation.

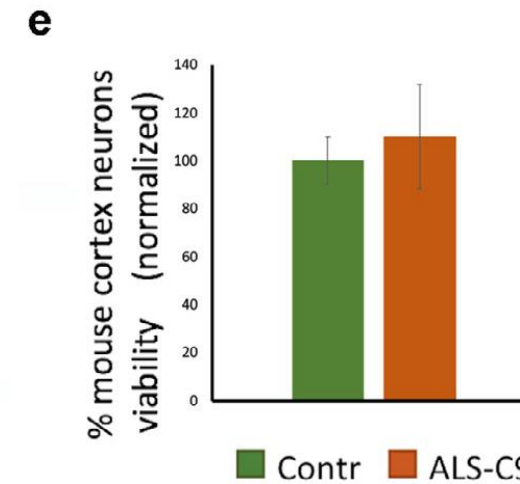
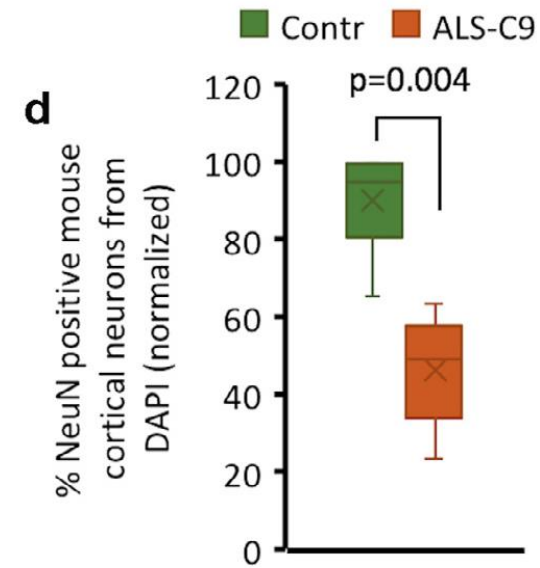
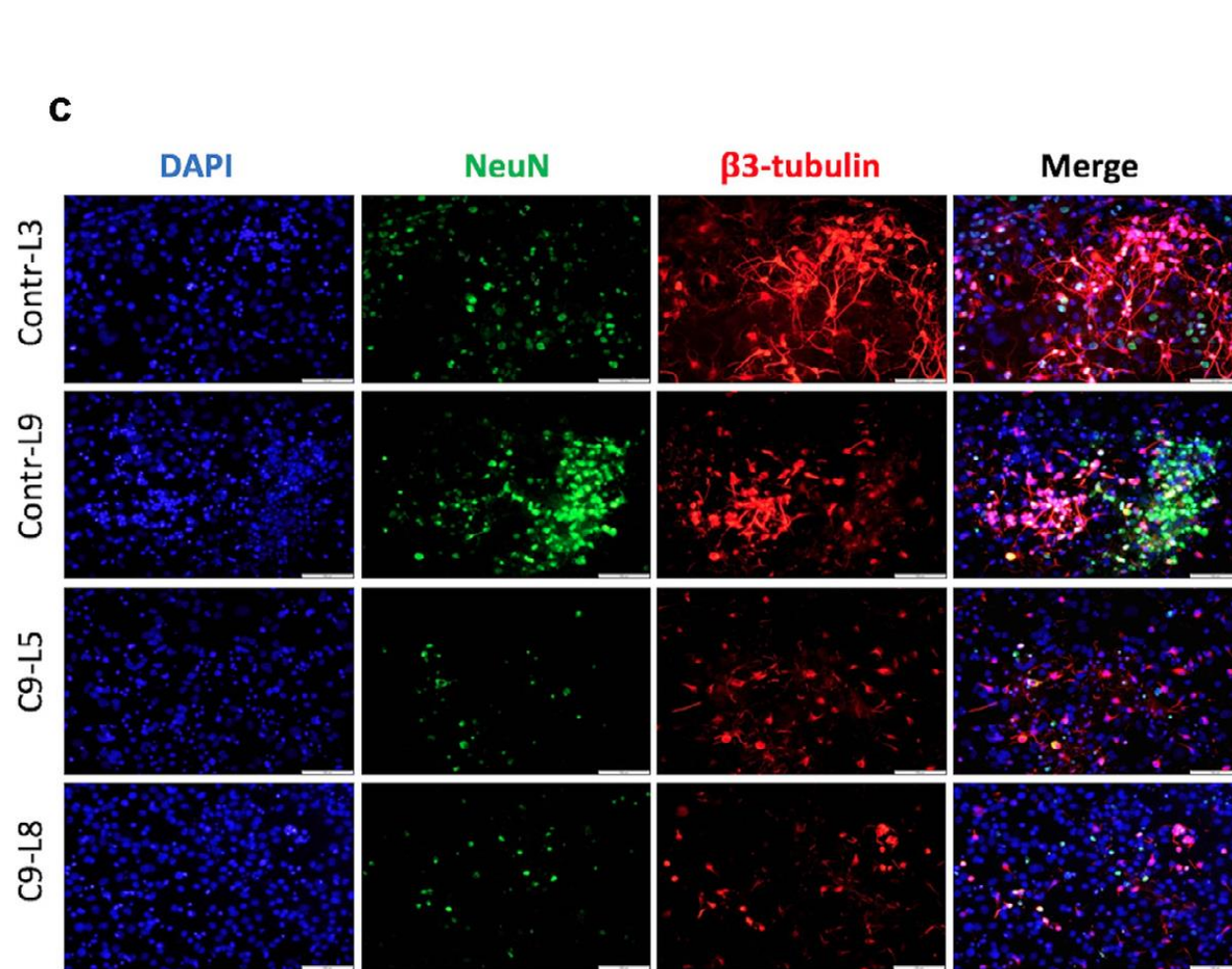


# Results



NOTE : Comparison with mouse model already studied

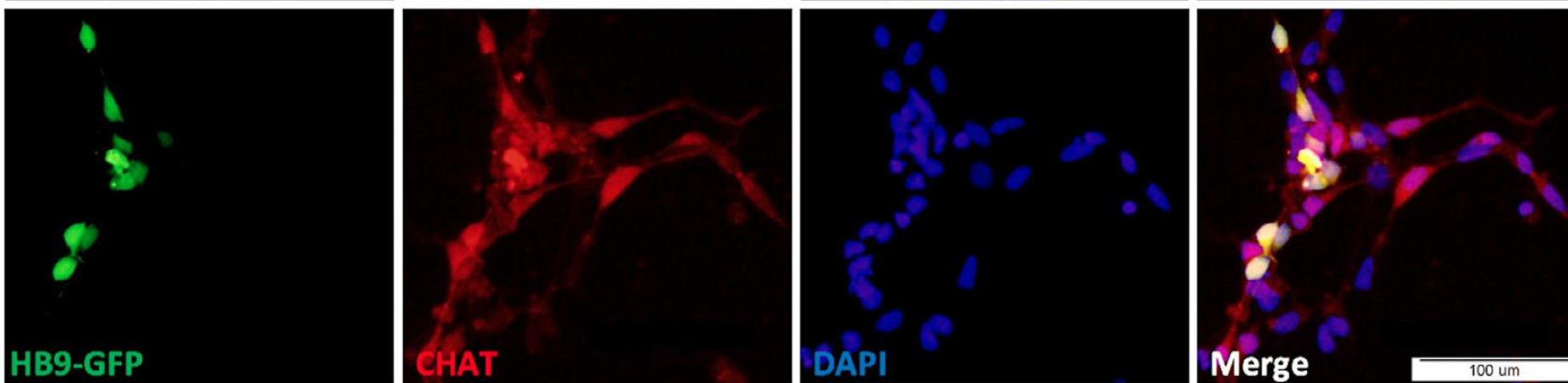
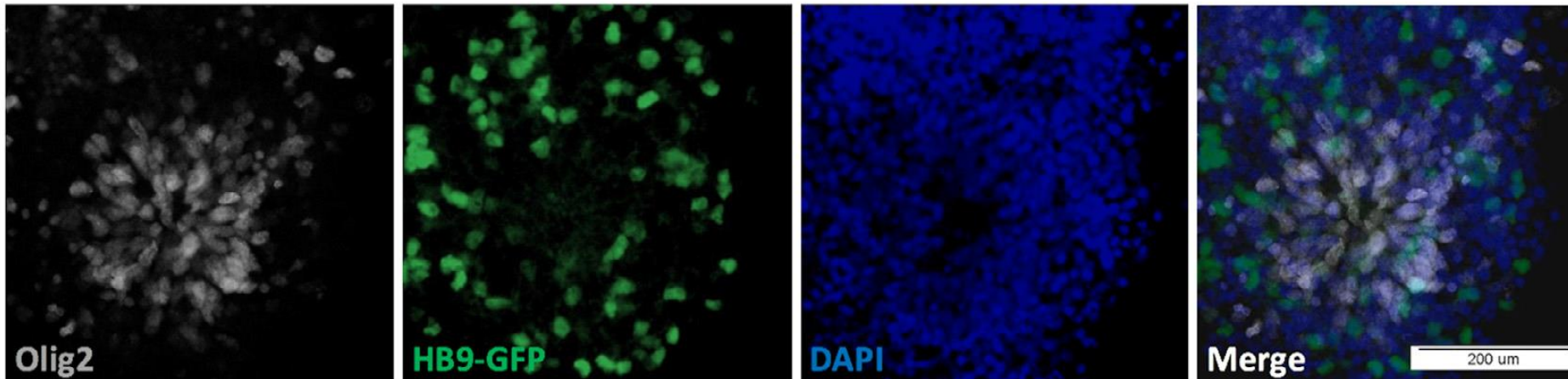
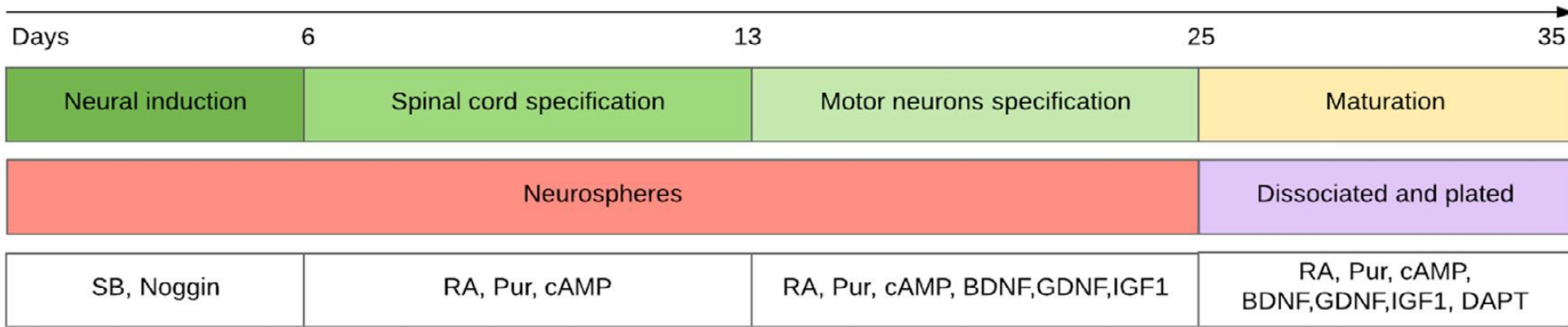
# Results



NOTE : Comparison with mouse model already studied

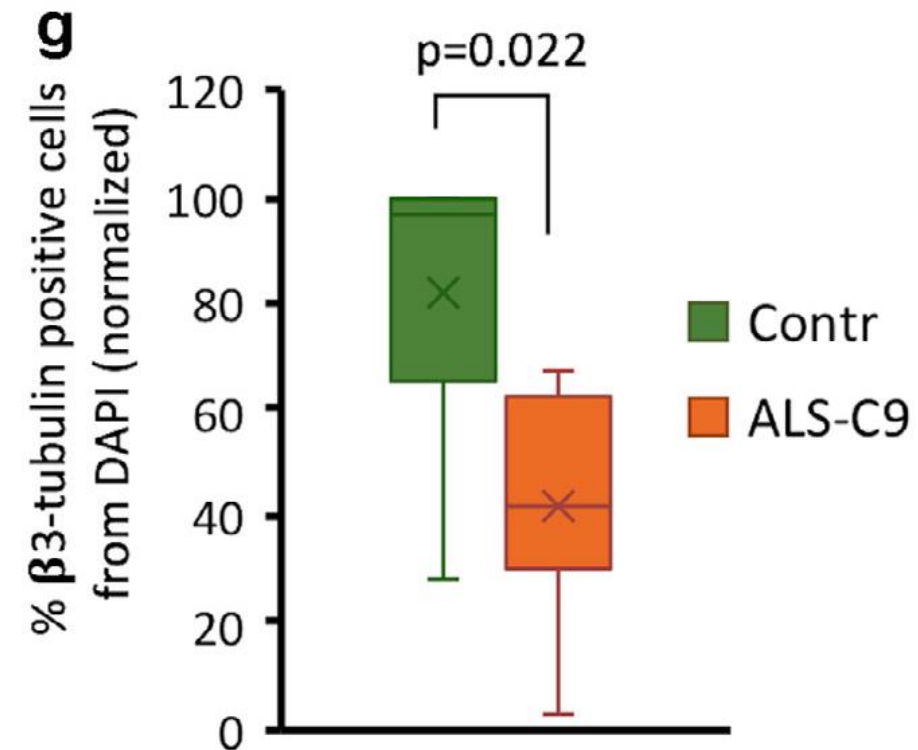
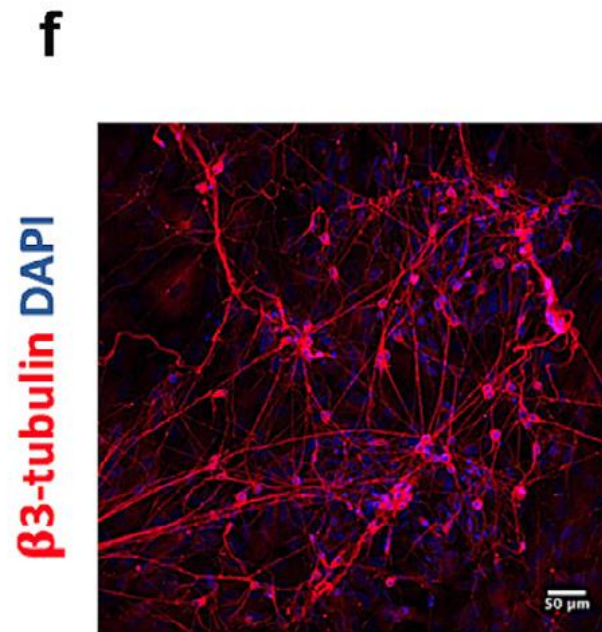
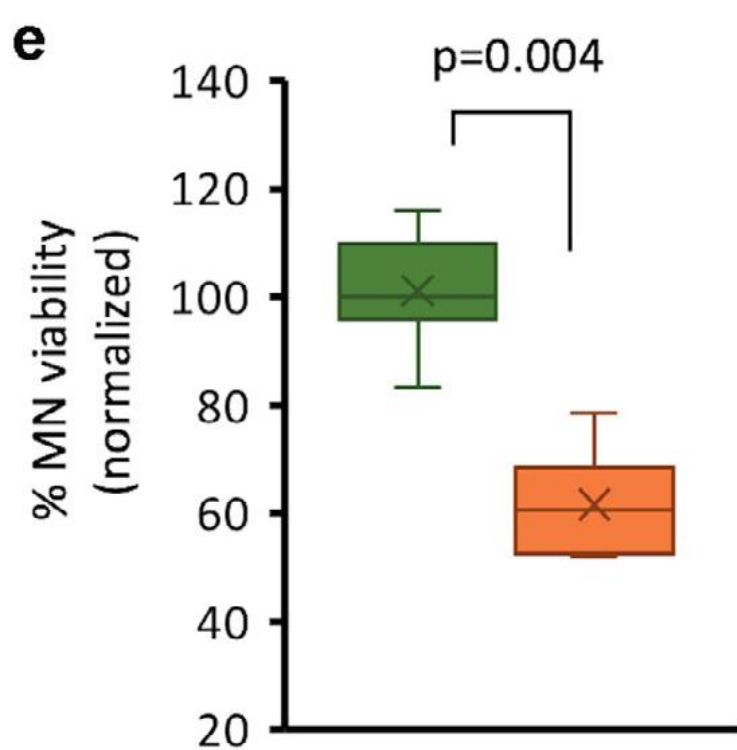
# Results

Generation of  
functional  
motorneurons.



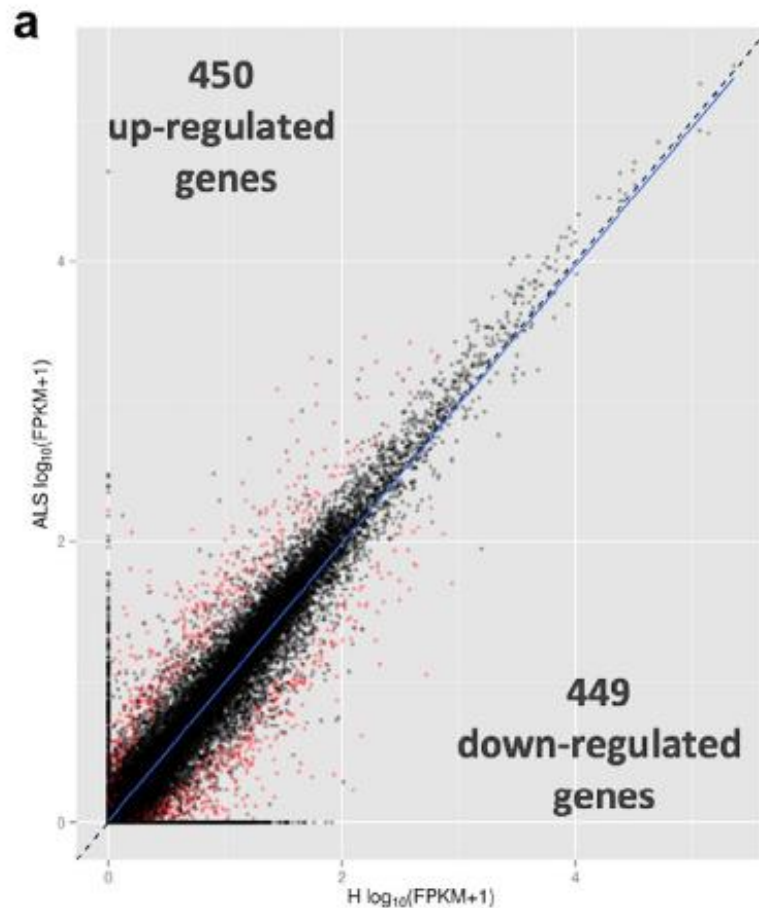


# Results

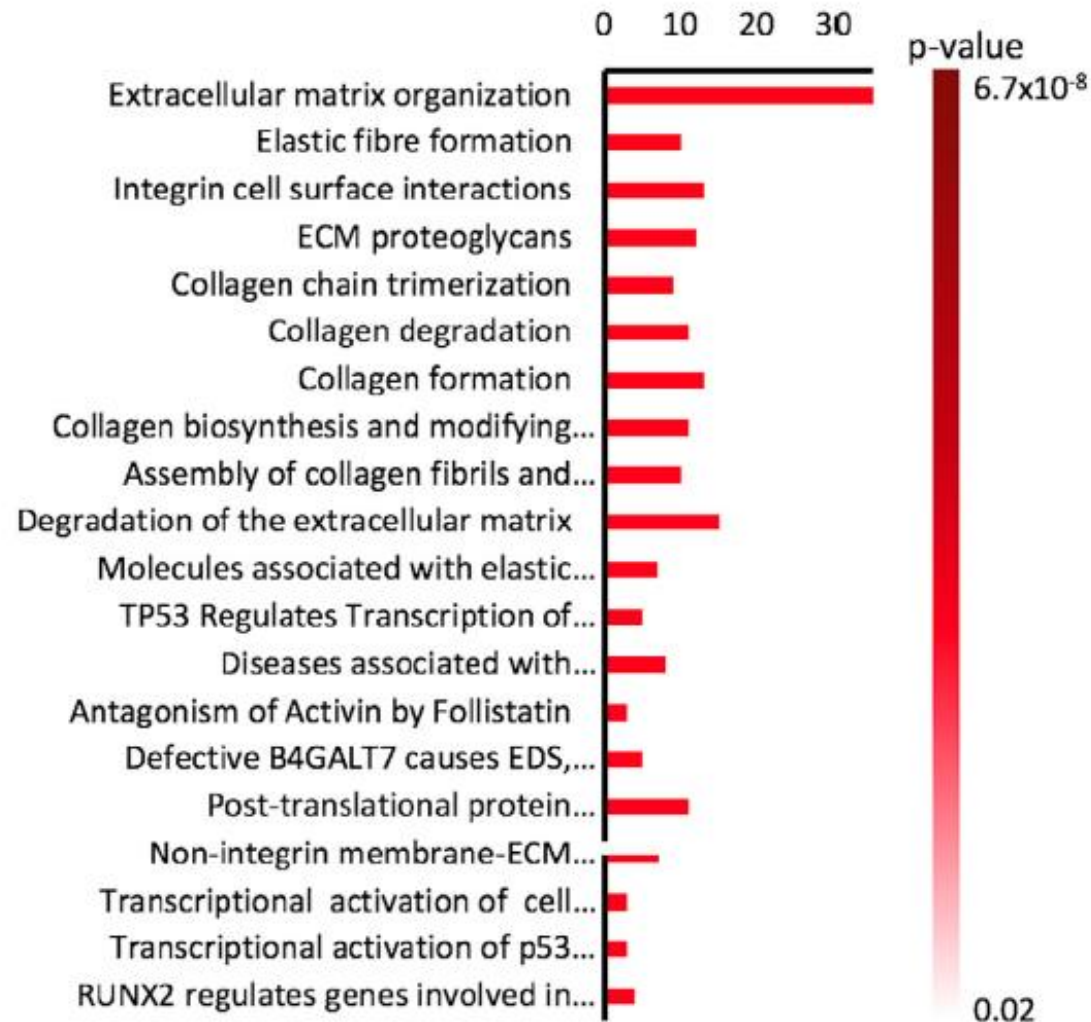


Toxicity of media conditioned by C9-mutated astrocytes to human iPSC-derived motorneurons.

# Results



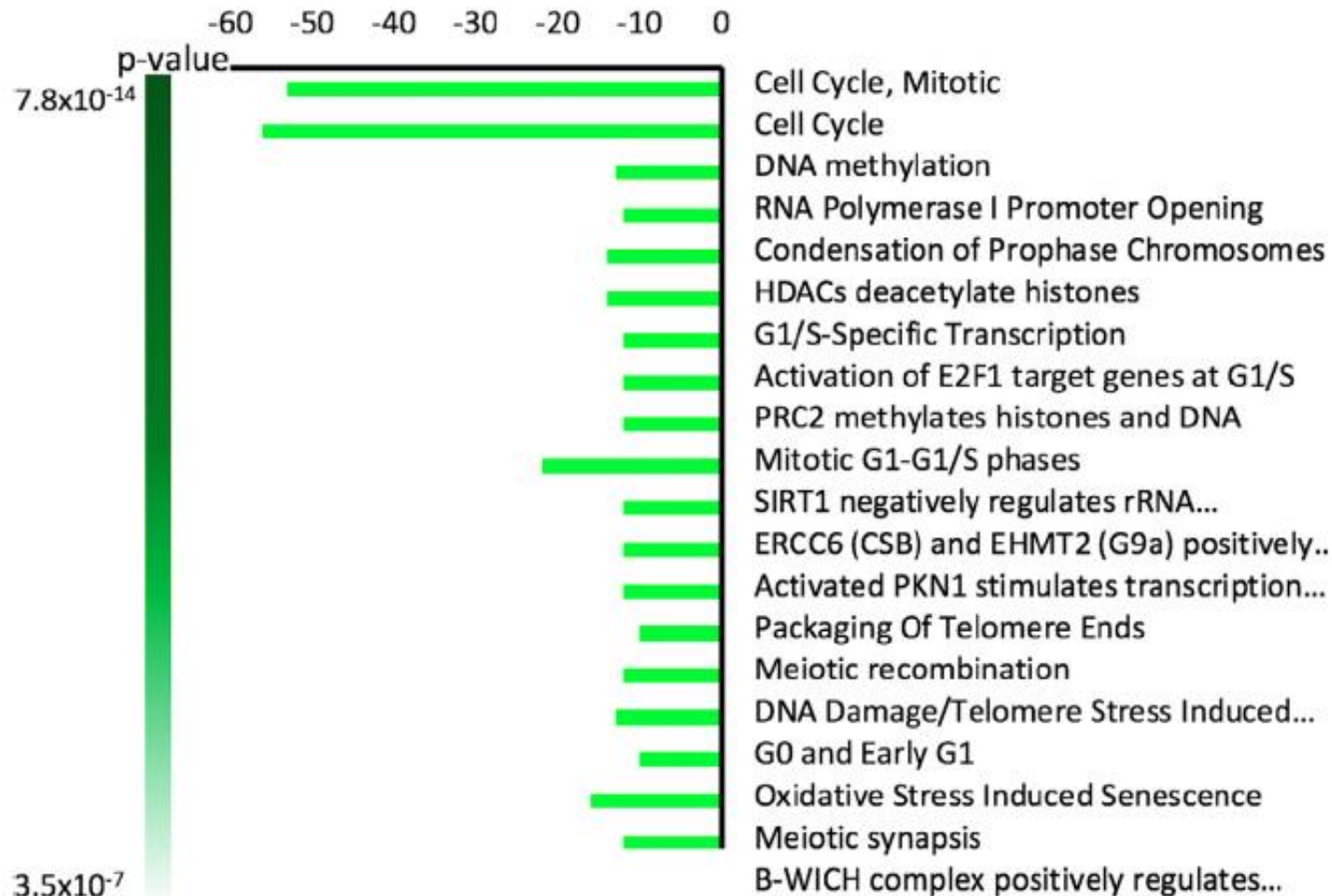
**b** Enriched pathways based on up-regulated genes



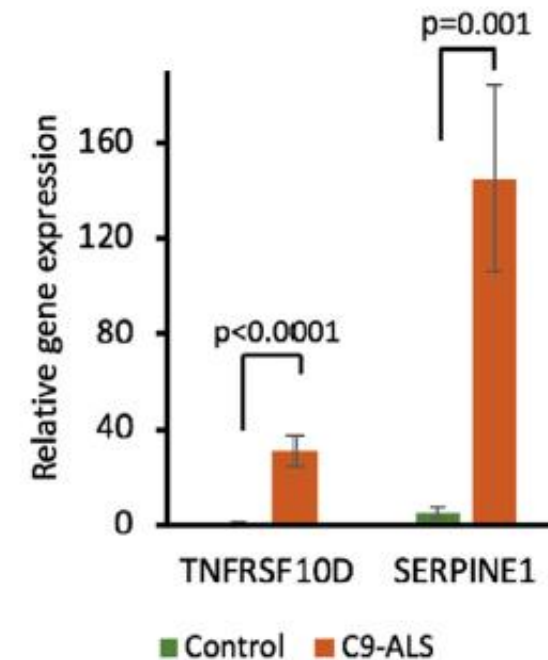
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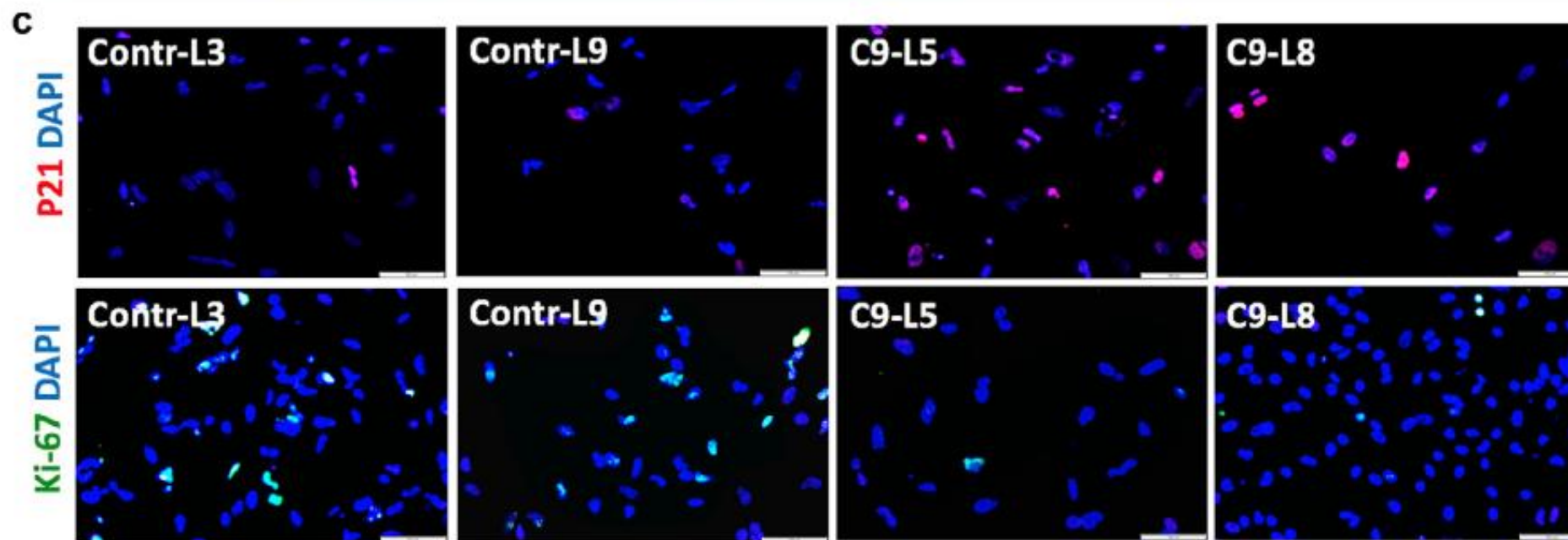
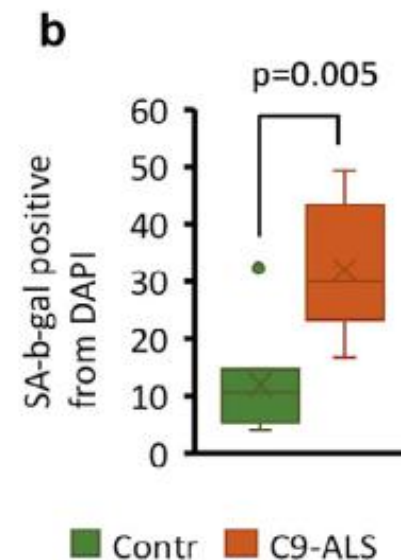
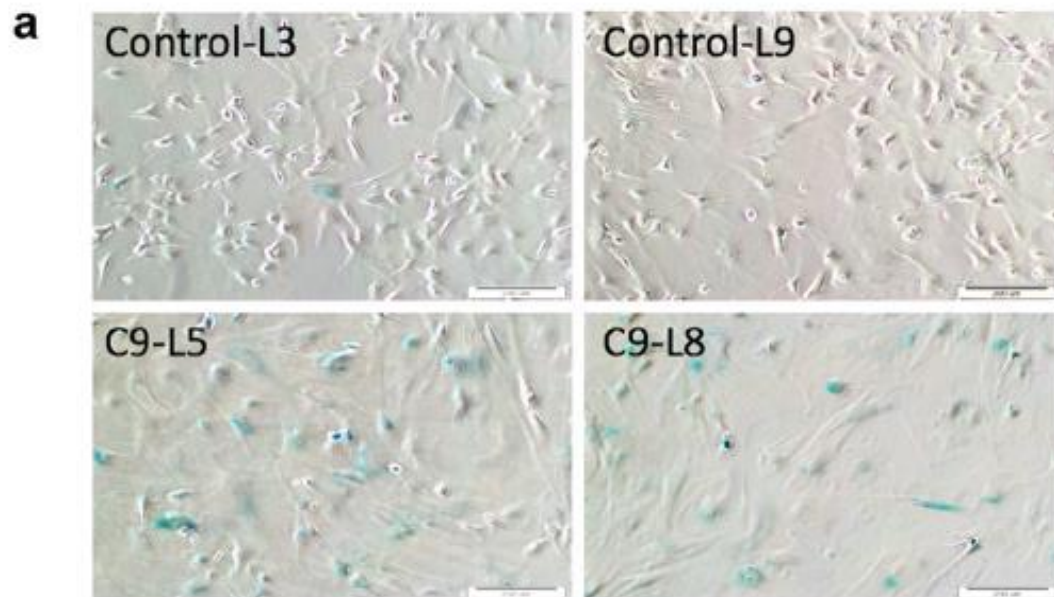
**c** Enriched pathways based on down-regulated genes



**d**

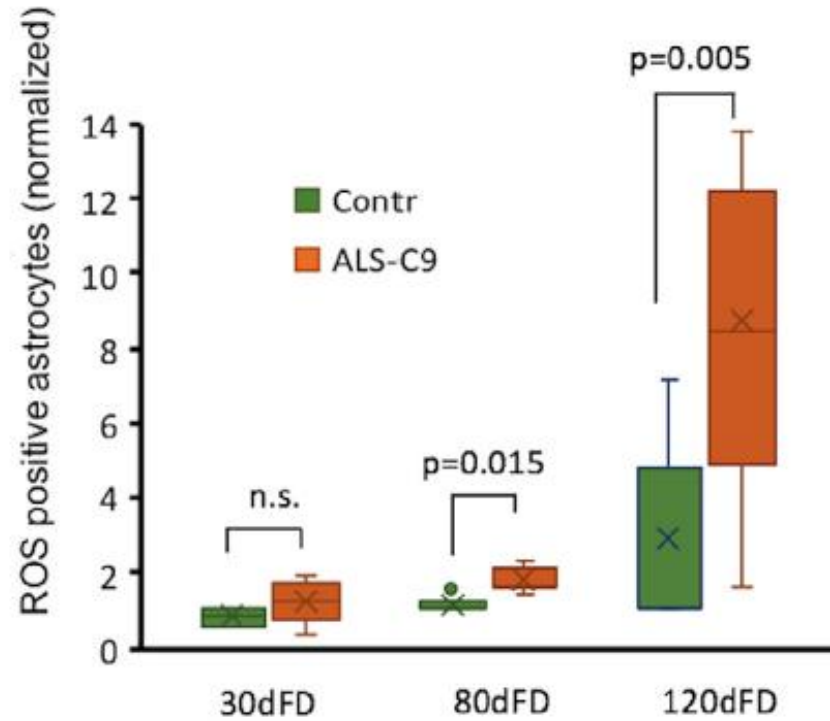
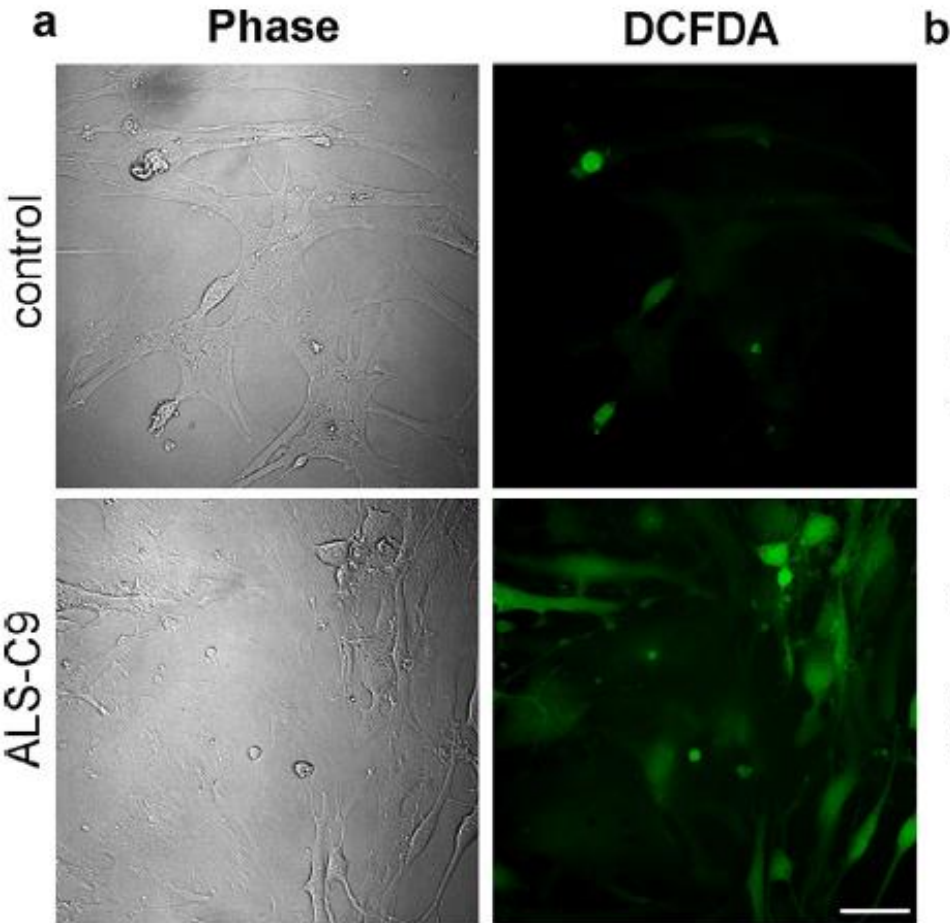






C9 mutated astrocytes acquire senescence phenotype at higher rates than non-mutated astrocytes.

# Results



C9 mutated astrocytes show higher percentage of ROS-positive cells and their secretome can induce higher ROS levels in MNs.



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