“Techniques in Cellular and Molecular Neurobiology”

International Master’s Degree in Neuroscience

Lesson 6

DIPARTIMENTO DI SCIENZE DELLA VITA

Gabriele Baj
gbaj@units.it

UNIVERSITÀ DEGLI STUDI DI TRIESTE

Published in final edited form as:
Cell. 2010 November 12; 143(4): 527–539. doi:10.1016/j.cell.2010.10.016.

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

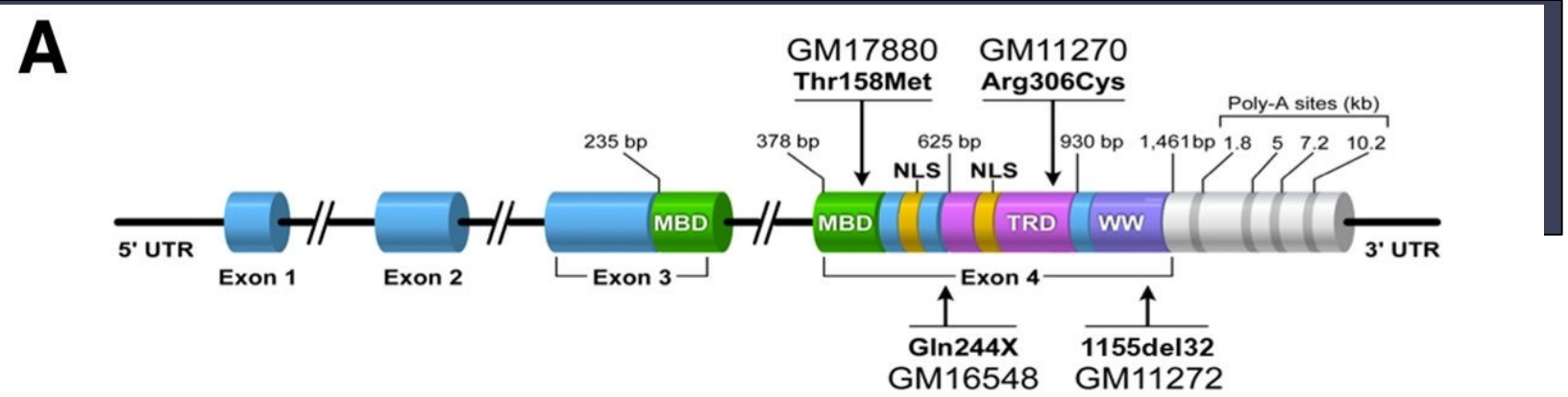
Maria C. N. Marchetto[1,†], **Cassiano Carromeu**[2,†], **Allan Acab**[2], **Diana Yu**[1], **Gene Yeo**[3], **Yangling Mu**[1], **Gong Chen**[4], **Fred H. Gage**[1], and **Alysson R. Muotri**[2,*]

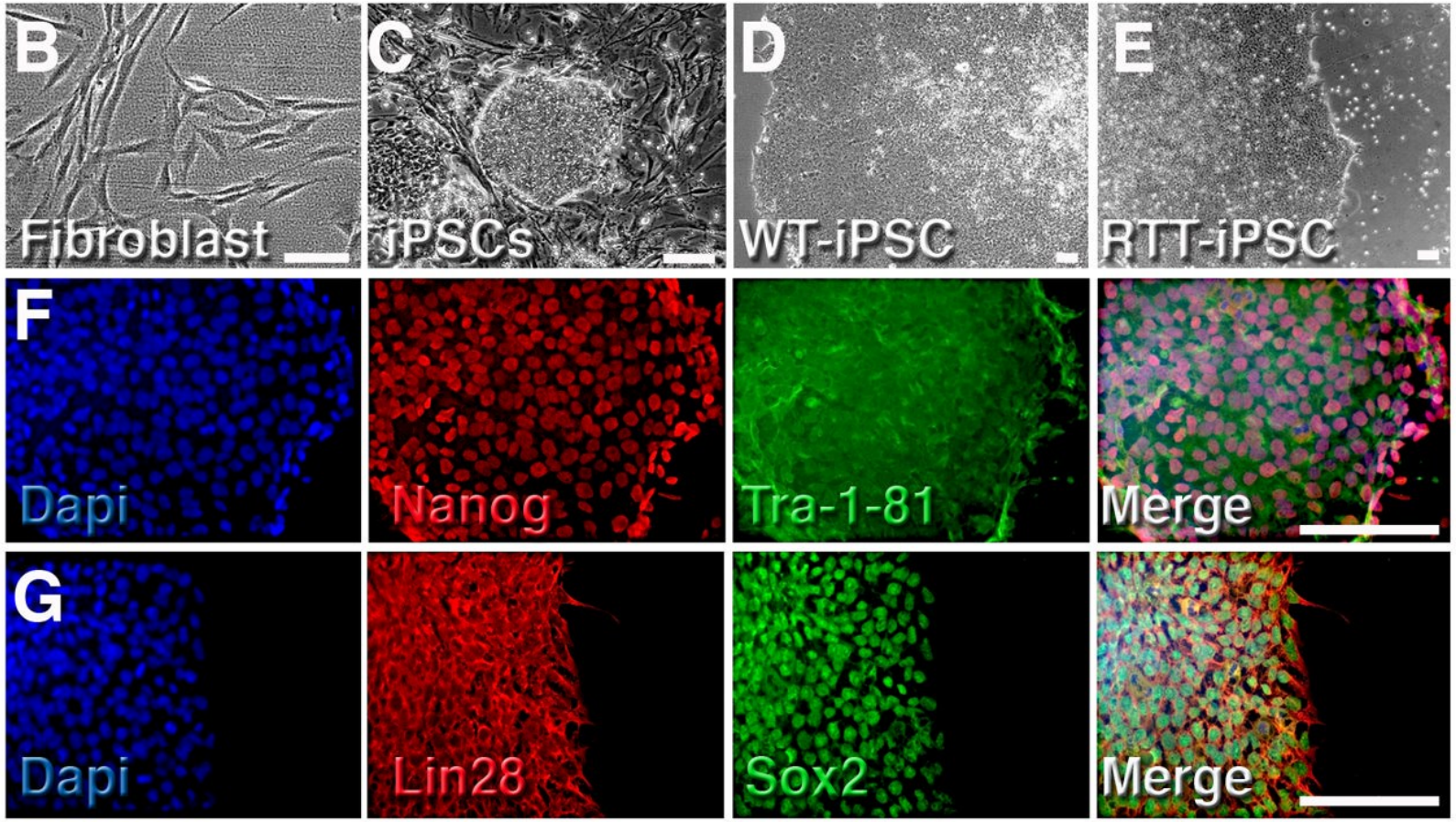
[1] The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

Background

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

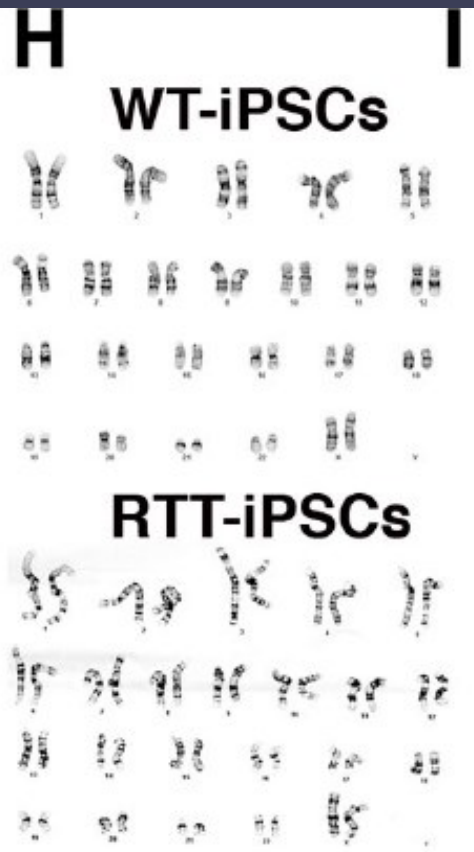
Autism spectrum disorders (ASD) are complex neurodevelopmental diseases in which different combinations of genetic mutations may contribute to the phenotype.
Using Rett syndrome (RTT) as an ASD genetic model, we developed a culture system using induced pluripotent stem cells (iPSCs) from RTT patients' 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.

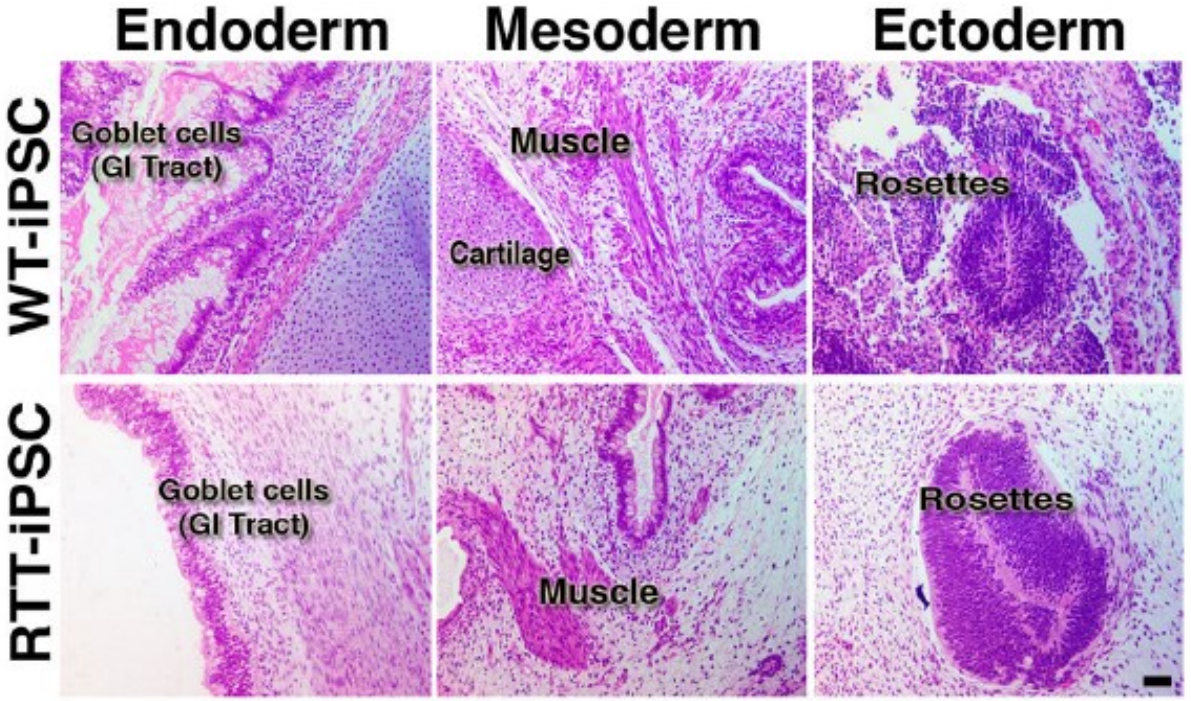




Generation of iPSCs. (A), Schematic representation of the MeCP2 gene structure and mutations used in this study

Results



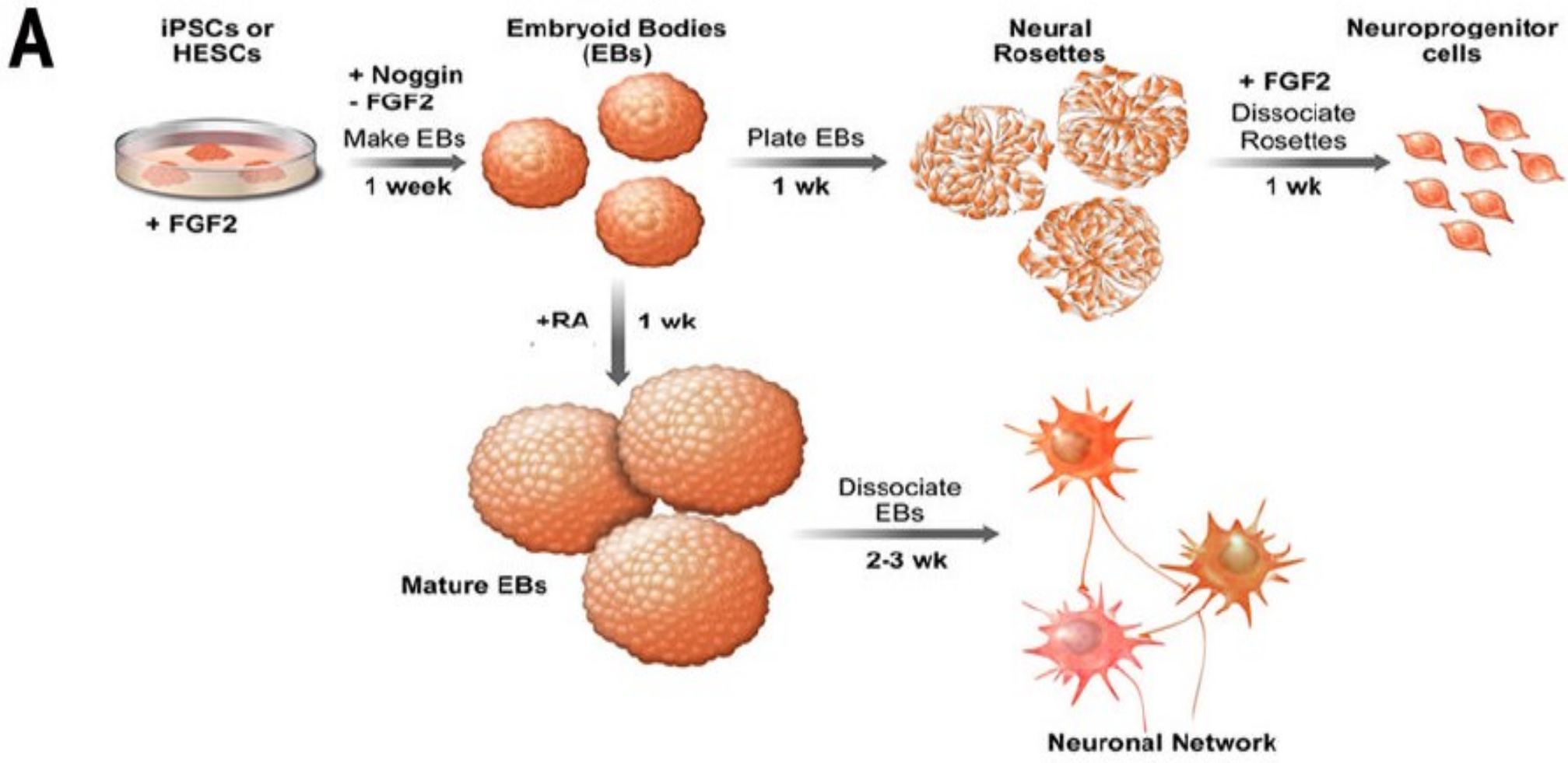


Representative images of teratoma sections.

NOTE : iPSCs are validated using animal models

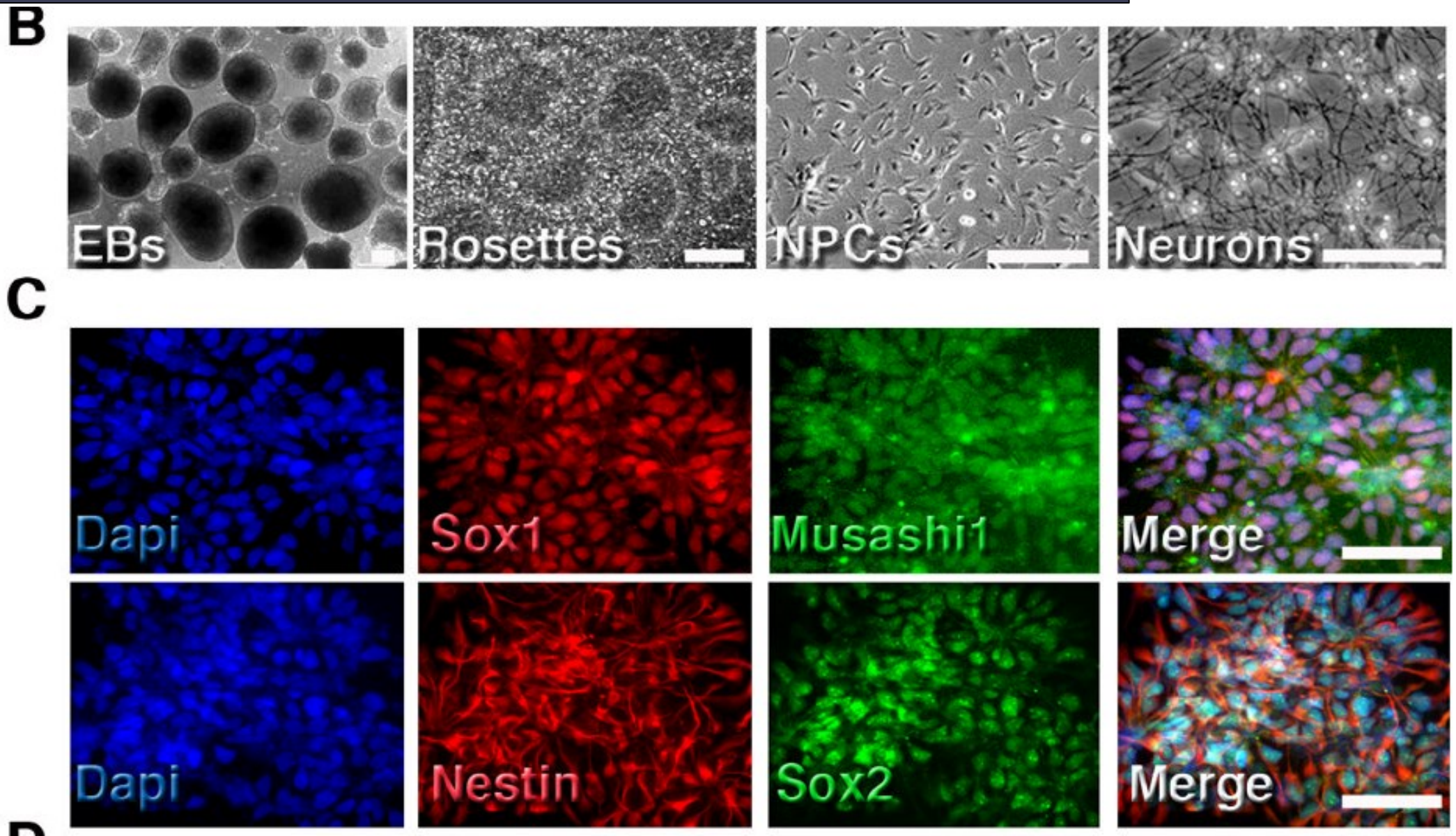
Results

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

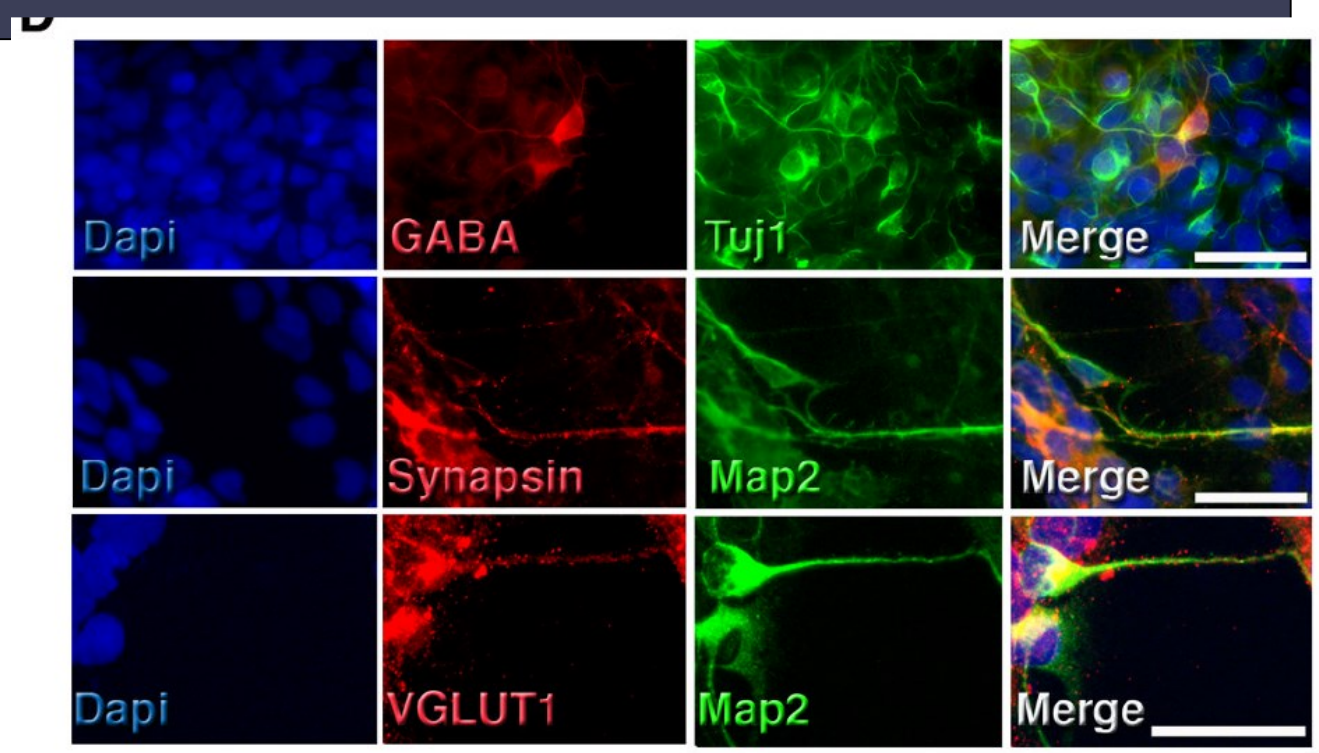


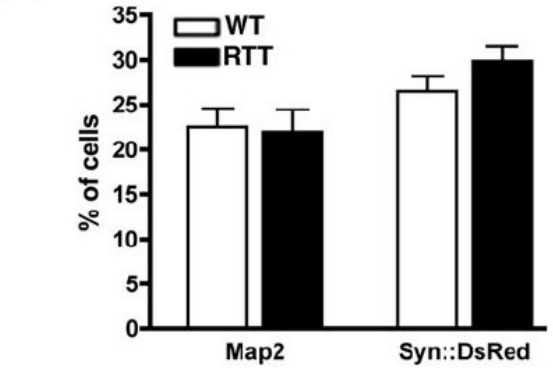
Results

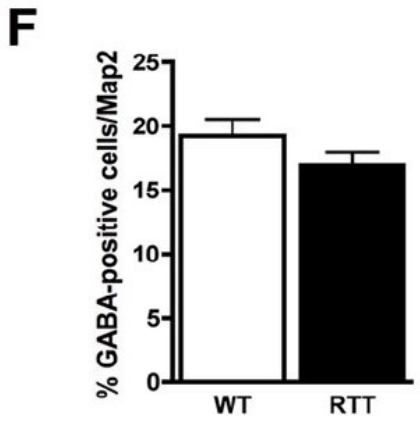
UNIVERSITÀ
DEGLI STUDI DI TRIESTE



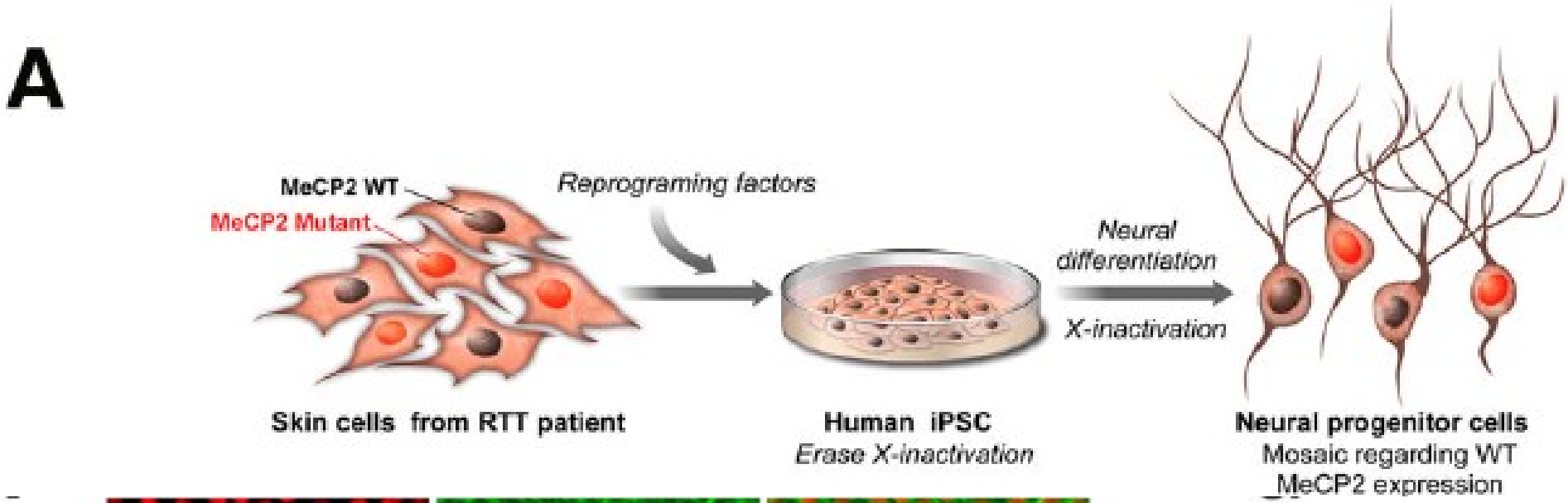
Results

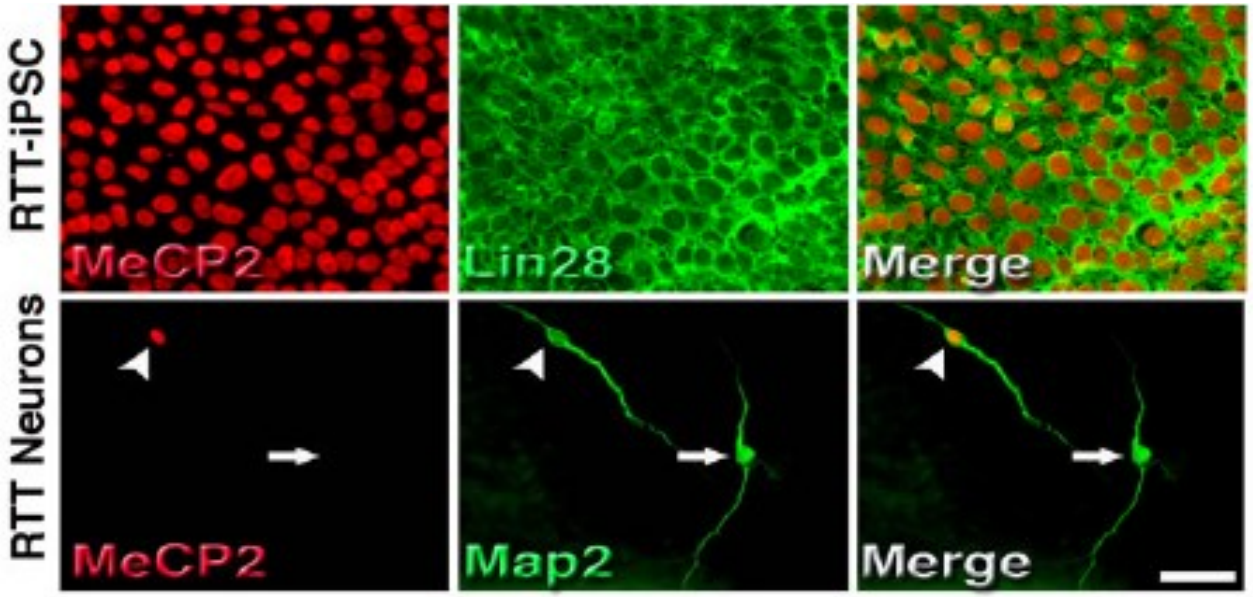

Dapi
GABA
Tuj1
Merge
Dapi
Synapsin
Map2
Merge
Dapi
VGLUT1
Map2
Merge

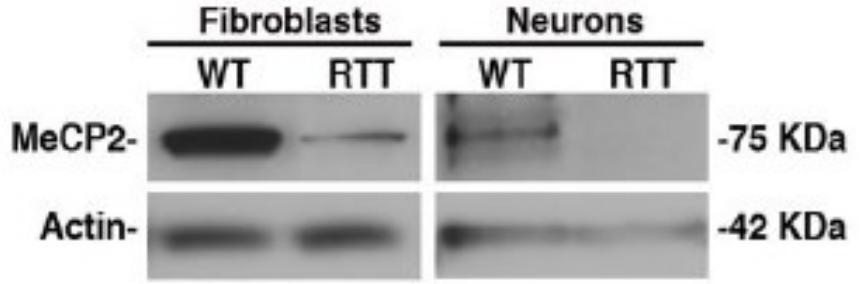

E
WT
RTT
% of cells
35
30
25
20
15
10
5
0
Map2
Syn::DsRed


F
% GABA-positive cells/Map2
25
20
15
10
5
0
WT
RTT

Results

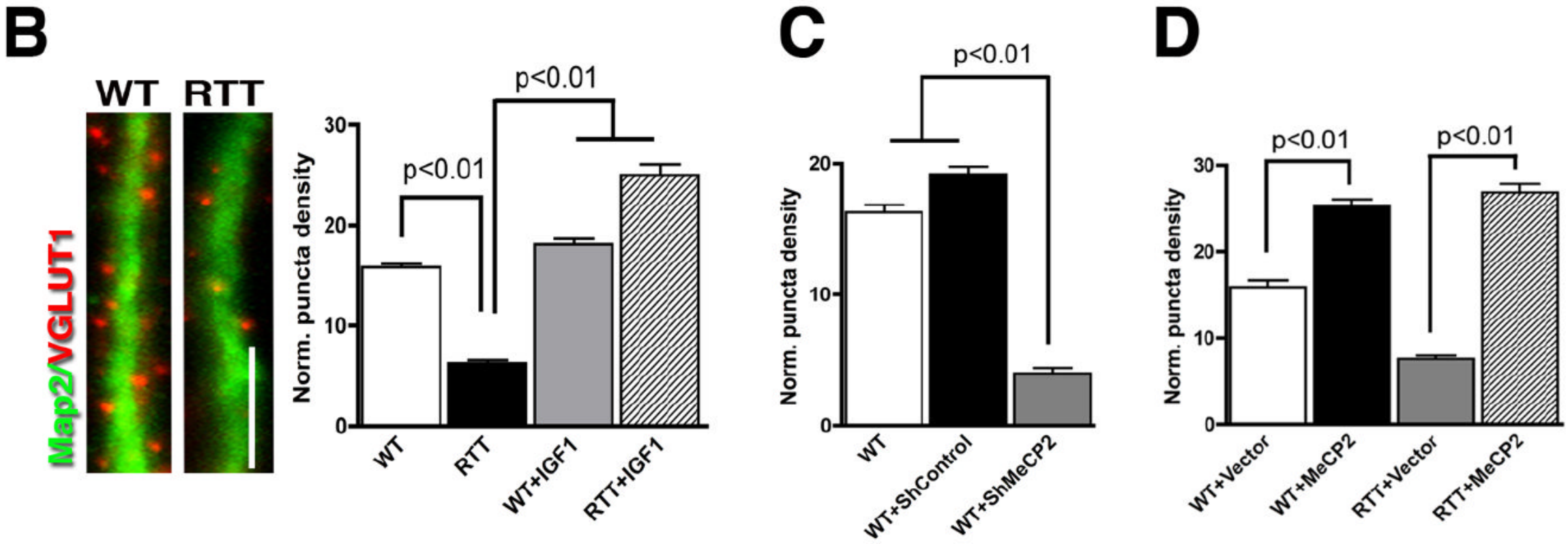

A
MeCP2 WT
MeCP2 Mutant
Reprograming factors
Neural differentiation
X-inactivation
Skin cells from RTT patient
Human iPSC
Erase X-inactivation
Neural progenitor cells
Mosaic regarding WT
MeCP2 expression


RTT-iPSC
MeCP2
Lin28
Merge
RTT Neurons
MeCP2
Map2
Merge


Fibroblasts
Neurons
WT
RTT
WT
RTT
MeCP2-
-75 KDa
Actin-
-42 KDa

Results

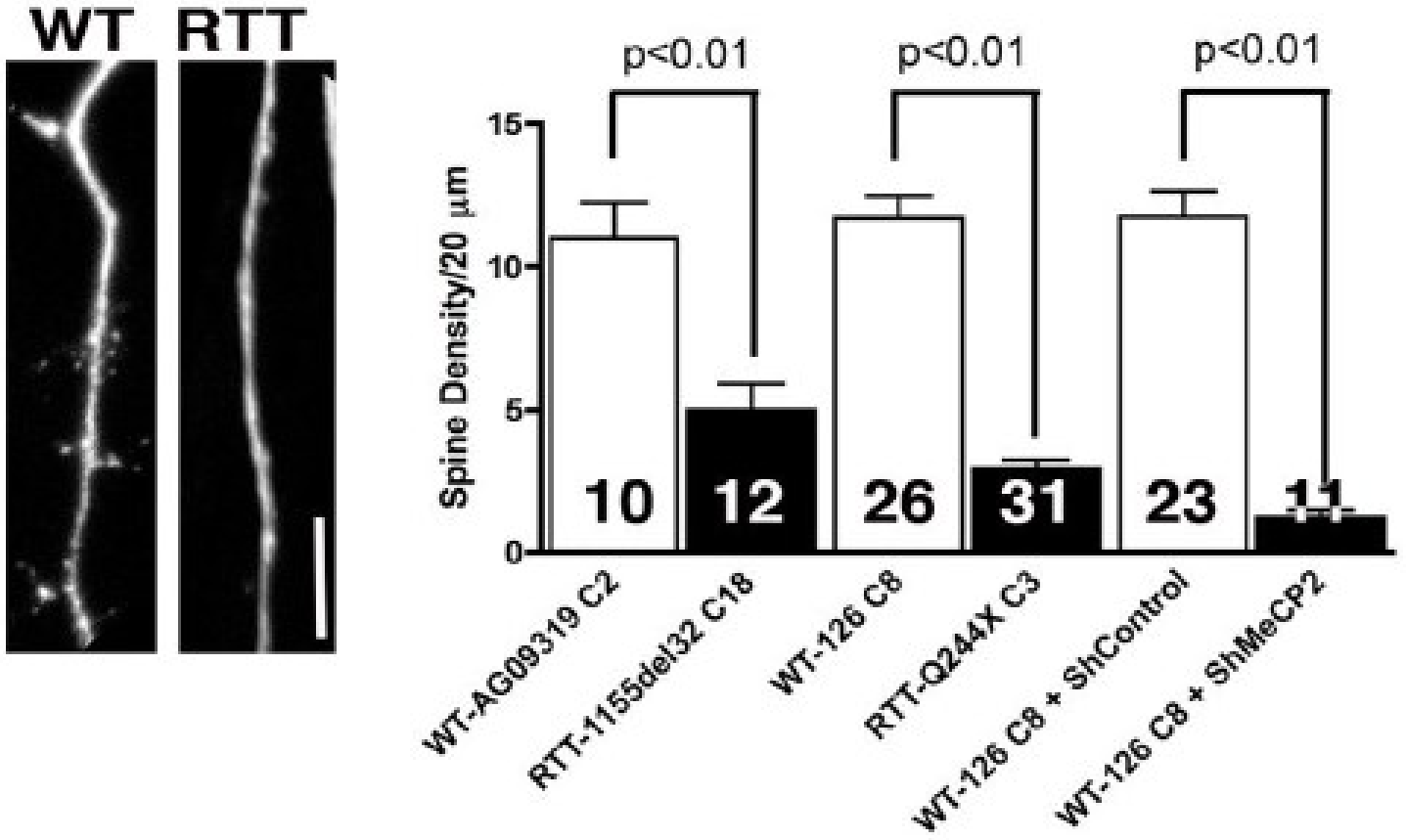
UNIVERSITÀ
DEGLI STUDI DI TRIESTE



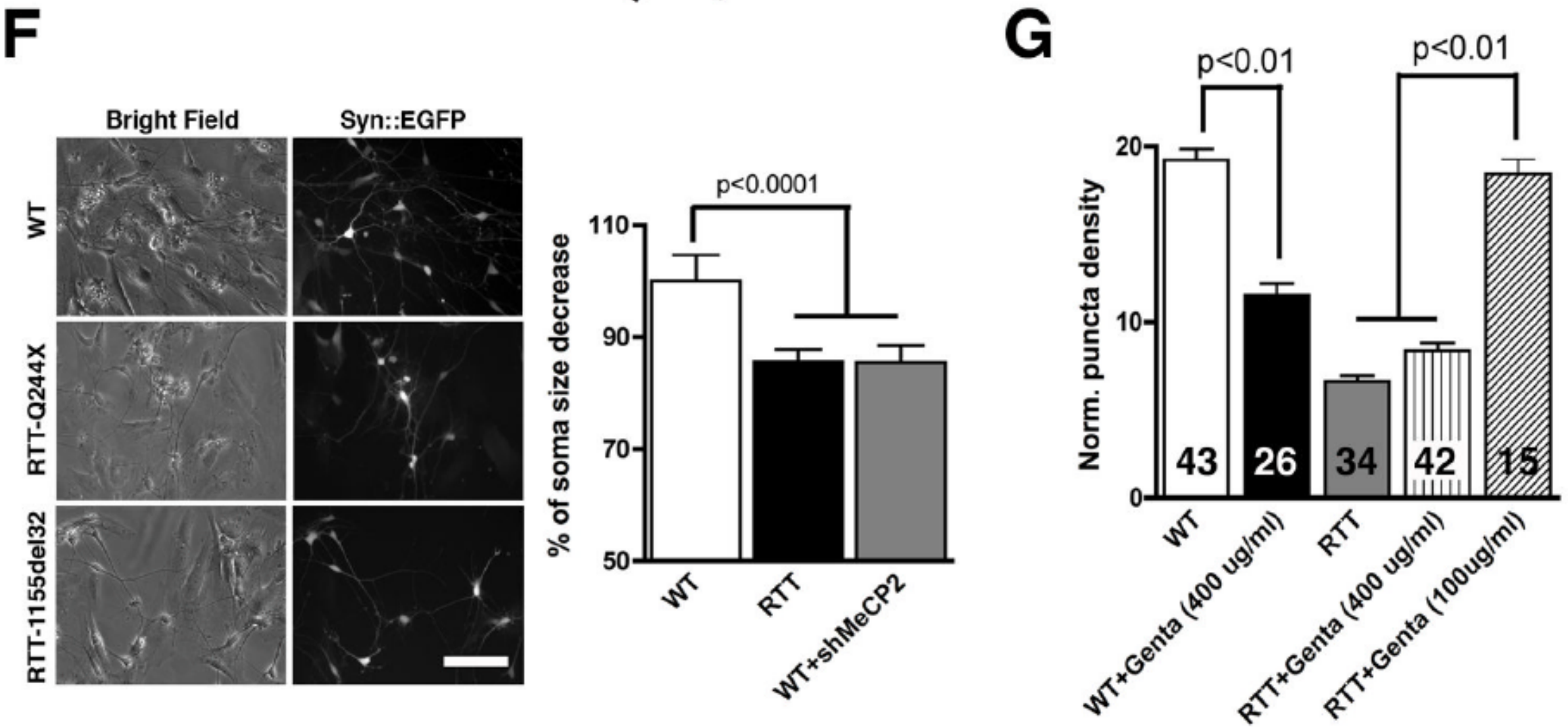
Results

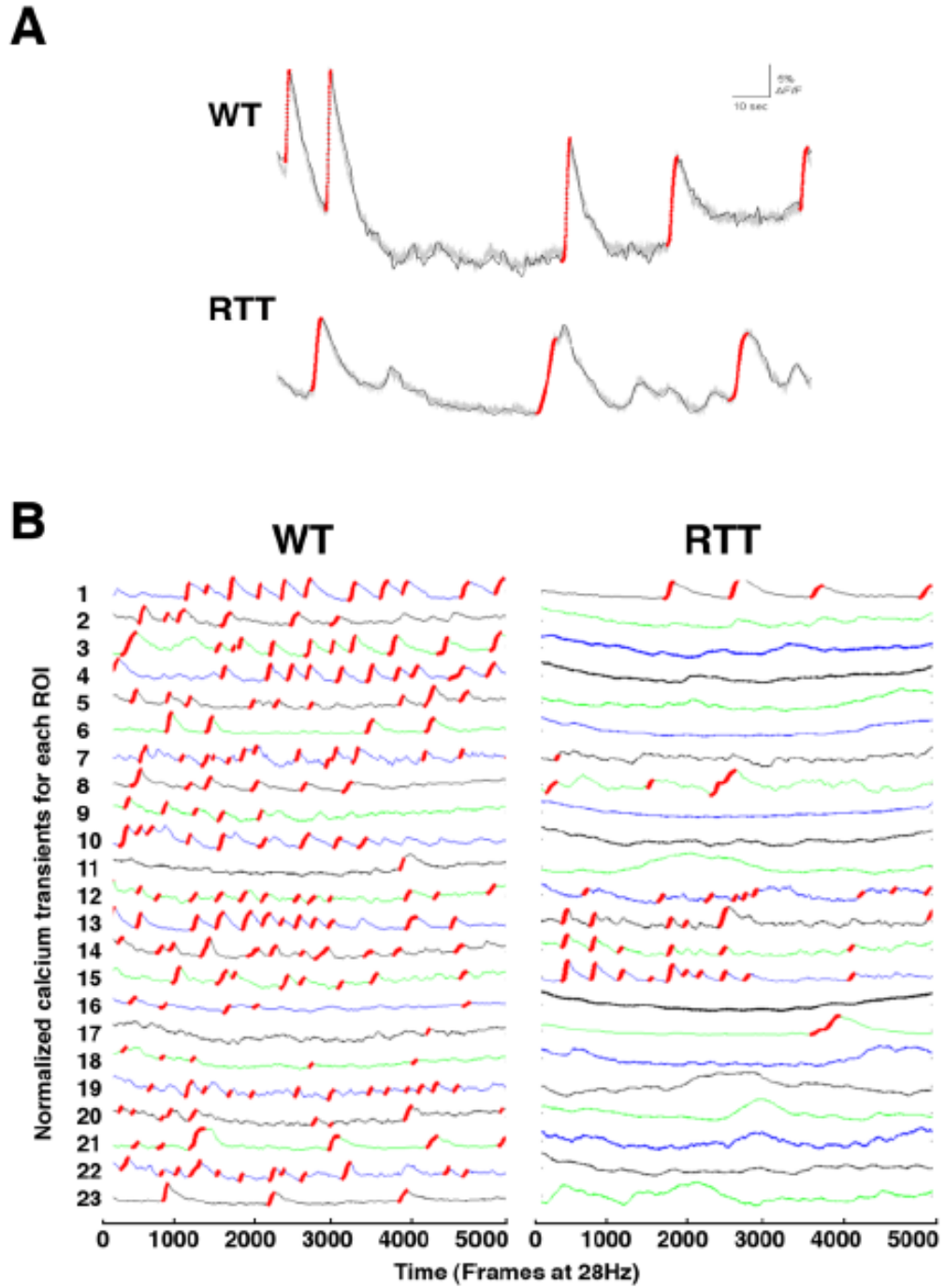
UNIVERSITÀ
DEGLI STUDI DI TRIESTE

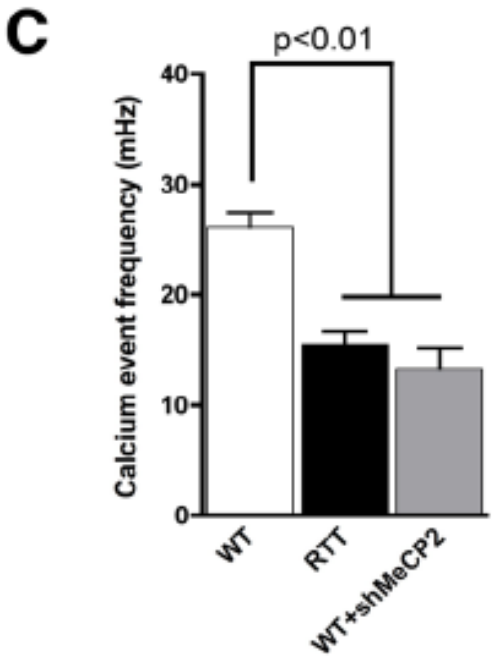
E

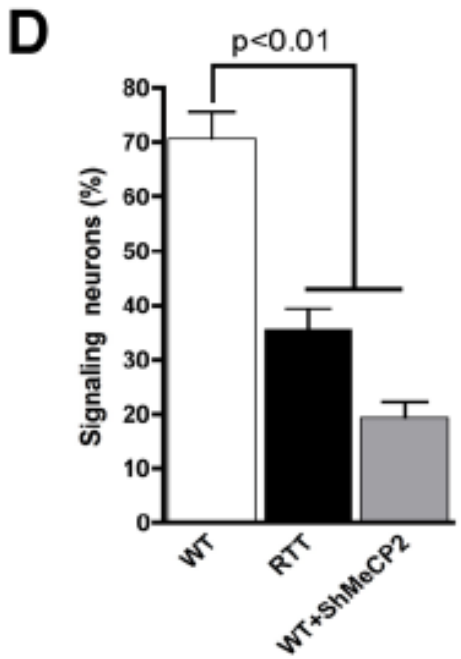


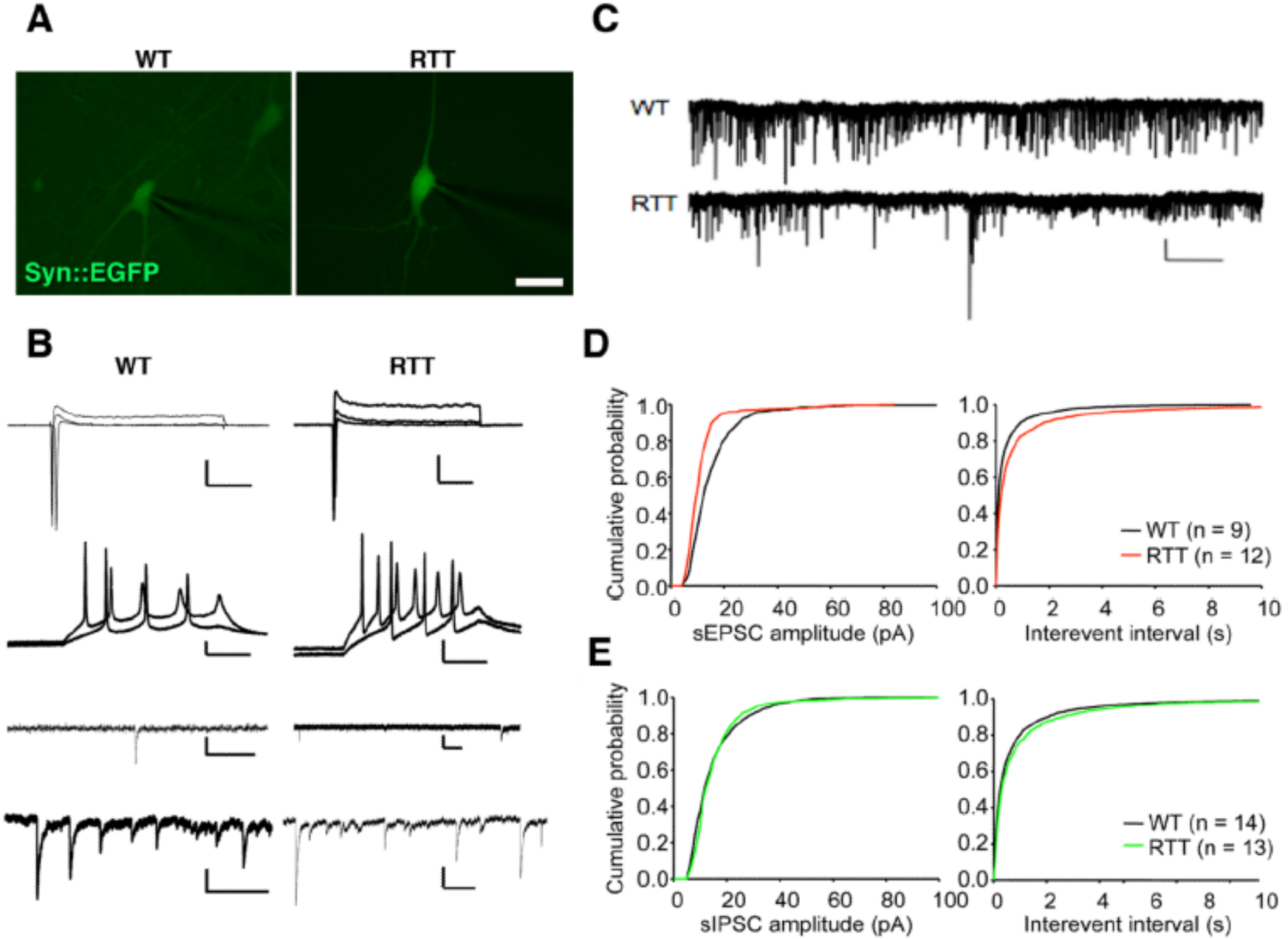
Results


F
Bright Field
Syn::EGFP
WT
RTT-Q244X
RTT-1155del32
p<0.0001
% of soma size decrease
110
90
70
50
WT
RTT
WT+shMeCP2
G
p<0.01
p<0.01
Norm. puncta density
20
10
0
43
26
34
42
15
WT
WT+Genta (400 ug/ml)
RTT
RTT+Genta (400 ug/ml)
RTT+Genta (100ug/ml)


A
WT
RTT
B
WT
RTT
Normalized calcium transients for each ROI
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
0
1000
2000
3000
4000
5000
Time (Frames at 28Hz)


C
p<0.01
Calcium event frequency (mHz)
0
10
20
30
40
WT
RTT
WT+shMeCP2


D
p<0.01
Signaling neurons (%)
0
10
20
30
40
50
60
70
80
WT
RTT
WT+ShMeCP2


A
WT
RTT
Syn::EGFP
B
WT
RTT
C
WT
RTT
D
Cumulative probability
sEPSC amplitude (pA)
Interevent interval (s)
WT (n = 9)
RTT (n = 12)
E
Cumulative probability
sIPSC amplitude (pA)
Interevent interval (s)
WT (n = 14)
RTT (n = 13)

PNAS

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

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

[a]University of Connecticut Stem Cell Institute and Departments of [b]Genetics and Developmental Biology and [c]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

Results

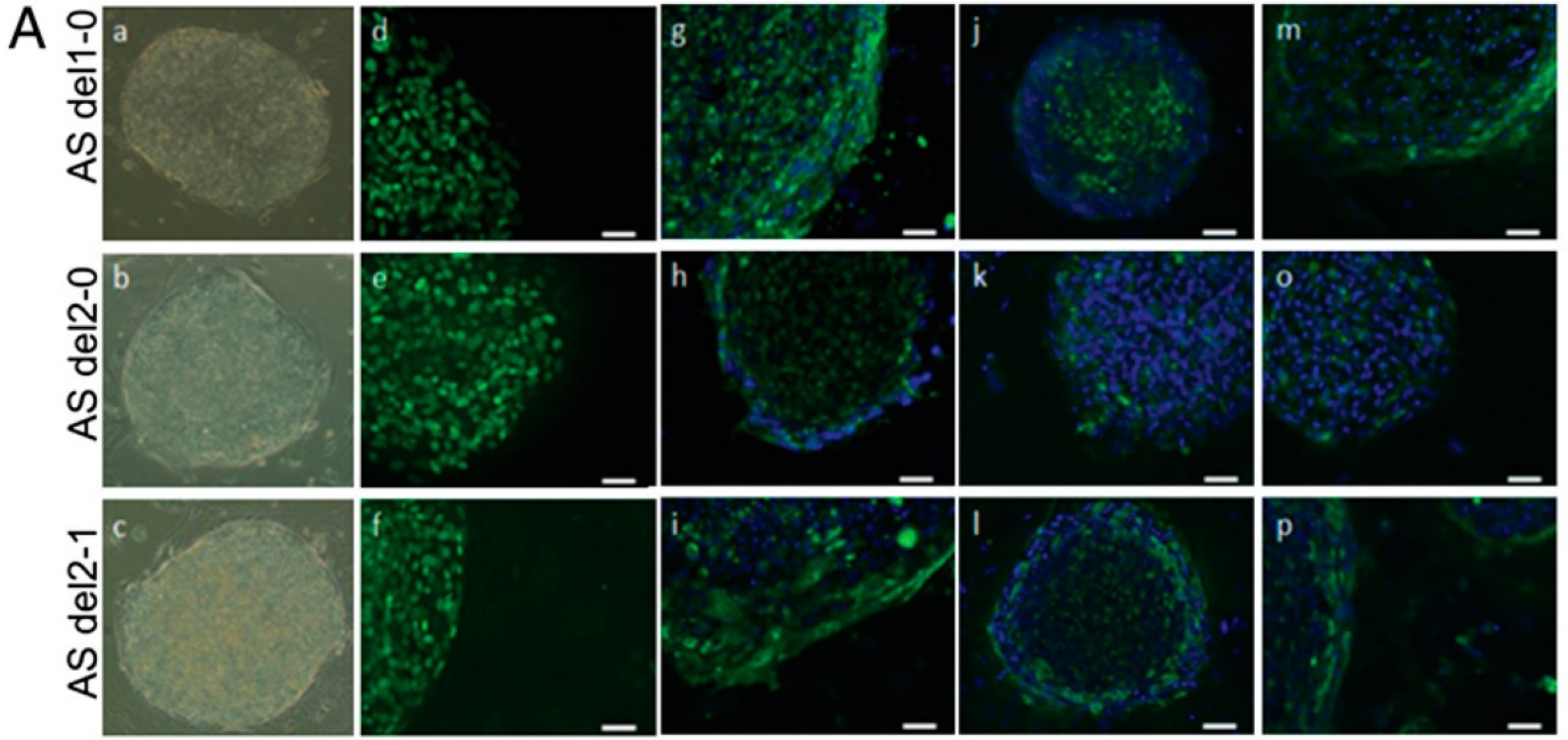
Background

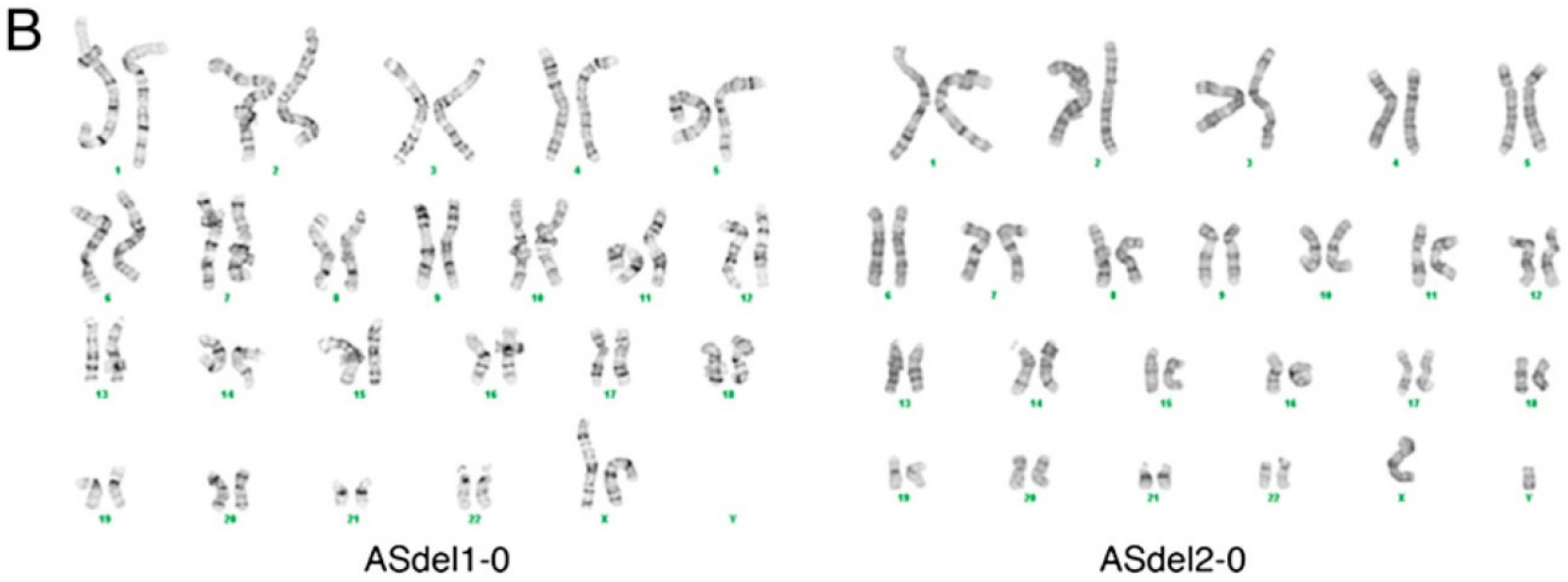
UNIVERSITÀ
DEGLI STUDI DI TRIESTE

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

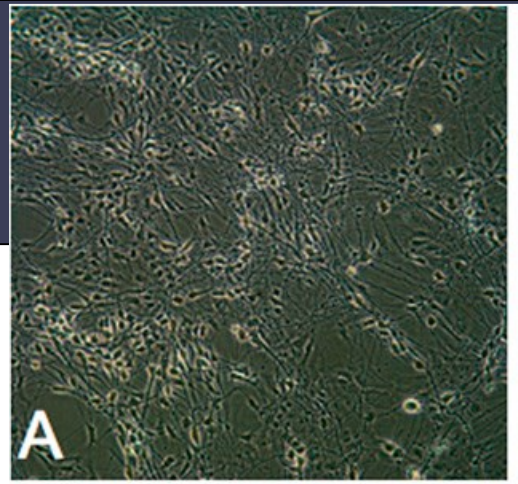
Biological question

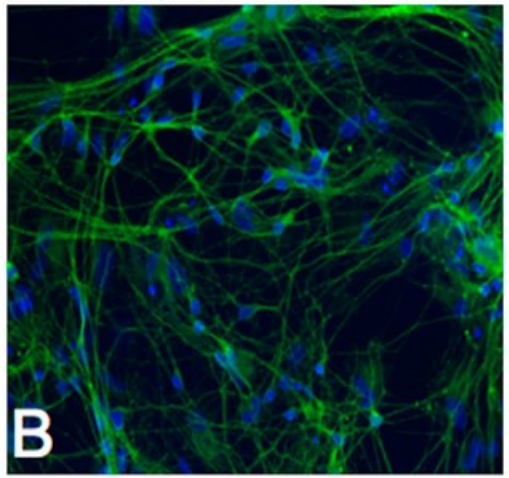
Can we generate a model of AS in vitro?

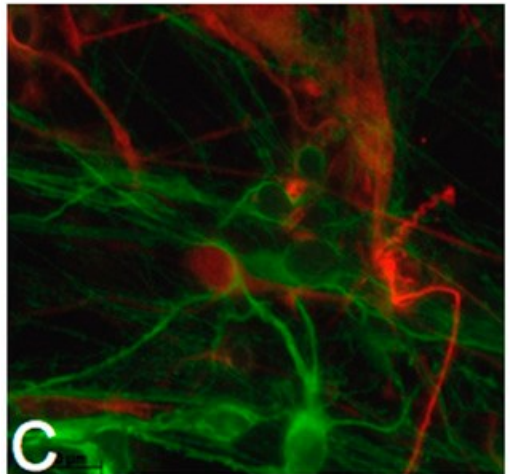


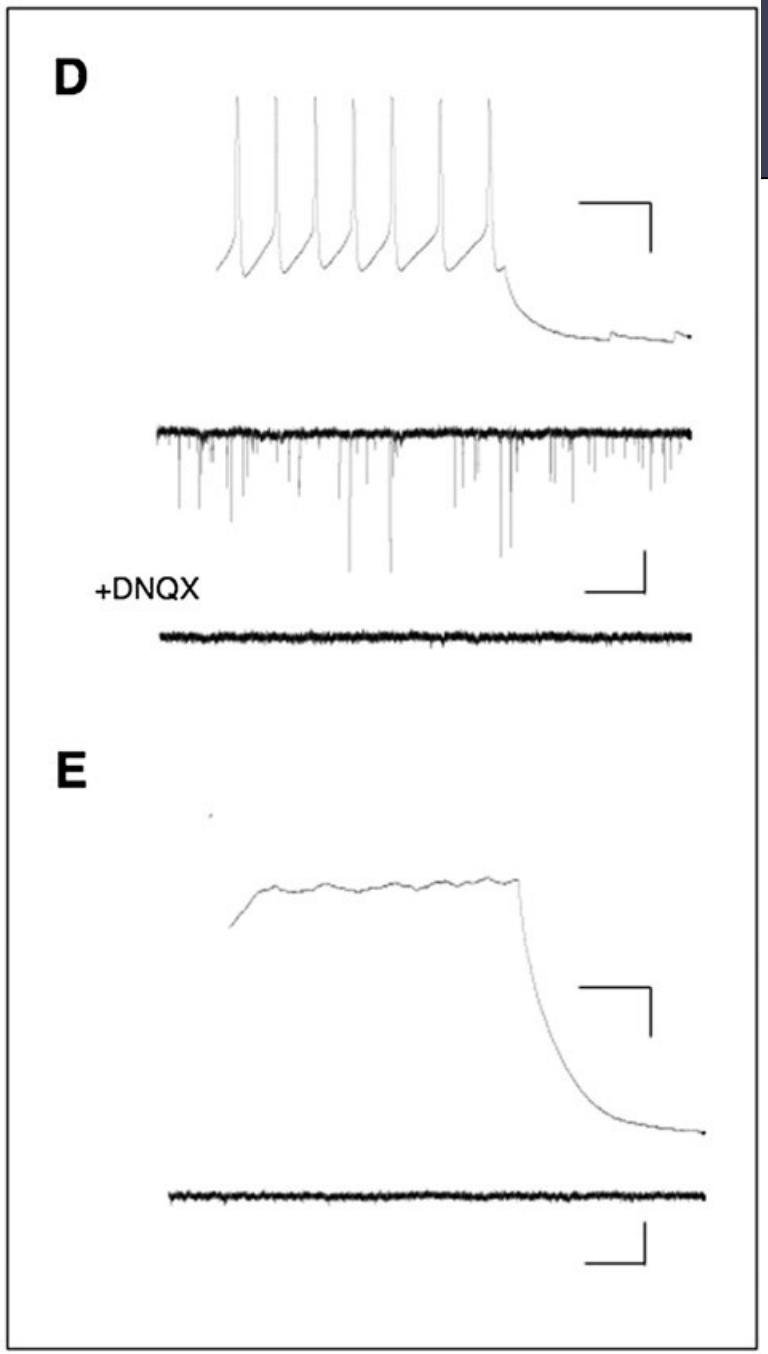


Immunocytochemistry for pluripotency markers on representative iPSC lines shows expression of NANOG (d–f), SSEA4 (g–i), TRA1-60 (j–l), and TRA1-81 (m–p).



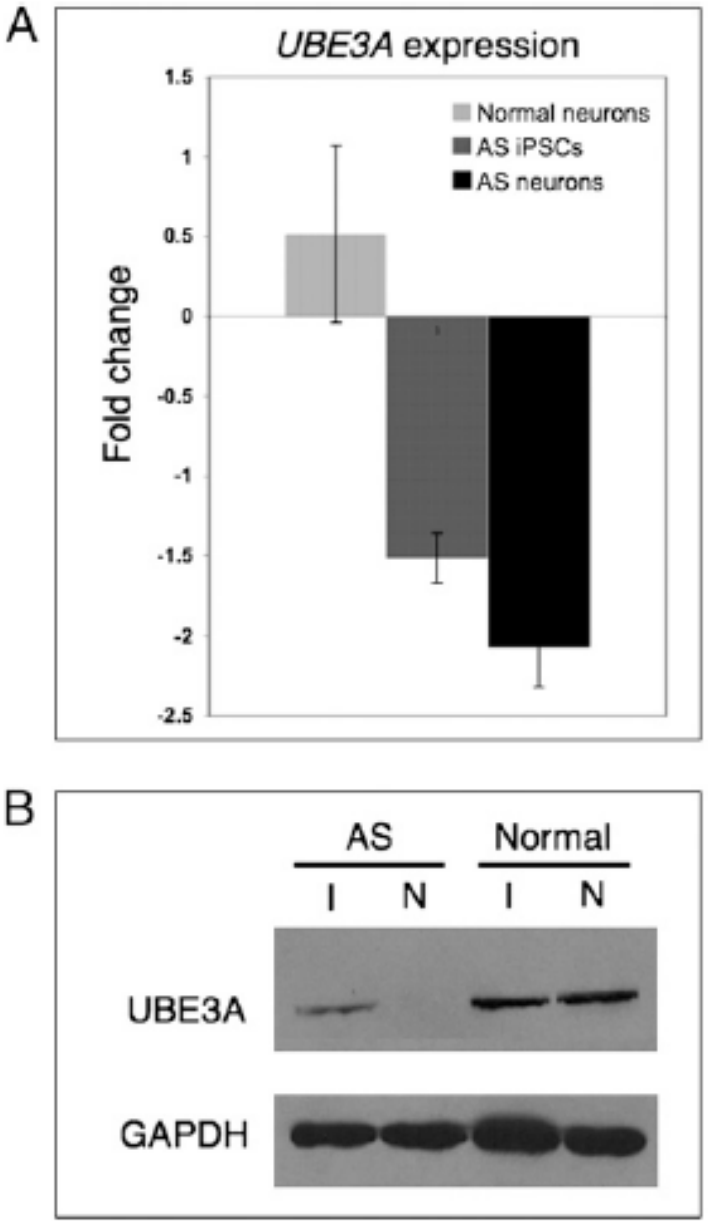






Functional neurons can be generated from AS iPSCs.

(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).

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

EBioMedicine 50 (2019) 274–289

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.elsevier.com/locate/ebiom

Research paper

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

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

Background

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

- 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.

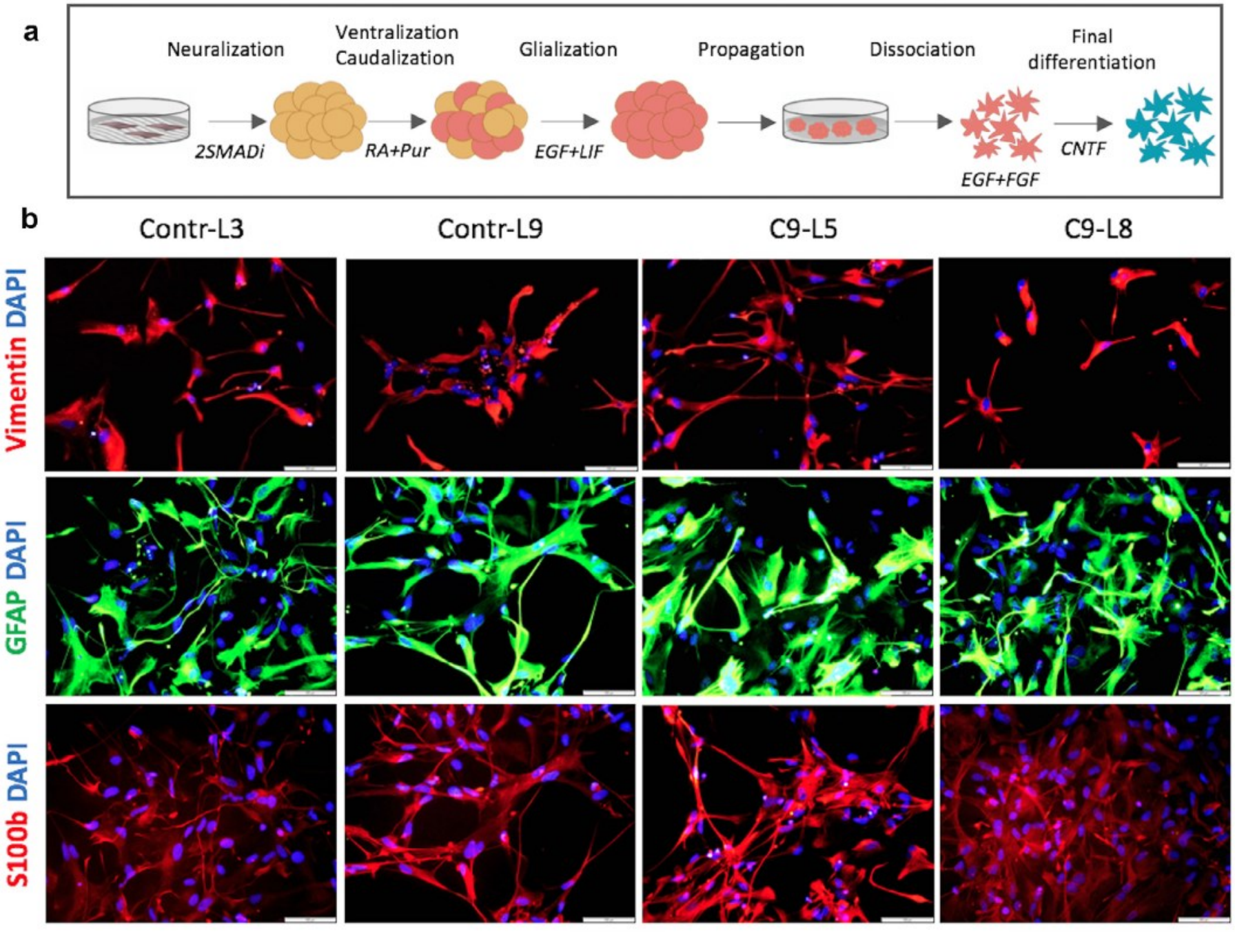
Biological question

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

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

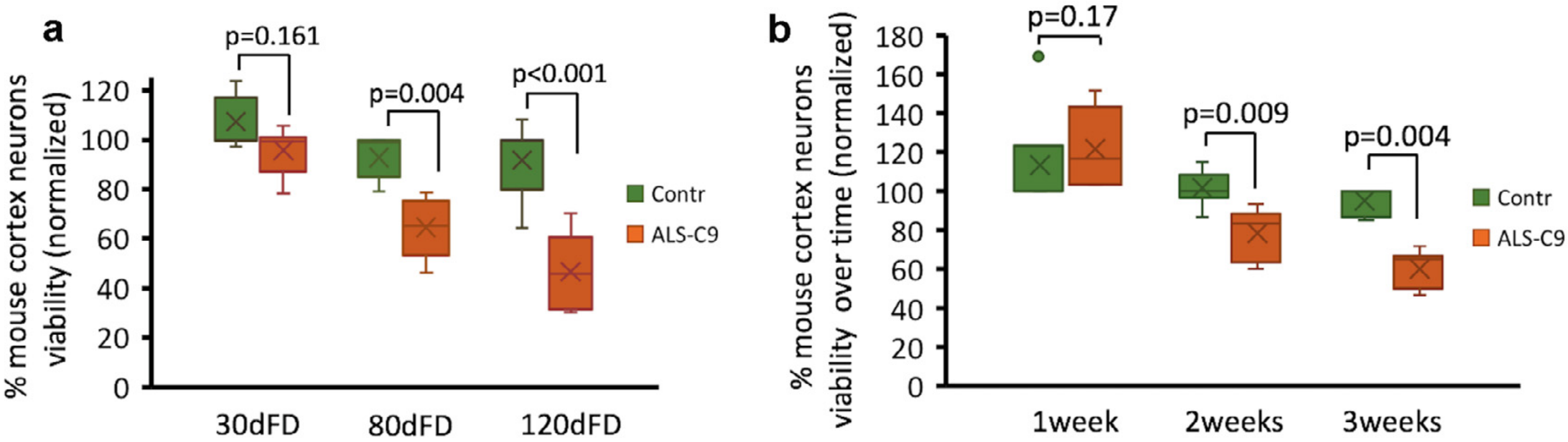


Generation of functional astrocytes from C9 mutated and control iPSClines.

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

Results

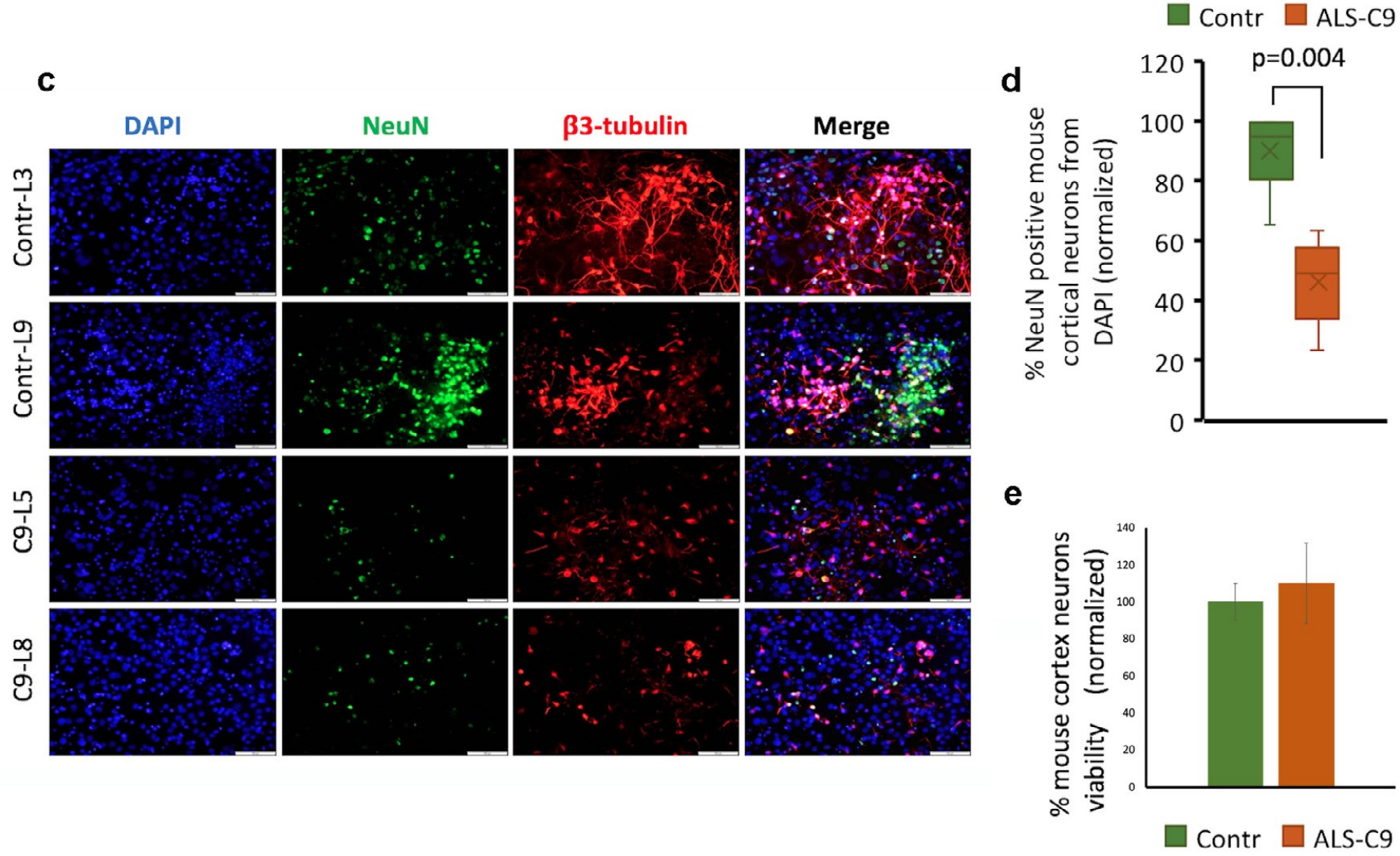
UNIVERSITÀ
DEGLI STUDI DI TRIESTE



NOTE : Comparison with mouse model already studied

Results

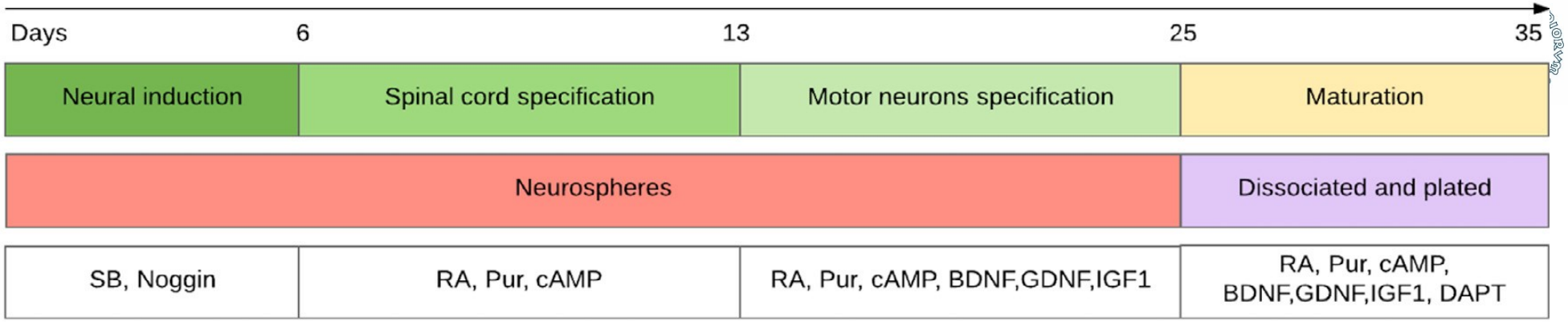
UNIVERSITÀ
DEGLI STUDI DI TRIESTE

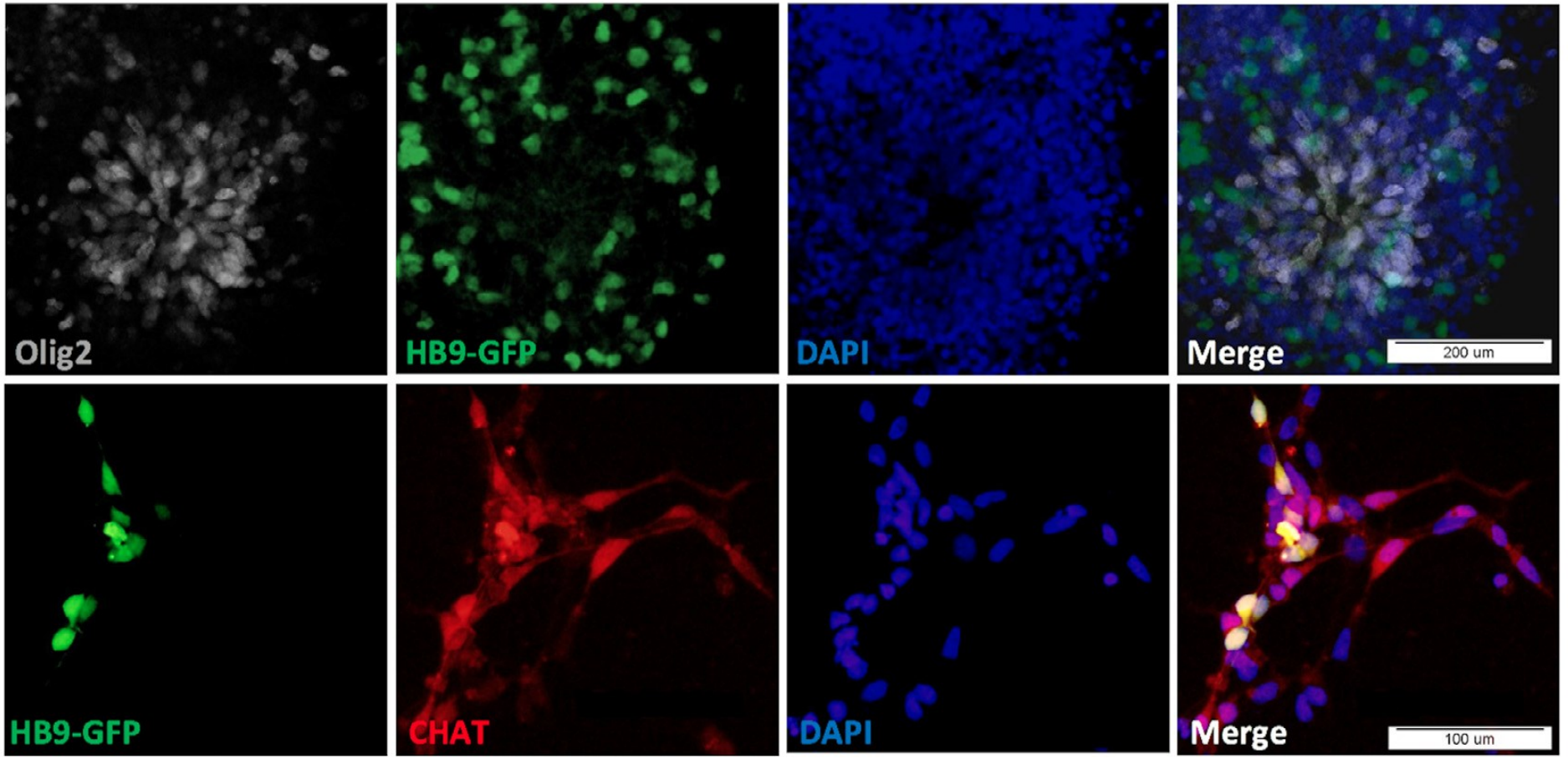


NOTE : Comparison with mouse model already studied

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

Results

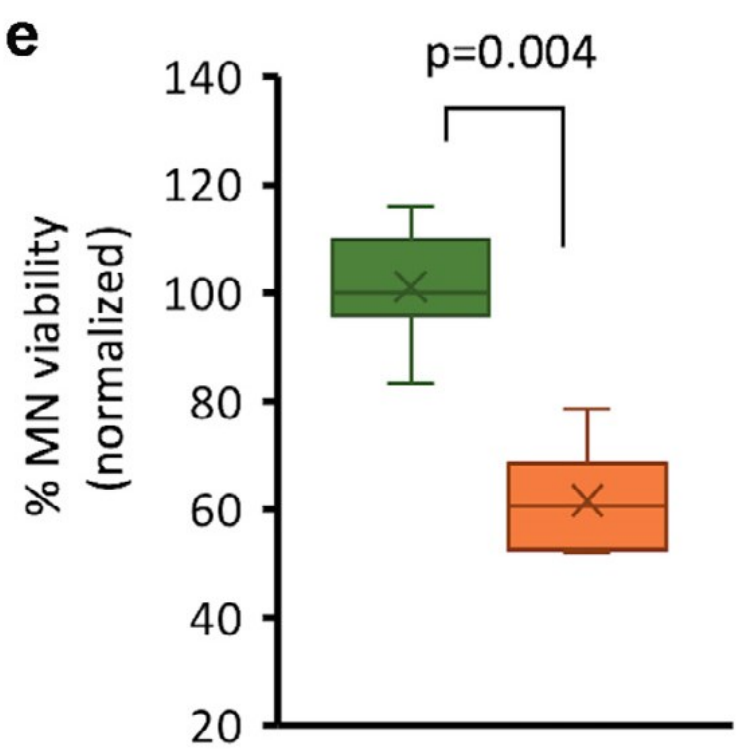


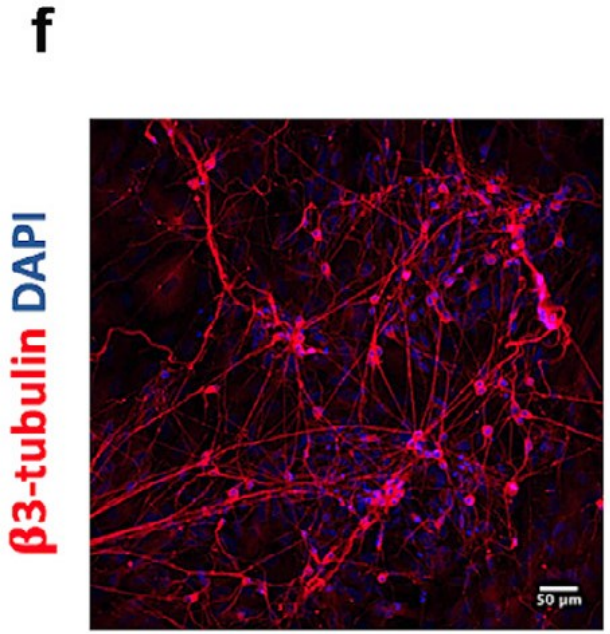


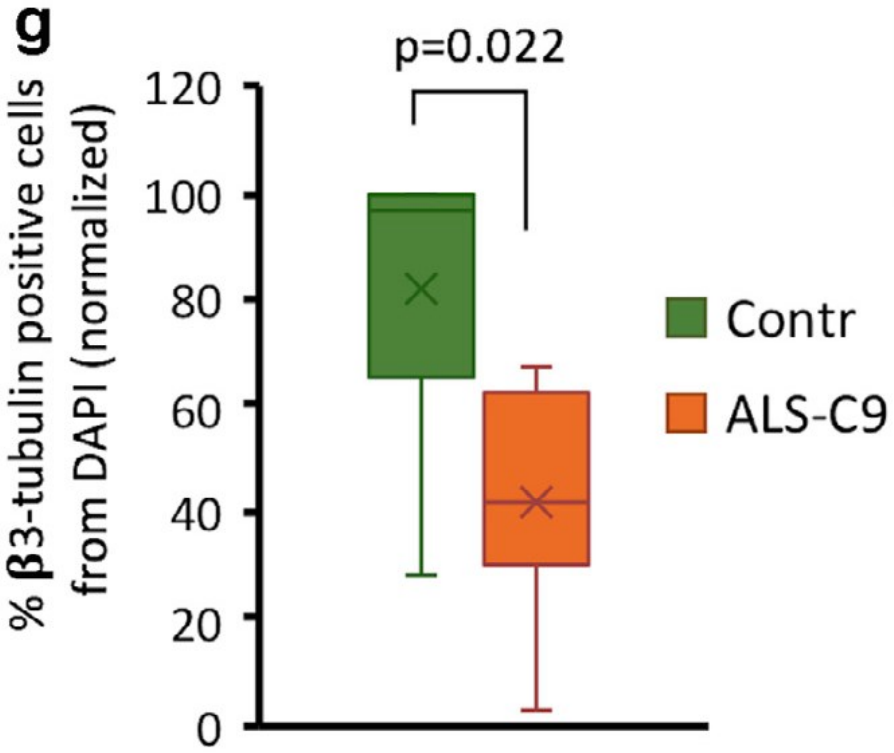
Generation of functional motorneurons.

Results

UNIVERSITÀ
DEGLI STUDI DI TRIESTE



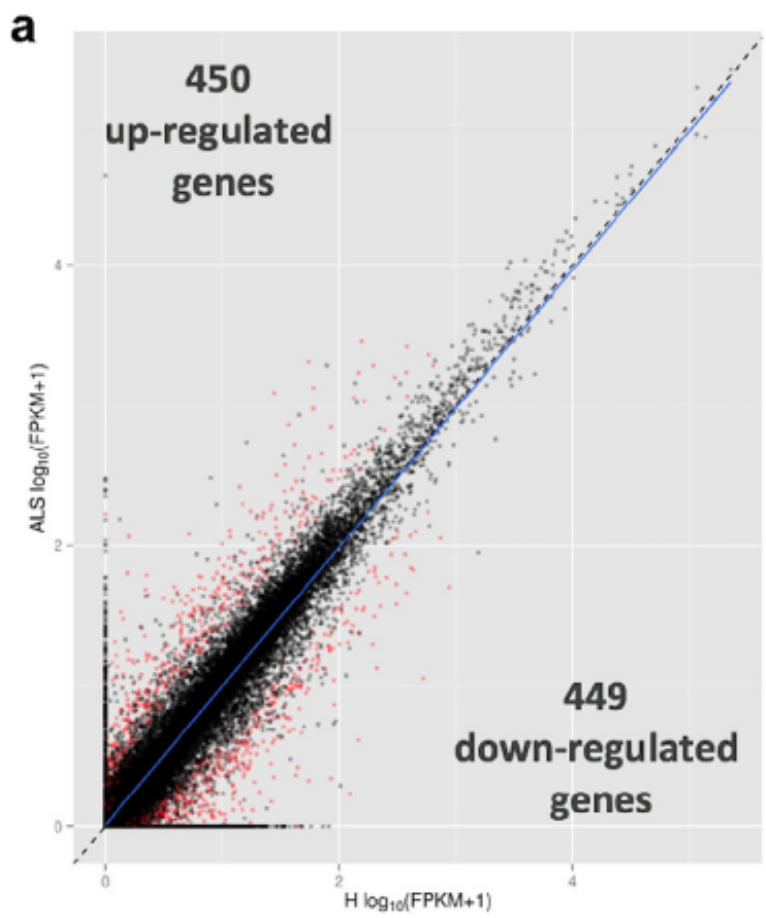


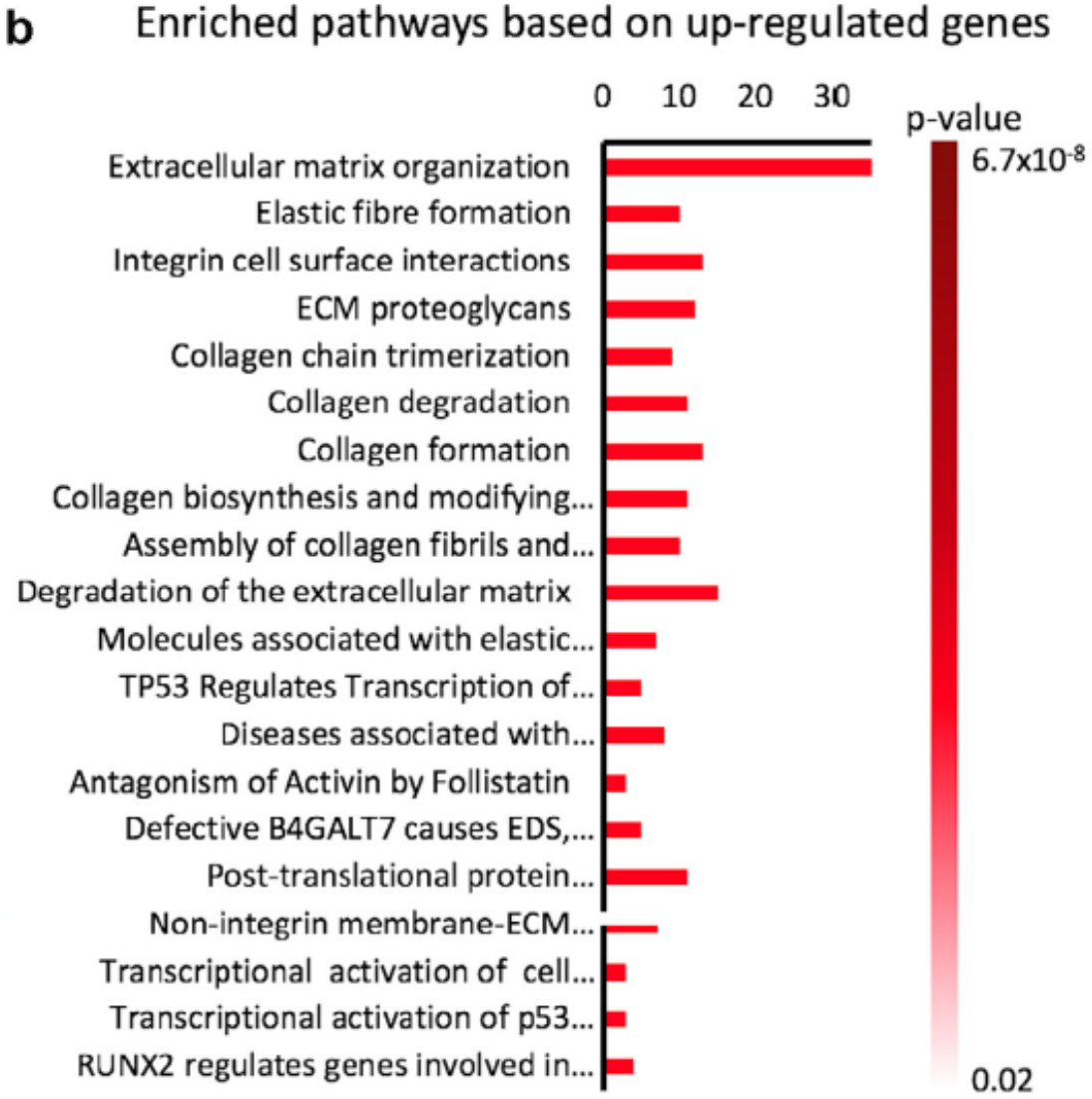


Toxicity of media conditioned by C9-mutated astrocytes to human iPSC-derived motorneurons.

Results

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

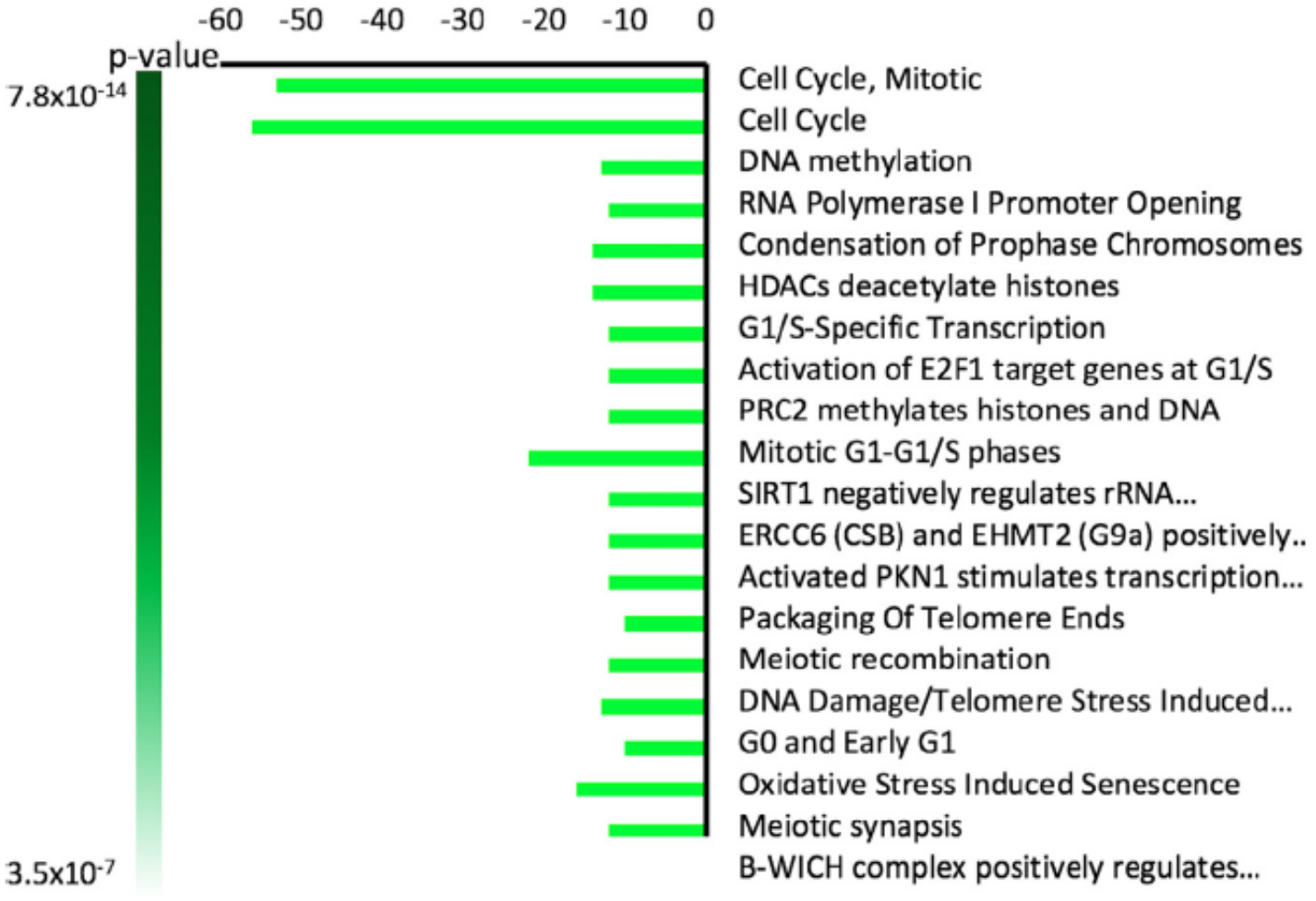




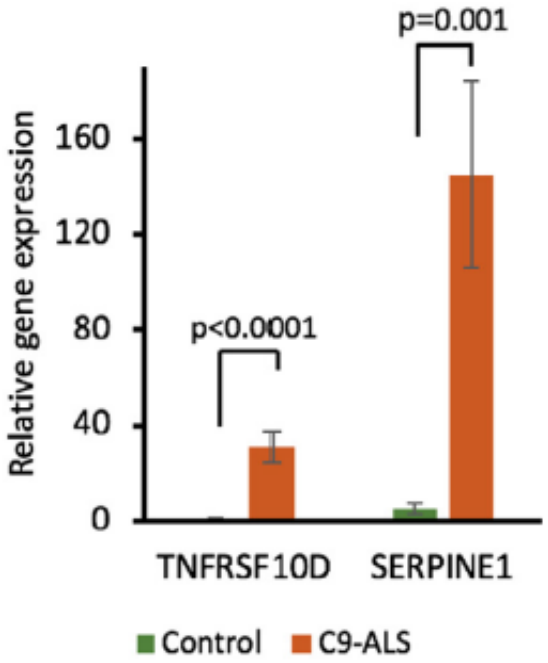
Results

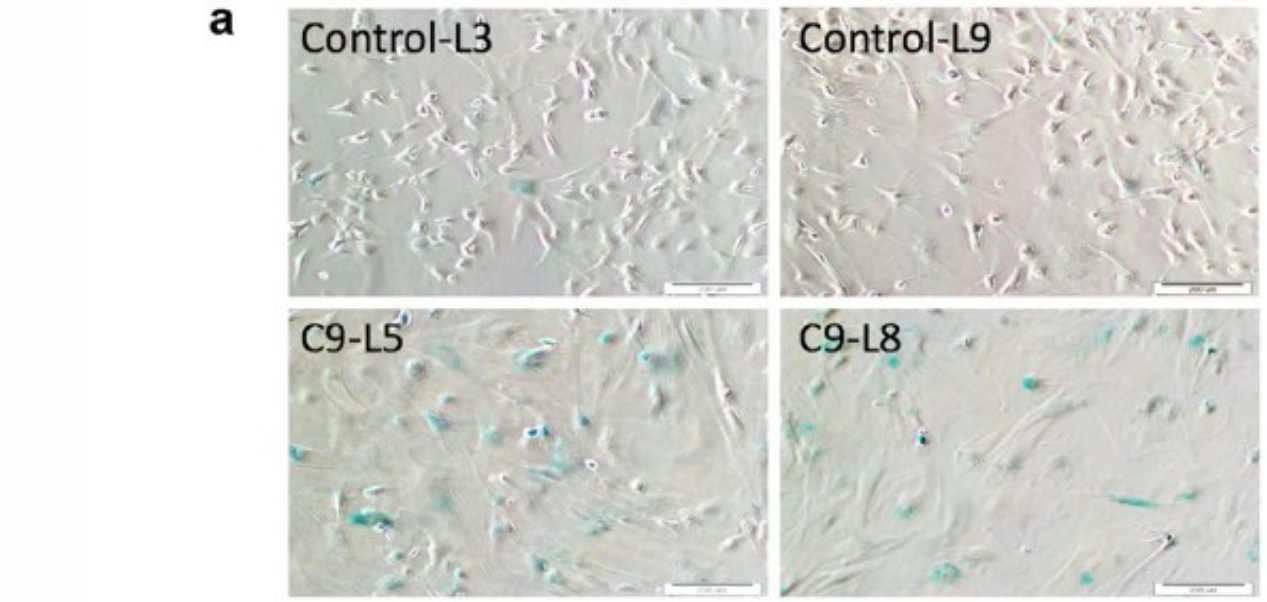
UNIVERSITÀ
DEGLI STUDI DI TRIESTE

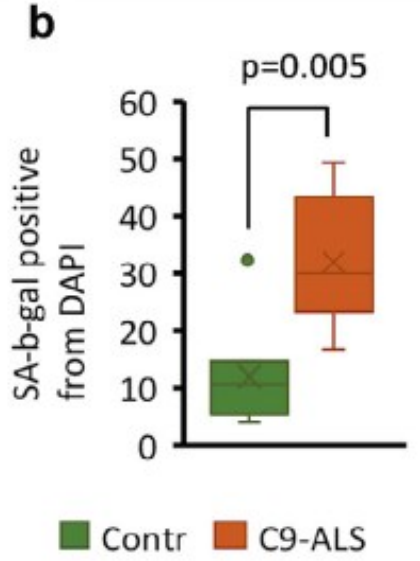
c Enriched pathways based on down-regulated genes

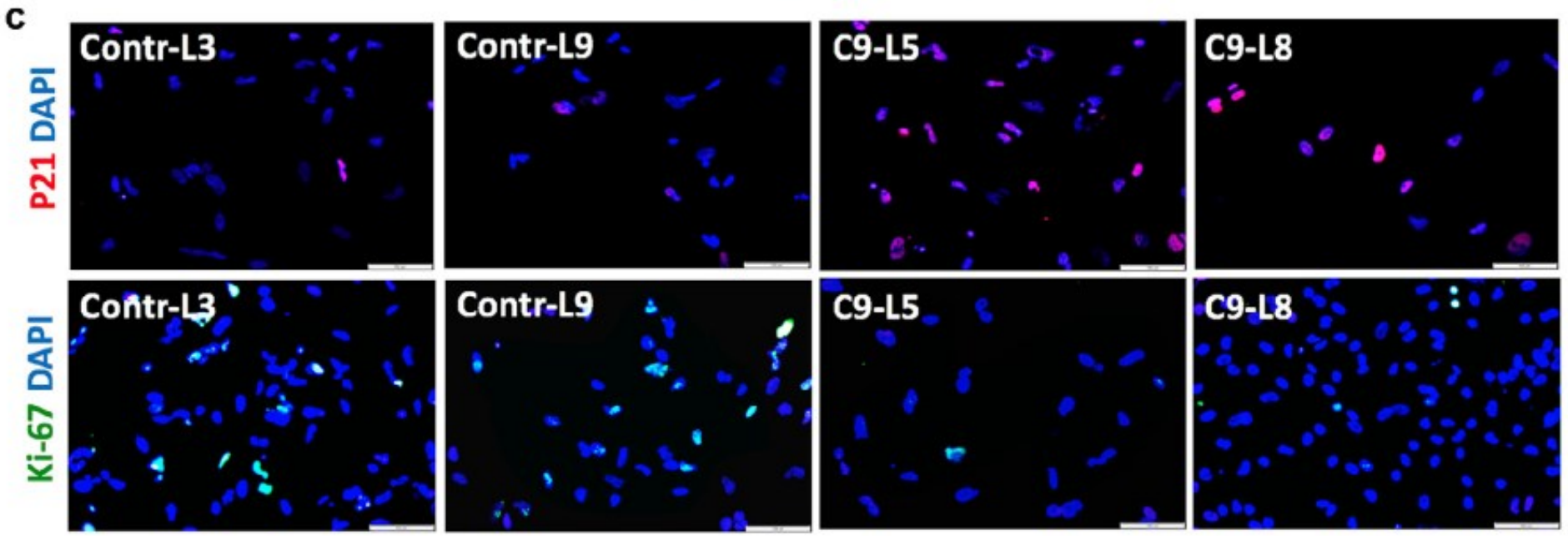


d





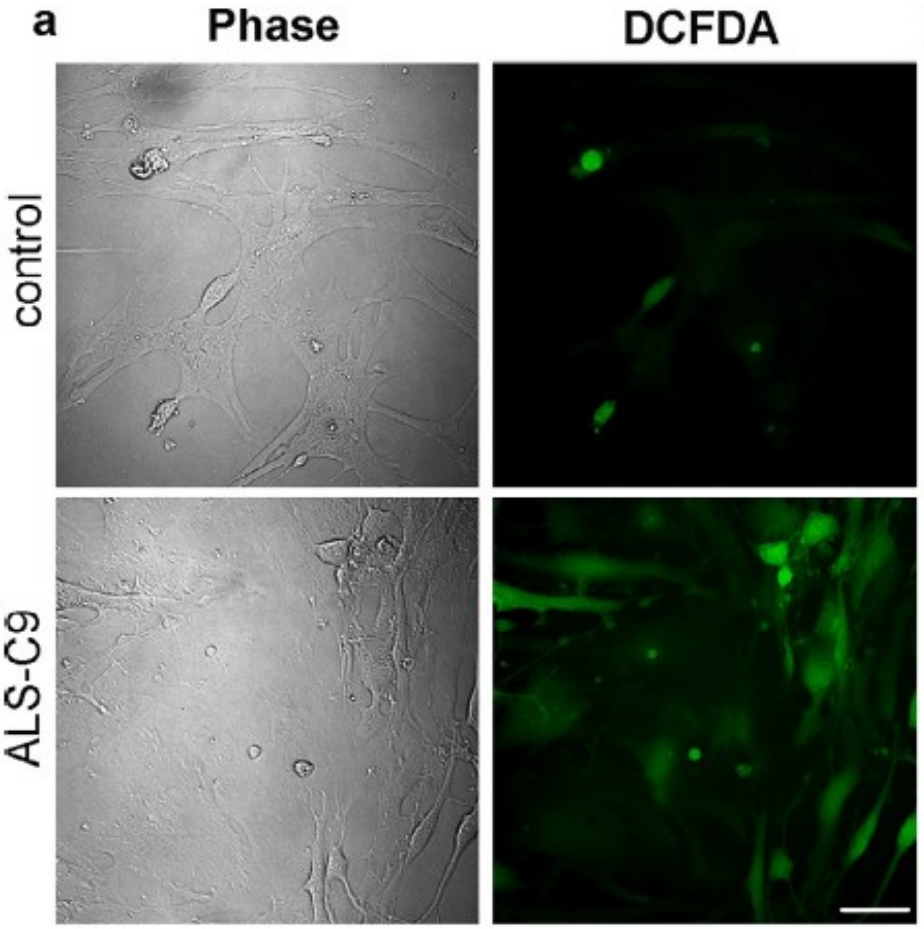


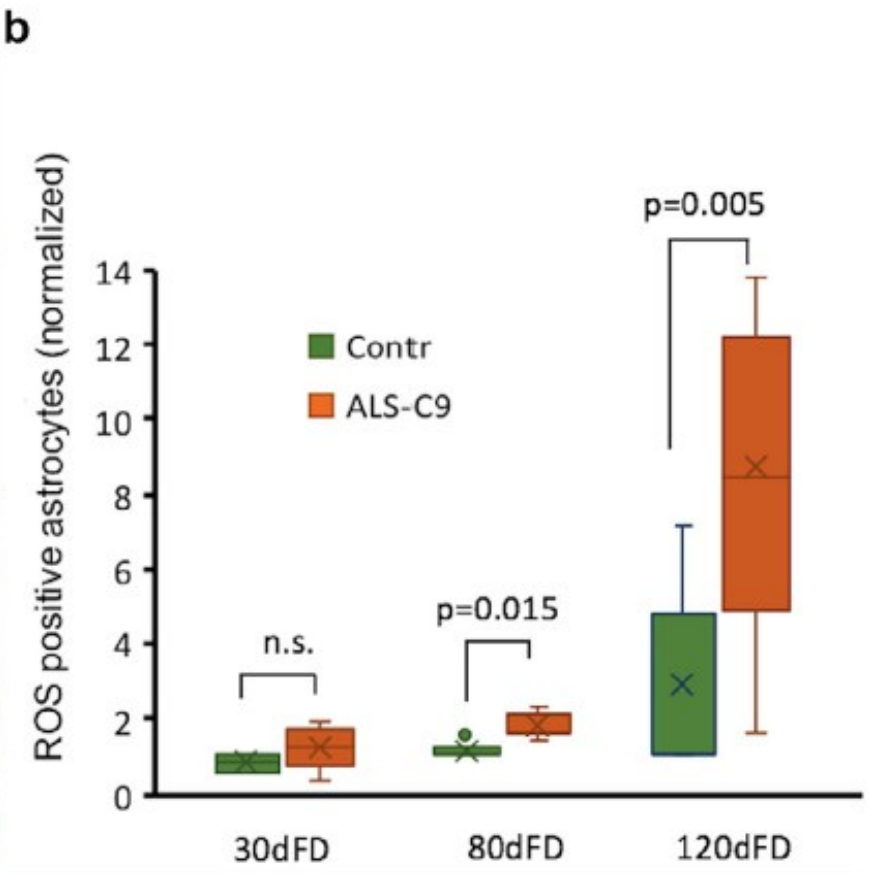


C9 mutated astrocytes acquire senescence phenotype at higher rates than non-mutated astrocytes.

Results

UNIVERSITÀ
DEGLI STUDI DI TRIESTE





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

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

UNIVERSITÀ
DEGLI STUDI DI TRIESTE