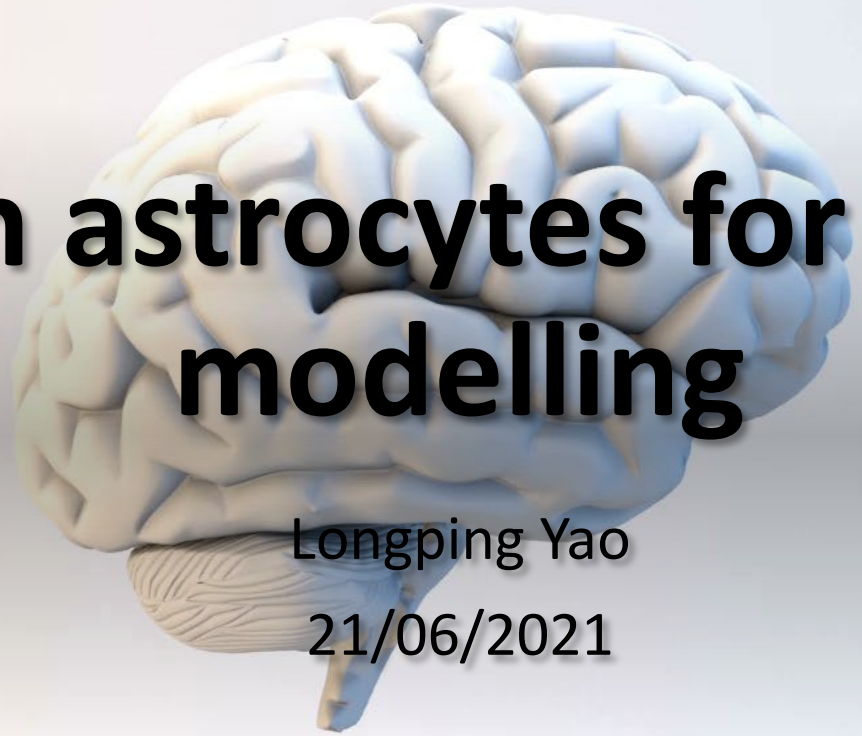
Human astrocytes for disease modelling



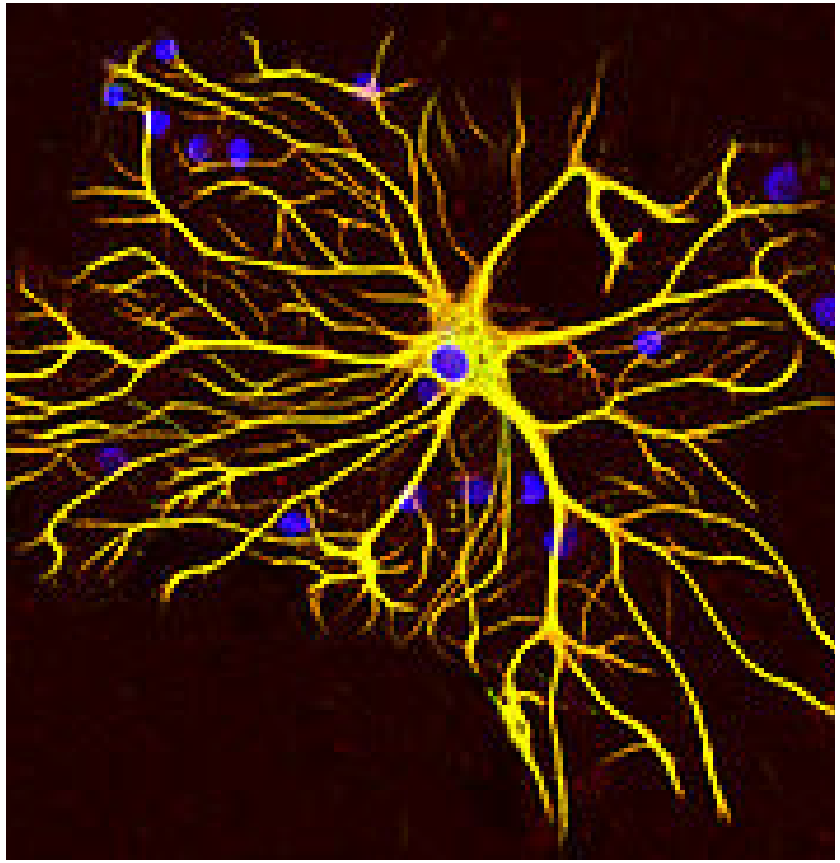
Longping Yao

21/06/2021

Background

- Astrocytes are a sub-type of glial cells in the central nervous system. They are also known as *astrocytic glial cells.* Star-shaped.

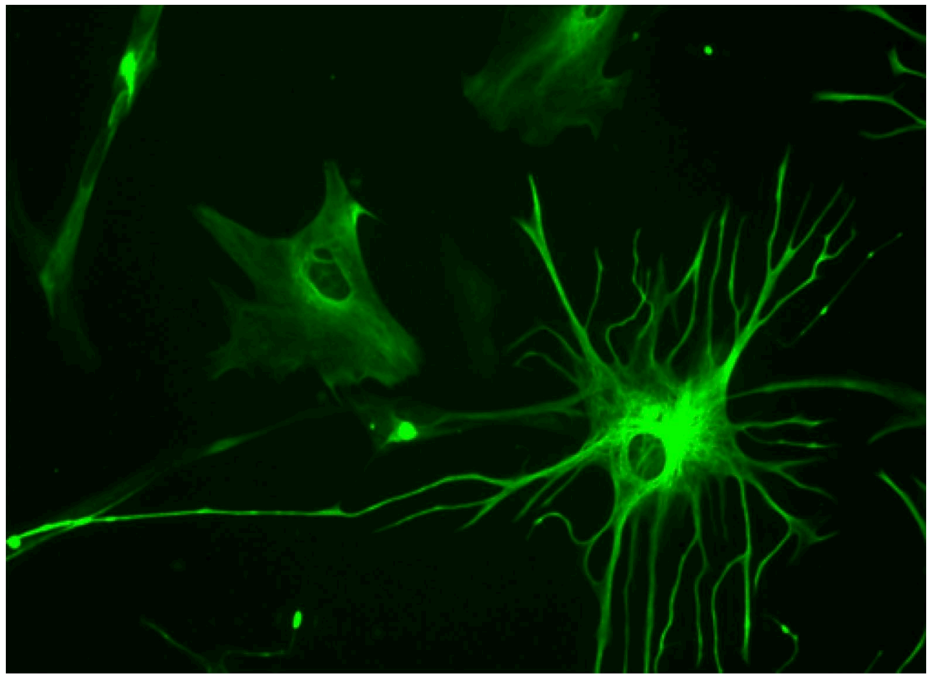
- Astrocytes in humans are more than twenty times larger than in rodent brains, and make contact with more than ten times the number of synapses.



Background

- Brain diseases are characterized by the active inflammatory state of the astrocytes, which is usually described as up-regulation of glial fibrillary acidic protein (GFAP), considered to be the primary indicator of astrogliosis.

- The proportion of astrocytes in the brain is not well defined; depending on the counting technique used, studies have found that the astrocyte proportion varies by region and ranges from 20% to 40% of all glia.



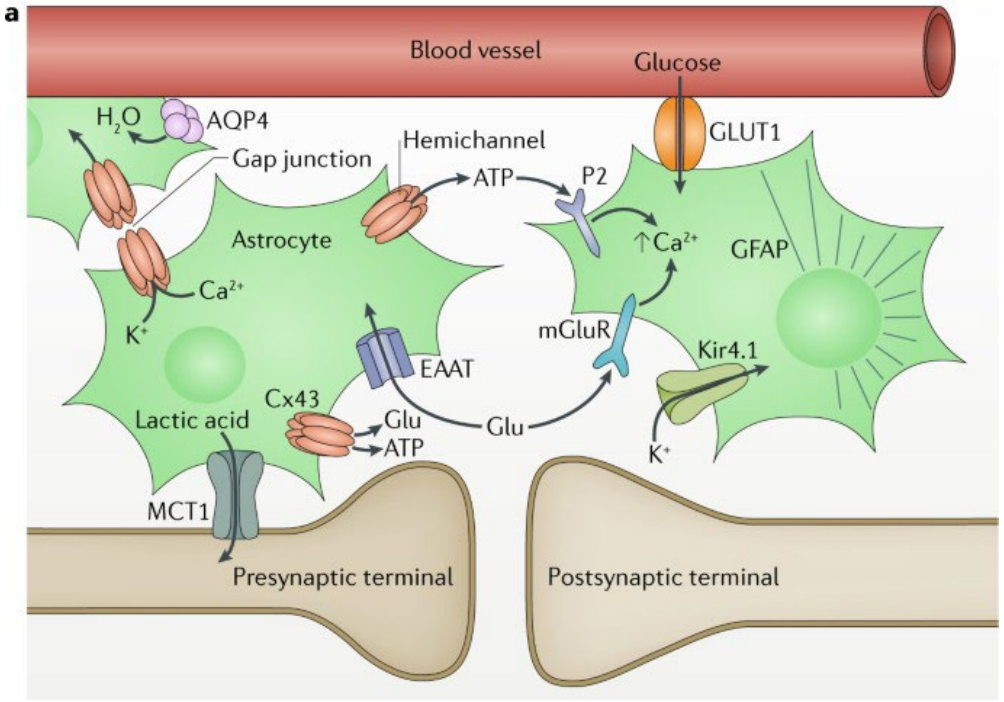
Verkhratsky A, etc. 2013

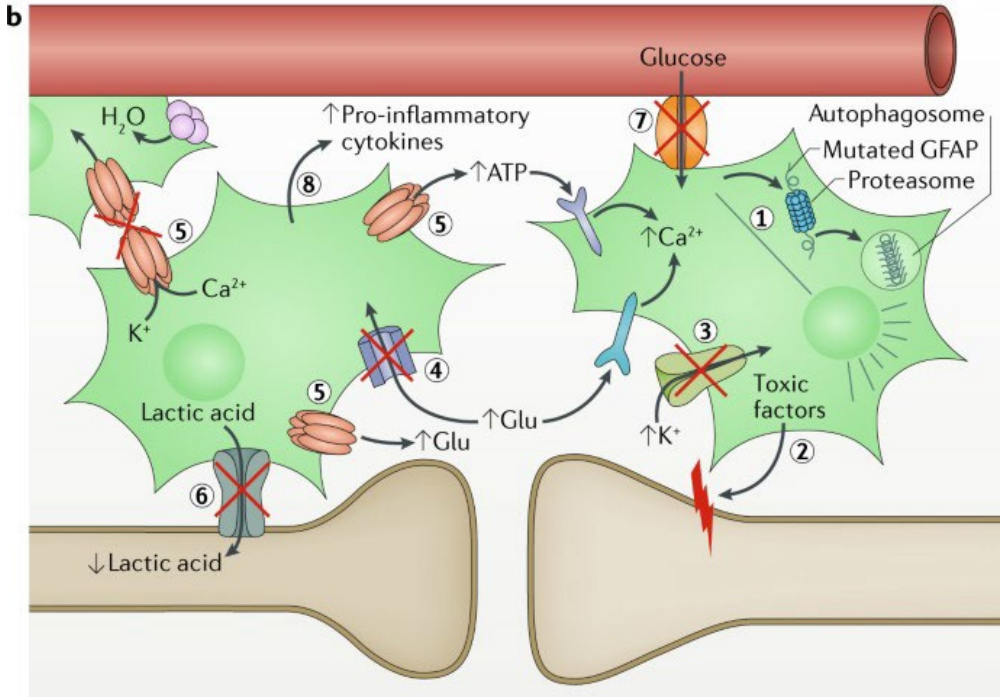
Function

Astrocytes have critical roles in preserving neurological function, from developmental regulation of synapse formation, elimination and maintenance.

- They are involved in the physical structuring of the brain. They are the most abundant glial cells in the brain that are closely associated with neuronal synapses.
- Metabolic support: They provide neurons with nutrients such as lactate.
- Regulation of synapse formation, elimination and maintenance to synaptic preservation in disease.
- Astrocytes express plasma membrane transporters such as glutamate transporters for several neurotransmitters, including glutamate, ATP, and GABA.
- Astrocytes contribute to the blood–brain barrier to maintain the CNS as an immune-privileged site, a function that is critical in the design of drug therapies that are intended to affect the CNS

Function


a
Blood vessel
Glucose
H_2O
AQP4
Gap junction
Hemichannel
ATP
P2
GLUT1
Astrocyte
$\uparrow Ca^{2+}$
GFAP
Ca^{2+}
K^+
mGluR
EAAT
Kir4.1
Cx43
Glu
ATP
Glu
Lactic acid
K^+
MCT1
Presynaptic terminal
Postsynaptic terminal


b
Glucose
H_2O
↑Pro-inflammatory cytokines
Autophagosome
Mutated GFAP
Proteasome
↑ATP
$\uparrow Ca^{2+}$
Ca^{2+}
K^+
Lactic acid
↑Glu
↑Glu
↑K^+
Toxic factors
↓Lactic acid

Classification

- Type 1 (A1): Antigenically Ran2^{+}, GFAP^{+}, FGFR3^{+}, A2B5^{-}. Complement 3 (C3), CFB, and MX1S are the most characteristic and significantly upregulated genes in A1 astrocytes. A1 astrocytes are induced by activated microglia and gain a neurotoxic function, resulting in neuron killing.

- Type 2: Antigenically A2B5^{+}, GFAP^{+}, FGFR3^{-}, Ran 2^{-}. A2 astrocytes upregulate many neurotrophic factors and strongly promote neuronal survival and tissue repair.

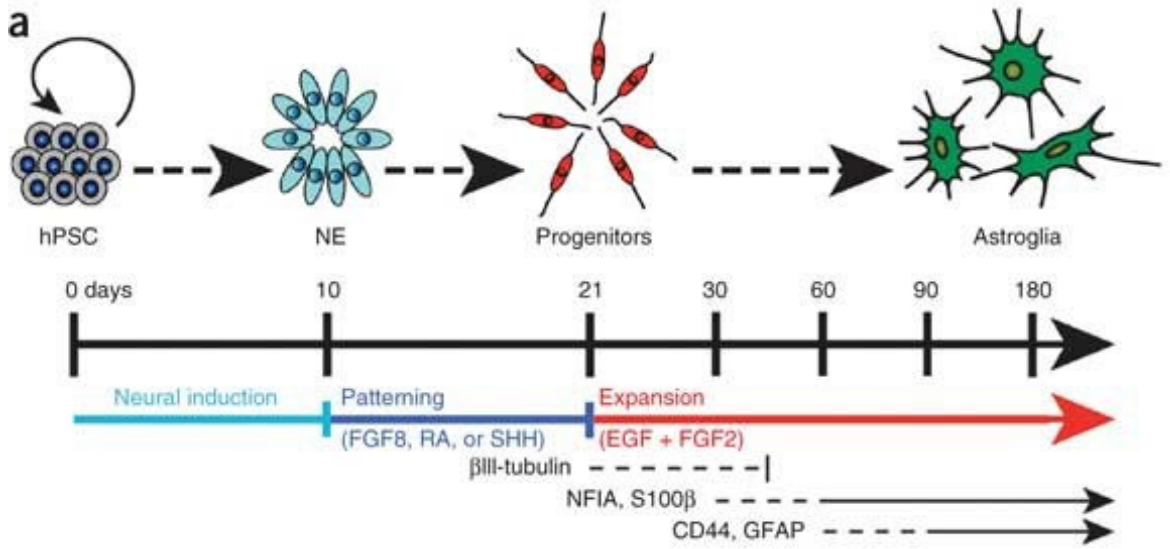
Liddelow et al., 2017

Huamn astrocytes in disease modeling

Human in vitro astrocyte models

Protocol 1

Immature astrocytes specifiable to the forebrain or spinal cord with FGF8 or RA, respectively, from hiPSCs using a 180-day protocol. Progenitors expanded with EGF + FGF2 for >150 days and then terminally differentiated with CNTF for 7 days. Astrocytes elicited electrophysiological responses to glutamate, propagated calcium waves upon mechanical stimulation, performed glutamate uptake and promoted synapse formation of co-cultured neurons.



FGF8, anterior patterning morphogen fibroblast growth factor 8

RA, retinoic acid

Krencik et al. (2011)

Human in vitro astrocyte models

Protocol 2

Human ES cell derived astrocytes obtained through a combination of BMP-mediated Smad and LIF-mediated JAK-STAT signalling. Neuroprotective properties of astrocyte conditioned media after exposure of human ES cell derived neurons to oxidative stress through glutathione-dependent and independent mechanisms.

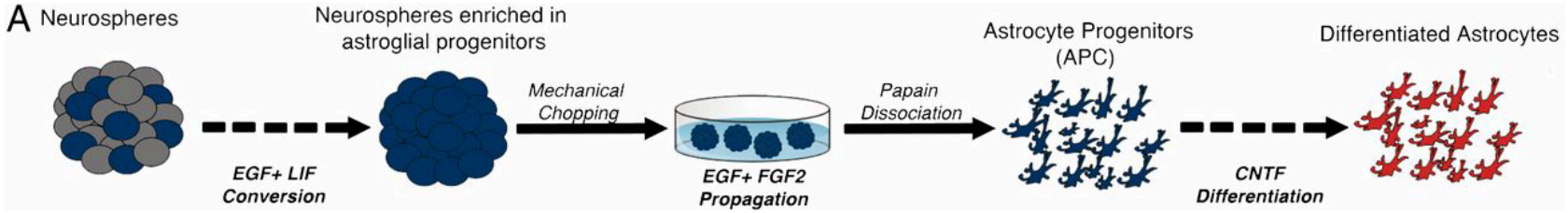
ES cell, Embryonic stem cell
BMP, Bone morphogenetic protein

Gupta et al. (2012)

Human in vitro astrocyte models

Protocol 3

Astrocytes from TARDBP mutant hiPSCs. NPCs grown in suspension as neurospheres, enriched with LIF and EGF (4−6wks) followed by expansion with EGF and FGF2 and then CNTF for terminal differentiation.



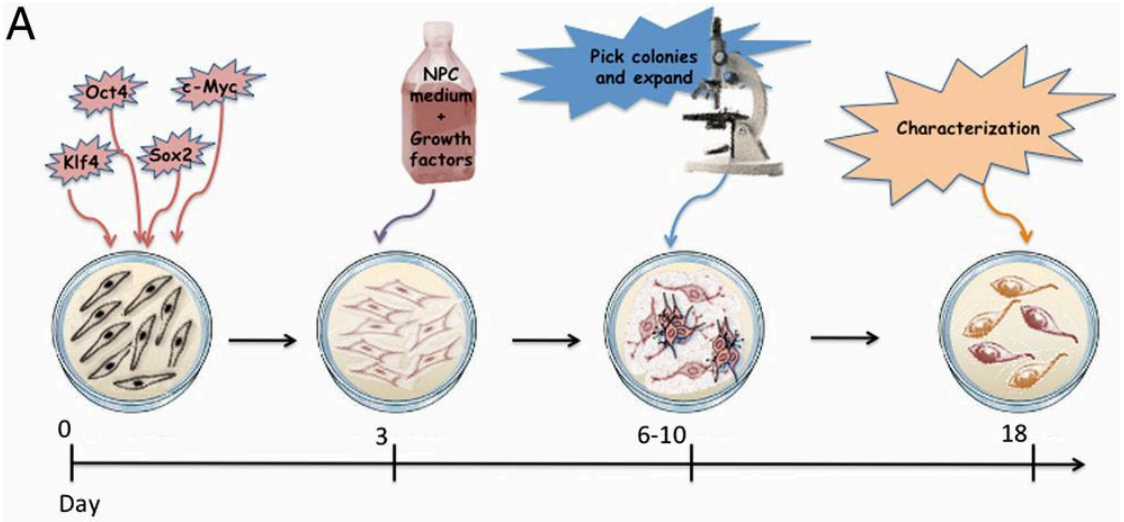
TARDBP, TAR DNA Binding Protein
NPCs, Neural Progenitor Cell

Serio et al. (2013)

Human in vitro astrocyte models

Protocol 4

Fibroblast derived astrocytes from SOD1A4V fALS, C9ORF72 fALS and sporadic ALS patients. Conversion to tripotent iNPCs through infection with Sox2, KLF4, Oct3/4, c-Myc, followed by switch to medium containing FGF2, EGF and heparin. Astrocytic differentiation initiated through seeding in NPC medium in fibronectin-coated dish, followed by 10% FBS and 0.3% N2.

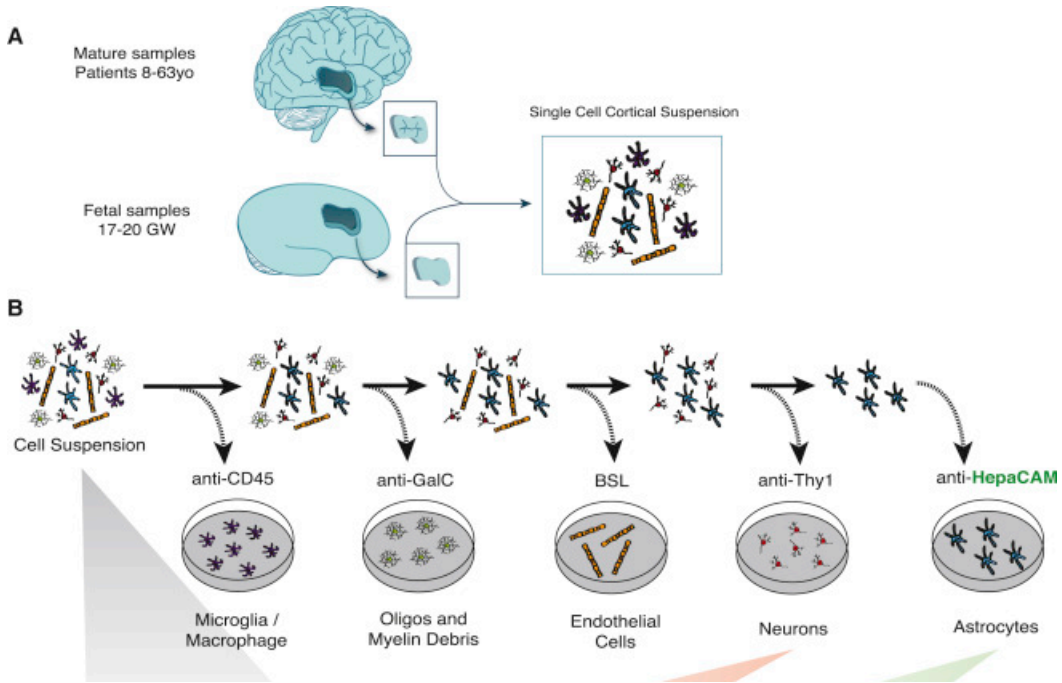


Meyer et al. (2014)

Human in vitro astrocyte models

Purification of astrocytes from fetal, juvenile and adult brains via immunopanning technique using anti-HepaCAM antibodies.

Protocol 5



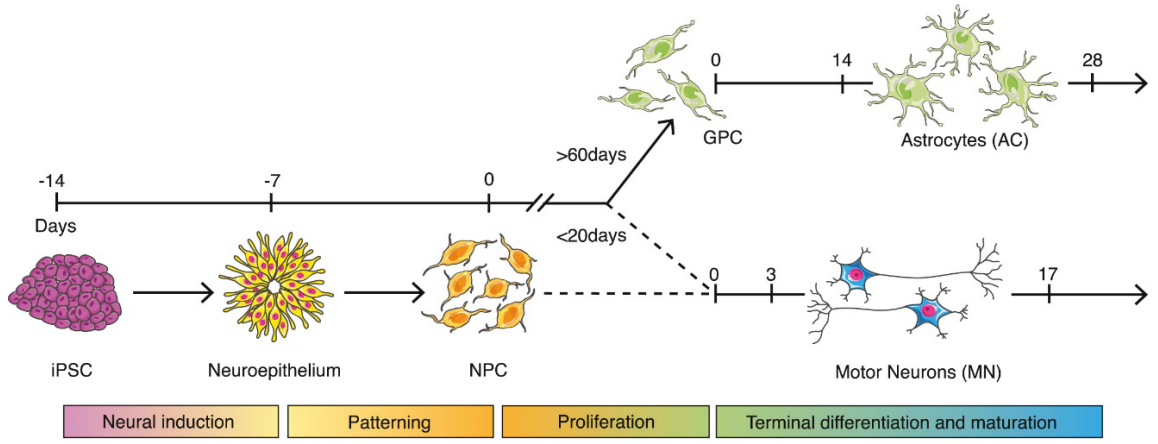
Zhang et al. (2016)

Human in vitro astrocyte models

iPSCs from VCP mutant fibroblasts. Astrocytes generated in monoculture throughout - FGF used to expand and BMP4 and LIF used to terminally differentiate.

Protocol 6

A



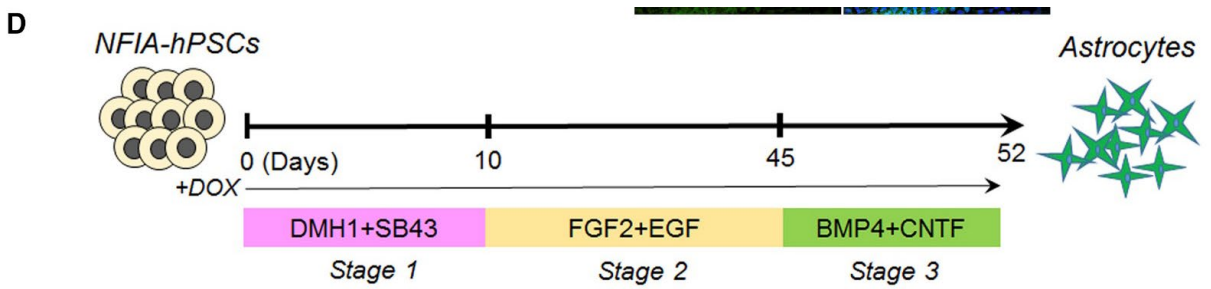
VCP, valosin-containing protein
GCP, gliogenic precursors

Hall et al. (2017)

Human in vitro astrocyte models

Protocol 7

Expression of NFIA and SOX9 speeds up iPSC derived astrocyte generation which display functional attributes including promoting neurite outgrowth, calcium waves after mechanical stimulation and glutamate uptake.



Li et al. (2018)

Advantages and disadvantages of human in vitro astrocyte models.

Model	Strengths	Limitations
hiPSC	**High purity**	Expensive
	Ability to self-renew	**Time consuming**
		Developmental model
ESCs	Same as hiPSCs	Same as hiPSCs plus ethical concerns
Transdifferentiated fibroblasts	**Preserved age of donor**	Limited supply
	Faster than stem cell-based protocols	**Reliant on expression of known factors**
		Currently reliant on serum affecting reactivity
Immunopanned primary astrocytes	**Ability to study cells exposed to *in vivo* cell-cell interactions**	Limited supply
	Can obtain from adult donors	
Immortalised cell line	**Fast**	Karyotype abnormalities
	Ability to self-renew	**Abnormal proliferative state**
		Currently reliant on serum affecting reactivity

Astrocytes in disease modeling- ALS

PNAS
Proceedings of the
National Academy of Sciences
of the United States of America

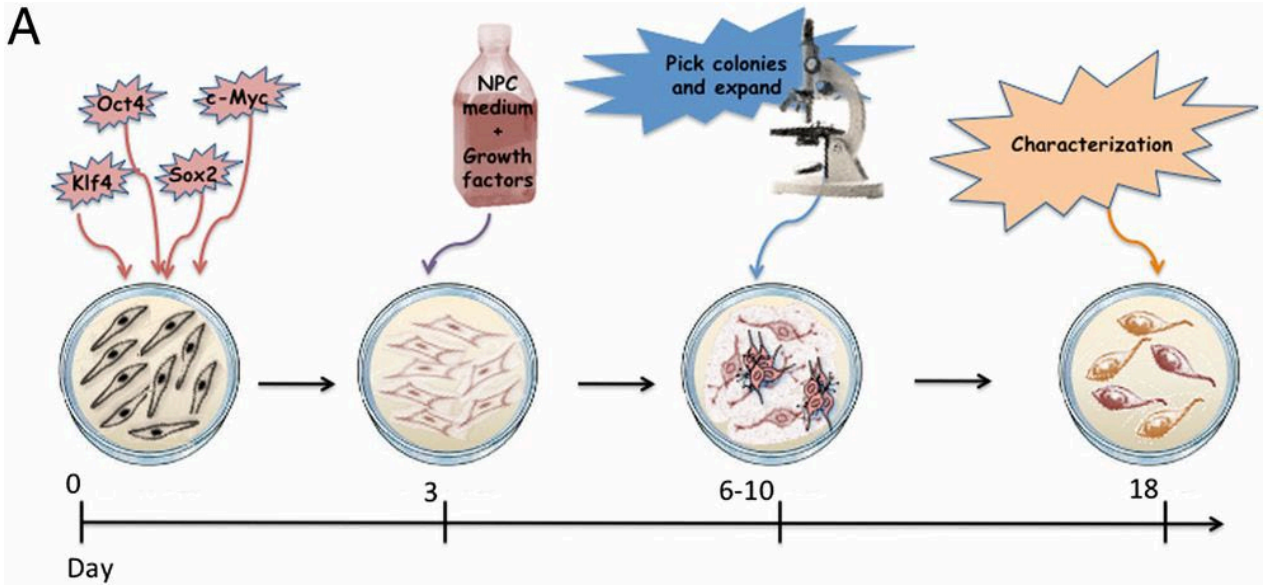
RESEARCH ARTICLE

Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS

Kathrin Meyer, Laura Ferraiuolo, Carlos J. Miranda, Shibi Likhite, Sohyun McElroy, Samantha …
+ See all authors and affiliations

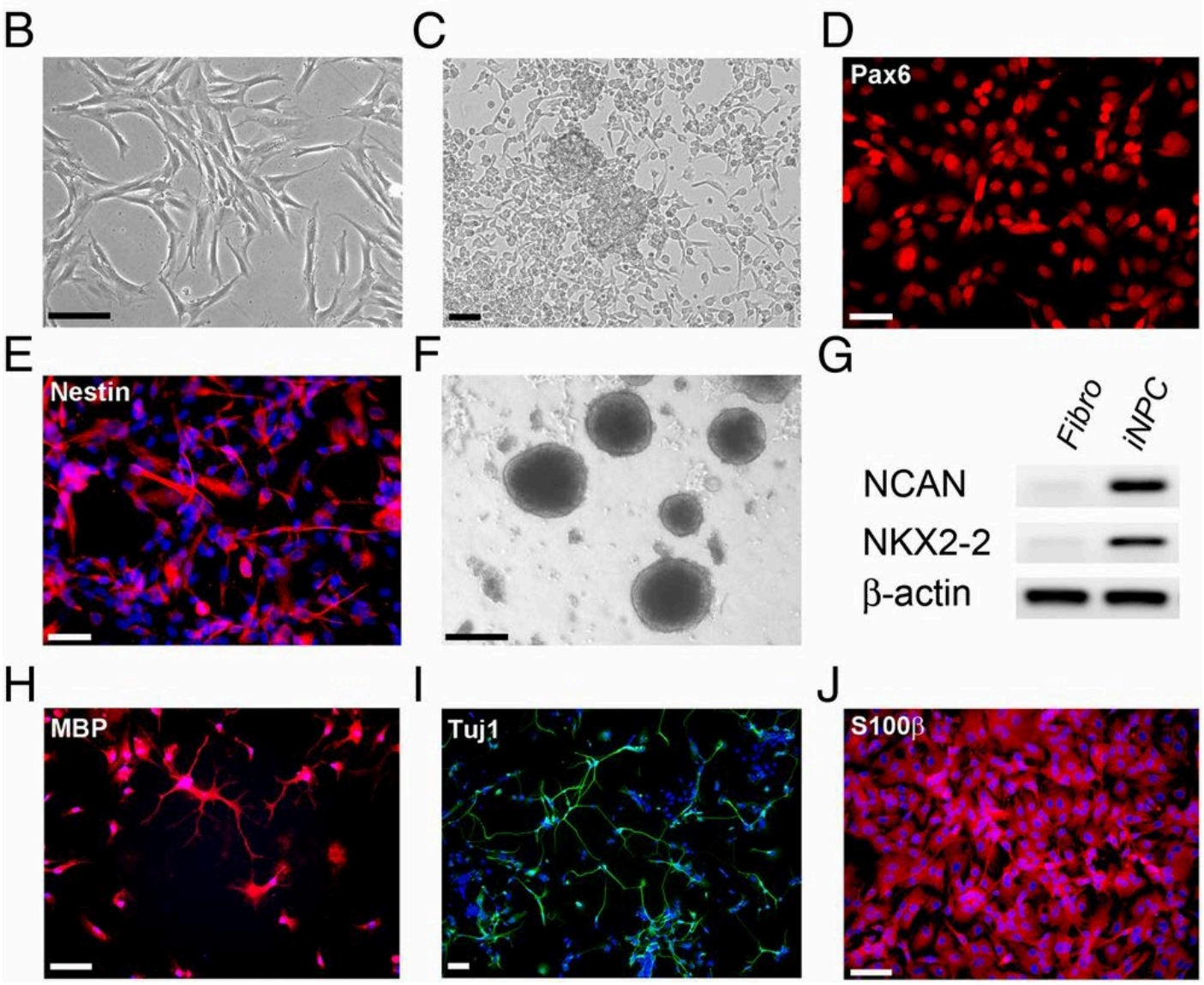
PNAS January 14, 2014 111 (2) 829-832; https://doi.org/10.1073/pnas.1314085111

Modeling-ALS

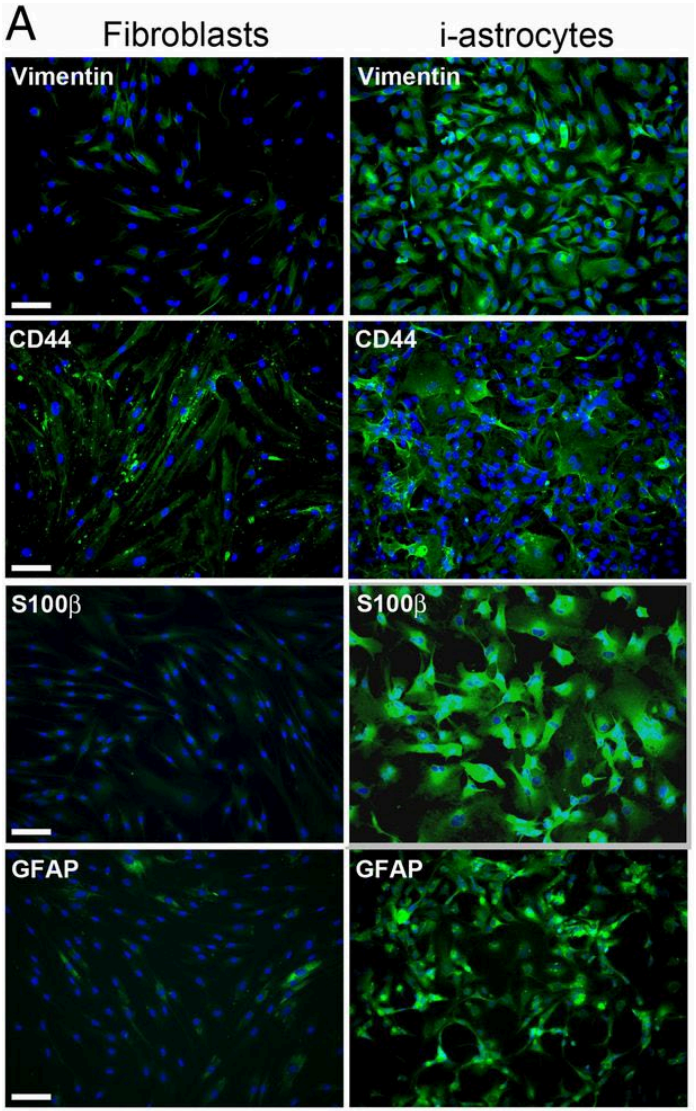


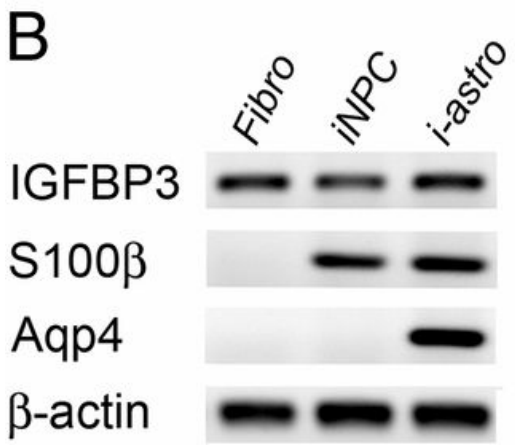
Direct conversion of human skin fibroblasts to tripotent iNPCs

Direct conversion of human skin fibroblasts to tripotent iNPCs



I-astrocytes express prototypic astrocyte markers





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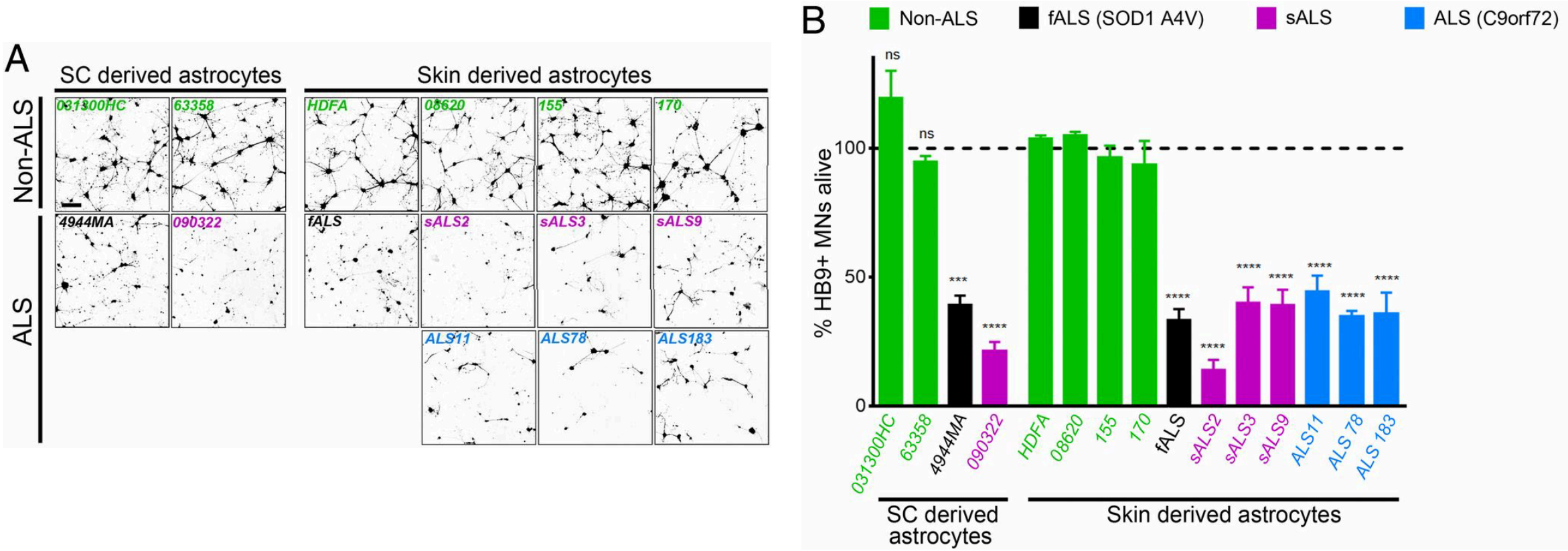
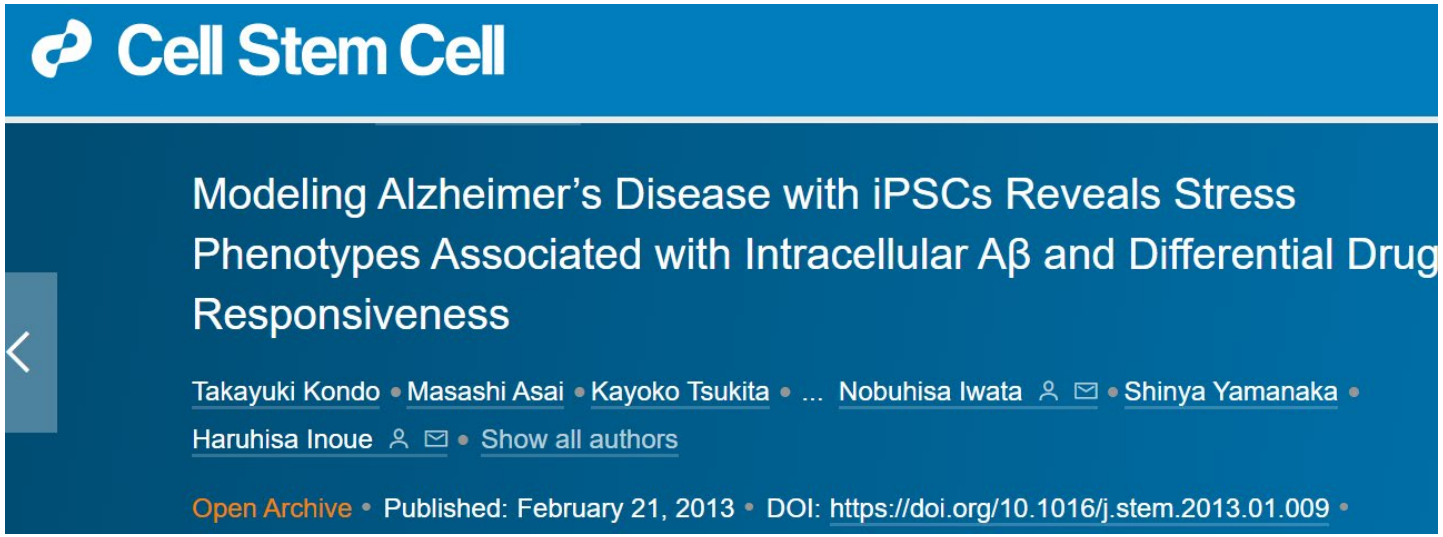
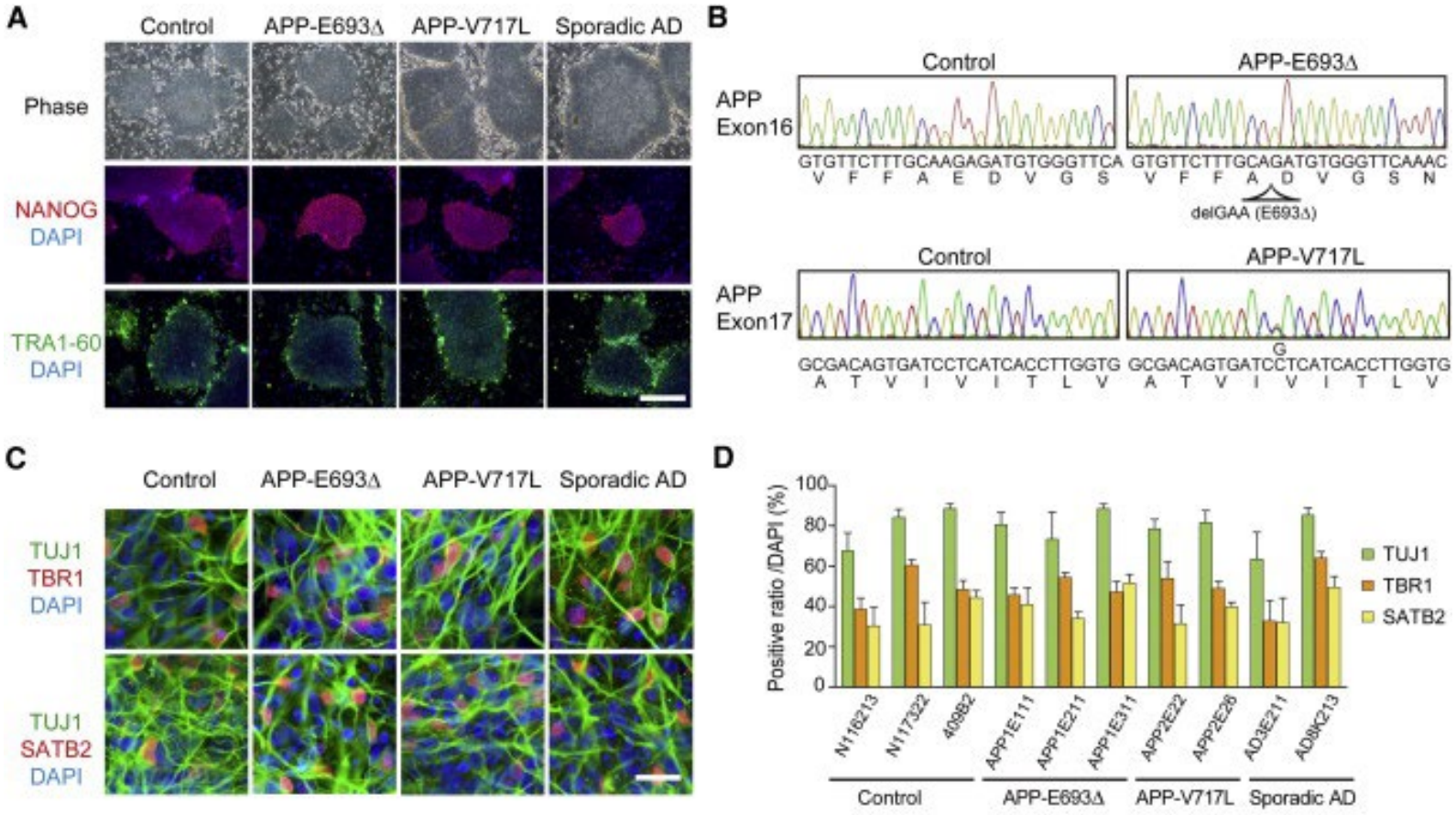




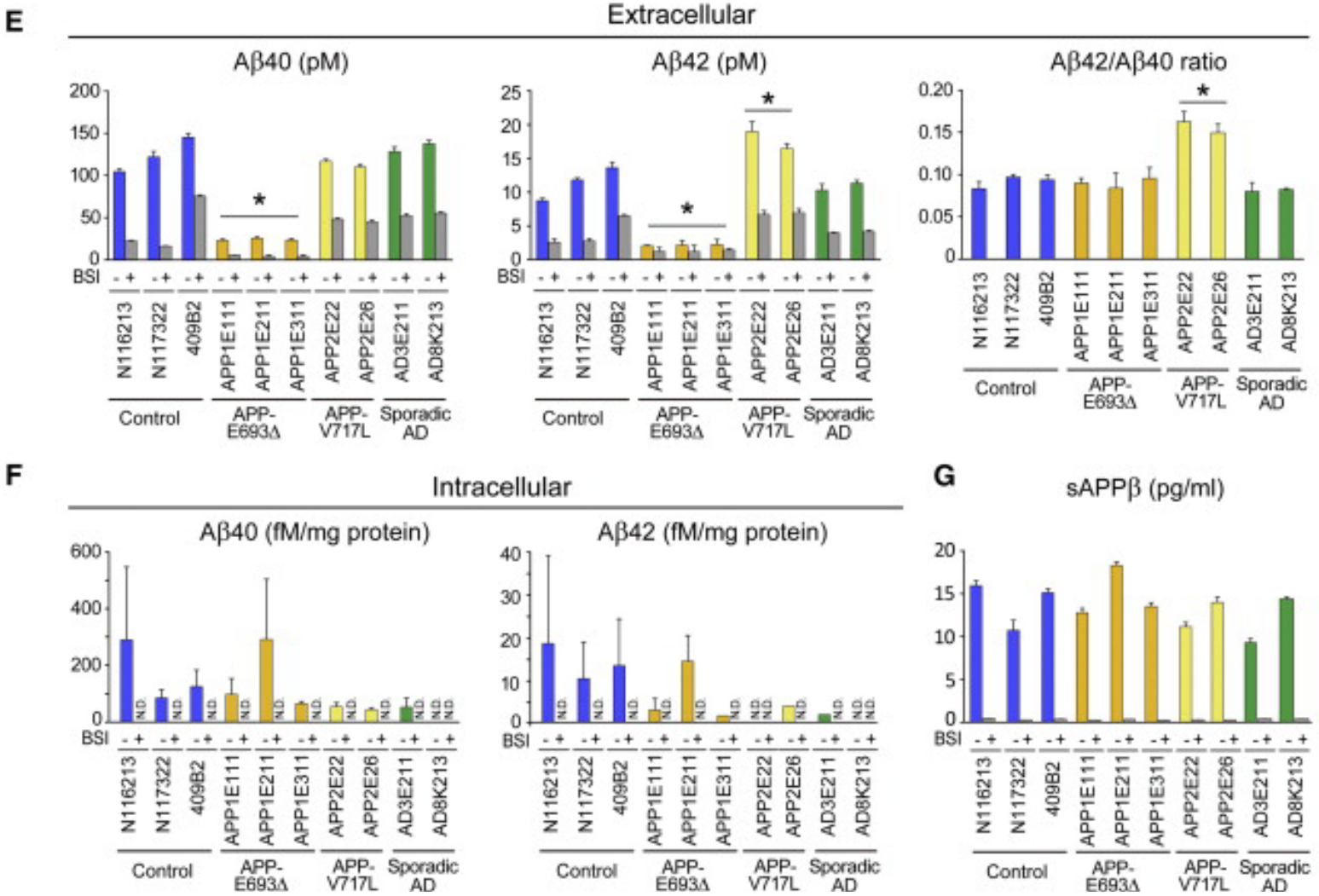
Summary

Oligomeric forms of amyloid-β peptide (Aβ) are thought to play a pivotal role in the pathogenesis of Alzheimer's disease (AD), but the mechanism involved is still unclear. Here, we generated induced pluripotent stem cells (iPSCs) from familial and sporadic AD patients and differentiated them into neural cells. Aβ oligomers accumulated in iPSC-derived neurons and astrocytes in cells from patients with a familial amyloid precursor protein (APP)-E693Δ mutation and sporadic AD, leading to endoplasmic reticulum (ER) and oxidative stress. The accumulated Aβ oligomers were not proteolytically resistant, and docosahexaenoic acid (DHA) treatment alleviated the stress responses in the AD neural cells. Differential manifestation of ER stress and DHA responsiveness may help explain variable clinical results obtained with the use of DHA treatment and suggests that DHA may in fact be effective for a subset of patients. It also illustrates how patient-specific iPSCs can be useful for analyzing AD pathogenesis and evaluating drugs.

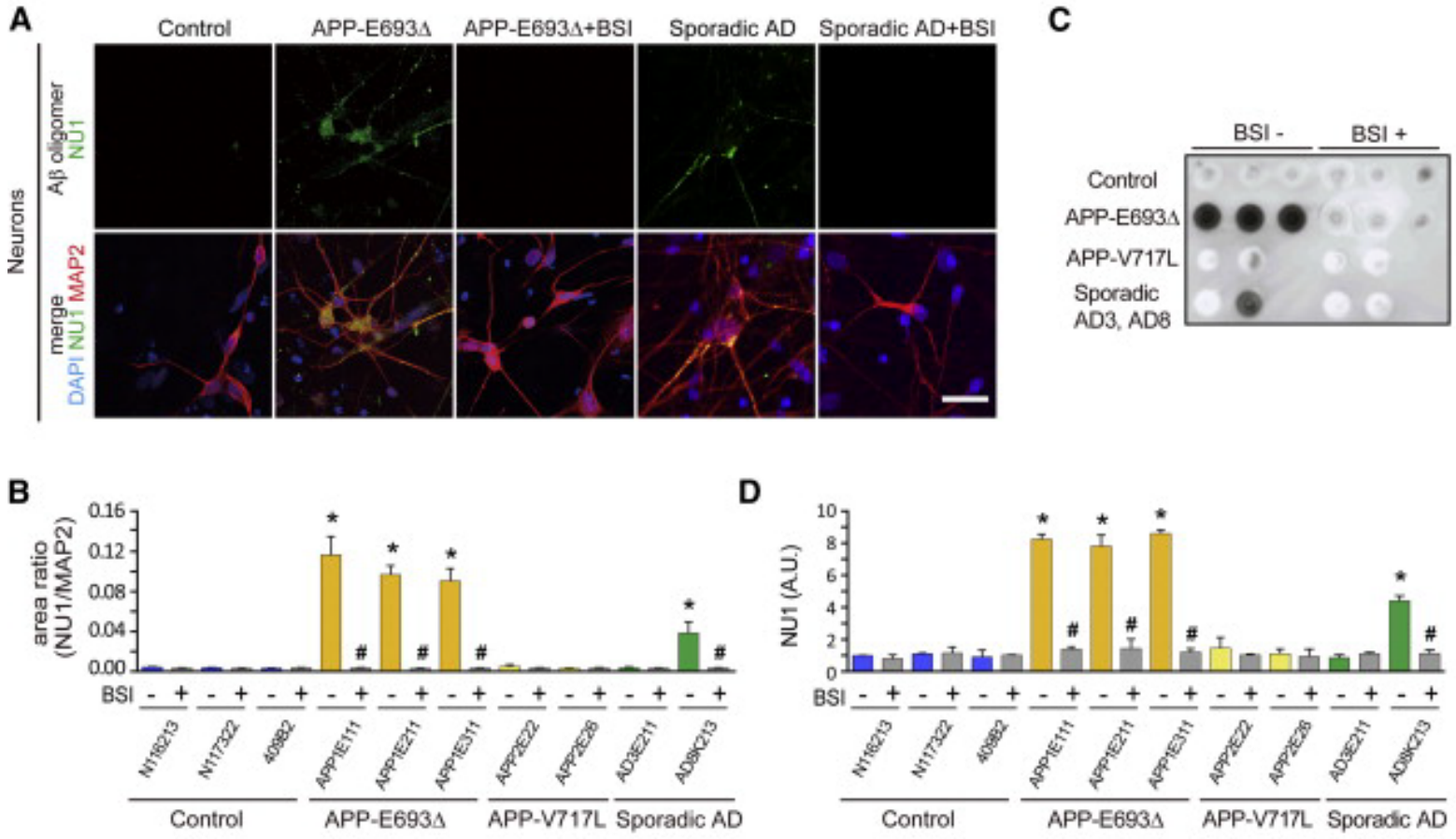
iPSC Generation and Cortical-Neuronal Differentiation



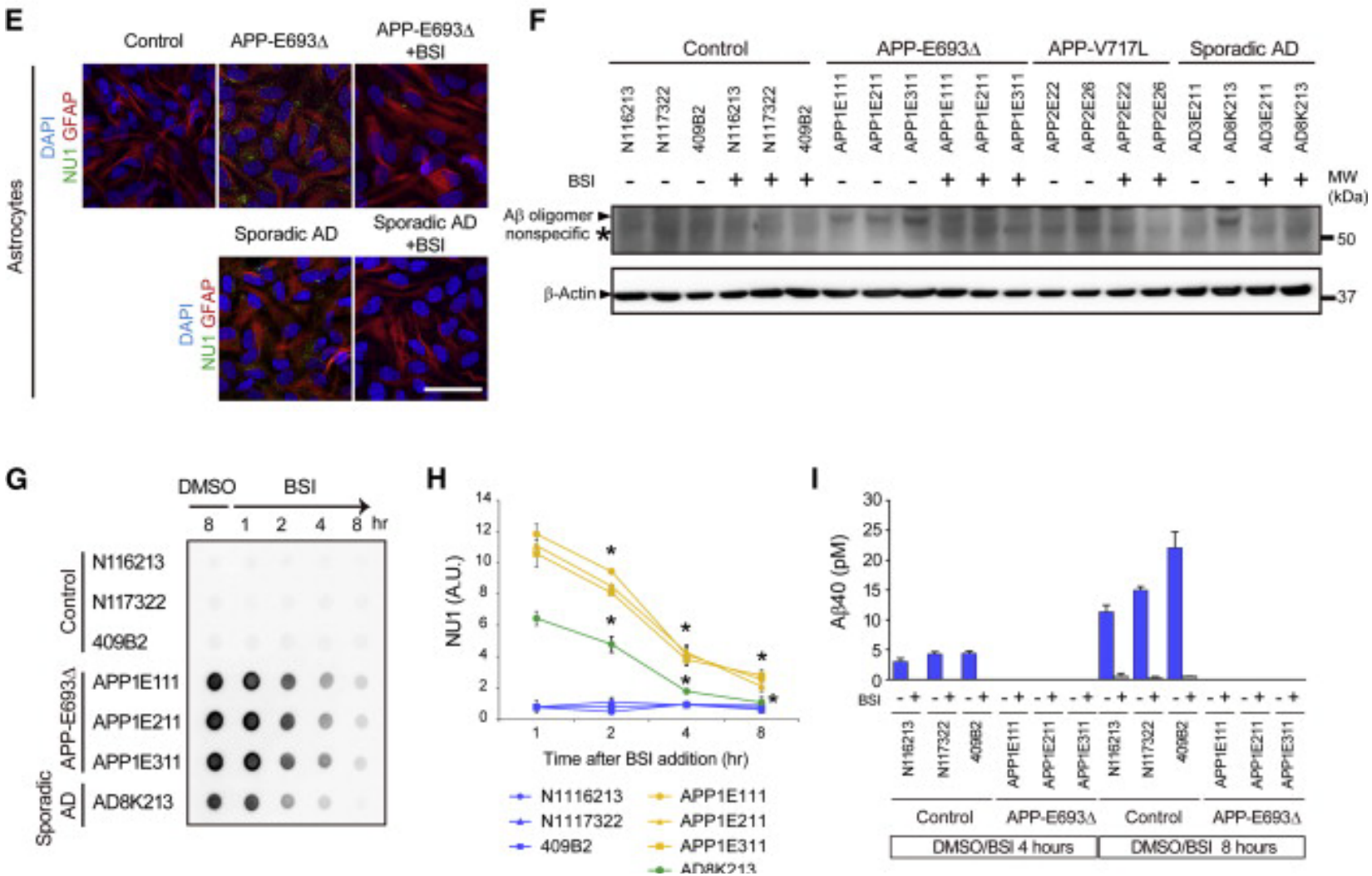
iPSC Generation and Cortical-Neuronal Differentiation



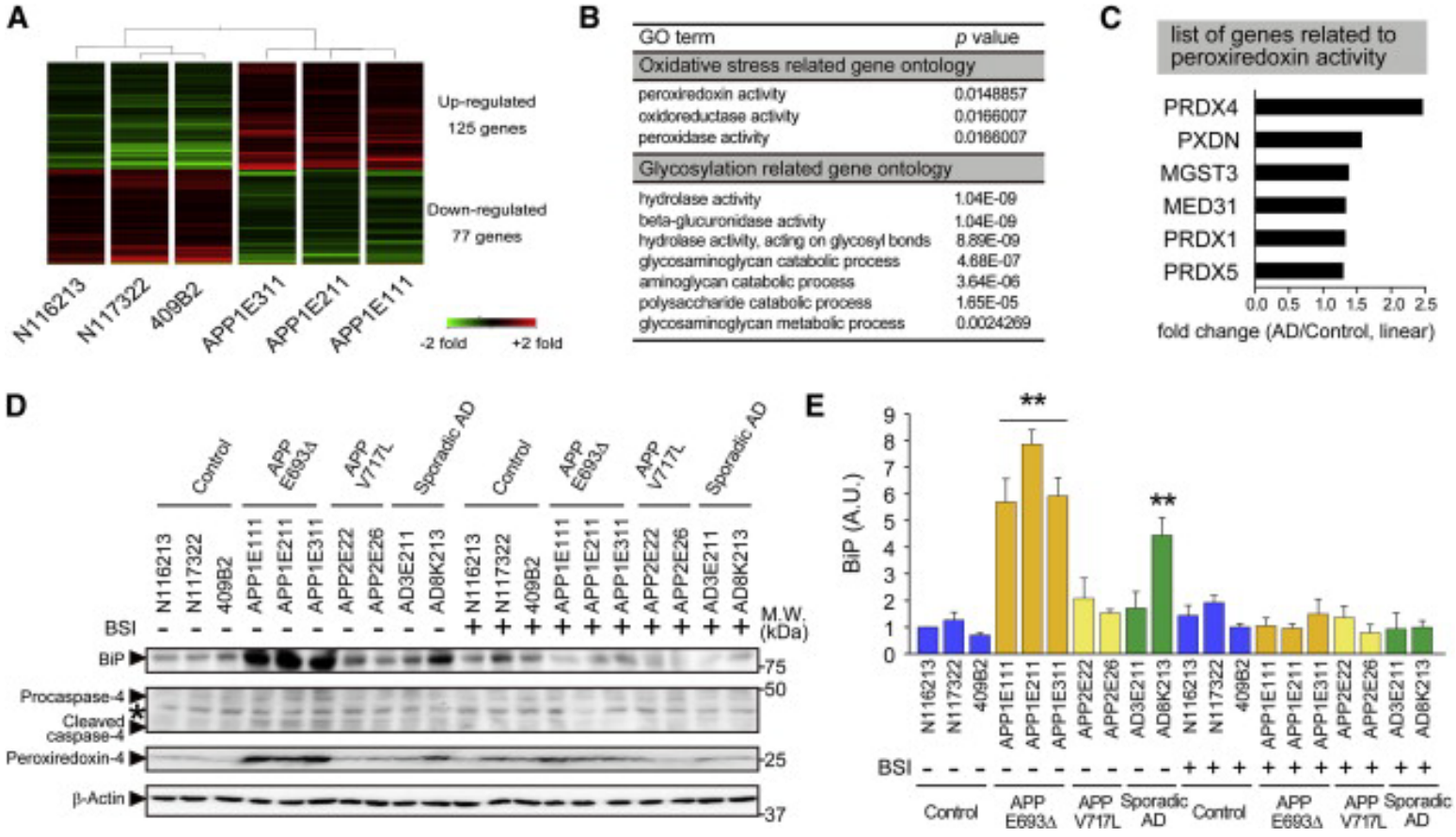
iPSC Generation and Cortical-Neuronal Differentiation



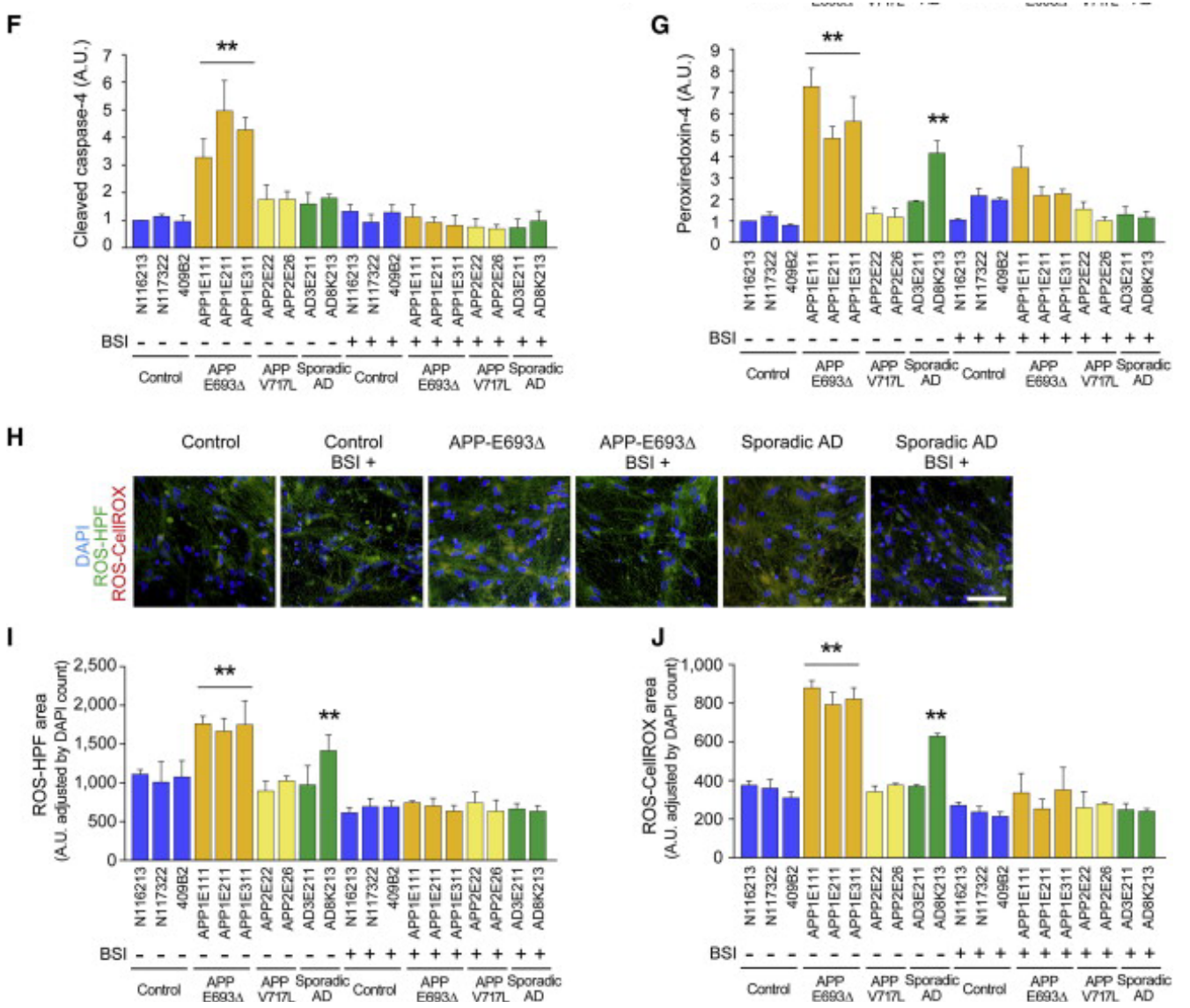
iPSC Generation and Cortical-Neuronal Differentiation



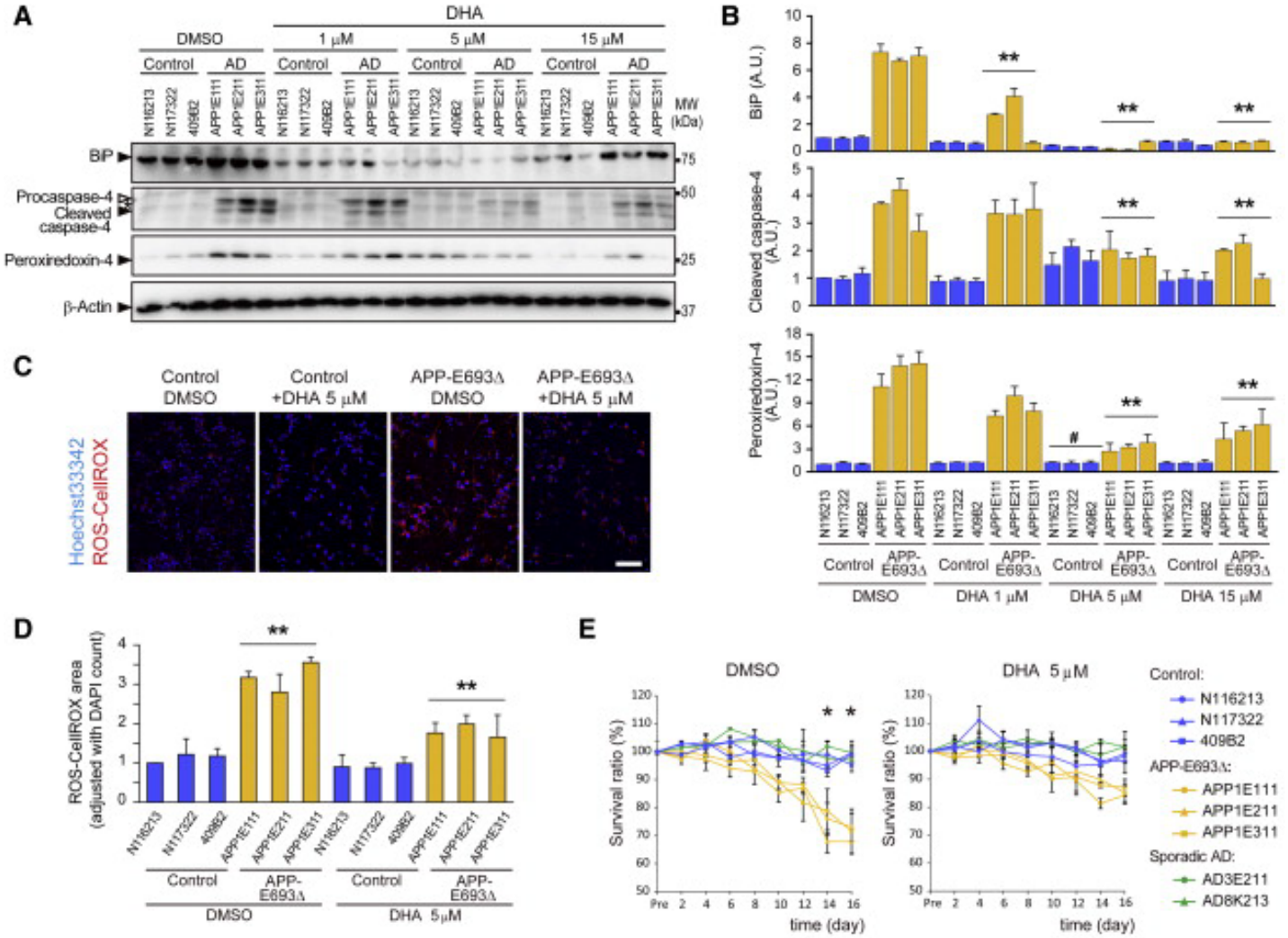
Cellular Stress Responses Caused By Intracellular Aβ Oligomers in AD iPSC-Derived Neural Cells



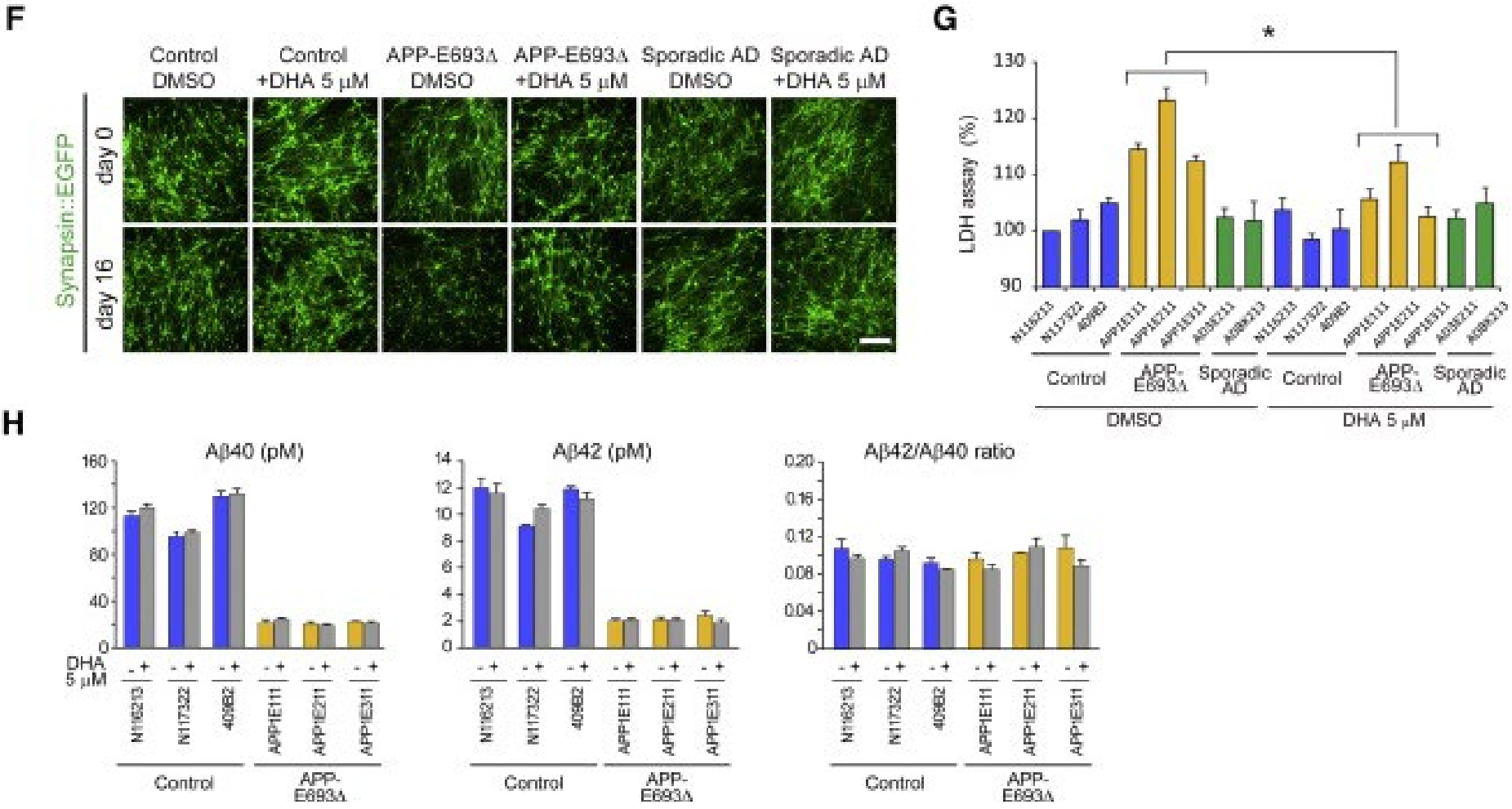
Cellular Stress Responses Caused By Intracellular Aβ Oligomers in AD iPSC-Derived Neural Cells



DHA-Alleviated Cellular Stress Caused By Intracellular Aβ Oligomers



DHA-Alleviated Cellular Stress Caused By Intracellular Aβ Oligomers



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RESEARCH ARTICLE | STEM CELLS

Drug Screening for ALS Using Patient-Specific Induced Pluripotent Stem Cells

Naohiro Egawa[1,2,*], Shiho Kitaoka[1,2,*], Kayoko Tsukita[1,2], Motoko Naitoh[3], Kazutoshi Takahashi[1], Takuya Yamamoto[1,4], Fumihiko Adachi[1], Takayuki Kondo[1,5], Keisuke Okita[1], Isao Asaka[1], Takashi Aoi[1], Akira Watanabe[1,4], Yasuhiro Yamada[1,4], Asuka Morizane[1,6], Jun Takahashi[1,6], Takashi Ayaki[5], Hidefumi Ito[5], Katsuhiro Yoshikawa[3], Satoko Yamawaki[3], Shigehiko Suzuki[3], Dai Watanabe[7], Hiroyuki Hioki[8], Takeshi Kaneko[8], Kouki Makioka[9], Koichi Okamoto[9], Hiroshi Takuma[10], Akira Tamaoka[10], Kazuko Hasegawa[11], Takashi Nonaka[12], Masato Hasegawa[12], Akihiro Kawata[13], Minoru Yoshida[14], Tatsutoshi Nakahata[1], Ryosuke Takahashi[5], Maria C. N. Marchetto[15], Fred H. Gage[15], Shinya Yamanaka[1,4,16] and Haruhisa Inoue[1,2,16,†]

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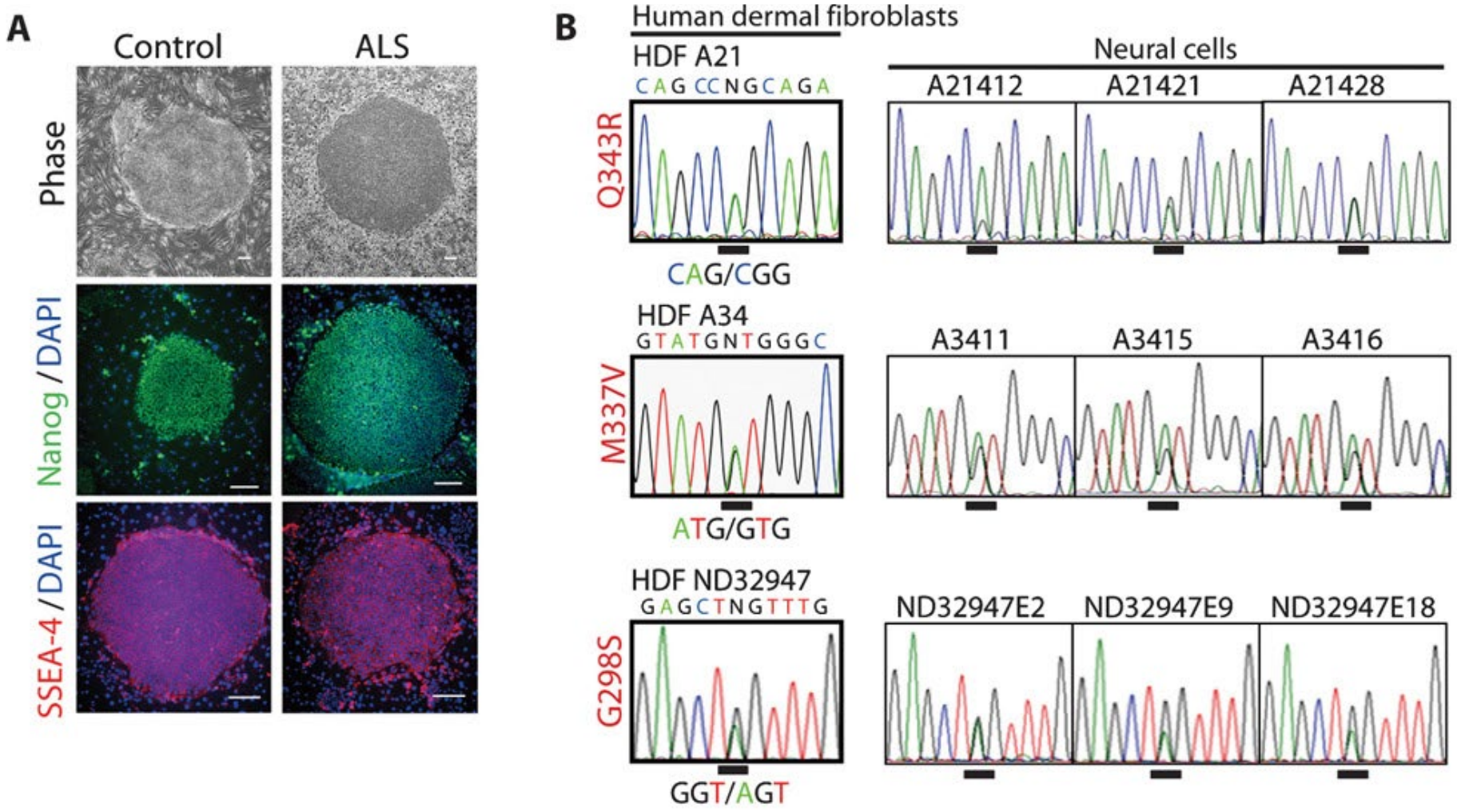
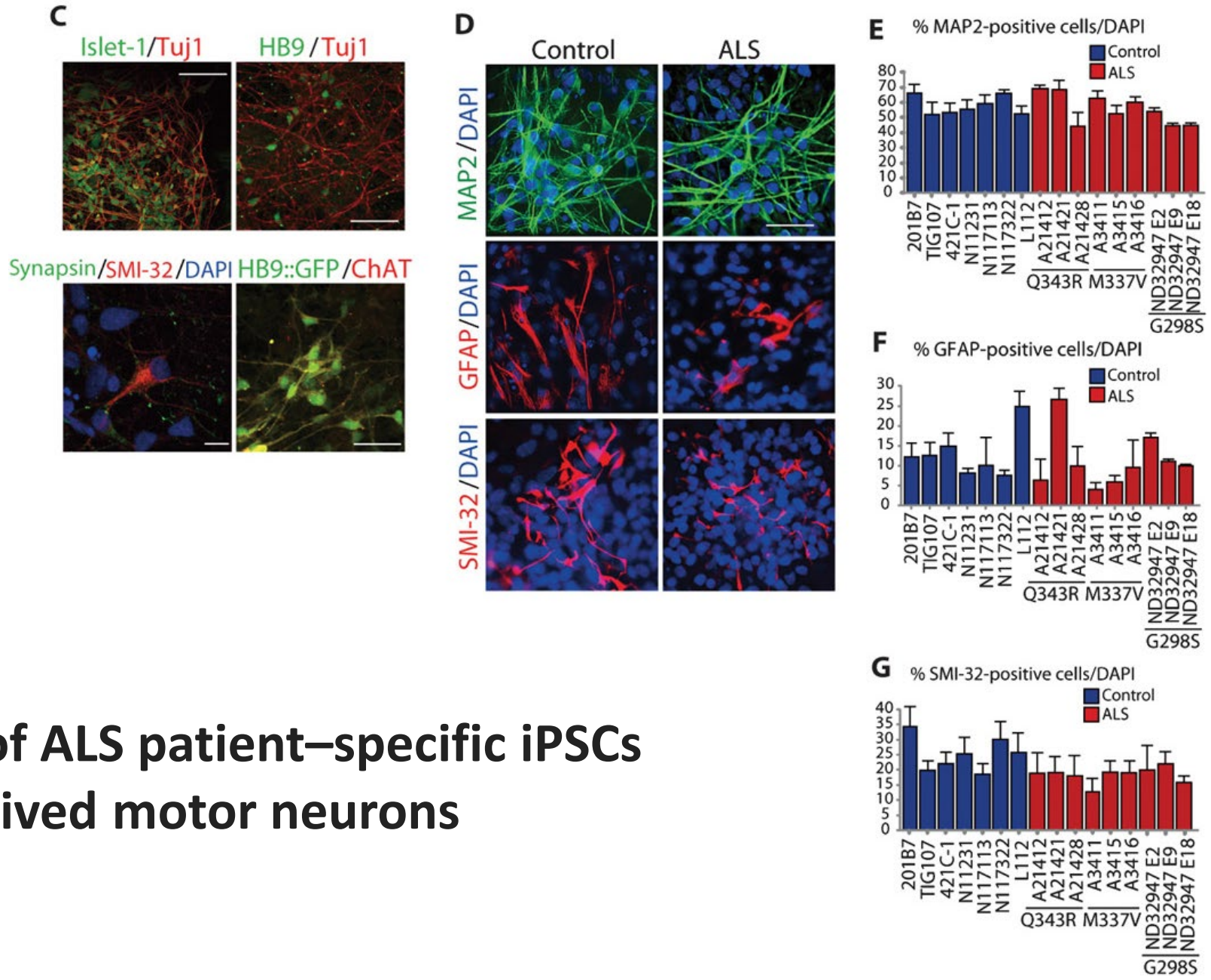
↵* These authors contributed equally to this work.

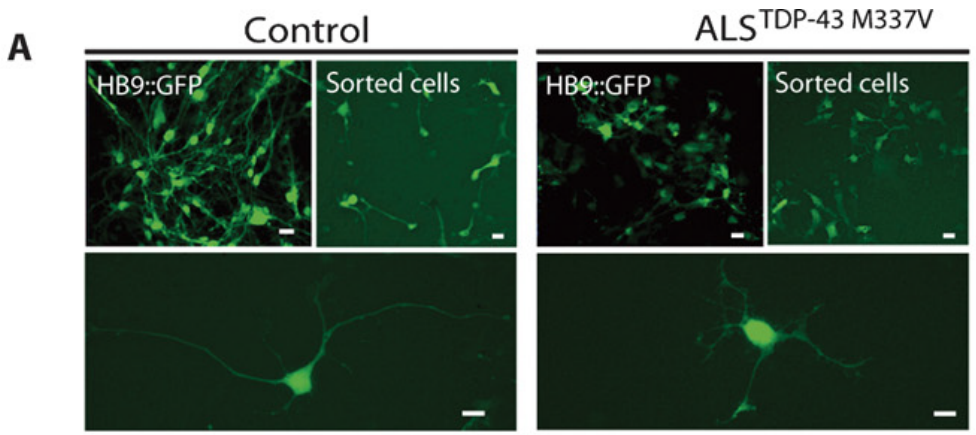
Generation of ALS patient–specific iPSCs and iPSC-derived motor neurons

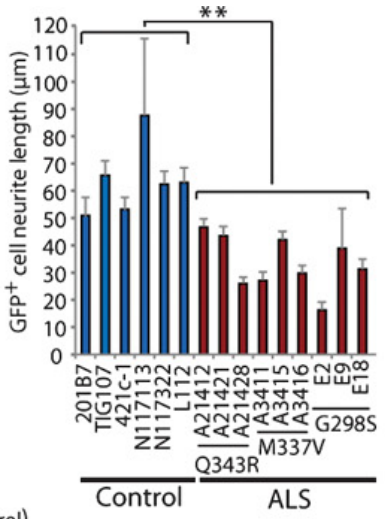


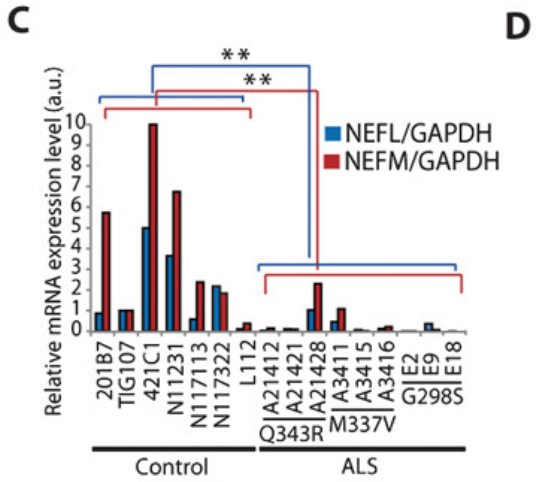


Generation of ALS patient–specific iPSCs and iPSC-derived motor neurons

Phenotypes of ALS iPSC–derived motor neurons







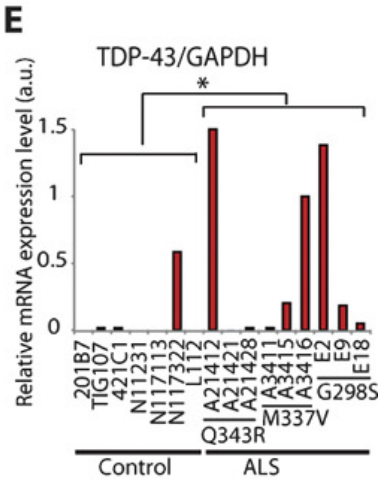
D

RNA metabolism-related Gene Ontology (ALS > Control)

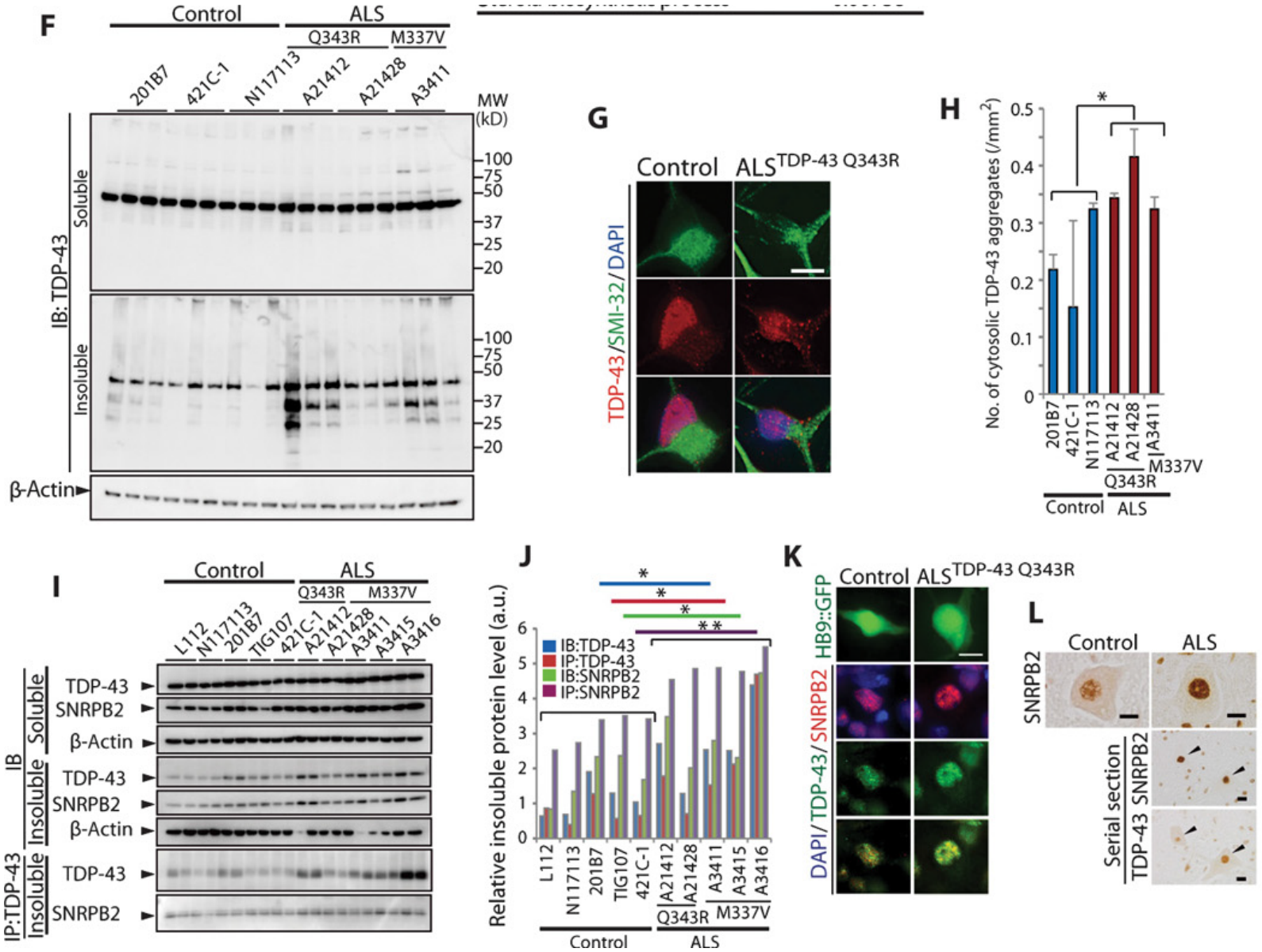
GO term	Corrected *P*
RNA binding	1.23E-36
RNA processing	2.67E-31
Ribonucleoprotein complex	2.20E-30
RNA splicing	2.77E-26
mRNA processing	1.27E-19
Spliceosomal complex	2.24E-18
Nuclear mRNA splicing via spliceosome	4.81E-15
RNA transport	1.53E-7
Transcriptional initiation	0.00942

Gene Ontology (ALS < Control)

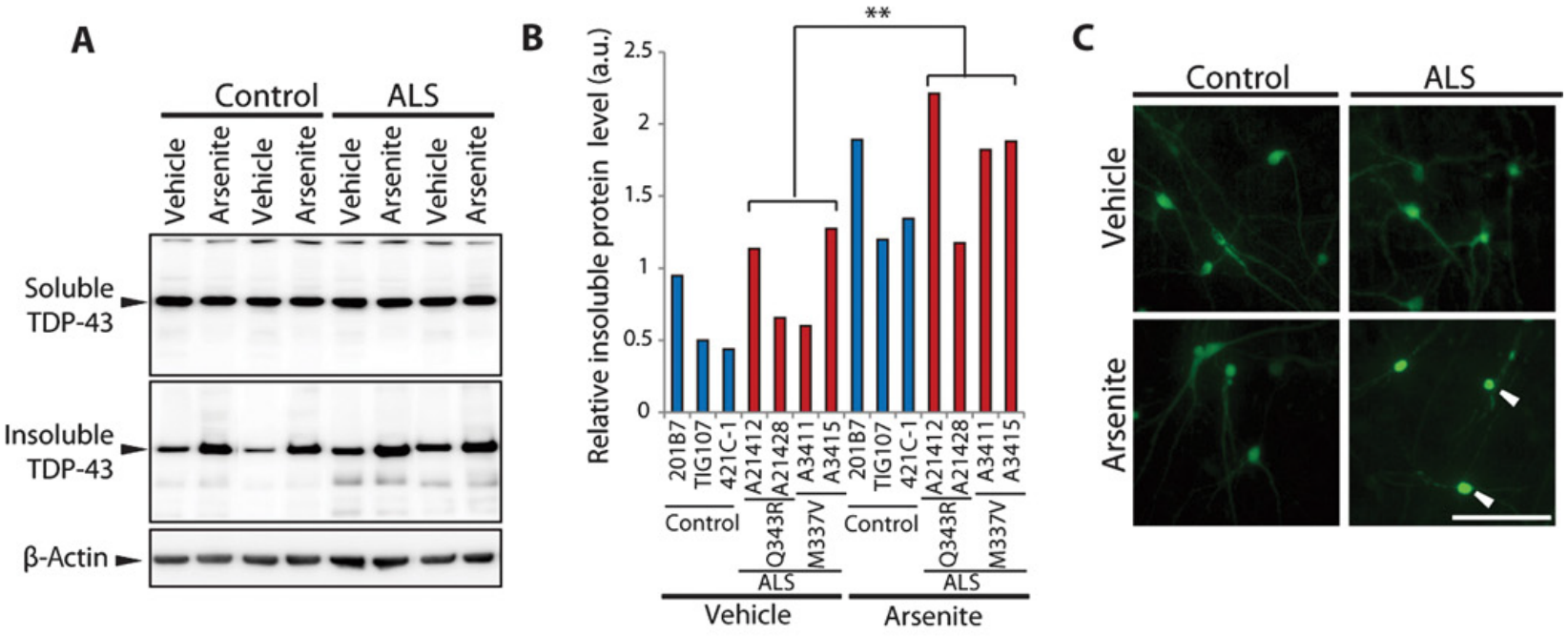
GO term	Corrected *P*
Sterol biosynthetic process	0.00118
Intermediate filament	0.00581
Intermediate filament cytoskeleton	0.00581
Steroid biosynthetic process	0.00738



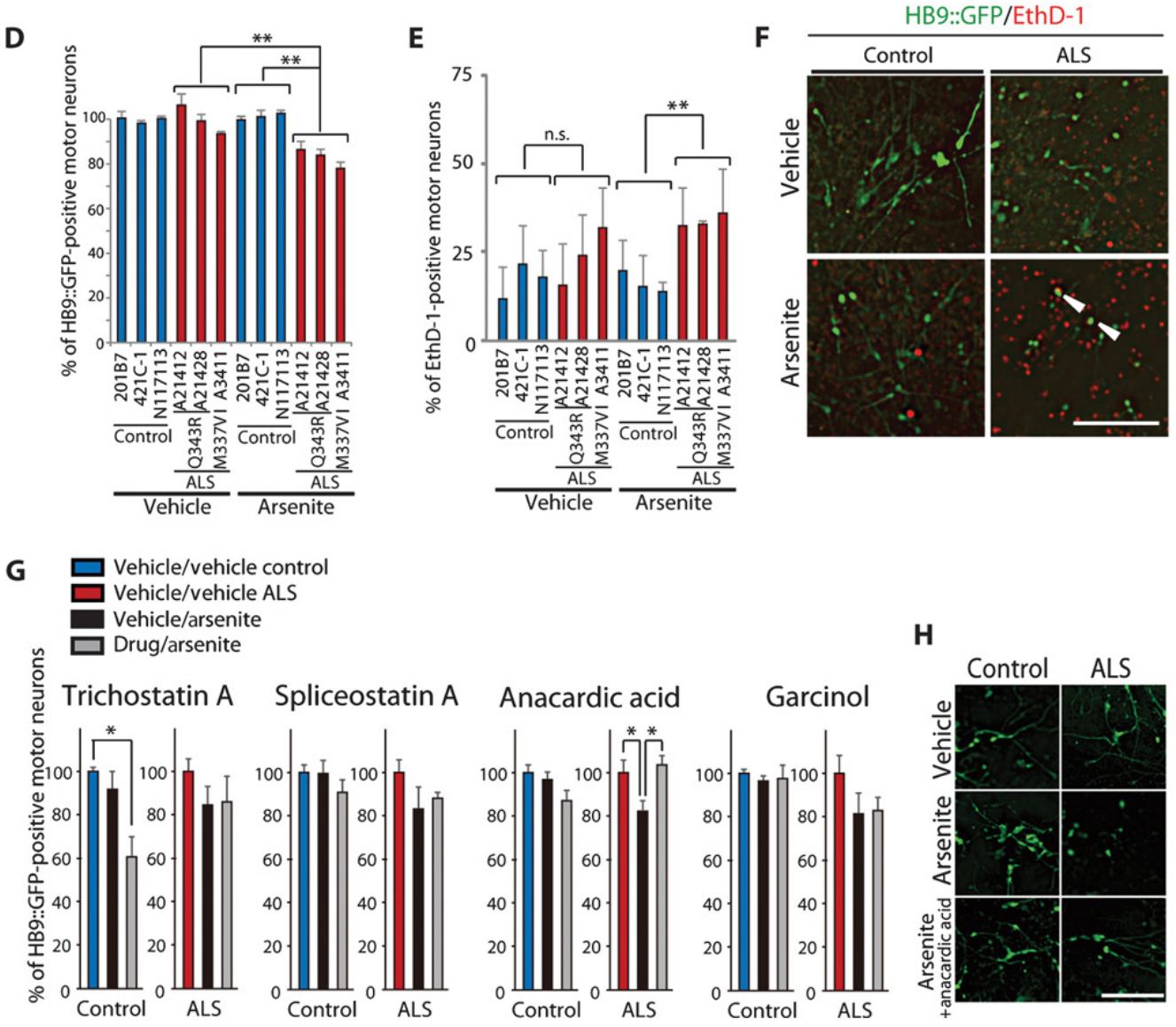
Phenotypes of ALS iPSC–derived motor neurons



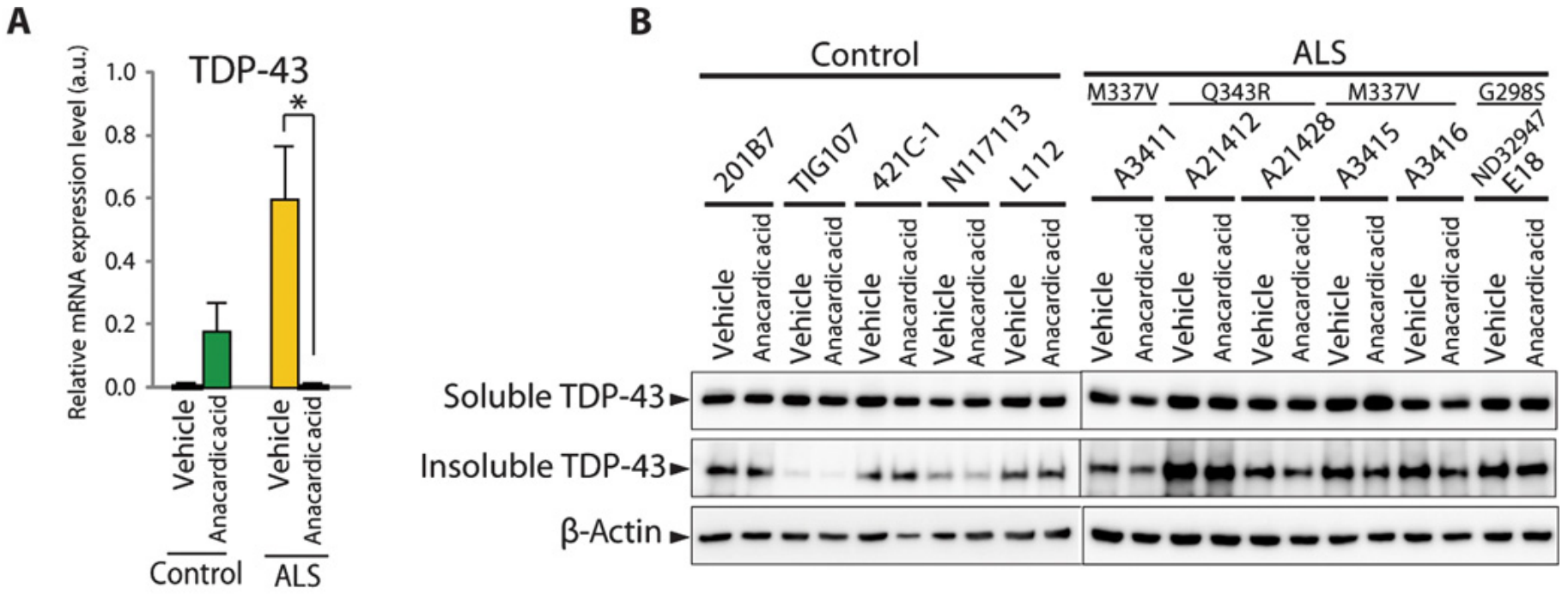
Arsenite-induced death of ALS and control iPSC–derived motor neurons



Arsenite-induced death of ALS and control iPSC–derived motor neurons



Anacardic acid–induced phenotypic changes in ALS and control motor neurons



Anacardic acid–induced phenotypic changes in ALS and control motor neurons

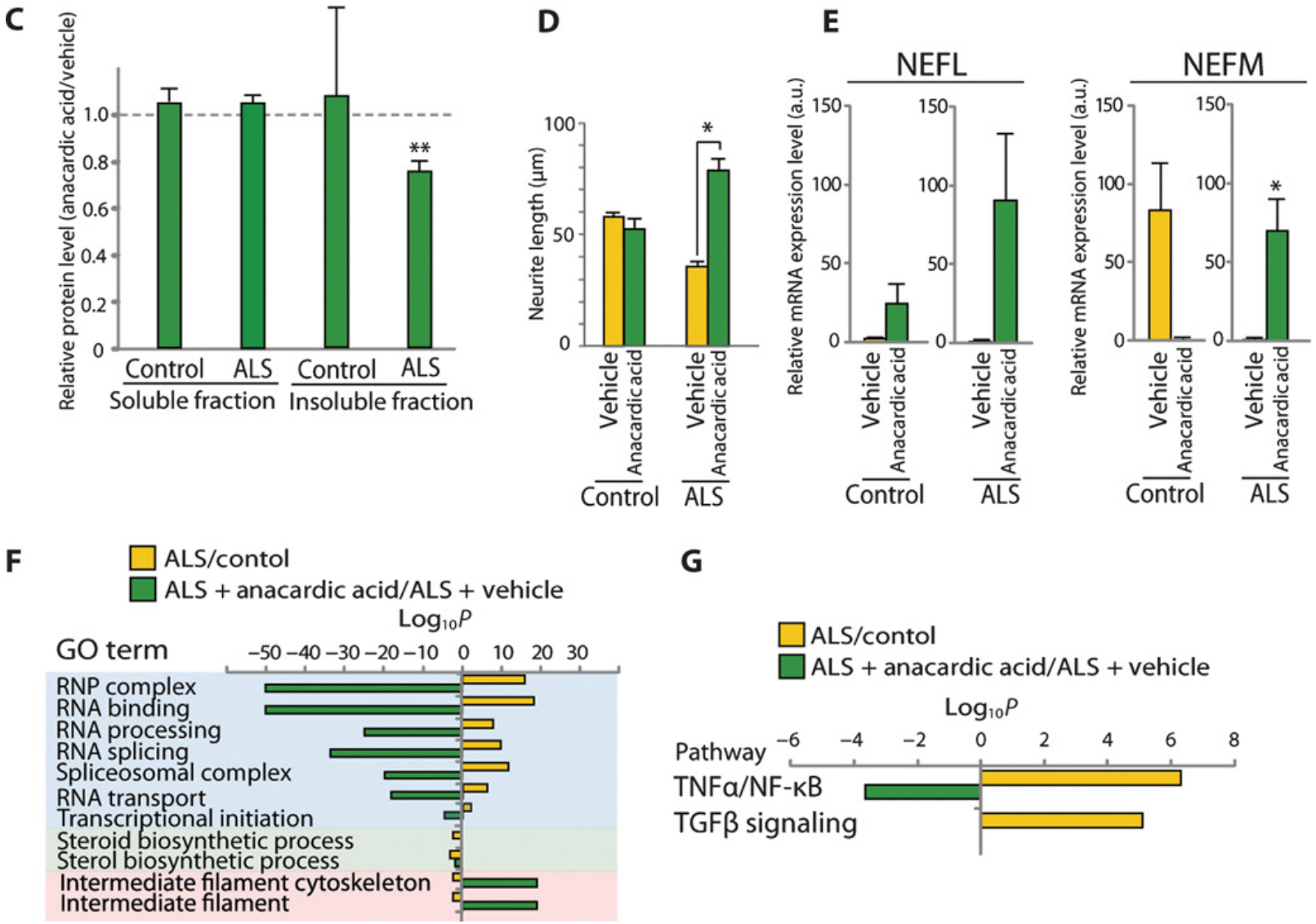


Table Molecular targets in astrocytes that are altered in neurological disease

Function of target	Proposed change in function in disease	Molecular target	Disease
Growth factors	Loss of function	IGF1	Rett syndrome
		BDNF	HD
		CCL5	HD
		NGF	ALS
Metabolic regulation	Loss of function	GLUT1 and MCT1	AD and ALS
		Energy metabolism	AD
		Mitochondrial dysfunction	HD, ALS and Rett syndrome
Homeostatic function	Gain of function	Connexins, gap junctions and hemichannels	Rett syndrome, AD, ALS and HD
		AQP4	Epilepsy
	Loss of function	Kir4.1	Rett syndrome, HD and epilepsy
Cytoskeleton	Loss of function	Microtubules	Rett syndrome
Glutamate receptor	Gain of function	Metabotropic glutamate receptors	ALS and AD
Glutamate transporter	Loss of function	GLT1	ALS, HD, AD and PD
Signalling pathways	Gain of function	Calcium signalling	AD and ALS
		Purinergic signalling	HD and AD
		JAK–STAT3	AD, PDand ALS
	Loss of function	MAPK	AD
		TGFβ	HD
		Cholesterol production	ALS, HD and AD
Oxidative stress	Loss of function	NRF2	HD, PD, AD and ALS
Inflammatory pathway	Gain of function	TNF NF-κB IFNγ	AD and ALS ALS and HD ALS and PD

Thank you!