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Supplemental Information

PSEN1 Mutant iPSC-Derived Model Reveals Severe Astrocyte Pathology in Alzheimer's Disease

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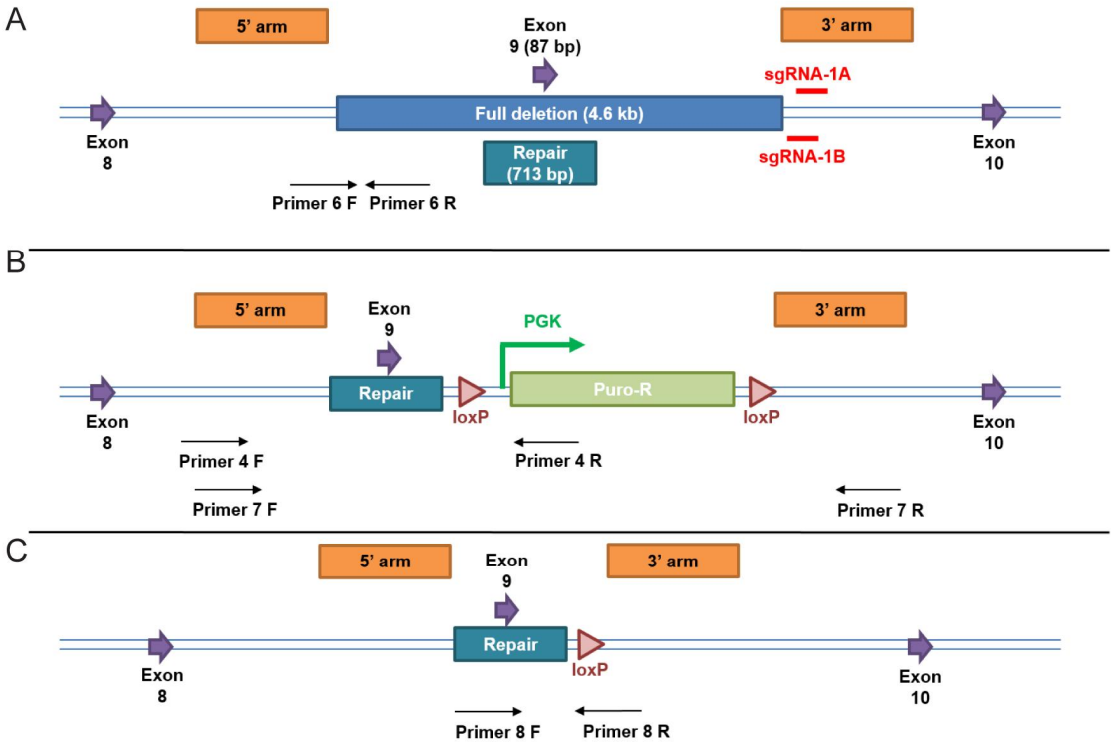
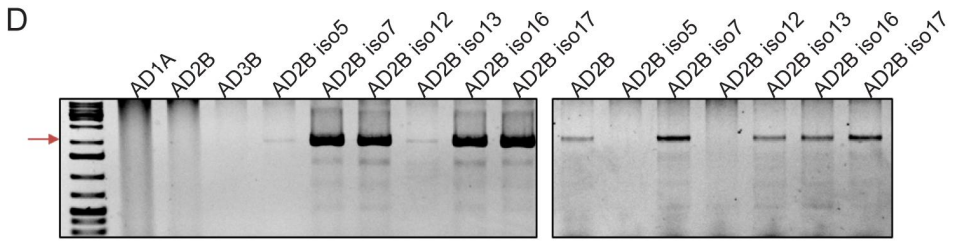
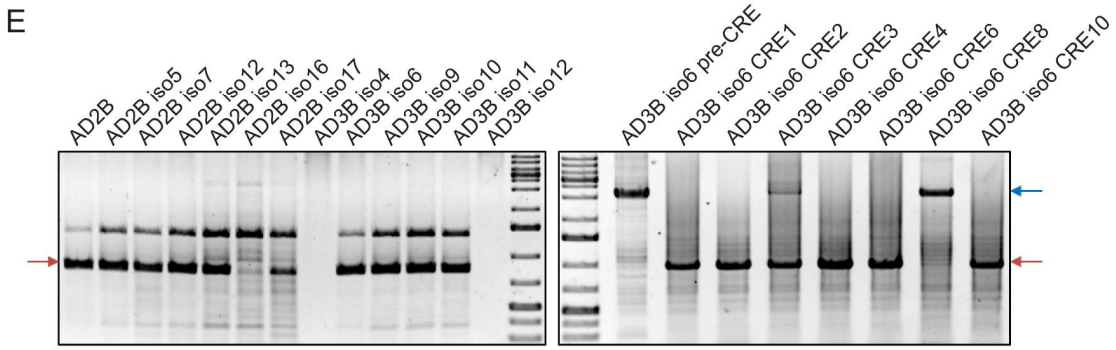
Figure S1


A
5' arm
Exon
9 (87 bp)
3' arm
sgRNA-1A
Full deletion (4.6 kb)
Exon
8
Repair
(713 bp)
sgRNA-1B
Exon
10
Primer 6 F
Primer 6 R
B
5' arm
Exon
9
PGK
3' arm
Repair
Puro-R
Exon
8
loxP
loxP
Exon
10
Primer 4 F
Primer 4 R
Primer 7 F
Primer 7 R
C
5' arm
Exon
9
3' arm
Repair
Exon
8
loxP
Exon
10
Primer 8 F
Primer 8 R


D
AD1A
AD2B
AD3B
AD2B iso5
AD2B iso7
AD2B iso12
AD2B iso13
AD2B iso16
AD2B iso17
AD2B
AD2B iso5
AD2B iso7
AD2B iso12
AD2B iso13
AD2B iso16
AD2B iso17


E
AD2B
AD2B iso5
AD2B iso7
AD2B iso12
AD2B iso13
AD2B iso16
AD2B iso17
AD3B iso4
AD3B iso6
AD3B iso9
AD3B iso10
AD3B iso11
AD3B iso12
AD3B iso6 pre-CRE
AD3B iso6 CRE1
AD3B iso6 CRE2
AD3B iso6 CRE3
AD3B iso6 CRE4
AD3B iso6 CRE6
AD3B iso6 CRE8
AD3B iso6 CRE10

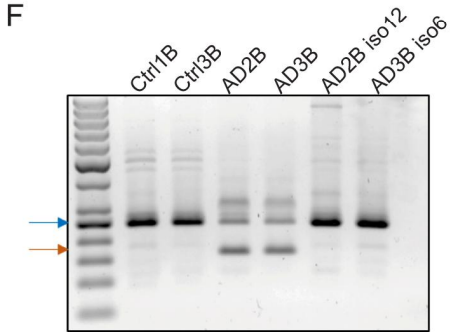

F
Ctrl1B
Ctrl3B
AD2B
AD3B
AD2B iso12
AD3B iso6

Figure S1. Schematic illustration of the generation of isogenic control lines. Related to Table 1 and Figures 1, 2, 5 and 6.

(A) The targeting strategy to correct the deletion of exon 9 in *PSEN1*. SgRNA pair was designed to target the + and – strands of DNA at the boundary of the full 4.6-kb full deletion. A homologous repair donor plasmid with 1 kb homology arms was designed to restore approximately 700 bp fragment containing exon 9 and the immediately-flanking intronic sequences, deemed sufficient for proper mRNA splicing without restoring the full-length intronic sequence.

(B) The targeted genomic region after successful electroporation. A LoxP-flanked PGK-puromycin cassette facilitates the selection of cells exhibiting homology directed repair at the *PSEN1* locus. PGK, phosphoglycerate kinase promoter; Puro-R, puromycin-resistance gene.

(C) The targeted genomic region after puromycin selection and CRE-recombinase treatment. To ensure proper *PSEN1* expression, the PGK-puromycin cassette was removed using CRE/LoxP recombination, leaving behind one of the LoxP sites.

(D) PCR genotyping. Transfected iPSC clones were screened for correct donor plasmid location (left gel), resulting in 2 kb product (red arrow; primer pair 4) and for exon 9 deletion (right gel) resulting in 2.1 kb product if the deletion is still present (red arrow; primer pair 7) and no product in the absence of deletion. Clones with correct plasmid location without the exon 9 deletion were chosen.

(E) PCR genotyping. Clones were screened for heterozygous repair of the exon 9 deletion allele (left gel), resulting in 0.8 kb product when the healthy allele remained intact (red arrow; primer pair 6). Successful CRE-recombination (right gel) was confirmed with primer pair 8, resulting in 1 kb product when successful (red arrow) or 2.5 kb product when unsuccessful (blue arrow). Clones with intact healthy allele and successful removal of puromycin-resistance cassette were chosen.

(F) Confirmation of the exon 9 inclusion in isogenic control lines at the transcript level. PCR products were amplified from cDNA. The correct product from exon 8 to exon 10 results in a 360 bp band (blue arrow) when the exon 9 is included and in a 273 bp band (red arrow) without exon 9. Control lines and isogenic control lines show only one band of 360 bp whereas AD lines show both bands as a sign of heterozygous deletion of exon 9.

Figure S2

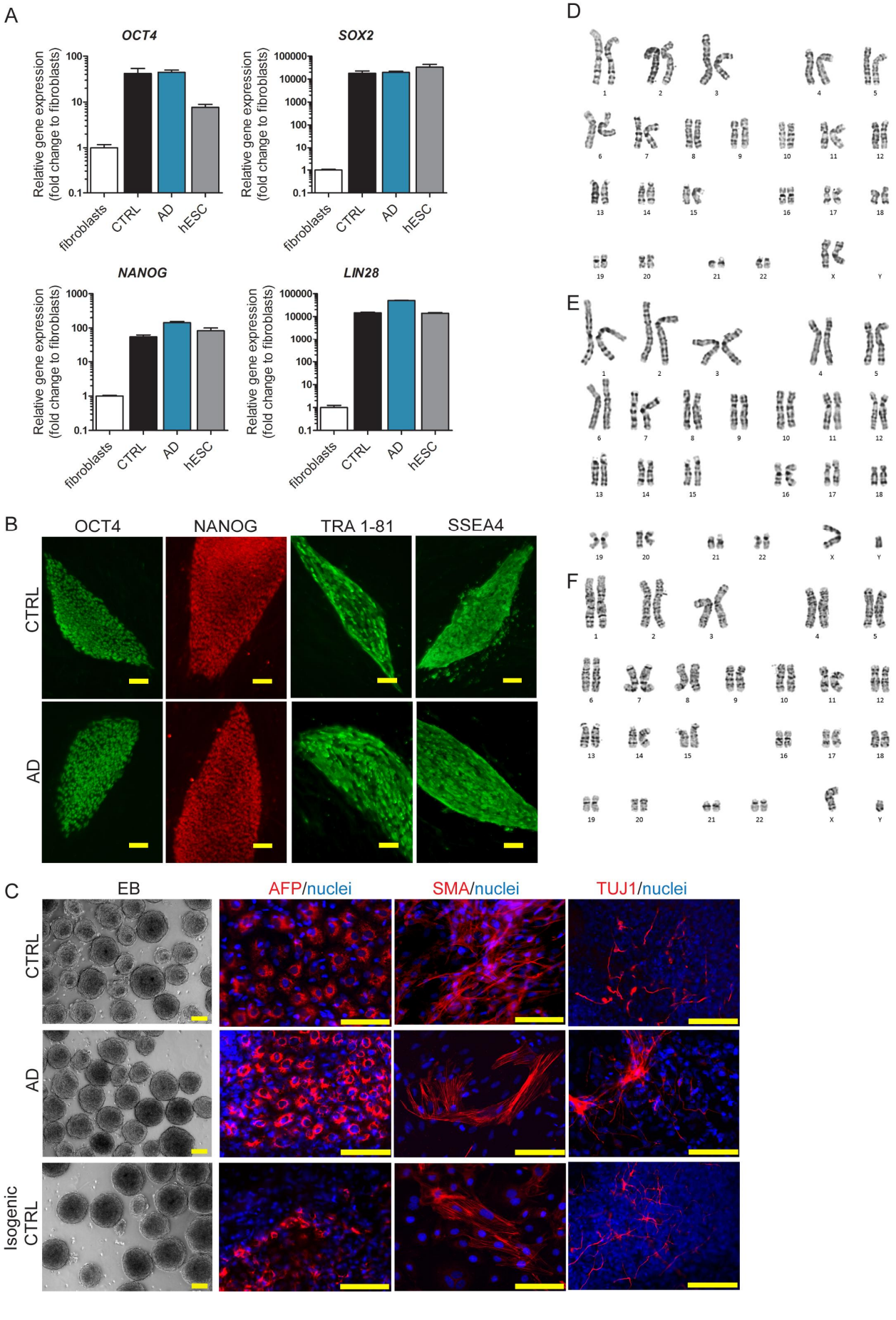

A
OCT4
SOX2
NANOG
LIN28
Relative gene expression (fold change to fibroblasts)
fibroblasts
CTRL
AD
hESC
B
OCT4
NANOG
TRA 1-81
SSEA4
CTRL
AD
C
EB
AFP/nuclei
SMA/nuclei
TUJ1/nuclei
CTRL
AD
Isogenic CTRL
D
E
F

Figure S2. Characterization of iPSC lines. Related to Table 1, Table S1 and all the Figures.
(A) Relative gene expression levels of *OCT4*, *SOX2*, *NANOG* and *LIN28* shown as fold change to fibroblasts. Representative data from one control (Ctrl3) and one AD patient (AD2) are shown. Fibroblasts are included as negative control and HS306 embryonic stem cells (hESCs) as positive control. Data are presented as mean ± SEM.
(B) Representative immunocytochemistry images of OCT4, NANOG, TRA 1-81 and SSEA4 from one control line and one AD line grown on human foreskin fibroblast feeders. Scale bars 100 μm.
(C) Representative bright field images of embryoid bodies (EB) grown in suspension for two weeks and representative immunocytochemistry images of alpha-fetoprotein (AFP, endoderm; red), smooth muscle actin (SMA, mesoderm; red) and beta III tubulin (TUJ1, ectoderm; red) from one control, one AD and one isogenic control line. Nuclei are stained with Hoechst. Scale bars 100 μm.
(D-F) Representative karyograms from one control (D), AD (E) and isogenic control (F) iPSC line showing normal euploid karyotypes (46,XX for D and 46, XY for E and F).
See also Table S1.

Figure S3

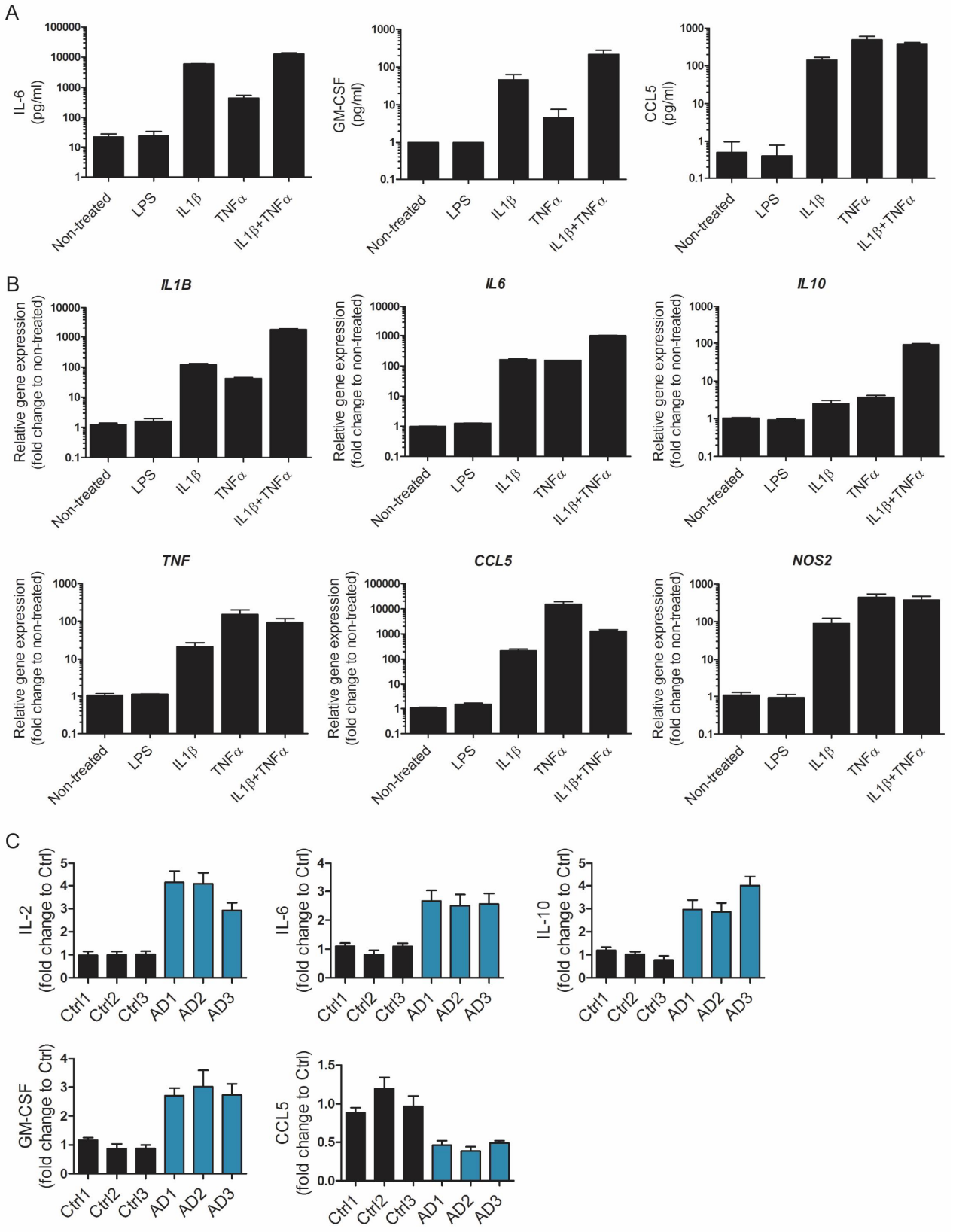

A
IL-6 (pg/ml)
GM-CSF (pg/ml)
CCL5 (pg/ml)
Non-treated
LPS
IL1β
TNFα
IL1β+TNFα
B
IL1B
IL6
IL10
TNF
CCL5
NOS2
Relative gene expression (fold change to non-treated)
C
IL-2 (fold change to Ctrl)
IL-6 (fold change to Ctrl)
IL-10 (fold change to Ctrl)
GM-CSF (fold change to Ctrl)
CCL5 (fold change to Ctrl)
Ctrl1
Ctrl2
Ctrl3
AD1
AD2
AD3

Figure S3. Cytokine profiles of iPSC-derived control astrocytes after different proinflammatory stimuli. Related to Figure 1 and Figure 4.

(A) Astrocytes were either left untreated or treated with 1 µg/ml LPS, 10 ng/ml IL1β, 50 ng/ml TNFα or a combination of IL1β and TNFα for 48 hours. Media was analyzed with CBA assay for the concentrations of secreted IL-6, GM-CSF and CCL5. Representative data from one control line are shown. Data are presented as mean ± SEM from two independent experiments.

(B) Relative gene expression levels of *IL1B*, *IL6*, *IL10*, *TNF*, *CCL5* and *NOS2* after 48 hours of treatment with 1 µg/ml LPS, 10 ng/ml IL1β, 50 ng/ml TNFα or a combination of IL1β and TNFα shown as fold change to untreated cells. Representative data from one control line are shown. Data are presented as mean ± SEM from two independent experiments.

(C) Quantification of IL-2, IL-6, IL-10, GM-CSF and CCL5 concentrations in the media after stimulation with TNFα (50 ng/ml) and IL1β (10 ng/ml) for 48 hours, using CBA assay. Results from individual donors are shown as fold change to control lines (Ctrl1, Ctrl2, Ctrl3). Data are presented as mean ± SEM from three independent experiments

Figure S4

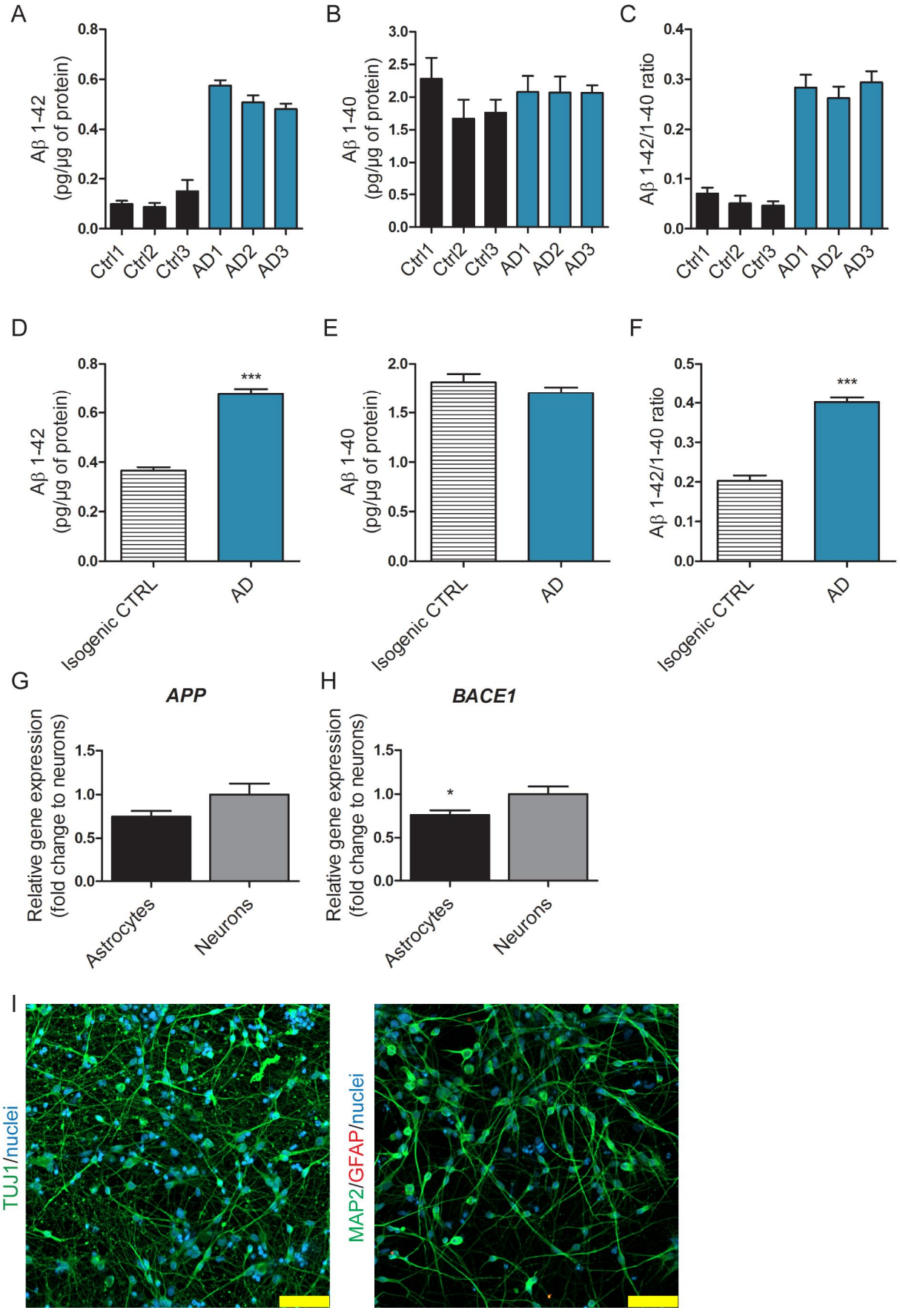

A
Aβ 1-42 (pg/µg of protein)
0.8
0.6
0.4
0.2
0.0
Ctrl1
Ctrl2
Ctrl3
AD1
AD2
AD3
B
Aβ 1-40 (pg/µg of protein)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
C
Aβ 1-42/1-40 ratio
0.4
0.3
0.2
0.1
0.0
D
***
Isogenic CTRL
AD
E
F
G
APP
Relative gene expression (fold change to neurons)
1.5
1.0
0.5
0.0
Astrocytes
Neurons
H
BACE1
*
I
TUJ1/nuclei
MAP2/GFAP/nuclei

Figure S4. *PSEN1 ΔE9* astrocytes and neurons have similar amyloidogenic properties. Related to Figure 2.
(A-C) Aβ 1-42 (A), Aβ 1-40 (B) and Aβ 1-42/1-40 ratio (C) were quantified from conditioned media (72 hours) and normalized to total protein content. Results are shown for individual donors as mean ± SEM from three independent experiments.
(D-F) Amounts of Aβ 1-42 (D), Aβ 1-40 (E) and Aβ 1-42/1-40 ratio (F) were quantified from conditioned media (72 hours) of iPSC-derived neurons matured for two weeks, with ELISA, and normalized to total protein content of each well. Data are presented as mean ± SEM from two isogenic pairs. (n=3 independent experiments, *** $p<0.001$)
(G) Relative gene expression levels of *APP* in matured astrocytes and neurons shown as fold change to iPSC-derived neurons. Data are presented as mean ± SEM. (Astrocytes n=14 lines, neurons n=9 lines)
(H) Relative gene expression levels of *BACE1* in matured astrocytes and neurons shown as fold change to iPSC-derived neurons. Data are presented as mean ± SEM. (Astrocytes n=14 lines, neurons n=9 lines, * $p<0.05$)
(I) Characterization of iPSC-derived neuronal cultures after 2 weeks of maturation. Representative immunocytochemistry images showing beta III tubulin (TUJ1; green) and MAP2 (green) positive neurons. GFAP (red) positive astrocytes were not detected. Nuclei were stained with Hoechst. Scale bars 50 µm.

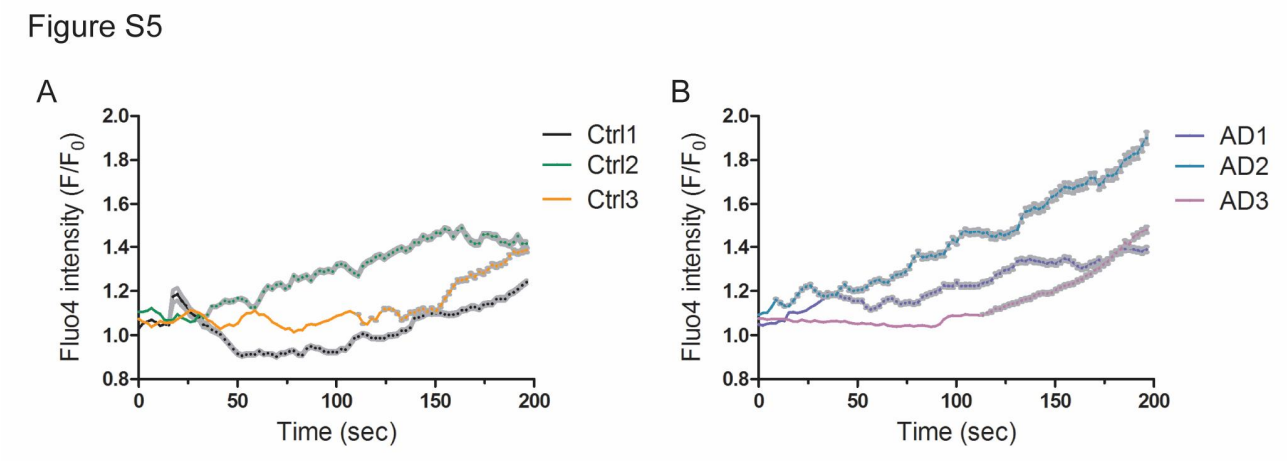


Figure S5. *PSEN1 ΔE9* astrocytes show increased Ca^{2+} leakage from the ER. Related to Figure 3.
(A) Average traces of control astrocytes from individual donors in the presence of 50 µM ryanodine, 100 µM 2APB and 1 µM thapsigargin. Results are shown as mean ± SEM (in gray). (Ctrl1 n=260, Ctrl2 n=289 and Ctrl3 n=265)
(B) Average traces of AD astrocytes from individual donors in the presence of 50 µM ryanodine, 100 µM 2APB and 1 µM thapsigargin. Results are shown as mean ± SEM (in gray). (AD1 n=157, AD2 n=274, AD3 n=109)

Table S1. Summary of the characterizations of iPSC lines used in this study. Related to Table 1 and all Figures.

iPSC line	Reprogramming method	Morphology	Pluripotency gene expression[a]	Pluripotency protein expression[b]	EB formation	Three germ layers[c]	Karyotype	Reference
Ctrl1A	SeV 1.0	+	+	+	+	+	normal	[d] (UEF-2B)
Ctrl1B	SeV 1.0	+	+	+	+	+	normal	[d] (UEF-2C)
Ctrl2A	Lenti	+	+	+	+	+	normal	[d] (UEF-3A)
Ctrl2B	SeV 1.0	+	+	+	+	+	normal	[d] (UEF-3B)
Ctrl3A	SeV 1.0	+	+	+	+	+	normal	This paper
Ctrl3B	SeV 1.0	+	+	+	+	+	normal	This paper
AD1A	SeV 1.0	+	+	+	+	+	normal	This paper
AD1B	SeV 1.0	+	+	+	+	+	normal	This paper
AD2A	SeV 2.0	+	+	+	ND	ND	normal	This paper
AD2B	SeV 2.0	+	+	+	+	+	normal	This paper
AD2B iso	-	+	ND	ND	+	+	normal	This paper
AD3A	SeV 2.0	+	+	+	+	+	normal	This paper
AD3B	SeV 2.0	+	+	+	ND	ND	normal	This paper
AD3B iso	-	+	ND	ND	+	+	normal	This paper

[a] Expression levels of *OCT4, SOX2, NANOG* and *LIN28* by qRT-PCR

[b] Expression of OCT4, NANOG, TRA 1-81 and SSEA4 by immunocytochemistry

[c] Expression of AFP (endoderm), SMA (mesoderm) and TUJ1 (ectoderm) by immunocytochemistry

[d] These lines have been previously characterized in Holmqvist et al., 2016

ND: not determined, EB: embryoid body

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Generation of isogenic control lines using CRISPR/Cas9. Related to Experimental procedures.
SgRNA pair was identified using the crispr.mit.edu design tool (sequences listed below) and the sgRNA sequences were cloned into a Cas9 sgRNA plasmid (Addgene #68463). The PL552 donor plasmid (Addgene #68407) with 1 kb homology arms, was designed to restore an approximately 700 bp fragment containing exon 9 and the immediately-flanking intronic sequence (350 bp on the 5' end of exon 9 and 250 bp on the 3' end of exon 9) of the full 4.6-kb deletion. Between the 3' intronic sequence and the 3' homology-directed repair arm is a LoxP-flanked PGK-puromycin resistance cassette to facilitate the selection of cells exhibiting HDR at the *PSEN1* locus. The hCas9_D10A plasmid was from Addgene (#41816).
Electroporations were performed using the Neon Transfection System (Invitrogen). iPSCs were cultured in E8 media on Matrigel coated plates and treated with 0.5 µM ROCK-inhibitor for 24 hours prior to electroporation. Cells were harvested with 0.5 mM EDTA, resuspended at $2x10^6$/100 µl, mixed with plasmid cocktail (6 µg hCas9_D10A, 10 µg donor, 2 µg each sgRNA), electroporated (1100 V, 20 ms and 2 pulses) and re-plated on Matrigel in E8 media with 5 µM ROCK-inhibitor. When colonies started to emerge (after 3-4 days), cells were treated with puromycin (0.25 µg/ml, Gibco) for 48-72 hours. Surviving cells were cultured in E8 media until colonies were of sufficient size for picking.
To remove the floxed PGK-puromycin resistance cassette, cells were treated with 10 µl of recombinant TAT-CRE recombinase (Millipore) in E8 media for 20 minutes at 37°C after which the cell were cultured as usual.
Genomic DNA was isolated using QuickExtract DNA Extraction Solution 1.0 (Epicentre).
PCR amplifications were performed using either Q5 high-fidelity polymerase (New England Biosystems) or Phusion high-fidelity polymerase (Thermo Scientific).
Sanger sequencing of the plasmids was performed at Quintara Biosciences and sequencing of the iPSC clones at the Genome Center of Eastern Finland.

Table of SgRNAs and primers used in the generation and screening of isogenic control lines.

Primer pair	Target	Sequence
	sgRNA 1A	TTTTCTATCAGGTTTACTAGTGG
	sgRNA 1B	GGAAAGAATTGCCACAAGCCTGG
1	5' homology arm (cloning)	F: GAAGTGGTTCACTCTGGGAGC R: CCCAGCCTGTTTATGGATTTCC
2	3' homology arm (cloning)	F: GGCCGCTGCTGCCATCAAGA R: TCTTCCTGTCTTCCCAGTGCCATA
3	Exon 9 donor (cloning)	F: TCACCATCTGAGGCTTTTGTGA R: ACCTTTCCATGCTGGTATTCTGT
4	5´ location confirmation	F: GCAAAATGCTCTTACTGACAGG R: ACCCGGTAGAATTTCGAGGT
5	3´ location confirmation	F: GGGAGGATTGGGAAGACAAT R: AGCATGCTCATTGTGCCATA
6	Intact normal allele	F: TCGCTGGTGGCTTCATTTCC R: GCAGCAAGCTGAGTCCAATC
7	Exon 9 deletion	F: GAAGTGGTTCACTCTGGGAGC R: TCTTCCTGTCTTCCCAGTGCCATA
8	CRE-removal confirmation	F: TCACCATCTGAGGCTTTTGTGA R: ACCTACATGCTCACAGACAATCA
9	Whole targeted area for sequencing	F: GCAAAATGCTCTTACTGACAGG R: ATGCTCATTGTGCCATAACTTG
10	Exons 8-10 at transcript level	F: GTGGCTGTTTTGTGTCCGAA R: TCCCATTCCTCACTGAACCC

Quantitative RT-PCR
RNA was extracted by RNeasy Mini Kit (Qiagen) followed by cDNA synthesis with Maxima reverse transcriptase (Life Technologies), or with TaqMan® Gene Expression Cells-to-C_T™ Kit (Applied Biosystems). The mRNA expression levels were determined by quantitative RT-PCR (StepOne Plus™ Real-Time PCR system; Life Technologies) using TaqMan assay mixes (Life Technologies) listed below. Expression levels were normalized to β-Actin (ACTB; Applied Biosystems) using Q-gene program (Equation 2).

Table of TaqMan assay mixes used for mRNA expression studies.

OCT4	Hs00742896_s1
NANOG	Hs02387400_g1
SOX2	Hs01053049_s1
LIN28	Hs00702808_s1
GFAP	Hs00909233_m1
S100B	Hs00902901_m1
SLC1A2	Hs01102423_m1
SLC1A3	Hs00188193_m1
AQP4	Hs00242342_m1
IL1B	Hs01555410_m1
IL6	Hs00985639_m1
IL10	Hs00961622_m1
TNF	Hs99999043_m1
CCL5	Hs00982282_m1
NOS2	Hs01075529_m1
APP	Hs00169098_m1
BACE1	Hs01121195_m1

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde and stained as previously described (Puttonen et al., 2013). Primary antibodies and dilutions are listed in Supplemental experimental procedures. Alexa Fluor 488 or 568 conjugated secondary antibodies (1:500, Thermo Fisher Scientific) were used for detection and nuclei were counterstained with Hoechst 33258 (Sigma).

Table of primary antibodies used for immunocytochemistry.

Target	**Dilution**	**Source**	**Catalog number**
OCT4	1:400	Chemicon	MAB4401
NANOG	1:100	R&D Systems	AF1997
TRA 1-81	1:200	Chemicon	MAB4381
SSEA4	1:400	Chemicon	MAB4304
AFP	1:500	Sigma	A8452
SMA	1:500	Sigma	A5228
TUJ1	1:2000	Covance	MMS-435P
GFAP	1:500	Dako	Z0334
S100β	1:1000	Abcam	ab11178
MAP2	1:400	Chemicon	MAB3418

Embryoid body assay

For embryoid bodies (EBs), iPSC colonies were mechanically lifted from Matrigel plates and cultured in suspension for 2 weeks using ultra-low attachment plates (Corning). The EBs were plated down on matrigel-coated coverslips to allow outgrowth of differentiated cells for 2 weeks. Media (1:1 mixture of E8-medium and ES-medium without bFGF) was changed every 2-3 days.

Karyotyping

iPSCs were arrested with 200 ng/ml KaryoMAX® Colcemid™ Solution (Invitrogen) and harvested. Karyotyping was performed at the Eastern Finland Laboratory Centre Joint Authority Enterprise (ISLAB, Kuopio, Finland) using Giemsa (G-banding) staining. A total of 20 metaphase cells were analyzed per line.

Western blotting

Western blotting was done as previously described (Viswanathan et al., 2011) with the following antibodies and dilutions: PS-1 (MAB5232, 1:1000; Chemicon), APP (A8717, 1:2000; Sigma) and GAPDH (ab8245, 1:15 000; Abcam).

Neuronal differentiation

Neuronal differentiation was carried out similarly to astroglial differentiation (described in the Experimental methods) until the neural progenitor sphere stage. To support neuronal enrichment, spheres were maintained in NDM media supplemented with 0.5 IU/ml heparin, 20 ng/ml bFGF and 10 ng/ml EGF. For neuronal maturation, spheres were dissociated with Accutase and plated on to poly-l-ornithine/Matrigel-coated dishes in NDM supplemented with 10 ng/ml BDNF and 10 ng/ml GDNF (both from Peprotech).

SUPPLEMENTAL REFERENCES

Puttonen, K.A., Ruponen, M., Kauppinen, R., Wojciechowski, S., Hovatta, O., and Koistinaho, J. (2013). Improved method of producing human neural progenitor cells of high purity and in large quantities from pluripotent stem cells for transplantation studies. Cell Transplant *22*, 1753-1766.