Supplementary Information

APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes across human iPSC-derived brain cell types

Yuan-Ta Lin, Jinsoo Seo, Fan Gao, Heather M. Feldman, Hsin-Lan Wen, Jay Penney, Hugh P. Cam, Elizabeta Gjoneska, Waseem K. Raja, Jemmie Cheng, Richard Rueda, Oleg Kritskiy, Fatema Abdurrob, Zhuyu Peng, Blerta Milo, Chung Jong Yu, Sara Elmsaouri, Dilip Dey, Tak Ko, Bruce A. Yankner and Li-Huei Tsai

Figure S1.

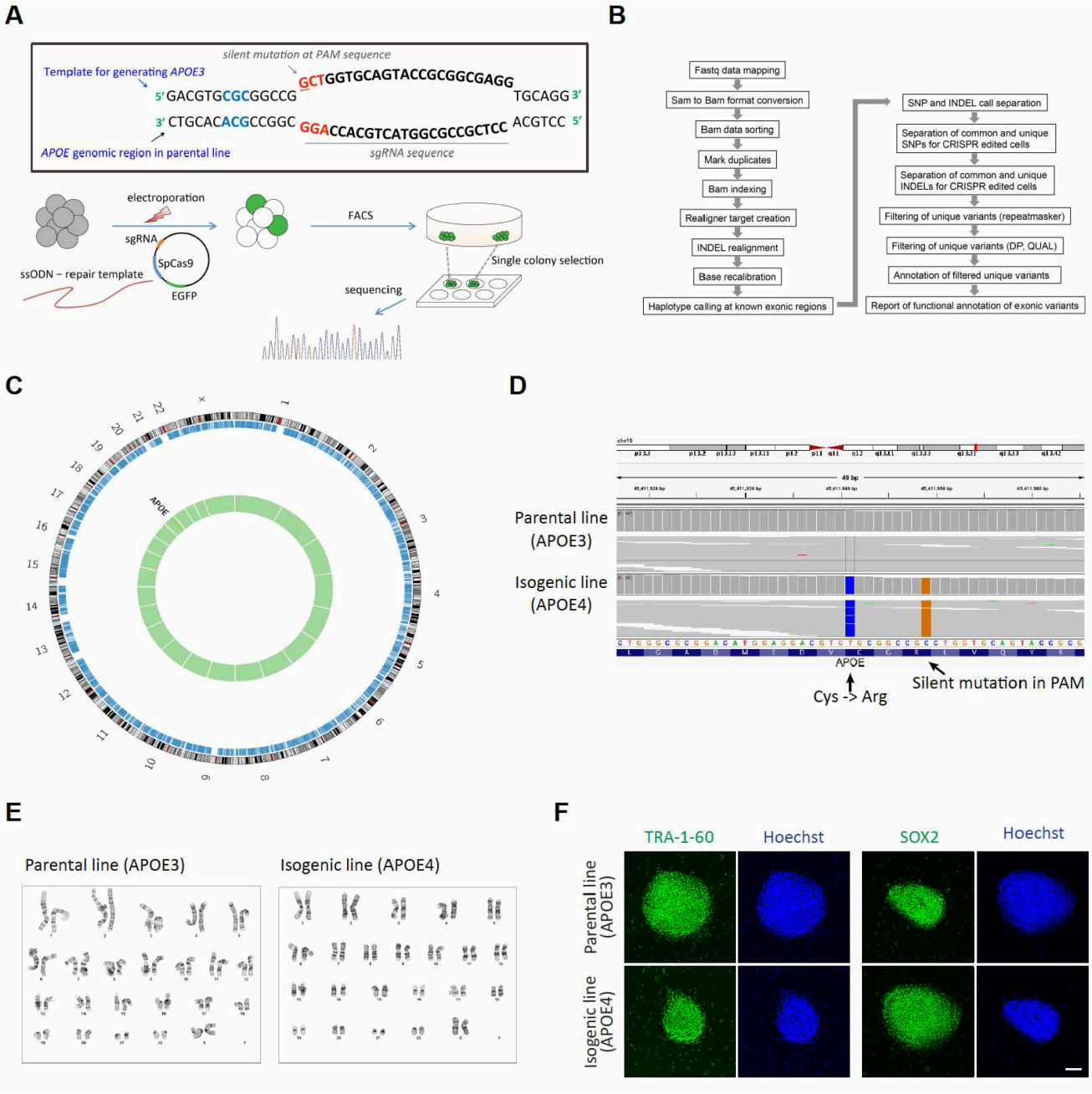
A
silent mutation at PAM sequence
Template for generating APOE3
GCTGGTGCAGTACCGCGGCGAGG
5′ GACGTGCGCGGCCG
TGCAGG 3′
3′ CTGCACACGCCGGC
GGACCACGTCATGGCGCCGCTCC
ACGTCC 5′
APOE genomic region in parental line
sgRNA sequence
electroporation
sgRNA
SpCas9
ssODN – repair template
EGFP
FACS
Single colony selection
sequencing
B
Fastq data mapping
Sam to Bam format conversion
Bam data sorting
Mark duplicates
Bam indexing
Realigner target creation
INDEL realignment
Base recalibration
Haplotype calling at known exonic regions
SNP and INDEL call separation
Separation of common and unique SNPs for CRISPR edited cells
Separation of common and unique INDELs for CRISPR edited cells
Filtering of unique variants (repeatmasker)
Filtering of unique variants (DP, QUAL)
Annotation of filtered unique variants
Report of functional annotation of exonic variants
C
APOE
D
Parental line (APOE3)
Isogenic line (APOE4)
APOE
Cys -> Arg
Silent mutation in PAM
E
Parental line (APOE3)
Isogenic line (APOE4)
F
TRA-1-60
Hoechst
SOX2
Hoechst
Parental line (APOE3)
Isogenic line (APOE4)

Figure S1 (Related to Figure 1). Generation of isogenic *APOE4* iPSCs.

(A) Schematics for CRISPR/Cas-9 genome editing. We designed a sgRNA targeting *APOE* gene near Cys112 coding region. Then, this sgRNA was integrated into a plasmid containing *S. pyogenes*-Cas9 coding gene and EGFP sequence. As a repair template, ssODN was designed to carry 200 nucleotides including coding sequence for Arg112 instead of Cys112, and silent mutation at PAM site to prevent additional DNA breaks in edited cells. iPSCs were electroporated to introduce the plasmid along with ssODN, and then only GFP-positive, Cas9-expressing cells were collected by FACS. Cells were then plated sparsely to allow single-cell colony formation. Each colony was inspected by Sanger sequencing to investigate whether Cys112 coding sequence was correctly replaced with Arg112 sequence.

(B) The pipeline for whole exome sequencing data to inspect potential off-target insertion/deletion or single nucleotide variation (SNV) by CRISPR/Cas9 genome editing.

(C) The genomes represented as concentric circles to show differential nucleotide sequence in isogenic *APOE4* iPSCs compared to parental *APOE3* iPSCs. The black outer ring depicts the human chromosomes and the blue ring represents a map of gene densities. The green ring indicates variant display background, and variant location (only *APOE* gene) shown as red-color line in the green circle.

(D) The genome browser shows two SNVs, *APOE3/4* coding locus and silent mutation insertion in PAM, in *APOE* gene of isogenic *APOE4* iPSCs compared to parental *APOE3* iPSCs.

(E) Both isogenic and parental iPSCs have normal karyotypes.

(F) Immunocytochemistry with TRA-1-60 and SOX2 antibodies in isogenic *APOE4* iPSCs and parental *APOE3* iPSCs. Scale bar = 80 μm.

Figure S2.

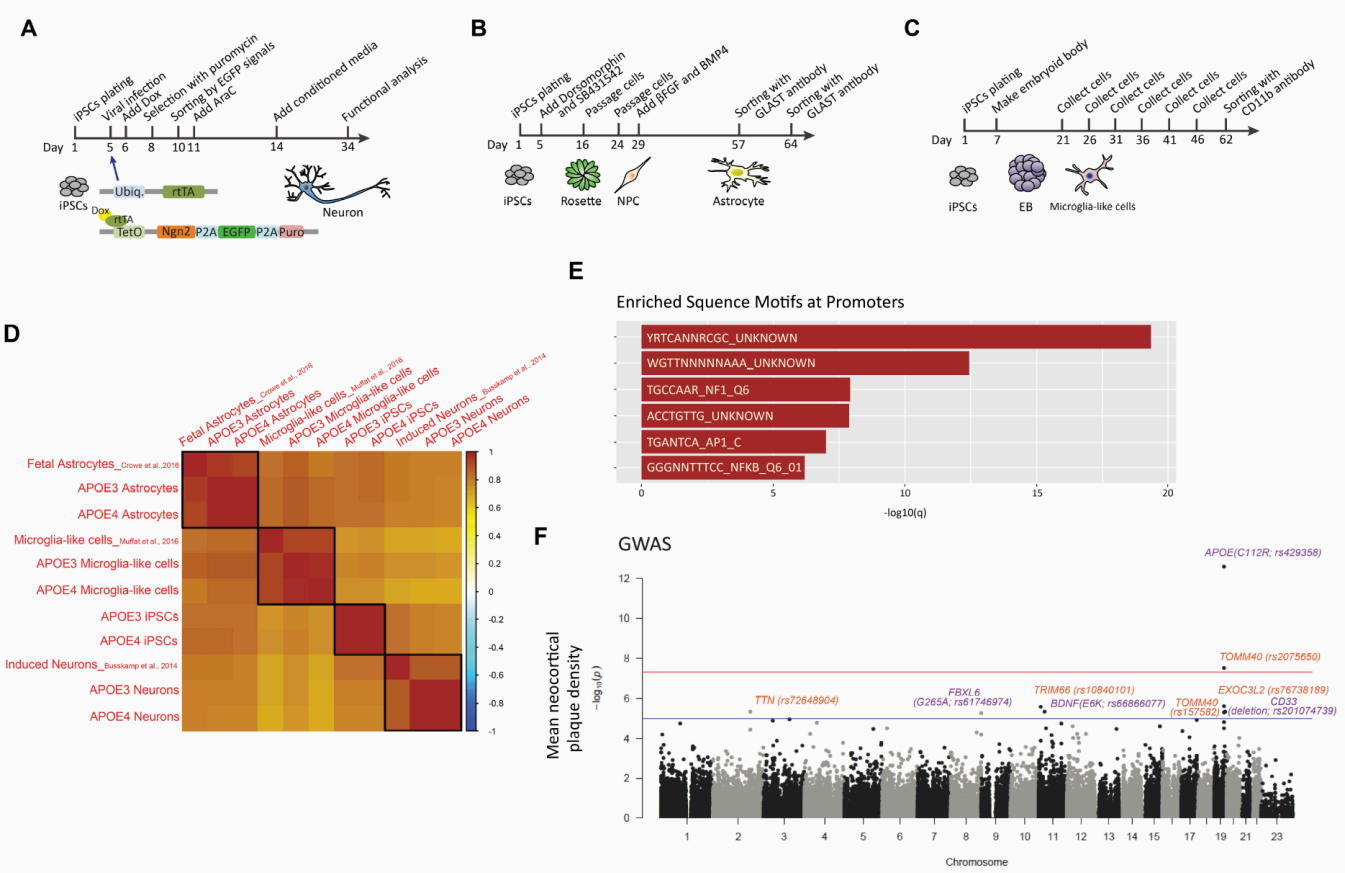

Figure S2 (Related to Figure 1). Generation of neurons, astrocytes and microglia-like cells from isogenic iPSCs carrying either *APOE3* or *APOE4* alleles.

(A-C) Schematics for generating iPSC-induced neurons, astrocytes and microglia-like cells.

(D) Hierarchical clustering of RNA-seq data from iPSC-derived neurons, astrocytes and microglia-like cells carrying either *APOE3* or *APOE4* together with publicly available cell type specific RNA-seq data from other groups (see methods). Color scale represents Pearson's correlation coefficient. (E) Genome-wide association of SNP variants identified from human brain exome-seq data with phenotypic mean neocortical plaque density. SNP variants with p-value < 1E-5 are highlighted. Nonsynonymous variants are colored in purple.

Figure S3.

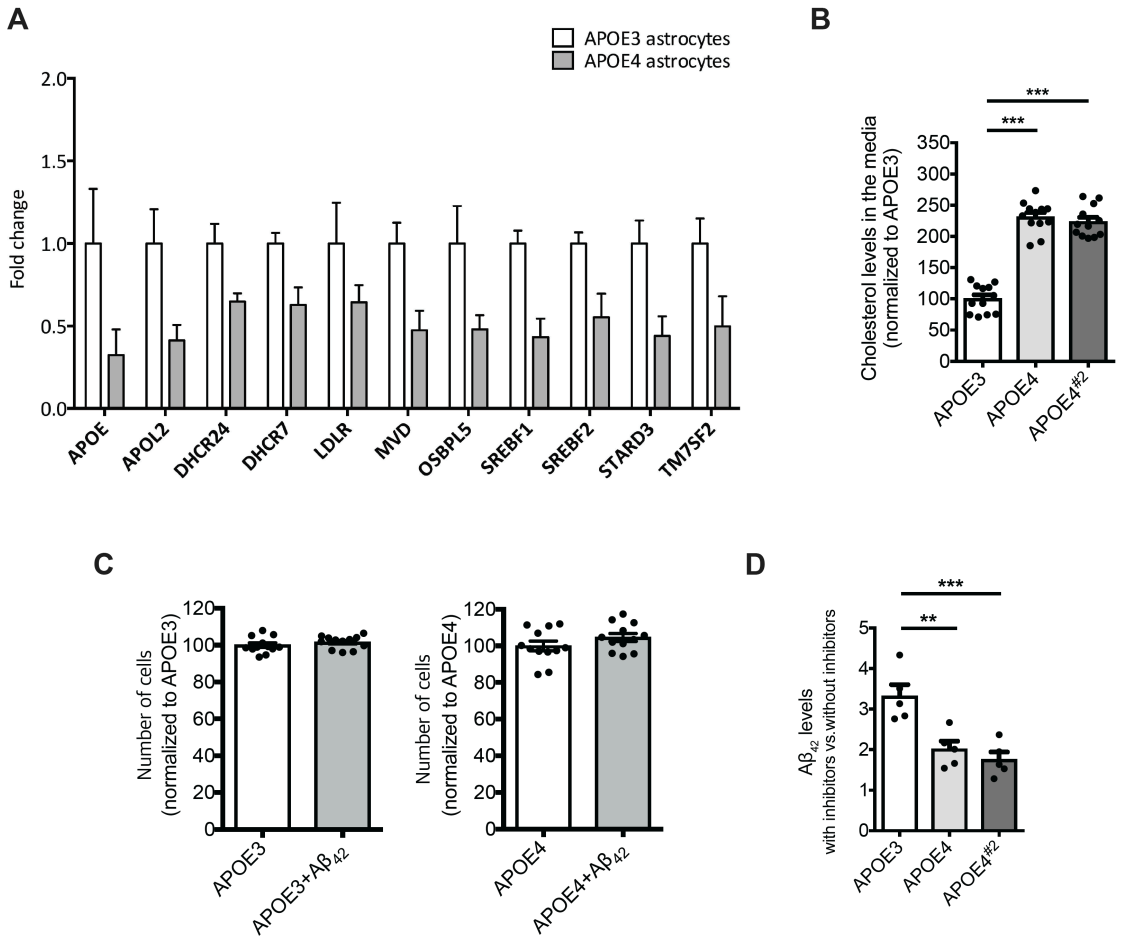

Figure S3 (Related to Figure 3). Altered lipid metabolism and impaired lysosome-dependent Aβ degradation in *APOE4* astrocytes compared to *APOE3* astrocytes.

(A) Decreased genes in the category of 'cholesterol/sterol metabolism and transport' by GO analysis in *APOE4* astrocytes (n=3, fold change: *APOE4* vs *APOE3*, q<0.05, error bars ± s.e.m.). (B) Extracellular cholesterol levels were measured in media from two independent *APOE4* isogenic astrocyte cultures and *APOE3* astrocyte cultures. n=12 from three independent cultures. (C) Number of cells in *APOE3* and *APOE4* astrocytes followed by $A\beta_{42}$ treatment (250 ng/ml for 2 days). n=12 from three independent cultures. (D) Lysosome-dependent $A\beta_{42}$ degradation in astrocytes induced from two independent *APOE4* iPSC clones and *APOE3* iPSC. n=5 from two independent cultures.

(***P*<0.01, ****P*<0.001 by Dunnett's test; error bars ± s.e.m.)

Figure S4.

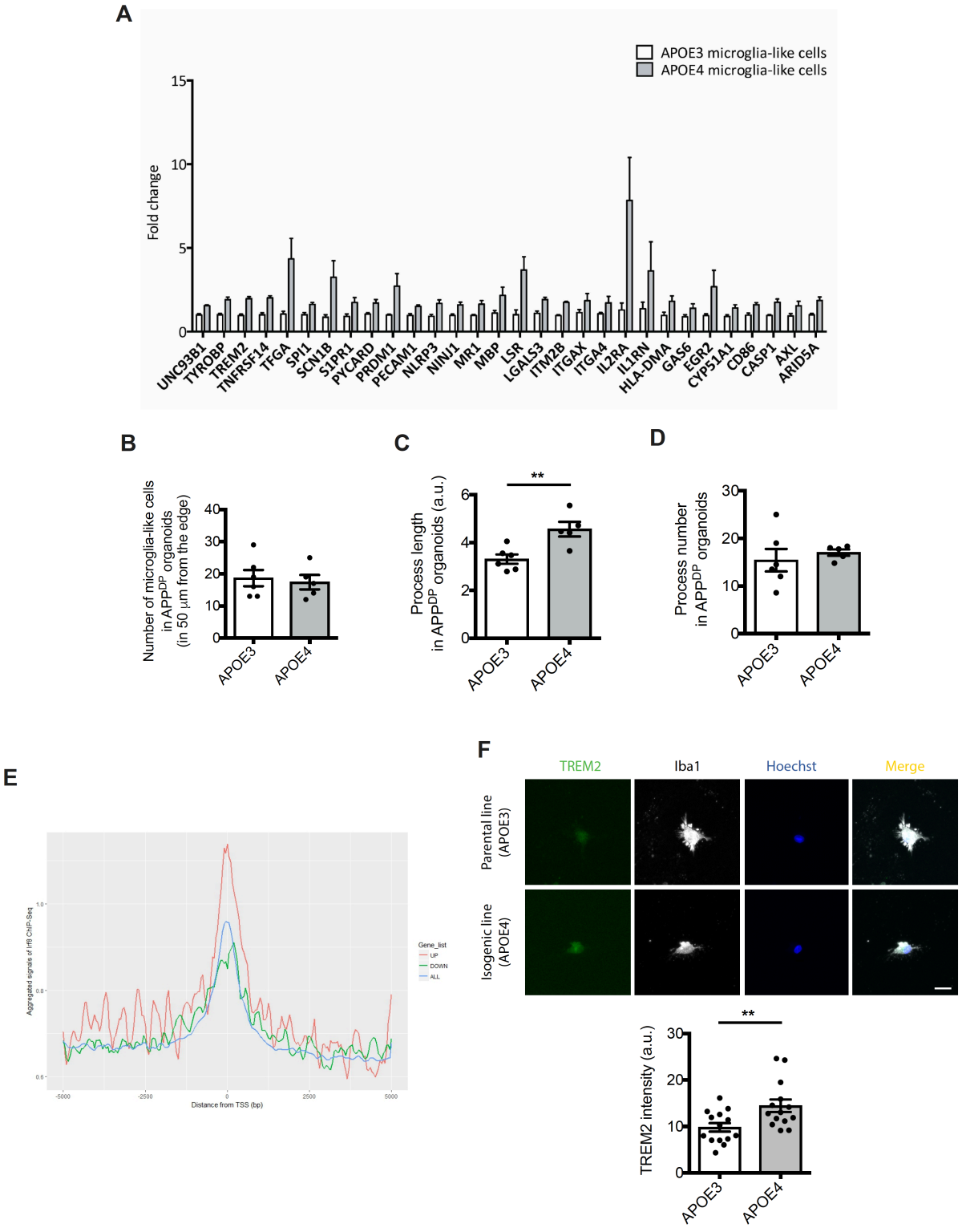
A
APOE3 microglia-like cells
APOE4 microglia-like cells
Fold change
UNC93B1 TYROBP TREM2 TNFRSF14 TFGA SPI1 SCN1B S1PR1 PYCARD PRDM1 PECAM1 NLRP3 NINJ1 MR1 MBP LSR LGALS3 ITM2B ITGAX ITGA4 IL2RA IL1RN HLA-DMA GAS6 EGR2 CYP51A1 CD86 CASP1 AXL ARID5A
B
Number of microglia-like cells in APP^DP organoids (in 50 µm from the edge)
APOE3
APOE4
C
Process length in APP^DP organoids (a.u.)
**
APOE3
APOE4
D
Process number in APP^DP organoids
APOE3
APOE4
E
Aggregated signals of Irf8 ChIP-Seq
Distance from TSS (bp)
Gene_list
UP
DOWN
ALL
F
TREM2
Iba1
Hoechst
Merge
Parental line (APOE3)
Isogenic line (APOE4)
TREM2 intensity (a.u.)
**
APOE3
APOE4

Figure S4 (Related to Figure 4). Pathological features in *APOE4* microglia-like cells.

(A) Increased genes in the category of 'inflammation of central nervous system' by Ingenuity Pathway Analysis in *APOE4* microglia-like cells (n=4, fold change: *APOE4* vs *APOE3*, q<0.05, error bars ± s.e.m.). (B) Number of microglia-like cells incorporated into APP^{DP} organoids. n=5~6 organoids. (C, D) Process length and number of microglia-like cells in APP^{DP} organoids were examined by immocytochemistry with Iba1 antibody. n=5~6 organoids. (E) Aggregation plot of average Irf8 ChIP-Seq intensity signals around gene transcription start sites (TSS). Average signals for mouse ortholog sets of up- and down-regulated genes in *APOE4* microglia-like cells were compared. Signals for all mouse annotated genes were also included in the plot. (F) Immunocytochemistry with TREM2 and Iba1 antibody in *APOE3* or *APOE4* microglia-like cells. Bar graphs represent relative immunoreactivity of TREM2 in *APOE3* or *APOE4* microglia-like cells. n=14 from three independent cultures. Scale bar = 20 μm.

(**P<0.01, by Student's t-test; error bars ± s.e.m.)

Figure S5.

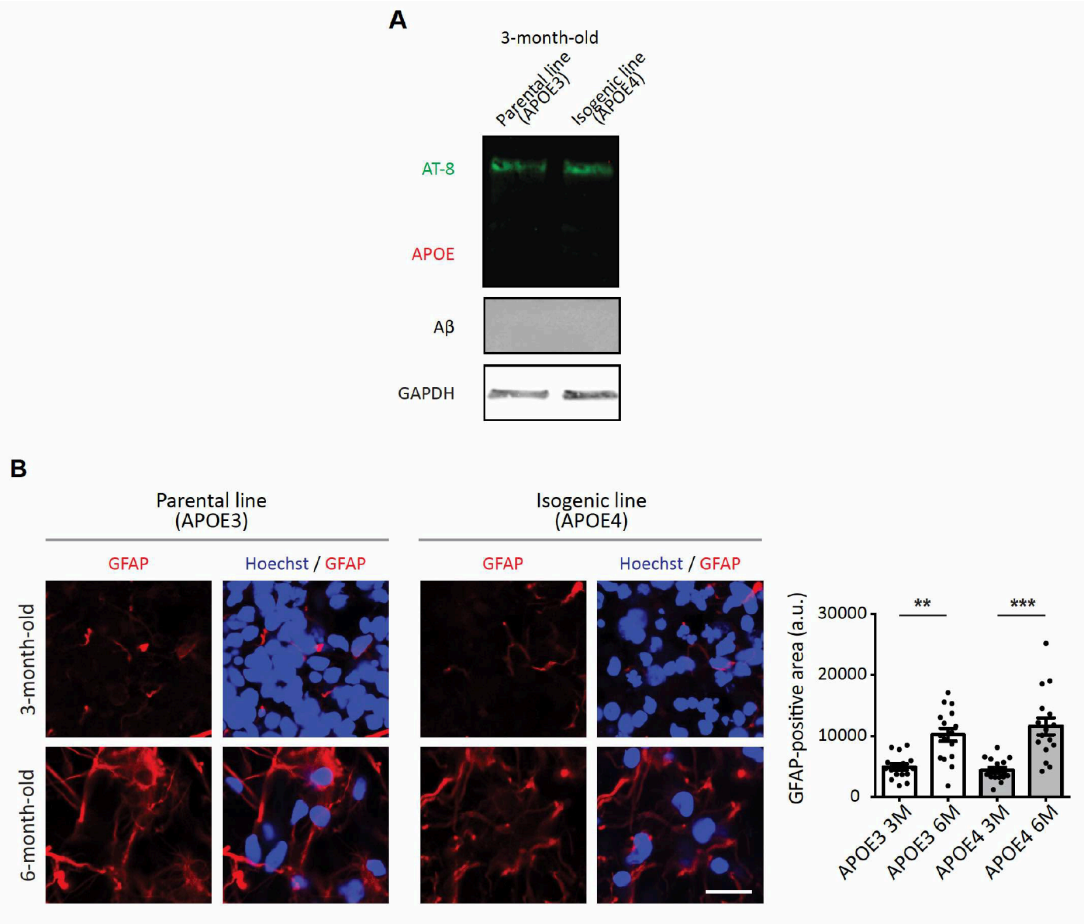

Figure S5 (Related to Figure 5). Increased GFAP-positive cells with age in iPSC-induced cerebral organoids.

(A) Three-month-old organoids from *APOE3* or *APOE4* iPSCs were lysed and subjected to the immunoblotting with antibodies against APOE, Aβ, AT-8 or GAPDH.

(B) Three-month and six-month-old organoids from *APOE3* or *APOE4* iPSCs were fixed and subjected to the immunostaining with GFAP antibody. A bar graph represents relative immunoreactivity of GFAP in organoids. Scale bar = 20 μm. n=16 images from 4~6 organoids. (P<0.0001 by ANOVA, **P<0.01, ***P<0.001 by Tukey's test; error bars ± s.e.m.)

Figure S6.

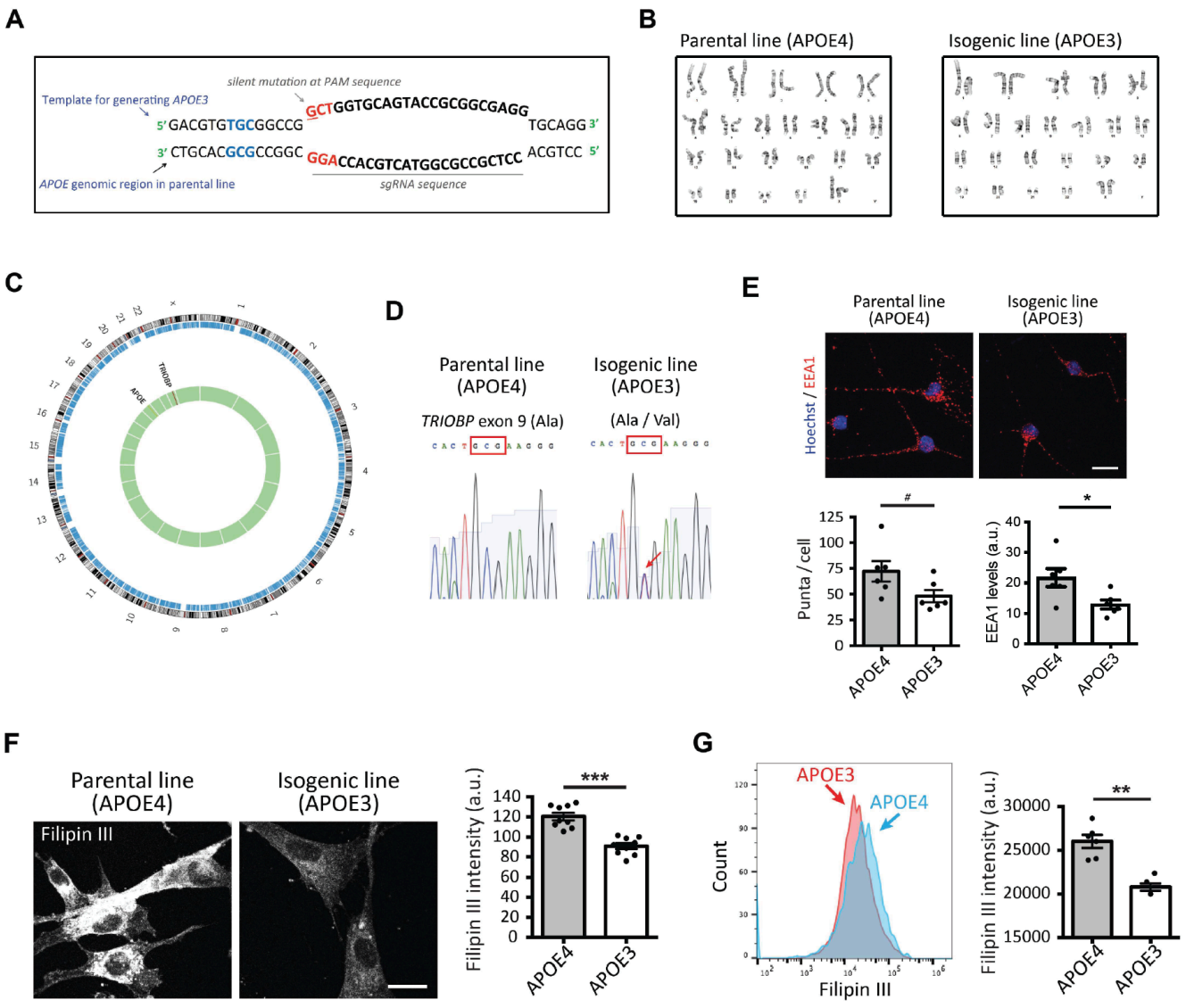

Figure S6 (Related to Figure 6). Converting *APOE4* to *APOE3* attenuates AD-related phenotypes in sAD iPSC-induced neurons, glia and organoids.

(A) The sequence of sgRNA and ssODN to generate isogenic *APOE3* iPSCs from sAD iPSCs carrying *APOE4* alleles.

(B) Both isogenic and parental iPSCs have normal karyotypes.

(C) The genome represented as concentric circles to show differential nucleotide sequence in isogenic *APOE3* iPSCs compared to parental *APOE4* iPSCs. The black outer ring depicts the human chromosomes and the blue ring represents a map of gene densities. The green ring indicates variant display background, and variant location (*APOE* and *TRIOBP* gene) shown as red-color line in the green circle.

(D) The variant in *TRIOBP* gene occurred in one allele and potentially results in replacement of the nonpolar Ala with nonpolar Val in exon 9.

(E) Immunocytochemistry with EEA1 antibody in iPSC-derived neurons from *APOE4* or *APOE3* iPSCs. Scale bar = 10 μm. n=6 from three independent cultures.

(F) iPSC-derived astrocytes were fixed and incubated with filipin III for 1hr followed by imaging. Scale bar = 10 μm. n=9 images from three independent cultures.

(G) iPSC-derived astrocytes were sorted based on the intensity of filipin III. n=3~6 per group.

($^{\#}P$=0.06, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 by Student's t-test; error bars ± s.e.m.)