Supplemental information

Cell-type-specific regulation of APOE and CLU

levels in human neurons

by the Alzheimer's disease risk gene SORL1

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Supplemental Information

Supplemental Figure and Table Legends

Supplemental Figure legends

Supp. Figure 1. Overview of iPSC differentiation protocols. Schematic of (A) iN, (B) iMGL, (C) iA, and (D) iEC differentiation protocol used in this study. For differentiation of iNs (neurons) and iAs (astrocytes), we used lentiviral transduction of cell type specific transcription factors to directly differentiate iPSCs into respective cell types. For differentiation of iMGLs (microglia) and iECs, non-viral differentiation methods were used. (E) Frequency distribution of the standard deviation of the log2 RNA expression level of all genes detected by RNAseq across cell type. (F) Shown are heatmaps of differentially expressed genes (DEGs) for a subset of pathways enriched in iNs, iAs, and/or iMGLs. Also shown are protein-level results for each pathway generated using TMT-MS. See also Supplemental Table S1 for full data set.

Supp. Figure 2. Expression of LOAD GWAS genes in SORL1 WT/KO iNs, iAs, iMGLs. (A) Heatmap of RNA expression levels of LOAD GWAS genes in iNs, iAs, and iMGLs. (B) Table of known LOAD GWAS genes differentially expressed in SORL1 WT/KO neurons, astrocytes, or microglia. (C-O) RNA levels (TPM) of LOAD GWAS genes in 5 different cell types (Only genes that are differentially expressed with SORL1 null (at least in one cell type) are graphed).

Supp. Figure 3. Generation of SORL1 KO iPSCs using CRISPR/Cas9. (A) Table showing the iPSC line source information. (B) Table with the information on the gRNAs used to target SORL1, and the edited sequence results for each clone used in this paper. (C) Sequence chromatogram of Line 2 WT and line 2 KO lines. (D-E) Table showing all the potential off-target loci identified by IDT CRISPR-Cas9 guide RNA design checker for (D) Cell line 1 and (E) Cell line 2. All potential off-target loci were sequenced and confirmed to be wild type for each of the lines. * For line#2, WT line showed a trend towards higher chromosome 20 dosage. Because this may indicate a low level of chromosome 20 duplication, we examined the RNAseq data from the resultant neurons. Examining all DEGs in our dataset in the line 2 comparison, 8.0% of genes detected were differentially expressed based on a cutoff of q<0.05. Within chromosome 20, only 6.5% of the genes detected were DEGs with the same cutoff, and only 24 of these were upregulated in wild type compared to SORL1 KO. These results suggest that wild type neurons in line 2 do not appear to express higher levels of chromosome 20 genes compared to their paired KO neurons.

Supp. Figure 4. SORL1 KO neurons show increased Aβ levels without altering APP levels. (A) Representative western blot of CLU protein expression in SORL1 WT and KO iNs. (B-C) Quantification of (B) CLUα chain/GAPDH and (C) CLU Precursor/GAPDH protein expression. (D-E) Representative western blot of APP protein expression in SORL1 WT and KO iNs in cell line 1 and 2. (F,J) Quantification of APP/GAPDH in iNs.(G,K) Aβ38 levels, (H,L) Aβ40 levels, and (I,M) Aβ 42/40 ratio in media generated from SORL1 WT and KO iNs in cell lines 1 and 2. Data show mean +/- SEM from three independent differentiations, for each differentiation, 3 wells were used for each group. Values are normalized to WT for each pair. Unpaired student's t test (two tailed).
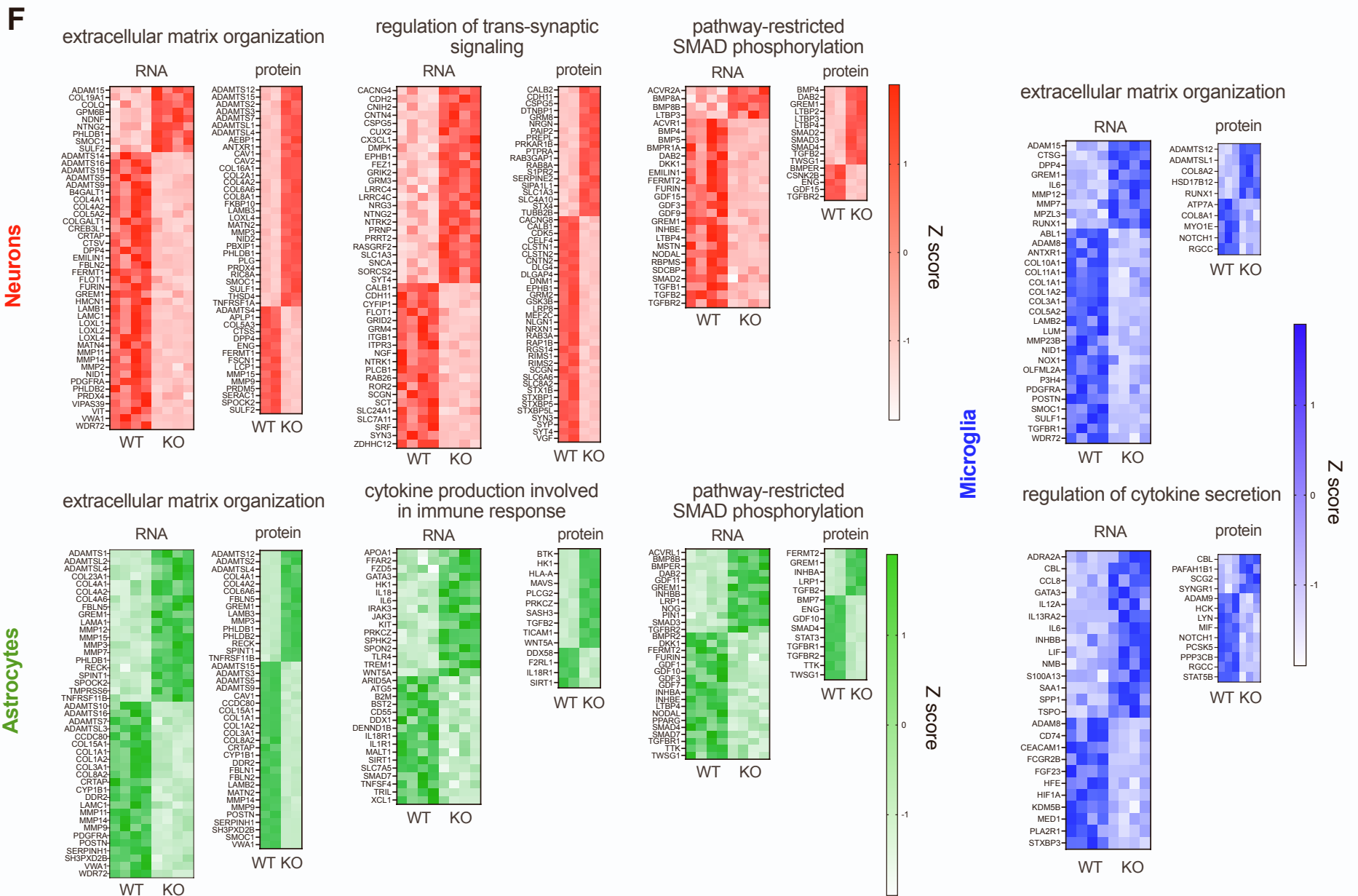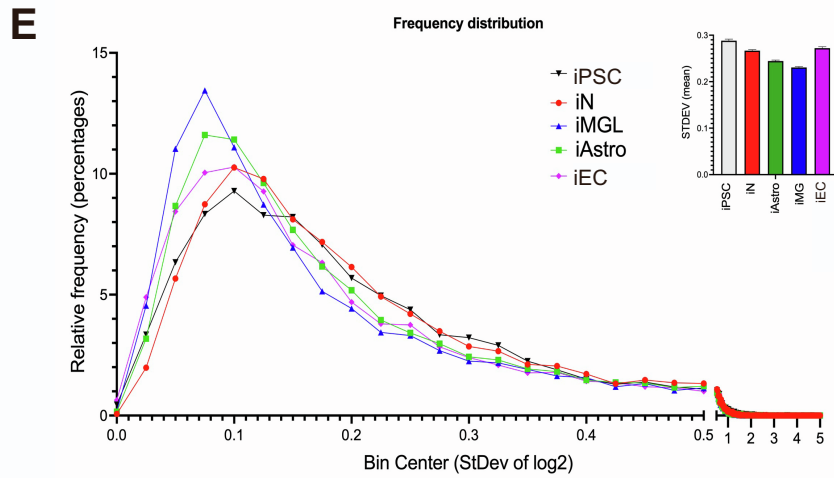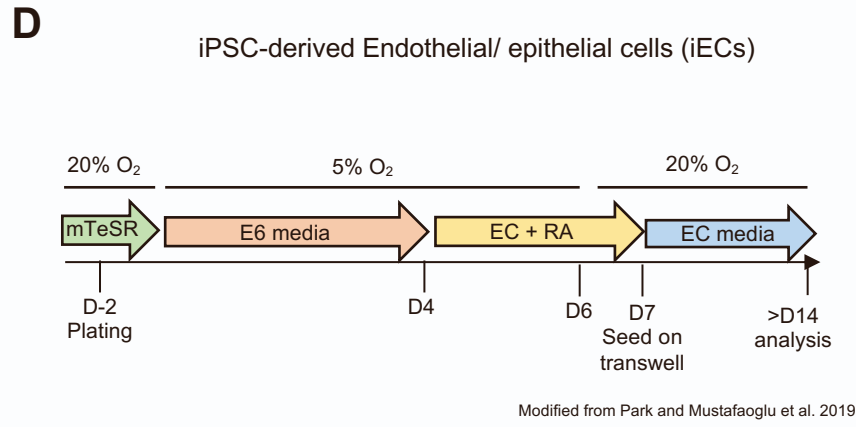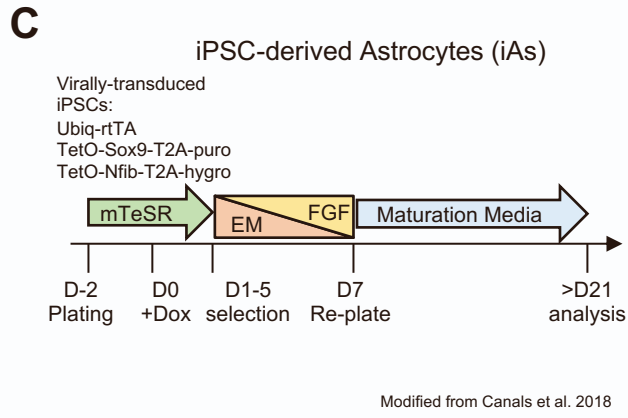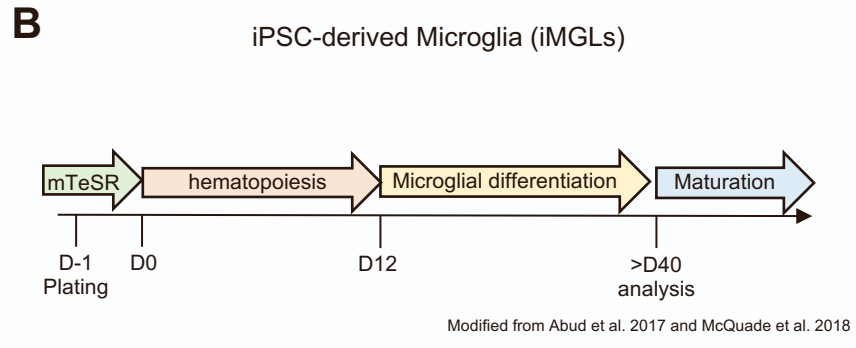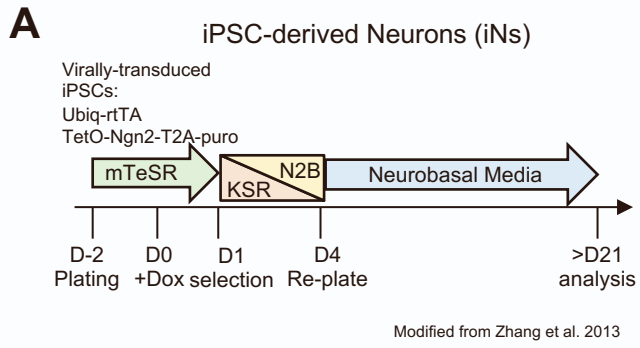
Supp. Figure 5. Loss of APOE does not affect SORL1 levels and p-tau levels in iNs. (A) Representative western blot of an isogenic set of iNs with APOE ε3/ε3, APOE ε4/ε4, and APOE KO genotypes. (B) Quantification of SORL1/GAPDH and (C) p-tau/tau. (D) Representative western blot of iNs that received APOE shRNA KD transduction. (E) Quantification of secreted APOE in the media, (F) SORL1/GAPDH, and (G) p-tau/tau. Data show mean +/- SEM from three independent differentiations, for each differentiation, 2-3 wells were used for each group. Values are normalized to APOE ε3/ε3 iNs or iNs with empty virus. One-way ANOVA with Tukey's multiple comparisons test.

Supp. Figure 6. Interrogation of candidate pathways associated with SORL1 loss-of-function. (A) Total Aβ levels after 72hr treatment of d17 iNs with 10uM or 20uM R33. Elevated Aβ levels in SORL1 KO iNs are partially rescued with 20 uM R33. (B) Aβ42/40 is not altered with R33 treatment. (C) Total Aβ levels in media of iNs after 72hr treatment with 100mM trehalose treatment. Elevated Aβ levels in SORL1 KO iNs are not rescued with trehalose. (D) Aβ42/40 is not altered with trehalose treatment. Data show mean +/- SEM from three differentiations, n=3 per differentiation for each line. Values are normalized to WT iNs treated with vehicle. One-way ANOVA with Tukey's multiple comparisons test.

Supp. Figure 7. SORL1 and APOE expression in iNs and iAs based on SORL1 and APOE genotype. (A,D) Western blot of iN d21 protein lysates and (G) iA d21 protein lysates from ROSMAP cohort. (B,C,E,F) SORL1 and APOE protein expression normalized to GAPDH categorized based on diagnosis. Data show mean +/- SEM. One-way ANOVA with Tukey's multiple comparisons test. (H,J) SORL1 protein levels and (I,K) APOE protein levels in iNs and iAs western blots from the ROSMAP cohort categorized based on APOE genotype. (L,N) SORL1 protein levels and (M,O) APOE protein levels in iNs and iAs western blots from the ROSMAP cohort categorized based on SORL1 5' haplotype. Data show mean +/- SEM. One-way ANOVA with Tukey's multiple comparisons test.
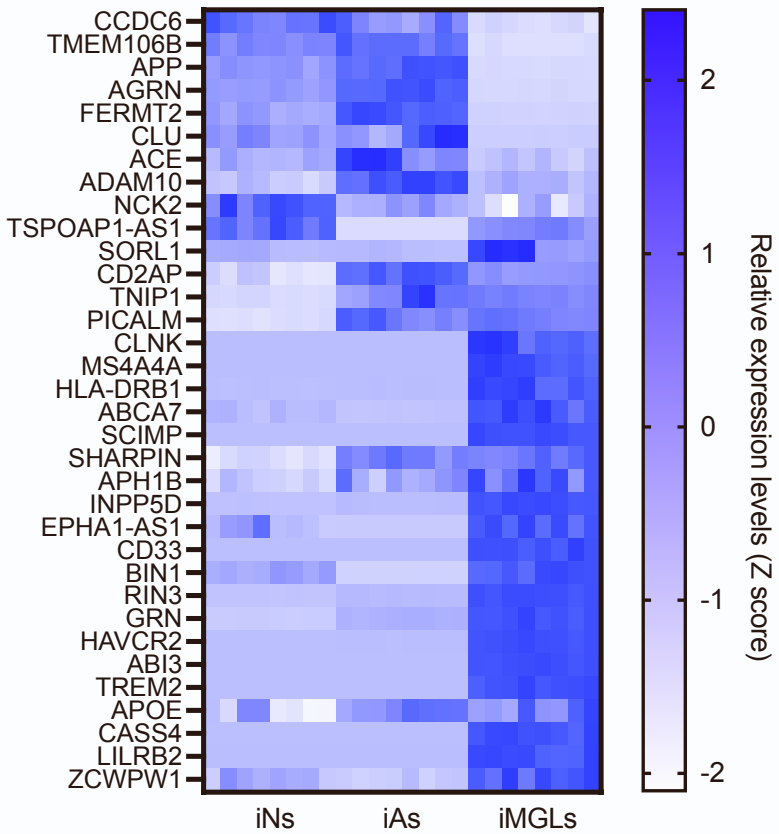
Supp. Figure 8. Association of SORL1, APOE, and CLU RNA levels in ROSMAP post-mortem brain tissue. (A,B) RNAseq from medial frontal cortex brain lysates of ROSMAP cohort. Shown are Pearson correlations between (A) SORL1 and APOE RNA levels (r=0.03, p=0.28) and (B) SORL1 and CLU RNA levels (r=0.47, p=0.0001).
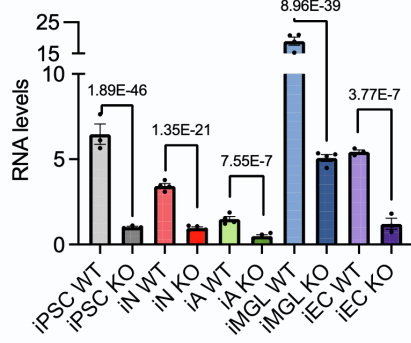
Supplemental Figure 1

A iPSC-derived Neurons (iNs)

Modified from Zhang et al. 2013

B iPSC-derived Microglia (iMGLs)

Modified from Abud et al. 2017 and McQuade et al. 2018

C iPSC-derived Astrocytes (iAs)

Modified from Canals et al. 2018

D iPSC-derived Endothelial/ epithelial cells (iECs)

Modified from Park and Mustafaoglu et al. 2019

E Frequency distribution

F

Supplemental Figure 2

A LOAD GWAS genes

B LOAD GWAS genes differentially expressed in SORL1 WT/KO iNs iAs and iMGLs

	INs	IAs	IMGLs
ABCA7			
ABI3			
ACE		Down in KO	
ADAM10			
AGRN			
APH1B			
APOE	Down in KO	Up in KO	
APP		Up in KO	
BIN1	Up in KO		Up in KO
CASS4			
CCDC6			
CD2AP	Down in KO		
CD33			
CLNK			
CLU	Down in KO	Up in KO	
EPHA1-AS1			
FERMT2	Down in KO	Down in KO	
GRN	Down in KO		
HAVCR2			
HLA-DRB1			
INPP5D	Down in KO		
LILRB2			
MS4A4A			Down in KO
NCK2			
PICALM		Down in KO	
RIN3			
SCIMP			
SHARPIN			
SORL1	Down in KO	Down in KO	Down in KO
TMEM106B			
TNIP1	Down in KO	Up in KO	
TREM2			
TSPOAP1-AS1			
ZCWPW1			

C SORL1 RNA
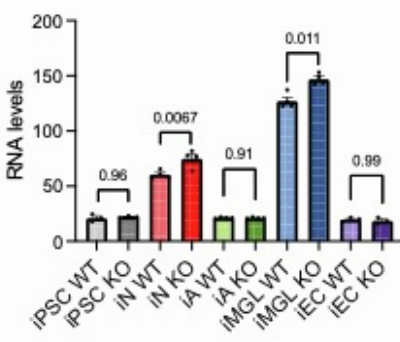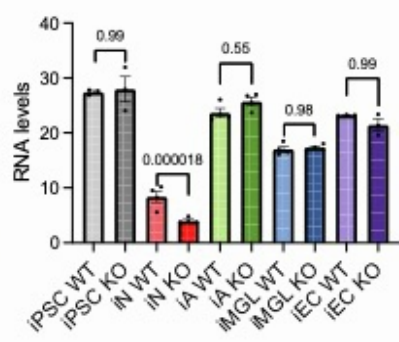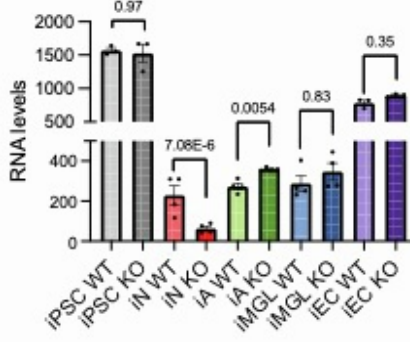
D BIN1 **E** CD2AP **F** APOE **G** CLU
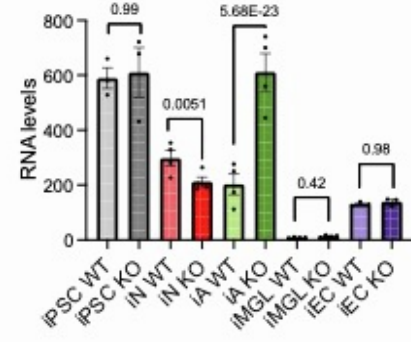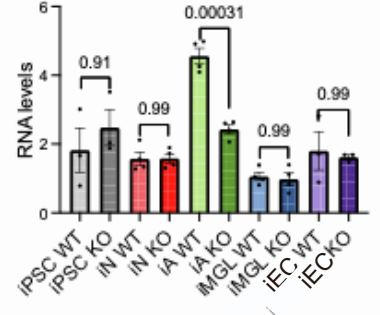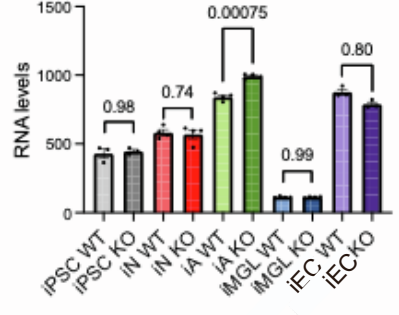
H ACE RNA **I** APP RNA **J** FERMT2 RNA **K** GRN RNA
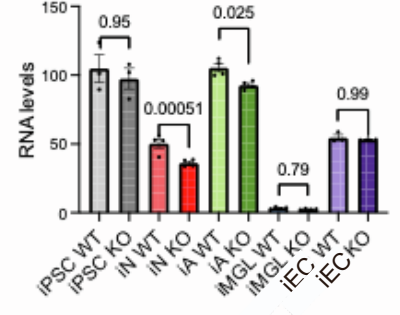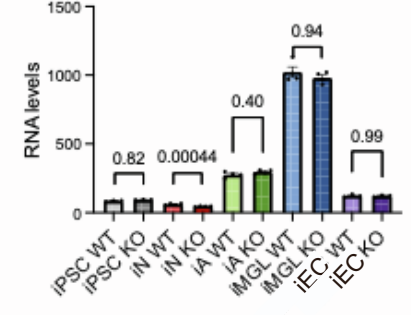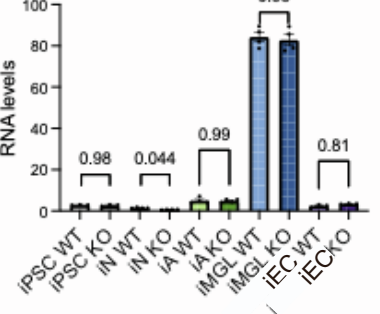
L INPP5D RNA **M** MS4A4A RNA **N** PICALM RNA **O** TNIP1 RNA
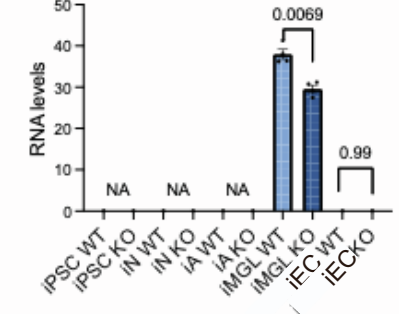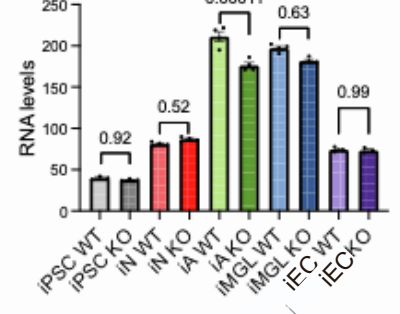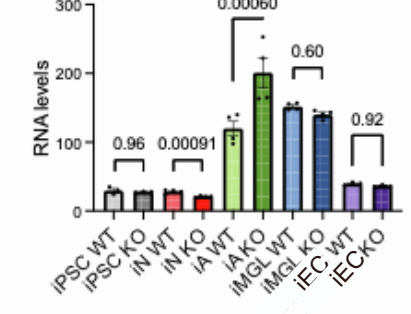
Supplemental Figure 3

A

Line name	iPSC Source	Sex	APOE genotype	Ethnicity	Publication
Line 1	Young lab, University of Washington	Male	ε3, ε4	>90% European ancestry (Levy et al. 2007)	Young et al. 2015 Mishra et al. 2022 Coriell Institute ID: GM25430
Line 2	Young-Pearse lab, Brigham and Women's Hospital	Female	ε3, ε3	>90% European ancestry	Muratore et al. 2014 Muratore et al. 2017

B

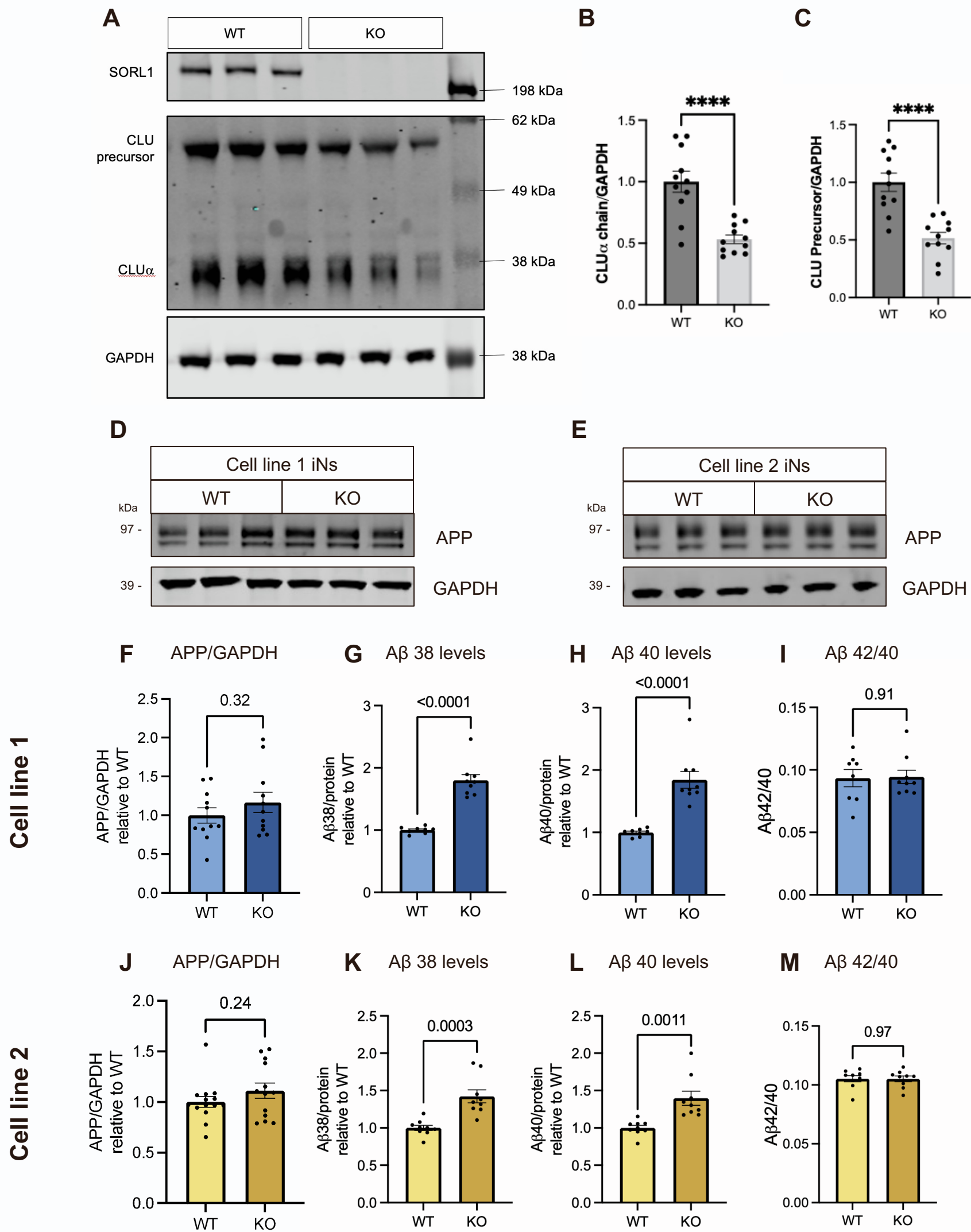|	SORL1 gRNA	Edited sequence	Genetic Analysis (Stem Cell Technologies Genetic Analysis kit)	Publication
Line 1 WT	ATTGAACGACATGAACCCTC	None	no significantly detected karyotype abnormalities	Knupp et al. 2020
Line 1 KO	ATTGAACGACATGAACCCTC	1 bp deletion	no significantly detected karyotype abnormalities	Knupp et al. 2020
Line 1 G511R	CTCTTGCATTTTAGGCTCAG	G511R	no significantly detected karyotype abnormalities	Mishra et al. 2022b
Line 2 WT	ATGTTCCTGAATCATGATCC	None	no significantly detected karyotype abnormalities*	Generated for this study
Line 2 KO	ATGTTCCTGAATCATGATCC	16 bp deletion	no significantly detected karyotype abnormalities*	Generated for this study

C

Line 2 WT

Line 2 KO
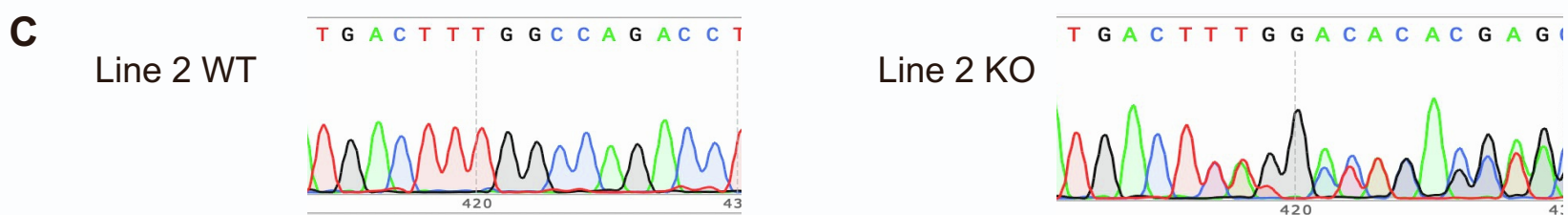
D

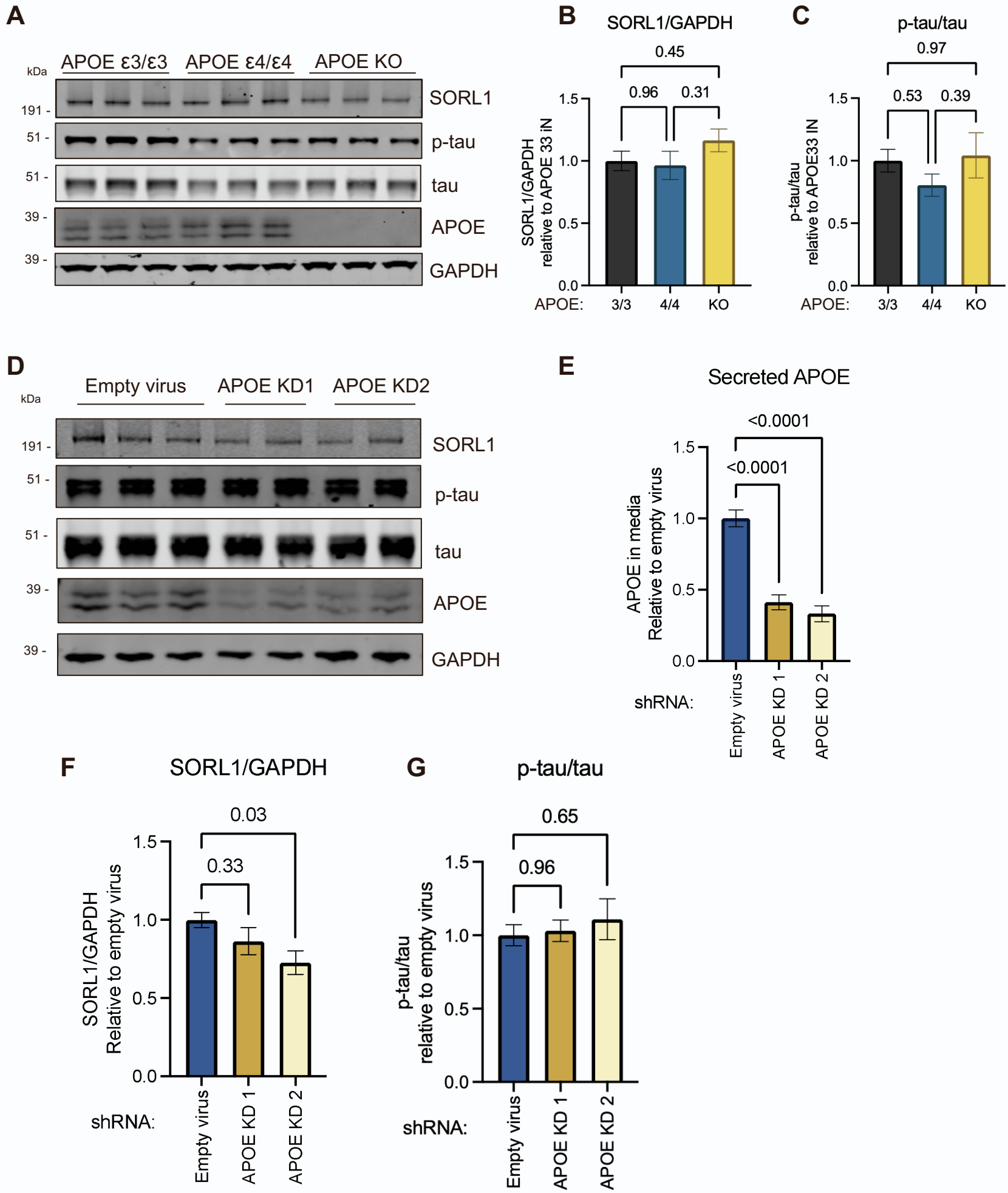| PCR Primer Sequences	Gene	Potential off-target sequence	PAM	Line1 WT	Line1 KO
CAT CTA ACT TCC AGC CTC TTC TC / AGA ACC CAA TTC TCC CTT TCC	SLC22A9	ACTGAAATACATGAACCGTC	GAG	WT	WT
CCA TGA TCT CCT CAA GCT GAA A / GAG ACA ACT GGG AAG CCA AA	SECISBP2	CTTGAACAAAATGAAGCCTC	AAG	WT	WT
AGC ACC TAT GTT GTT CTC ACT C / GTC AGC CTG AAC TCT CCT TTC	LOC107984387	TCTGAACGAGATGAACCTTC	CAG	WT	WT
GCG ATC TCT GGT CGA GAA TAA A / CCA GGT CTT TAC AGA GGC TAA C	E2F8	ATTGGACTACTTGAACCATC	GGG	WT	WT
TGG AAG GAA GCA GTT TGG AG / GAA CGA CAC CTC TGG GAT TAA A	LOC105375202	ATTGAACCAC-TGAACGCTC	CAG	WT	WT

E

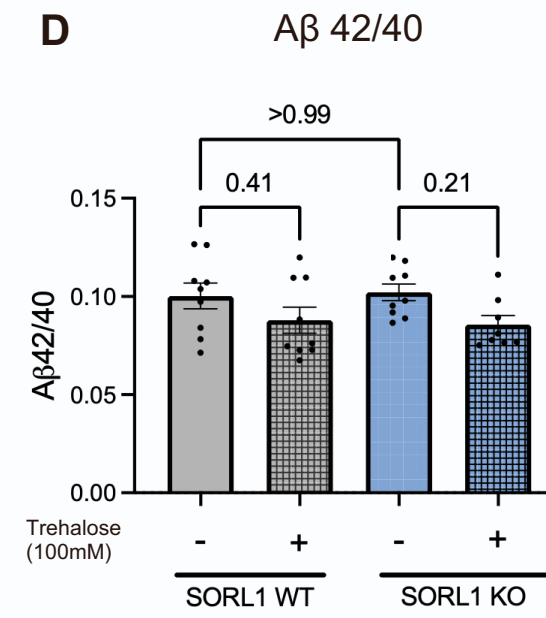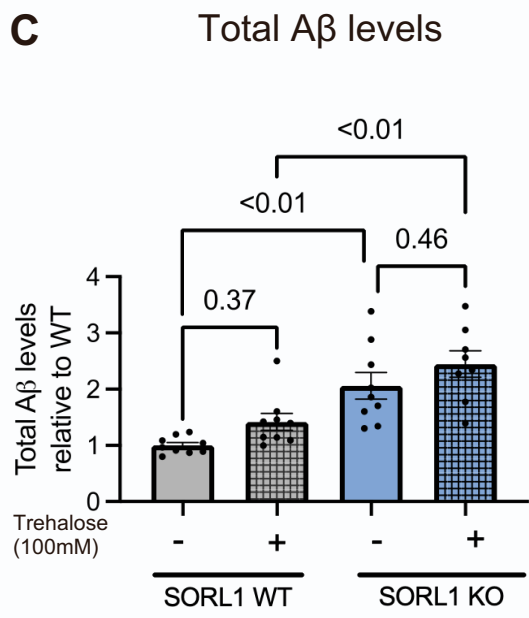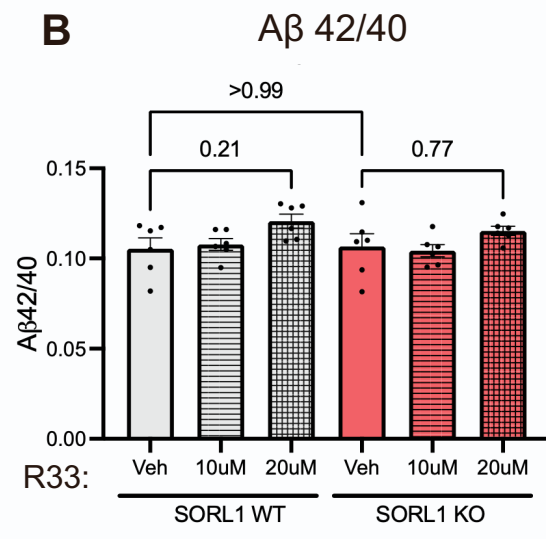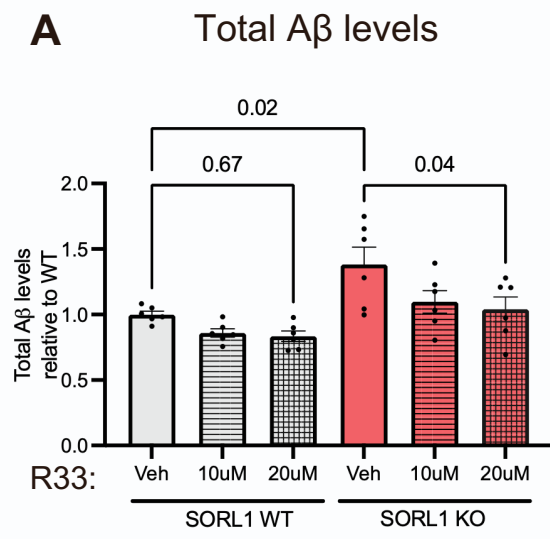| PCR Primer Sequences	Gene	Potential off-target sequence	PAM	Line2 WT	Line2 KO
CAG GGC CAC ATA GTG AGT TAA G / GAG TAT CTC CAG GGA CTG AGA A	TNFSF15	GTCTTCCTGAACCATAATCC	TGG	WT	WT
CCT AGC CCT CTT TCC TCT GAT A / GTT CCC TCC TCC TCC TCT T	ZNF428	ACGATCCTGAAT-ATGATCC	TGG	WT	WT
GAA GCC CTC CAC TTA CAA TCA / TCA CTG CGA AAC CTT ACC TAA A	GPNMB	ATG-TCCTGATTCATGATCC	TAG	WT	WT
CCG TTC TAT CCT TTC CCG TAT C / GAG AGC TGC TTC TTG GTC TAT C	SLX4IP	CTGTTCCTGTCCCATGATCC	CAG	WT	WT
TGG CAA ACT GGA TGA GGA TTA C / TAG CAC ATT GCA TCC CAA GAG	DICER1	ATGTTCCTG-ATCATACTCC	AGG	WT	WT
GTT CAG GAG GAA GGT GTG ATG / TGA TCA GCT GTG AGT GAT GTG	MRM3	AAGTTCCTGAAGCATCATCC	TGG	WT	WT
ACA GAG GAT GTG TGG GAA AG / CGC CTG AGG AAG TCC ATA AA	OSBP	ATGCCCCTGAATCATAATCT	TAG	WT	WT
GGG CTA TCT CTA AGG TGC TAC T / CAG GGA AAT GGG TGG ATC AAT	ADAMTS19	ATGTTCATGAA-CATGATCA	AAG	WT	WT

Supplemental Figure 4

Supplemental Figure 5.

Supplemental Figure 6

A Total Aβ levels

B Aβ 42/40

C Total Aβ levels

D Aβ 42/40

Supplemental Figure 7

Supplemental Figure 8

A

ROSMAP brain RNAseq

Pearson r = 0.03
p-value = 0.28

B

ROSMAP brain RNAseq

Pearson r = 0.47
p-value = 0.0001