Supplemental Information:

Figure S1-S4

Table S1-S4

Supplemental Experimental Procedures

Supplemental References

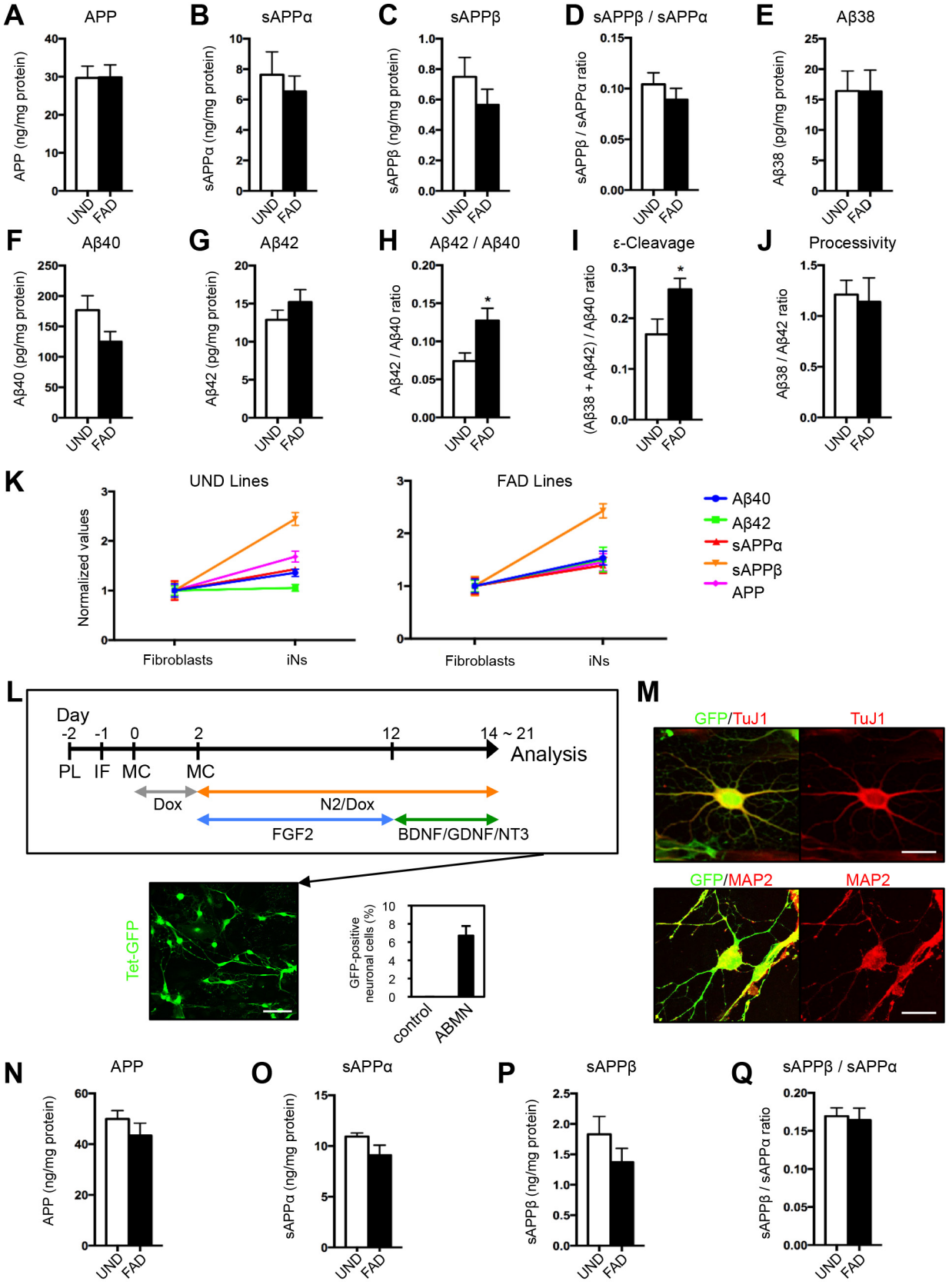
A
APP
APP (ng/mg protein)
B
sAPPα
sAPPα (ng/mg protein)
C
sAPPβ
sAPPβ (ng/mg protein)
D
sAPPβ / sAPPα
sAPPβ / sAPPα ratio
E
Aβ38
Aβ38 (pg/mg protein)
F
Aβ40
Aβ40 (pg/mg protein)
G
Aβ42
Aβ42 (pg/mg protein)
H
Aβ42 / Aβ40
Aβ42 / Aβ40 ratio
I
ε-Cleavage
(Aβ38 + Aβ42) / Aβ40 ratio
J
Processivity
Aβ38 / Aβ42 ratio
UND
FAD
K
UND Lines
FAD Lines
Normalized values
Fibroblasts
iNs
Aβ40
Aβ42
sAPPα
sAPPβ
APP
L
Day
-2 -1 0 2 12 14 ~ 21
Analysis
PL IF MC MC
Dox
N2/Dox
FGF2
BDNF/GDNF/NT3
Tet-GFP
GFP-positive neuronal cells (%)
control
ABMN
M
GFP/TuJ1
TuJ1
GFP/MAP2
MAP2
N
APP
O
sAPPα
P
sAPPβ
Q
sAPPβ / sAPPα

Figure S1, Related to Figure 1 and 2: APP processing in fibroblasts and hiN cells from FAD patients.
(A-J) Levels of APP (A), sAPPα (B), sAPPβ (C), the sAPPβ/sAPPα ratio (D), Aβ38 (E), Aβ40 (F), Aβ42 (G), the Aβ42/Aβ40 ratio (H), ε-cleavage (I), and processivity (J) are shown. These data represent an independent analysis of fibroblast cultures from patients with FAD PSEN mutations or unaffected controls (UND), presented as further replication of the findings. **(K)** Comparison of the levels of Aβ40, Aβ42, sAPPα, sAPPβ, and APP between fibroblasts (as presented in Figure S1A, B, C, F, and G) and hiNs (as presented in Figure 2 of UND and FAD lines). n = 3 lines per group. **(L)** Timeline of hiN direct conversion from fibroblasts. PL, plating; IF, lentiviral infection; MC, medium change; Dox, doxycycline. Representative picture (green) and respective quantification of GFP-positive hiNs (the ratio of GFP-positive neuronal morphology cell per fibroblast plated). Bar, 20 μm. **(M)** Converted hiNs were positive for neuronal markers such as TuJ1 and MAP2. Bars, 10 μm. **(N-Q)** The levels of APP (N), sAPPα (O), sAPPβ (P), the sAPPβ/sAPPα ratio (Q) in hiN cells as in Figure 2. Fibroblast lines used in these studies are shown in Table S1.

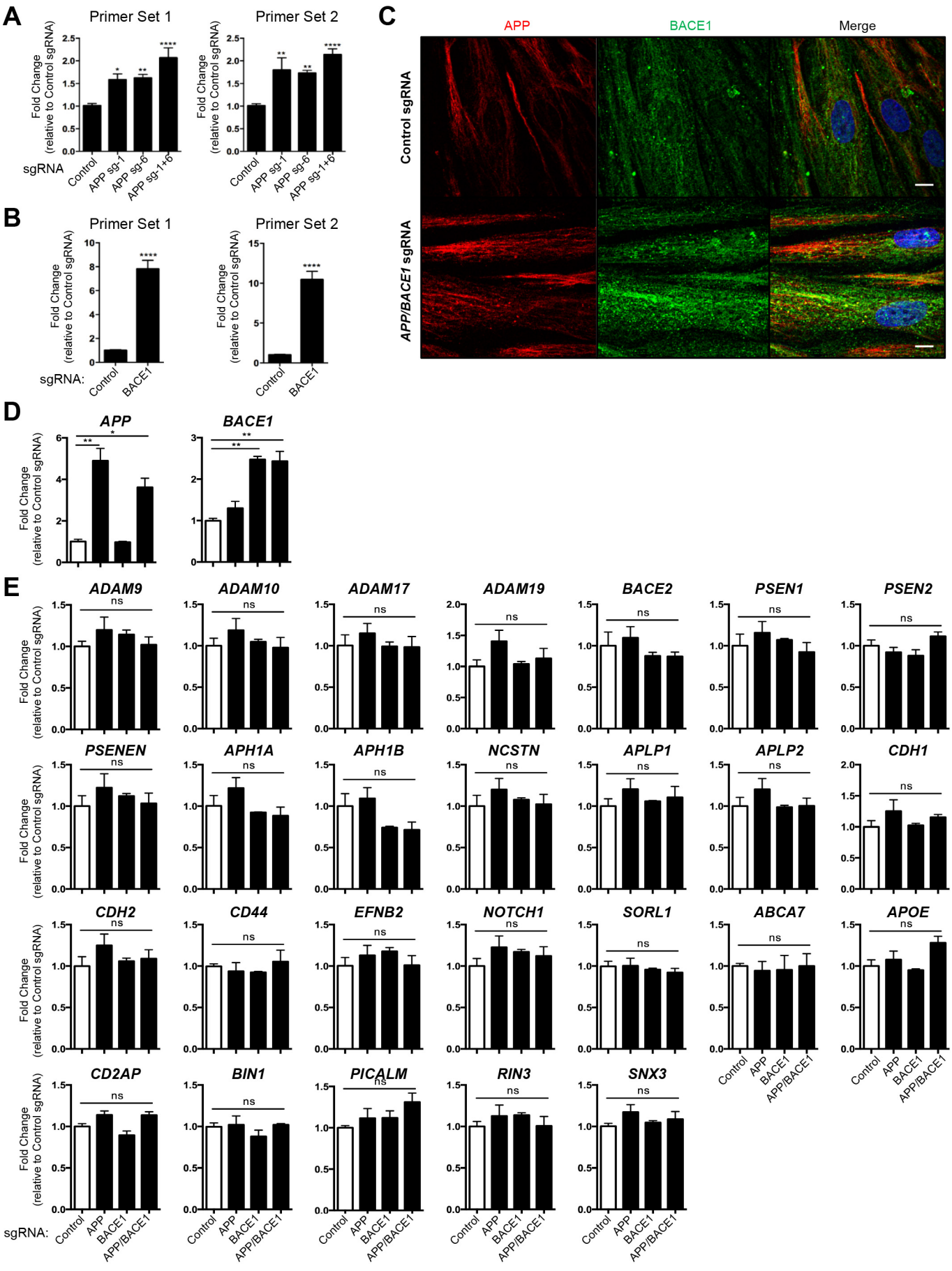
A
Primer Set 1
Primer Set 2
Fold Change (relative to Control sgRNA)
sgRNA
Control
APP sg-1
APP sg-6
APP sg-1+6
B
Primer Set 1
Primer Set 2
Fold Change (relative to Control sgRNA)
sgRNA:
Control
BACE1
C
APP
BACE1
Merge
Control sgRNA
APP/BACE1 sgRNA
D
APP
BACE1
Fold Change (relative to Control sgRNA)
E
ADAM9
ADAM10
ADAM17
ADAM19
BACE2
PSEN1
PSEN2
PSENEN
APH1A
APH1B
NCSTN
APLP1
APLP2
CDH1
CDH2
CD44
EFNB2
NOTCH1
SORL1
ABCA7
APOE
CD2AP
BIN1
PICALM
RIN3
SNX3
ns
Control
APP
BACE1
APP/BACE1
sgRNA:

Figure S2, Related to Figure 3: Characterization of SAM-activated cells.
(A) Additional elevation of endogenous *APP* expression by simultaneous treatment of *APP* sg-1 and sg-6 (sg-1+6), as quantified by qPCR analysis using two different primer sets. **(B)** Elevation of endogenous *BACE1* expression by treatment of *BACE1* sg-3, as quantified by qPCR analysis using two different primer sets. The primer sequences for *APP* or *BACE1* are shown in Table S3. Fold change is relative to control sgRNA. n = 3 fibroblast lines. **(C)** Higher magnification images of APP and BACE1 in SAM activated or control fibroblasts show no obvious change in protein distribution and some co-localization of APP and BACE1. Bars, 10 μm. **(D)** Specific elevation of *APP* or *BACE1* expression by treatment of *APP* or *BACE1* sgRNA, respectively, as quantified by qPCR analysis. Each expression level was normalized by *GAPDH* expression. **(E)** No off-target elevation of the other 26 genes associated with APP/Aβ processing. No significant activation relative to control sgRNA treatment was observed. Each expression level was normalized by *GAPDH* expression. The primer sequences are shown in Table S3. Fold change is relative to control sgRNA. n = 3 independent wells from HEK293T cell lines transfected with SAM plasmids.

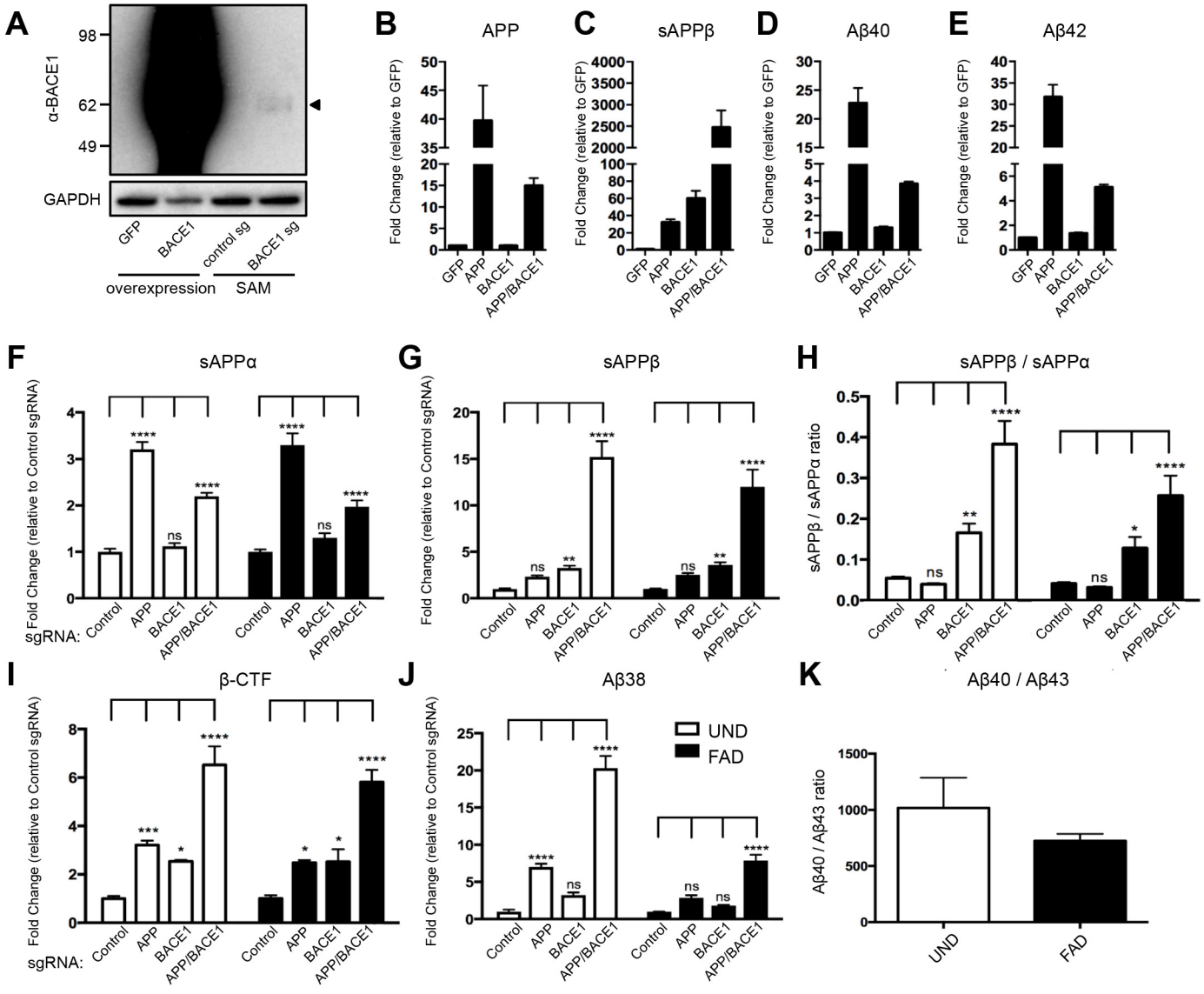

Figure S3, Related to Figure 4: APP processing in SAM-activated fibroblasts from FAD patients.
(A) Comparison of lentiviral overexpression and SAM activation of BACE1. Lentiviral overexpression of *BACE1* resulted in much higher levels of expression than SAM activation. BACE1 protein, arrowhead. **(B-E)** The levels of APP (B), sAPPβ (C), Aβ40 (D), and Aβ42 (E), as determined by ELISA, in UND fibroblast cultures with lentiviral overexpression of GFP, APP, BACE1, or APP/BACE1. Fold change is relative to GFP overexpression. **(F-J)** The levels of sAPPα (F), sAPPβ (G), the sAPPβ/sAPPα ratio (H), β-CTF (I) and Aβ38 (J) in UND and FAD fibroblasts, as determined by ELISA, in the presence of SAM activation of *APP*, *BACE1* or both together. Fold change is relative to control sgRNA (F, G, I, and J). **(K)** The ratio of Aβ40/Aβ43 on SAM activation with *APP* and *BACE1* in UND and FAD fibroblasts.

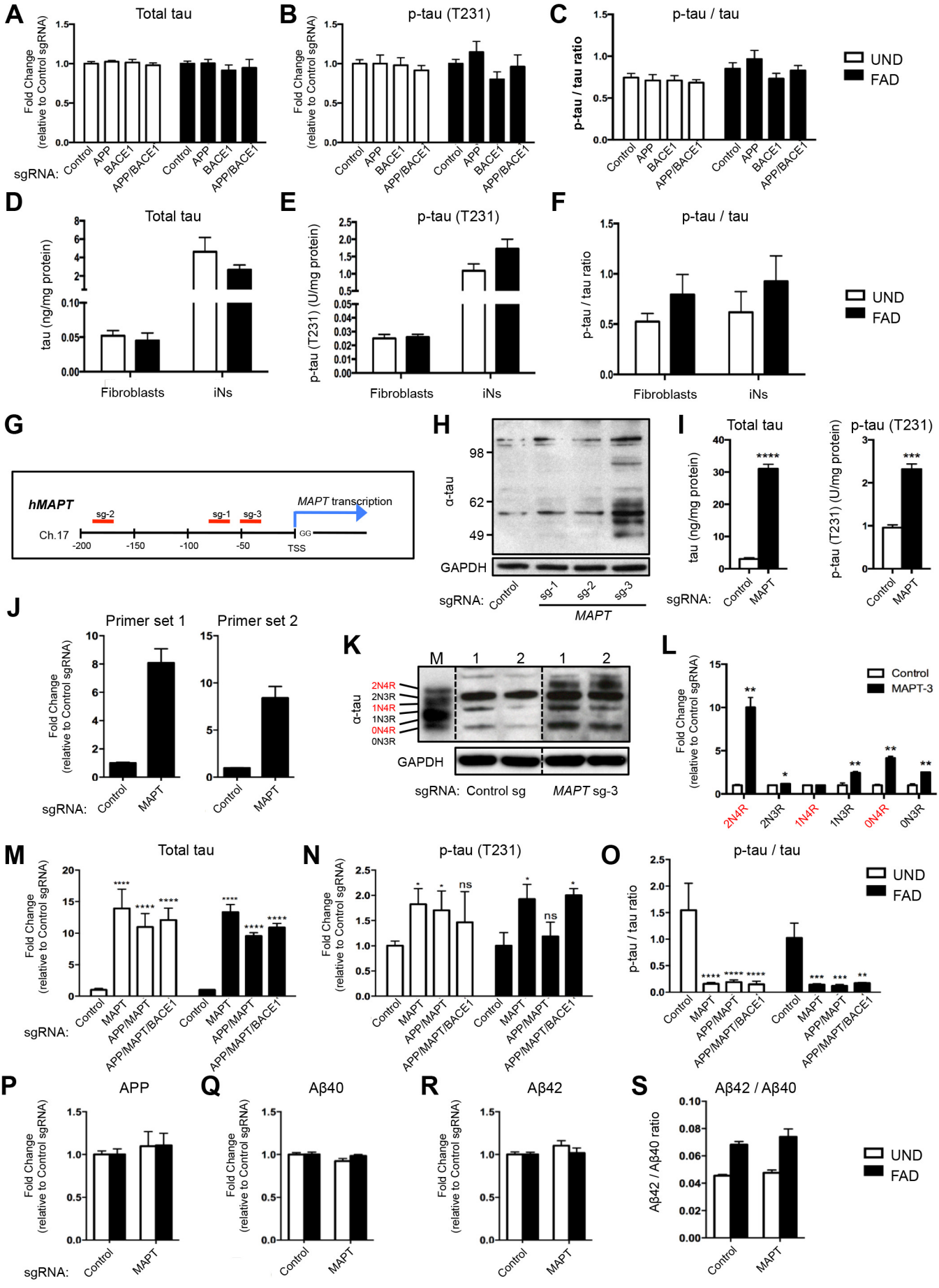
A
Total tau
Fold Change (relative to Control sgRNA)
sgRNA: Control APP BACE1 APP/BACE1 Control APP BACE1 APP/BACE1
B
p-tau (T231)
C
p-tau / tau
p-tau / tau ratio
UND
FAD
D
Total tau
tau (ng/mg protein)
Fibroblasts
iNs
E
p-tau (T231)
p-tau (T231) (U/mg protein)
F
p-tau / tau
G
hMAPT
sg-2
sg-1
sg-3
MAPT transcription
Ch.17
-200
-150
-100
-50
GG
TSS
H
α-tau
98
62
49
GAPDH
sgRNA: Control sg-1 sg-2 sg-3
MAPT
I
Total tau
tau (ng/mg protein)
sgRNA: Control MAPT
p-tau (T231)
p-tau (T231) (U/mg protein)
J
Primer set 1
Primer set 2
Fold Change (relative to Control sgRNA)
sgRNA: Control MAPT
K
M 1 2 1 2
2N4R
2N3R
1N4R
1N3R
0N4R
0N3R
α-tau
GAPDH
sgRNA: Control sg MAPT sg-3
L
Control
MAPT-3
Fold Change (relative to Control sgRNA)
2N4R
2N3R
1N4R
1N3R
0N4R
0N3R
M
Total tau
Fold Change (relative to Control sgRNA)
sgRNA: Control MAPT APP/MAPT APP/MAPT/BACE1
N
p-tau (T231)
O
p-tau / tau
p-tau / tau ratio
P
APP
Q
Aβ40
R
Aβ42
S
Aβ42 / Aβ40
Aβ42 / Aβ40 ratio

Figure S4, Related to Figure 4: No alteration in tau accumulation by SAM-activated fibroblasts.
(A-C) SAM activation of APP and/or BACE1 unaltered the levels of total tau (A) and p-tau (T231) (B) in UND and FAD cultures, as determined by ELISA. The ratios of p-tau/tau were unchanged in UND and FAD cultures (C). **(D-F)** Comparison of the levels of total tau (D) and p-tau (T231) (E), quantified by ELISA, showed a marked increase in hiNs relative to fibroblasts, but no significant differences between UND and FAD lines. The ratios of p-tau/tau were unchanged (F). **(G)** Design of individual sgRNAs for *MAPT*. Three sgRNAs (red) were designed within the first 200 bp upstream the TSS. **(H)** Screening of *MAPT* sgRNAs by Western Blotting. *MAPT* sgRNAs (sg-1 to -3) elevated endogenous tau levels relative to control sgRNA (3.6- to 19.5-fold). **(I)** Elevation of endogenous tau and p-tau (T231) by treatment of *MAPT* sg-3, as quantified by ELISA. n = 3 fibroblast lines. **(J)** Elevation of endogenous *MAPT* expression by treatment of *MAPT* sgRNA-3, as quantified by qPCR analysis using two different primer sets. The primer sequences for *MAPT* are shown in Table S3. n = 3 fibroblast lines. **(K)** Different tau isoforms were induced by treatment of *MAPT* sgRNA-3. Control sgRNA treatment expressed only 3R-tau isoform (0N3R, 1N3R, and 2N3R), whereas *MAPT* sgRNA-3 treatment induced 4R-tau isoforms (0N4R and 2N4R) as well as 3R-tau. **(L)** Quantification of tau protein isoforms by Western Blotting. n = 3 fibroblast cultures. **(M-O)** SAM activation of *APP*, *MAPT*, *APP*/*MAPT*, or *APP*/*BACE1*/*MAPT* in FAD and UND fibroblasts. Activation of *MAPT* transcription in FAD and UND fibroblasts led to a greater increase in total tau (M) than in p-tau (T231) (N), and thus the p-tau/tau ratio was decreased (O) in FAD and UND cells. n = 3 lines per group. **(P-S)** SAM activation of *MAPT* expression did not change the levels of APP (P), Aβ40 (Q), Aβ42 (R), and the Aβ42/Aβ40 ratio (S) relative to control sgRNA both in UND and FAD fibroblasts. n = 3 lines per group. Increased APP and APP cleavage products did not appear to modify tau accumulation, and tau accumulation did not appear to modify APP processing.

Table S1, Related to Figures 1, 2, and 4

List of human fibroblast lines used in this study

	line	affected	gender	age	ethnicity	mutation	origin
	HDFa	no	female	53	unknown	-	Thermo Fisher Scientific
UND	AG07871	no	female	49	Italian	-	Coriell Institute
	AG07926	no	female	adult	Italian	-	Coriell Institute
	AG08125	no	male	64	Italian	-	Coriell Institute
FAD	AG06840	FAD	male	56	Canadian	*PSEN1* A246E	Coriell Institute
	AG07768	FAD	female	31	Canadian	*PSEN1* A246E	Coriell Institute
	AG09908	FAD	female	81	German	*PSEN2* N141I	Coriell Institute

AG07768 *PSEN1* mutation was determined by sequencing analysis.

List of human fibroblast lines used in Figure 1 and 2

	Experiment 1	*Experiment 2*
		replication study
UND	AG07871	AG07871
	AG07926	AG07926
	AG08125	HDFa
FAD	AG06840	AG06840
	AG07768	AG07768
	AG09908	AG09908
Corresponding Figure	Figure 1B-K Figure 2G (Fibroblasts)	Figure 2A-F Figure 2G (iNs) Figure S1, S4D-F

Table S2, Related to Figure 3

List of sgRNA used in initial screening

Gene Name	Transcript ID	sgRNA	TSS Distance	Guide RNA Sequence (5'- to -3')	WB	qPCR
Control	-	-	-	CTGAAAAAGGAAGGAGTTGA	-	-
APP	NM_001204302	sg-1	120	GGGGGCGCGTGGGGTGCAGG	*2.3X*	*2.8X*
		sg-2	141	GGCCGTCGGCCGGGGAGCGG	*1.2X*	*2.0X*
		sg-3	70	CGGGGCGCGAGGGCCCCTCC	*0.8X*	*1.7X*
		sg-4	47	CGCGAGCGGGCGCAGTTCCC	*1.5X*	*2.0X*
		sg-5	26	AGACCCCTAGCGGCGCCGCC	*0.8X*	*2.5X*
		sg-6	5	TCTCGGGTGCCGAGCGGGGT	*2.6X*	*4.2X*
		sg-7	92	CCCACAGGTGCACGCGCCCT	-	*1.8X*
		sg-8	162	ACTGTTCACGAAGCCCAGGT	-	*1.5X*
	NM_001136131	sg-9	84	AGAGGTGGGGCAGGCGTTTC	-	*1.6X*
		sg-10	56	CCGCATTTCGTTTTTTCTCT	-	*1.5X*
		sg-11	175	ACGTTGGGGGTTAAAAAATG	-	*1.5X*
		sg-12	114	TGAGAACGAGTGAAGCTTAA	-	*1.3X*
	NM_001136016	sg-13	134	GGCTGTGGAGAAGGAACTGC	-	*1.4X*
		sg-14	92	TTCCTTTTGTAGGATTTTCT	-	*1.5X*
		sg-15	113	GGCACAAAGCCAAGTGTCAG	-	*1.8X*
		sg-16	24	GCCAACTTCTAAGCTAACAA	-	*1.4X*
		sg-17	54	AGACAGAGTTCCGGAGGCTT	-	-
		sg-18	160	GTCAACCTTGGATGGAATTT	-	*1.6X*
BACE1	NM_138971	sg-1	148	TCCGTCTGGCCCTTCCCGCC	*19.6X*	-
		sg-2	73	GGCAATCCGGCTTCCGGGGC	*27.2X*	-
		sg-3	102	GTCTGGGATGCCCTCTGCCG	*30.9X*	
MAPT	NM_005910	sg-1	60	GCGGTCAGCGCCGCGGCCTG	*4.2X*	-
		sg-2	171	GGCCATGCGGGGTCGGGGCA	*3.5X*	-
		sg-3	33	GCGGGAGGGGGACCGCGAAA	*19.4X*	

HDFa was used in the WB and qPCR screening (WB, no replicate: qPCR, 3 technical replicates). *APP* sg-1/sg-6, *BACE1* sg-3, and *MAPT* sg-3 (shown in *pink*) were further validated and characterized by qPCR and ELISA using 3 independent fibroblast lines (see Figure 3D-E, 3G, S2A-B, S4I-J).

Table S3, Related to Figure 3

List of primers used in the qPCR analysis for sgRNA screening

Gene		Primer Set 1	Primer Set 2
APP	forward:	5'- CAA GCA GTG CAA GAC CCA TC -3'	5'- GCC CTG CGG AAT TGA CAA G-3'
	reverse:	5'- AGA AGG GCA TCA CTT ACA AAC TC -3'	5'- CCA TCT GCA TAG TCT GTG TCT G -3'
BACE1	forward:	5'- ACC AAC CTT CGT TTG CCC AA -3'	5'- TCT GTC GGA GGG AGC ATG AT -3'
	reverse:	5'- TCT CCT AGC CAG AAA CCA TCA G -3'	5'- GCA AAC GAA GGT TGG TGG T -3'
MAPT	forward:	5'- CCA AGT GTG GCT CAT TAG GCA -3'	5'- GAG TCC AGT CGA AGA TTG GGT -3'
	reverse:	5'- CCA ATC TTC GAC TGG ACT CTG T -3'	5'- GGC GAG TCT ACC ATG TCG ATG -3'
GAPDH	forward:	5'- AAG GTG AAG GTC GGA GTC AAC-3'	———
	reverse:	5'- GGG GTC ATT GAT GGC AAC AAT A -3'	

List of primers used in the qPCR analysis for the off-targeting study

Gene	Forward primer	Reverse primer
ADAM9	5'- TCC ATT GCT CTT AGC GAC TGT -3'	5'- GGG GTT CAA TCC CAT AAC TCG -3'
ADAM10	5'- ATG GGA GGT CAG TAT GGG AAT C -3'	5'- ACT GCT CTT TTG GCA CGC T -3'
ADAM17	5'- GTG GAT GGT AAA AAC GAA AGC G -3'	5'- GGC TAG AAC CCT AGA GTC AGG -3'
ADAM19	5'- GGG AGC CTG GAT GGA CAA G -3'	5'- AGC TTT GAG TGG ATG CTT TTC TC -3'
BACE1	5'- TCT GTC GGA GGG AGC ATG AT -3'	5'- GCA AAC GAA GGT TGG TGG T -3'
BACE2	5'- GGA GAT GCT GAT CGG GAC C -3'	5'- AGT ACG TGT CTA TGT AGG AGT GC -3'
PSEN1	5'- TGG CTA CCA TTA AGT CAG TCA GC -3'	5'- CCC ACA GTC TCG GTA TCT TCT -3'
PSEN2	5'- AGT GTG TGA TGA GCG GAC G -3'	5'- ACT GGG CAG TGT TCT CTC CAT -3'
APH1A	5'- TTT TTC GGC TGC ACT TTC GTC -3'	5'- TGC GAC CAG GAT GAT AAC GC -3'
APH1B	5'- CGA GCC GTT GCG TAT CAT CTT -3'	5'- CCA AAC AAG GGA CGA AAT CAG T -3'
PSENEN	5'- TCT TCT GGG TGA TAG TGC TCA -3'	5'- CCA GGG GTA TGG TGA AGG AGA -3'
NCSTN	5'- AAT AAA ACA GCT CCC TGT GTT CG -3'	5'- ACT ACG TGG ATA ACC CCT GTG -3'
APP	5'- CAA GCA GTG CAA GAC CCA TC -3'	5'- AGA AGG GCA TCA CTT ACA AAC TC -3'
APLP1	5'- GGA CCA ATG TGA GAG TTC AAC C -3'	5'- GAG CCA CAG GGT AAG AGC A -3'
APLP2	5'- TGA GCC TCA AAT CGC AAT GTT -3'	5'- CCT GTT GGA TCA GGT TCC CAT -3'
CDH1	5'- CGA GAG CTA CAC GTT CAC GG -3'	5'- GGG TGT CGA GGG AAA AAT AGG -3'
CDH2	5'- TCA GGC GTC TGT AGA GGC TT -3'	5'- ATG CAC ATC CTT CGA TAA GAC TG -3'
CD44	5'- CTG CCG CTT TGC AGG TGT A -3'	5'- CAT TGT GGG CAA GGT GCT ATT -3'
EFNB2	5'- TAT GCA GAA CTG CGA TTT CCA A -3'	5'- TGG GTA TAG TAC CAG TCC TTG TC -3'
NOTCH1	5'- TGG ACC AGA TTG GGG AGT TC -3'	5'- GCA CAC TCG TCT GTG TTG AC -3'
SORL1	5'- CAA GGT GTA CGG ACA GGT TAG T -3'	5'- CCA ATG CCA GGC TAT CTC G -3'
ABCA7	5'- CCC TCA ACT GGT ACG AGG CTA -3'	5'- AGC TTC CGG GTA AAA GGT GTA -3'
APOE	5'- GTT GCT GGT CAC ATT CCT GG -3'	5'- GCA GGT AAT CCC AAA AGC GAC -3'
BIN1	5'- TGA GCA GTG CGT CCA GAA TTT -3'	5'- CGA TCT TGT TTG CCT CAT CCC -3'
CD2AP	5'- AAG TTG GGA CTG TTT CCC TCA -3'	5'- TTC TTG TCC GAA GTT TCA CAG AG -3'
PICALM	5'- TGA CAG AGG TGA TAT ACC AGA CC -3'	5'- TGC TTT TCC CTT TCA TCC ACT TT -3'
RIN3	5'- TTT CCT TCT CTG AAC GAA AGC TC -3'	5'- ACA CAG TAG AAC GCA ATC AAT CT -3'
SNX3	5'- CCA AGC CGC AGA ACC TGA AT -3'	5'- GAC CCT GAT TTC GTA AGT GGT G -3'
GAPDH	5'- GGA GCG AGA TCC CTC CAA AAT -3'	5'- GGC TGT TGT CAT ACT TCT CAT GG -3'

Supplemental Experimental Procedures

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-APP[22C11]	Millipore	Cat#MAB348; RRID:AB_94882
Rabbit polyclonal anti-BACE1	Abcam	Cat#ab10716; RRID:AB_297419
Mouse monoclonal anti-Tau [HT7]	Thermo Fisher Scientific	Cat#MN1000; RRID:AB_2314654
Rabbit monoclonal anti-GAPDH [14C10]	Cell Signaling Technology	Cat#2118; RRID:AB_561053
Rabbit polyclonal anti-BACE1	Thermo Fisher Scientific	Cat#PA1-757; RRID:AB_325863
Donkey polyclonal anti-mouse IgG (H+L), HRP-conjugated	Jackson ImmunoResearch	Cat#715-036-151; RRID:AB_2340774
Donkey polyclonal anti-rabbit IgG (H+L), HRP-conjugated	Jackson ImmunoResearch	Cat#711-035-152; RRID:AB_10015282
Goat anti-rabbit IgG (H+L), Oregon Green488-conjugated	Thermo Fisher Scientific	Cat#O-11038; RRID:AB_2539798
Goat anti-mouse IgG (H+L), Alexa Fluor555-conjugated	Thermo Fisher Scientific	Cat#A-21422; RRID: AB_2535844
Bacterial and Virus Strains		
One Shot Stbl3 Chemically Competent E. coli	Thermo Fisher Scientific	Cat#C737303
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
poly-L-Lysine solution	Travigen	Cat#343820001
CaCl2 solution	Sigma-Aldrich	Cat#21115
HEPES buffered saline, 2x concentrate	Sigma-Aldrich	Cat#51558
Forskolin	LC Laboratories	Cat#F9929
D-MEM	Thermo Fisher Scientific	Cat#11995065
Lenti-X concentrator	Takara Clontech	Cat#631232
Fetal Bovine Serum	Thermo Fisher Scientific	Cat#10082147
Penicillin and Streptomycin (10,000 U/ml)	Thermo Fisher Scientific	Cat#15140122
Platimun SuperFi DNA Polymerase	Thermo Fisher Scientific	Cat#12351-010
0.1% gelatin solution	Millipore	Cat#ES006B
Doxycycline	Sigma-Aldrich	Cat#9891
D-MEM/F12 (1:1)	Thermo Fisher Scientific	Cat#10565-018
N2 supplement	Thermo Fisher Scientific	Cat#17502-048
bFGF	R&D Systems	Cat#233-FB/CF
BDNF	R&D Systems	Cat#248-BD/CF
GDNF	R&D Systems	Cat#212-GD/CF

NT3	R&D Systems	Cat#267-N3/CF
T4 Polynucleotide Kinase	New England Biolabs	Cat#M0201
Fast Digest Esp3I (BsmBI)	Thermo Fisher Scientific	Cat#FD0454
T7 DNA Ligase	Enzymatics	Cat#L6020F
BSA	New England Biolabs	Cat#B9000S
Hemadimethrine bromide (polybrene)	Sigma-Aldrich	Cat#H9268
RIPA buffer	Thermo Fisher Scientific	Cat#89900
Protease inhibitor cocktail	Sigma-Aldrich	Cat#P8340
Phosphatase inhibitor cocktail	Thermo Fisher Scientific	Cat#78420
NuPAGE 4-12% Bis-Tris Protein Gel	Thermo Fisher Scientific	Cat#NP0322
NuPAGE MES SDS Running Buffer	Thermo Fisher Scientific	Cat#NP0002
NuPAGE Transfer Buffer	Thermo Fisher Scientific	Cat#NP0006-1
SuperSignal West Pico Chemiluminescent Substrate	Thermo Fisher Scientific	Cat#34080
SuperSignal West Dura Chemiluminescent Substrate	Thermo Fisher Scientific	Cat#34076
16% paraformaldehyde solution	Electron microscopy Sciences	Cat#15710
Phosphate-Buffered Saline (PBS)	Thermo Fisher Scientific	Cat#10010023
Triton X-100	Sigma-Aldrich	Cat#93443
Normal Goat Serum	Jackson ImmunoResearch	Cat#005-000-121
DAPI solution	Thermo Fisher Scientific	Cat#62248
Critical Commercial Assays		
RNeasy Mini Kit	Qiagen	Cat#74104
SuperScript III First-Strand Synthesis System for RT-PCR	Thermo Fisher Scientific	Cat#18080-051
pENTR/D-TOPO cloning Kit	Thermo Fisher Scientific	Cat#K240020
Fast SYBR Green Cells-to-Ct Kit	Thermo Fisher Scientific	Cat#4402956
Power STBR Green PCR master Mix	Thermo Fisher Scientific	Cat#4367659
V-PLEX Aβ Peptide Panel 1 (6E10) Kit	Meso Scale Discovery	Cat#K15200E
sAPPα/sAPPβ Kit	Meso Scale Discovery	Cat#K15120E
Phospho(Thr231)/Total Tau Kit	Meso Scale Discovery	Cat#K15121D
APP Human ELISA Kit	Thermo Fisher Scientific	Cat#KHB0051
BACE1 Assay Kit	Immuno-Biological Laboratories	Cat#JP27752
Human Amyloidβ (1-43) (FL) Assay Kit	Immuno-Biological Laboratories	Cat#JP27710
APP β-CTF ELISA	IBL America	Cat#27776
Pierce BCA Protein Assay Kit	Thermo Fisher Scientific	Cat#23225

Deposited Data		
Experimental Models: Cell Lines		
Human Dermal Fibroblasts, Adult (HDFa)	Thermo Fisher Scientific	Cat#C0135C; LOT1668134
Human Fibroblast: Female, 49-yo	Coriell Institute	AG07871
Human Fibroblast: Female, Adult	Coriell Institute	AG07926
Human Fibroblast: Male, 64-yo	Coriell Institute	AG08125
Human Fibroblast: Male, 56-yo: FAD	Coriell Institute	AG06840
Human Fibroblast: Female, 31-yo: FAD	Coriell Institute	AG07768
Human Fibroblast: Female, 81-yo: FAD	Coriell Institute	AG09908
HEK293T cell line	Sigma-Aldrich	Cat#12022001
Experimental Models: Organisms/Strains		
Oligonucleotides		
Primers for sgRNA plasmid construction, see Table S2	Integrated DNA Technologies	see Table S2
Primers for qPCR analysis, see Table S3	Integrated DNA Technologies	see Table S3
Recombinant DNA		
pMD2.G	Trono lab	Addgene #12259
psPAX2	Trono lab	Addgene #12260
pTet-O-FUW-Ascl1	Vierbuchen et al., 2010	Addgene #27150
pTet-O-FUW-Brn2	Vierbuchen et al., 2010	Addgene #27151
pTet-O-FUW-Myt1l	Vierbuchen et al., 2010	Addgene #27152
pTet-O-FUW-Neurod1	Vierbuchen et al., 2010	Addgene #30129
pTet-O-FUW-EGFP	Vierbuchen et al., 2010	Addgene #30130
FUW-M2rtTA	Hockemeyer et al., 2008	Addgene #20342
lenti-dCas9-VP64_Blast	Konermann et al., 2015	Addgene #61425
lenti-MS2-P65-HSF1_Hygro	Konermann et al., 2015	Addgene #61426
lenti-sgRNA(MS2)_zeo backbone	Konermann et al., 2015	Addgene #61427
Software and Algorithms		
sgRNA Design tool, Cas9 Activators with SAM	Konermann et al., 2014	http://sam.genome-engineering.org
PrimerBank, MGH-PGA	Wang et al., 2012	http://pga.mgh.harvard.edu/primerbank
GraphPad PRISM 6.0	GraphPad Software	http://www.graphpad.com/scientific-software/prism/
Other		
0.45 µm PVDF syringe-filter	Millipore	Cat#SLHV033RS
24 well ibiTreat µ-Plate	ibidi	Cat#82406
PVDF membrane	Thermo Fisher Scientific	Cat#LC2005

QuantStudio 6 Flex real-time PCR system	Thermo Fisher Scientific	equipment
MicroAmp Optical 384-Well Reaction Plate	Thermo Fisher Scientific	Cat#4309849
MSD Sector Imager 2400	Meso Scale Discovery	equipment
UVmax plate reader	Molecular Devices	equipment
Andor Revolution XDi Spinning Disk Confocal System	ANDOR	equipment

Fibroblasts

Human dermal fibroblasts from seven individuals were used in this study (Table S1). All lines were derived from de-identified, banked tissue samples. HDFa was purchased from Thermo Fisher Scientific (#C0135C, LOT1668134). The other lines were obtained from the Coriell Institute for Medical Research. Human dermal fibroblasts were maintained on 0.1% gelatin-coated dishes in D-MEM (#11995065, Thermo Fisher Scientific) containing 10% or 15% fetal bovine serum (FBS) (#10082147, Thermo Fisher Scientific) and 100 units/ml penicillin and 100 μg/ml streptomycin (#15140122, Thermo Fisher Scientific).

Mutation in the PSEN1 gene

Total RNA was extracted from human fibroblast line AG07768 by RNeasy Mini Kit (#74104, Qiagen), and the cDNA library was synthesized by random hexamers using SuperScript III First-Strand Synthesis System for RT-PCR (#18080-051, Thermo Fisher Scientific). Whole *PSEN1* sequence was amplified by Platinum SuperFi DNA Polymerase (#12351-010, Thermo Fisher Scientific) and sub-cloned into the pENTR/D-TOPO vector by directional TOPO cloning (K240020, Thermo Fisher Scientific). The point-mutation corresponding to A246E in the *PSEN1* gene was identified by Sanger sequencing analysis.

Plasmids

pMD2.G and psPAX2 were gifts from Didier Trono (Addgene plasmid #12259 and #12260, respectively). pTet-O-FUW-Ascl1, pTet-O-Brn2, pTet-O-Myt1l, pTet-O-Neurod1, pTet-O-FUW-EGFP (Addgene plasmid #27150, #27151, #27152, #30129, #30130, respectively) were gifts from Marius Wernig (Vierbuchen et al., 2010; Pang et al., 2011). FUW-M2rtTA was a gift from Rudolf Jaenisch (Addgene plasmid #20342) (Hockemeyer et al., 2008). Lenti dCas9-VP64_Blast, lenti MS2-P65-HSF1_Hygro, and lenti sgRNA(MS2)_zeo backbone were gifts from Feng Zhang (Addgene plasmid #61425, #61426, and #61427, respectively) (Konermann et al., 2015). pENTR/D-TOPO (#K2400-20) and pLenti6.3/V5-DEST (#V53306) vectors were purchased from Thermo Fisher Scientific.

Lentivirus production

Lentiviral particles were produced using the calcium-phosphate transfection method. HEK293T cells (#12022001, Sigma-Aldrich) were plated onto a poly-L-Lysine (#343820001, Travigen) coated 10-cm dish at the density of $5x10^6$ cells/dish in D-MEM (#11995065, Thermo Fisher Scientific) containing 10% FBS (#10082147, Thermo Fisher Scientific). On the following day, 10 μg of lentiviral

plasmid mixture containing 4 μg of psPAX2 (#12260, Addgene), 2 μg of pMD2.G (#12259, Addgene), and 2 μg of gene-specific lentiviral vector in 0.25 M $CaCl_2$ solution (#21115, Sigma-Aldrich) were mixed with an equal volume of 2x HEPES-buffered saline (#51558, Sigma-Aldrich), and used to transfect the HEK293T cells. After 20 hours, the culture medium was replaced to 10 ml D-MEM containing 10% FBS and 10 μM forskolin (#F9929, LC Laboratories), and incubated for another 72 hours. The culture medium was then collected and centrifuged at 2,600 rpm for 5 min to remove the cell debris. The viral supernatants were filtrated with 0.45 μm PVDF syringe-filter (#SLHV033RS, Millipore). One-third volume (3.4 ml) of Lenti-X concentrator (#631232, Takara Clontech) was added to the viral supernatant, and incubated at 4°C for overnight. The supernatant was centrifuged at 2,600 rpm at 4°C for 45 min, and the pellet was re-suspended in 1/20x volume (500 μl) of D-MEM containing 10% FBS and 8 μg/ml polybrene (#H9268, Sigma-Aldrich). Concentrated viral particles were stored at -80°C.

hiN generation from human fibroblasts

Conversion of human fibroblasts into neuronal cells was driven by forced expression of Ascl1, Brn2, Myt1l, and Neurod1 according to a prior publication with minor modifications (Vierbuchen et al., 2010; Pang et al., 2011). Briefly, human fibroblasts were plated onto a 0.1% gelatin-coated dish at the density of $0.7x10^4$ cells/cm^2 in D-MEM containing 10% FBS. On the next day (Day-0), concentrated lentivirus particles including pTet-O-FUW-Ascl1, pTet-O-Brn2, pTet-O-Myt1l, pTet-O-Neurod1, pTet-O-FUW-EGFP (#27150, #27151, #27152, #30129, #30130 Addgene) and FUW-M2rtTA (#20342, Addgene) were directly applied into the fibroblast culture medium and incubated for 24 hours (Hockemeyer et al., 2008). The medium was subsequently replaced to fresh D-MEM containing 10% FBS and 8 μg/ml doxycycline (#9891, Sigma-Aldrich) (Day-1). At Day-2, the medium was replaced to fresh D-MEM/F12 (1:1) media containing N2 supplement (#10565-018, 17502-048, Life Technologies), 10 ng/ml basic fibroblast growth factor (bFGF: #233-FB/CF, R&D Systems) and 8 μg/ml doxycycline. At Day-12, the medium was replaced to fresh D-MEM/F12 (1:1) media containing N2 supplement (#10565-018, 17502-048, Life Technologies), 10 ng/ml BDNF, GDNF, and NT3 (#248-BD/CF, #212-GD/CF, #267-N3/CF, R&D Systems) and 8 μg/ml doxycycline. Half volume of culture medium was changed every 2 to 3 days.

Western Blotting analysis

Cells were harvested in RIPA buffer (#89900, Thermo Fisher Scientific) containing protease inhibitor cocktail (#P8340, Sigma-Aldrich) and phosphatase inhibitor cocktail (#78420, Thermo Fisher Scientific), sonicated for a few seconds on ice, and centrifuged at 15,000 rpm at 4°C for 30 min. Ten μg of protein lysates were subjected to SDS-PAGE using NuPAGE 4-12% Bis-Tris Protein Gel (#NP0322, Thermo Fisher Scientific) in NuPAGE MES SDS Running Buffer (#NP0002, Thermo Fisher Scientific) at 200 volts for 52 min and transferred to a

0.45 µm pore-size PVDF membrane (#LC2005, Thermo Fisher Scientific) in NuPAGE Transfer Buffer (#NP0006-1, Thermo Fisher Scientific) at 30 Volts for 65 min. Blots were incubated with blocking buffer (20 mM Tris, 137 mM NaCl, [pH7.6], 0.1% Tween 20, 5% non-fat skim milk) at room temperature for 60 min and probed with the primary antibody diluted in blocking buffer at 4°C overnight. The primary antibodies used were: anti-GAPDH [14C10] (#2118, Cell Signaling Technology), anti-APP [22C11] (#MAB348, Millipore), anti-BACE1 (#ab10716, Abcam), and anti-tau [HT7] (#MN1000, Thermo Fisher Scientific). For APP CTF detection, 10 µg of protein lysates were subjected to SDS-PAGE using Novex 10-20% Tricine Protein Gel (#EC6625BOX, Thermo Fisher Scientific) in Novex Tricine SDS Running Buffer (#LC1675, Thermo Fisher Scientific) at 125 volts for 2 hrs and transferred to a 0.2 µm pore-size nitrocellulose membrane (#LC2000, Thermo Fisher Scientific) in Novex Tris-Glycine Transfer Buffer (#LC3675, Thermo Fisher Scientific) at 25 Volts for 60 min. The primary antibody used was: anti-APP C-Terminal Fragment (#802801, BioLegend). Blots were incubated with the appropriate HRP-conjugated secondary antibodies (#715-036-151, #711-036-152, Jackson ImmunoResearch) diluted in blocking buffer at room temperature for 60 min, and visualized using the SuperSignal West Pico Chemilumininescent Substrate (#34080, Thermo Fisher Scientific) or SuperSignal West Dura Chemilumininescent Substrate (#34076, Thermo Fisher Scientific).

Gene expression analysis by quantitative real-time PCR (qPCR)

RNA extractions and reverse transcriptions to cDNA for quantitative gene expression analyses in fibroblasts or HEK293T cells were performed using the Fast or Power SYBR® Green Cells-to-C_T™ Kit (#4402956, #4402955, Thermo Fisher Scientific) according to the manufacturer's protocol. Real-time PCR analysis of 2 ~ 8 µl of cDNA products were performed in triplicates and probed with two different primer pairs (250 nM final concentration) for each transcript of interest with Power SYBR® Green PCR Master Mix (#4367659, Thermo Fisher Scientific). The primer sequences for the individual genes were obtained from the PrimerBank (https://pga.mgh.harvard.edu/primerbank) (Wang et al., 2012) and are listed in Table S3. Samples were applied in MicroAmp® Optical 384-Well Reaction Plates (#4309849, Thermo Fisher Scientific) and analysed in a QuantStudio™ 6 Flex real-time PCR system (Thermo Fisher Scientific). Relative expression levels were normalized to the housekeeping gene *GAPDH* and calculated using the comparative C_T method.

Immunofluorescence analysis

Cells were fixed with 4% paraformaldehyde (PFA) (#15710, Electron Microscopy Sciences) in phosphate-buffered saline (PBS) at room temperature for 10 min and subsequently washed with 10 mM Tris-HCl (pH 7.4) saline buffer. Cells were then permeabilized and blocked in PBS with 0.1 % Triton-X100 and 3 % normal goat serum (NGS) for 10 min. Primary antibodies were diluted in PBS supplemented with 1 % NGS and added to the cells for an overnight incubation at 4ºC. Cells were subsequently washed and incubated with the secondary

antibodies for 1h at room temperature. The primary antibodies used in the study were: anti-APP [22C11] (#MAB348, Millipore) and anti-BACE1 (#PA1-757, Thermo Fisher Scientific). The secondary antibodies used in the study were: Oregon Green 488-conjugated goat anti-rabbit IgG (H+L) antibody (#O-11038, Thermo Fisher Scientific) and Alexa Fluor 555-conjugated goat anti-mouse IgG (H+L) antibody (#A-21422, Thermo Fisher Scientific). Cell nucleus was counter-stained with DAPI solution (#62248, Thermo Fisher Scientific). Images were acquired using an Andor Revolution XDi Spinning Disk Confocal System. Total brightness of APP or BACE1 was quantified as the signal subtracted background fluorescence and doubly normalized to the cell number and control sgRNA.

Statistical Analysis

Statistical analyses were performed using the GraphPad PRISM 6.0 software. Values are expressed as means ± SEM. Statistical differences were tested using unpaired t tests for two samples and a two-way ANOVA combined with Turkey's post-hoc test for multiple samples. Experiments were conducted with a minimum of 3 independent cell cultures from different individuals per UND and FAD group. Statistical differences are indicated by *p*-values of * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$.

Supplemental References

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Wang X, Spandidos A, Wang H, Seed B (2012) PrimerBank: a PCR primer database for quantitative gene expression analysis, 2012 update. Nucleic acids research 40:D1144-1149.