SUPPLEMENTARY INFORMATION (Wang C. et al.)

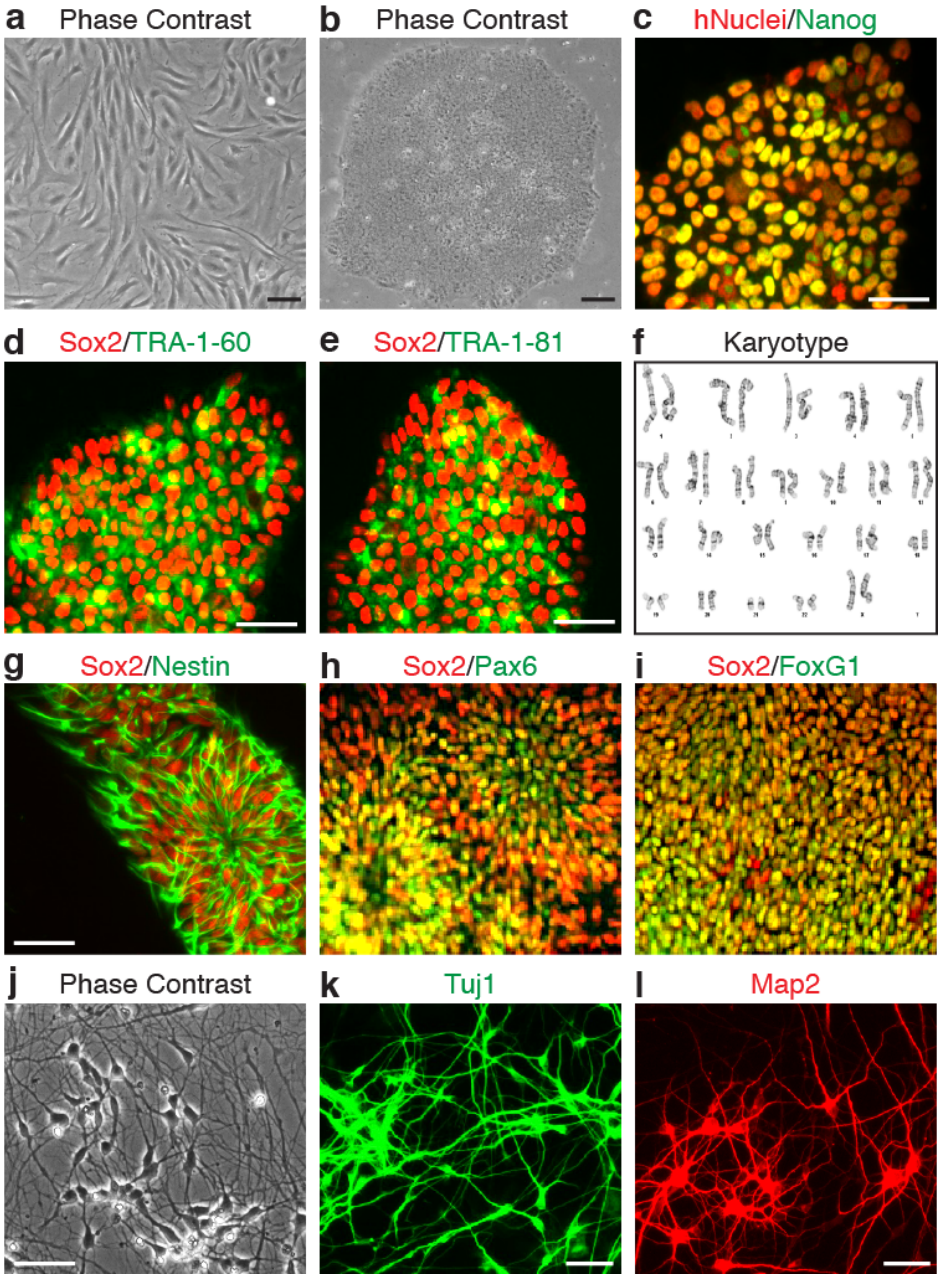
Supplementary Table 1. General Information of Subjects from Whom hiPSC Lines Were Generated

	Patient Characteristics		
hiPSC Line	Age (years)	Gender	AD Status
ApoE3/3-A	66	Female	No
ApoE3/3-B	64	Female	No
ApoE3/3-C	71	Male	No
ApoE4/4-A	64	Female	AD
ApoE4/4-B	62	Male	AD
ApoE4/4-C	70	Female	AD
Isogenic ApoE3/3	64	Female	AD
$ApoE^{-/-}$	43	Male	No

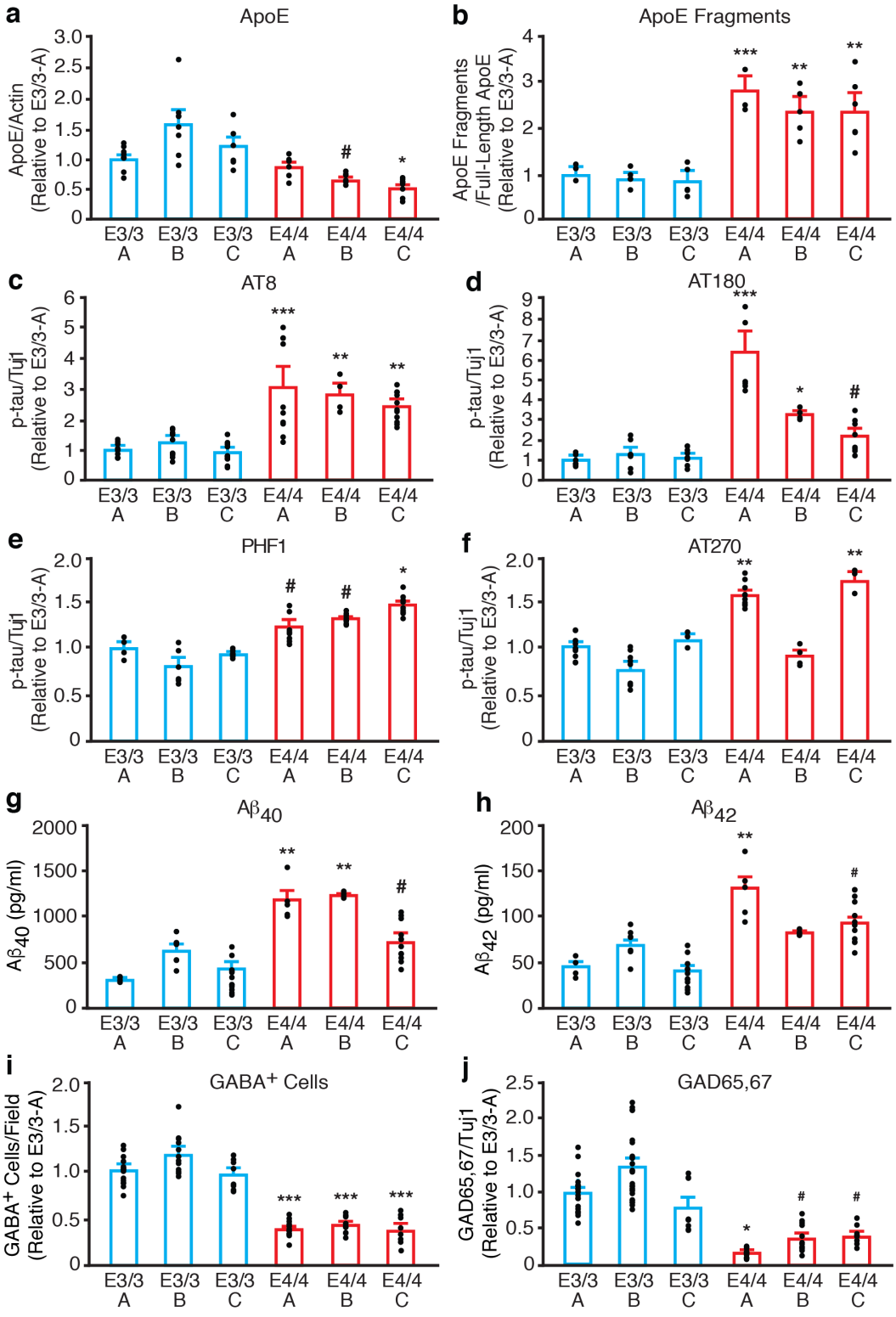
Supplementary Table 2. cDNA Sequence Analysis of Alzheimer's Disease Related Genes in the Parental ApoE4/4-hiPSC and the Isogenic ApoE3/3-hiPSC Lines

	DNA Sequence Characteristics		
Gene	Parental E4/4 Line	Isogenic E3/3 Line	Difference with E4/4 Line
APP	Normal	Normal	No
MAPT (Tau)	Normal	Normal	No
BACE1	Normal	Normal	No
PS1	Normal	Normal	No
NPLOC4*	Normal	Normal	No

*The coding sequence of the NPLOC4 gene was sequenced because it was predicted as a potential off-target gene of the gene editing approach.

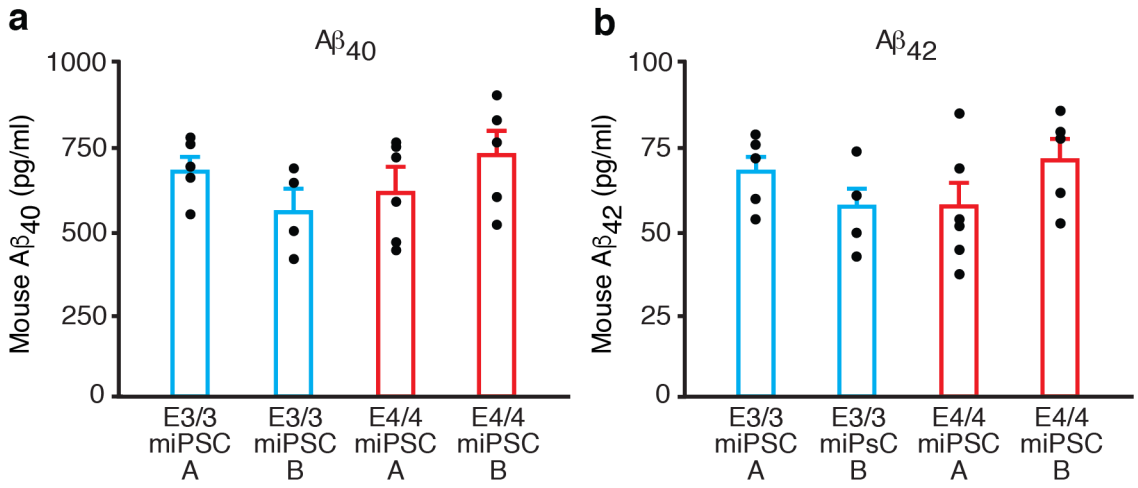


Supplementary Figure 1. Representative images of the generation and characterization of hiPSC lines. (**a, b**) Phase-contrast images of human fibroblasts (a) from a subject homozygous for apoE4/4 and the derived hiPSC clone (b) cultured under feeder-free conditions. Scale bar, 100 μm. (**c–f**) Immunostaining for pluripotent stem cell markers (c–e) in hiPSCs from a subject homozygous for apoE4/4 and the karyotype (f). Scale bar, 50 μm. (**g–i**) Immunostaining for neural stem cell markers in neural progenitors derived from apoE4/4-hiPSCs. Scale bar, 50 μm. (**j**) Phase-contrast image of neurons differentiated from apoE4/4-hiPSCs. Scale bar, 50 μm. (**k, l**) Immunostaining for neuronal markers Tuj1 (k) and Map2 (l) in neurons differentiated from apoE4/4-hiPSCs. Scale bar, 50 μm. The experiments were repeated independently over three times with similar results.

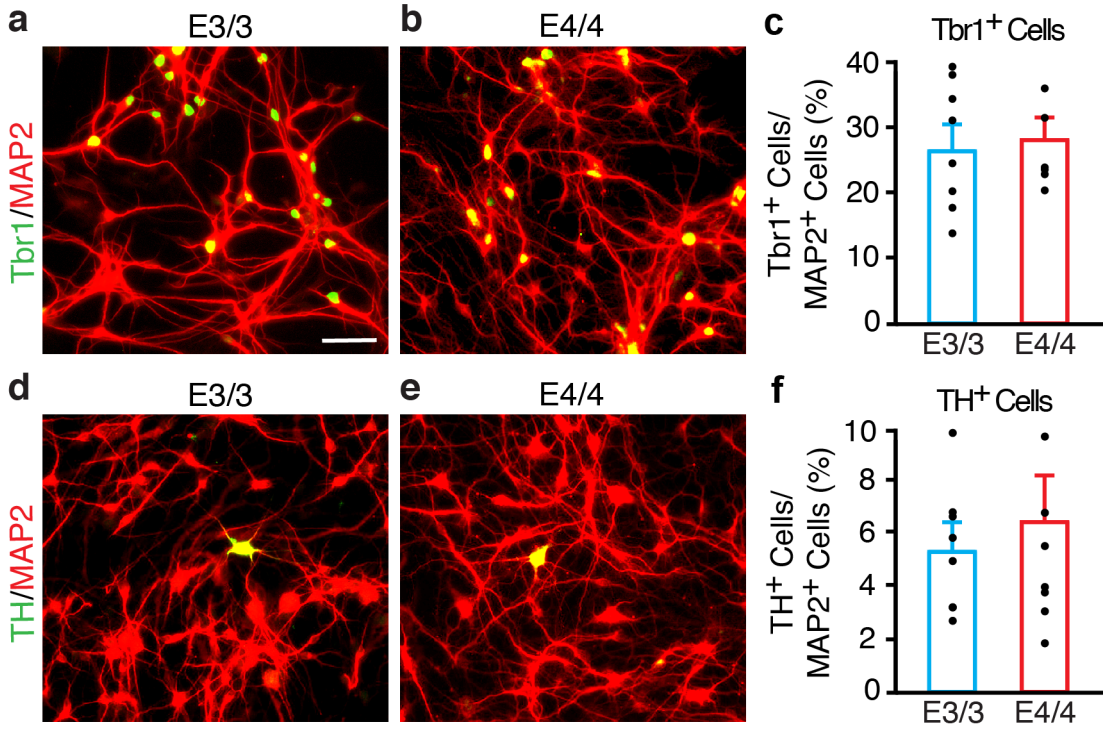


Supplementary Figure 2. Phenotypic analyses of neuronal cultures derived from individual hiPSC lines with different apoE genotypes. (**a**) Quantification of full-length apoE in neuronal lysates derived from different apoE3/3-hiPSC and apoE4/4-hiPSC lines. Values are normalized

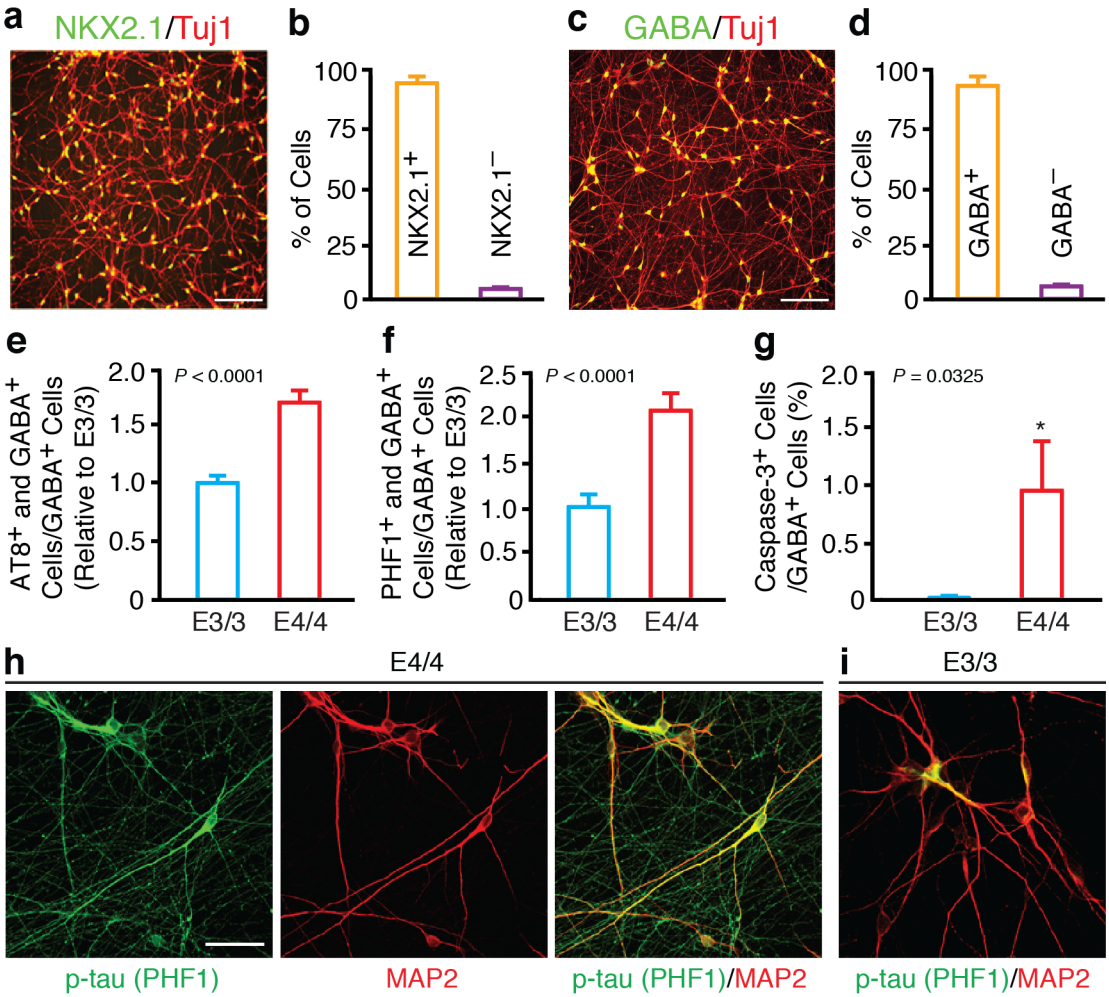
to E3/3-A line. n = 9 (E3/3-A), n = 8 (E3/3-B), n = 6 (E3/3-C), n = 6 (E4/4-A), n = 6 (E4/4-B), and n = 8 (E4/4-C) biologically independent samples. (**b**) Quantification of apoE fragments in neuronal lysates derived from different apoE3/3-hiPSC and apoE4/4-hiPSC lines. Values are normalized to E3/3-A line. n = 3 (E3/3-A), n = 5 (E3/3-B), n = 5 (E3/3-C), n = 3 (E4/4-A), n = 5 (E4/4-B), and n = 6 (E4/4-C) biologically independent samples. (**c**) Quantification of p-tau levels (AT8) in neuronal lysates derived from different apoE3/3-hiPSC and apoE4/4-hiPSC lines. Values are normalized to E3/3-A line. n = 10 (E3/3-A), n = 10 (E3/3-B), n = 11 (E3/3-C), n = 10 (E4/4-A), n = 4 (E4/4-B), and n = 11 (E4/4-C) biologically independent samples. (**d**) Quantification of p-tau levels (AT180) in neuronal lysates derived from different apoE3/3-hiPSC and apoE4/4-hiPSC lines. Values are normalized to E3/3-A line. n = 7 (E3/3-A), n = 7 (E3/3-B), n = 8 (E3/3-C), n = 6 (E4/4-A), n = 4 (E4/4-B), and n = 8 (E4/4-C) biologically independent samples. (**e**) Quantification of p-tau levels (PHF1) in neuronal lysates derived from different apoE3/3-hiPSC and apoE4/4-hiPSC lines. Values are normalized to E3/3-A line. n = 4 (E3/3-A), n = 6 (E3/3-B), n = 7 (E3/3-C), n = 8 (E4/4-A), n = 8 (E4/4-B), and n = 9 (E4/4-C) biologically independent samples. (**f**) Quantification of p-tau levels (AT270) in neuronal lysates derived from different apoE3/3-hiPSC and apoE4/4-hiPSC lines. Values are normalized to E3/3-A line. n = 10 (E3/3-A), n = 10 (E3/3-B), n = 3 (E3/3-C), n = 10 (E4/4-A), n = 4 (E4/4-B), and n = 3 (E4/4-C) biologically independent samples. (**g, h)** $A\beta_{40}$ (g) and $A\beta_{42}$ (h) levels in culture medium of neurons derived from different apoE3/3-hiPSC and apoE4/4-hiPSC lines. n = 4 (E3/3-A), n = 7 (E3/3-B), n = 12 (E3/3-C), n = 5 (E4/4-A), n = 4 (E4/4-B), and n = 12 (E4/4-C) biologically independent samples. (**i**) Quantification of GABA-positive cells per field in neuronal cultures derived from different apoE3/3-hiPSC and apoE4/4-hiPSC lines. Values are normalized to E3/3-A line. n = 16 fields from E3/3-A with total of 4001 $GABA^+$ neurons counted, n = 12 fields from E3/3-B with total of 3368 $GABA^+$ neurons counted, n = 8 fields from E3/3-C with total of 1956 $GABA^+$ neurons counted), n = 12 fields from E4/4-A with total of 1224 $GABA^+$ neurons counted, n = 12 fields from E4/4-B with total 1369 GABA+ neurons counted, and n = 8 fields from E4/4-C with total of 840 $GABA^+$ neurons counted. (**j**) Quantification of ratio of GAD65/67 to Tuj1 in neuronal lysates derived from different apoE3/3-hiPSC and apoE4/4-hiPSC lines. Values are normalized to E3/3-A line. n = 20 (E3/3-A), n = 25 (E3/3-B), n = 9 (E3/3-C), n = 14 (E4/4-A), n = 12 (E4/4-B), and n = 9 (E4/4-C) biologically independent samples. All values are expressed as mean ± SEM. Differences among groups were determined with one-way ANOVA followed with Tukey's multiple comparison test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus all three E3/3 lines. $^{\#}p < 0.05$ versus at least two out of the three E3/3 lines.



Supplementary Figure 3. ApoE4 does not increase Aβ production in neurons derived from miPSC lines. (**a, b**) Mouse $A\beta_{40}$ (a) and $A\beta_{42}$ (b) levels in culture medium of neurons derived from apoE3/3-miPSC and apoE4/4-miPSC lines. n = 5 (E3/3-miPSC-A), n = 4 (E3/3-miPSC-B), n = 6 (E4/4-miPSC-A), and n = 5 (E4/4-miPSC-B) biologically independent samples. All values are expressed as mean ± SEM. Differences among groups were determined with one-way ANOVA followed with Tukey's multiple comparison test. No comparison reaches statistical significance.

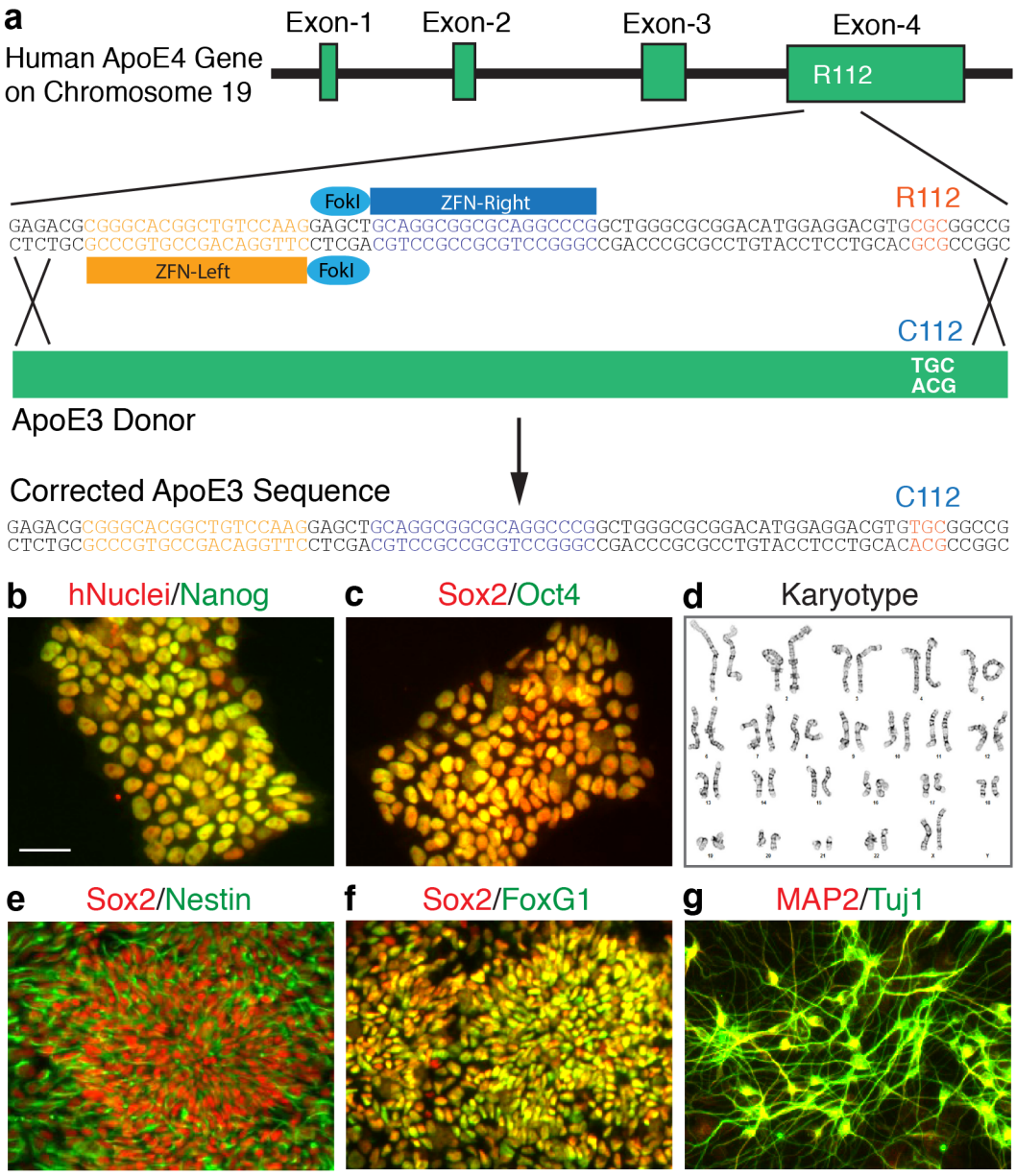


Supplementary Figure 4. ApoE4 does not affect Tbr1-positive glutamatergic and TH-positive dopaminergic neurons derived from hiPSC lines. (**a, b**) Anti-Tbr1 and anti-MAP2 double immunostaining of neuronal cultures derived from apoE3/3-hiPSCs and apoE4/4-hiPSCs. (**c**) Quantification of percentage of $Tbr1^+$ glutamatergic neurons in neuronal cultures derived from apoE3/3-hiPSCs and apoE4/4-hiPSCs. n = 8 fields from E3/3 with total of 1127 $MAP2^+$ neurons counted, n = 6 fields from E4/4 with total of 889 $MAP2^+$ neurons counted. (**d, e**) Anti-TH and anti-MAP2 double immunostaining of neuronal cultures derived from apoE3/3-hiPSCs and apoE4/4-hiPSCs. (**f**) Quantification of percentage of TH^+ dopaminergic neurons in neuronal cultures derived from apoE3/3-hiPSCs and apoE4/4-hiPSCs. n = 8 fields from E3/3 with total of 1017 $MAP2^+$ neurons counted, n = 8 fields from E4/4 with total of 916 $MAP2^+$ neurons counted. In c and f, values are expressed as mean ± SEM. Differences between groups were determined with the unpaired two-sided *t* test. No comparison reaches statistical significance. Scale bar, 50 μm.

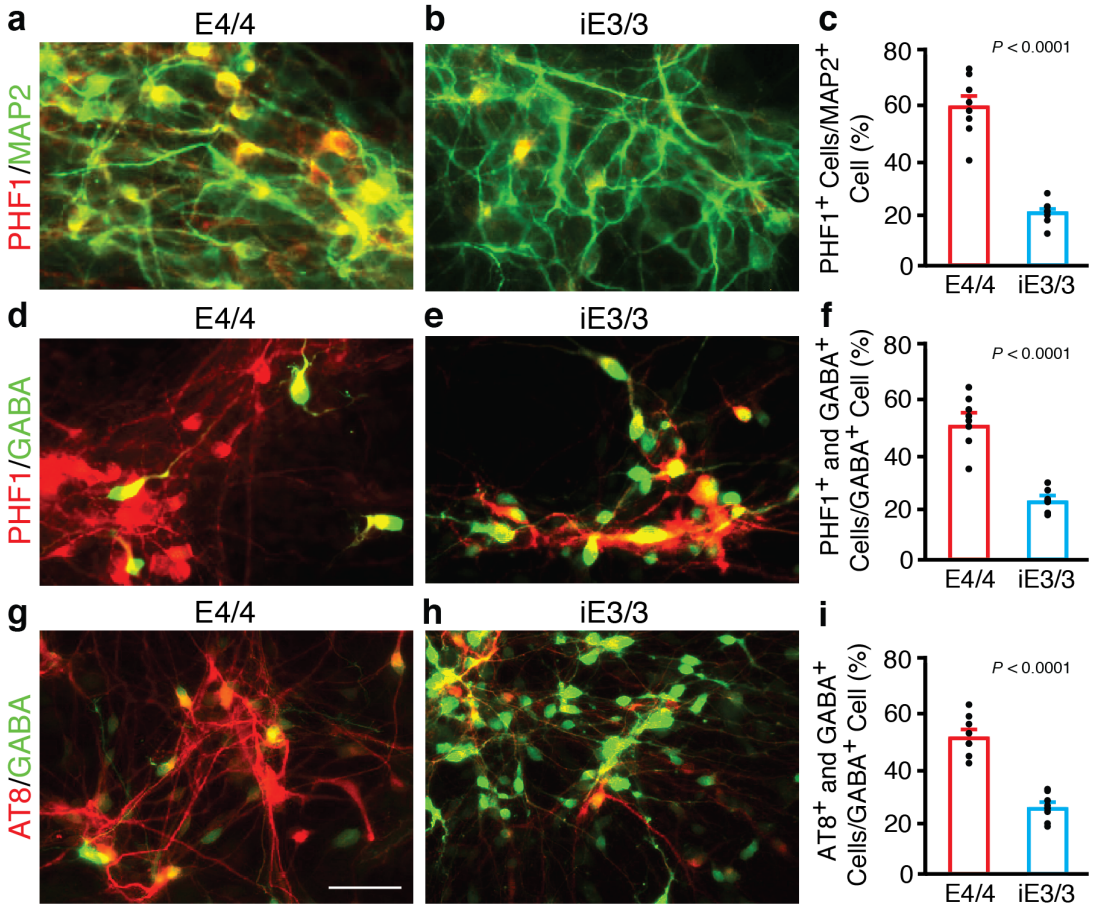


Supplementary Figure 5. ApoE4 causes GABAergic neuron degeneration in hiPSC-derived neuronal culture. (**a**) Generation of MGE cells positive for transcription factor NKX2.1 and neuronal marker Tuj1 from hiPSCs. Scale bar, 100μm. The experiment was repeated independently over three times with similar results. (**b**) Flow cytometry quantification of the percentage of $NKX2^+$ cells in hiPSC-derived MGE GABAergic progenitors. n = 12 (3 repeats of 4 hiPSC lines). (**c**) Generation of GABAergic neurons positive for GABA and neuronal marker Tuj1. Scale bar, 100μm. The experiment was repeated independently over three times with similar results. (**d**) Flow cytometry quantification of the percentage of $GABA^+$ cells in hiPSC-derived GABAergic neuron culture. n = 12 (3 repeats of 4 hiPSC lines). (**e**) Immunocytochemical quantification of the percentage of $AT8^+$ and $GABA^+$ cells relative to the total number of $GABA^+$ cells in GABAergic neuron cultures derived from apoE3/3-hiPSCs and apoE4/4-hiPSCs. Values are normalized to E3/3. n = 12 fields from E3/3 (2 hiPSC lines with total of 342 neurons counted), n =12 fields from E4/4 (2 hiPSC lines with total of 412 neurons counted). (**f**) Immunocytochemical quantification of the percentage of $PHF1^+$ and $GABA^+$ cells relative to the total number of $GABA^+$ cells in GABAergic neuron cultures derived from apoE3/3-hiPSCs and apoE4/4-hiPSCs. Values are normalized to E3/3. n = 12 fields from E3/3 (2 hiPSC lines with total of 364 neurons counted), n = 12 fields from E4/4 (2 hiPSC lines with total of 436 neurons counted). (**g**) Immunocytochemical quantification of the percentage of Caspase-

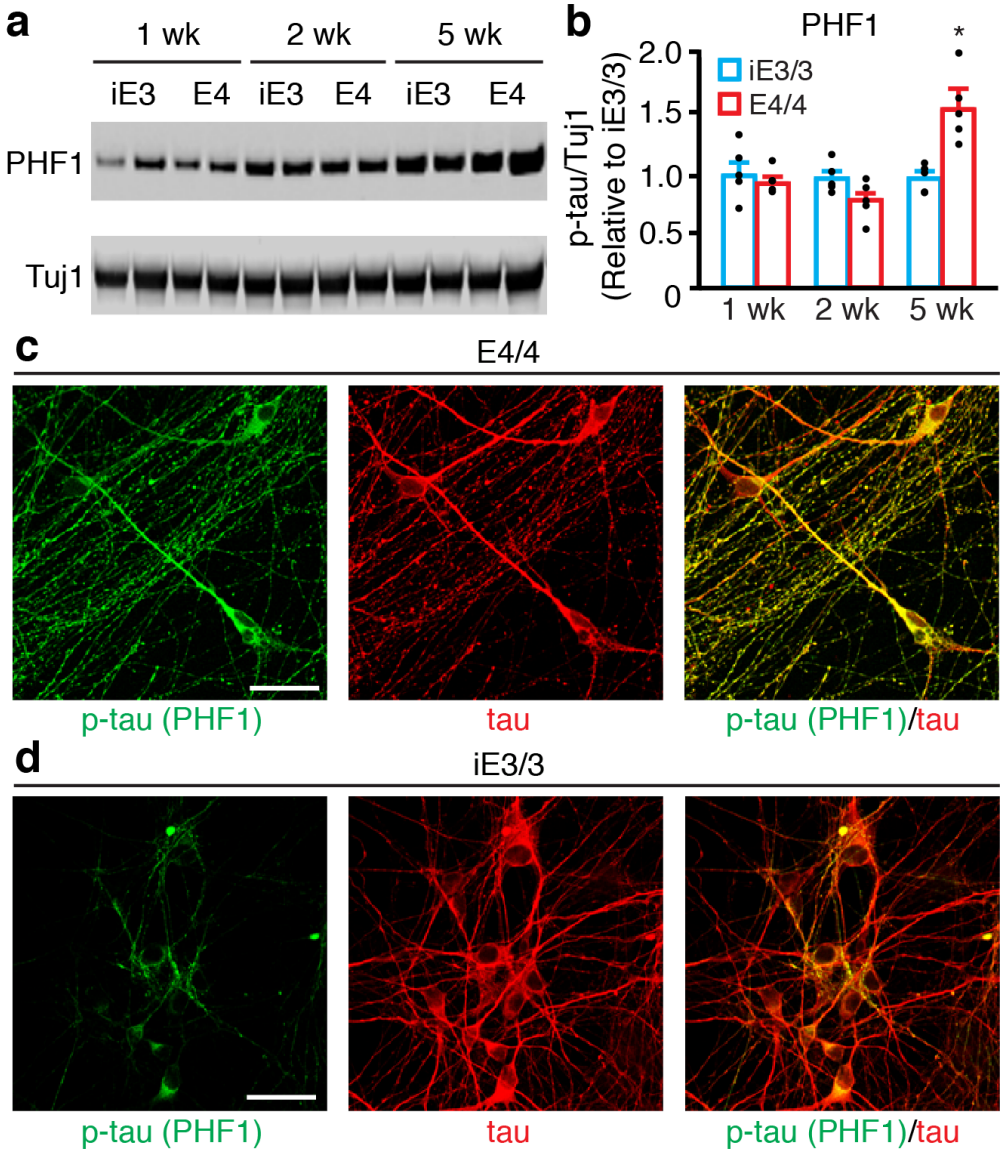
3^+ and $GABA^+$ cells relative to the total number of $GABA^+$ cells in GABAergic neuron cultures derived from apoE3/3-hiPSCs and apoE4/4-hiPSCs. n = 12 fields from E3/3 (2 hiPSC lines with total of 339 neurons counted), n = 12 fields from E4/4 (2 hiPSC lines with total of 444 neurons counted). (**h, i**) Double immunostaining for p-tau (PHF1) and MAP2 in GABAergic neuron culture derived from apoE4/4-hiPSCs (h) and apoE3/3-hiPSCs (i). Scale bar, 25 μm. The experiments were repeated independently over three times with similar results. All values are expressed as mean ± SEM. Differences between groups were determined with the unpaired two-sided *t* test.



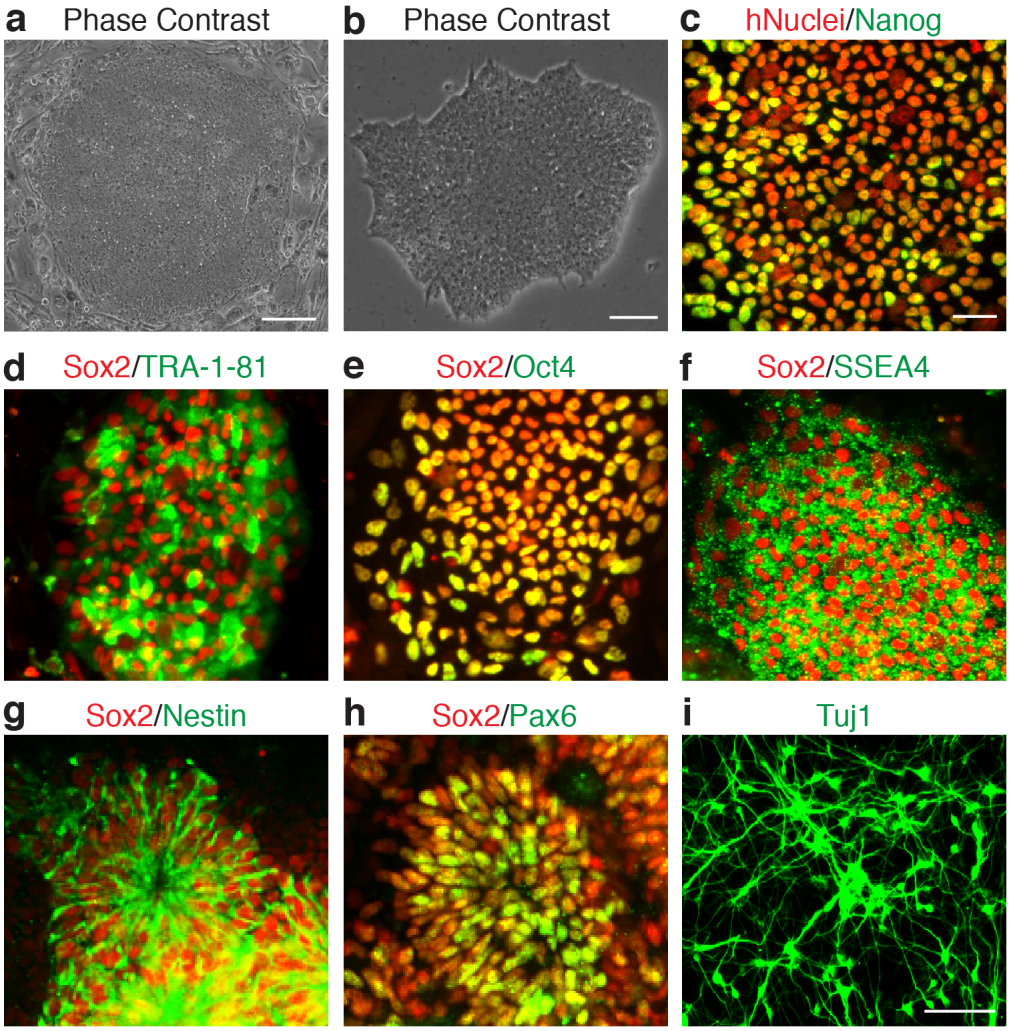
Supplementary Figure 6. Generation and characterization of isogenic apoE3/3-hiPSC lines from a parental apoE4/4-hiPSC line. (**a**) Schematic of a ZFN pair targeting the sequence around the codon (CGC) for Arg-112 (R112) in exon 4 of the human apoE4 gene and its cleavage site. The codon (TGC) for Cys-112 (C112) in exon 4 of the human apoE3 gene is indicated in the apoE3 donor and the corrected apoE3 sequence. (**b–d**) Immunostaining for pluripotent stem cell markers (b, c) in isogenic apoE3/3-hiPSCs and the karyotype (d). (**e, f**) Immunostaining for neural stem cell markers in isogenic apoE3/3-hiPSC-derived neural progenitors. (**g**) Immunostaining for neuronal markers Tuj1 and Map2 in neurons differentiated from the isogenic apoE3/3-hiPSCs. Scale bar, 50 μm (b, c, and e–g). Each experiment in b–g was repeated independently over three times with similar results.



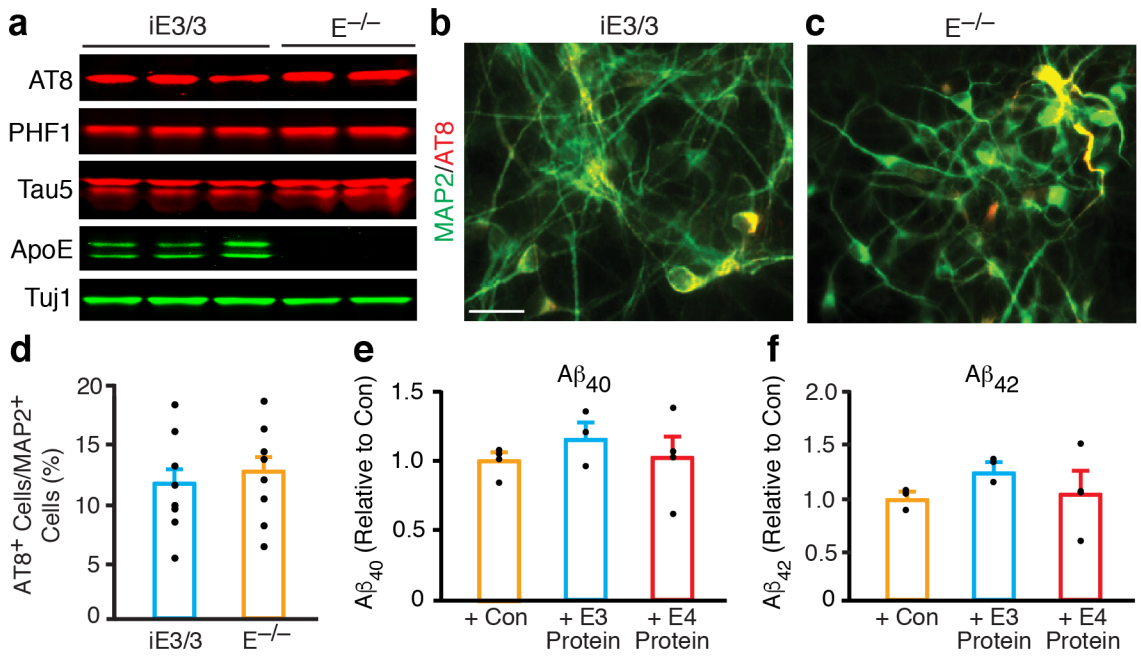
Supplementary Figure 7. Conversion of apoE4 to apoE3 by gene editing decreases the number of p-tau-positive neurons derived from isogenic apoE3/3-hiPSCs. (**a, b**) Immunostaining for MAP2 and p-tau (PHF1) in neuronal cultures derived from parental apoE4/4-hiPSCs (a) and isogenic apoE3/3-hiPSCs (iE3/3) (b). (**c**) Quantification of the percentage of MAP2-positive neurons that are also positive for p-tau (PHF1) in neuronal cultures derived from parental apoE4/4-hiPSCs and isogenic apoE3/3-hiPSCs. n = 8 fields from E4/4 with total of 2428 $MAP2^+$ neurons counted, n = 8 fields from iE3/3 with total of 2445 $MAP2^+$ neurons counted. (**d, e**) Immunostaining for GABA and p-tau (PHF1) in neuronal cultures derived from parental apoE4/4-hiPSCs (d) and isogenic apoE3/3-hiPSCs (e). (**f**) Quantification of the percentage of GABA-positive neurons that are also positive for p-tau (PHF1) in neuronal cultures derived from parental apoE4/4-hiPSCs and isogenic apoE3/3-hiPSCs. n = 8 fields from E4/4 with total of 176 $GABA^+$ neurons counted, n = 8 fields from iE3/3 with total of 254 $GABA^+$ neurons counted). (**g, h**) Immunostaining for GABA and p-tau (AT8) in neuronal cultures derived from the parental apoE4/4-hiPSCs (g) and the isogenic apoE3/3-hiPSCs (h). (**i**) Quantification of the percentage of GABA-positive neurons that are also positive for p-tau (AT8) in neuronal cultures derived from the parental apoE4/4-hiPSCs and the isogenic apoE3/3-hiPSCs. n = 8 fields from E4/4 with total of 107 $GABA^+$ neurons counted), n = 8 fields from iE3/3 with total of 344 $GABA^+$ neurons counted. Scale bar, 50 μm (a, b, d, e, g, and h). All values are expressed as mean ± SEM. Differences between groups were determined with the unpaired two-sided *t* test.



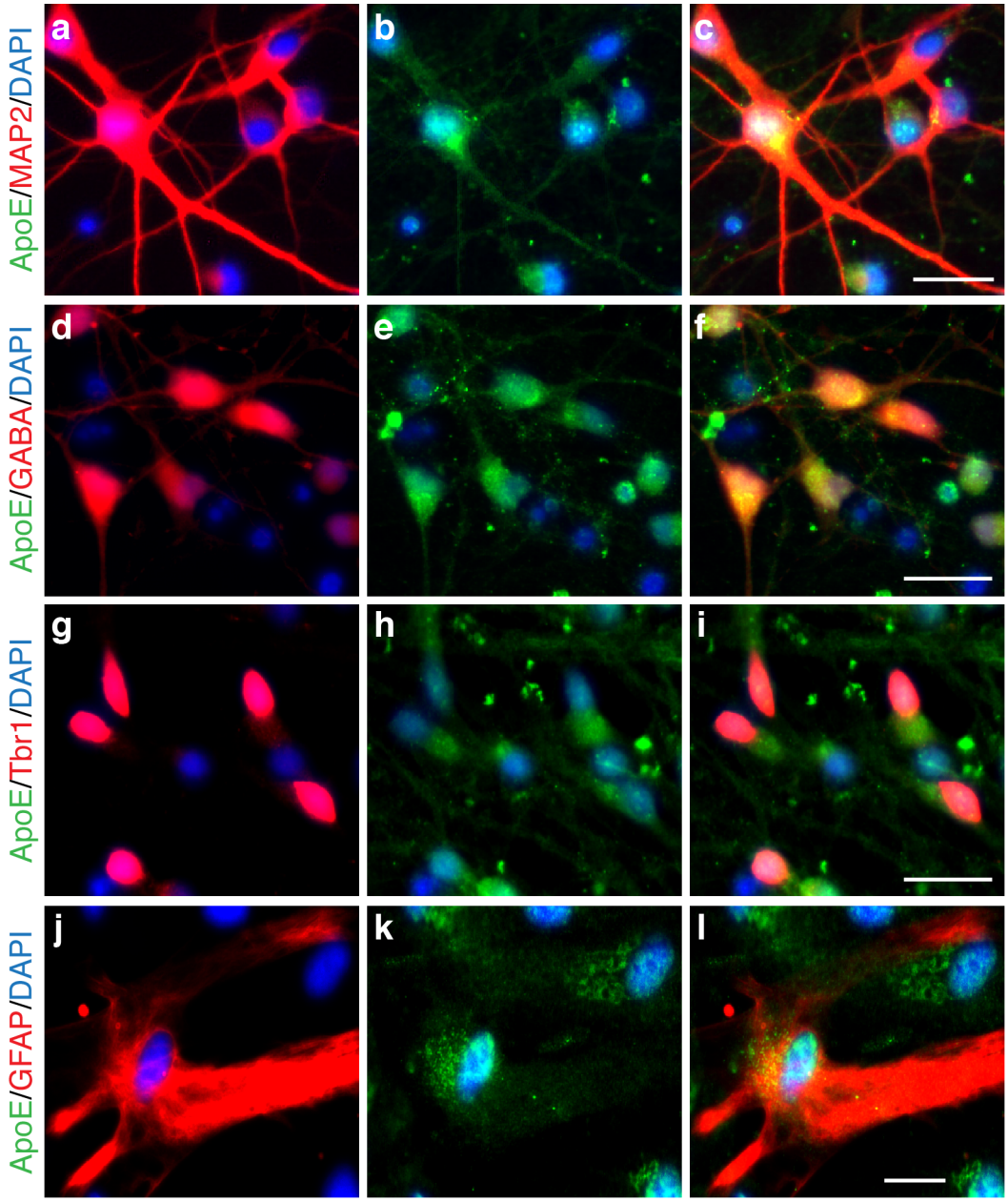
Supplementary Figure 8. AD-related pathologies in human apoE4/4 GABAergic neurons are specifically induced by apoE4. (**a**) Western blot analysis of p-tau (PHF1) in lysates of GABAergic neurons derived from isogenic apoE3/3-hiPSCs (iE3/3) and the parental apoE4/4-hiPSCs (E4/4). (**b**) Quantification of p-tau (PHF1) in lysates of GABAergic neurons derived from isogenic apoE3/3-hiPSCs (iE3/3) and the parental apoE4/4-hiPSCs (E4/4). Values are normalized to iE3/3. n = 5 (iE3/3) and n = 5 (E4/4) biologically independent samples. (**c, d**) Double immunostaining for p-tau (PHF1) and total tau in GABAergic neurons derived from the parental apoE4/4-hiPSCs (E4/4) (c) and the isogenic apoE3/3-hiPSCs (iE3/3) (d). Scale bar, 25μm. The experiments were repeated independently over three times with similar results. All values are expressed as mean ± SEM. Differences among groups were determined by two-way ANOVA followed with Sidak's multiple comparisons test in b. *$p<0.05$ versus iE3/3 at 5 weeks.



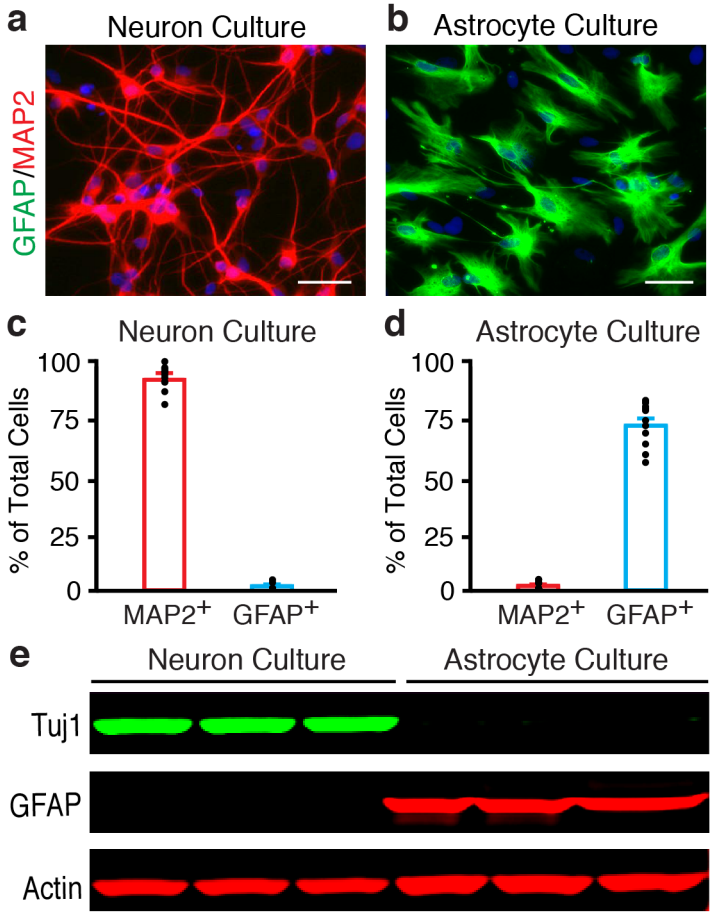
Supplementary Figure 9. Generation and characterization of hiPSC lines from an apoE-deficient subject. (**a, b**) Phase-contrast images of hiPSC clones cultured on feeders (a) or without feeders (b) derived from an apoE-deficient subject. Scale bar, 100 μm. (**c–f**) Immunostaining for pluripotent stem cell markers in apoE-deficient hiPSCs. (**g, h**) Immunostaining for neural stem cell markers in apoE-deficient hiPSC-derived neural progenitors. Scale bar in c, 50 μm (c–h). (**i**) Immunostaining for neuronal marker Tuj1 in differentiated neurons derived from the apoE-deficient hiPSCs. Scale bar, 100 μm. The experiments were repeated independently over three times with similar results.



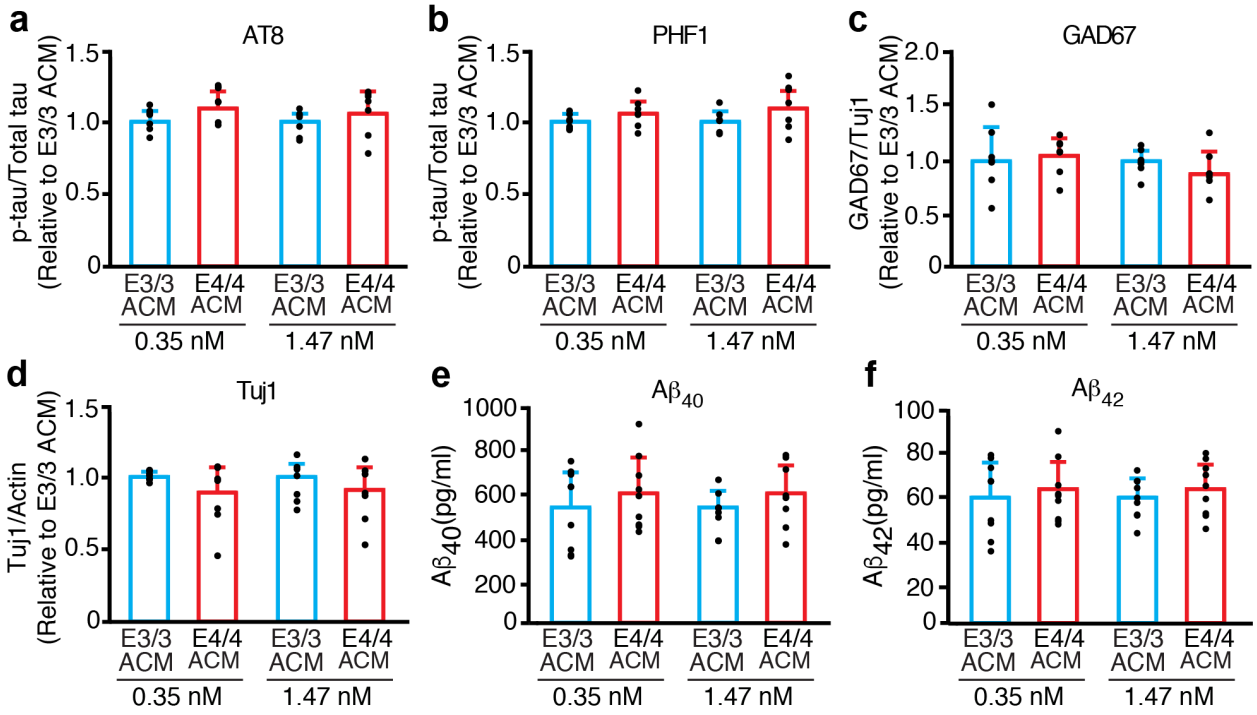
Supplementary Figure 10. Phenotypic analyses of neuronal cultures derived from apoE-deficient hiPSCs. (**a**) Western blot analysis of p-tau (AT8 and PHF1) and apoE in lysates of neurons derived from isogenic apoE3/3-hiPSCs (iE3/3) and apoE-deficient ($E^{-/-}$) hiPSCs. The experiments were repeated independently twice with similar results. (**b, c**) Immunostaining for MAP2 and p-tau (AT8) in neuronal cultures derived from the isogenic apoE3/3-hiPSCs (iE3/3) (b) and the apoE$^{-/-}$ hiPSCs (c). Scale bar, 25 μm. (**d**) Quantification of the percentage of MAP2-positive neurons that are also positive for p-tau (AT8) in neuronal cultures derived from the isogenic apoE3/3-hiPSCs and the apoE$^{-/-}$ hiPSCs. n = 8 fields from iE3/3 with total of 1691 MAP2$^+$ neurons counted, n = 8 fields from $E^{-/-}$ with total of 1398 MAP2$^+$ neurons counted. (**e**) $A\beta_{40}$ levels in culture medium of apoE-null neurons treated with control (+Con), purified recombinant apoE3 (+E3 protein, 220 nM), or purified recombinant apoE4 (+E4 protein, 220 nM). Values are normalized to +Con. n = 4 (+Con), n = 3 (+E3 protein), and n = 4 (+E4 protein) biologically independent samples. (**f**) $A\beta_{42}$ levels in culture medium of apoE-null neurons treated with control (+Con), purified recombinant apoE3 (+E3 protein, 220 nM), or purified recombinant apoE4 (+E4 protein, 220 nM). Values are normalized to +Con. n = 4 (+Con), n = 3 (+E3 protein), and n = 4 (+E4 protein) biologically independent samples. All values are expressed as mean ± SEM. Differences between groups were determined with the unpaired two-sided *t* test in d. Differences among groups were determined with one-way ANOVA followed with Tukey's multiple comparison test in e and f. No comparison reaches statistical significance.



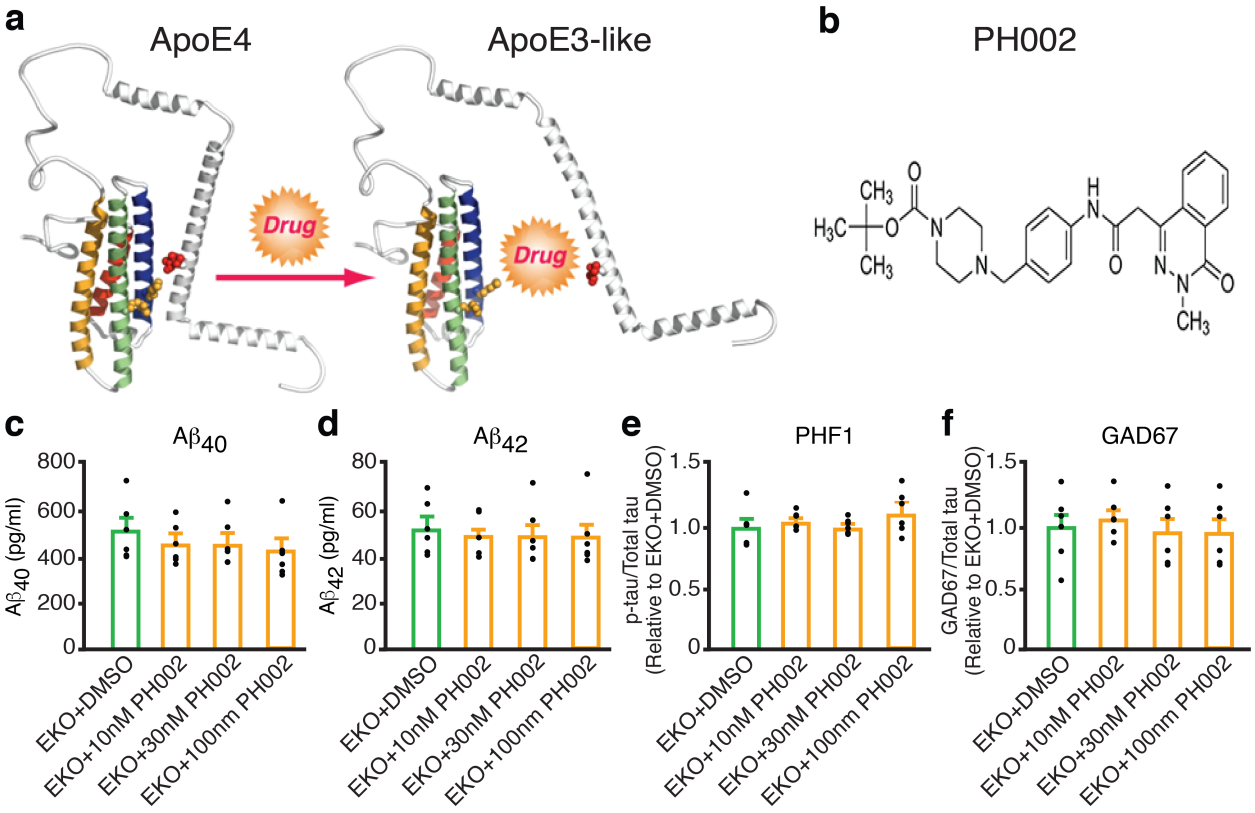
Supplementary Figure 11. Expression of apoE in different types of neurons and astrocytes derived from hiPSCs. (**a–c**) Anti-apoE and anti-MAP2 (general neuronal marker) double immunostaining of neurons derived from hiPSCs. (**d–f**) Anti-apoE and anti-GABA (GABAergic neuronal marker) double immunostaining of neurons derived from hiPSCs. (**g–i**) Anti-apoE and anti-Tbr1 (glutamatergic neuronal marker) double immunostaining of neurons derived from hiPSCs. (**j–l**) Anti-apoE and anti-GFAP (astrocytic marker) double immunostaining of astrocytes derived from hiPSCs. Scale bars are 25 μm. The experiments were repeated independently over three times with similar results.



Supplementary Figure 12. Characterization of highly pure neuronal and astrocytic cultures derived from hiPSCs. (**a**) Anti-MAP2 and anti-GFAP double immunostaining of highly pure neuronal cultures derived from hiPSCs. (**b**) Anti-MAP2 and anti-GFAP double immunostaining of astrocytic cultures derived from hiPSCs. (**c**) Quantification of $MAP2^+$ and $GFAP^+$ cells in highly pure neuronal cultures derived from hiPSCs. n=12 fields with total of 326 cells counted. (**d**) Quantification of $MAP2^+$ and $GFAP^+$ cells in astrocytic cultures derived from hiPSCs. n=12 fields with total of 388 cells counted. (**e**) Western blot analysis of Tuj1, GFAP, and actin in highly pure neuronal and astrocytic cultures derived from hiPSCs. The experiments were repeated independently over three times with similar results. In c and d, values are expressed as mean ± SEM. Scale bars are 50 μm.



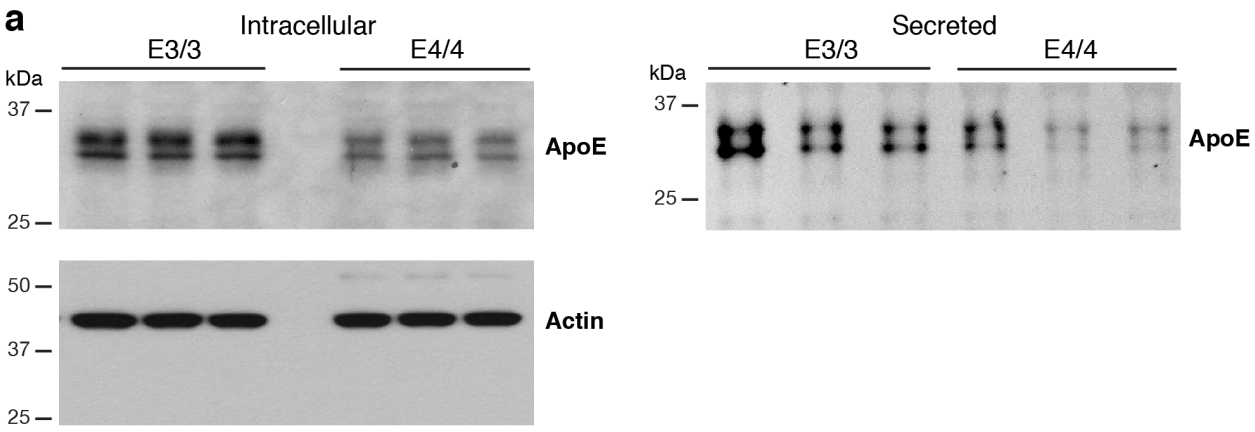
Supplementary Figure 13. hiPSC-derived astrocyte-conditioned medium with apoE3 (E3/3-ACM) or apoE4 (E4/4-ACM) had no effect on p-tau levels, GABAergic neurons, and Aβ production in neuronal cultures derived from apoE$^{-/-}$ hiPSCs. (**a, b**) Quantification of p-tau levels, as determined by AT8 (a) and PHF1 (b) western blots, in apoE$^{-/-}$ hiPSC-derived neurons treated with E3/3-ACM or E4/4-ACM containing 0.35 nM or 1.47 nM of apoE for 7 days. n = 7 (E3/3-ACM) and n = 7 (E4/4-ACM) biologically independent samples. (**c**) Quantification of GABAergic neurons, as determined by GAD67 western blot, in apoE$^{-/-}$ hiPSC-derived neuronal cultures treated with E3/3-ACM or E4/4-ACM containing 0.35 nM or 1.47 nM of apoE for 7 days. n = 7 (E3/3-ACM) and n = 7 (E4/4-ACM) biologically independent samples. (**d**) Quantification of levels of general neuronal marker Tuj1, as determined by western blot, in apoE$^{-/-}$ hiPSC-derived neurons treated with E3/3-ACM or E4/4-ACM containing 0.35 nM or 1.47 nM of apoE for 7 days. n = 7 (E3/3-ACM) and n = 7 (E4/4-ACM) biologically independent samples. (**e, f**) Quantification of Aβ$_{40}$ and Aβ$_{42}$ levels in medium of apoE$^{-/-}$ hiPSC-derived neurons treated with E3/3-ACM or E4/4-ACM containing 0.35 nM or 1.47 nM of apoE for 7 days. n = 9 (E3/3-ACM) and n = 9 (E4/4-ACM) biologically independent samples. In a–d, values are normalized to E3/3-ACM. All values are expressed as mean ± SD. Differences between groups were determined with the unpaired two-sided *t* test. No comparison reaches statistical significance.

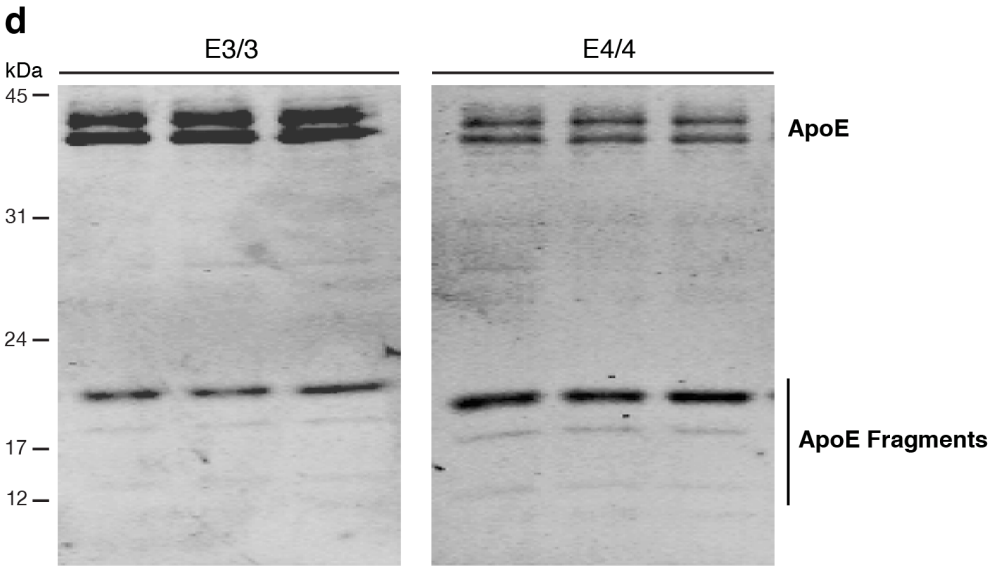


Supplementary Figure 14. ApoE4 domain interaction, a small-molecule apoE4 structure corrector, and its effects on AD-related pathologies in apoE$^{-/-}$ (EKO) hiPSC-derived neurons. (**a**) Schematic of apoE4 domain interaction mediated by the ionic interaction between arginine-61 in the N-terminus and glutamic acid-255 in the C-terminus of the molecule. This interaction can be blocked by small-molecule apoE4 structure correctors, which convert apoE4 to an apoE3-like molecule. (**b**) Chemical structure of the small-molecule apoE4 structure corrector PH002. (**c–f**) Quantification of Aβ$_{40}$ (c), Aβ$_{42}$ (d), p-tau (PHF1) (e), and GAD67 (f) in apoE$^{-/-}$ (EKO) hiPSC-derived neurons treated with DMSO (control) or different doses of PH002 for 7 days. n = 6 biologically independent samples for each treatment group. In e and f, values are normalized to EKO+DMSO. All values are expressed as mean ± SEM. Differences among groups were determined with one-way ANOVA followed with Tukey's multiple comparison test in c–f. No comparison reaches statistical significance.

Supplementary Figure 15

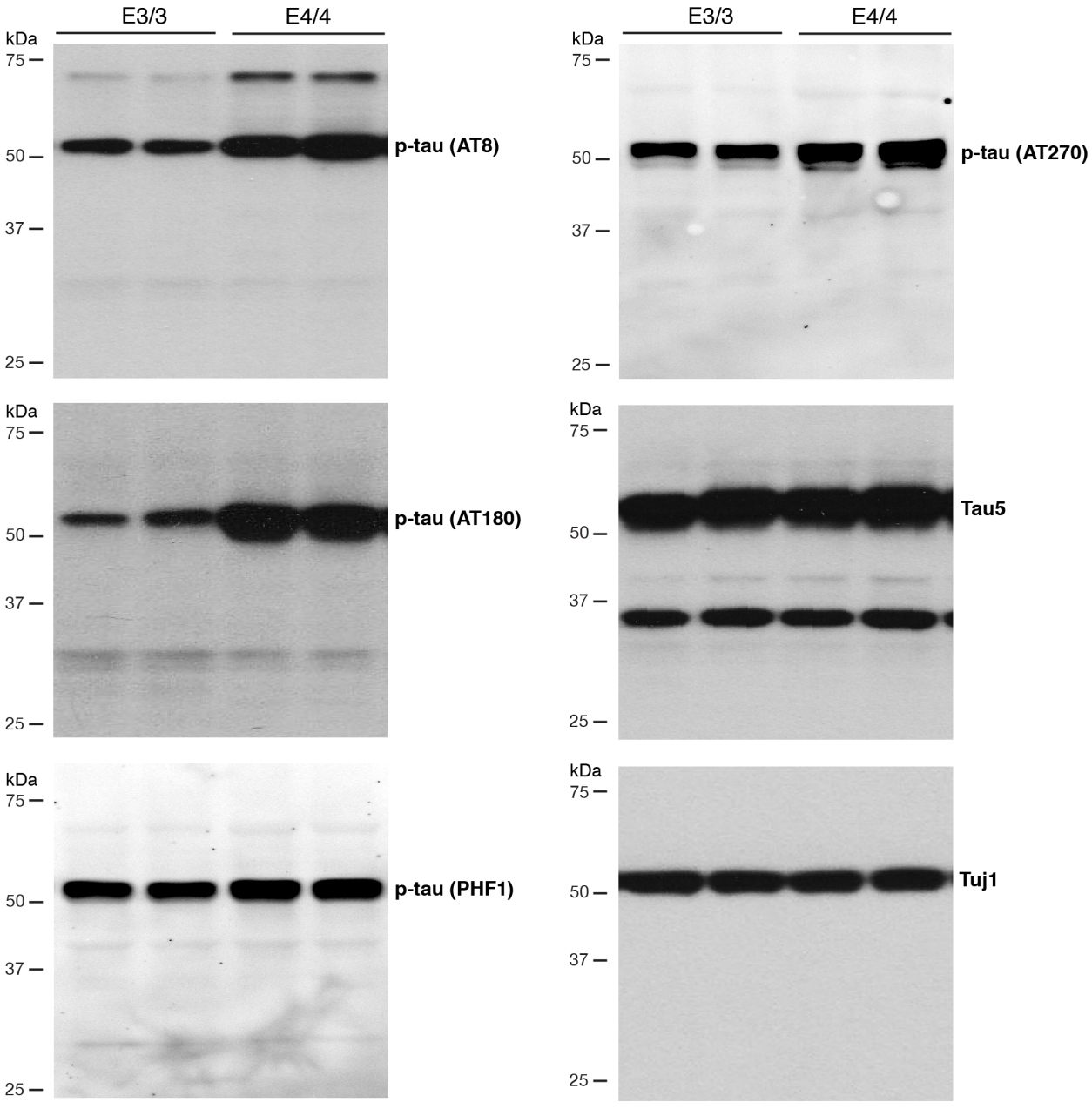
Figure 1a and 1d





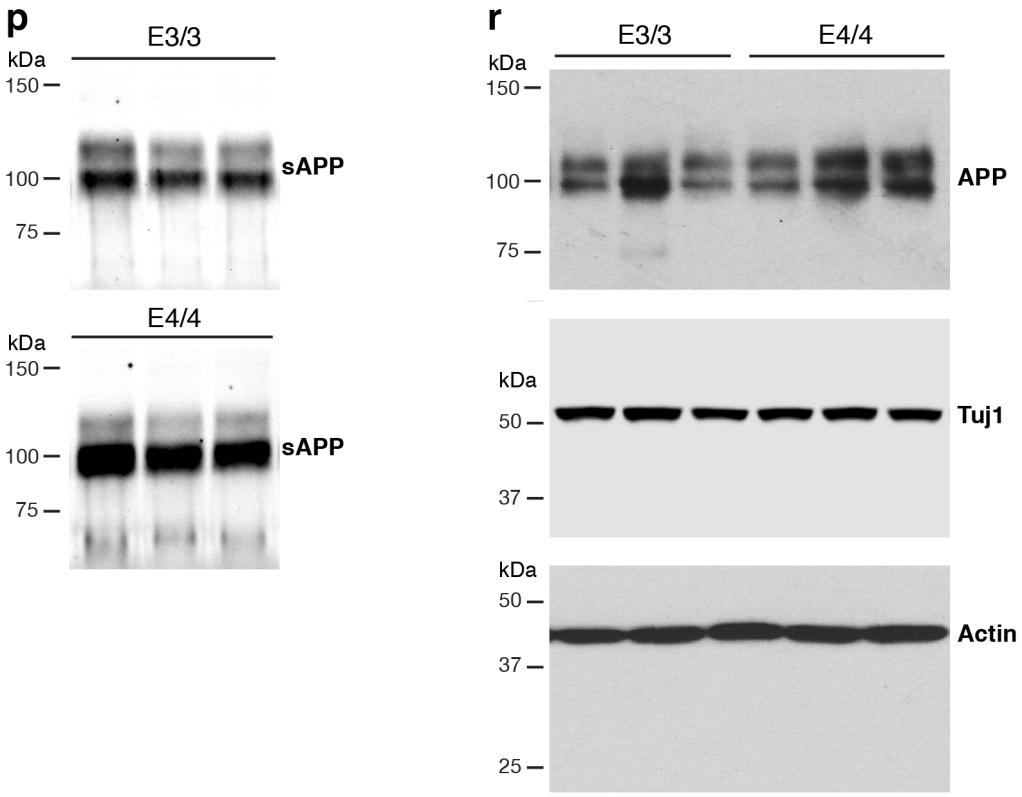
Supplementary Figure 15 (Continued)

Figure 1f



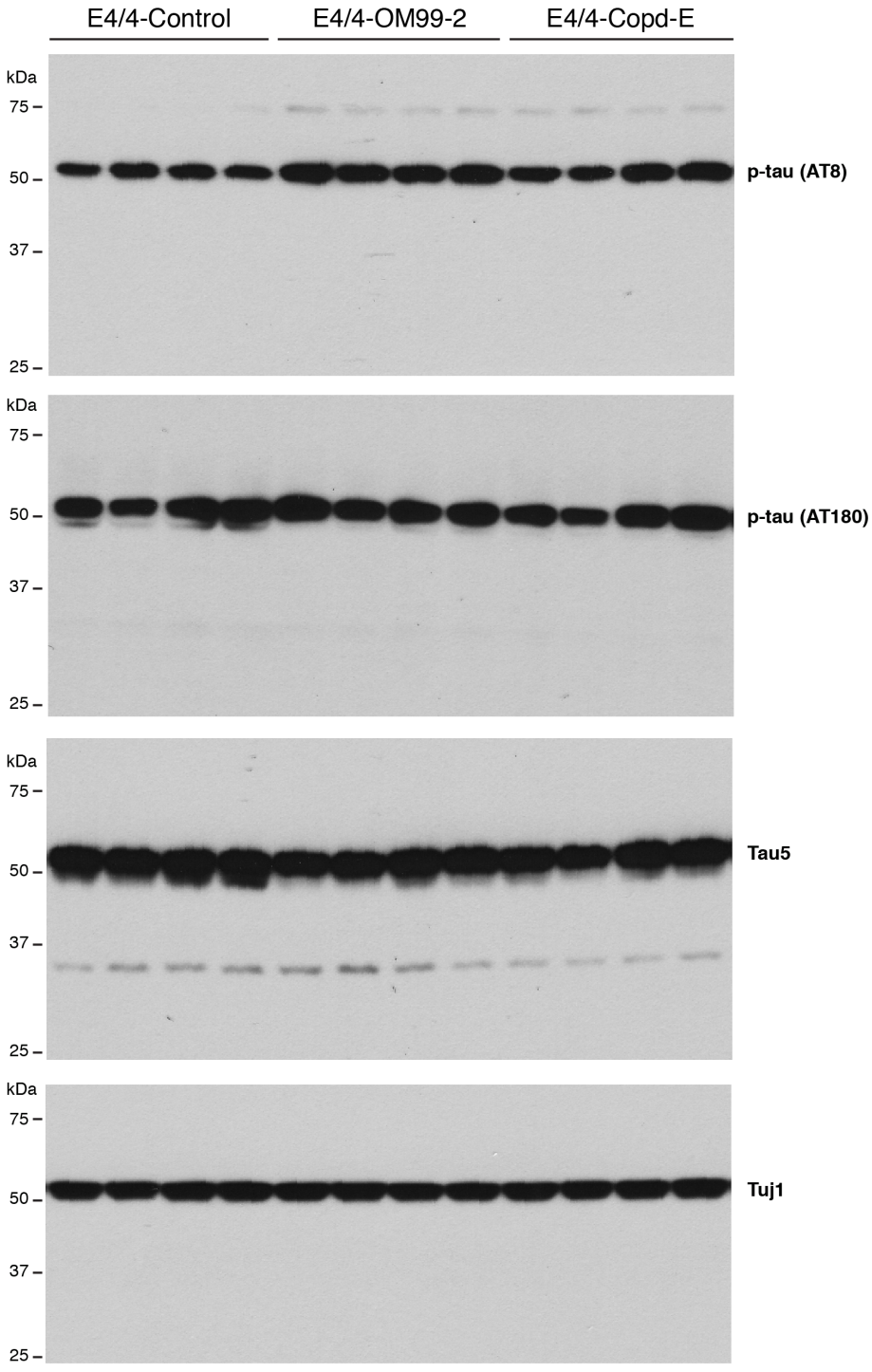
Supplementary Figure 15 (Continued)

Figure 1p and 1r



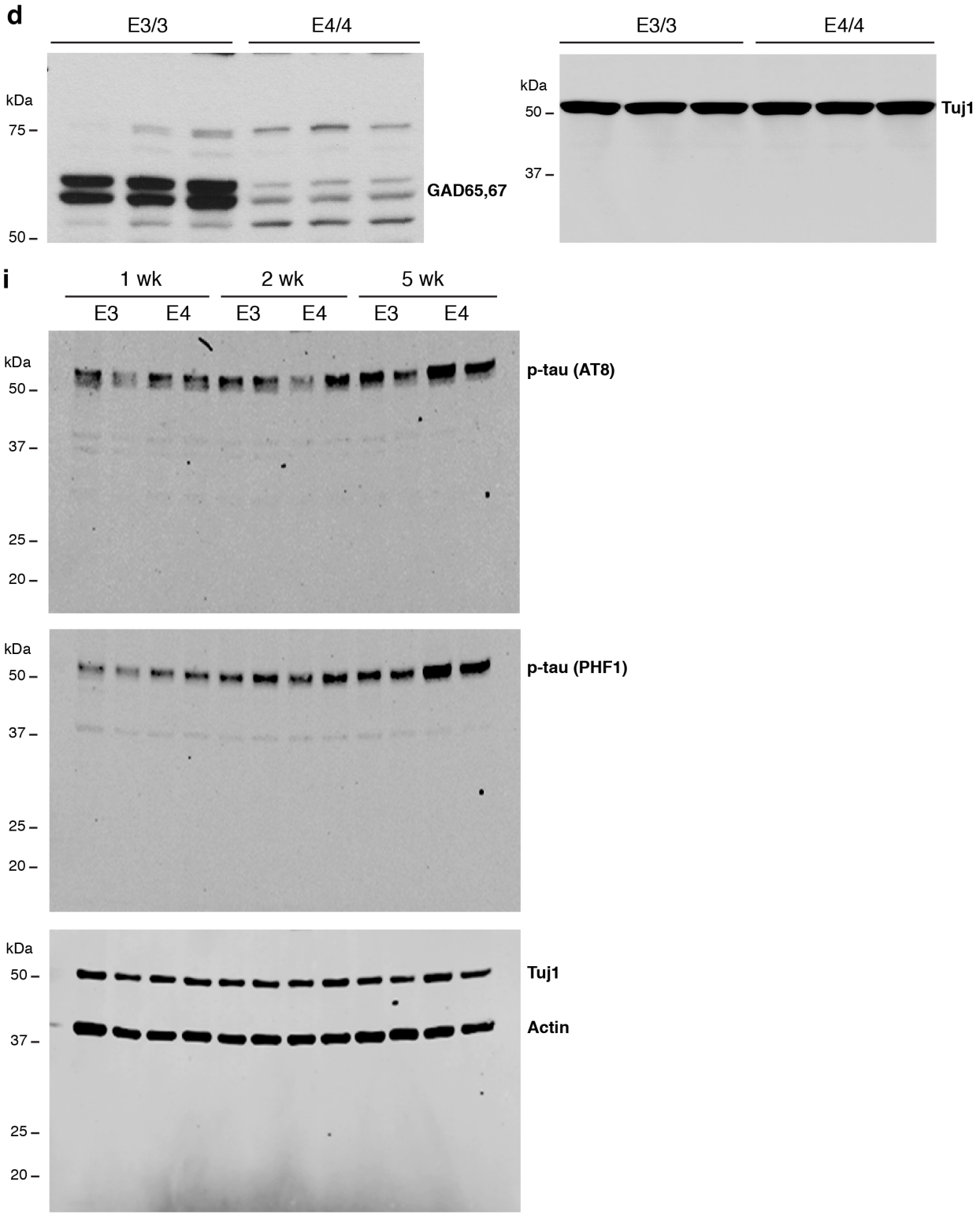
Supplementary Figure 15 (Continued)

Figure 2c



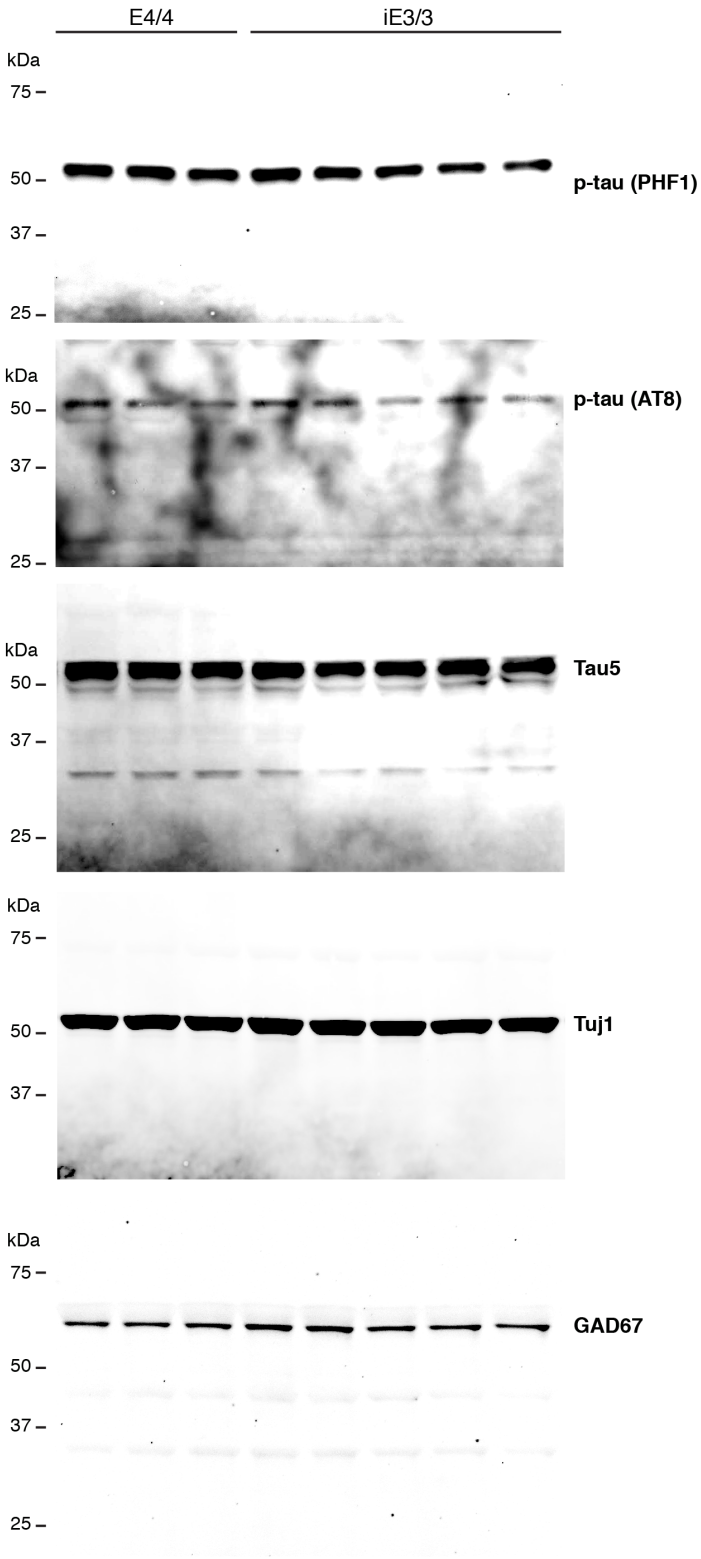
Supplementary Figure 15 (Continued)

Figure 3d and 3i



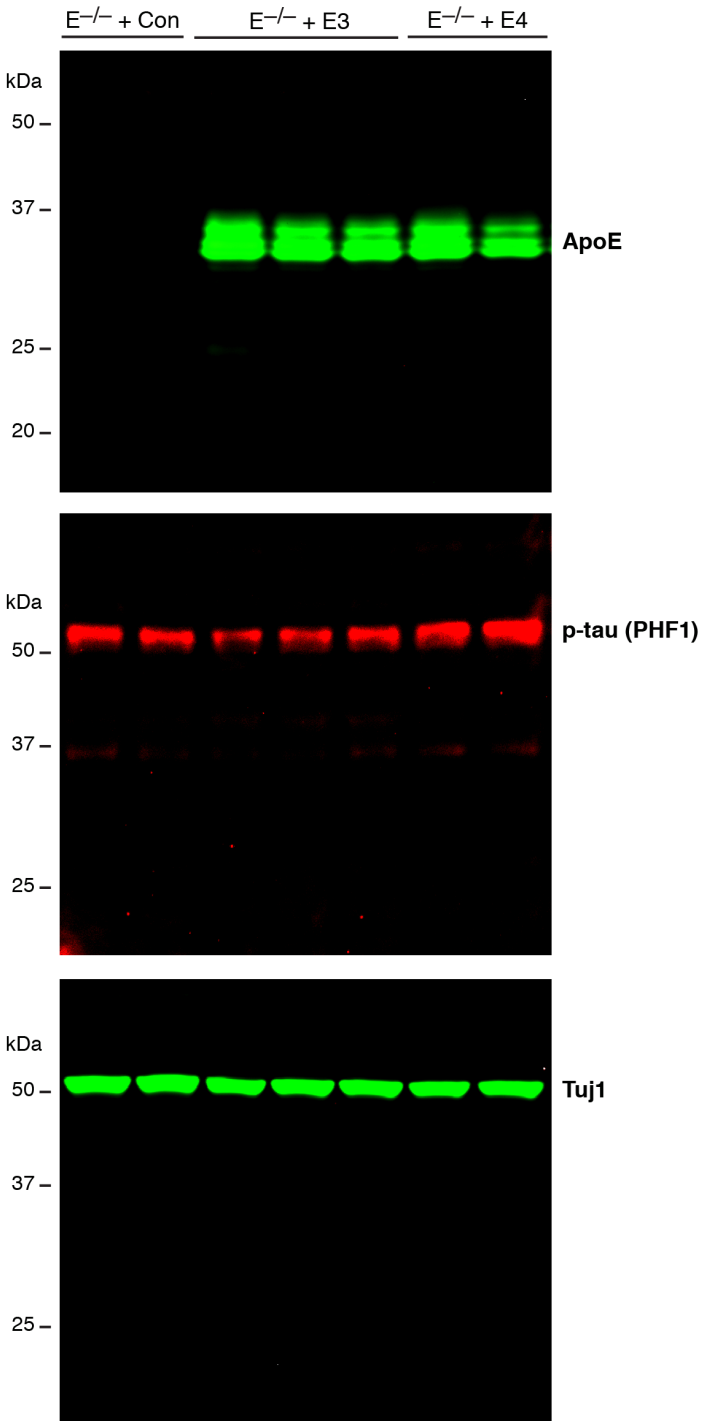
Supplementary Figure 15 (Continued)

Figure 4e



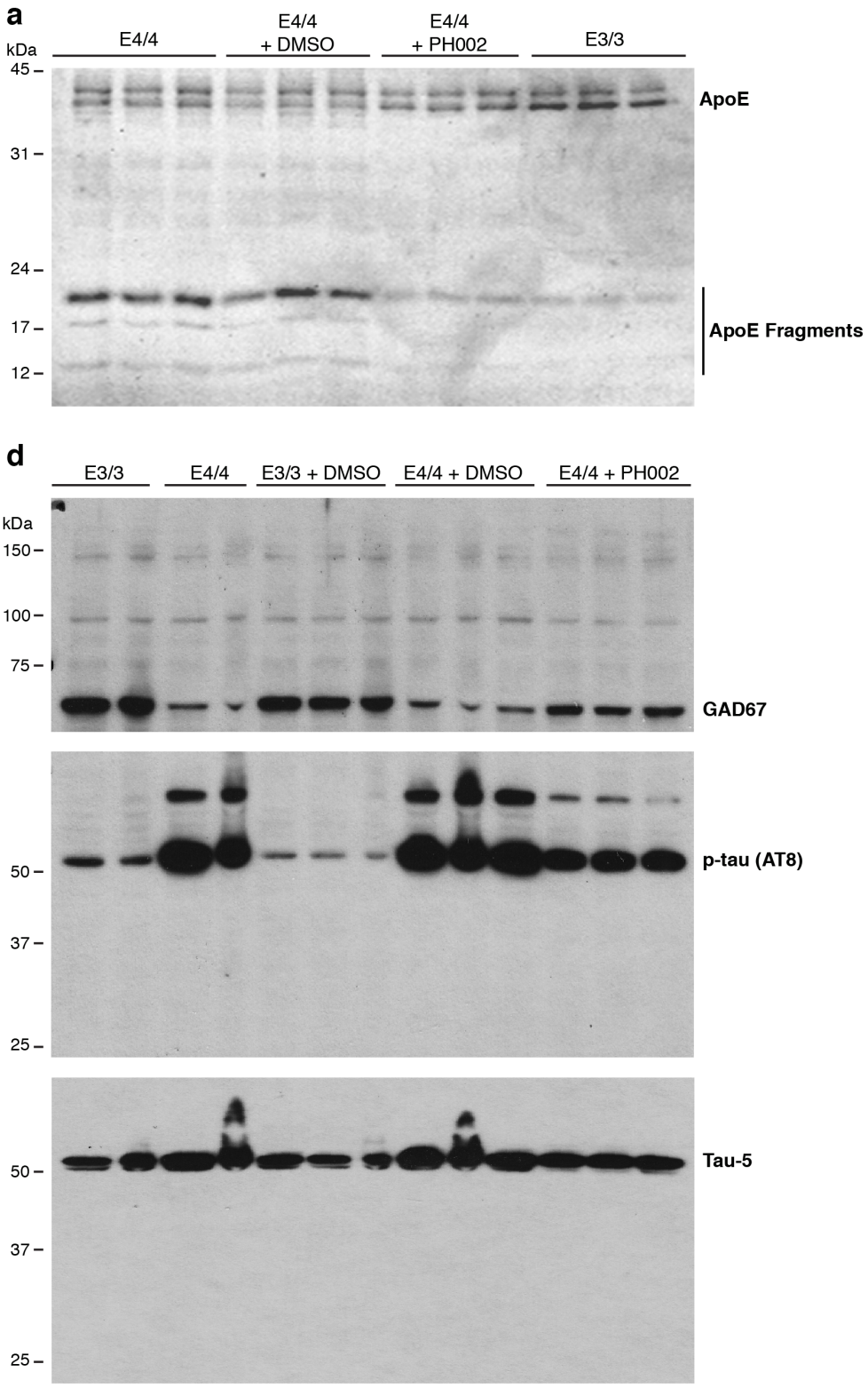
Supplementary Figure 15 (Continued)

Figure 5h



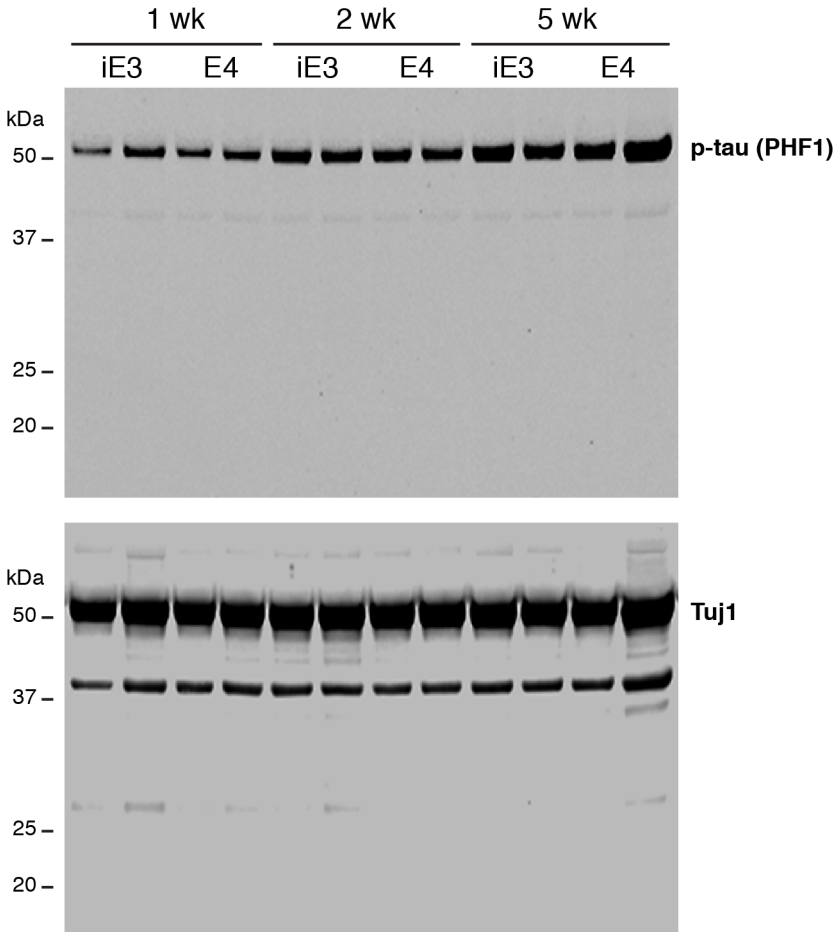
Supplementary Figure 15 (Continued)

Figure 6a and 6d



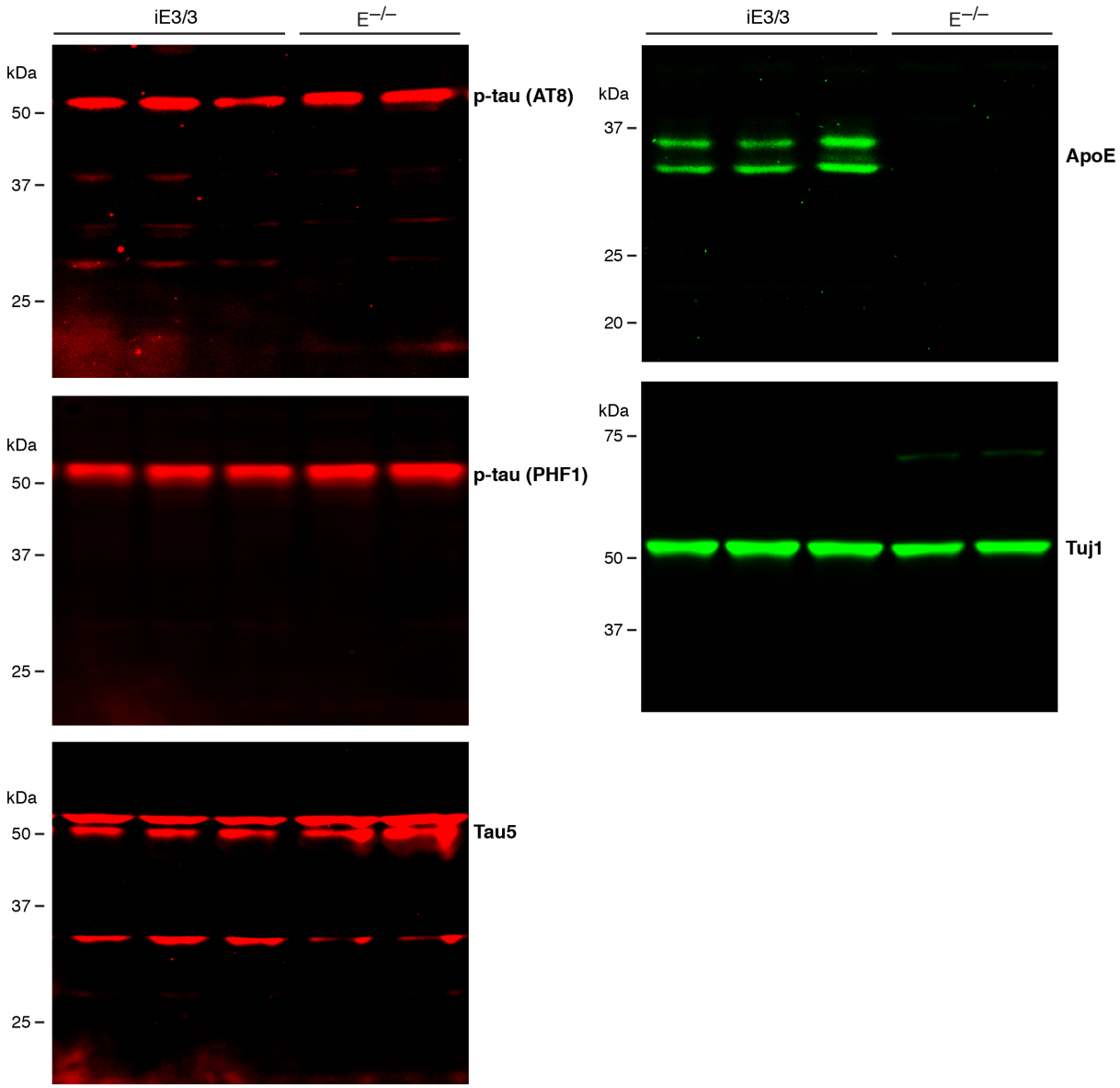
Supplementary Figure 15 (Continued)

Supplementary Figure 8a



Supplementary Figure 15 (Continued)

Supplementary Figure 10a



Supplementary Figure 15 (Continued)

Supplementary Figure 12e