Supplementary Figure-S3 (Young-Pearse)

**A**

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**B**

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**C**

Western blot

\[
\frac{APP_{\alpha}}{APP_{\text{total}}} \text{ for Control and fAD}
\]

*P < 0.005*
Supplementary Figure-S6 (Young-Pearse)

A

B

C

D

E

F

G
Supplementary Figure-S7 (Young-Pearse)

A

![Diagram showing time points for aggregates, primitive NE, definitive NE, and neuronal differentiation with annotations for total Tau phenotype and phospho-Tau phenotype, as well as AW7 early and late treatment.]

B

![Bar chart showing Aβ protein levels with PI and AW7 in control and IAD conditions.]

C

![Graph showing ELISA results for Total Tau/NEP2 with PI and AW7 in control and IAD conditions.]

D

![Graph showing Western Blot results for Total Tau/NEP2 with PI and AW7 in control and IAD conditions.]
Supplementary Figure S1. Characterization of human iPSC lines generated in this manuscript.

A) Microscopy images are shown for 2 fAD1 and 2 fAD2 iPSC lines. iPSCs were stained for Alkaline Phosphatase or else immunostained for Oct4, NANOG, SSEA3, SSEA4, or TRA-1-60. B) RNA templates purified from iPSC lines were first reversed-transcribed to cDNA (+) or without reverse transcription (-), followed by PCR for markers listed. 2 fAD1 and 2 fAD2 iPSC lines are shown. C) To test for pluripotency, each line was differentiated using an embryoid body protocol for 15 days, and RT-PCR was utilized to test for the ability to generate all three germ layers. 2 fAD1 and 2 fAD2 iPSC lines are shown. Karyotyping results from fAD 2 clone b (D) and fAD 1 clone a (E) performed by Cell Line Genetics.

Supplementary Figure S2. Microelectrode array (MEA) recordings show spontaneous activity from mature iPSC-derived neurons.

Control and fAD lines were plated at day 24 and co-cultured with astrocytes for up to 21 days. A) A 50 sec voltage trace from a representative electrode is shown for 2 control and 2 fAD lines. Raw traces were low-pass filtered with a Gaussian filter with cutoff frequency of approximately 200 Hz. Single unit waveforms (3 ms, right column) are representative examples from each raw trace. B) Raster plots for above cell lines showing synaptic events over time were plotted without alteration. 8 representative electrodes were selected from 1 well of recording, from 2 control and 2 fAD lines, respectively. Each raster grouping shows examples of low and high activity.
Supplementary Figure S3. Confirmation of effects of V717I on cleavage of APP using alternate methodologies.

Control and fAD APP V717I lines were differentiated to neuronal fates. A) Western blot showing APP and CTFs for control and fAD lines treated with the γ-secretase inhibitor compound E (48 hrs, 50nM). B) Representative Western blots for APPsα (1736) and APPs total (22C11) of conditioned media from neurons derived from a single control and single fAD line at day 50-60 in two different experiments. Quantification of densitometry is shown in C. Error bars represent SEM, control n=8 wells, fAD n=8 wells. Two-tailed t-tests for each comparison were performed **p<0.01.

Supplementary Figure S4. FAD mutation (APP V717I) in forebrain neuronal cells leads to increased Aβ42 and Aβ38 production, shown relative to total protein and shown by differentiation round.

Control and fAD iPSC lines were differentiated for 40-60 days to neuronal fates. Media conditioned on the cells for the final 48 hrs was collected, and Aβ40 (A), Aβ42 (B), and Aβ38 (C) were detected in a single well using a multiplex ELISA (MesoScale Discoveries) and normalized to total protein. Error bars represent SEM, control n=4, APP V717I (fAD) n=19 *p<0.05. D) Control and fAD iPSC lines were differentiated for 100 days to neuronal fates. Media was collected and cells lysed, and Aβ measured in both using the same ELISA. Error bars represent SEM, control n=5, APP V717I (fAD) n=5, * p<0.05, ** p<0.01, *** p<0.001. ND=not detected. Aβ ELISA data shown is broken down by round of differentiation (E) and by ELISA plate (F). Letter codes denote separate differentiation experiments and dates correspond to separate ELISA runs. Each
Supplementary Figure S5. APP V717I mutation increases Aβ42 and Aβ38 generation as well as increases APPsβ generation in HEK cells.

HEK cells were transiently transfected with APP V717I or WT APP. 24 hours later, cells were treated with DAPT (5 µM) or vehicle for 24 hours, followed by collection of media and lysis of cells. A-G) In the media, Aβ40 (C), Aβ42 (B), and Aβ38 (D) were detected using a multiplex ELISA, and APPsα (F) and APPsβ (G) were detected using a duplex ELISA. Ratios for Aβ42/40 and APPsα/β are shown in A and E, respectively. H-K) HEK cells were transiently transfected with either APPV717I or WT APP and subsequently treated with DMSO or DAPT (5µM). APP (H), total CTFs (I) and β-CTFs (J) were quantified by densitometry from Western blot analysis (K). Error bars represent SEM. For A-G, n=6 and for H-K, n=3. Two-tailed t-tests for each comparison were performed * p<0.05, ** p<0.01, *** p<0.001. ND=not detected.

Supplementary Figure S6. Examination of cleavage of APP following γ-secretase and β-secretase inhibitor treatment in HEK cells.

HEK cells were transiently transfected with APP V717I or WT APP. 24 hours later, cells were treated with DAPT (5 µM), Compound E (50 nM), L-685,458 (1.5 µM) or vehicle for 24 hours (A-E), or else with C3 (0.3 µM) and vehicle (F,G) for 24 hours. After treatment, conditioned media were collected and cells lysed. A-D) In the media, Aβ38 (A), Aβ40 (B), and Aβ42 (C) were detected using a multiplex ELISA, and the ratio of APPsα/APPsβ was detected using a duplex ELISA (D). Western blots were performed on
the lysates to confirm equal levels of expression of APP (E). F) After C3 or vehicle treatment, the ratio of APPsα/APPsβ was detected using a duplex ELISA, as well as the ratio of Aβ42/40 (G). For A-D black asterisks show significance vs. WTAPP DMSO and green asterisks show significance vs. APPV717I DMSO. In D, WTAPP and APPV717I data are independently normalized to 100% of their respective DMSO levels. Error bars represent SEM, n=3 for all columns. Two-tailed t-tests for each comparison were performed * p<0.05, ** p<0.01, *** p<0.001.

**Supplementary Figure S7. Aβ-specific antibodies do not rescue the Tau phenotype beyond the neural progenitor critical period.**

A) Timeline schematic of Tau phenotypes and antibody treatments. The total Tau phenotype is observed from day 24 to the latest time-point characterized, day 100. The phospho-Tau phenotype is only observed at the late time-point (D100). Two time-frames for antibody treatment were examined: days 20-35 and days 45-55. B-D) Control and fAD neurons (day 45) were treated with the Aβ-specific antibody AW7 and compared to preimmune serum for 15 days. B) ELISA data for Aβ is shown, following pull-down of Aβ. C,D) Treatment with a polyclonal antibody (AW7) was compared to treatment with its preimmune serum. ELISA data for total Tau (C) and quantification of total Tau by densitometry from Western blot (D) is shown. Fresh neural differentiation media with antibody was applied every three days. * p<0.05, *** p<0.001. PI=preimmune. N.D.=not detected. Error bars = SEM.