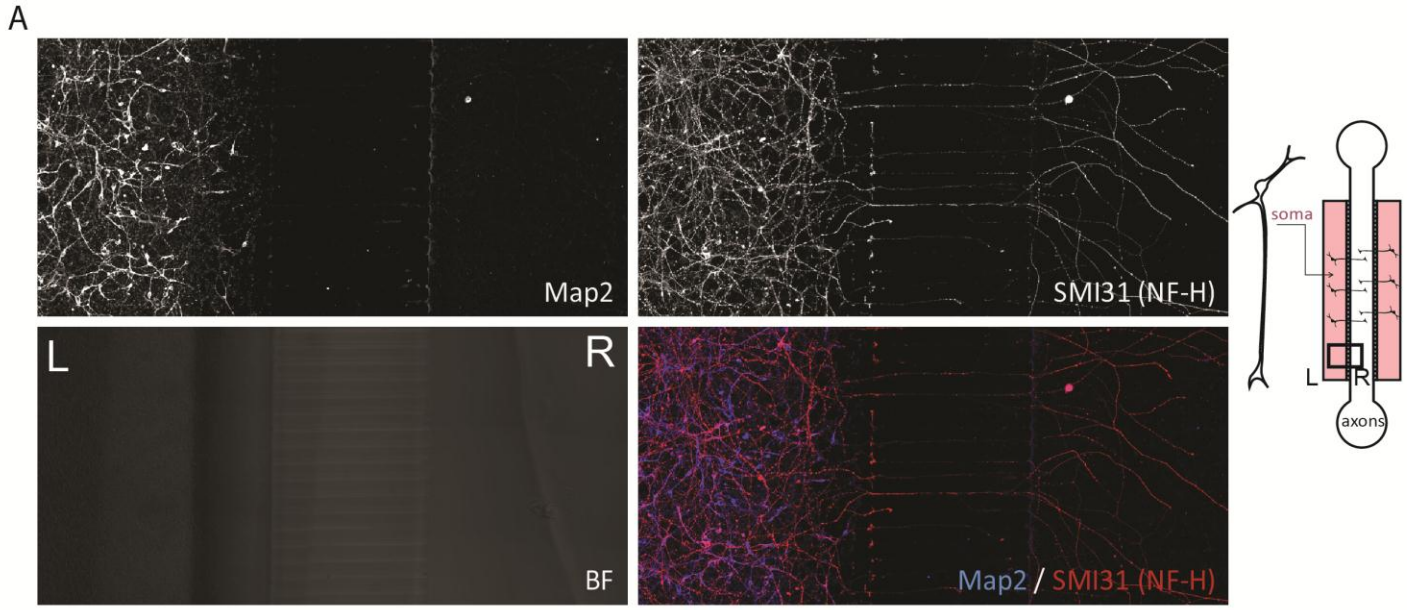
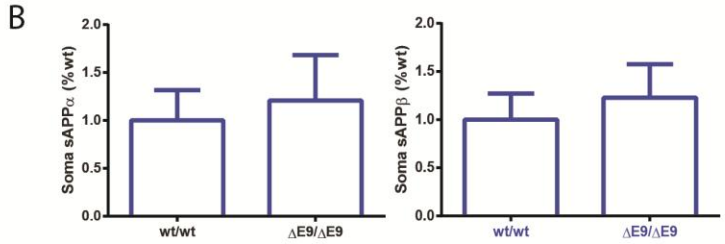

A
Map2
SMI31 (NF-H)
L
R
BF
Map2 / SMI31 (NF-H)
soma
L
R
axons


B
Soma sAPPα (%wt)
Soma sAPPβ (%wt)
2.0
1.5
1.0
0.5
0.0
wt/wt
ΔE9/ΔE9

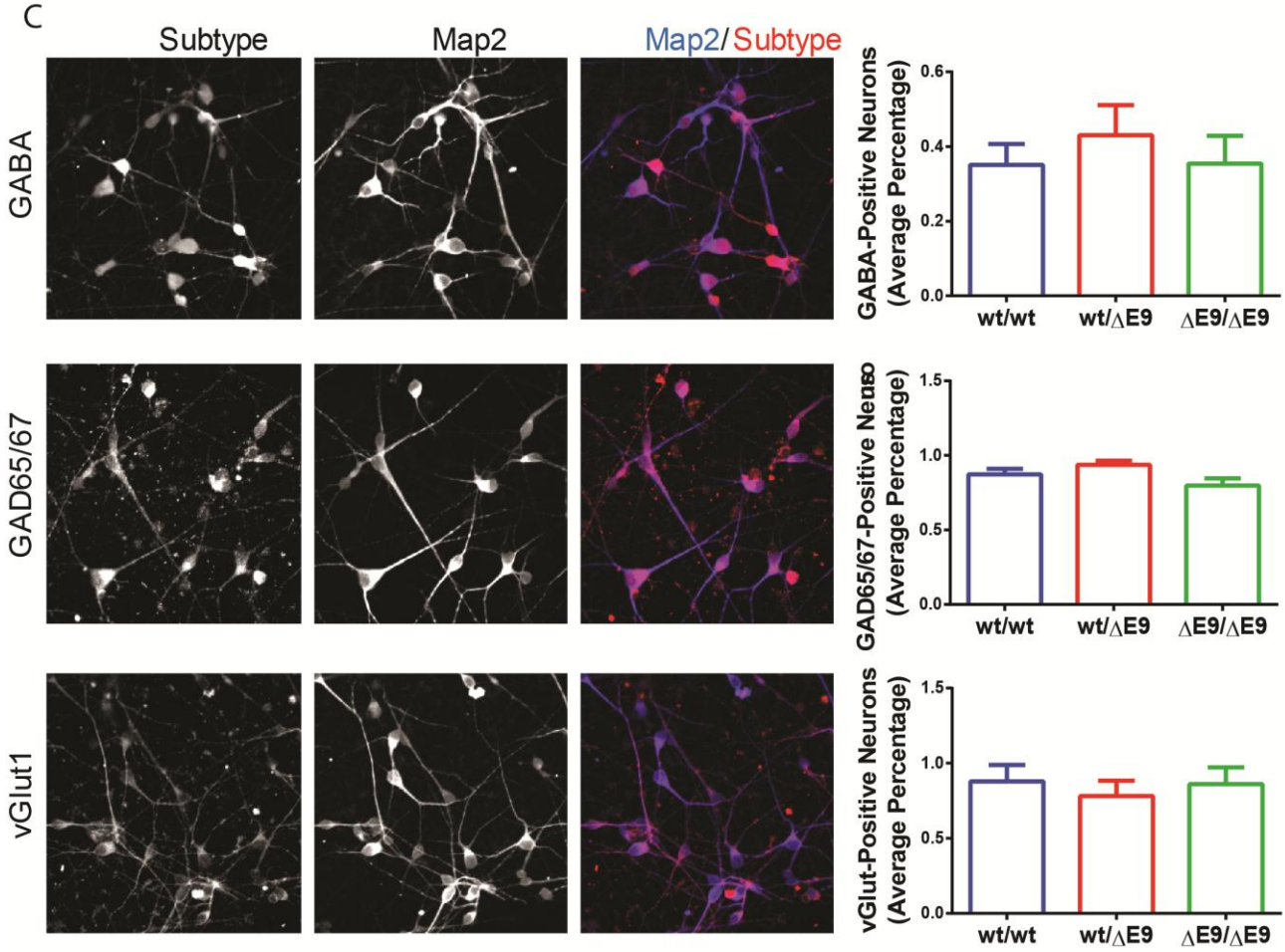

C
Subtype
Map2
Map2/Subtype
GABA
GAD65/67
vGlut1
GABA-Positive Neurons (Average Percentage)
GAD65/67-Positive Neurons (Average Percentage)
vGlut-Positive Neurons (Average Percentage)
0.6
0.4
0.2
0.0
1.5
1.0
0.5
wt/wt
wt/ΔE9
ΔE9/ΔE9

Figure S1. Neuronal Subtype Quantification in hIPSC-Derived PS1 Genotypes, Related to Figure 1

A) Axons grown in microfluidic devices express the axonal marker SMI31, neurofilament-H (NF-H), but not the somatodendritic marker Map2. Black box in lower left of compartment schematic indicates where images were taken. B) Quantification of total sAPP α and β levels from the soma side of the compartment. B) Examples of purified neurons stained with Map2 and GABA, GAD65/67, or vGlut1. Quantification of neuronal subtype percentages. Data represent the average of 12 $PS1^{wt/wt}$, 12 $PS1^{wt/\Delta E9}$ and 12 $PS1^{\Delta E9/\Delta E9}$ biological replicates. None of the stains were statistically different (GABA: $PS1^{wt/\Delta E9}$ $p = 0.4284$, $PS1^{\Delta E9/\Delta E9}$ $p = 0.9682$; GAD65/67 $PS1^{wt/\Delta E9}$ $p = 0.1934$, $PS1^{\Delta E9/\Delta E9}$ $p = 0.2401$; vGlut1: $PS1^{wt/\Delta E9}$ $p = 0.5284$, $PS1^{\Delta E9/\Delta E9}$ $p = 0.9165$).

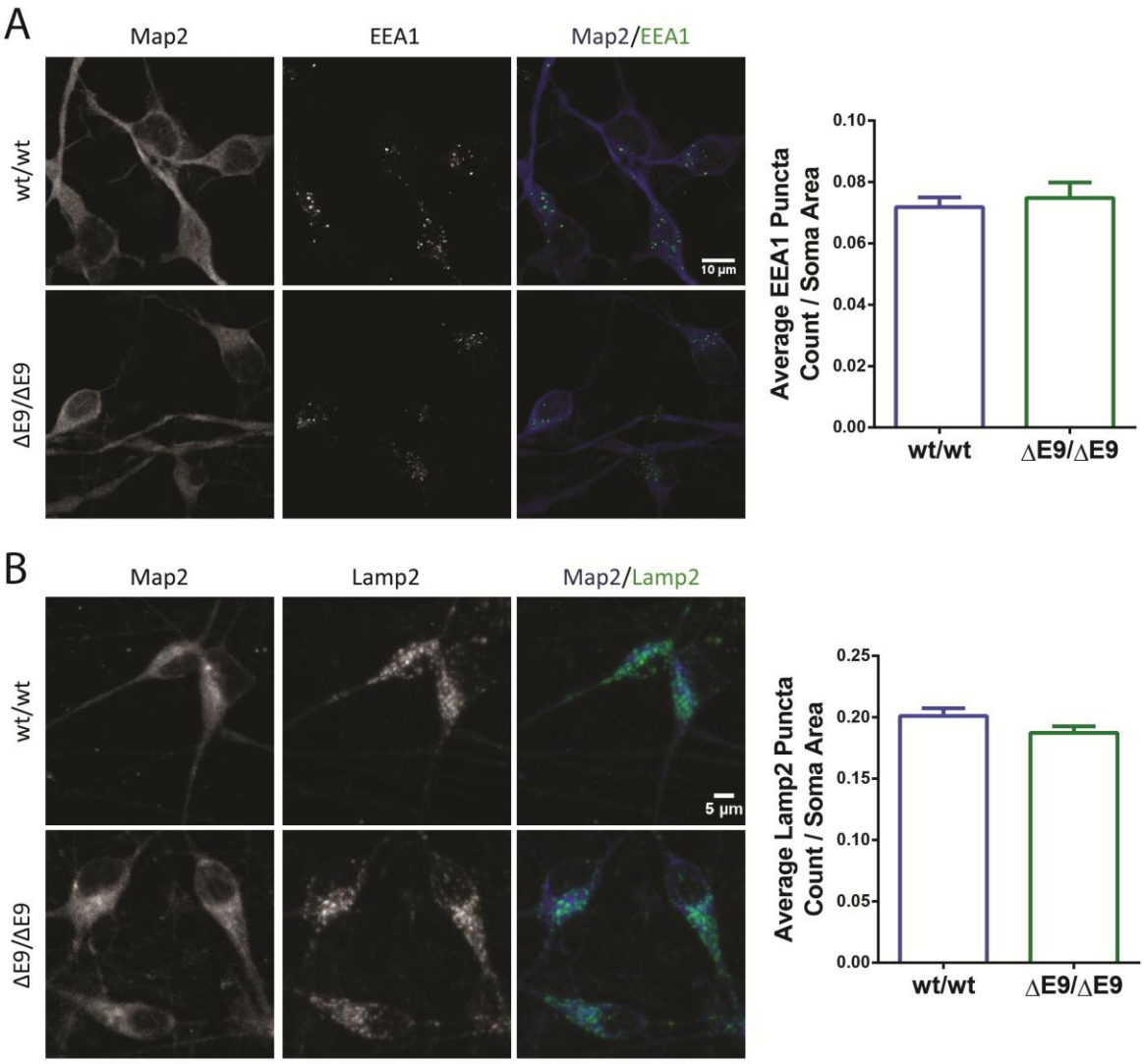


Fig. S2

Figure S2. Quantification of EEA1 and LAMP2, Related to Figure 2

A) EEA1 (BD biosciences) staining in PS1 iPSC-derived neurons with example images of $PS1^{wt/wt}$ and $PS1^{\Delta E9/\Delta E9}$ co-stained with the somatodendritic marker Map2. Average soma count is depicted normalized to cell area. ($p = 0.4475$) Data represent the average of 4 $PS1^{wt/wt}$ and 3 $PS1^{\Delta E9/\Delta E9}$ biological replicates. B) Lamp2 staining in PS1 iPSC-derived neurons with example images of $PS1^{wt/wt}$ and $PS1^{\Delta E9/\Delta E9}$ co-stained with the somatodendritic marker Map2. Average soma count is depicted normalized to cell area. ($p = 0.1081$) Data represent the average of 3 $PS1^{wt/wt}$ and 3 $PS1^{\Delta E9/\Delta E9}$ biological replicates.

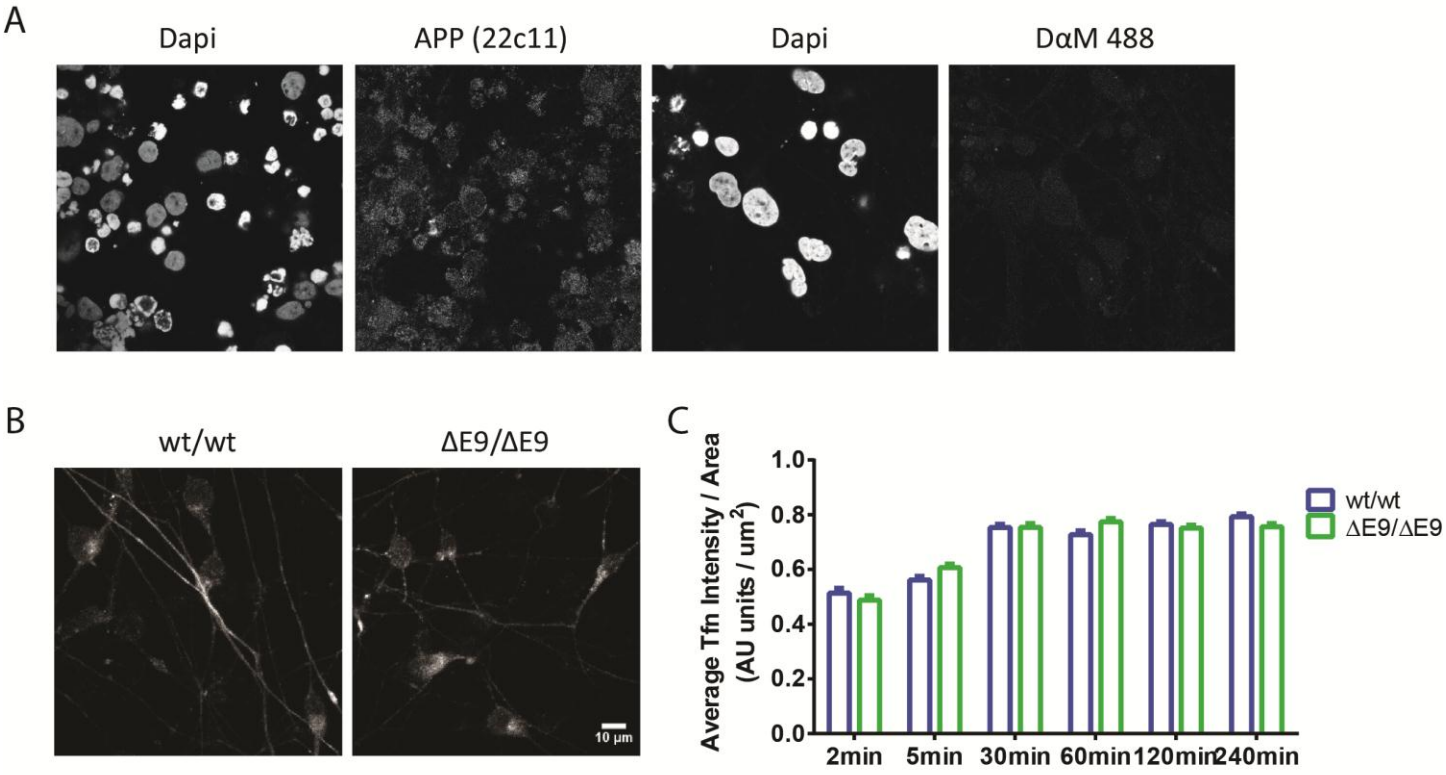


Fig. S3

Figure S3. Uptake of a Non-Specific Antibody and Quantification of Transferrin Endocytosis, Related to Figure 3

A) Images of neurons treated with either the APP (22c11) antibody or DαM 488 for 4 hours. There is minimal uptake of DαM 488 after 4 hours. B) Transferrin endocytosis in $PS1^{wt/wt}$ and $PS1^{\Delta E9/\Delta E9}$ neurons. Neurons were allowed to internalize alexa-647 conjugated transferrin for the indicated times, fixed, and analyzed for soma intensity. C) Quantification of images of transferrin endocytosis.(ANOVA p = 0.6640) Data represent the average of 8 $PS1^{wt/wt}$ and 8 $PS1^{\Delta E9/\Delta E9}$ biological replicates.

A

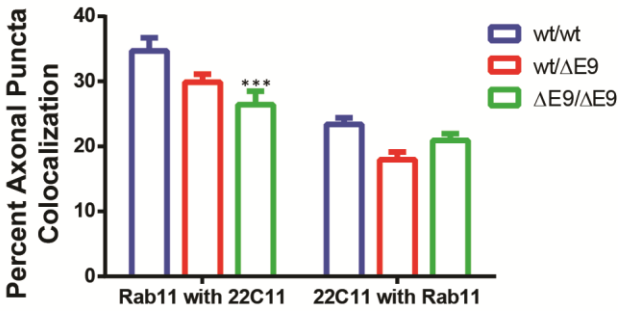


Fig. S4

Figure S4: Colocalization of Rab11 and APP, Related to Figure 4

A) Quantification of average percent colocalization per axon of Rab11 with 22C11 and 22C11 with Rab11 in $PS1^{wt/wt}$, $PS1^{wt/dE9}$, and $PS1^{dE9/dE9}$ hIPSC-derived neurons. Note that the percent of Rab11 with 22C11 decreases in $PS1^{dE9/dE9}$, as expected if 22C11 density is decreasing, but the percentage of 22C11 with Rab11 remains unchanged. Data represent the average of 4 biological replicates per genotype. (Rab11 with 22C11: $PS1^{wt/\Delta E9}$ p = 0.0649, $PS1^{\Delta E9/\Delta E9}$ p =0.0005; 22C11 with Rab11: $PS1^{wt/\Delta E9}$ p = 0.0568, $PS1^{\Delta E9/\Delta E9}$ p = 0.2831)

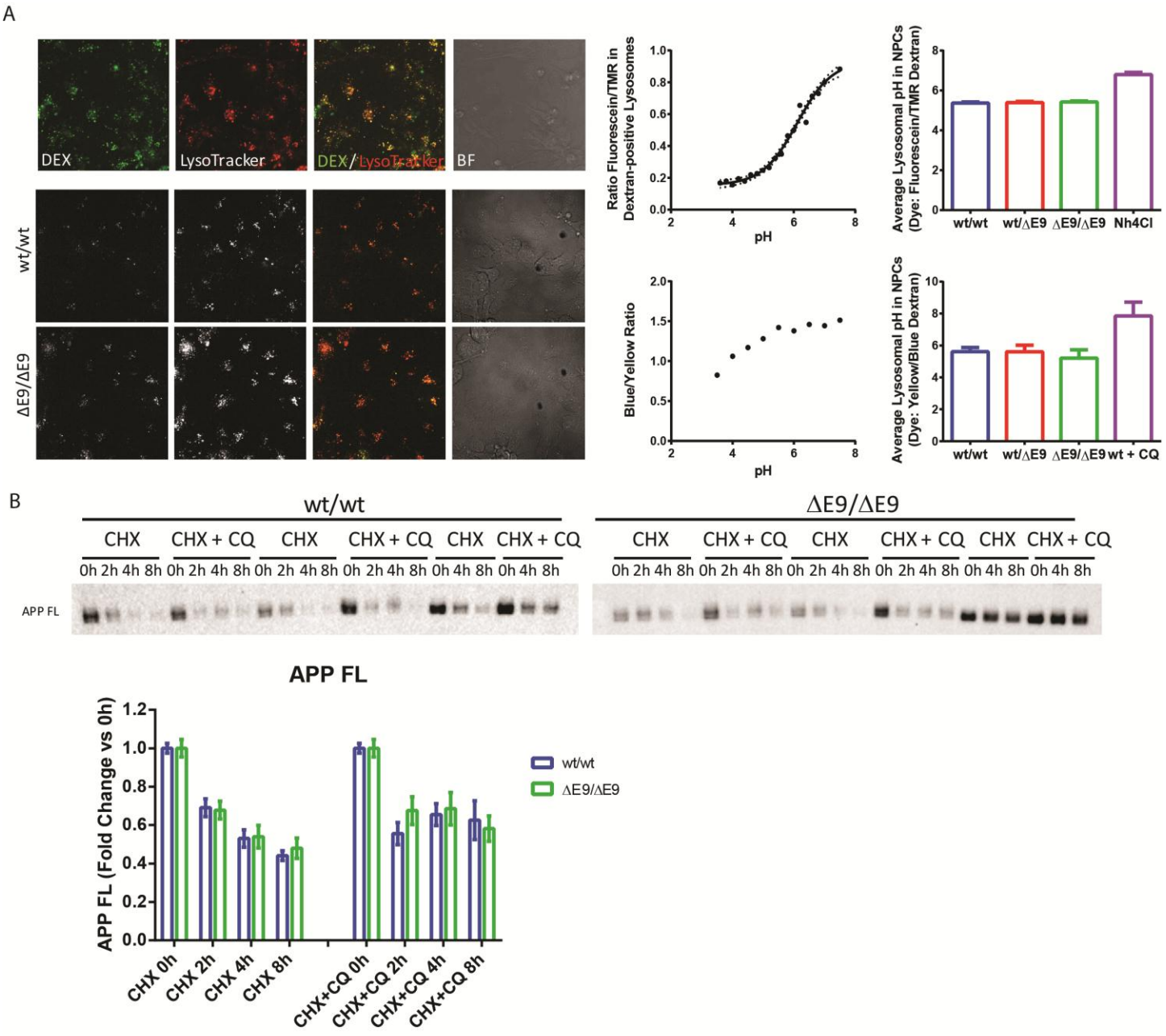


Figure S5. PS1$^{\Delta E9}$ Mutations do not have Altered Lysosomal pH or APP Protein Degradation, Related to Figure 5

A) PS1$^{\Delta E9}$ NPCs were incubated with Fl/TMR Dextran or Lysosensor Yellow/Blue Dextran to label lysosomes. Example images of Fl/TMR Dextran are shown. Lysosomal pH was measured using these ratiometric probes and plotting the experimental values on a standard curve (shown in graphs). Neither Fl/TMR Dextran (ANOVA $p < 0.0001$; PS1$^{wt/\Delta E9}$ p = n.s., PS1$^{\Delta E9/\Delta E9}$ p = n.s., PS1$^{wt/wt}$+NH4Cl $p < 0.0001$) nor Lysosensor Yellow/Blue Dextran (ANOVA $p < 0.0001$; PS1$^{wt/\Delta E9}$ p = n.s., PS1$^{\Delta E9/\Delta E9}$ p = n.s., PS1$^{wt/wt}$+NH4Cl $p < 0.0001$) were

statistically significant for lysosomal pH in $PS1^{\Delta E9}$ neurons. B) Neurons were differentiated for 5 weeks and then treated with cycloheximide (CHX) with and without chloroquine (CQ) for the indicated times. Samples were harvested and run on a Western and probed for APP. Rate of APP degradation was not different in $PS1^{\Delta E9}$ neurons. Data represent the average of 8 biological replicates per genotype. (ANOVA $p = 0.5360$; CHX 0h $p = 1.0$, CHX 2h $p = 0.9014$, CHX 4h $p = 0.9073$; CHX 8h $p = 0.6345$; CHX+CQ 0h $p = 1.0$, CHX+CQ 2h $p = 0.2301$, CHX+CQ 4h $p = 0.7068$; CHX+CQ 8h $p = 0.5896$)

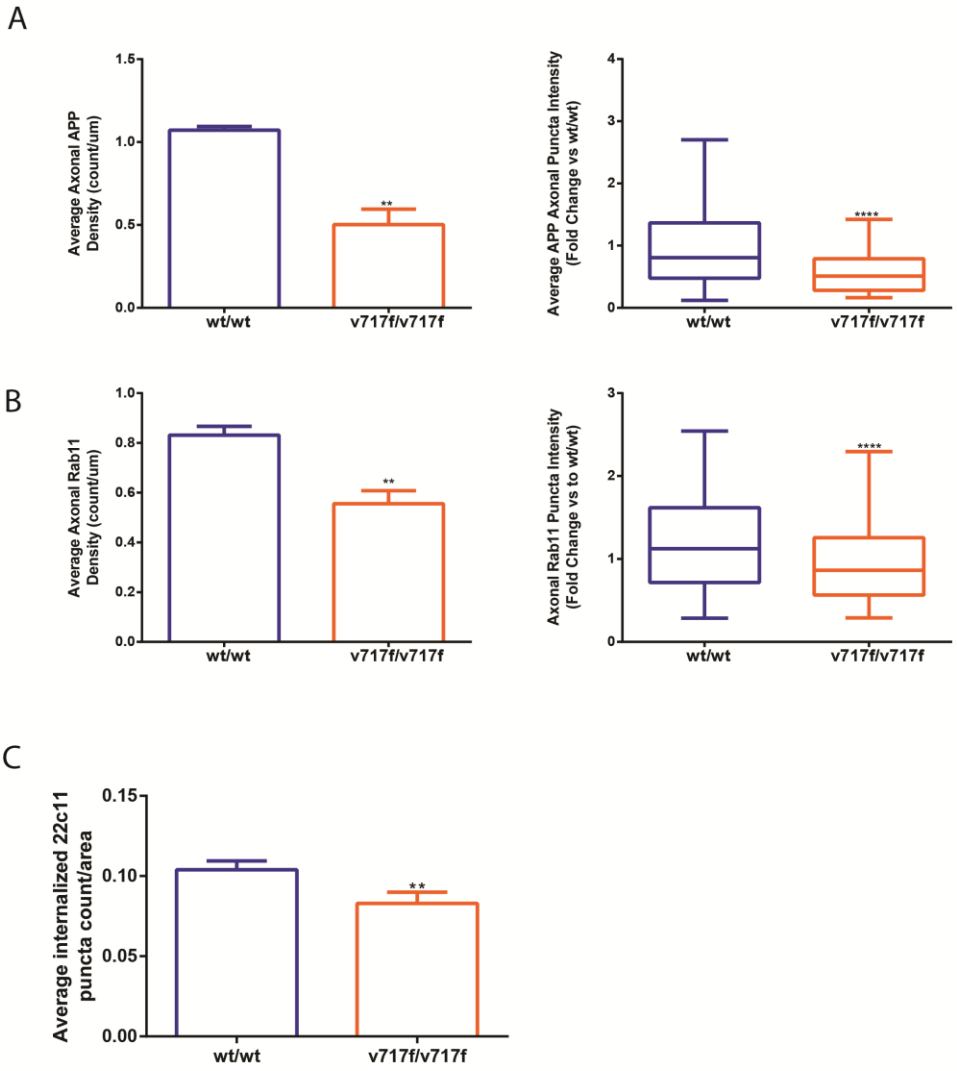


Figure S6. Quantification of Select Measures in APP^{v717f} Mutant hIPSC-Derived Neurons, Related to Figure 7

A) Average axonal APP density (APP$^{v717f/v717f}$, p = 0.0002) and intensity (APP$^{v717f/v717f}$, p <.00001) are reduced in APP^{v717f} mutant neurons compared to APP$^{wt/wt}$. Data represent 6 APP$^{wt/wt}$ and 3 APP$^{v717f/v717f}$ biological replicates. (B) Average axonal Rab11 density (APP$^{v717f/v717f}$, p = 0.0033) and intensity (APP$^{v717f/v717f}$, p < 0.0001) are reduced in APP^{v717f} mutant neurons compared to APP$^{wt/wt}$. Data represent 6 APP$^{wt/wt}$, and 3 APP$^{v717f/v717f}$ biological replicates. C) Endocytosis of 22C11 is reduced in APP^{v717f} mutant neurons at 240min. (APP$^{v717f/v717f}$, p = 0.0190) Data represent 4 APP$^{wt/wt}$, 3 APP$^{v717f/v717f}$ biological replicates**.**

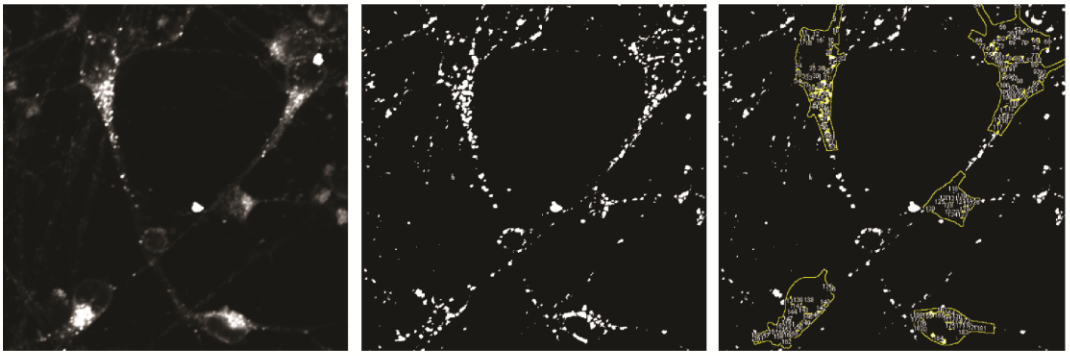
A

SomatoDendrite_ROI

Map2 B/W Mask ROIs

B

PunctaInROIAnalysis



Puncta Stain (LDL) B/W Puncta Mask Puncta ROIs

C

Text File Name: Puncta_Summary

Slice	Count	TotalArea	AverageSize	%Area
Rd_IB7_A1_001.tif	2	2.7574	1.3787	0.9185
Rd_IB7_A1_001.tif	6	3.9637	0.6606	1.1429
Rd_IB7_A1_001.tif	4	1.7234	0.4308	0.9268
Rd_IB7_A1_001.tif	2	3.1882	1.5941	2.4899
Rd_IB7_A1_002.tif	22	28.1768	1.2808	6.7920
Rd_IB7_A1_003.tif	12	13.0544	1.0879	6.9591
Rd_IB7_A1_003.tif	13	12.6236	0.9710	4.6222

Text File Name: Puncta_Results

PunctaID	Size	Intensity	StDev	Min	Max	x	y
Rd_IB7_A1_001.tif:0001-0225	2.4127	32039.4464	18490.6674	8287	65535	25.4158	46.7803
Rd_IB7_A1_001.tif:0002-0227	0.3447	15470.8750	3919.3119	10840	21773	28.5663	47.2473
Rd_IB7_A1_001.tif:0003-0156	0.3447	16818.8750	4360.1810	9800	22600	59.7013	32.4582
Rd_IB7_A1_001.tif:0004-0176	0.7324	23555	2108.9892	19190	27079	57.6730	36.6233
Rd_IB7_A1_001.tif:0005-0185	0.7324	19023.4118	2176.7977	15592	22683	43.1433	38.3937
Rd_IB7_A1_001.tif:0006-0197	1.2494	22825.4483	2106.1480	17677	27271	48.0087	40.9586
Rd_IB7_A1_001.tif:0007-0206	0.1293	17189	1666.7102	16083	19106	50.4041	42.7933

D

Text File Name: Rd_IB7_A_001.tif_ROIIntensity

Soma ID	Area	Mean	StDev	Min	Max
Rd_IB7_A_001.tif:2963-0034	121.7980	4515.0534	4429.0481	16	21038
Rd_IB7_A_001.tif:2964-0054	267.8953	3695.6052	5601.8429	20	65535
Rd_IB7_A_001.tif:2965-0192	105.0815	4434.5158	4664.2077	9	43447
Rd_IB7_A_001.tif:2966-0244	168.3285	3207.6783	3304.9807	15	19479
Rd_IB7_A_001.tif:2967-0331	189.0088	3559.1828	3033.5720	17	21541
Rd_IB7_A_001.tif:2968-0437	127.7005	3752.1167	3586.7898	17	21636

Figure S7: Automated Detection and Analysis of Map2-Positive Soma

A) Purified hIPSC-derived neurons are stained with the somatodendritic marker Map2 and thresholded using intensity to generate a binary mask image. ROIs are automatically identified by the script SomatoDendrite_ROI. B) Further analysis can be done on a parallel quantification channel to count puncta and quantify puncta area. An example of puncta staining for LDL is depicted in the first field. Automatic ImageJ thresholding allows for robust detection of many puncta within the soma ROIs identified in 2.1.A. C) Example

text files of the puncta counts per soma (Puncta_Summary) as well as individual puncta data including size and intensity (Puncta_Results). D) An example text file of soma intensity measurements obtained from the MeasureIntensityinROI script.

Supplemental Methods:

Cell Culture:

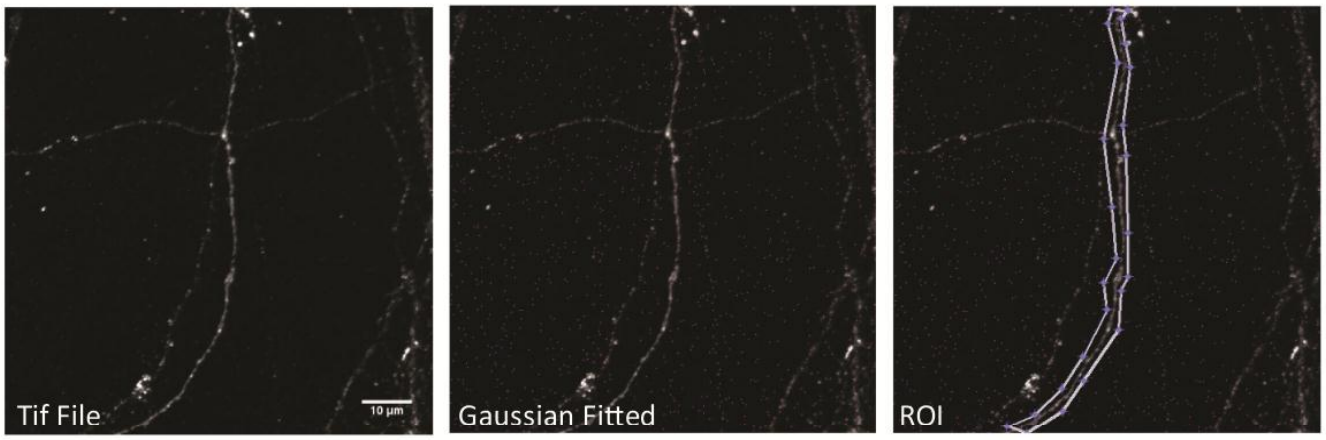
Isogenic iPSCs and NPCs were derived as previously described (Israel et al., 2012; Woodruff et al., 2013; Yuan et al., 2011). Purified neurons were generated by differentiating NPCs for 3 weeks in medium containing DMEM:F12 + Glutamax, .5x N2 (Life Technologies), .5x B27 (Life Technologies), and 1x Pen/Strep on plates coated with 20ug/mL poly-ornithine (PO) and 5µg/mL laminin (L) (both from Sigma). Media was replaced twice per week. After the 3-week differentiation, neurons were purified by fluorescence activated cell sorting (FACS, BD Biosciences). Cells were stained with CD184, CD44, CD24 (all from BD Bioscience) and cells that were CD184-, CD44- and CD24+ were selected and plated on PO/L coated plates. Purified neurons were cultured in the same medium as above with the addition of 0.5mM dbCAMP (Sigma), 20ng/mL brain-derived neurotrophic factor, and 20ng/mL glial cell line-derived neurotrophic factor (both from Peprotech). Cells that were grown in microfluidic compartments were differentiated for 3 weeks and then dissociated and re-plated in compartments. Transcytosis experiments were performed after cells had been in microfluidic compartments for 7-10 days.

Lysosomal pH Measurements

To measure lysosomal pH by microscopy, NPCs or neurons were grown in 96 well imaging plates and incubated with Dextran, fluorescein and tetramethylrhodamine (Fl/TMR) (250 µg/ml, Invitrogen D1951) or Lysosensor Yellow/Blue Dextran (Invitrogen, L-22460) for 2-6 hours. Dextran was chased into the lysosomes for >12 hours. Red/green or Yellow/Blue wavelengths were captured simultaneously in live cells. Cells incubated with dextran Fl/TMR were imaged on a confocal microscope in warm media. Imaging time was limited to half an hour per plate. A custom ImageJ program identified all red puncta greater than 0.2 um in size (pH-insensitive dye) and then the ratio of green/red mean intensity per puncta was determined. Cells incubated with Lysosensor Yellow/Blue Dextran were imaged on a plate reader. Cells were excited at 352 nm and simultaneously measured for Yellow and Blue fluorescence. To generate a standard curve, wild-type cells were treated with pH-

calibrated buffers and the average green/red or yellow/blue ratio per image was fit to a standard curve (See Figure S3A). (Diwu et al., 1999; Lee et al., 2010)

Axon Puncta Quantification



An example of a neuronal axon stained with APP and the Gaussian-fitted puncta identified using a previously established Matlab program (developed by the Guo lab). An axonal ROI is manually generated to identify axons and Gaussian-fitted puncta are counted and analyzed for intensity.

Automated Image Analysis:

In order to perform unbiased, quantitative analysis of fluorescent images, a series of ImageJ scripts were developed. In all soma immunofluorescence experiments, neurons were labeled with chicken anti-Map2. The Map2 channel served to delineate and identify soma borders (Figure S7A). The SomatoDendrite_ROI script used thresholding to generate black and white mask images, which was then used to identify soma bodies 50-200 um^2 in size. Zip files corresponding to the soma ROIs from each image were then used to quantify fluorescent intensity in unthresholded images (Figure S7B). To quantify neuronal puncta, a separate script, PunctaInROIAnalysis, was developed that used the thresholding and Analyze Particles features of ImageJ to automatically segment and count puncta in the ROIs generated from SomatoDendrite_ROI (Figure S7C and D). Example output text files can be seen for PunctaInROIAnalysis (Figure S7C) and MeasureIntensityInROI (Figure S7D) scripts.

Axonal puncta quantification

To determine axonal puncta densities, a previously developed MATLAB program was used to identify diffraction-limited, overlapping spots. When the resolution limit exceeds the distance separating two labeled-proteins, the superposition of the channel images creates a single local maximum. Current automatic detection and localization methods fail to take into account the limitations of diffraction-limited data (Anthony and Granick, 2009; Goulian and Simon, 2000; Jaqaman et al., 2008). From previous work in the lab, we had a MATLAB-based program that made use of a Gaussian fitting algorithm to identify punctate features in an axon (Jaqaman et al., 2008; Szpankowski et al., 2012). Briefly, the algorithm scans an unprocessed, undeconvolved image and identifies local maxima, while accounting for local background. The program then iteratively fits one or multiple Gaussian functions onto each local maxima “seed” and estimates the point source location for each acquired Gaussian amplitude (Jaqaman et al., 2008). An example of the Gaussian Fitted file is depicted in supplemental methods where the point source of detected maxima are indicated by pink dots. Densities were calculated by counting Gaussian Fitted dots and normalizing to axonal length. Colocalization is based on a previous publication from our lab (Szpankowski et al., 2012). Briefly, for all analyses, the cutoff for subpixel colocalization was set to a 300-nm radius based on the optics, resolution limit, and relevant physical size of the vesicles and motor subunits (Encalada et al., 2011). Thus, local maxima in channel 2 that were within 300-nm of the seed channel (channel 1) were considered to be co-localized while those outside of that radius were not.