

Supplementary Materials for

Altered expression of Presenilin2 impacts endolysosomal homeostasis and synapse function in Alzheimer's disease-relevant brain circuits

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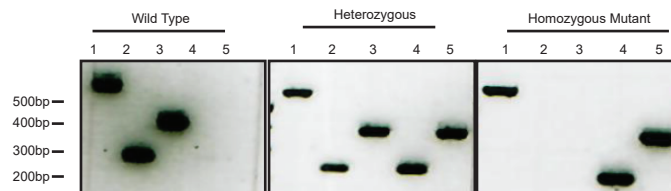
Supplementary figures S1 - S6
Source data for the respective figures

Exon 5 Exon 6 Exon 7 Exon 8

S I Y T P F T E D T P S V G Q R L L N S V L **N** T L I M I S V I V V M T I F L V V L Y
C AGC ATC TAC ACG CCA TTC ACT GAG GAC ACA CCC TCG GTG GGC GAG CGC CTC CTC AAC TCC GTG CTT AAC ACC CTC ATC ATG ATC AGC GTC ATC GTG GTT ATG ACC ATC TTC TTG GTG GTC CTC TAC AA

S I Y T P F T E D T P S V G Q R L L N S V L I T L I M I S V I V V M T I F L V V L Y
C AGC ATC TAC ACG CCA TTC ACT GAG GAC ACA CCG GTG GGC CAG CGC CTC CTC AAC TCT GTC CTG ATC ACC CTC ATC ATG ATC AGC GTC ATC GTG GTT ATG ACC ATC TTC TTG GTG GTG CTC TAC AA

Primer set 1: 615bp band whole PSEN2 gene
Primer set 2: 266bp if Wild Type; no band if mutant
Primer set 3: 395bp if Wild Type; no band if mutant
Primer set 4: Wild Type no band; 240bp if mutant
Primer set 5: Wild Type no band; 395bp if mutant



C

mPSEN2 539
Black6_Sanger 392
Quality scores
Trace data
FADPSN2_Sanger 392
Quality scores
Trace data
Consensus CGCCCTTCAC GGAGGACACG CCCTCGGTGG GCCAGCGGCT CCTCAACTCC GTGCTTAACA CCCTCATCAT GATCAGCGTC ATCGTAGTCA
Conservation 100.0% 0.0% 2.0%
Sequence logo

Western blot analysis showing protein levels of PSEN2 (17kDa), NCT (98kDa), and Actin (49kDa) in Wild Type, PSEN2KO, and FADPSEN2 mice. The blots show bands for each protein across the three genotypes. PSEN2 is present in Wild Type and FADPSEN2 but absent in PSEN2KO. NCT and Actin serve as loading controls and are present in all samples.

e 6Months Insoluble

%Ag Peptide

Legend: APP NL G F (dark purple), APPxPSEN2KO (dark blue), APPxPSEN2 (light blue)

Mass-to-Charge Ratio	APP NL G F (%)	APPxPSEN2KO (%)	APPxPSEN2 (%)	n
37	0.6452	0.6259	0.6859	3
38	0.9270	0.7689	0.9693	4
40	0.6252	0.3228	0.9940	3
42	0.8944	0.7031	0.9680	4

	3 Months	6 Months	9 Months	12 Months	18 Months
APP NL G F					
APPxPSEN2KO					
APPxPSEN2					

Cortex

Legend:
 ■ APP NL G F
 ■ APPxPSEN2KO
 ■ APPxPSEN2

Group	Genotype	n	% Area Covered Thioflavin (approx.)
Group 1	APP NL G F	3	0.05
	APPxPSEN2KO	3	0.05
	APPxPSEN2	3	0.05
Group 2	APP NL G F	3	0.15
	APPxPSEN2KO	3	0.15
	APPxPSEN2	3	0.15
Group 3	APP NL G F	3	0.25
	APPxPSEN2KO	3	0.25
	APPxPSEN2	3	0.25
Group 4	APP NL G F	9	0.35
	APPxPSEN2KO	9	0.35
	APPxPSEN2	9	0.35
Group 5	APP NL G F	12	0.55
	APPxPSEN2KO	12	0.55
	APPxPSEN2	12	0.55
Group 6	APP NL G F	18	1.05
	APPxPSEN2KO	18	1.05
	APPxPSEN2	18	1.05

Statistical significance (p-values):
 Group 1 vs 2: 0.9520
 Group 1 vs 3: 0.0045
 Group 2 vs 3: 0.5360
 Group 3 vs 4: 0.0004
 Group 4 vs 5: <0.0001
 Group 5 vs 6: 0.0051
 Group 6 vs 5: 0.0724
 Group 6 vs 6: 0.9489
 Group 6 vs 5: 0.0007

Fig. S1 Generation of FAD-PSEN2 knock-in mice

a. Schematic of the targeted exon and sequence. In red nucleotides representing the mutated base in the N141I mutation, in blue the silent mutations introduced to prevent recutting by the cas9 enzyme. **b.** representative DNA gels showing genotyping strategy. **c.** Sanger sequencing indicating the altered base pairs in the FADPSEN2 mouse compared to murine PSEN2 (NC_000067.7) and C57BL/6J sequence, showing the integration of the donor and homozygosity of the mouse colony. **d.** Western blot on brain lysate and immunoprobed for PSEN2, nicastrin with actin as loading control. **e.** MSD ELISA indicating percent of each peptide represented in insoluble brain fractions of 6 month old mice **f.** Representative images of coronal cortical slices of APPKI, APPxPSEN2KO and APPxFADPSEN2 mice stained for thioflavin-s over time and quantified in **g.** normalized to brain size. Number of mice (N) indicated in the bar and number of slices analyzed (n) indicated by number of symbols. All graphs are mean $\pm$ SEM, and include p-values. Scale bars are indicated in the relevant panels.

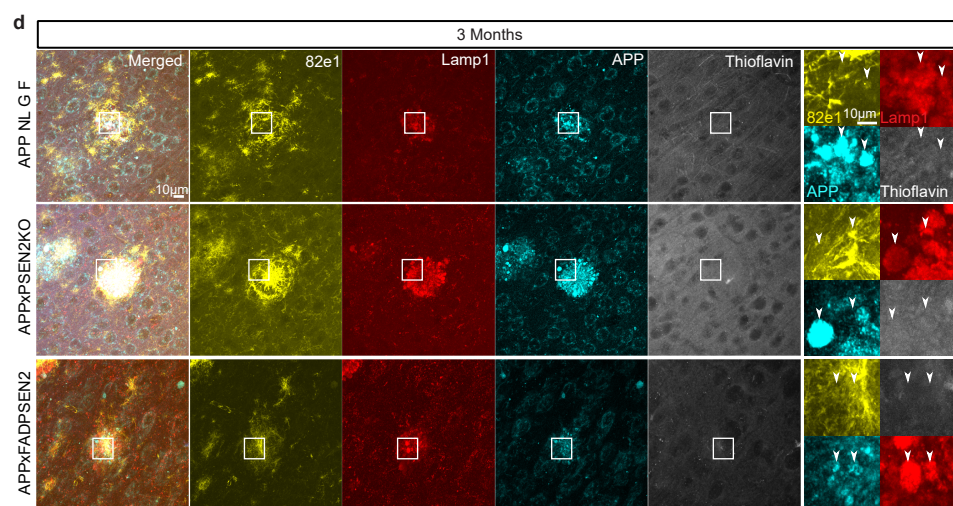
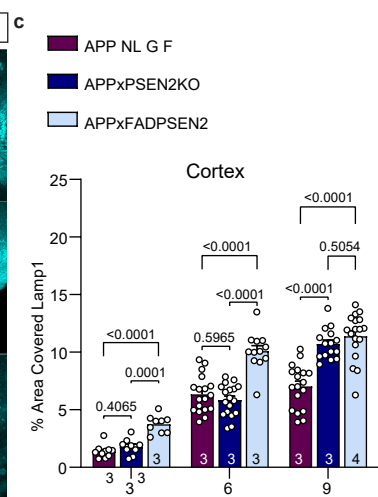
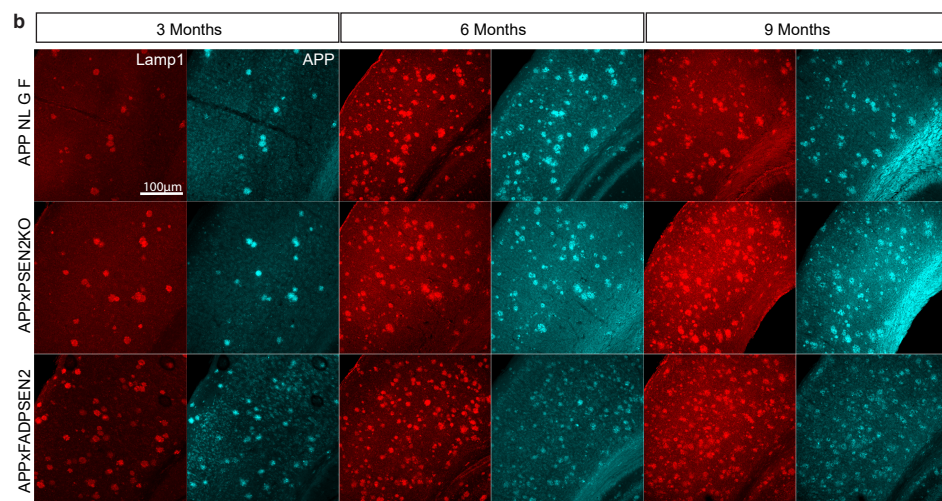
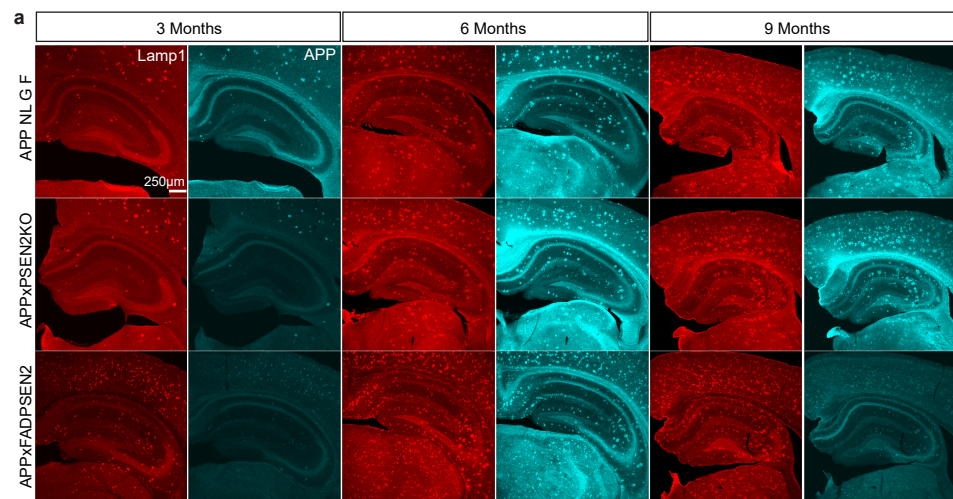
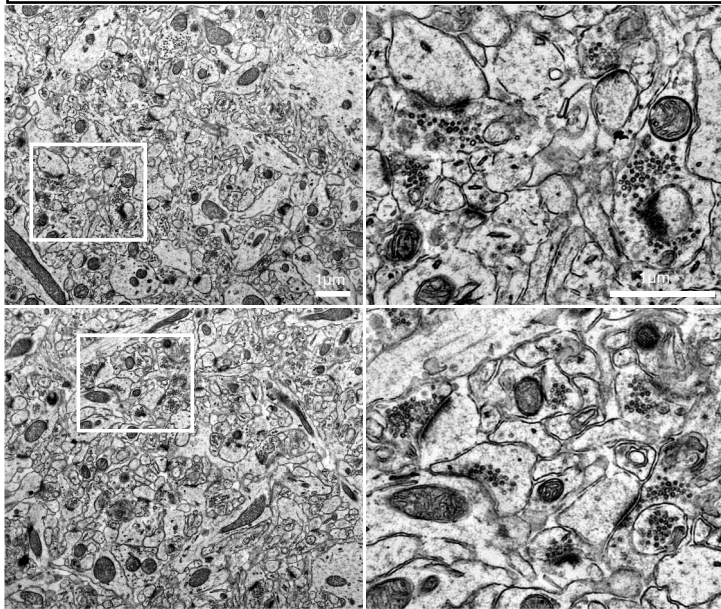


Fig. S2 Dystrophic neurite pathology is worsened in PSEN2 deficient and FADPSEN2 mutant mouse brain

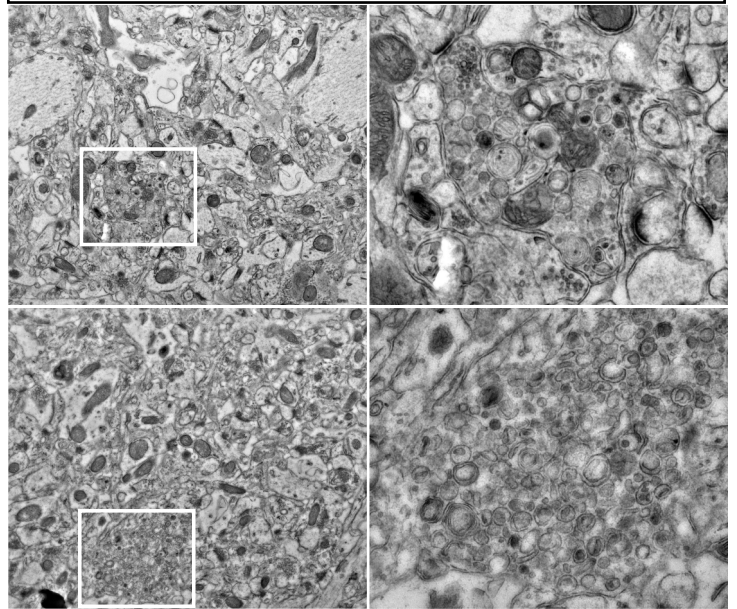
Immunostaining of coronal hippocampal (**a**) and cortical (**b**) slices of APPKI, APPxPSEN2KO and APPxFADPSEN2 mice over time for APP-CTF/ full length APP and LAMP1, next to thioflavin. **c**. Quantification of LAMP1+ area as a percent of total cortical area. **d**. Maximum projected Z-stack of zoomed in on a dystrophic neurite labelled with LAMP1, APP-CTF/A β (82e1) and APP-CTF/APP full length (Y188). A two-way ANOVA analysis with Tukey's correction for multiple testing compared to APPKI was used for analysis. Scale bars and p-values are indicated in the images. All graphs are mean $\pm$ SEM.

a

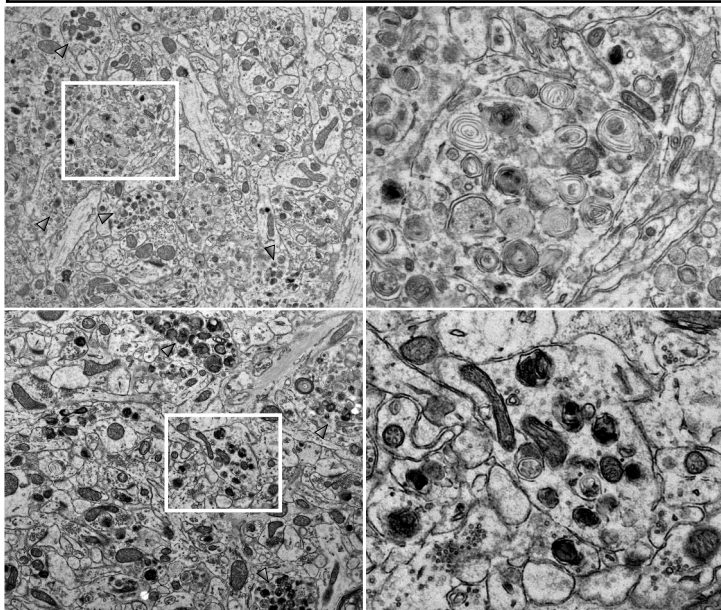
Wild Type



APP NL G F



APPxPSEN2KO



APPxFADPSEN2

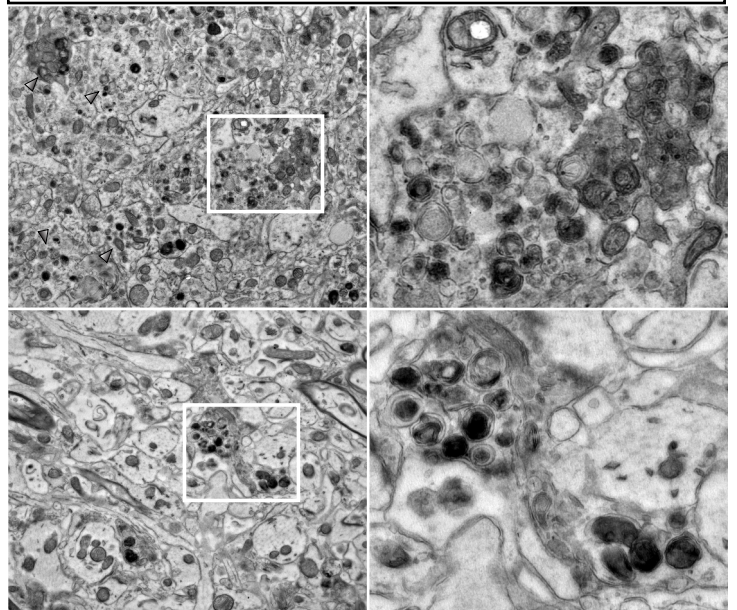


Fig. S3 Ultrastructural analysis of dystrophic neurites in hippocampal regions

Representative TEM images of dystrophic neurites observed in hippocampus for the different genotypes. Representative images were selected from three different mice analyzed per genotype. Whereas absent in wild-type, and observed in APPKI, in APPxPSEN2KO and APPxFADPSEN2 they appear larger and with accumulating degradative compartments, including multilamellar bodies and numerous darker organelles, likely representing autophagosomes and autolysosomes. For each genotype, two overviews with a zoomed inset are displayed. Scale bars = 1 μ m for all images.

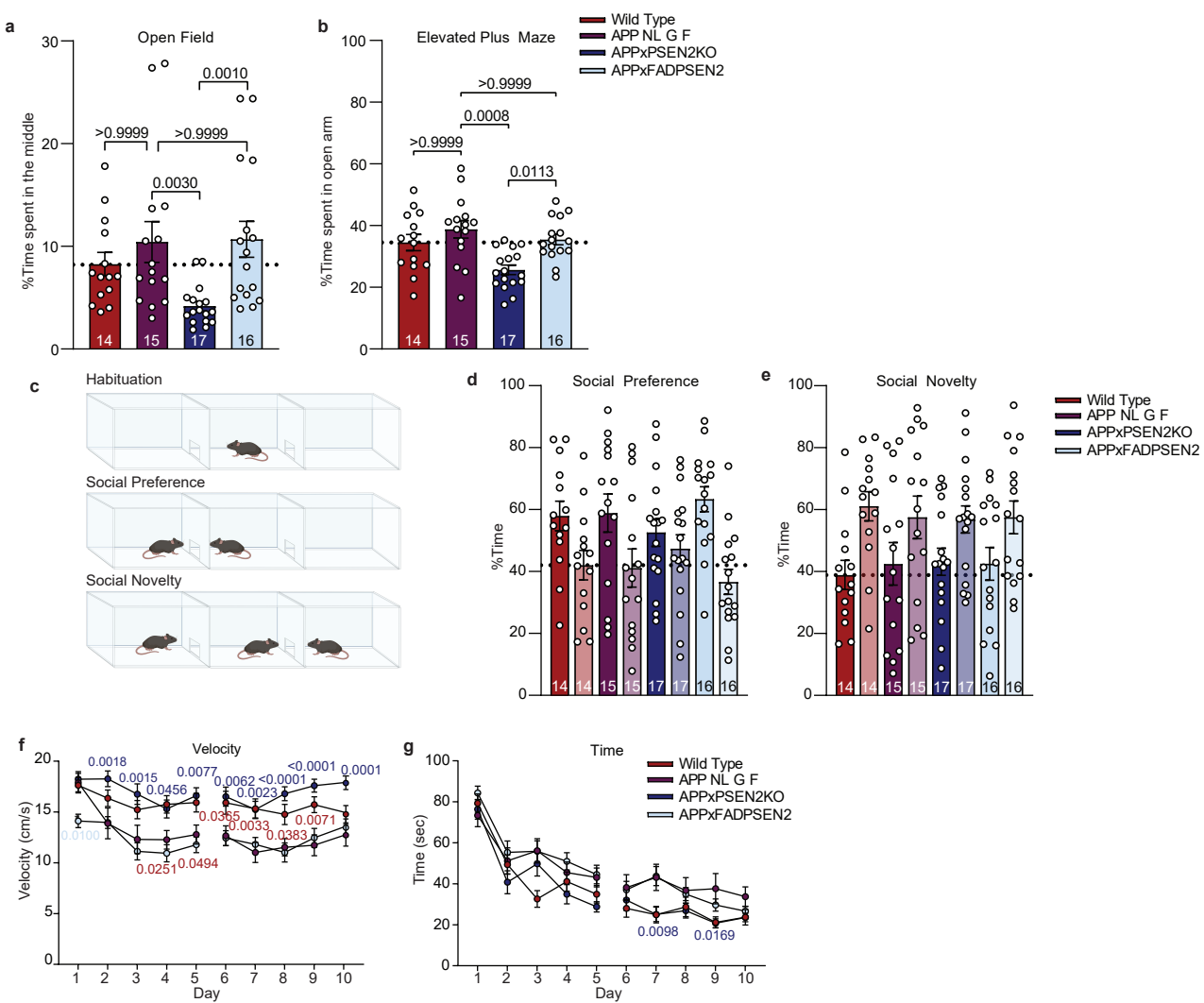


Fig. S4 Behavioral characterization of PSEN2 AD models

a. Average percent of total time spent in the middle in an open-field paradigm **b.** Average percentage of total time spent in open arms during the elevated plus maze paradigm. Statistical analysis for a and b was by one-way ANOVA with Tukey's correction for multiple testing comparing all genotypes. **c.** Schematic of the three consecutive phases of social preference/social novelty paradigm. **d.** Percentage of time spent in close proximity to the mouse containing chamber (dark bar) compared to the empty side (opaque bar) **e.** Percentage of total time spent in close proximity to the novel mouse (opaque bar) compared to the known mouse (dark bar). **f.** Average swimming velocity per genotype across the 10 training days. Statistical analysis was performed using two-way ANOVA with Dunnett's correction for multiple testing always compared to APP NL G F **g.** Average time taken to reach the platform per genotype across the 10 different training days. Statistical analysis was by two-way ANOVA using Dunnett's test to correct for multiple testing comparing all genotypes. For both f-g, wild type N=14, APP NL G F N=15, APPxPSEN2KO N= 17, APPxFADPSEN2 N= 16. All graphs are mean $\pm$ SEM, with p-values indicated in the panels.

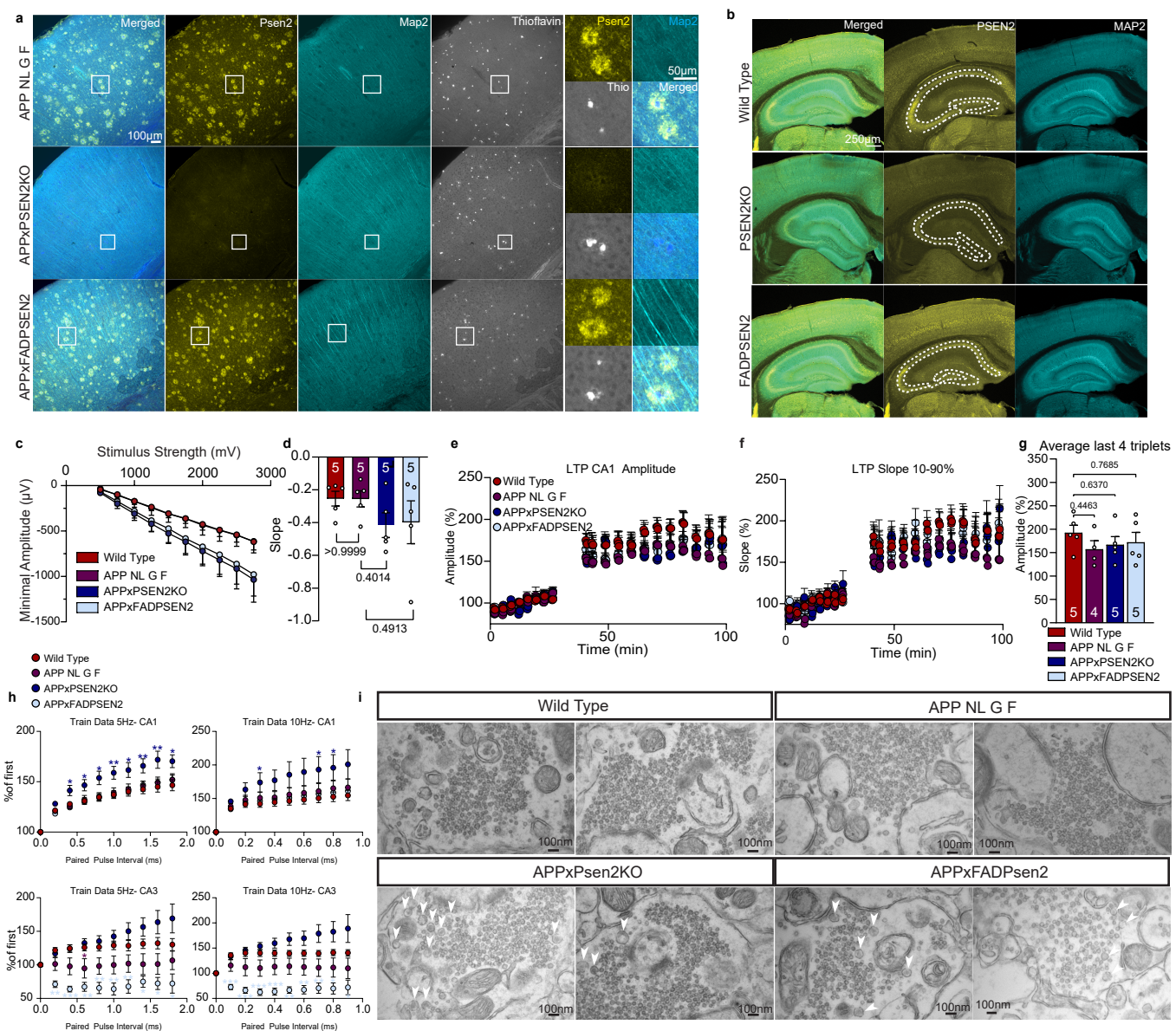


Fig. S5 CA3-localized PSEN2 modulates synaptic function and morphology

a. Representative images of coronal cortex slices immunostained for PSEN2 and MAP2, next to Thioflavin in an APPKI background. **b.** Representative images of coronal cortex slices immunostained for PSEN2 and MAP2, next to Thioflavin in a C57BL/6J background. For both a-b, representative images were selected from stainings performed on 3 different mice per genotype. **c.** Input-output curve showing response amplitude to set stimuli. **d.** Slopes calculated from the input-output curves using a linear regression model (as represented in (c)) from each individual experiment. Slopes were statistically analyzed using one-way ANOVA with Dunnett's test for multiple comparison compared to APPKI. **e-g.** fEPSPs were recorded from Schaeffer collateral CA1 synapses in 6 month old wild-type, APPKI, APPxPSEN2KO and APPxFADPSEN2 mice. After 30min of baseline recordings, LTP was induced by applying two trains of 75hz stimulation and fEPSPs were recorded for another 50min; **e** represents the amplitude and **f** the slope. **g.** Average of the last 4 triplet amplitude percentages compared using oneway ANOVA with Dunnett's test for multiple comparisons compared to wild-type. For e-g wild type N=5, APP NL G F N=4, APPxPSEN2KO N=5 APPxFADPSEN2 N=5. **h.** Train stimulations in both CA1 pyramidal cells and CA3 mossy fibers represented as % of the first recorded amplitude. Statistical analysis was performed using one way-ANOVA with Dunnett's correction for multiple testing. Genotypes were always compared to wild type. Stars indicating significance are colored according to the corresponding genotype. Actual p-values CA1 5Hz 0.4ms*=0.0189, 0.6ms*=0.0258, 0.8ms*=0.0141, 1ms**=0.0058, 1.2ms*=0.0179, 1.4ms**=0.0085, 1.6ms**=0.0083, 1.8ms*=0.0136. CA1 10Hz 0.3ms*=0.0498, 0.7ms*=0.0464, 0.8ms*=0.0483. CA3 5Hz 0.2ms**=0.0015, 0.4ms***=0.0008, 0.6ms*=0.0394, **=0.0017, 0.8ms**=0.0013, 1ms**=0.0011, 1.2ms**=0.0040, 1.4ms*=0.0103, 1.6ms*=0.0133, 1.8ms*=0.0300. CA3 10Hz 0.1ms***=0.0008, 0.2ms***=0.0008, 0.3ms***=0.0005, 0.4ms***=0.0007, 0.5ms**=0.0016, 0.6ms**=0.0023, 0.7ms**=0.0071, 0.8ms*=0.0109, 0.9ms*=0.460. Number of mice (N) and cells (n) recorded for CA1: Wild-type N=5 n=10, APPKI N=5 n=9, APPxPSEN2KO N=5 n=10, APPxFADPSEN2 N=5 n=9. Number of mice (N) and cells (n) recorded for CA3: Wild-type N=5 n=9, APPKI N=4 n=5, APPxPSEN2KO N=5 n=7, APPxFADPSEN2 N=3 n=4. **i.** Representative TEM images depicting presynaptic vesicle clustering across the different genotypes. White arrowheads indicate enlarged vesicles, particularly more abundant in the APPxPSEN2KO and APPxFADPSEN2 mouse brain. All graphs are mean $\pm$ SEM, and include p-values. Scale bars are indicated in the relevant panels.

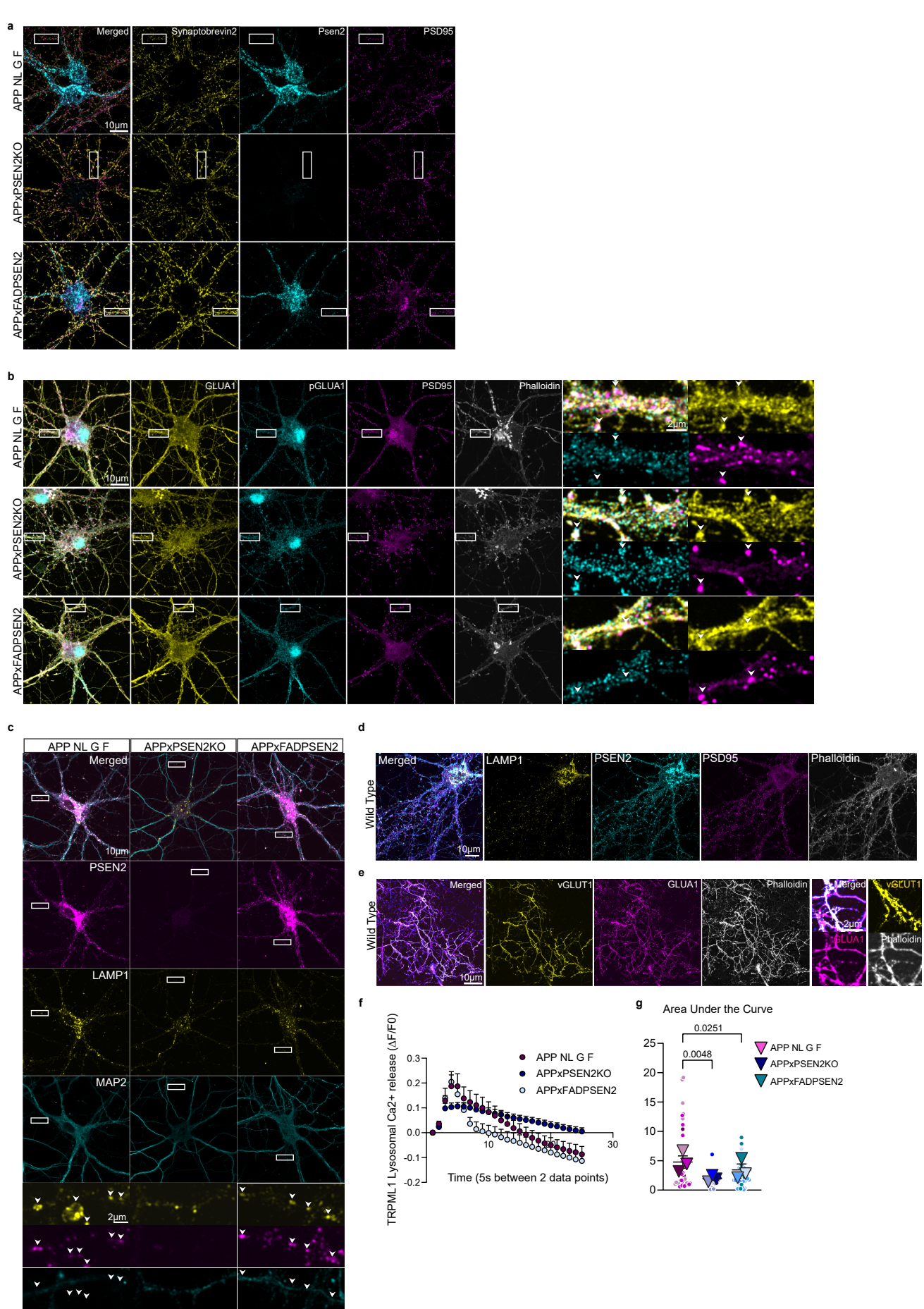


Fig. S6 Altering PSEN2 expression affects synapse features in hippocampal neurons

a. Representative images of DIV14 hippocampal neurons immunostained for synaptobrevin2, PSD95 and PSEN2. Zoomed insets highlight PSEN2's close proximity to synapses are displayed in figure 6. **b.** Representative images of neurons immunostained for GLUA1, phosphorylated GLUA1 (pGLUA1) and PSD95, next to Phalloidin. Zoomed insets show GLUA1 and pGLUA1 in close proximity to PSD95+ puncta (white arrowheads). **c.** Representative images of DIV14 hippocampal neurons immunostained for PSEN2, LAMP1 and MAP2. Zoomed insets indicate PSEN2+ LE/Lys within MAP2-negative axons. For a-c, representative images were selected from stainings performed on three different cultures. **d.** Representative images of wild-type DIV14 hippocampal neurons immunostained for LAMP1, PSEN2 and PSD95. **e.** Representative images of surface labelling of vGLUT1, GLUA1, combined with phalloidin to label neurons. For d-e, representative images were selected from stainings of two different coverslips from one culture. **f.** Lysosomal Ca²⁺ release over time upon stimulation with the TRML1 agonist, MLSA1, and quantified in **g.** as area under the curve per genotype. Statistical analysis was done using a one-way ANOVA with Dunnett's correction for multiple comparisons compared to APPKI. All graphs are mean $\pm$ SEM, and include p-values. Scale bars are indicated in the relevant panels.

PSEN2-CTF

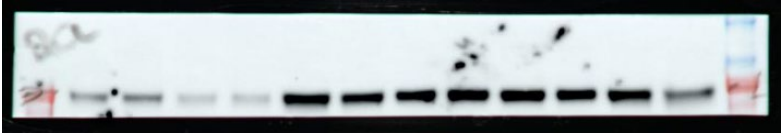
PSEN2-CTF- further enhanced

17kDa



Nicastrin

98kDa



Actin

38kDa

