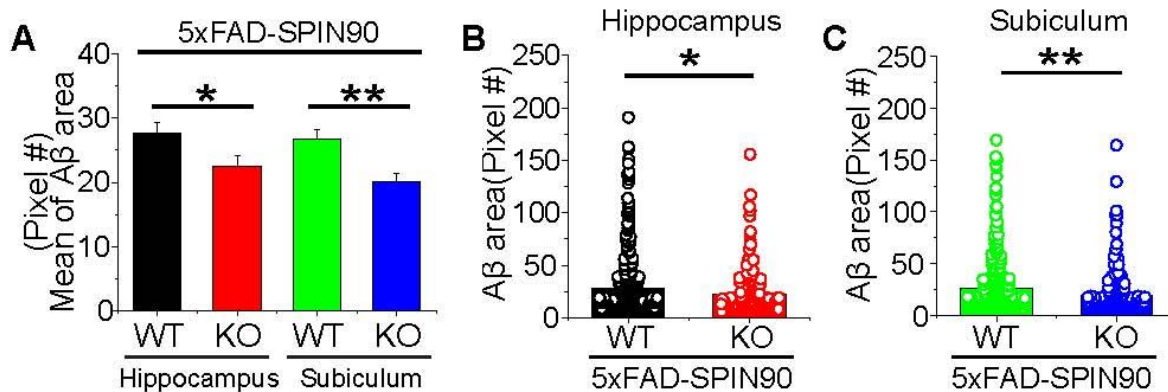


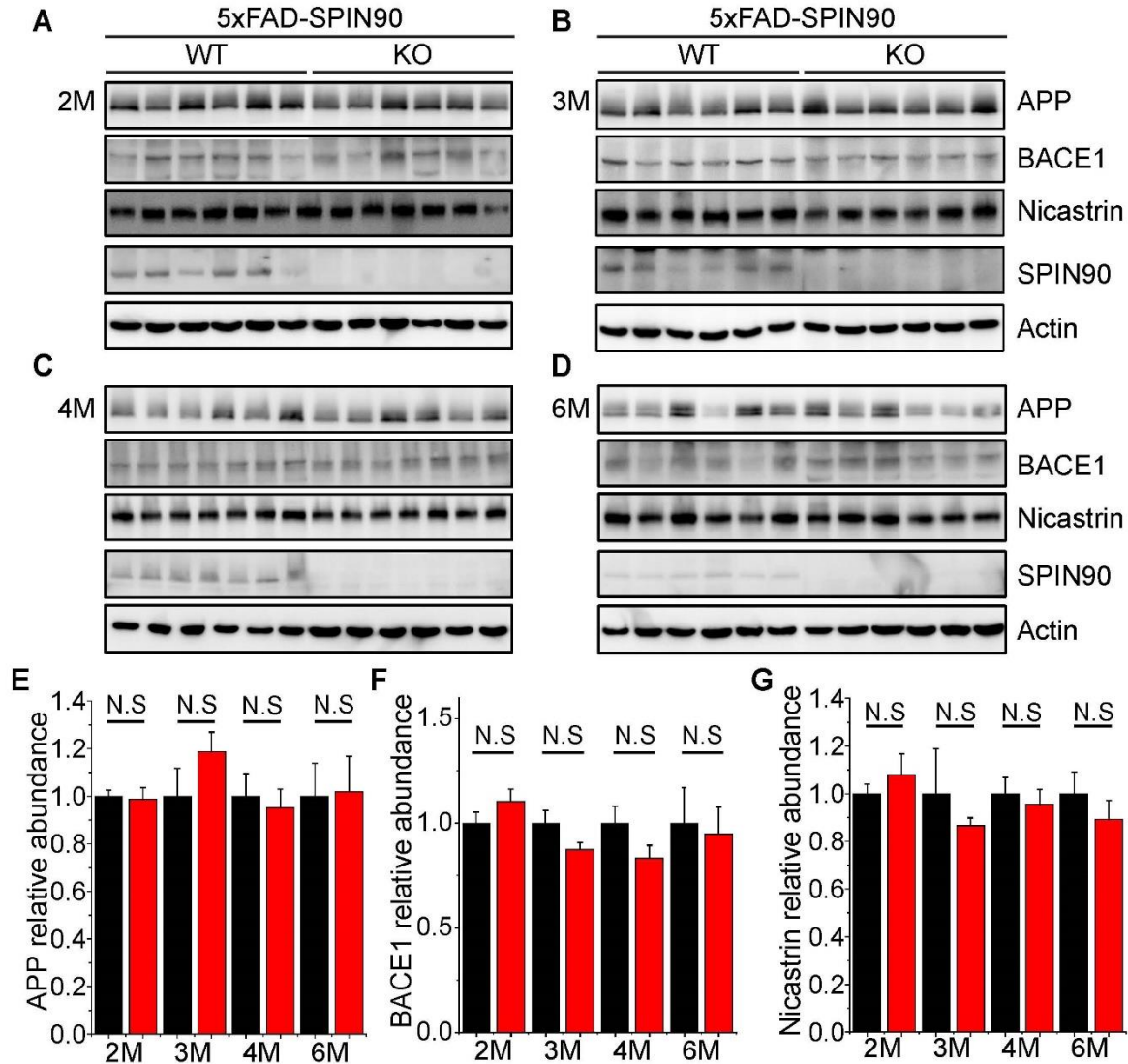
Supplementary Material

1 Supplementary Data

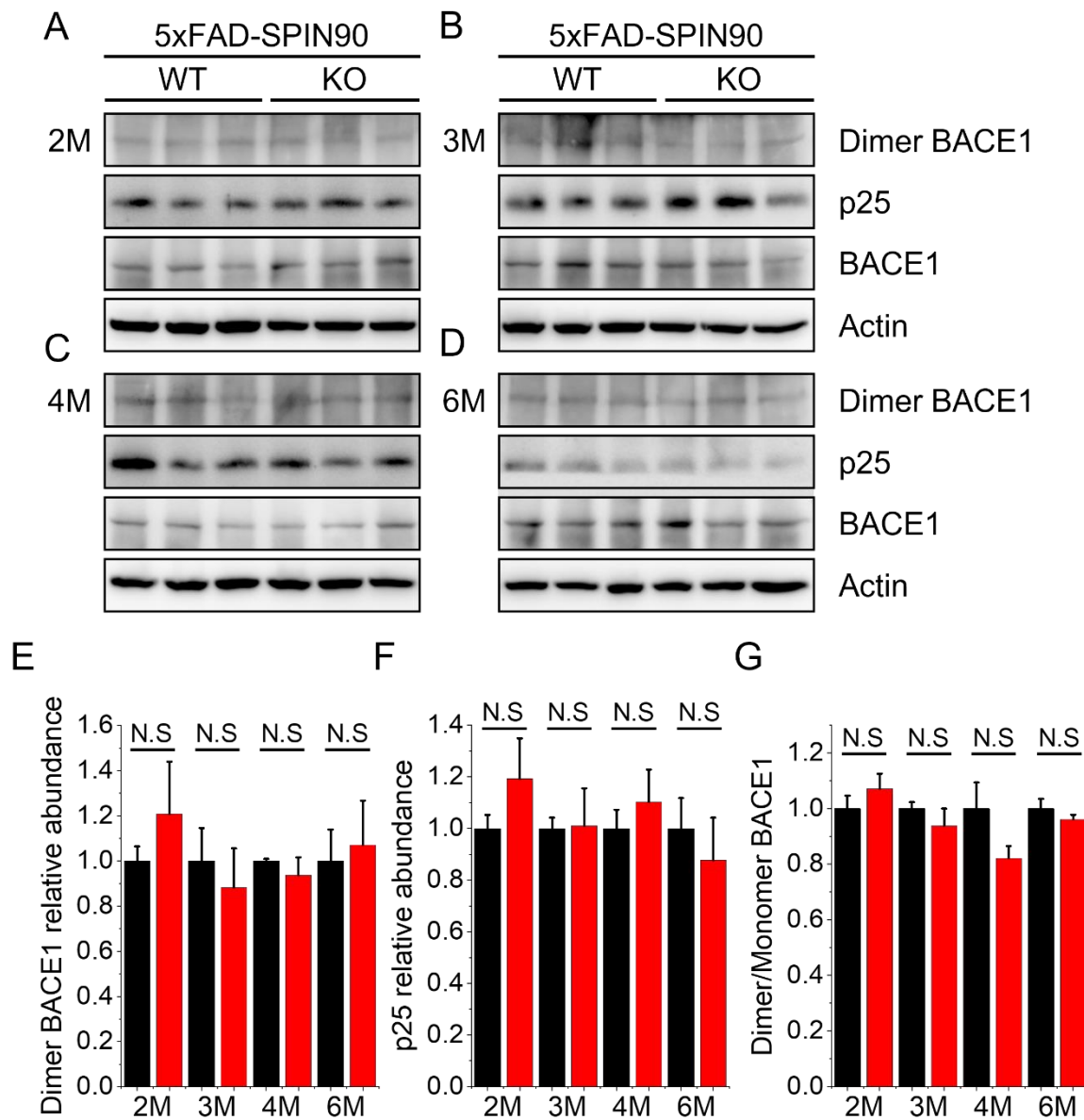
1.1 Supplementary Figures



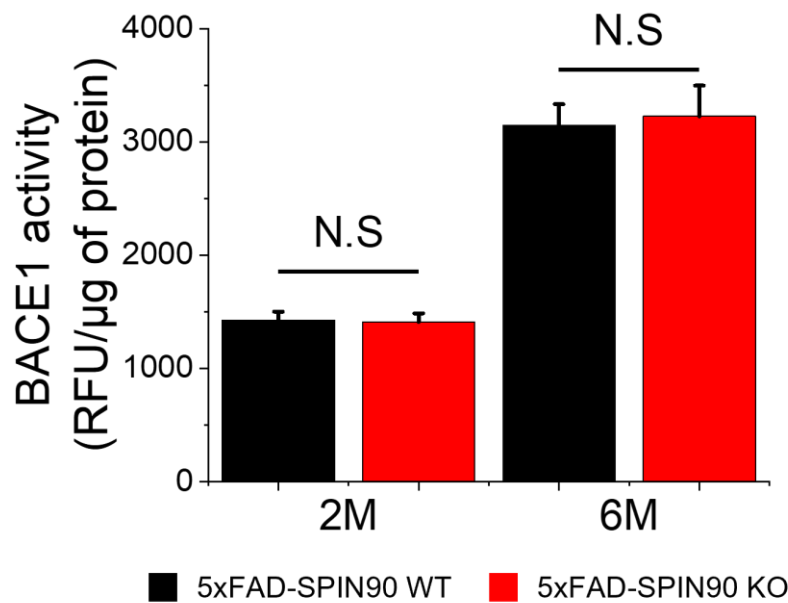
Supplementary Figure S1. Comparative sizes of areas of Aβ deposition in 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO mice. (A) Mean sizes of Aβ plaques in the hippocampus (27.71 ± 1.52 vs. 22.44 ± 1.70 pixels), and subiculum ($26.76 \pm 1.45\%$ vs. $20.08 \pm 1.37\%$) of 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO mice ($n=6$ each). (B,C) Overlay of individual Aβ plaque sizes (open circles) with mean Aβ plaque sizes in the (B) hippocampus and (C) subiculum of 5xFAD-SPIN90 WT ($n=395$ and $n=344$) and 5xFAD-SPIN90 KO ($n=169$ and $n=237$) mice. * $p < 0.05$, ** $p < 0.01$.



Supplementary Figure S2. Relative levels of expression of APP, BACE1, and Nicastrin in the brains of 5xFAD-SPIN90 WT and of 5xFAD-SPIN90 KO mice. (A-D) Representative western blots of APP, BACE1, and Nicastrin protein expression in the brains of 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO mice at **A**: 2 months; **B**: 3 months; **C**: 4 months; and **D**: 6 months. Brains were isolated from 6 mice at each time point and homogenized. Results are representative of three identical experiments. (E-G) Relative expression of APP (**E**), BACE1 (**F**), and Nicastrin (**G**) in 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO brains at 2, 3, 4, and 6 months. All band intensities were normalized to the intensities of actin bands in the same samples.

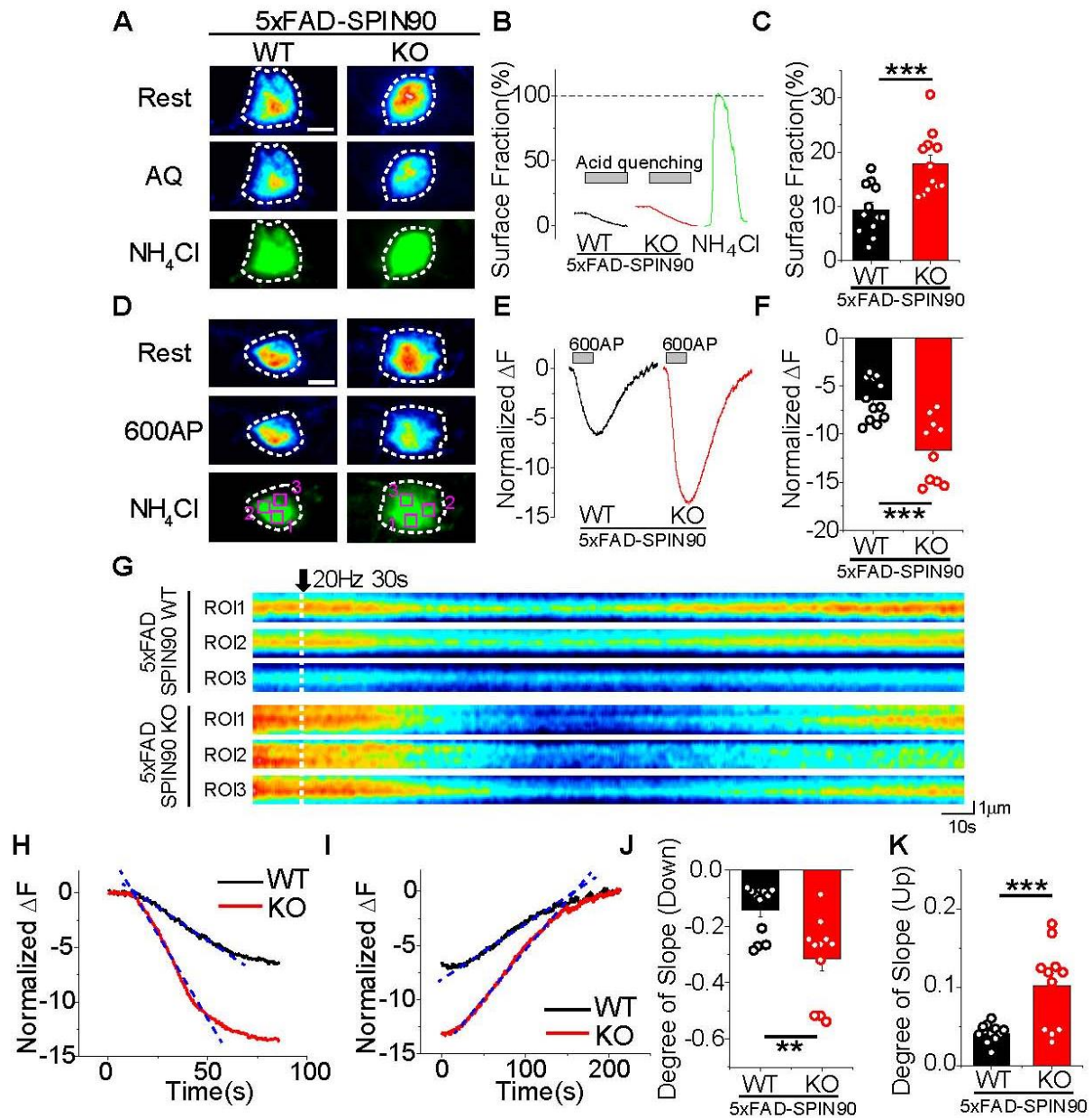


Supplementary Figure S3. The status of BACE1 protein in brains of 5xFAD-SPIN90 WT and KO mice. (A-D) Representative western blots of dimer BACE1, p25, and BACE1 protein expression in the brains of 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO mice at **A**: 2 months; **B**: 3 months; **C**: 4 months; and **D**: 6 months. Brains were isolated from 3 mice at each time point and homogenized. Results are representative of three identical experiments. (E-G) Relative expression of dimer BACE1 (E), p25 (F), and the ratio of dimer/monomer BACE1 (G) in 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO brains at 2, 3, 4, and 6 months. All band intensities were normalized to the intensities of actin bands in the same samples.



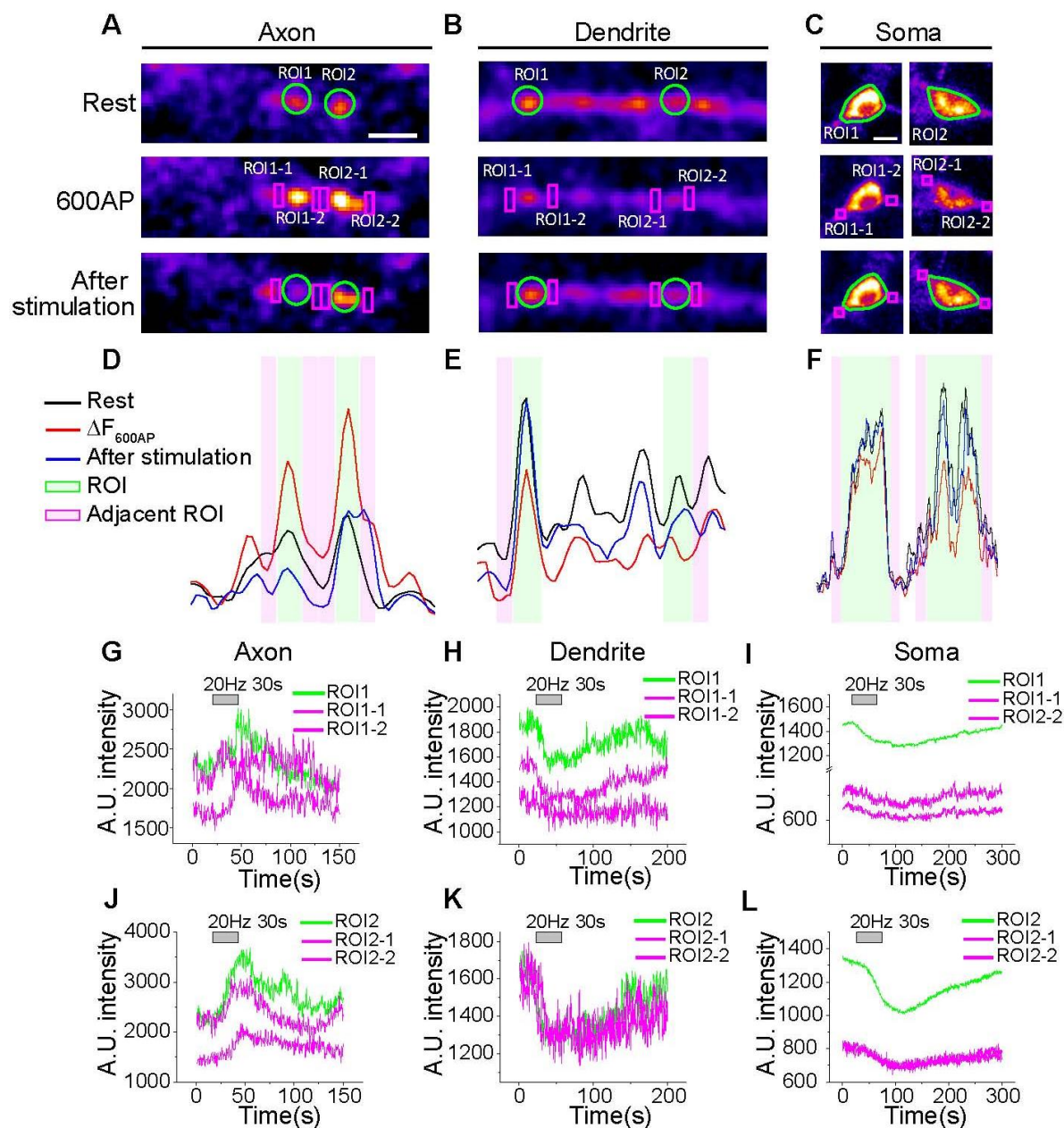
Supplementary Figure S4. BACE1 activity in the brains of 5xFAD-SPIN90 WT and KO mice.

BACE1 activity in the brains of 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO mice at 2 months and 6 months was measured using β -secretase activity fluorometric assay kits. Brains were isolated from mice at corresponding time points (2 months and 6 months) and homogenized. Results are representative of three identical experiments. [2M: 5xFAD-SPIN90WT] = 1426.85 ± 74.19 RFU/ μ g of protein (n=4 mice), [2M: 5xFAD-SPIN90KO] = 1410.55 ± 74.32 RFU/ μ g of protein (n=4 mice), [6M: 5xFAD-SPIN90WT] = 3145.89 ± 186.90 RFU/ μ g of protein (n=3 mice), [6M: 5xFAD-SPIN90KO] = 3225.21 ± 271.05 RFU/ μ g of protein (n=3 mice).

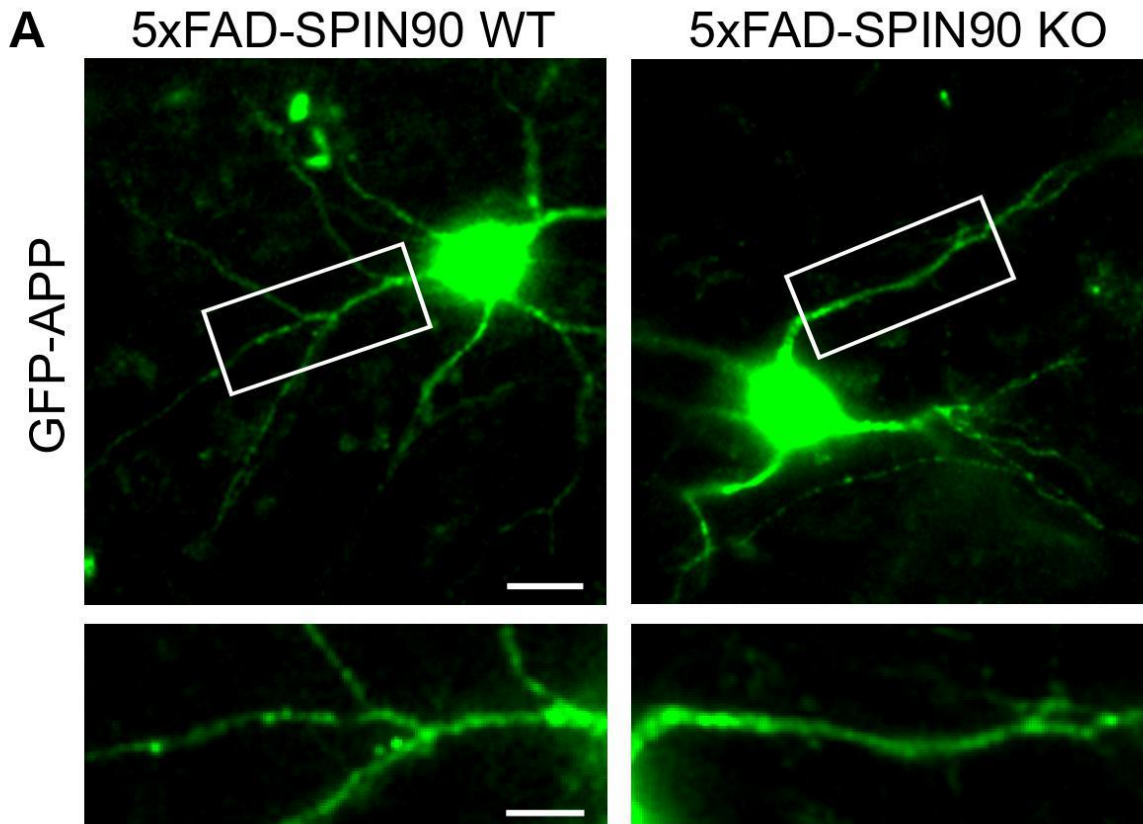


Supplementary Figure S5. Distribution and trafficking of APP in the soma is influenced by SPIN90 depletion in 5xFAD neurons. (A) Representative images of pH-APP in the soma of 5xFAD-SPIN90 WT (left) and 5xFAD-SPIN90 KO (right) brains at rest (top), during acid quenching (middle), and during application of NH_4Cl (bottom). Scale bar: 5 μm . (B) Representative traces of pH-APP in the soma in response to acid quenching in 5xFAD-SPIN90 WT (black) and 5xFAD-SPIN90 KO (red) neurons. (C) Mean surface fractions of pH-APP in the soma of 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO neurons. [Surface fraction: soma]_{5xFAD-SPIN90WT} = 9.40 ± 1.33 % (n=12 cells), [Surface fraction: soma]_{5xFAD-SPIN90KO} = 17.85 ± 1.66 % (n= 12 cells). (D) Representative images of pH-APP in the soma of 5xFAD-SPIN90 WT (left) and 5xFAD-SPIN90 KO (right) neurons at rest (top), after application of 600AP (middle), and after application of NH_4Cl (bottom). Scale bar: 5 μm . (E) Representative trace of pH-APP at axons in response to 600AP in 5xFAD-SPIN90 WT (black) and

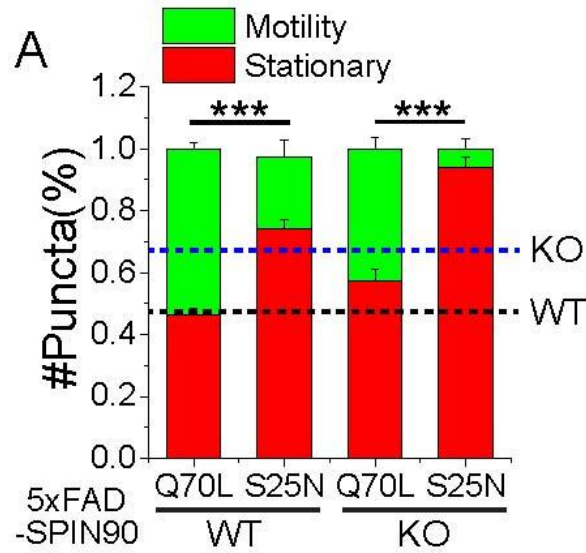
5xFAD-SPIN90 KO (red) neurons. **(F)** Mean peak amplitudes of endocytosis in the soma of 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO neurons. [600AP: soma]_{5xFAD-SPIN90WT} = -6.43 ± 0.62 % (n=12 cells), [600AP: soma]_{5xFAD-SPIN90KO} = -11.68 ± 1.06 % (n=10 cells). **(G)** Kymographs of pH-APP in the corresponding areas of the soma in response to 600APs in 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO neurons. **(H-K)** Representative traces of pH-APP with linear fit-line during **(H)** and after **(I)** stimulation of the soma in 5xFAD-SPIN90 WT (black) and 5xFAD-SPIN90 KO (red) mice. **(J)** Mean slope of the soma, as determined by linear fitting, during stimulation of 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO neurons. [Degree of slope: soma]_{5xFAD-SPIN90WT} = -0.14 ± 0.026 (n=12 cells); [Degree of slope: soma]_{5xFAD-SPIN90KO} = -0.31 ± 0.045 (n=11 cells). **(K)** Mean slope of the soma, as determined by linear fitting, after stimulation of 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO neurons. [Degree of slope: soma]_{5xFAD-SPIN90WT} = 0.04 ± 0.004 (n=12 cells); [Degree of slope: soma]_{5xFAD-SPIN90KO} = 0.10 ± 0.02 (n=11 cells). **p<0.005, ***p<0.001.



Supplementary Figure S6. Activity-dependent recycling of pH-APP between the endosomes and the surface. (A-C) Representative images of pH-APP at axons (A), dendrites (B), and soma (C) in 5xFAD-SPIN90 KO neurons at rest (top), at the peak of 600AP stimulation (middle) and at the end of stimulation (bottom). Green circles indicate regions of interest (ROI); pink squares indicate regions adjacent to ROIs. (D-F) Line scan profile from figure (A-C). (G-L) Fluorescence traces of pH-APP in response to 600AP of ROIs in axons (G,J), dendrites (H,K), and soma (I,L).



Supplementary Figure S7. APP is not accumulated in dendrites. (A) Representative images of GFP-APP at dendrites. Scale bar: 10 μ m(top), 5 μ m(bottom).



Supplementary Figure S8. Rab11 participates in axonal trafficking of APP. (A) Quantification of normalized mean stationary and mobile APP in 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO neurons in the presence of Rab11-Q70L or S25N. [stationary APP]_{5xFAD-SPIN90WT} with Rab11A-Q70L = 0.47 ± 0.02 (n=3 cells); [mobile APP]_{5xFAD-SPIN90WT} with Rab11A-Q70L = 0.53 ± 0.02 (n=3 cells); [stationary APP]_{5xFAD-SPIN90WT} with Rab11A-S25N = 0.74 ± 0.03 (n=6 cells); [mobile APP]_{5xFAD-SPIN90WT} with Rab11A-S25N = 0.24 ± 0.05 (n=6 cells); [stationary APP]_{5xFAD-SPIN90KO} with Rab11A-Q70L = 0.57 ± 0.04 (n=7 cells); [mobile APP]_{5xFAD-SPIN90KO} with Rab11A-Q70L = 0.43 ± 0.04 (n=7 cells); [stationary APP]_{5xFAD-SPIN90KO} with Rab11A-S25N = 0.94 ± 0.03 (n=5 cells); [mobile APP]_{5xFAD-SPIN90KO} with Rab11A-S25N = 0.06 ± 0.03 (n=5 cells). ***p<0.001.