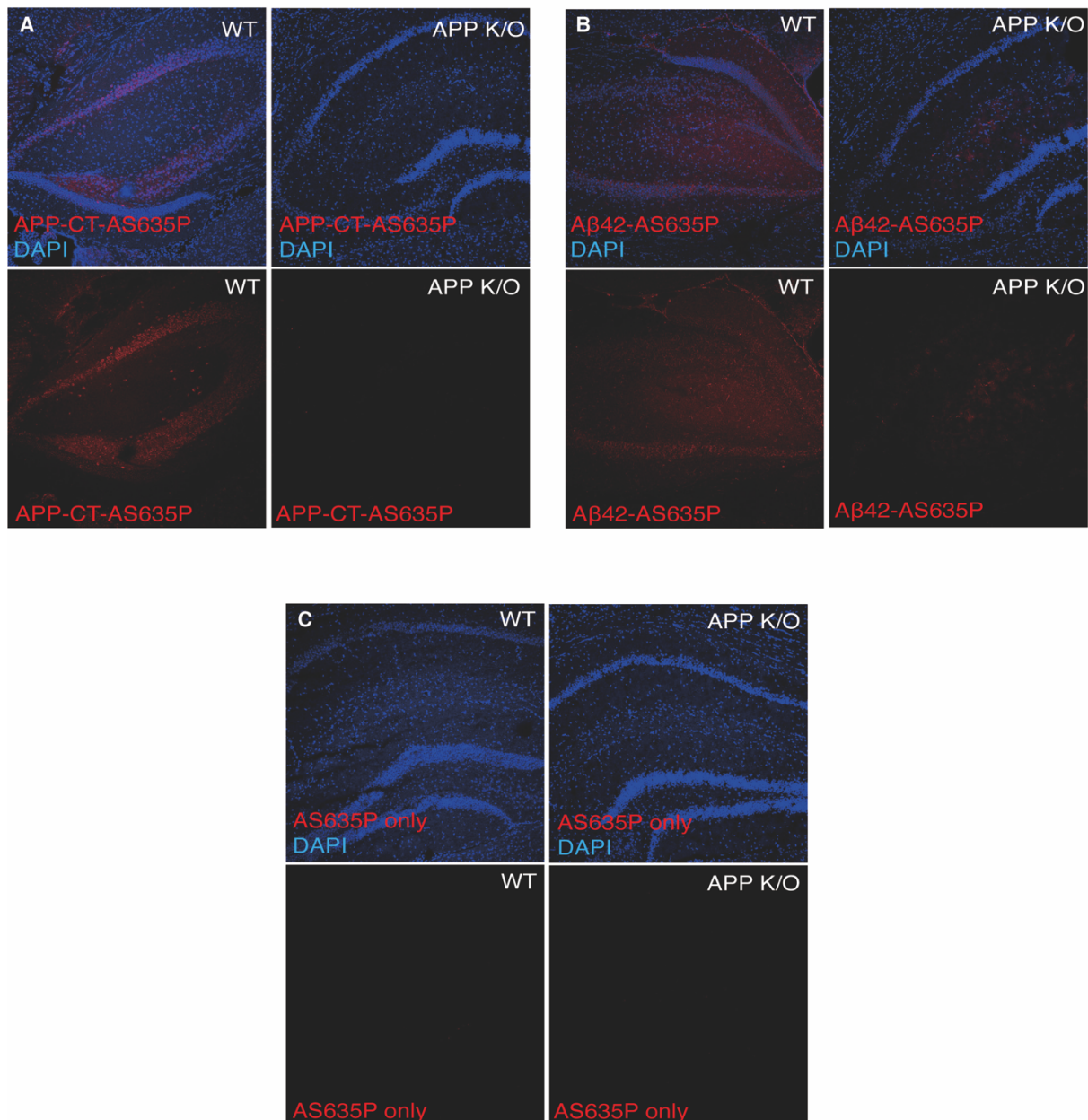


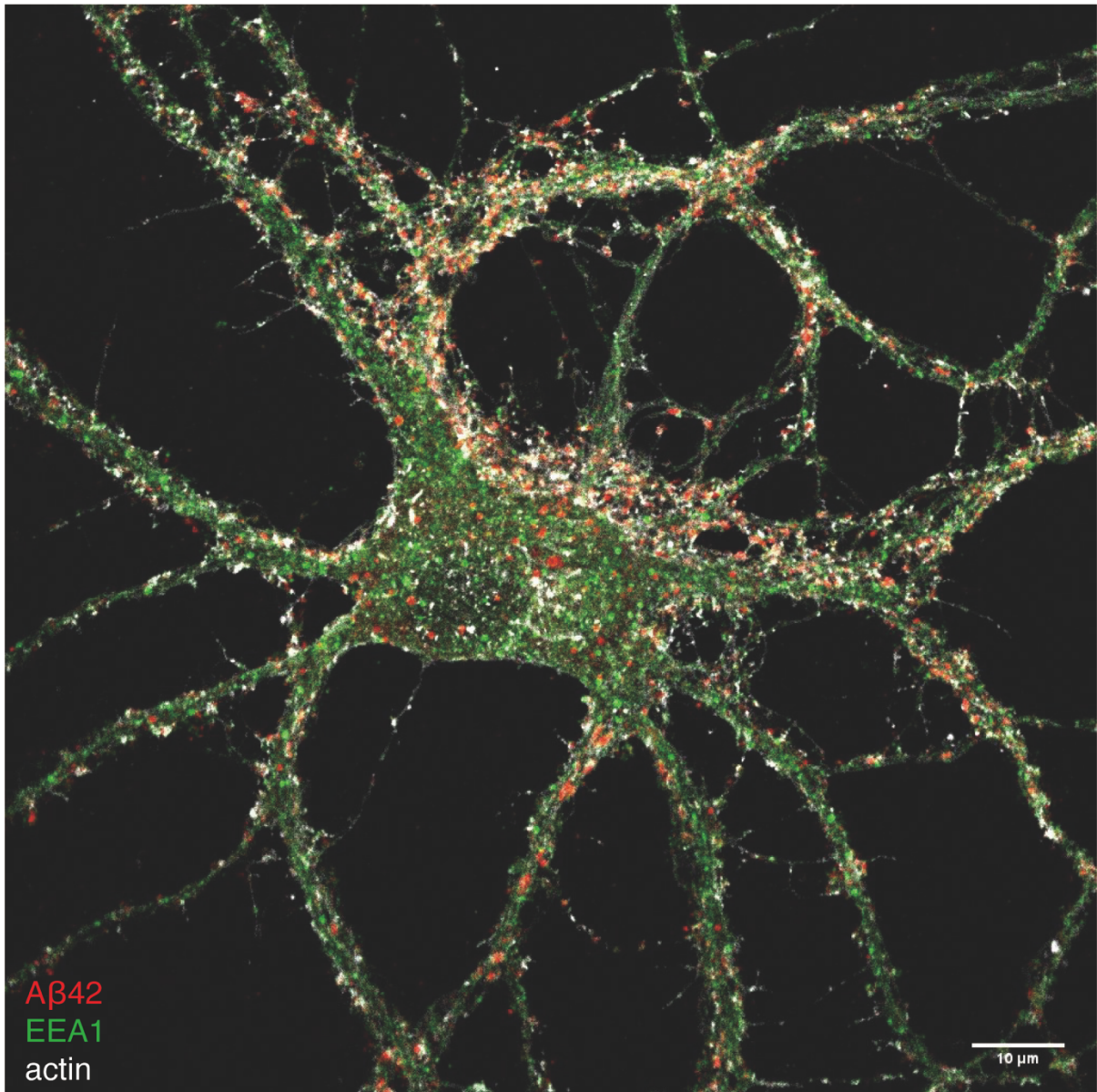
Supplementary Material

A Super-Resolved View of the Alzheimer's Disease-Related Amyloidogenic Pathway in Hippocampal Neurons

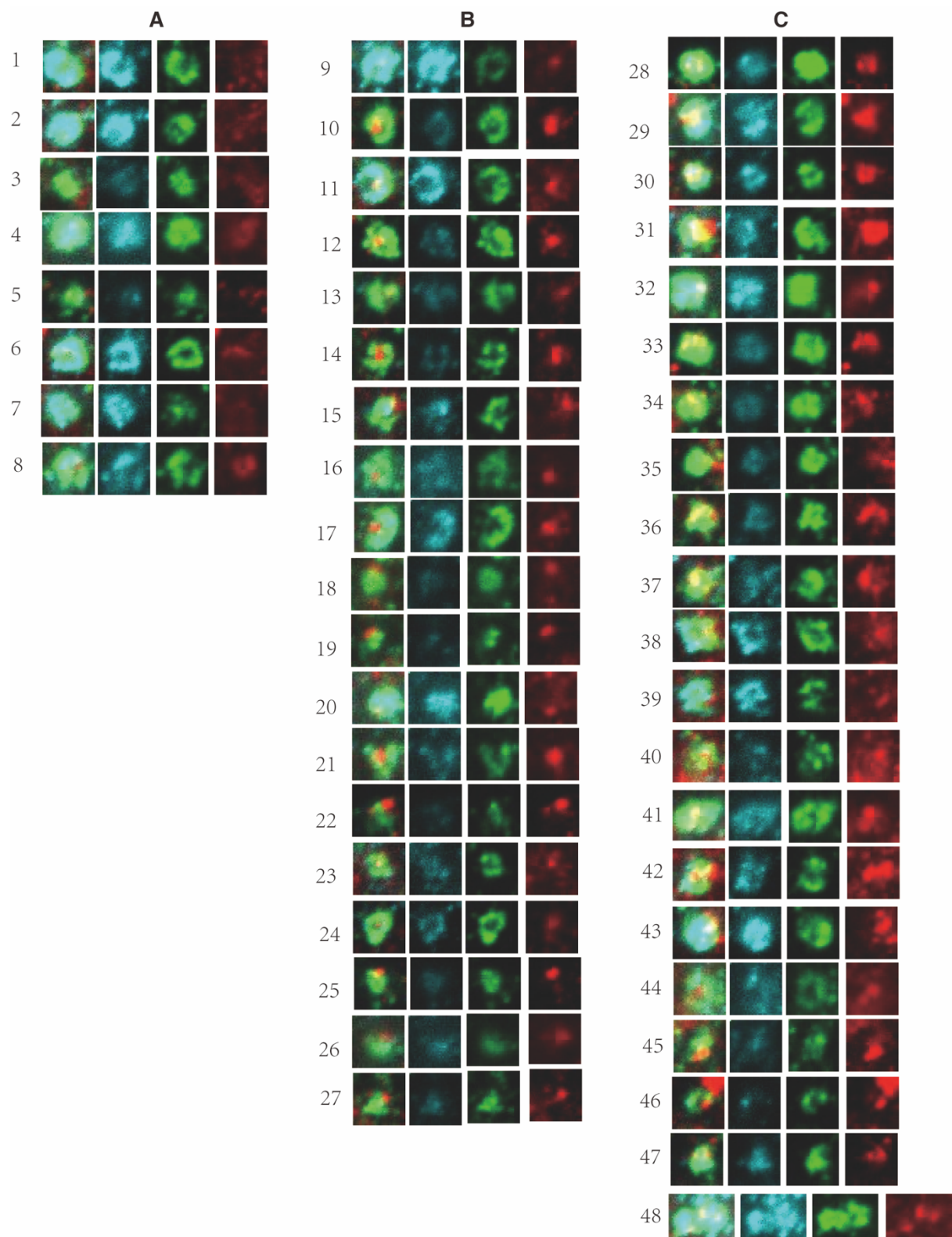
Supplementary Figure 1. Validation of APP and A β antibodies using APP knockout (K/O) mice. Brains from 18-month-old WT or APP K/O mice brain were stained with anti-APP-CT or anti-A β ₄₂ and DAPI. Abberior Star 635P (AS635P) conjugated secondary antibody was used. Samples were imaged by confocal microscopy with 10x magnification. A) Anti-APP-CT, B) Anti-A β ₄₂, and C) negative controls lacking the primary antibody.



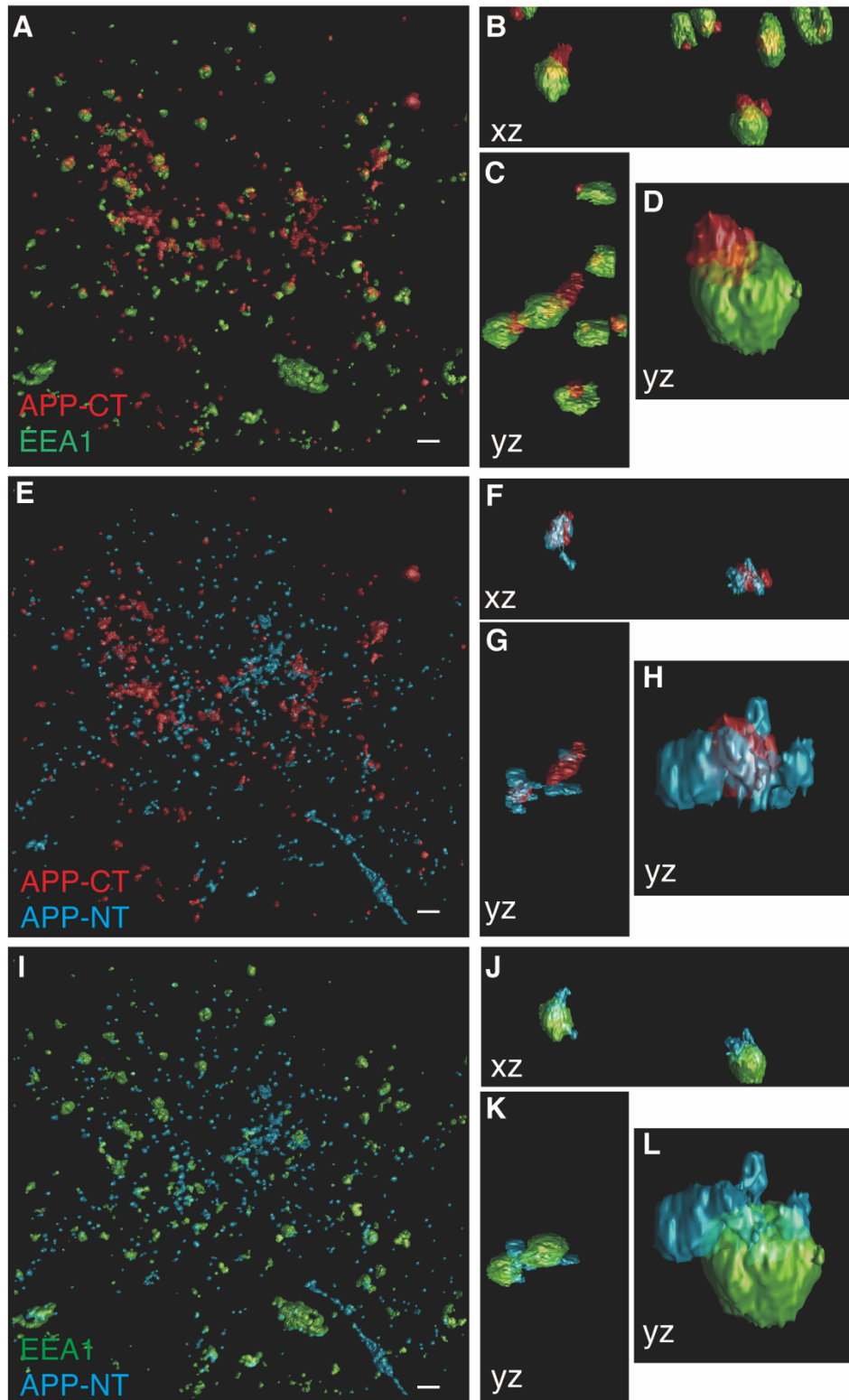
Supplementary Figure 2. Large view STED image of a hippocampal neuron. Image of a typical neuron from our mouse primary hippocampal neuron cultures. A β_{42} (red) and EEA1 (green) were imaged by STED channels and actin (white) was imaged by a confocal channel.



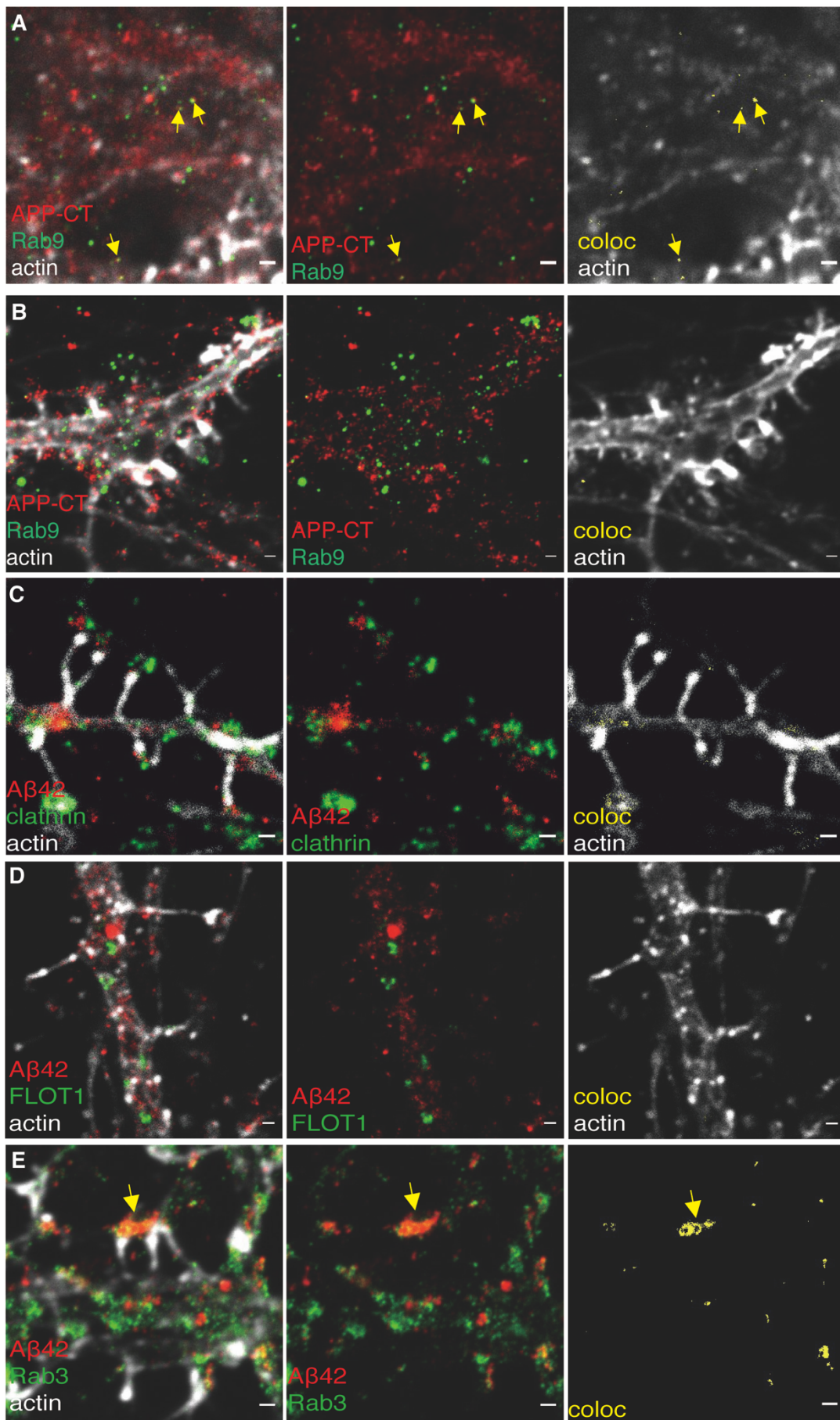
Supplementary Figure 3. Zoomed-in STED images of A β PP and A β PP fragments in early endosomes in hippocampal neurons. APP-CTF is shown in red, EEA1 is shown in green and APP-NTF is shown in cyan. Panel A, B, and C shows no detectable APP-CTF, one APP-CTF cluster, and several APP-CTF clusters, respectively.



Supplementary Figure 4. 3D STED images of APP/APP-CTF, NTF, or EEA1 staining in soma. A) APP-CT and EEA1. B, C) zoomed-in images show two other directions (xz, yz) for Fig. 3A. D) Another direction (yz) for Fig. 3B. E) APP-CT and APP-NT. F, G) Zoomed-in images show two other directions (xz, yz) for Fig. 3C. H) Another direction (yz) for Fig. 3D. I) APP-NT and EEA1. J, K) Zoomed-in images show two other directions (xz, yz) for Fig. 3E. L) Another direction (yz) for Fig. 3F. Scale bars for all: 500 nm.



Supplementary Figure 5. STED images of APP/APP-CTF or A β ₄₂ and subcellular markers. Immunolabelling and 2-channel STED imaging was used to visualize the subcellular localization of APP/APP-CTF or A β ₄₂ and subcellular markers in hippocampal neurons. A third confocal channel was used to image the actin cytoskeleton (phalloidin staining). A) APP-CTF and Rab9 in soma. Yellow arrows point at areas with colocalization. B) Staining of APP-CTF and Rab9 in neurites. C) Staining of A β ₄₂ and clathrin in neurites. D) Staining of A β ₄₂ (red) and flotillin-1 (green). E) Staining of A β ₄₂ (red) and Rab3 (green). Scale bars: 500 nm.



Supplementary Figure 6. Live cell labelling of hippocampal neurons with HiLyteFluor 647-labeled $A\beta_{42}$. Primary hippocampal neurons were treated with 100 nM HiLyteFluor 647-labeled $A\beta_{42}$ (HF- $A\beta_{42}$) for 48 hours (A) or 8 hours (B), fixed and imaged by confocal microscopy with Airyscan detector. Scale bar for all pictures: 500 nm. A) Images showed no colocalization of internalized $A\beta_{42}$ (magenta) with VAMP2 (green) in pre-synapses. Staining of actin (white). B) Images showed little colocalization of internalized $A\beta_{42}$ (magenta) with clathrin (green) in pre-synapse (white), but no colocalization with VAMP2 antibody.

