LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Figure S1. Antibodies to N- and C-terminal epitopes of APP show strict specificity for their cognate epitopes. Examples for antibody AB5352 (recognizing a C-terminal epitope) and antibody 22C11 (recognizing a N-terminal epitope) are shown. (**A**) Control immunolabeling experiment where the primary antibodies were omitted. Background labeling due to the secondary antibodies is minimal. The field includes cells with rounded or flattened soma, and neurites with their terminals. A phase contrast micrograph (Phase) is provided. (**B, C**) Dual labeling experiments of CAD cells in the presence of excess polypeptides that encompass either a 12 amino-acid region centered on Thr^{668} (+ Short APP_C peptide) or the entire APP cytoplasmic domain (+ Long APP_C peptide; see diagram in **D**). Antibody 22C11 was used either preadsorbed on APP (22C11**; **C**), or preadsorbed on BSA (22C11*; **B**). Note that the antibody preadsorbed on APP (22C11**), a procedure that removes the anti-APP immunoreactive species from the IgG fraction, shows no labeling (**C**). Incubation in the presence of excess, long APP_C peptide blocks labeling with antibody AB5352 (**B**), while incubation with excess, short APP_C peptide does not (**C**). The red arrow in (**B**) shows accumulation of N-terminal epitopes at the neurite terminal; white arrows show localization of N-terminal epitopes to an extended compartment surrounding the nucleus. (**D**) Diagram showing the position of the epitopes recognized by the antibodies (black lines), and the regions encompassed by the polypeptides used in the competition experiments (purple lines). Scale bars, 50 μm (**A**); 20 μm (**B, C**).

Supplementary Figure S2. APP-derived NTFs, not full-length APP, localize to cytoskeletal filaments. (**A**) APP-derived NTFs (detected with antibodies recognizing epitopes in the

ectodomain of APP: R8, Alz 90, 22C11; see Fig. 1A) localize to trains of closely spaced, vesicle-like particles. The filamentary distribution of APP NTFs is easily detected within enlarged segments of thin neurites (varicosities and bifurcations; bottom right images, short arrows), and often results from closely spaced trains of vesicles (long arrows, and inset). The long arrow in the bottom left image points to the region of interest shown in the inset. (**B**) Silencing BACE with siRNA eliminates the filamentous labeling with antibody 22C11. CAD cells were transfected with BACE-specific siRNA plus GFP, to mark the transfected cells. Scale bars, 10 µm (two bottom right images, and inset, in **A**); 20 µm (two bottom left images in **A**); 20 µm (upper images in **A**); 50 µm (**B**).

Supplementary Figure S3. The filamentous distribution detected with antibodies to N-terminal epitopes of APP (APP_N) is specific to NTFs of APP. (**A**) The distribution of amyloid precursor-like protein 2 (APLP2; detected with anti-APLP2 antibody) in CAD cells is distinct from that of APP_N (detected with antibody 22C11), both in the soma (left images) and the neurites (right images). (**B**) Antibodies to APP_N (22C11) show punctate distribution in $APP^{-/-}$ mouse neuronal cells, which is different than the filamentous distribution detected in APP containing neurons (compare with Fig. 3). This faint immunolabeling is likely due to the potential crossreactivity of antibody 22C11 with APLP1 and APLP2. An antibody to the Aβ region of rodent APP, not conserved in APLP1 and APLP2, does not show detectable immunolabeling. Scarce, punctate labeling is seen with antibodies to the C-terminal domain of APP (APP_C). Scale bars, 20 µm.

Supplementary Figure S4. The N-terminal epitopes of APP reside inside membrane-bounded compartments. (**A, B**) Lack of immunolabeling with antibodies to APP NTFs (antibody 22C11)

in CAD cells treated with saponin, prior to formaldehyde fixation (**B**), and in cells fixed, but not permeabilized (**A**). In **B**, two examples are shown. (**C**) Typical distribution of N- and C-terminal epitopes of APP (APP_N and APP_C) in cells fixed with formaldehyde, and permeabilized with Triton X-100 (our normal fixation/permeabilization procedure). Note that the antibody to the ectodomain of APP detects cell surface APP in nonpermeabilized and saponin permeabilized cells. The antibody to the C-terminal domain of APP, exposed to the cytoplasm, detects the cognate epitopes in cells treated with saponin (**B**) or Triton X-100 (**C**), but not in nonpermeabilized cells (**A**). Scale bars, 20 μm.

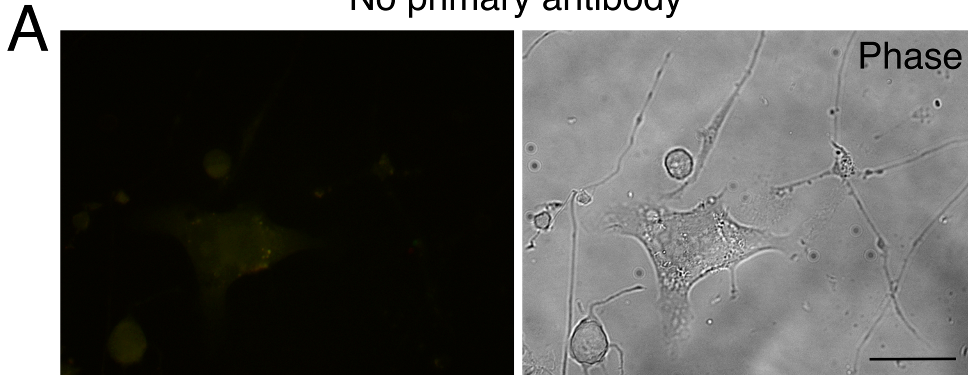
Supplementary Figure S5. Exogenous expression of Fe65 promotes accumulation of CTFs, but not NTFs in neurites. CAD cells were transfected with Myc-tagged Fe65 plus GFP, and immunostained for epitopes from the C-terminal (APP_C; C9 antibody; **A**), or N-terminal (APP_N; 22C11 antibody; **B**) region of APP. Arrows point to the long neurite of a transfected cell. (**C**) Quantitation of the effect of expression of Fe65 on the accumulation of CTFs and NTFs in neurites. The graph shows the change in frequency of cells with increased levels of neuritic CTFs or NTFs, in Fe65-Myc transfected CAD cells, compared to nontransfected control cells. C-terminal and N-terminal epitopes of APP were detected by immunocytochemistry with antibodies C9 and 22C11, respectively (see Materials and Methods). Bars represent SEM. (**D**) Exogenous expression of JIP-1-FLAG does not alter the filamentous distribution of NTFs. Note that the distribution of JIP-1-FLAG (detected with an anti-FLAG antibody) is very different from the distribution of the NTFs. Scale bars, 50 μm (**A, B**); 25 μm (**D**).

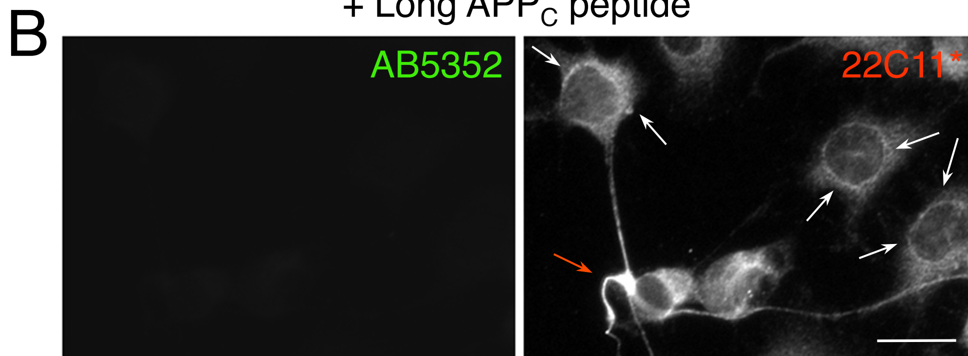
Supplementary Figure S6. Disruption of the filamentary distribution of NTFs by cold methanol. (**A**) Treatment with cold methanol eliminates the filamentary distribution of APP N-terminal epitopes (APP_N; detected with antibody 22C11) in CAD cells (right image). An image from a control experiment with normal, formaldehyde fixation, is shown at left. (**B**) Unlike for APP_N, cold methanol treatment does not alter the distribution pattern of APP C-terminal epitopes (APP_C; antibody AB5352). The bottom image in (**B**) is a higher magnification view (rotated anti-clockwise 90º) of the cell indicated by an arrow in the top left image. Scale bars, 20 µm.

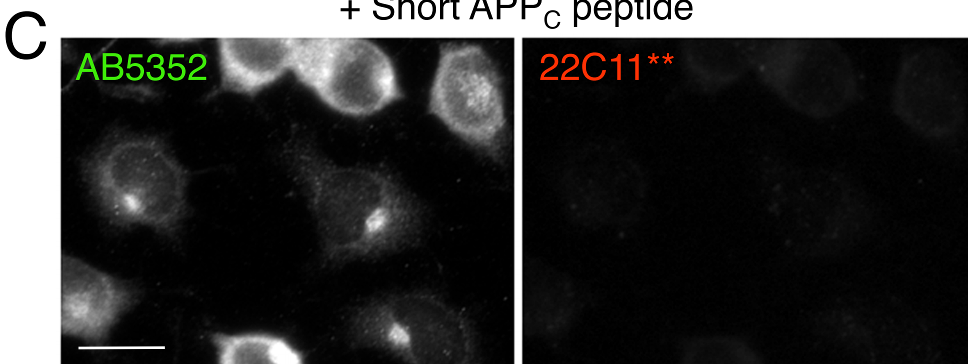
Supplementary Figure S7. Different fate of N- and C-terminal tags of dual-tagged APP in CAD cells expressing CFP-APP-YFP at high and low levels. (**A**) Extensive colocalization of CFP (red color) and YFP (green color) in CAD cells expressing CFP-APP-YFP at high levels. Note that, at this level of expression, the dual tagged APP uncharacteristically penetrates into the filopodia-like processes that emanate laterally from the soma and the neurites. Also note that the C-terminal tag, YFP, massively accumulates at the neurite terminal, a situation that is atypical for the C-terminal epitopes of endogenous APP, or for the YFP tag in cells expressing CFP-APP-YFP at low levels. (**B**) The distribution of the N-terminal tag, CFP, mimics more faithfully the distribution of immunoreactivity detected with antibodies (in this case, antibody 22C11) raised to N-terminal APP epitopes (APP_N). The arrow points to accumulation of endogenous NTFs at the neurite terminal of a nontransfected cell. (**C**) Partial segregation of CFP from YFP in the neurites of CAD cells expressing CFP-APP-YFP at low-to-moderate levels (two examples are shown). At comparable levels of fluorescence intensity of the two tags in the soma, more CFP is detected at the process terminal, compared to YFP (bottom images). (**D**) Test of two-color alignment of the microscope. Note the perfect overlap of images, acquired through the YFP and

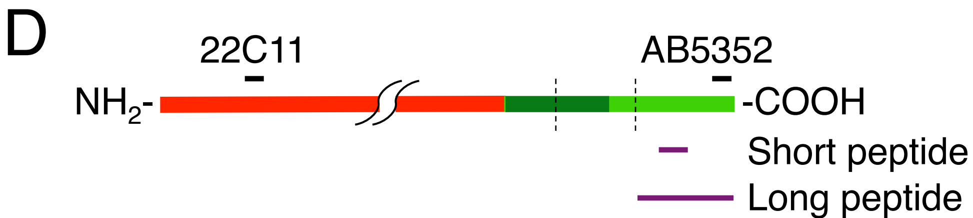
CFP channels, of CAD cells expressing pmaxGFP, which is detectable in both channels. (**E**) Proximal ligation assay (PLA) testing for interaction between FLAG (rabbit antibody) and Myc (mouse antibody), in CAD cells transfected with FLAG-APP-Myc. The left images show a general view of cells, with little or no PLA signal detected. The right images show a cell with pronounced PLA signal. Note that the dots (representing PLA signal) detected in the neurites are randomly scattered, and localize to the outer regions of the thick process. Strong signal is also detected in the soma, particularly at sites adjacent to the plasma membrane. In control experiments, where the anti-FLAG antibody was omitted, no PLA signal was detected (data not shown). Phase contrast micrographs (Phase) are provided. The right image is an overlay of the fluorescence (PLA signal) and phase contrast micrographs, clearly showing the localization of the PLA signal in the proximity of the cell membrane. The arrow points to the neurite terminal, devoid of PLA signal. Scale bars, 20 µm (**A-D**); 50 µm (**E**).

Supplementary Figure S1

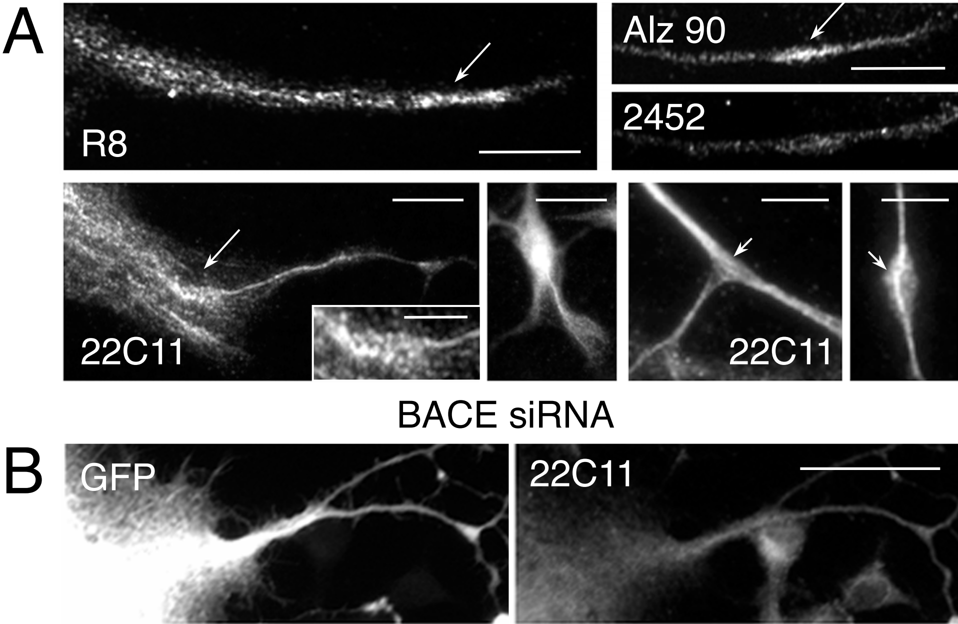








Supplementary Figure S2



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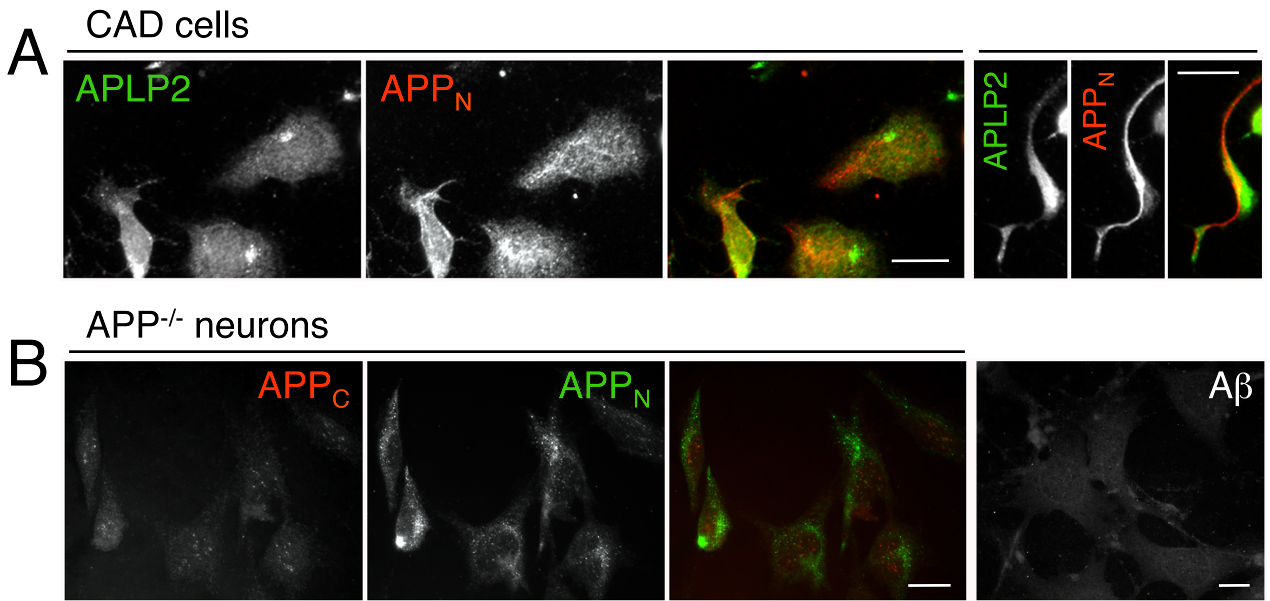

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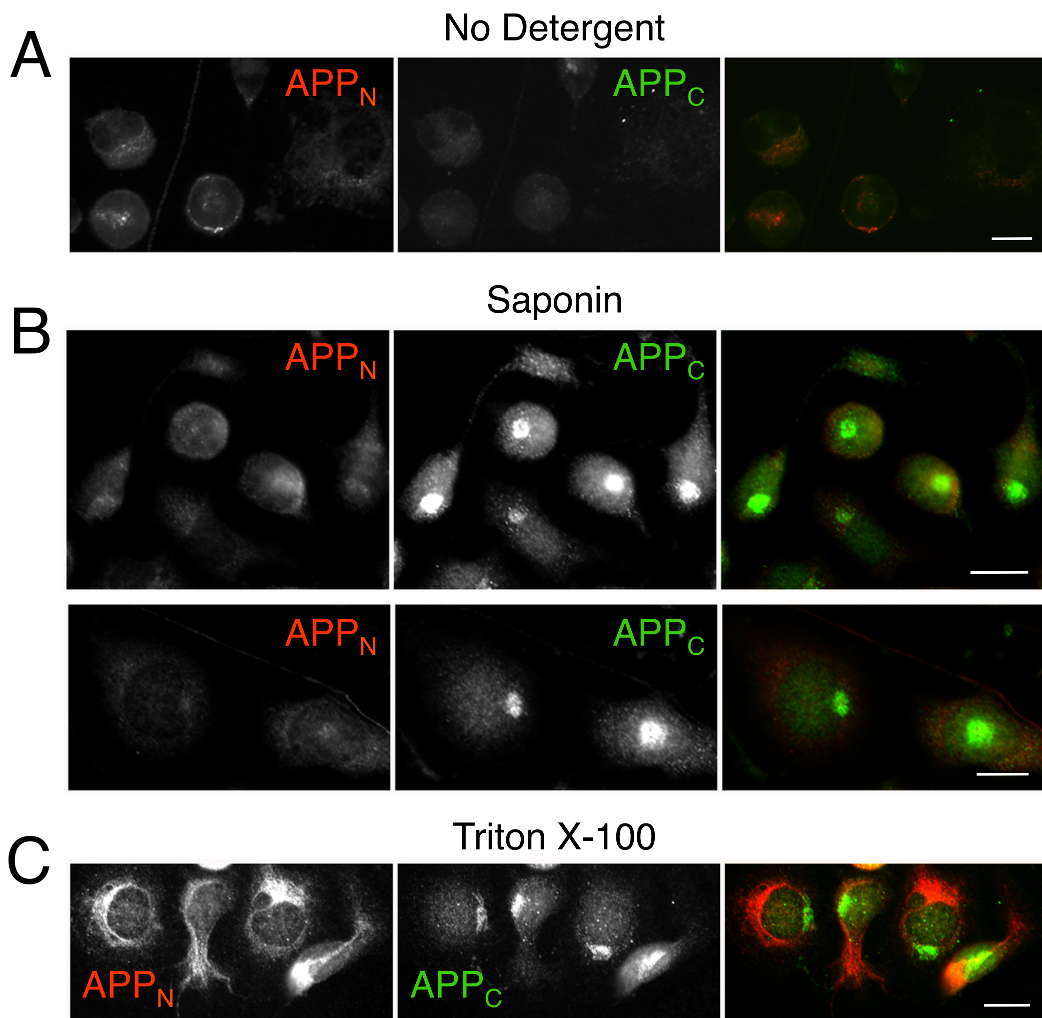

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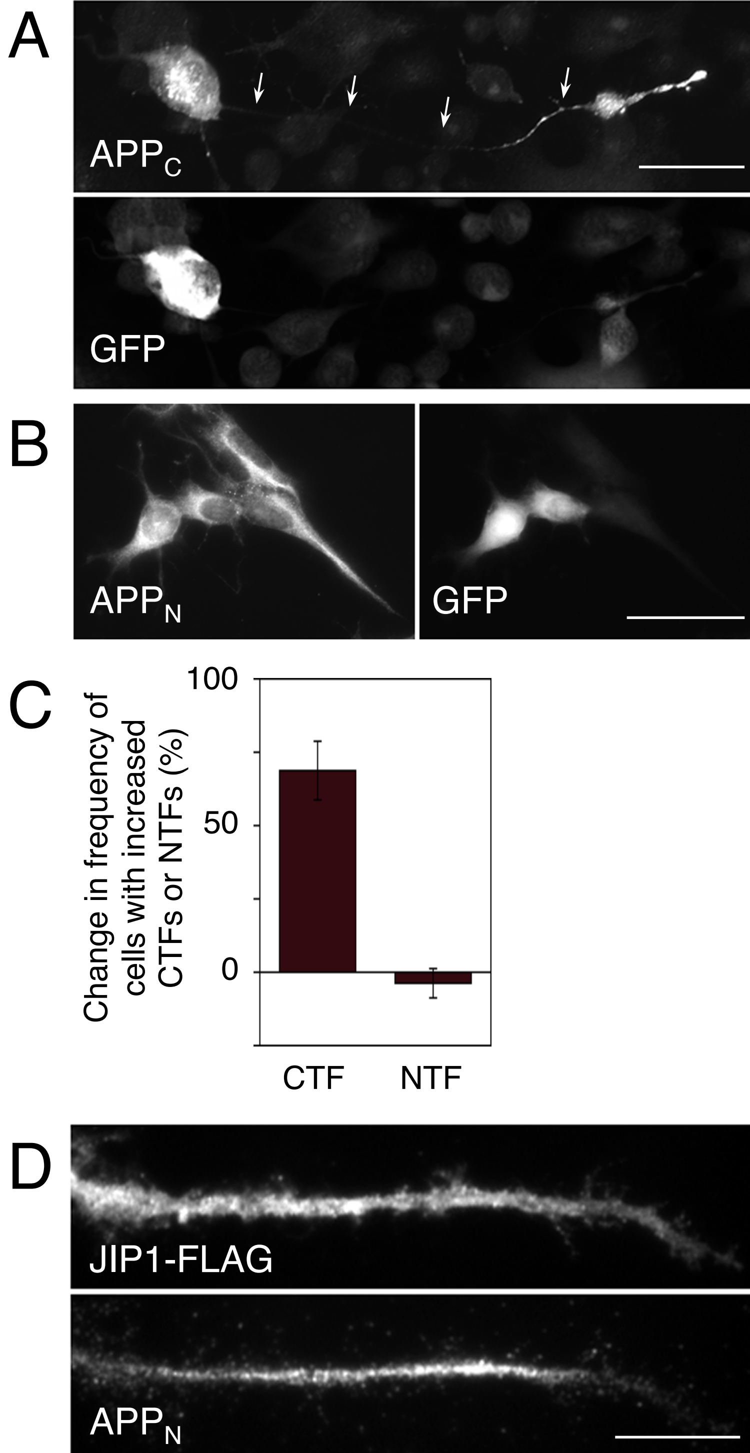
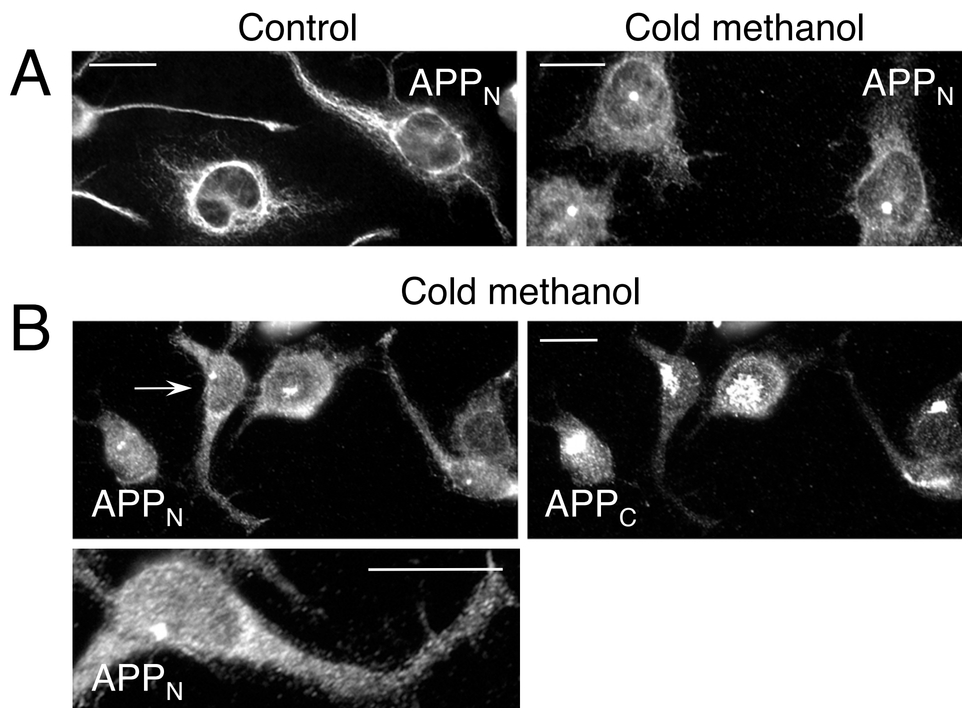
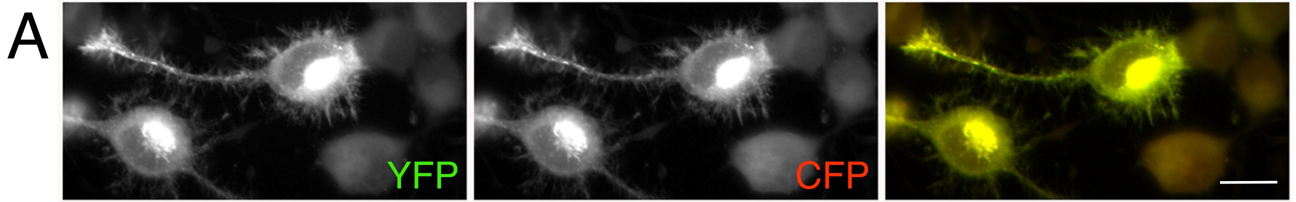


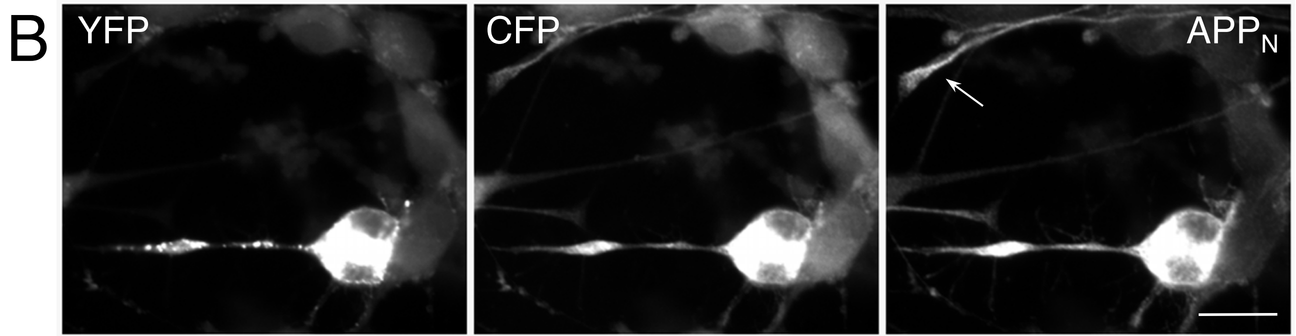
Supplementary Figure S6

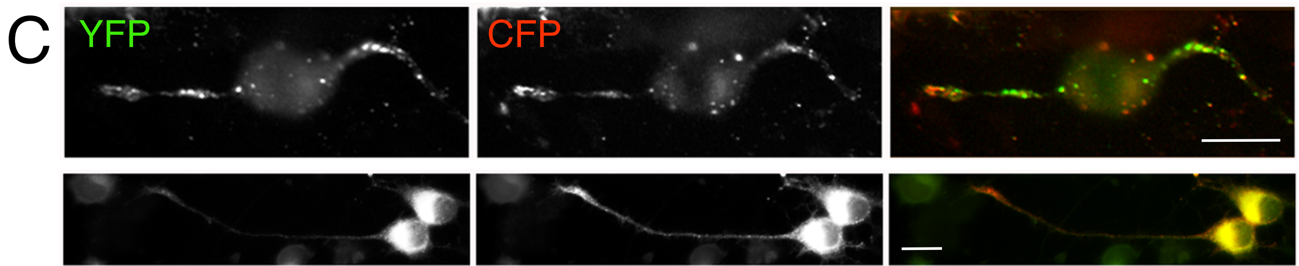


Supplementary Figure S7

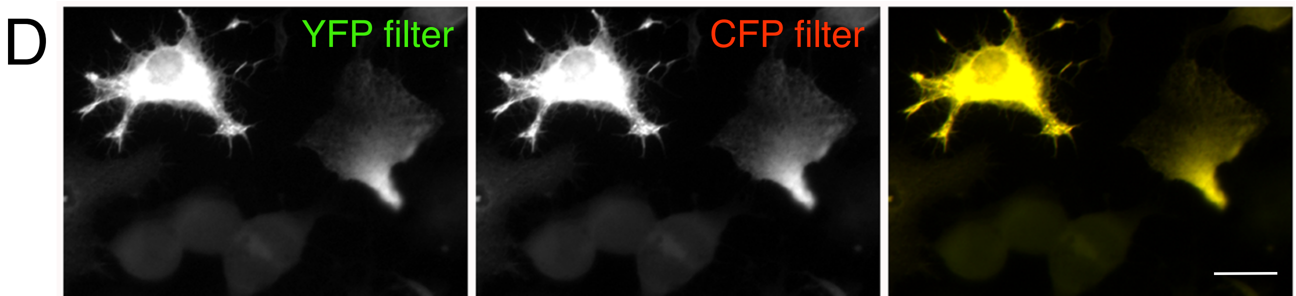
Transfected with CFP-APP-YFP







Transfected with pmaxGFP



PLA: FLAG + Myc, in cells transfected with FLAG-APP-Myc