Supplemental Information

Experimental procedures:

Experimental procedures for culturing PC12M cells and BFCN neurons, live cell imaging, immunostaining and immunoblotting are all described in detail in Materials and Methods. For siRNA knockdown against APP, procedures supplied by the manufacture (Sigma) were followed.

Supplemental Figures:

Fig S1. APP processing by β-, α- and γ-secretase and expression patterns of APP-GFP, APP mutants or C99 and C83 in PC12M cells.

A: A diagram showing the different APP processing pathways and the resulting APP proteolytic products (β-, α-CTF, AICD, P3, Aβ species). PC12M cells were transfected with indicated plasmids and live-imaging results showed different pattern of GFP-positive intracellular structures (**B, C**). In **D,** PC12M cells were co-transfected with APP-GFP/mCherry $Rab5^{S34N}$ and C99-GFP/mCherry $Rab5^{S34N}$. Images from live-imaging results showed smaller GFP-positive puncta compared to single transfected cells. The number and size of GFP-positive puncta was quantified using ImageJ and the results are shown **(E, F),** and the size distribution of Rab5-positive endosomes is shown in **G**. All corresponding *p* values (***: $p<0.001$; **: $p<0.01$) are calculated using a student t-test.

Fig S2. Overexpression of APP^{SWE} and Rabex5 induced hyperactivation of Rab5 and expression of C99 inhibited NGF-induced activation of Erk1/2 in PC12M cells.

PC12M cells were transfected with the indicated plasmids: EGFP, C99-GFP or C83-GFP in serum free DMEM. 24 hrs post transfection, lysates were assayed for activated Rab5 using the GTP-agarose pulldown method. Representative blots for the level of activated Rab5 and total Rab5 are shown in **A**. In **B**, PC12M cells were transfected with the indicated plasmids: EGFP, C99-GFP or C83-GFP in serum-free DMEM. 24 hrs after transfection, NGF (50ng/ml) was used to treat the cells for 0, 5, 30min. Cell lysates were harvested and analyzed by SDS-PAGE/immunoblotting with indicated antibodies. Total-Erk and GAPDH were also blotted with specific antibodies as loading controls. **C**: The levels of pErk1/2 in cells expressing either C99-GFP or C83-GFP were normalized against pErk1/2 in cells expressing EGFP (n=4; n.s.: non-significant; **: $p<0.05$; ***: $p<0.01$). All corresponding *p* values are calculated using a student t-test.

Fig S3. Expression of C99-GFP suppresses NGF-induced pCREB signals.

Rat E18 BFCNs were cultured in microfluidic chamber and transfected with EGFP, C99-GFP or C83 GFP at DIV6. NGF was removed from the cell body chamber while the axonal chamber was supplied with 50 ng/ml NGF for 48 hrs. Neurons were processed for immunostaining with a specific antibody to pCREB. Representative images are shown for p-CREB staining of rat E18 BFCNs transfected with EGFP(**A**), C99-GFP(**B**) and C83-GFP (**C**). The signal for pCREB in neurons expressing C99-GFP shows a marked decrease.

Fig S4. Expression of mCD8-GFP, Rab5 constructs alone did not induce axonal blockages in *Drosophila* larval segmental nerves.

A, Representative images of *Drosophila* 3rd instar larval segmental nerves immuno-stained with the synaptic vesicle marker cystein string protein (CSP) in F1 larvae expressing mCD8-GFP (1), YFPRab5^{WT}(2), YFPRab5^{CA}(3), and YFPRab5^{DN}(4). Axonal blockages were quantitated within the entire larvae for each genotype as in **Fig 12**. The average number of axonal blocks for each genotype is shown in **B**. The data for ApplGal4, human APP695 were also included for comparison.

Supplemental videos

Movie S1. A representative movie showing the movement of QD-NGF in the axon of untransfected rat E18 BFCNs. The image series was captured at 1 frame/sec.

Movie S2. A representative movie showing the movement of QD-NGF in the axon of rat E18 BFCNs transfected with EGFP. The image series was captured at 1 frame/sec.

Movie S3. A representative movie showing the movement of QD-NGF in the axon of rat E18 BFCNs transfected with C99-GFP. The image series was captured at 1 frame/sec.

Movie S4. A representative movie showing the movement of QD-NGF in the axon of rat E18 BFCNs transfected with C83-GFP. The image series was captured at 1 frame/sec.

Movie S5. A representative movie showing the movement of QD-NGF in the axon of rat E18 BFCNs pretreated with 0.1% DMSO (vehicle for GSI). The image series was pseudo-colored magenta and captured at 1 frame/sec. The Scale bar = 10 μm.

Movie S6. A representative movie showing the movement of QD-NGF in the axon of rat E18

BFCNs pretreated with 1.0 μM GSI for 2 hrs. The image series was pseudo-colored magenta and captured at 1 frame/sec. Scale bar = 10 μm.

Fig S1 Xu et al., 2016

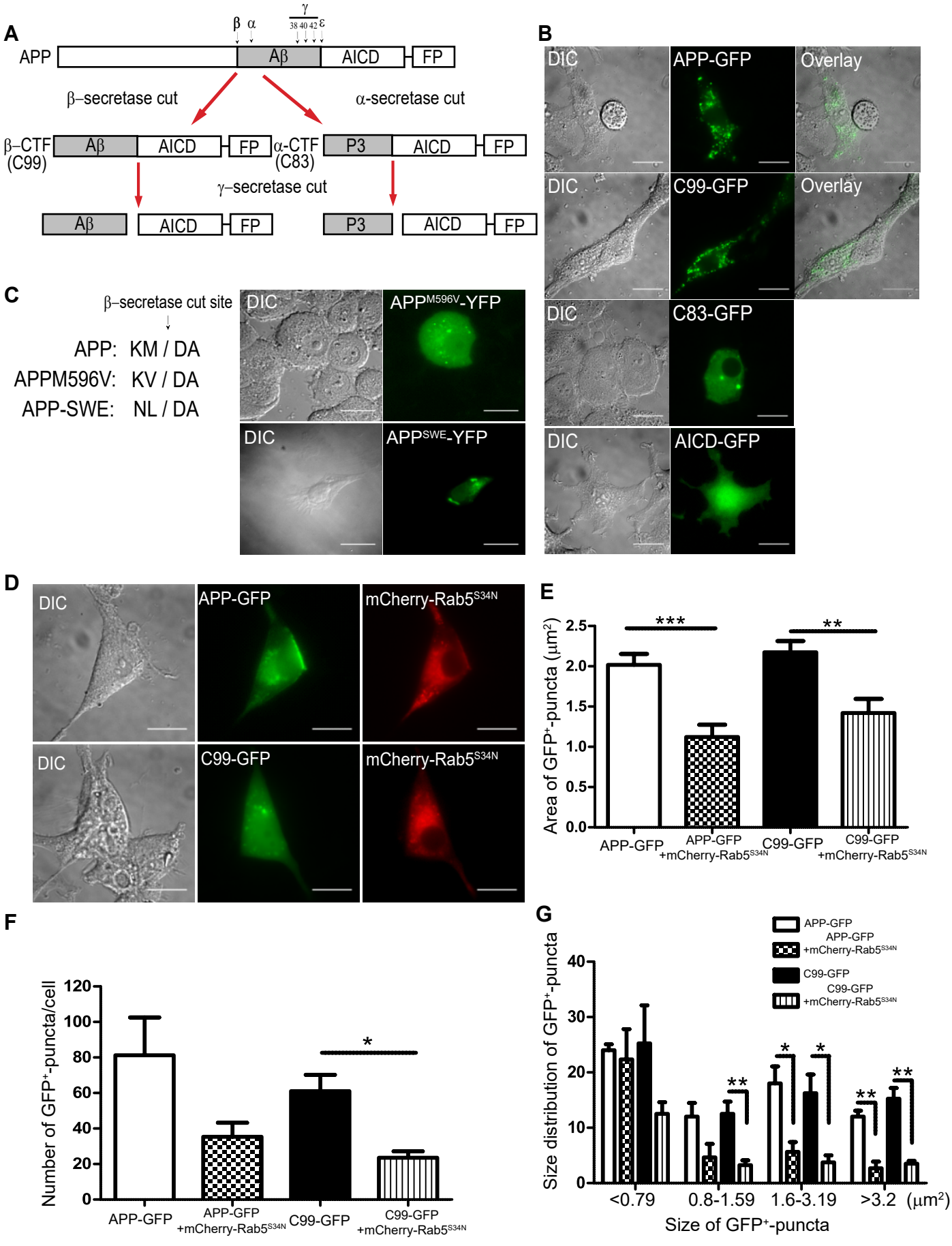

Fig S2 Xu et al., 2016

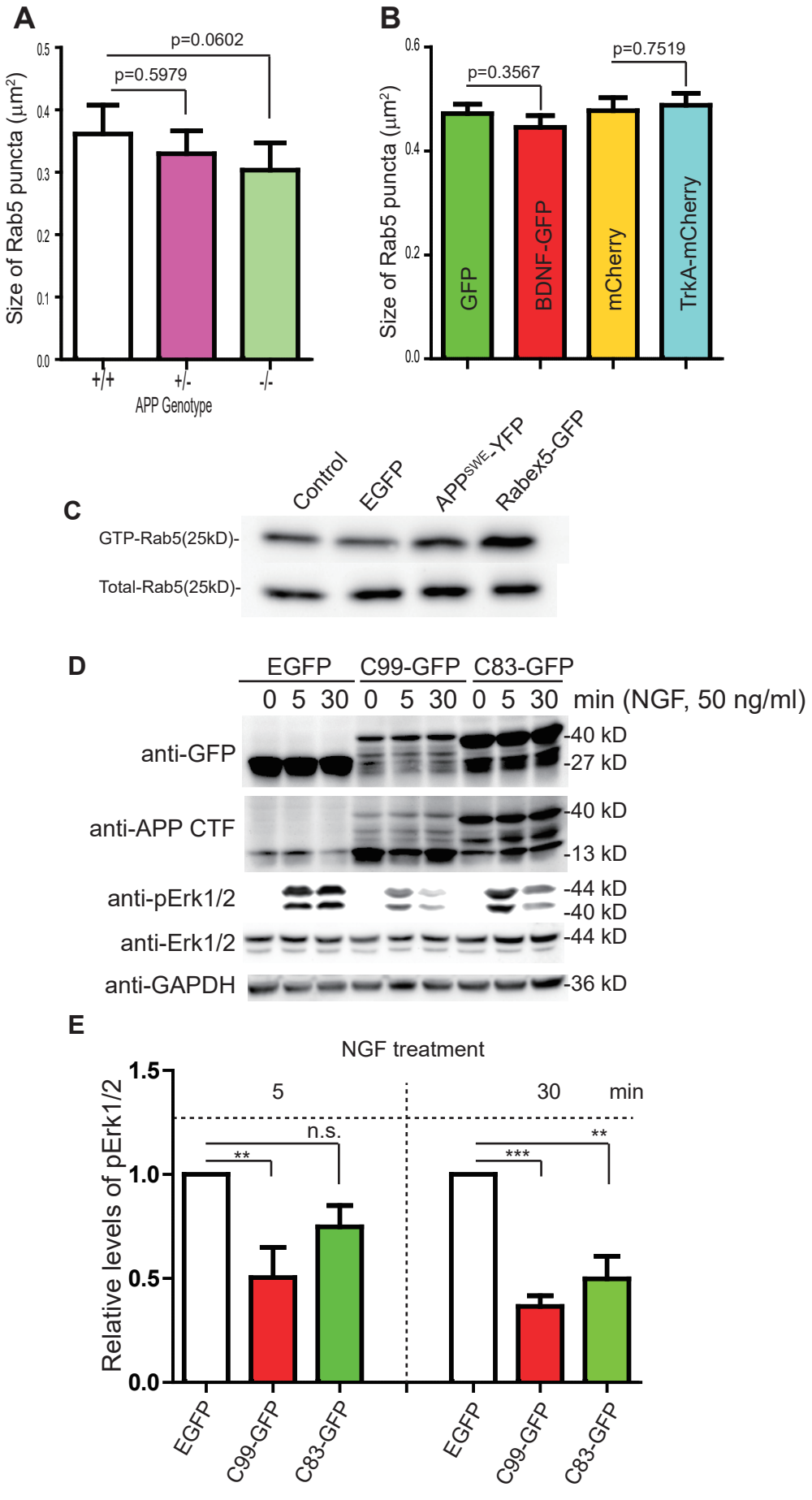

Fig S3 Xu et al., 2016

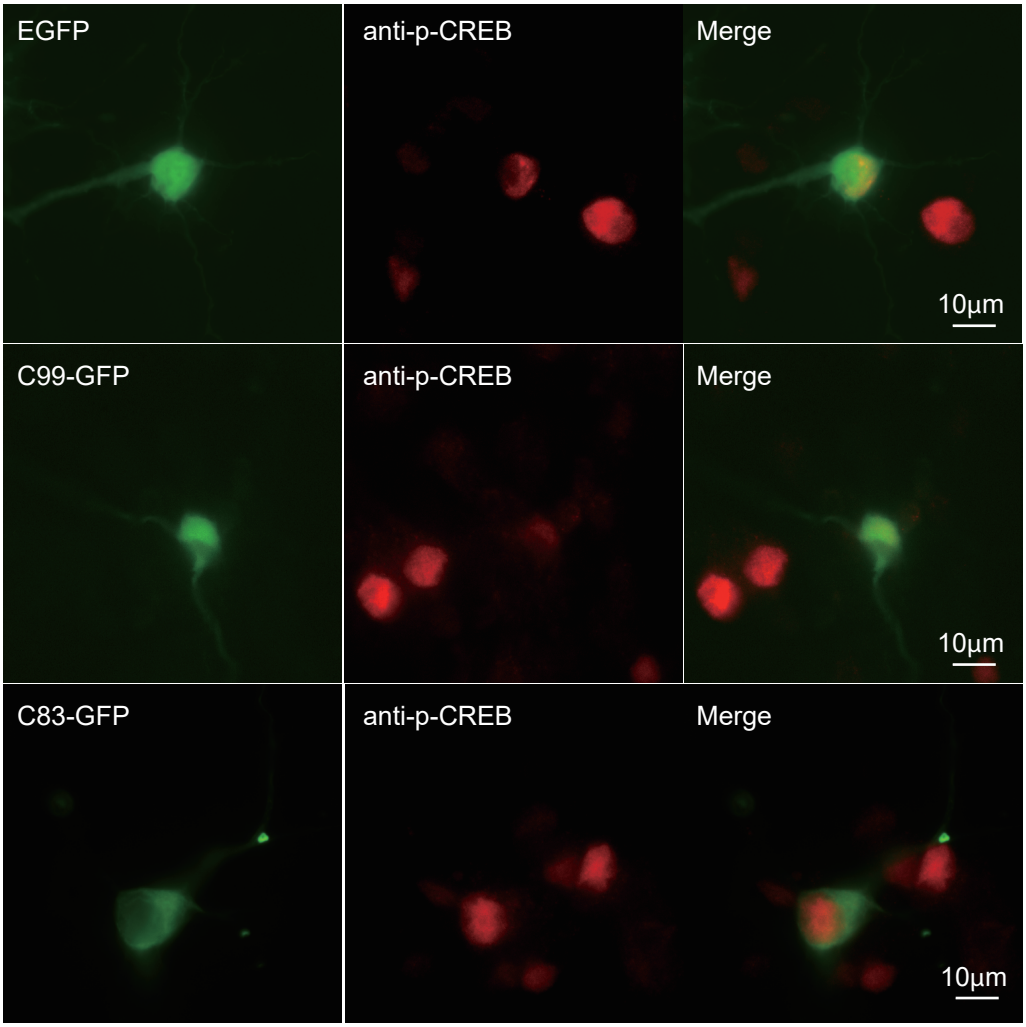

Fig S4, Xu et al., 2016