SUPPLEMENTAL DATA

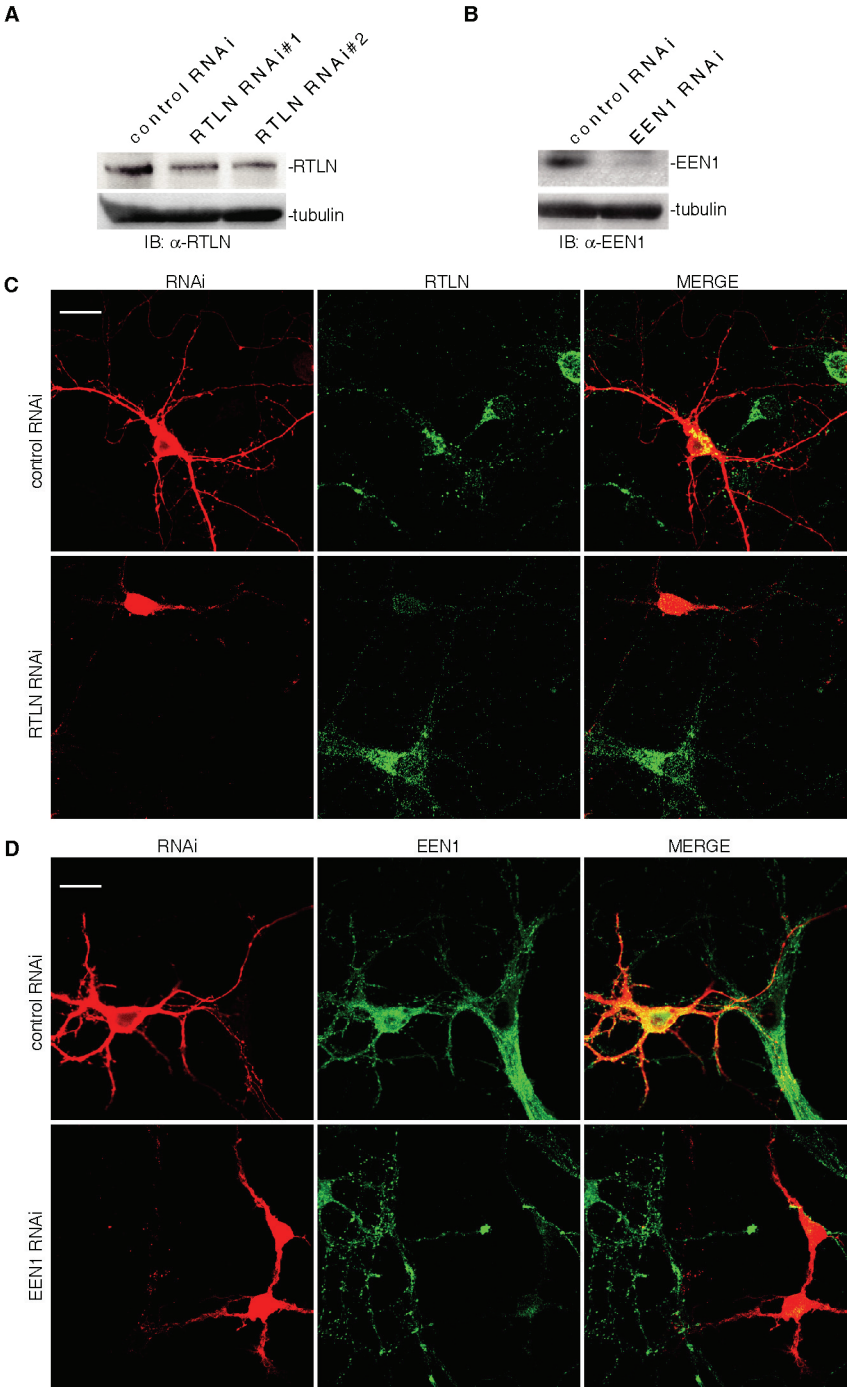

Figure S1. Retrolinkin and endophilin A1 shRNA leads to specific knockdown in their protein levels in hippocampal neurons, respectively. (A) Mouse hippocampal neurons were infected with lentivirus expressing shRNA for 7 days. Total RTLN and tubulin were detected by Western blotting of whole cell lysates. (B) Hippocampal neurons were infected with lentivirus expressing shRNA for 7 days. Total EEN1 and tubulin were detected by Western blot of whole cell lysates. (C) Hippocampal neurons transfected with construct coexpressing dsRed and RTLN shRNA for 4 days were fixed and stained with antibodies to dsRed and RTLN. (D) Hippocampal neurons infected with construct coexpressing dsRed and EEN1

shRNA for 4 days were fixed and stained with antibodies to dsRed and EEN1, followed by the appropriate fluorescently labeled secondary antibodies. Scale bar, 10 μm. RTLN, retrolinkin; EEN1, endophilin A1.

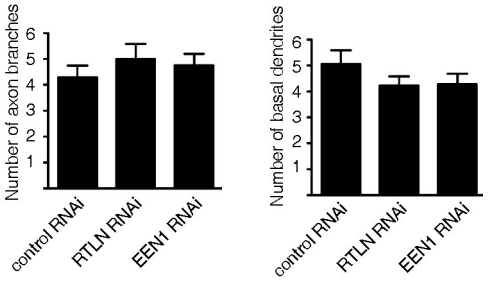

Figure S2. Depletion of retrolinkin or endophilin A1 in hippocampal neurons does not affect axon branching and number of dendrites. Hippocampal neurons were transfected at DIV1 with shRNA constructs coexpressing RFP and shRNA and fixed at DIV5, followed by immunostaining with the anti-RFP Ab. Shown are average number of axon branches and dendrites per transfected neuron (± SEM) of 40-60 neurons. RTLN, retrolinkin; EEN1, endophilin A1.

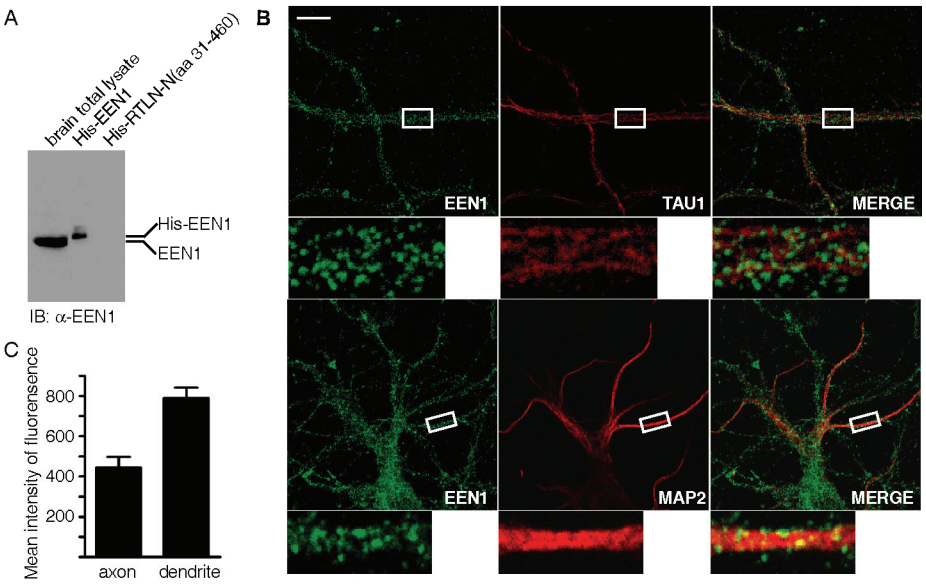

Figure S3. Distribution of endophilin A1 in hippocampal neurons. (A) Western blot probed with Guinea pig anti-EEN1 antibody. (B) Immunofluorescence staining on cultured mouse hippocampal neurons with antibodies to EEN1 and TAU1 or MAP2. Scale bar, 10 μm. (C) Background subtracted, mean intensity of EEN1 fluorescence in primary dendrites and axons. Measurement of fluorescent intensity is expressed in arbitrary units per square area in both axons and dendrites. All of the images were obtained in the same settings below saturation at a resolution of 1,024x1,024 pixels (12 bit) (n = 12-20). EEN1, endophilin A1.

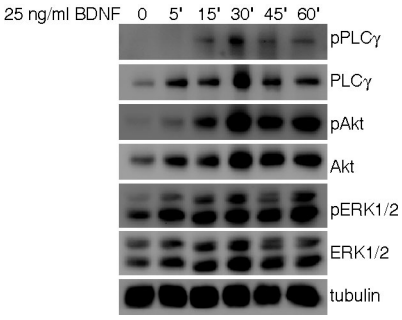

Figure S4. Time course of BDNF-induced activation of PLC-γ, Akt, and ERK1/2 in cortical neurons. Mouse cortical neurons were treated with BDNF for 0, 5, 15, 30, 45 and 60 min. Immunoblots of whole cell protein extracts were probed with antibodies to total and phosphorylated proteins. Western blotting for α-tubulin demonstrates equal loading of protein samples.

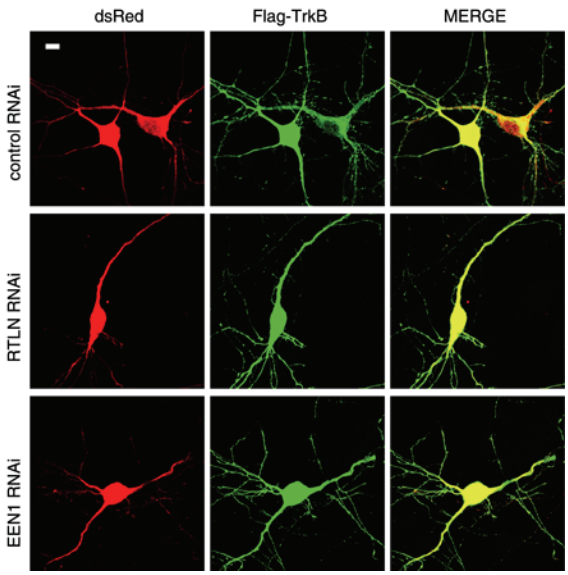

Figure S5. Expression of Flag-TrkB in hippocampal neurons. DIV3 hippocampal neurons were cotransfected with Flag-TrkB and shRNA expressing constructs for 3 days. Cells were fixed and immunostained with antibodies to Flag and dsRed, followed by the appropriate fluorescently labeled secondary antibodies. Scale bar, 10 μm. RTLN, retrolinkin; EEN1, endophilin A1.

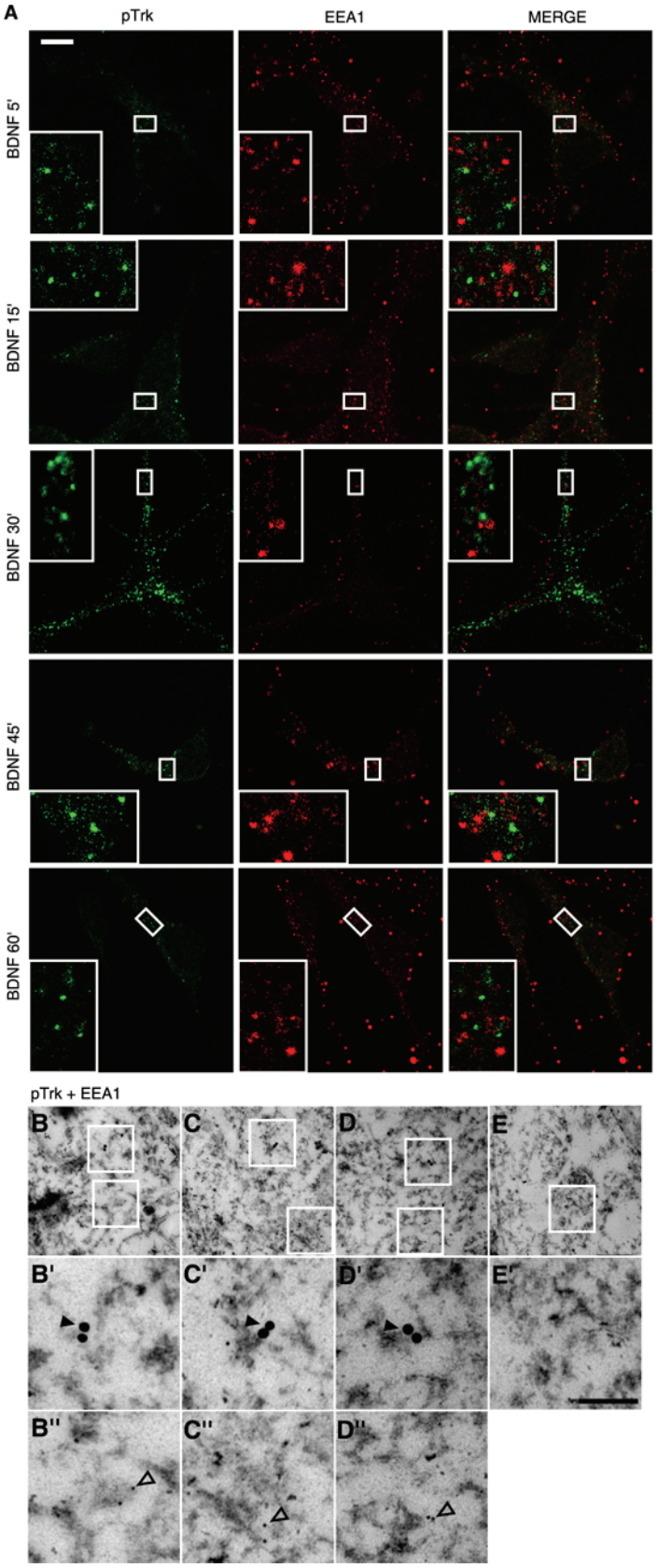

Figure S6. Little colocalization between pTrk and EEA1-positive endosomes. (A) DIV10 hippocampal neurons were starved for 2 h, stimulated with 25 ng/ml BDNF for 5, 15, 30, 45

and 60 min and double-stained with antibodies to EEA1 and TrkB or pTrk. Scale bar, 10 μm. (B-D) Representative examples of double immunogold labeling of adult mouse hippocampus with antibodies to pTrk (18 nm) and EEA1 (6 nm). (B', B'', C', C'', D' and D'') Higher magnification of boxed areas in (B), (C) and (D), respectively. pTrk: open triangle. EEA1: solid triangle. (E) Negative control with secondary antibodies only. (E') Higher magnification of boxed area in (E). Scale bar, 350 nm in (B-E), and 100 nm in (B'-E').