Supplemental Materials

Molecular Biology of the Cell

Xu et al.

Supplemental Figures:

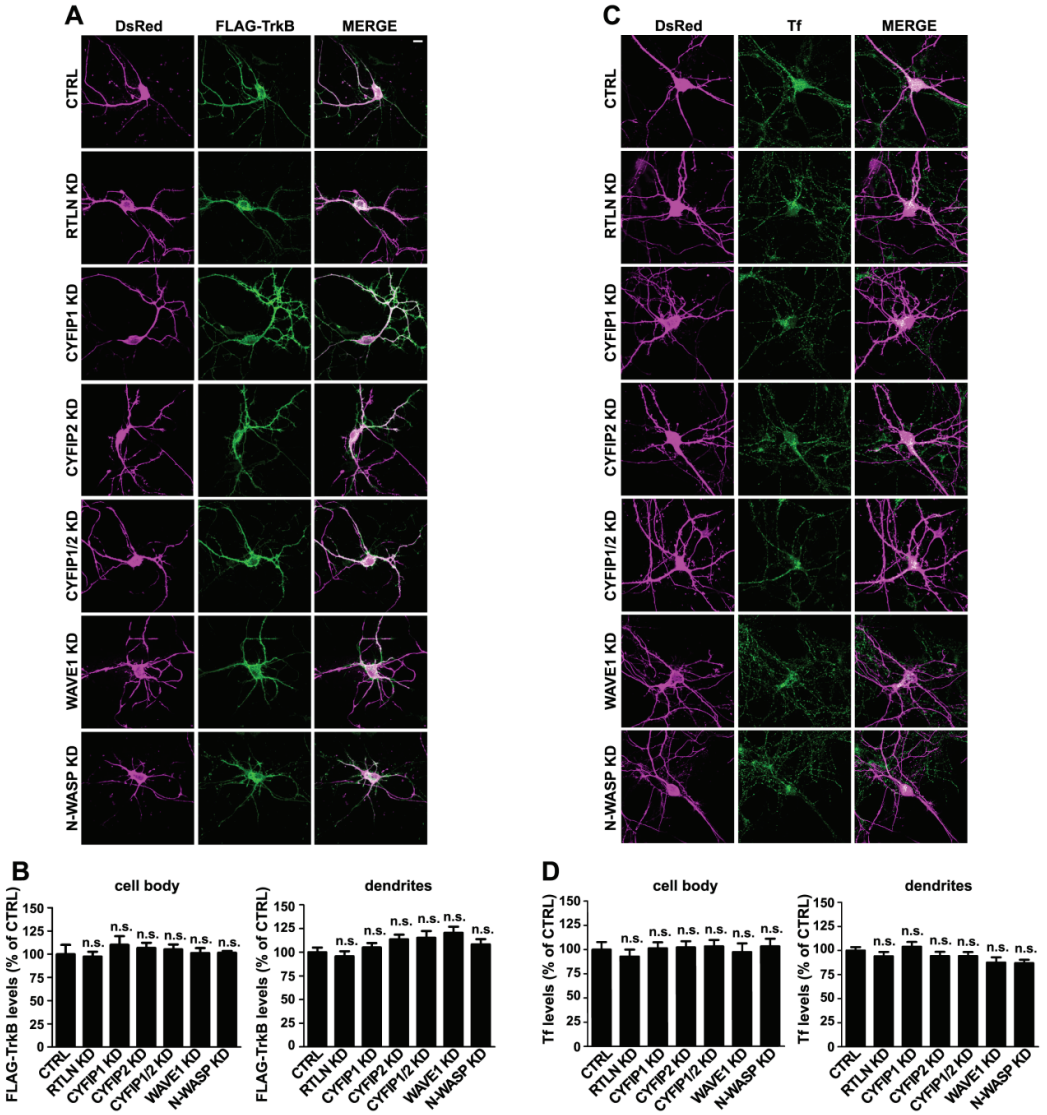

Figure S1: The surface expression levels of FLAG-TrkB and transferrin binding to cell surface did not change significantly in knockdown neurons. Related to Figure 4. (A) Hippocampal neurons were cotransfected with FLAG-TrkB and shRNA expressing constructs at DIV4. Neurons were fixed and immunostained with anti-FLAG antibody at DIV8. For surface staining of FLAG-TrkB, neurons were incubated with primary antibody without membrane permeabilization after PFA fixation. (B) Quantification of mean fluorescence intensity of FLAG-TrkB signals in cell body and dendrites from neurons in A (3 dendritic segments/neuron, 15 μm/segment, 20 neurons/group). Data represent mean ± SEM. *N* = 2 independent experiments. (C) Hippocampal neurons were transfected with shRNA constructs at DIV4, incubated with 10 μg/ml Alexa Fluor 488-conjugated transferrin (Tf) for 15 min at 37°C at DIV8. Neurons were fixed without acid stripping. (D) Quantification of mean fluorescence intensity of Tf signals in cell body and dendrites from neurons in C (3 dendritic segments/neuron, 15 μm/segment, 20 neurons/group). Data represent mean ± SEM. *N* = 2 independent experiments. n.s., not significant. Scale bar, 10 μm.

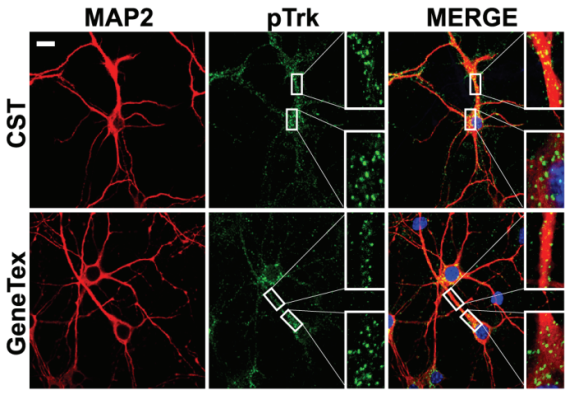

Figure S2: Testing of antibodies for immunofluorescence staining of activated Trk/phospho-Trk (Tyr-490). Hippocampal neurons were starved for 2 h, stimulated with BDNF (25 ng/ml) for 30 min, fixed with 4% PFA and double-stained with antibodies against MAP2 and pTrk (Tyr-490) from CST (cat # 9141S) and GeneTex (cat # GTX21445). DAPI was used for nuclear staining. Boxed regions are enlarged to show the punctate staining pattern of pTrk. Scale bar, 10 μm.

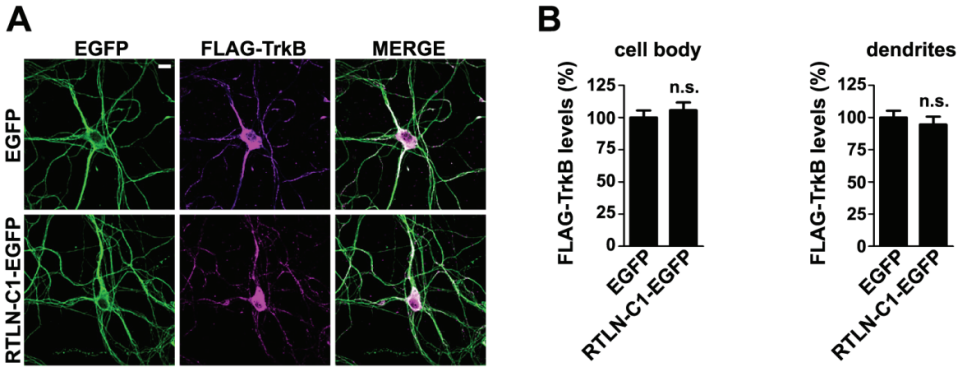

Figure S3. Overexpression of RTLN-C1-EGFP in neurons does not affect the surface expression levels of FLAG-TrkB. Related to Figure 7. (A) Hippocampal neurons were cotransfected with constructs expressing FLAG-TrkB and EGFP or RTLN-C1-EGFP at DIV4. Neurons were fixed and surface stained with anti-FLAG antibody at DIV8. (B) Quantification of mean fluorescence intensity (MFI) of FLAG-TrkB signals in cell body and dendrites from neurons in A (3 dendritic segments/neuron, 15 μm/segment, 20 neurons/group). Data represent mean ± SEM (*N* = 2). n.s., not significant. Scale bar, 10 μm.

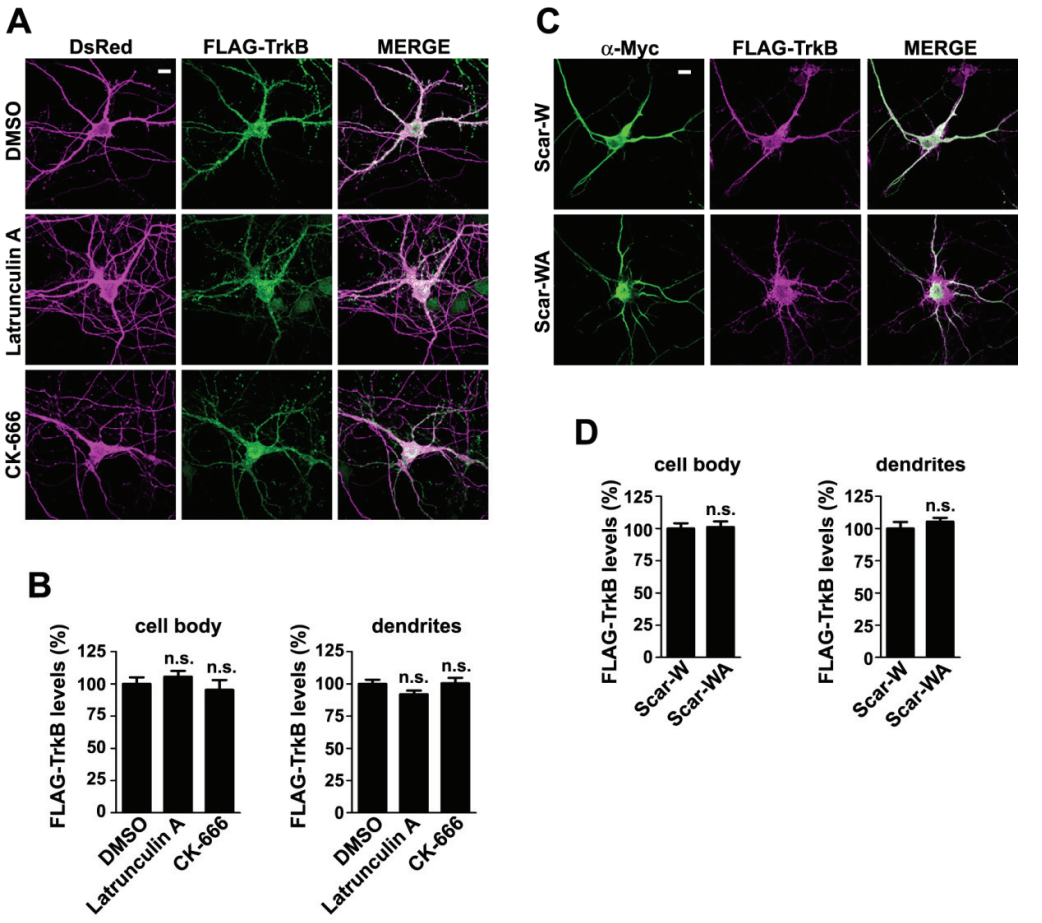

Figure S4. Surface expression levels of FLAG-TrkB did not change upon latrunculin A or CK-666 treatment, or overexpression of Scar-WA. Related to Figure 8. Hippocampal neurons cotransfected with constructs expressing FLAG-TrkB and DsRed were treated with latrunculin A (1 μM) for 1 h or CK-666 (200 μM) for 2 h, fixed and surface stained with anti-FLAG antibody. (B) Quantification of mean fluorescence intensity (MFI) of FLAG-TrkB signals in cell body and dendrites from neurons in A (3 dendritic segments/neuron, 15 μm/segment, 20 neurons/group). (C) Hippocampal neurons were cotransfected with constructs expressing FLAG-TrkB and Scar-W or Scar-WA at DIV4. On DIV8 neurons were fixed and surface stained with anti-FLAG antibody, followed by permeabilization with 0.4 % Tx-100 and staining with anti-Myc antibody. (D) Quantification of mean fluorescence intensity (MFI) of FLAG-TrkB signals in cell body and dendrites from neurons in C (3 dendritic segments/neuron, 15 μm/segment, 20 neurons/group). Data represent mean ± SEM (*N* = 2). n.s., not significant. Scale bar, 10 μm.

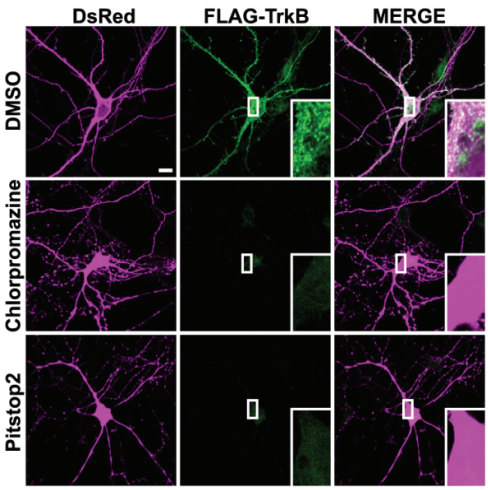

Figure S5. Surface expression of Flag-TrkB was not detectable in neurons treated with CME inhibitors. Related to Figure 9. DIV4 hippocampal neurons were cotransfected with constructs expressing FLAG-TrkB and DsRed for 72 h. Neurons were starved with MEM for 2 h at 37°C and incubated with chlorpromazine (25 µM) for 30 min or Pitstop2 (10 µM) for 15 min at 37°C, fixed and surface stained with anti-FLAG antibody. Scale bar, 10 µm.

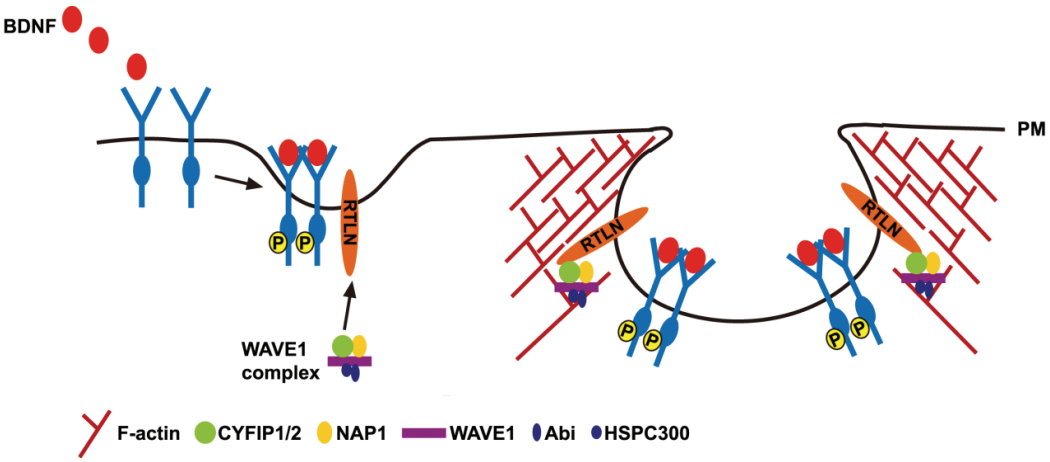

Figure S6: Model of proposed mechanism by which retrolinkin recruits the WAVE1 complex to mediate BDNF-TrkB endocytosis. Upon binding to BDNF, TrkB receptors self-dimerize and are phosphorylated. Binding of the ligand also induces receptor-mediated endocytosis. Through direct interaction with CYFIP1/2, retrolinkin recruits the WAVE1 complex and promotes actin nucleation at the BDNF-TrkB endocytic site in the plasma membrane (PM). Polymerized actin/F-actin drives membrane shape changes and promotes endocytosis.