Supporting Information

Philippidou et al. 10.1073/pnas.1015981108

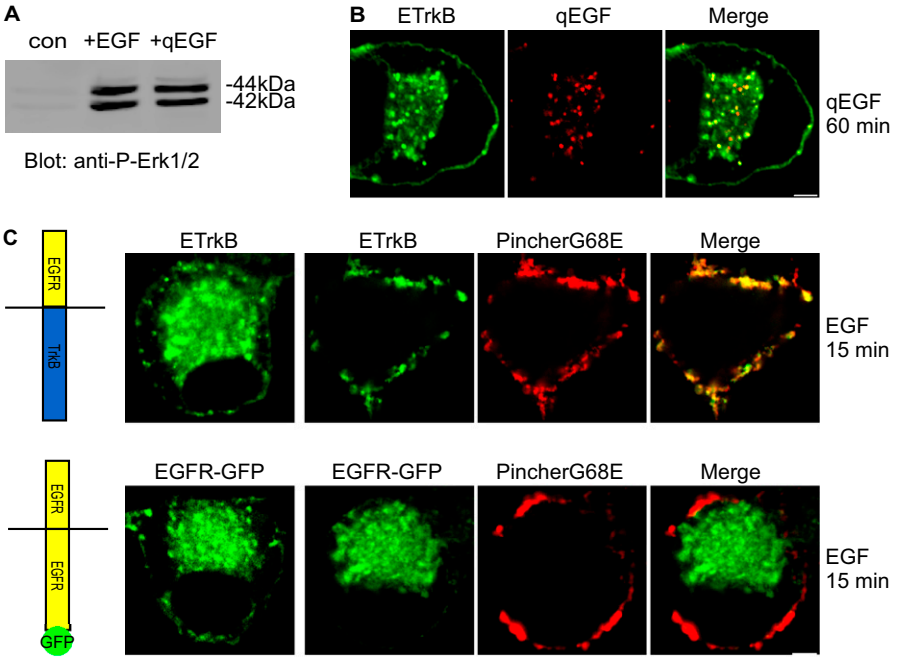

Fig. S1. Using the EGFR/TrkB receptor (ETrkB) chimera and EGF-Quantum dot 605 (qEGF) to monitor and manipulate Trk retrograde signaling. (*A*) Western blot analysis of PC12 cell extracts treated with 20 ng/mL EGF (*Center*) or 20 ng/mL qEGF (*Right*) or left untreated (*Left*). Cell lysates were probed with an anti–phospho-Erk1/2 antibody. (*B*) Mass cultures of superior cervical ganglia (SCG) neurons were infected with an adenovirus containing ETrkB, the chimeric EGF-binding TrkB receptor, and treated with qEGF for 60 min. Cells were fixed and stained with an antibody against the extracellular domain of EGFR. ETrkB (anti-EGFR, green) and qEGF (red) were visualized. (Scale bar, 2 μm.) (*C*) Mass cultures of SCG neurons were infected with an adenovirus containing either ETrkB (*Upper*) or EGFR-GFP (*Lower*) alone (*Left*) or together with PincherG68E (three *Right* panels), treated with EGF for 15 min, fixed and stained. ETrkB (anti-EGFR, green, *Upper*), EGFR (GFP, green, *Lower*), and PincherG68E (anti-Pincher, red) were visualized. (Scale bar, 2 μm.)

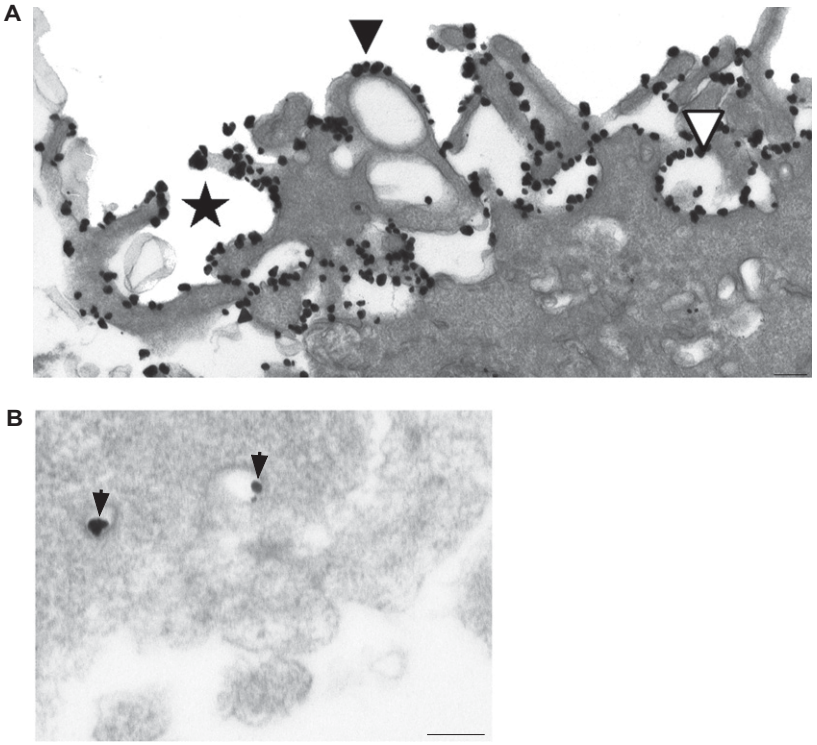

Fig. S2. The qEGF bound to ETrkB or EGFR is internalized in distinct endosomes. Hippocampal neurons were infected with an adenovirus containing ETrkB (*A*) or EGFR (*B*) for 48 h, starved, treated with qEGF for 20 min, fixed, and processed for electron microscopy (EM) with Quantum dot silver enhancement (*Materials and Methods*). (*A*) The qEGF accumulated in membrane ruffles (star), endocytosing ruffles (arrowhead), and internalized structures (open arrowhead). (Scale bar, 200 nm.) (*B*) The qEGF is seen in clathrin-coated vesicles (arrows), as evidenced by their small size and the appearance of a thick clathrin coat. (Scale bar, 100 nm.)

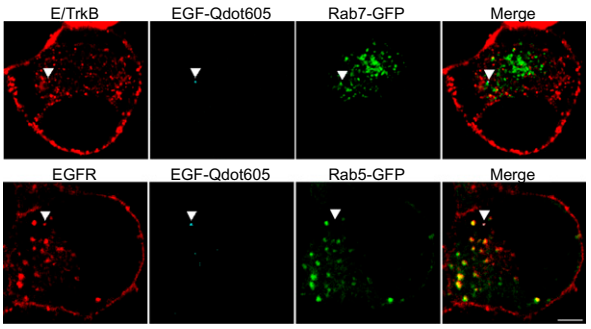

Fig. S3. Retrogradely transported ETrkB and EGFR endosomes are differentially processed by Rab5 and Rab7. Chamber-cultured SCG neurons were infected with either ETrkB together with Rab7-GFP (*Upper*) or EGFR together with Rab5-GFP (*Lower*) adenoviruses. After 2 d, the distal axons were treated with qEGF for 2 h. ETrkB (anti-EGFR, red, *Upper*), EGFR (anti-EGFR, red, *Lower*), qEGF (cyan), Rab5-GFP (GFP, green, *Upper*), Rab7 (GFP, green, *Lower*). Arrowheads point to receptor/qEGF complexes. (Scale bar, 2 μm.)

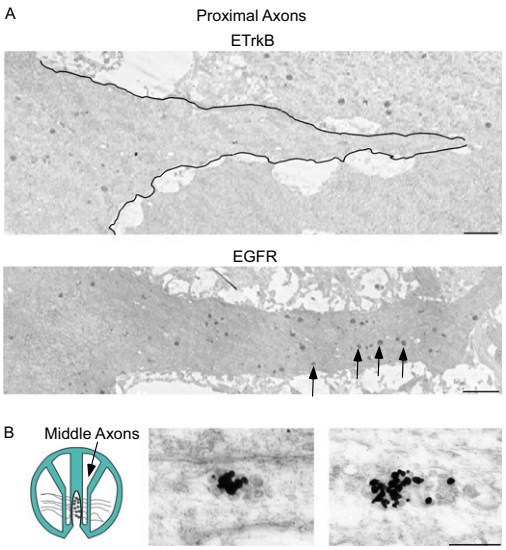

Fig. S4. Lysosomal processing of retrogradely transporting EGFR-multivesicular bodies (MVBs), but not ETrkB-MVBs, begins in axons. (*A*) SCGs grown in compartmentalized cultures were infected with an adenovirus expressing either ETrkB (*Upper*) or EGFR (*Lower*). After 2 d, cells were starved and the distal axons incubated with EGF for 2 h, fixed, and processed for EM as described in *Materials and Methods*. Proximal axons of EGFR- (*Lower*), but not ETrkB- (*Upper*) expressing neurons, show a high density of lysosomes (arrows). (*B*) SCGs grown in compartmentalized cultures with three compartments (cell body, middle axons, and distal axons; see schematic, *Left*) were infected with an EGFR-expressing adenovirus and treated with qEGF for 2 h. The middle axon compartment was then processed for EM with Quantum dot silver enhancement. (Scale bars: 2 μm in *A*, 200 nm in *B*.)

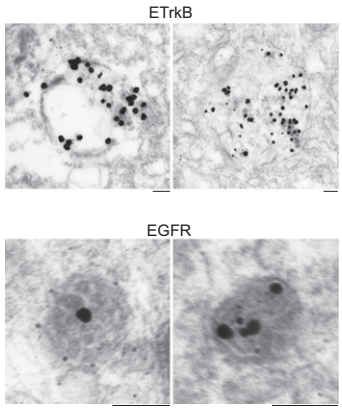

Fig. S5. ETrkB, but not EGFR, MVBs resist lysosomal processing. SCG neurons grown in compartmentalized cultures were infected with an adenovirus expressing either ETrkB (*Upper*) or EGFR (*Lower*). After 2 d, the distal axons were treated with EGF conjugated to fluoro-nanogold (EGF-nG) for 30 min at 4 °C, washed, and then incubated at 37 °C for 12 h, fixed, and processed for EM with gold enhancement as described in *Materials and Methods*. (Scale bars, 100 nm.)