Supplemental Materials

Molecular Biology of the Cell

Li et al.

Supplemental Figure legends

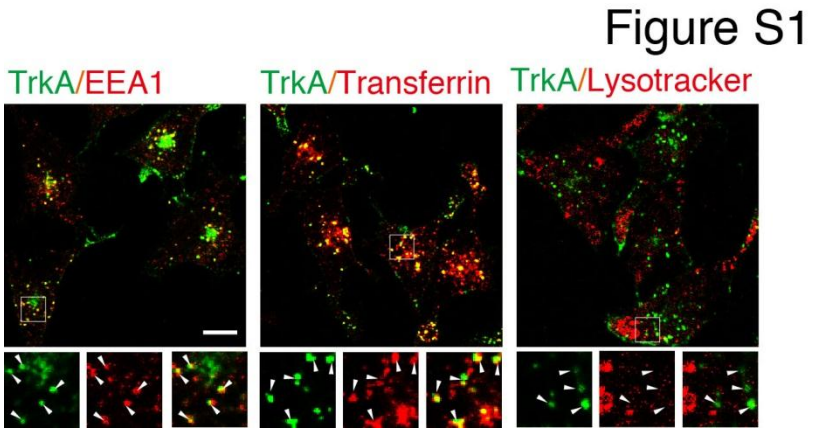

Figure S1. Comparison of TrkA distribution with endosomal and lysosomal markers.

Confocal microscopy images comparing the distribution of cell-surface-labeled TrkA receptors (5C3 antibodies) internalized for 15 min in PC12 (615) cells with the endosomal markers, endogenous EEA1, Alexa-594 conjugated transferrin (internalized for 15 min), and the lysosomal marker lysotracker. The insets show regions of higher magnification. Arrowheads indicate TrkA-labeled vesicles. Scale bar, 10 µm.

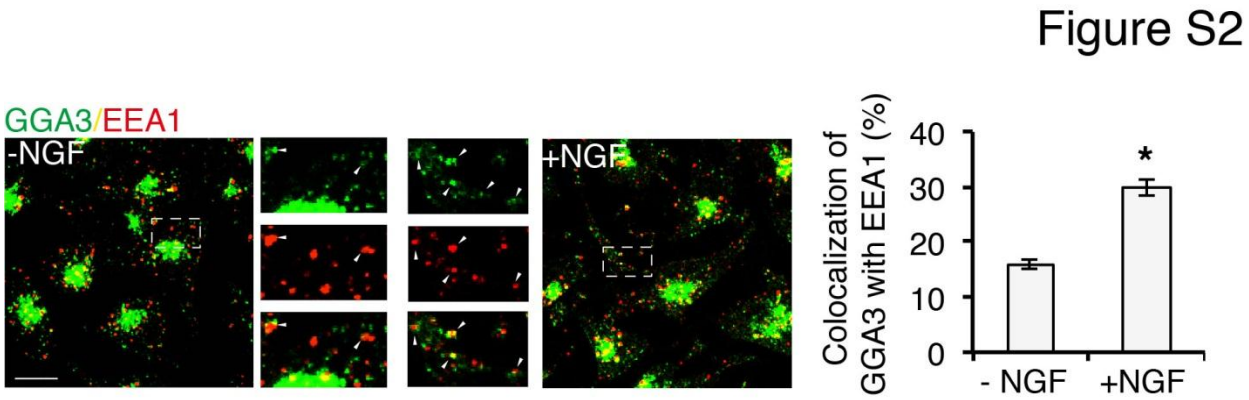

Figure S2. GGA3 recruitment on early endosomes in response to NGF.

Confocal microscopy images comparing the distributions of endogenous GGA3 and EEA1 in PC12 (615) cells incubated with or without 10 ng/ml NGF for 15 min. Insets show regions of higher magnification. Arrowheads indicate co-localization. Scale bar, 10 µm. The histogram depicts the

quantification of the degree of overlap between GGA3 and EEA1 from 3 independent experiments. Student's *t* test, *$p<0.05$.

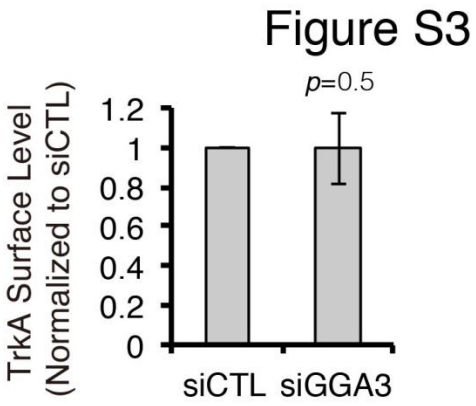

Figure S3. Comparison of cell-surface TrkA levels in unstimulated control and GGA3-depleted PC12(615) cells. Cell-surface TrkA in unstimulated cells (no NGF) was detected using the biotinylation assay described in Figure 2A and biotinylated TrkA was quantified densitometrically from 10 independent experiments and was normalized to siRNA control cells (siCTL). Student's *t* test, *$p<0.05$.

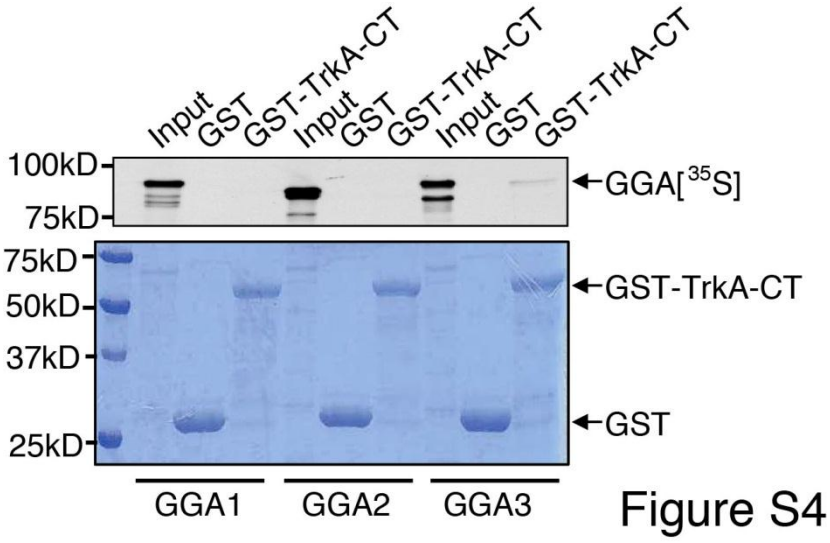

Figure S4. TrkA cytoplasmic tail interacts directly with GGA3 but not with GGA1 and GGA2.

In vitro translated, ^{35}S-labeled GGA1, GGA2 and GGA3 were incubated with GST or GST-TrkA. Bound proteins were resolved by SDS–PAGE and detected by autoradiography. 8% input is shown. The lower panel is Coomassie staining showing GST-tagged proteins.

Figure S5

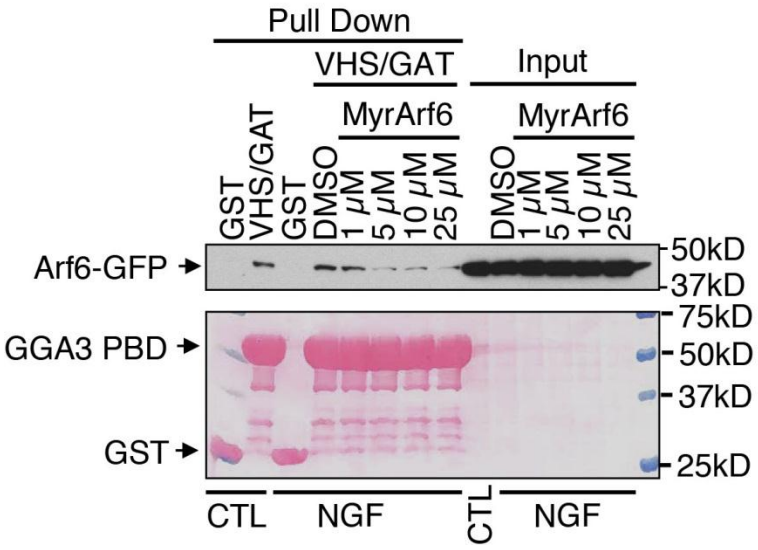

Figure S5. Concentration-dependent inhibitory effect of the MyrArf6 peptide on Arf6 activation. Lysates from PC12(615) cells expressing Arf6-GFP and stimulated with NGF in the presence of vehicle (DMSO) or 1, 5, 10 or 25 μM Myr-Arf6 peptide were incubated with GST or GST- GGA3 PBD, which specifically interacts with the active (GTP-bound) form of Arf6. Bound proteins were then analyzed by western blotting with anti-GFP.

Figure S6

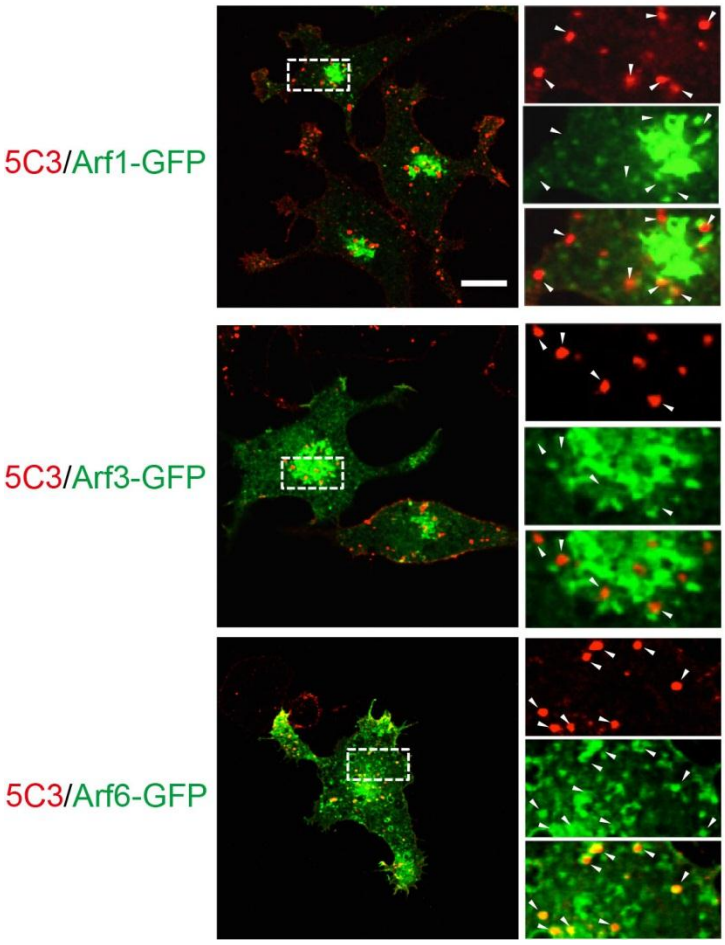

Figure S6. Internalized TrkA co-localizes specifically with Arf6.

Confocal microscopy images comparing the distribution of internalized TrkA with Arf1, Arf3 and Arf6. PC12 (615) cells transfected with GFP tagged Arf1, Arf3 and Arf6 were surface labeled with 5C3 and then driven for internalization for 15 min. Insets show regions of higher magnification. Arrowheads indicate co-localization. Scale bar, 10 μm.

Figure S7

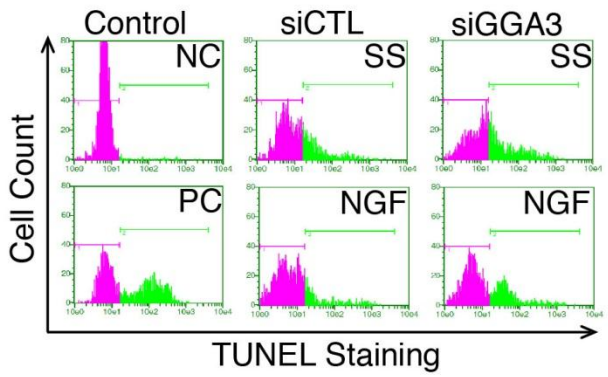

Figure S7. Flow cytometry analysis of apoptosis in control and GGA3 depleted PC12(615) cells. PC12(615) cells untreated or treated with control siRNA or GGA3 siRNA were serum starved (SS) or stimulated with 10 ng/ml NGF for 24 h. Apoptotic cells were assessed by detection of DNA fragmentation using the Guava® TUNEL kit Assay and analyzed using by the Guava EasyCyte flow cytometer. Green region in the graphs indicates the apoptotic cells and pink regions represent non-apoptotic cells. Negative control (NC) and positive control (PC) (supplied by Guava technologies) were used to verify the reagent performance and set the analysis markers delineating the negative and positive apoptotic cell populations.

Figure S8

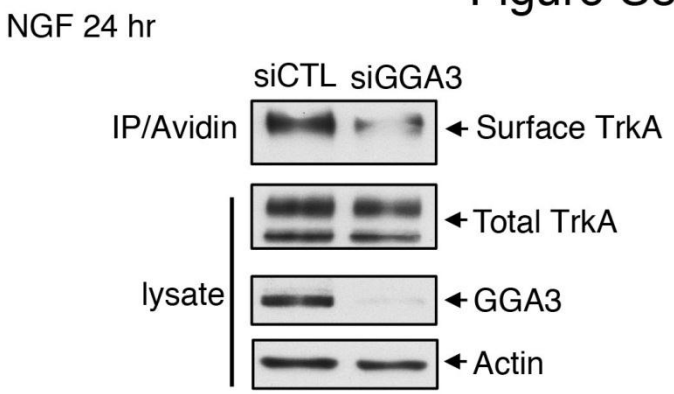

Figure S8. Comparison of cell-surface TrkA levels in control and GGA3-depleted PC12 cells following prolonged NGF stimulation. Control and GGA3-depleted PC12(615) cells were stimulated with 10 ng/ml NGF for 24 h at 37℃and then surface biotinylated at 4℃to label cell-surface proteins.

Biotinylated proteins were collected with avidin and immunoblotted with TrkA antibodies. Total TrkA receptors were detected by immunoblotting total cell lysates.

Figure S9

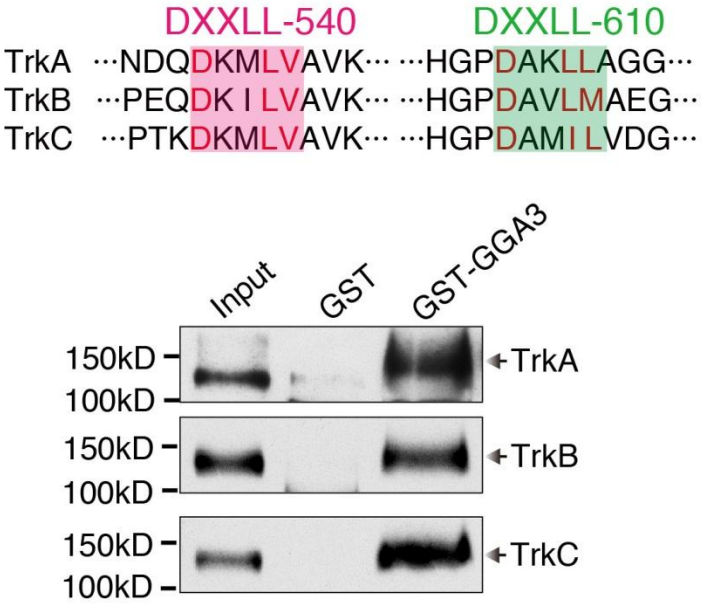

Figure S9. GGA3 binds to TrkA, TrkB and TrkC

(A) Presence of the two putative DXXLL motifs (DXXLL-540 and DXXLL-610) in TrkA, TrkB and TrkC cytoplasmic tails.

(B) GST pull-down experiment demonstrating that GGA3 interacts with TrkA, TrkB and TrkC. HEK293T cells transfected with wild type TrkA, TrkB and TrkC were incubated with GST alone or GST-GGA3. Bound proteins were resolved by SDS–PAGE and detected by WB.