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Supplemental Information

Combined kinesin-1 and kinesin-3 activity drives axonal trafficking of TrkB receptors in Rab6 carriers

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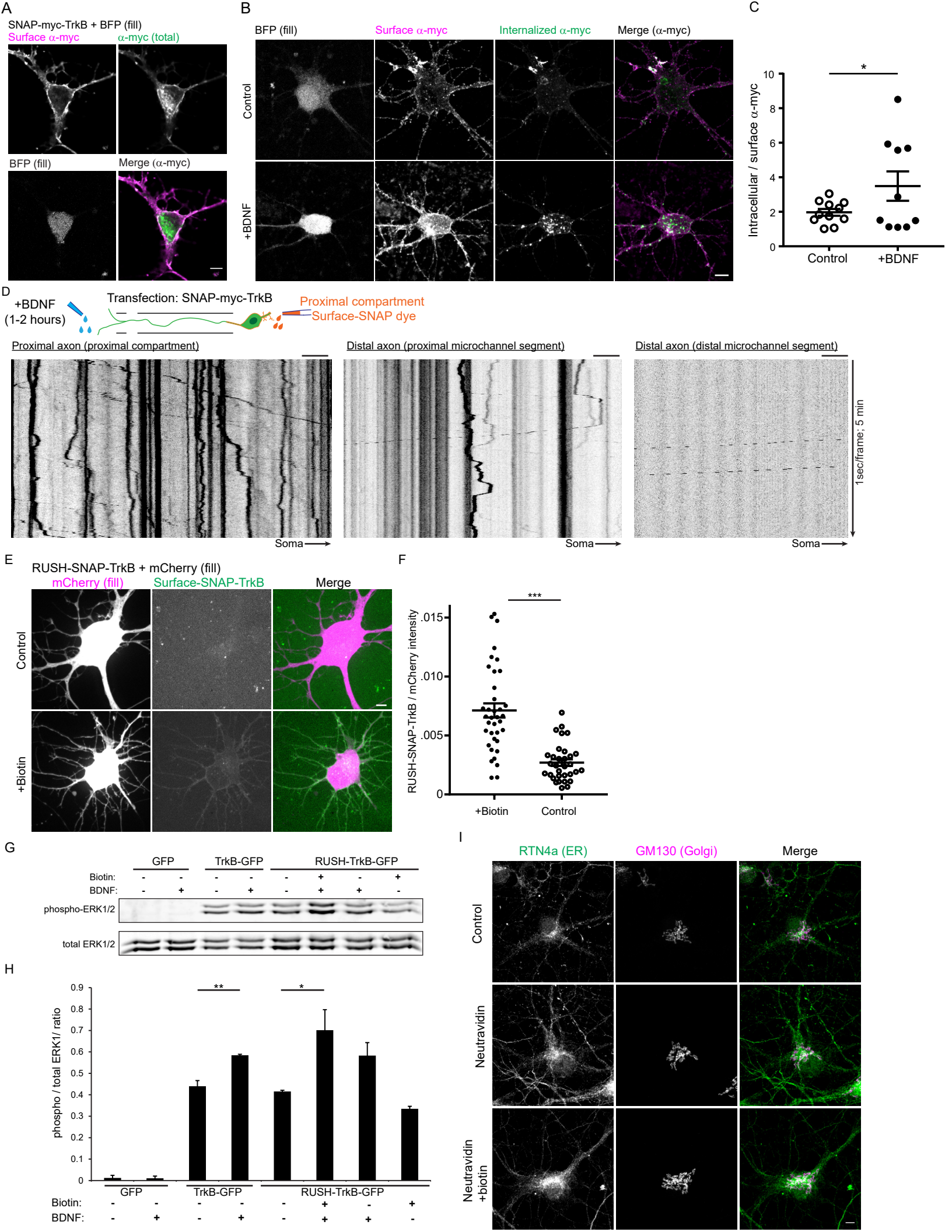

A
SNAP-myc-TrkB + BFP (fill)
Surface α-myc
α-myc (total)
BFP (fill)
Merge (α-myc)
B
BFP (fill)
Surface α-myc
Internalized α-myc
Merge (α-myc)
Control
+BDNF
C
Intracellular / surface α-myc
Control
+BDNF
D
+BDNF
(1-2 hours)
Transfection: SNAP-myc-TrkB
Proximal compartment
Surface-SNAP dye
Proximal axon (proximal compartment)
Distal axon (proximal microchannel segment)
Distal axon (distal microchannel segment)
Soma
1sec/frame; 5 min
E
RUSH-SNAP-TrkB + mCherry (fill)
mCherry (fill)
Surface-SNAP-TrkB
Merge
Control
+Biotin
F
RUSH-SNAP-TrkB / mCherry intensity
+Biotin
Control
G
GFP
TrkB-GFP
RUSH-TrkB-GFP
Biotin:
BDNF:
phospho-ERK1/2
total ERK1/2
H
phospho / total ERK1/ ratio
GFP
TrkB-GFP
RUSH-TrkB-GFP
Biotin:
BDNF:
I
RTN4a (ER)
GM130 (Golgi)
Merge
Control
Neutravidin
Neutravidin +biotin

Figure S1: Additional controls for SNAP-TrkB and RUSH-TrkB expression and trafficking dynamics, Related to Figure 1.

(A&B) Neurons were transfected with SNAP-myc-TrkB and cytosolic tagBFP2. (A) After 24 hours, neurons were surface labeled with anti-myc then fixed and permeabilized for whole-cell myc staining (B) To measure internalization, neurons were pulsed labeled with surface myc antibody then incubated with BDNF or control media for 30 mins followed by fixation and incubation secondary antibodies before and after permeabilization to capture surface and internalized SNAP-myc-TrkB, respectively. Images are confocal slices based on 2 independent experiments. **(C)** Ratio of internalized to surface SNAP-myc-TrkB was measured from cells stained as in (B). Data are based on 11 & 10 cells based on 2 independent experiments. *=Student's t-test value p-value<0.05. **(D)** Neurons were plated in microfluidic chambers to assay proximal to distal transcytosis of SNAP-TrkB as presented in Figure 1F&I. Surface labeling of SNAP-TrkB was carried in the proximal compartment and BDNF was added to the distal compartment for 1-2 hours prior to live imaging of axons crossing from the proximal to distal compartments. Segments of the proximal axon and along the axon were captured at 1sec/ frame for 5 minutes. Representative kymographs depict anterograde (white arrowheads) and retrograde (black arrowheads) transport of SNAP-TrkB carriers. Images are based on 3 independent experiments. **(E&F)** Neurons were co-transfected with RUSH-SNAP-TrkB and mCherry (fill) and pre-treated with biotin or control media for 2.5 hours before surface-SNAP labeling. Neurons were promptly imaged to probe the amount of RUSH-SNAP-TrkB in the plasma membrane. (E) Representative max-projection images of soma z-stack. (F) Mean SNAP-TrkB intensity in the outline of max-projection image were measured and normalized to cytosolic mCherry signal. Plot lines depict are based on 2 independent experiments on 37 & 32 neurons in control and +biotin conditions, respectively. ***= Student's t-test p-value <0.001. **(G)** HEK cells expressing TrkB-GFP, RUSH-TrkB-GFP and GFP only as negative control were treated with biotin for 120 mins, followed by 30 min incubation with BDNF then lysed and processed for WB analysis of ERK1/2 activation using antibodies against phosphorylated ERK1 (pThr202) & ERK2 (pTyr204) and total ERK1/2. (**H)** Ratios of phosphoERK1/2 to total ERK1/2 bands from 3 independent experiments. * & ** = Student's T-test p<0.05 & p<0.01, respectively. **(I)** Neurons were treated with either control media or Neutravidin for 48 hours, then with or without biotin for 45 mins and immunolabeled against RTN4a (ER) and GM130 (Golgi). Images are based of 3 independent experiments. All scale bars=5 µm. Data in graphs are mean± S.E.M.

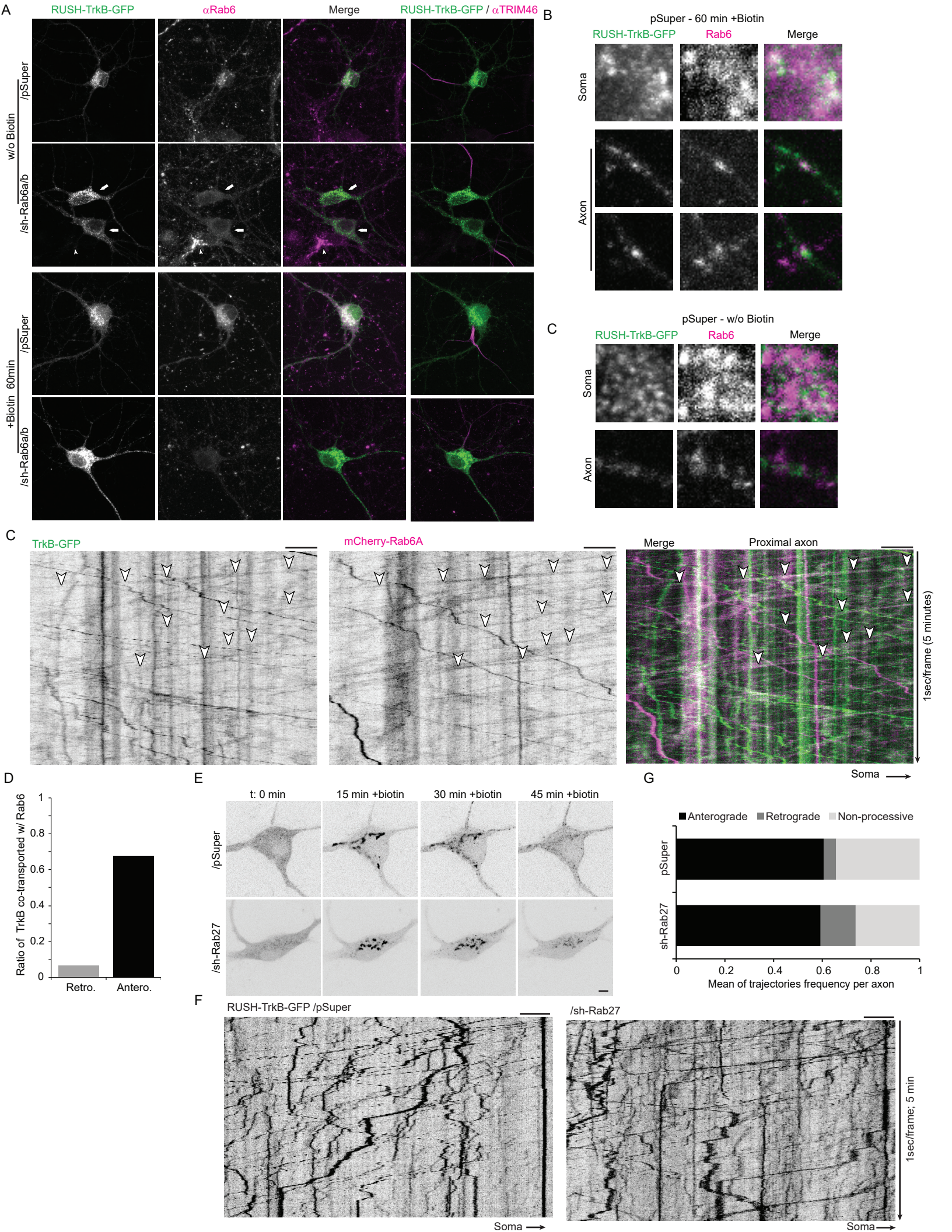

A
RUSH-TrkB-GFP
αRab6
Merge
RUSH-TrkB-GFP / αTRIM46
w/o Biotin
/pSuper
/sh-Rab6a/b
+Biotin 60min
/pSuper
/sh-Rab6a/b
B
pSuper - 60 min +Biotin
RUSH-TrkB-GFP
Rab6
Merge
Soma
Axon
C
pSuper - w/o Biotin
RUSH-TrkB-GFP
Rab6
Merge
Soma
Axon
C
TrkB-GFP
mCherry-Rab6A
Merge
Proximal axon
1sec/frame (5 minutes)
Soma
D
Ratio of TrkB co-transported w/ Rab6
1
0.8
0.6
0.4
0.2
0
Retro.
Antero.
E
t: 0 min
15 min +biotin
30 min +biotin
45 min +biotin
/pSuper
/sh-Rab27
F
RUSH-TrkB-GFP /pSuper
/sh-Rab27
1sec/frame; 5 min
Soma
Soma
G
Anterograde
Retrograde
Non-processive
pSuper
sh-Rab27
0
0.2
0.4
0.6
0.8
1
Mean of trajectories frequency per axon

Figure S2: Additional validation experiments on axon transported TrkB interaction with Rab6 and Rab27, Related to Figure 5.

(A) Neurons were co-transfected with RUSH-TrkB-GFP and shRNA against Rab6a & Rab6b or pSuper control vector and treated with control or biotin supplemented media for 60 minutes followed by fixation and immunostained for Rab6 and TRIM46 to visualize the axon initial segment (AIS). Neurons expressing the shRab6a/b show diminished Rab6 staining are marked by arrows. Arrowhead marks a neighboring cell not expressing GFP. Scale bar= 5µm **(B&C)** Zoom-in images of soma and axon areas showing co-localization between Rab6 and secreted RUSH-TrkB in the soma peri-nuclear region and in puncta along the axon in biotin and untreated neurons expressing RUSH-TrkB-GFP and pSuper control. Scale bar= 2µm. **(D)** Proximal axon segments of neurons co-expressing TrkB-GFP and mRFP-Rab6A were live-imaged for 5 minutes at 1 frame/sec. Arrowheads mark trajectories of co-transported TrkB and Rab6-positive carriers. **(D)** Co-localization ratio of TrkB+Rab6 to total TrkB trajectories analyzed in the anterograde and retrograde direction. Data is pooled from 109 retrograde and 131 anterograde trajectories, from 12 separate axons in 3 independent experiments. **(E-G)** Neurons were co-transfected with pSuper control or shRNA constructs against Rab27A & Rab27B and RUSH-TrkB-GFP. Live imaging was carried 2-3 days after transfection. **(E)** Soma was imaged in the course of 45 mins following the addition of biotin at 1 min/frame. **(F&G)** Proximal axons were imaged at a time window of 45-90 minutes after biotin addition at a frame rate of 1 sec/frame for 5 mins. Kymograph trajectories were manually traced and quantified to measure the ratio of anterograde, retrograde and non-processive carriers. Data presented are mean ratio per cell, based on 12 (pSuper) & 11 (shRab27) axons from 3 independent experiments.

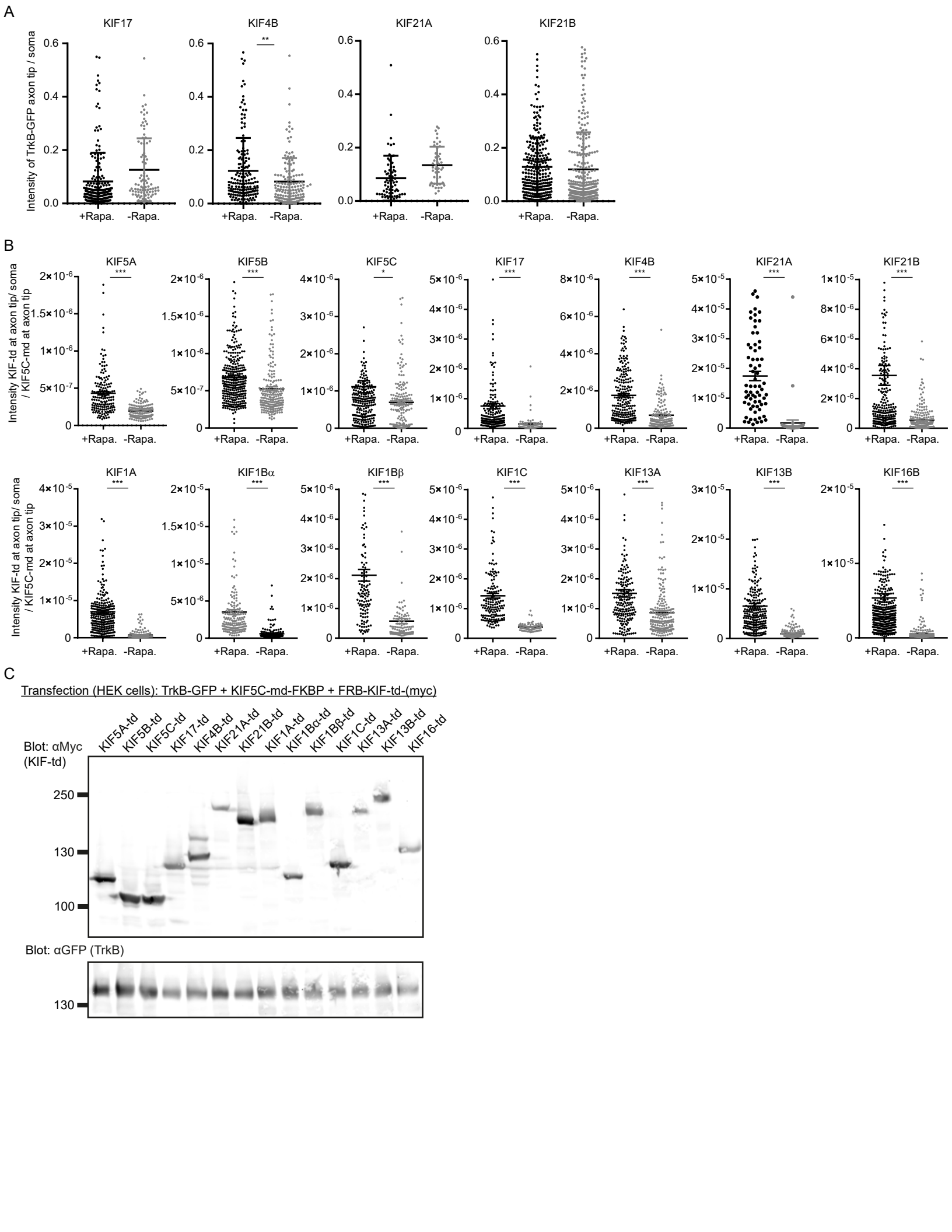
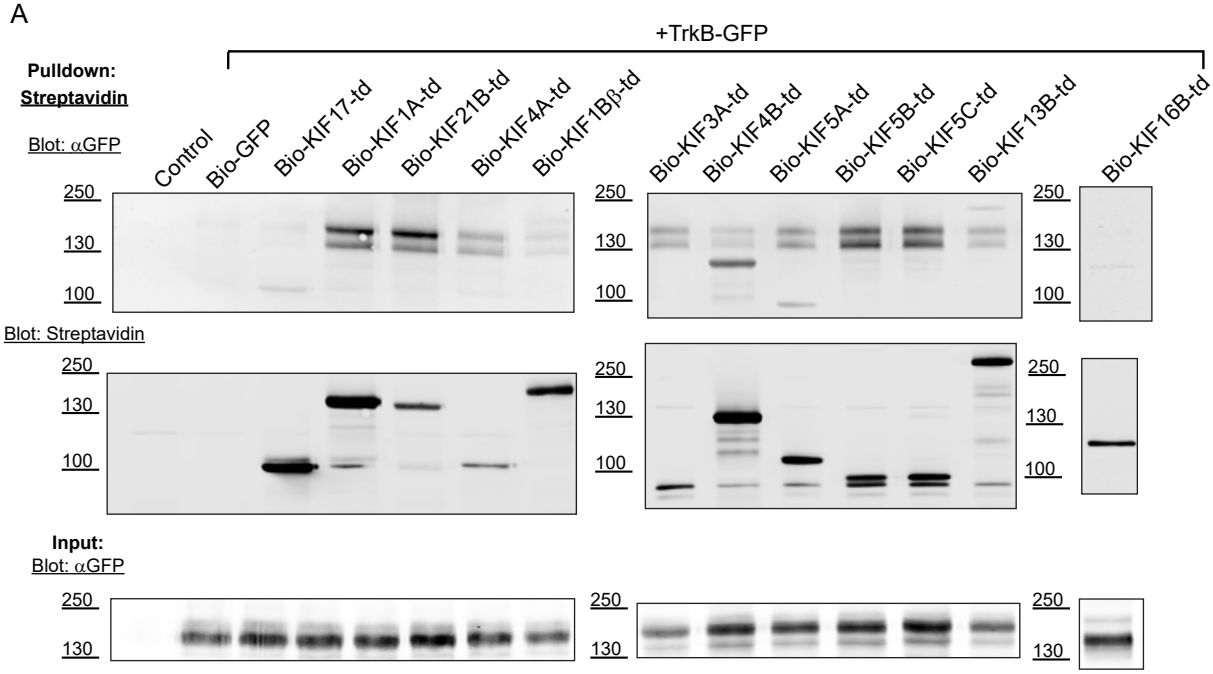

A
KIF17
KIF4B
KIF21A
KIF21B
Intensity of TrkB-GFP axon tip / soma
**
+Rapa.
-Rapa.
B
KIF5A
KIF5B
KIF5C
KIF17
KIF4B
KIF21A
KIF21B
KIF1A
KIF1Bα
KIF1Bβ
KIF1C
KIF13A
KIF13B
KIF16B
Intensity KIF-td at axon tip/ soma / KIF5C-md at axon tip
***
*
C
Transfection (HEK cells): TrkB-GFP + KIF5C-md-FKBP + FRB-KIF-td-(myc)
KIF5A-td
KIF5B-td
KIF5C-td
KIF17-td
KIF4B-td
KIF21A-td
KIF21B-td
KIF1A-td
KIF1Bα-td
KIF1Bβ-td
KIF1C-td
KIF13A-td
KIF13B-td
KIF16-td
Blot: αMyc (KIF-td)
250
130
100
Blot: αGFP (TrkB)
130

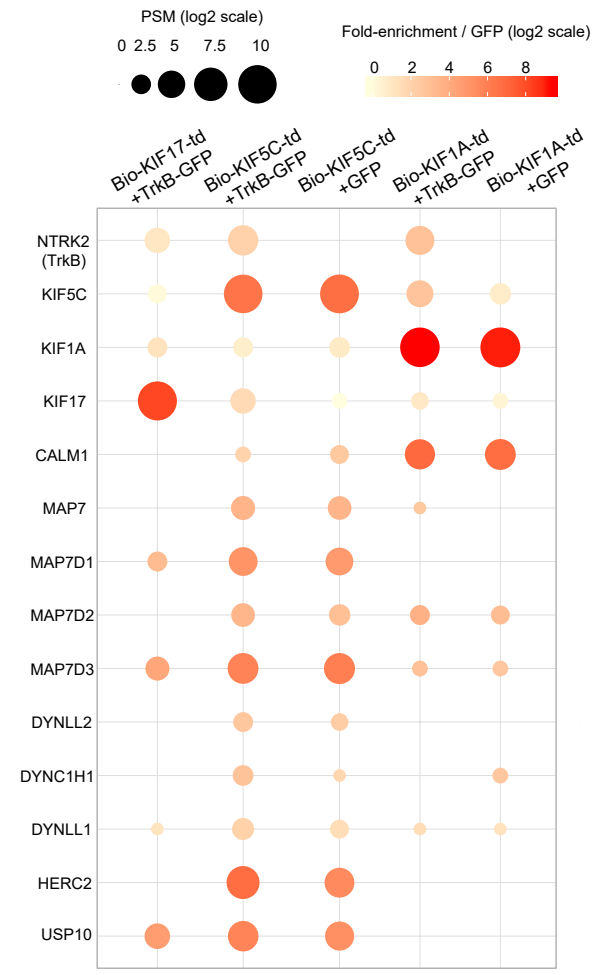
Figure S3: Additional control data of Split Kinesin Assay, Related to Figure 6.

Split kinesin assay was carried in neurons as described in Figure 4B **(A)** TrkB-GFP axon tip enrichment in neurons tested for its interaction with tail domains of Kinesin-2 & Kinesin-4 families. Scatter plot of TrkB-GFP mean intensity in individual axon tips normalized to the mean intensity in its corresponding soma. Lines depict mean and S.D. ** = Two-sided Student's t-test, $p<0.01$. Data points were collected from the following number of axon tip: KIF17: 197 & 97, KIF4B: 161 & 139, KIF21A: 67 & 46, KIF21B: 267 & 276 for +Rapa & -Rapa conditions, respectively. **(B)** Validation of kinesins tail-domain recruitment to axon tips in split kinesin assays presented in (A) and in Figure 4E-F. Axon tips and their respective soma were imaged and intensity of KIF-td and KIF5C-md was measured. KIF-td signal at the axon tip was divided by KIF-td signal in the soma and KIF5C-md signal in the tip to calculate the relative enrichment of KIF-td at the tip in rapalog vs. control conditions. Lines depict mean and S.D. * & *** = Two-sided Student's t-test, $p<0.05$ & $p<0.001$. Data points were collected from the following number of axon tip: KIF5A:160 & 147, KIF5B: 323& 219, KIF5C: 241 & 217, KIF17: 199 & 70, KIF4B: 228 & 140, KIF21A: 68 & 46, KIF21B: 267 & 263, KIF1A: 277 & 213, KIF1Ba: 173 & 168, KIF1Bb: 120& 111, KIF1C: 160 & 86, KIF13A: 189 & 231, KIF13B: 237 & 230, KIF16B: 389 & 297 for +Rapalog & -Rapalog conditions, respectively. **(C)** HEK cells were transfected with SKA constructs, lysed and analysed by SDS-PAGE and western-blot against myc and GFP-tags to compare relative KIF- td and TrkB-GFP respective expression between different KIF-td constructs. Blot shown is representative of 4 independent experiments.

A



B



C

Gene name / PD condition	Bio-GFP +TrkB-GFP	PSM Bio-Kif17-td +TrkB-GFP	Bio-Kif5c-td +BirA +TrkB-GFP	Bio-Kif5c-td +BirA +GFP	Bio-Kif1a-td +BirA +TrkB-GFP	Bio-Kif1a-td +BirA +GFP
NTRK2 (TrkB)	12	20	72	0	49	0
KIF5C	7	5	1064	1157	28	8
KIF1A	3	6	6	7	1515	1719
KIF17	4	1262	19	3	4	3
CALM1	0	1	3	5	68	83
MAP7	0	0	14	13	2	1
MAP7D1	0	6	46	35	1	0
MAP7D2	0	0	13	8	6	5
MAP7D3	0	14	81	87	3	3
HERC2	0	0	157	62	0	0
USP10	0	20	75	50	0	0

D

Gene name / PD condition	Bio-Kif16-td +TrkB-GFP	Bio-Kif5c-td +BirA +TrkB-GFP	Bio-Kif5c-td +BirA +GFP	Bio-Kif1a-td +BirA +TrkB-GFP	Bio-Kif1a-td +BirA +GFP
NTRK2 (TrkB)	52	0	36	0	48
KIF5C	1	1	652	551	35
KIF1A	0	0	1	1	1467
KIF16B	1695	1898	5	20	27
CALM1	1	1	1	2	123
MAP7	0	0	7	5	4
MAP7D1	2	1	29	28	2
MAP7D2	1	1	4	2	9
MAP7D3	2	2	55	60	5
HERC2	0	0	108	67	0
USP10	16	2	103	97	0

Figure S4: Validation of Kinesin-TrkB interaction, Related to Figure 6.

(A) HEK cells co-expressing TrkB-GFP, BirA and specified Bio-tagged kinesin-tail domain constructs (Bio-KIF) or TrkB-GFP, BirA and bio-GFP as negative control were lysed and processed for a Streptavidin based pulldown. The pulldown elution and total lysate fraction were probed with an antibody against GFP to detect TrkB-GFP and with Streptavidin to detect the specific biotinylated kinesin-tail domain. **(B)** Pull down eluates of HEK cells expressing the constructs specified in the x-axis were collected as described in (A). Samples were processed for MS proteomic analysis. The bubble plot represents the protein abundance (PSM), depicted as bubble size and enrichment over TrkB-GFP +GFP (negative control condition) depicted as bubble color. **(C & D)** of PSM values of selected proteins detected in Bio-Kif-td pulldown (PD) mass-spectrometry analyses #1 & #2.