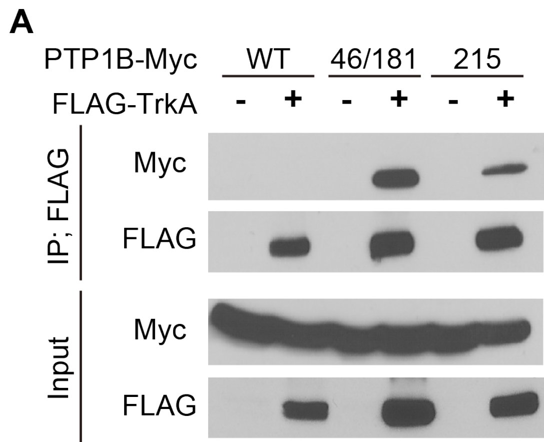
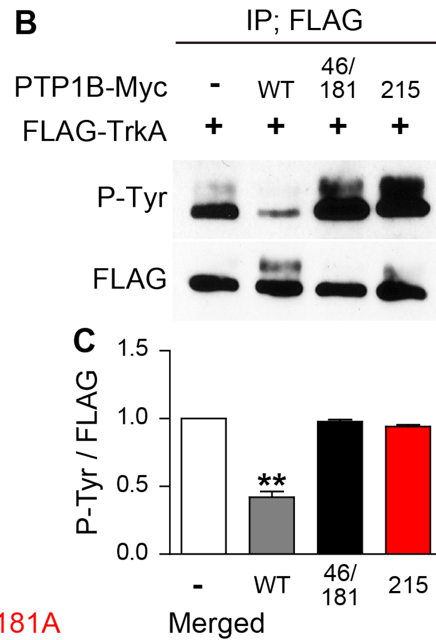

A
PTP1B-Myc
WT
46/181
215
FLAG-TrkA
- + - + - +
IP; FLAG
Myc
FLAG
Input
Myc
FLAG


B
IP; FLAG
PTP1B-Myc
-
WT
46/181
215
FLAG-TrkA
+ + + +
P-Tyr
FLAG
C
P-Tyr / FLAG
1.5
1.0
0.5
0.0
**
-
WT
46/181
215

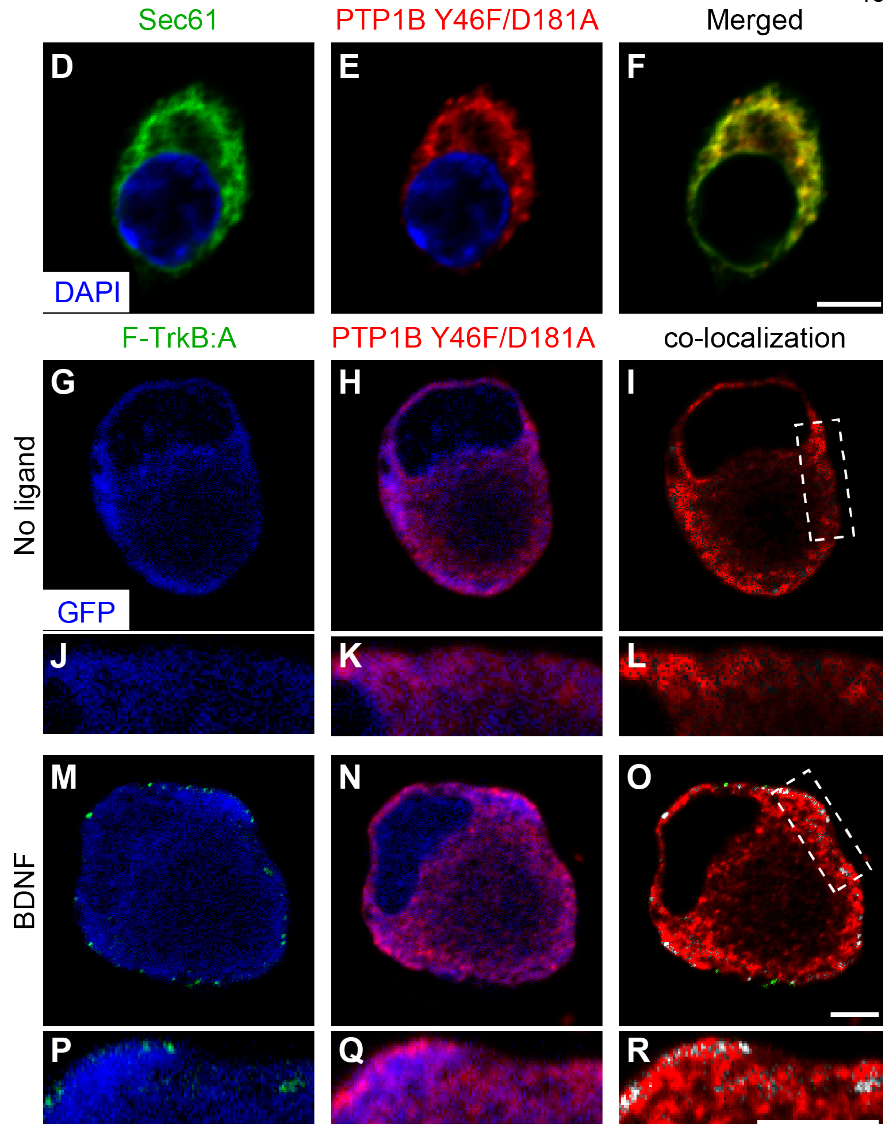
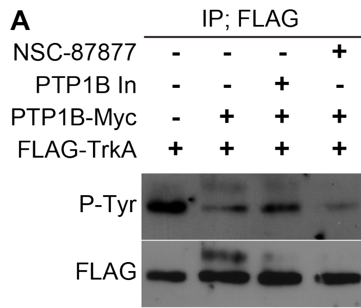
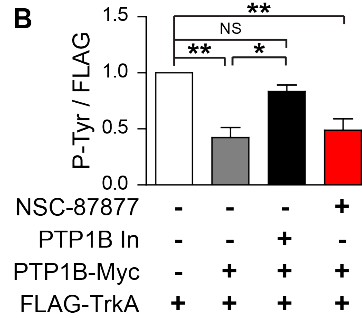
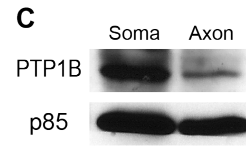

Sec61
PTP1B Y46F/D181A
Merged
D
E
F
DAPI
F-TrkB:A
PTP1B Y46F/D181A
co-localization
G
H
I
No ligand
GFP
J
K
L
M
N
O
BDNF
P
Q
R

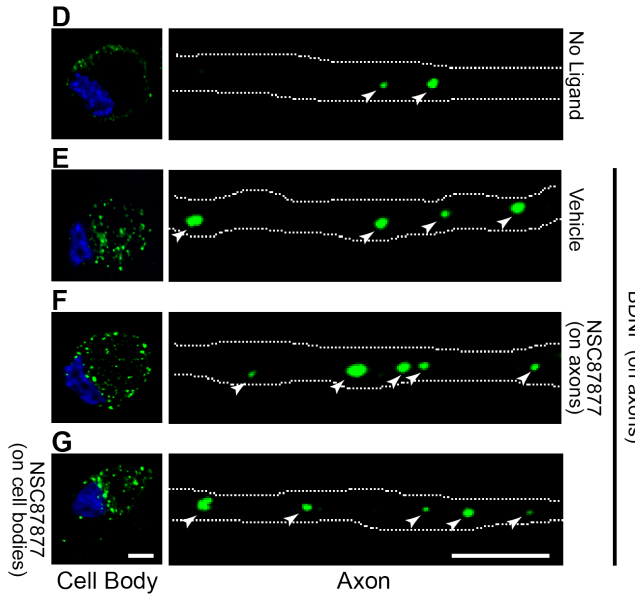
Figure S1. PTP1B interacts with, and dephosphorylates TrkA, in cell bodies, Related to Figure 3

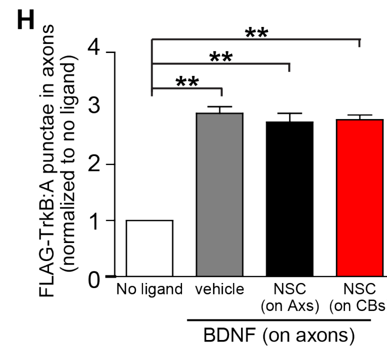
(A) Co-immunoprecipitation of FLAG-TrkA with Myc-tagged $PTP1B^{Y46F/D181A}$ and $PTP1B^{C215S}$ substrate trapping mutants, but not $PTP1B^{WT}$, in HEK293T cells. (B) FLAG-TrkA tyrosine phosphorylation is suppressed by $PTP1B^{WT}$, but not $PTP1B^{Y46F/D181A}$ and $PTP1B^{C215S}$ mutants. HEK293T cell lysates were subjected to FLAG immunoprecipitation, and immunoblotting with an anti-pY20 phosphotyrosine antibody. (C) Densitometric quantification of P-TrkA levels normalized to FLAG. $^{**}p<0.01$ compared to all other conditions, one-way ANOVA and Tukey-Kramer post-hoc test. Results are mean ± SEM from 3 independent experiments. (D-F) Sub-cellular localization of mCherry-$PTP1B^{Y46F/D181A}$ and GFP-Sec61 in cultured sympathetic neurons. (G-R) Co-localization of soma surface-derived FLAG-TrkB:A and mCherry-$PTP1B^{Y46F/D181A}$ PTP1B, even after stripping of surface-bound FLAG antibodies. Representative images are shown in (G-R). Magnified images of boxed areas in (I, O) are shown in lower panels (J-L, P-R). Co-localization of FLAG-TrkB:A and PTP1B is shown in white, using Image J co-localization highlighter (I, L, O, R). FLAG-TrkB:A infected neurons are identified by GFP co-expression. Scale bars, 5 µm.









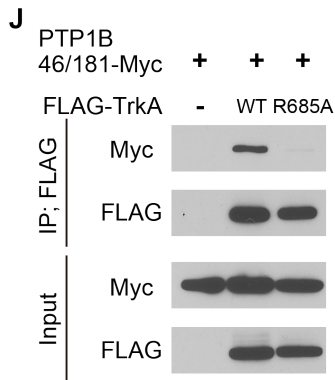


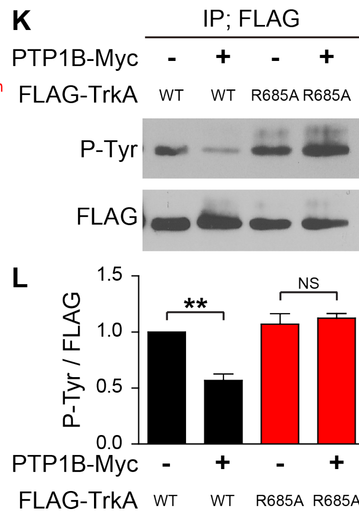
I

rat TrkA WT 678-IYSTDYYRVGGRTMLPIRWMPPE-700

PTP1B recognition motif +
Trk receptor kinase activation domain

rat TrkA R685A 678-IYSTDYYAVGGRTMLPIRWMPPE-700





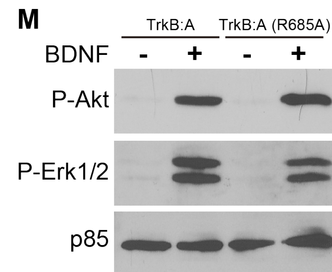
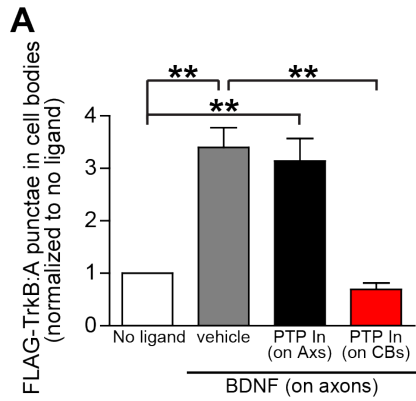
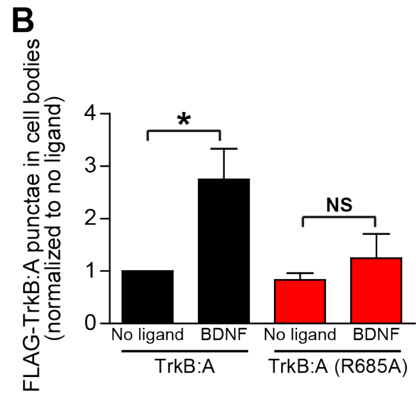


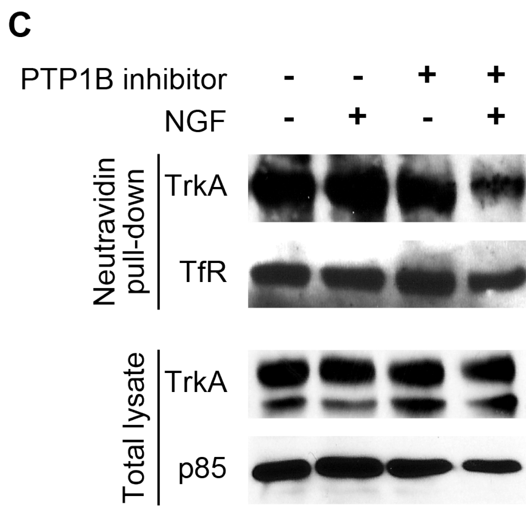
Figure S2. Specificity of PTP1B activity in regulating soma-to-axon transcytosis of Trk receptors, Related to Figure 4

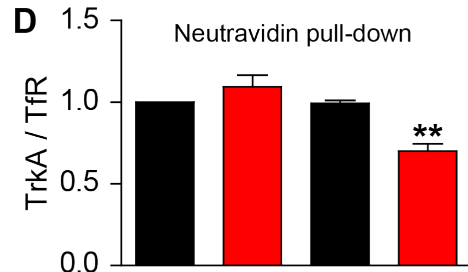
(A) PTP1B-induced dephosphorylation of FLAG-TrkA receptors is attenuated by the PTP1B inhibitor (compound II, 1 μM), but not by the SHP tyrosine phosphatase inhibitor (NSC-87877, 10 μM). HEK293T cell lysates were subjected to FLAG immunoprecipitation and immunoblotting with an anti-pY20 phosphotyrosine antibody. (B) Densitometric quantification of P-TrkA levels normalized to FLAG. **$p<0.01$ compared to all other conditions, two-way ANOVA and Tukey-Kramer post-hoc test. Results are mean ± SEM from 4 independent experiments. (C) PTP1B is expressed both in soma and axons. Cultured sympathetic neurons were grown in compartmentalized chambers, lysates prepared from cell body and axon compartments and immunoblotted with anti-PTP1B, followed by stripping and reprobing for p85 for normalization. (D-G) The SHP inhibitor, NSC-87877, added locally either to cell body or axon compartments, has no effect on ligand-induced transcytosis of soma surface-derived FLAG-TrkB:A receptors. Arrowheads indicate soma surface-derived FLAG-labeled receptors in axons. Nuclei were stained by DAPI (blue). Scale bars, 5 μm. (H) Quantification of FLAG-Trk punctae in axons. **$p<0.01$ relative to “no ligand” condition, two-way ANOVA and Tukey-Kramer post-hoc test. Results are means ± SEM from 3 independent experiments. 20 axons were counted per experiment. (I) Generation of TrkA^{R685A} mutant receptors by point mutation of the PTP1B recognition motif “DYYR” located in the TrkA kinase activation domain to “DYYA”. (J) FLAG-TrkA^{WT}, but not FLAG-TrkA^{R685A}, receptors interact with Myc-tagged PTP1B$^{Y46F/D181A}$ substrate trapping mutant in HEK293T cells. HEK293T cell lysates were subjected to FLAG immunoprecipitation and immunoblotting

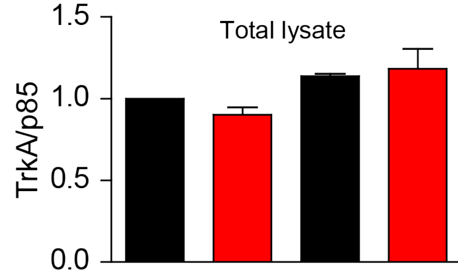
with either anti-Myc or anti-FLAG antibodies. (K) Tyrosine phosphorylation of FLAG-TrkA^{WT}, but not TrkA^{R685A}, receptors is suppressed by PTP1B^{WT}. The R685A mutation does not interfere with TrkA kinase activity since mutant TrkA^{R685A} receptors show tyrosine phosphorylation upon over-expression. HEK293T cell lysates were subjected to FLAG immunoprecipitation, and immunoblotting with an anti-pY20 phosphotyrosine antibody. (L) Densitometric quantification of P-TrkA levels normalized to FLAG. **$p<0.01$, two-way ANOVA and Tukey-Kramer post-hoc test. Results are mean ± SEM from 3 independent experiments. (M) FLAG-TrkB:A^{R685A} receptors are able to mediate BDNF-induced Akt and Erk1/2 phosphorylation similar to control FLAG-TrkB:A receptors in cultured sympathetic neurons. Representative immunoblotting images are shown from 2 independent experiments.

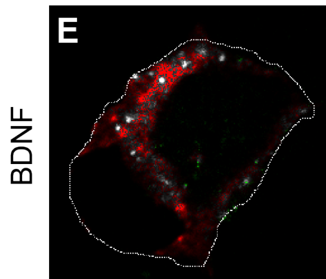

A
FLAG-TrkB:A punctae in cell bodies (normalized to no ligand)
**
**
**
No ligand
vehicle
PTP In (on Axs)
PTP In (on CBs)
BDNF (on axons)

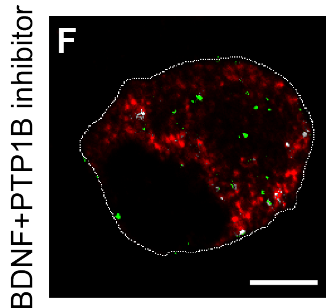

B
FLAG-TrkB:A punctae in cell bodies (normalized to no ligand)
*
NS
No ligand
BDNF
No ligand
BDNF
TrkB:A
TrkB:A (R685A)


C
PTP1B inhibitor
NGF
Neutravidin pull-down
TrkA
TfR
Total lysate
TrkA
p85


D
Neutravidin pull-down
TrkA / TfR
**


Total lysate
TrkA/p85
PTP1B inhibitor
NGF


FLAG-TrkB:A / Rab11
Co-localization
E
BDNF


F
BDNF+PTP1B inhibitor

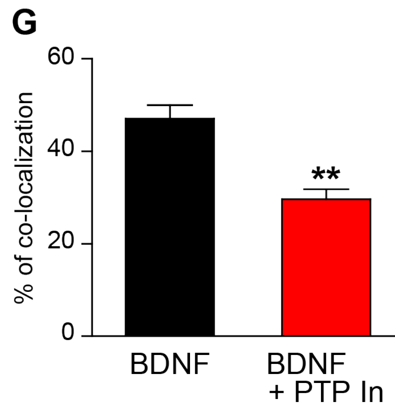
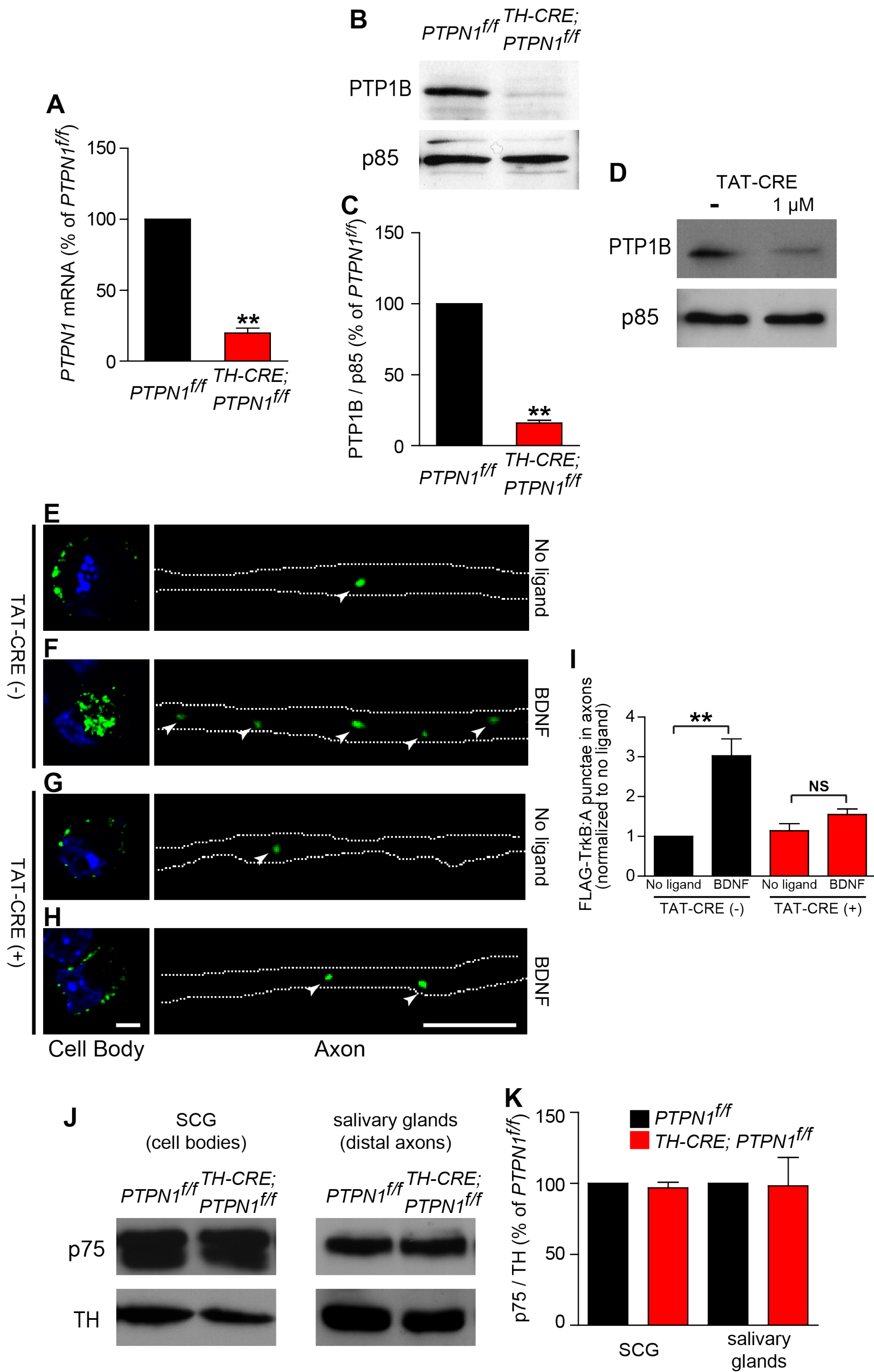

G
% of co-localization
**
BDNF
BDNF + PTP In

Figure S3. PTP1B inhibition results in degradation of surface-derived Trk receptors, Related to Figure 5

(A) Cell body-specific inhibition of PTP1B prevents ligand-induced intracellular accumulation of soma surface-derived FLAG-Trk receptors. Cell body compartments were live-labeled with FLAG antibody and distal axons were stimulated with BDNF (100 ng/ml, 4 hr) in the presence of the PTP1B inhibitor added to cell body or axon compartments. Representative images are shown in Figures 4B-E. $^{**}p<0.01$ relative to "no ligand" condition, two-way ANOVA and Tukey-Kramer post-hoc test. Results are means ± SEM from 3 independent experiments. 15 cell bodies were counted per experiment. (B) Soma surface-derived mutant FLAG-TrkB:A^{R685A} receptors that are unable to undergo PTP1B-mediated dephosphorylation do not accumulate in cell bodies in response to ligand stimulation of axons. Representative images are shown in Figures 4G-J. $^{*}p<0.05$ relative to "no ligand of TrkB:A" condition, two-way ANOVA and Tukey-Kramer post-hoc test. Results are means ± SEM from 3 independent experiments. 15 cell bodies were counted per experiment. (C,D) Prolonged PTP1B inhibition promotes degradation of surface TrkA receptors. Surface proteins in mass cultures of sympathetic neurons were biotin-labeled, followed by NGF stimulation (100 ng/ml, 1.5 hr) in the presence or absence of the PTP1B inhibitor. Biotinylated proteins were precipitated by neutravidin and subjected to immunoblotting for TrkA, followed by stripping and reprobing for the transferrin receptor (TfR). Supernatants were immunoblotted for p85 to normalize for total protein amounts. (D) Normalized intensities of TrkA/TfR (neutravidin pull-downs) or TrkA/p85 (total lysates). $^{**}p<0.01$ relative to all other conditions by two-way ANOVA and Tukey-Kramer test. Results are means ± SEM from 3 independent

experiments. (E-G) Soma-specific inhibition of PTP1B decreases co-localization of soma surface-derived Trk receptors with Rab11 in BDNF-treated neurons. Co-localization of FLAG-TrkB:A and Rab11 is shown in white using Image J co-localization highlighter. Scale bar, 5 μm. (G) Quantification of co-localization between TrkB:A and Rab11. **$p<0.01$, *t*-test. Results are mean ± SEM from 3 independent experiments. Total 45 neurons were analyzed.



A
B
C
D
E
F
G
H
I
J
K
PTPN1 mRNA (% of PTPN1f/f)
PTPN1f/f
TH-CRE; PTPN1f/f
PTP1B
p85
PTP1B / p85 (% of PTPN1f/f)
TAT-CRE
1 µM
No ligand
BDNF
TAT-CRE (-)
TAT-CRE (+)
Cell Body
Axon
FLAG-TrkB:A punctae in axons (normalized to no ligand)
NS
SCG (cell bodies)
salivary glands (distal axons)
p75
TH
p75 / TH (% of PTPN1f/f)
SCG
salivary glands

Figure S4. Conditional deletion of PTP1B in sympathetic neurons, Related to Figure 6

(A, B) PTP1B deletion in P0.5 *TH-CRE; PTPN1*$^{f/f}$ SCG, determined by qPCR or western blot analyses. *PTPN1* mRNA levels were normalized to *GAPDH* expression. Representative western blots are shown in (B). (C) Densitometric quantification of PTP1B protein normalized to p85. **$p<0.01$, n=5 mice per genotype for (A) and 3 mice per genotype for (C). (D) PTP1B levels are attenuated by delivery of TAT-CRE to *PTPN1*$^{f/f}$ sympathetic neuron cultures. (E-H) CRE-mediated PTP1B deletion decreases ligand-induced transcytosis of FLAG antibody-bound Trk receptors in compartmented cultures of *PTPN1*$^{f/f}$ sympathetic neurons. Arrowheads indicate soma surface-derived FLAG-labeled receptors in axons. Nuclei were stained by DAPI (blue). Scale bars, 5 μm. (I) Quantification of FLAG-Trk punctae in axons. **$p<0.01$ relative to "no ligand" of control neurons, two-way ANOVA and Tukey-Kramer post-hoc test. Results are means ± SEM from 3 independent experiments. 20 axons were counted per experiment. (J) Conditional *PTPN1* deletion in sympathetic neurons does not alter p75 protein levels in axon terminals innervating salivary glands or in cell bodies residing in superior cervical ganglia (SCG). Sympathetic ganglia and salivary glands from P0.5 *TH-CRE;PTPN1*$^{f/f}$ and control *PTPN1*$^{f/f}$ mice were harvested and immunoblotted for p75, followed by stripping and re-probing for Tyrosine Hydroxylase (TH). (K) Densitometric quantification of p75 levels normalized to TH. Results are means ± SEM from 5 mice per genotype.