SUPPLEMENTARY INFORMATION

Figure S1. Specificity of the anti-PTP1B antibody. Fibroblasts null for PTP1B were transfected with GFP (upper panels), GFP-PTP1B (middle panels), or co-transfected with GFP plus TC-PTP (bottom panels). Twenty four hours after transfection cells were fixed and processed for detection of anti-PTP1B. Note that anti-PTP1B detects the GFP-PTP1B wild type but not the TC-PTP. Bar, 25 μm.

Figure S2. Co-localization analysis of PTP1B and different vesicle and ER markers. Hippocampal neurons of stage 2-3 were fixed and processed for double immunofluorescence detection of N-cadherin/PTP1B and synapsin/PTP1B. The fluorescent labels were color-encoded in the panels. Note the lack of co-localization between PTP1B and both vesicle markers, N-cadherin and synapsin. Selected squares (yellow dashes) were enlarged regions In another set of experiments hippocampal neurons were transfected with GFP-SERCA, and immunolabeled for PTP1B. Selected squares (yellow dashes) near the cell margin were enlarged for better visualization. Note the PTP1B punctate (red dots) over the ER tubules displayed by GFP-SERCA. Bars, 5 μm.

Figure S3. PTP1B and GFP-PTP1B co-align with tyrosinated microtubules. Upper panels: Hippocampal neurons at stage 2-3 were fixed and processed for double immunofluorescence to detect tyrosinated microtubules (Tyr-MTs, green) and PTP1B (red). Note the co-alignment of PTP1B punctate with microtubules extending to the periphery of the growth cone (arrows). Lower panels: Hippocampal cells in suspension

were electroporated with GFP-PTP1B and then plated. At stage 2-3 were fixed and labeled for tyrosinated microtubules. Note the co-alignment of GFP-PTP1B extensions and tyrosinated microtubules projecting to the periphery (arrows). A few microtubules which have no GFP-PTP1B associated are also marked (arrowheads). Bar, 5 μm.

Figure S4. PTP1B function in axonal length. Hippocampal neurons electroporated with GFP-PTP1B or GFP-PTP1B-CS were plated and developed until reaching stage 3. Then, cells were fixed and processed for immunofluorescence detection of tau-1 (red label). Note that neurons expressing the GFP-PTP1B (WT) extend long axons that are indistinguishable from those in non transfected neurons (CO). In contrast, neurons expressing the GFP-PTP1B-CS (CS) display much shorter axons. Bar, 30 μm. The graphic below shows the quantifications (control, CO, n = 27; WT, n = 17; CS, n = 10). Error bars represent s.e.m. The asterisk indicates $p < 0.01$ (one-way Anova with Dunnett's post-test).

Figure S5. Dynamics of GFP-PTP1B in a growth cone in contact with a neurite. This figure is the companion of the movie-S2. Hippocampal neurons were co-electroporated with DsRed and GFP-PTP1B. Neurons were plated on a glass-bottom dish and grow until they polarize. The figure shows a cell aggregate at the left upper corner containing several neurons transfected and projecting processes to the right. One of these processes (only DsRed channel is shown) shows a lamellar growth cone projecting free to the substrata (white arrow) and a branch making contact with a neurite (yellow arrow). Bar, 10 μm.

Movie S1. Dynamics of GFP-PTP1B processes in a growth cone. Hippocampal neurons were co-transfected with GFP-PTP1B and DsRed. White arrows indicate GFP-PTP1B processes extending into transient filopodia-like structures. Not all growth cone protrusions are invaded by GFP-PTP1B, yellow arrows indicate such events. Images in the time-lapse were taken every 10 sec from a total time of 10 min. Movie runs at 6 fps; movie frame, 250 x 325 pixels.

Movie S2. Dynamics of GFP-PTP1B in a growth cone partly in contact with a neurite. This movie is a companion of the Figure S5. Hippocampal neurons were co-transfected with GFP-PTP1B and DsRed. The movie starts with a merge image that corresponds to the first frame of the series. Only the GFP-PTP1B fluorescence is shown here. Yellow arrow points the tip of a branch of the growth cone in contact with a neurite. White arrow points the lamellar growth cone interacting with the substrata. Note the GFP-PTP1B processes projecting to the peripheral zone of the growth cone at the lamellar contact-free region, and their subsequent retrograde translocation. The GFP-PTP1B at the branch in contact does not show significant variations. Images were taken every 20 sec from a total time of 13 min. Movie runs at 6 fps; movie frame, 250 x 252 pixels.

Figure S1

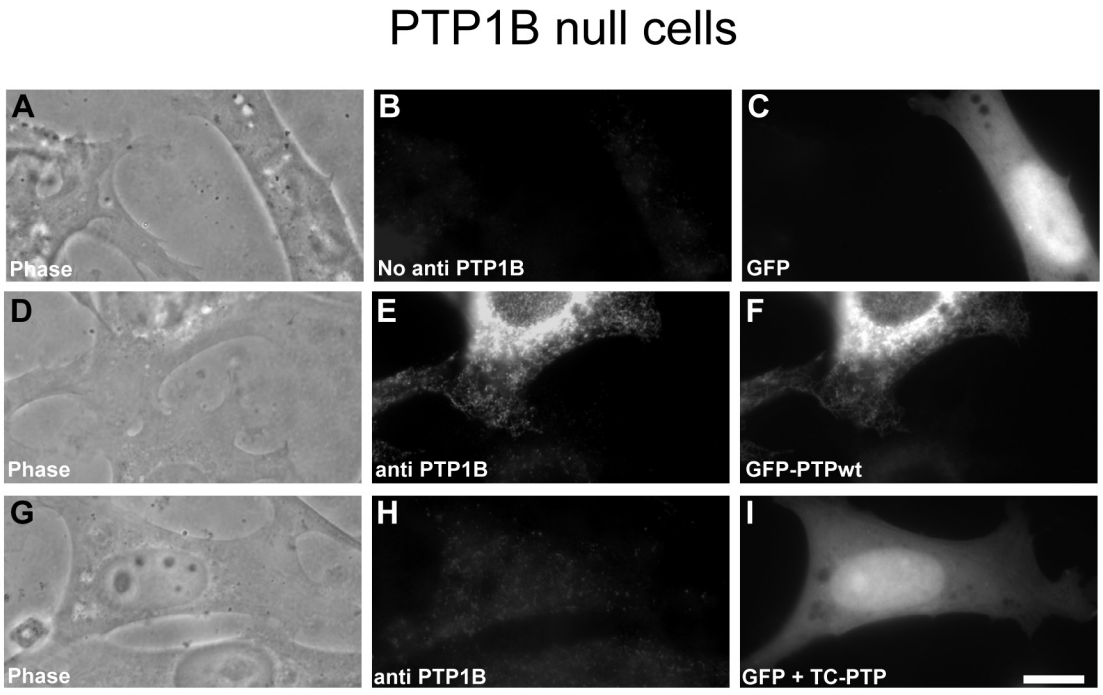

PTP1B null cells
A
Phase
B
No anti PTP1B
C
GFP
D
Phase
E
anti PTP1B
F
GFP-PTPwt
G
Phase
H
anti PTP1B
I
GFP + TC-PTP

Figure S2

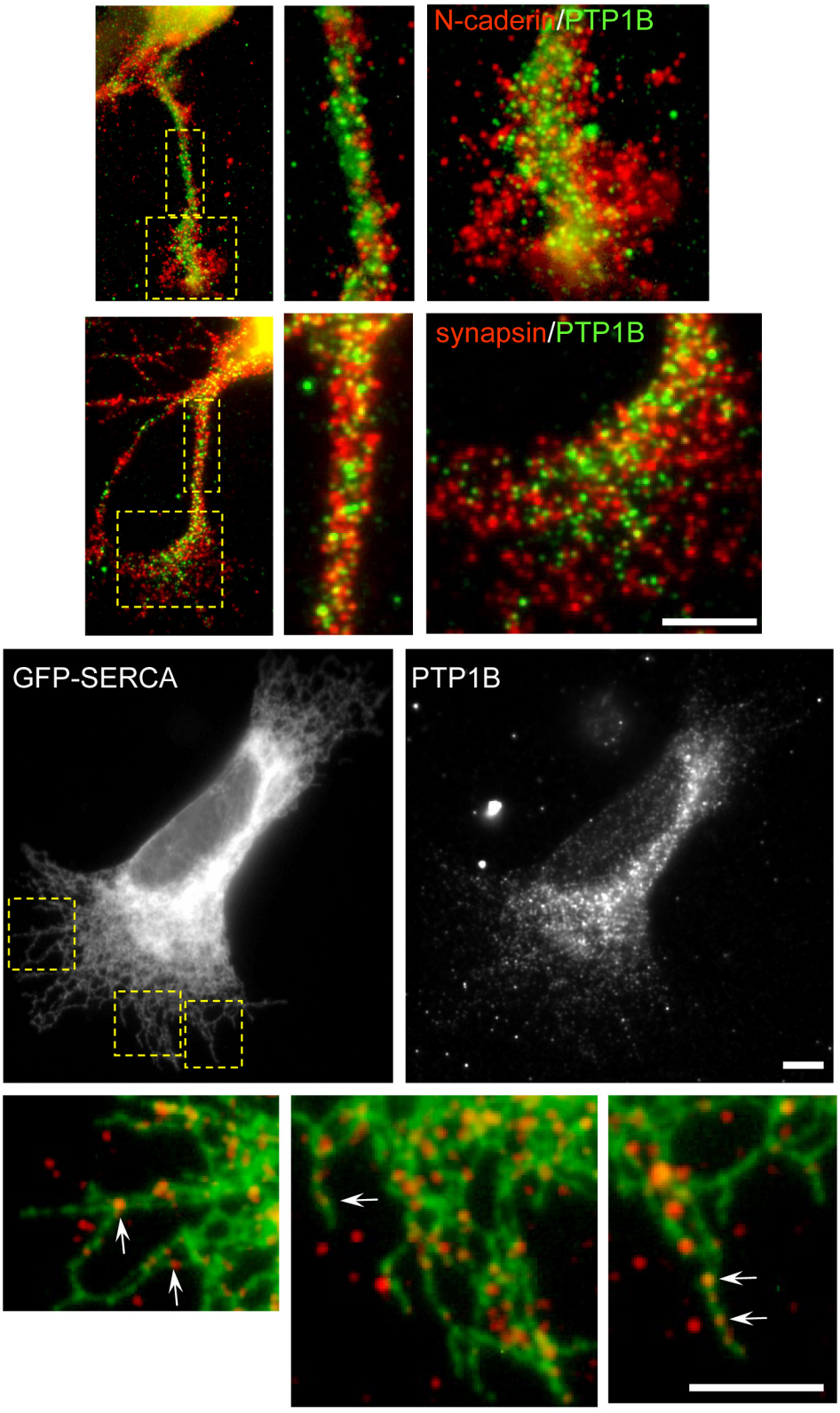


Figure S3

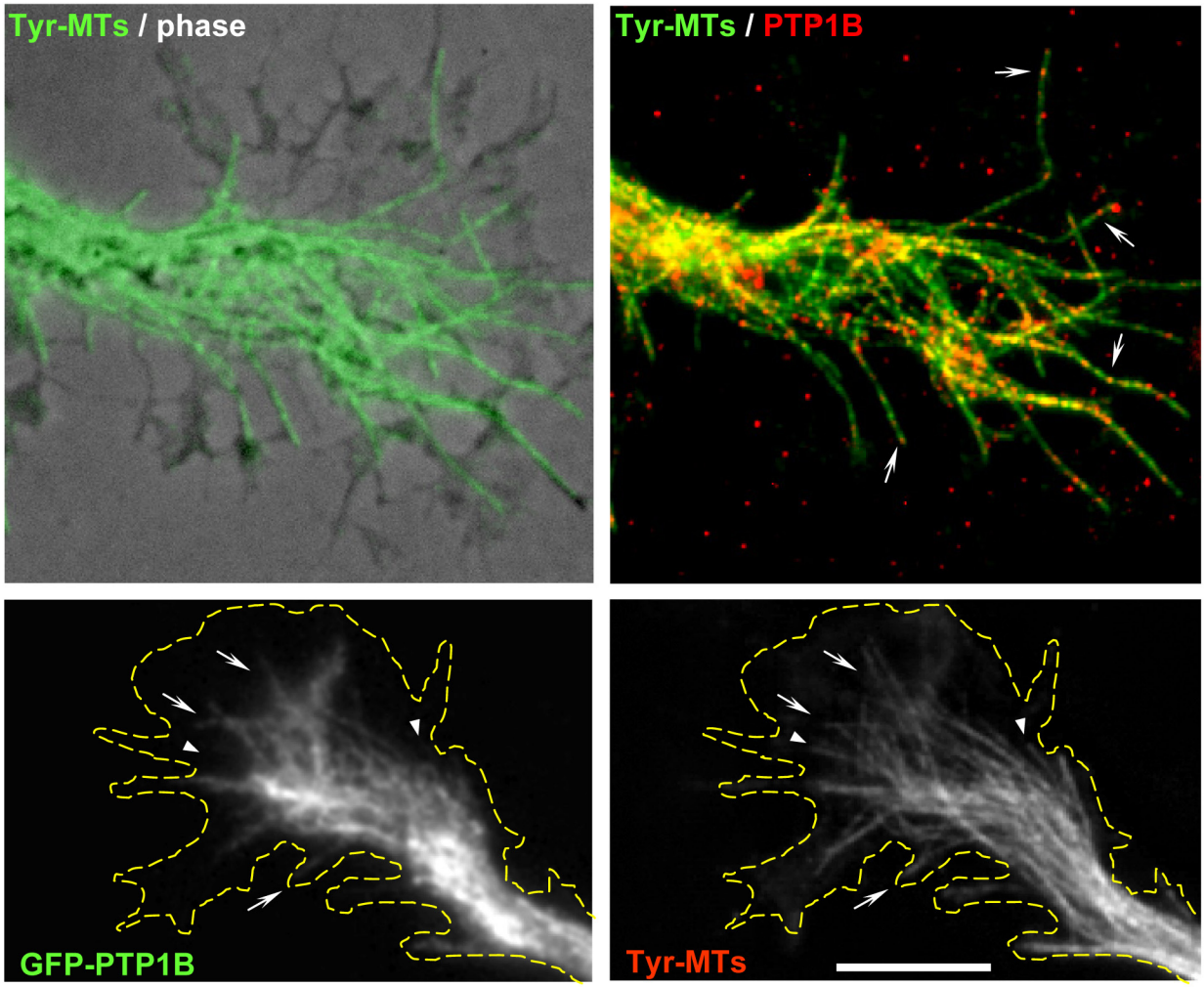
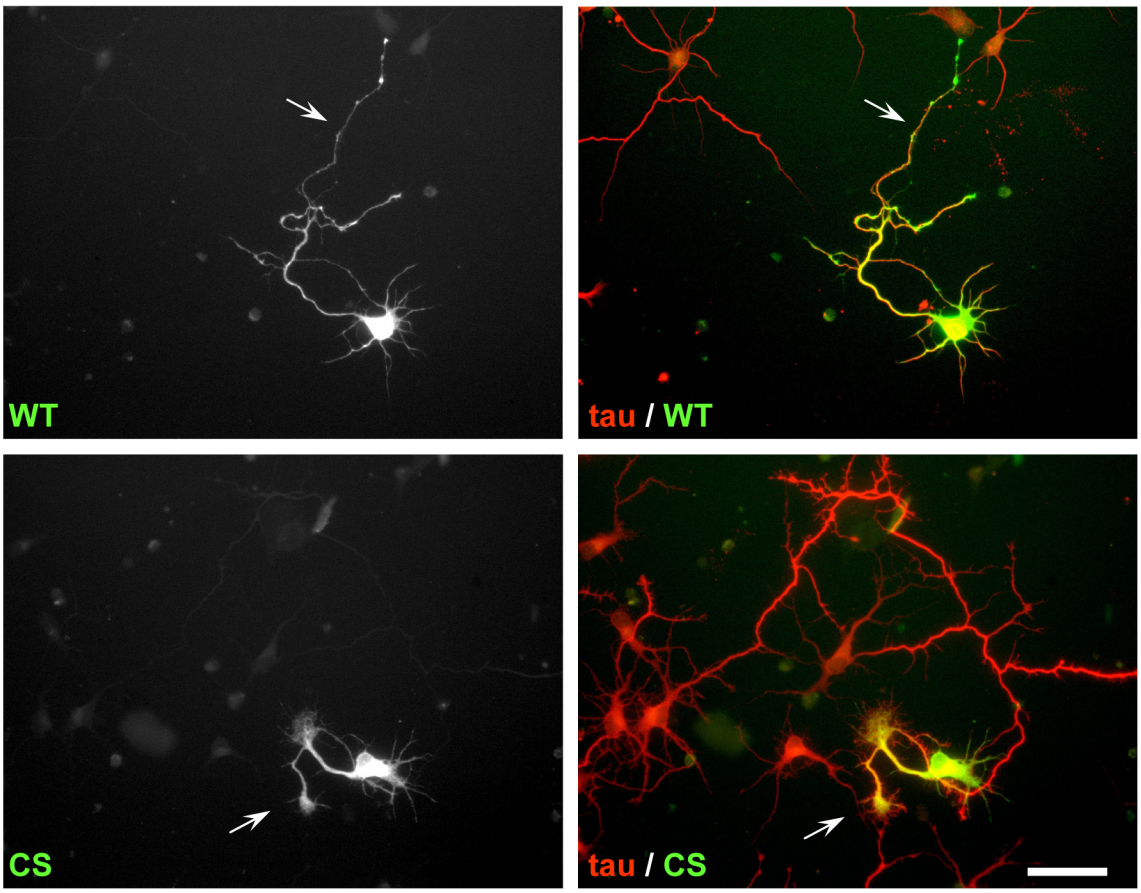


Figure S4



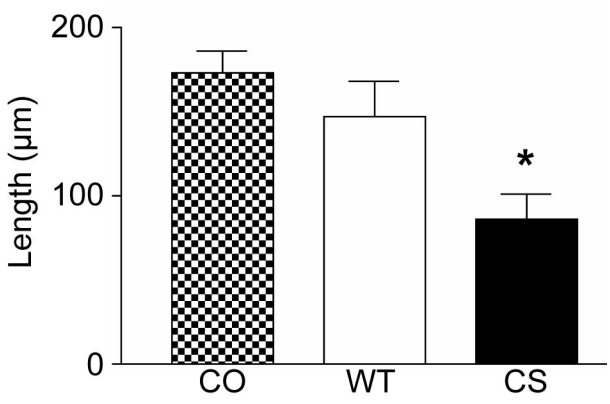


Figure S5