

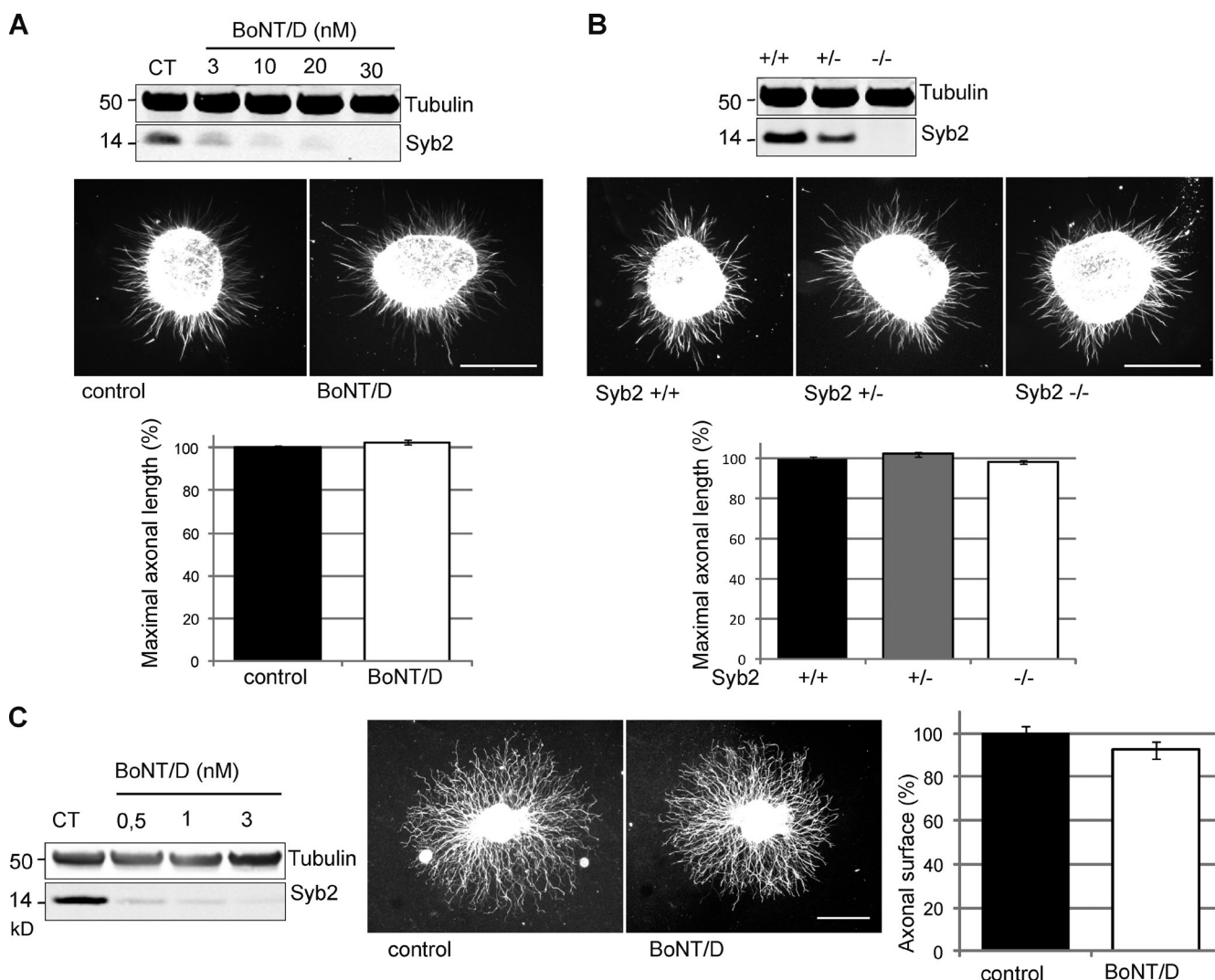
Zylbersztejn et al., <http://www.jcb.org/cgi/content/full/jcb.201106113/DC1>

Figure S1. Inactivation of Syb2 in E15 cortical or E13 DRG explants does not affect axonal growth. (A, top) E15 cortical explants were grown on poly-L-lysine and laminin-coated dishes with increasing concentrations of BoNT/D in the medium. After 15 h of treatment, tubulin and Syb2 were detected by Western blotting in cell lysates. (A, middle) Representative images of E15 cortical explants grown in plasma matrix for 24 h with or without 30 nM BoNT/D in the medium. (A, bottom) The quantification of maximal axonal length displayed as mean \pm SEM values (error bars) for each condition (control, $n = 89$; BoNT/D, $n = 85$ explants). (B, top) E15 cortex from Syb2^{+/+}, Syb2^{+/-}, and Syb2^{-/-} embryos were analyzed by Western blotting to demonstrate the complete loss of Syb2 in Syb2^{-/-} embryos. (B, middle) Representative images of E15 cortical explants from Syb2^{+/+}, Syb2^{+/-}, and Syb2^{-/-} embryos grown in plasma matrix. (B, bottom) The quantification of maximal axonal length displayed as mean \pm SEM values (error bars) for each genotype (Syb2^{+/+}, $n = 100$; Syb2^{+/-}, $n = 121$; Syb2^{-/-}, $n = 174$ explants). (C, left) Western blot analysis of Tubulin and Syb2 after cleavage of Syb2 with increasing concentrations of BoNT/D in the DRG explants. (C, middle) Representative images of DRGs grown in collagen gel with or without 3 nM BoNT/D toxin added to the culture medium. (C, right) Quantification of total axonal surface of DRG explants in control or 3 nM BoNT/D condition expressed as means \pm SEM (error bars; control, $n = 48$; BoNT/D, $n = 57$ explants). Bars, 200 μ m.

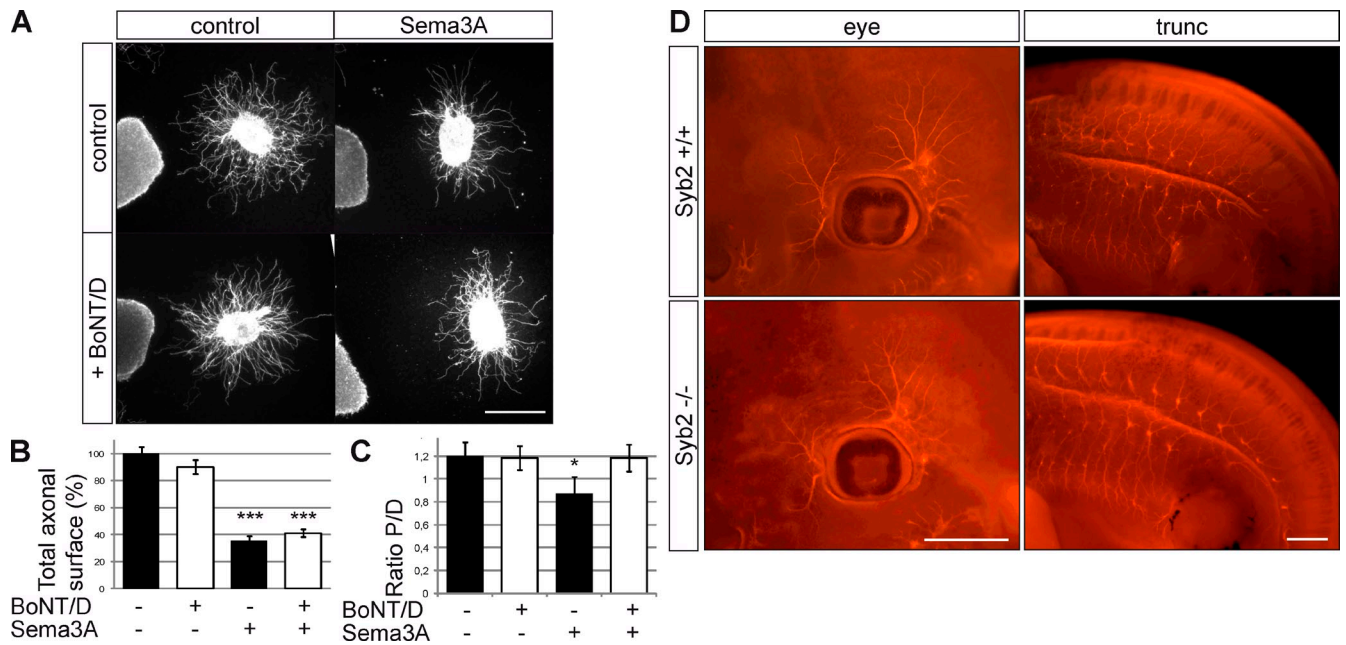


Figure S2. **Lack of peripheral nervous system defect in *Syb2*^{-/-} embryos.** (A) E13 DRG explants were grown in matrix supplemented with 100 ng/ml NGF in front of mock- or Sema3A-secreting cell aggregates with or without BoNT/D in the medium. Bar, 200 μ m. (B and C) In each condition, total axonal surface and the ratio of proximal over distal axonal areas was quantified (error bars indicate means \pm SEM; control, $n = 13$; control + BoNT/D, $n = 13$; Sema3A, $n = 16$; Sema3A + BoNT/D, $n = 18$ explants). Note that repulsion, but not growth, was inhibited by BoNT/D. Previous studies have revealed that Sema3A also competes with NGF for binding to TrkA and inhibits axonal outgrowth of DRG neurons. This result suggests that Syb1/2 are involved in Sema3A-mediated guidance, which is dependent on Nrp1/PlexA1 but not in Sema3A-induced TrkA-dependent axonal growth inhibition. *, $P < 0.05$; ***, $P < 0.0005$ by Student's t test. (D) E12 *Syb2*^{+/+} and *Syb2*^{-/-} embryos were stained in toto with neurofilament 2H3 antibody ($n = 3$ embryos each). Bars, 500 μ m.

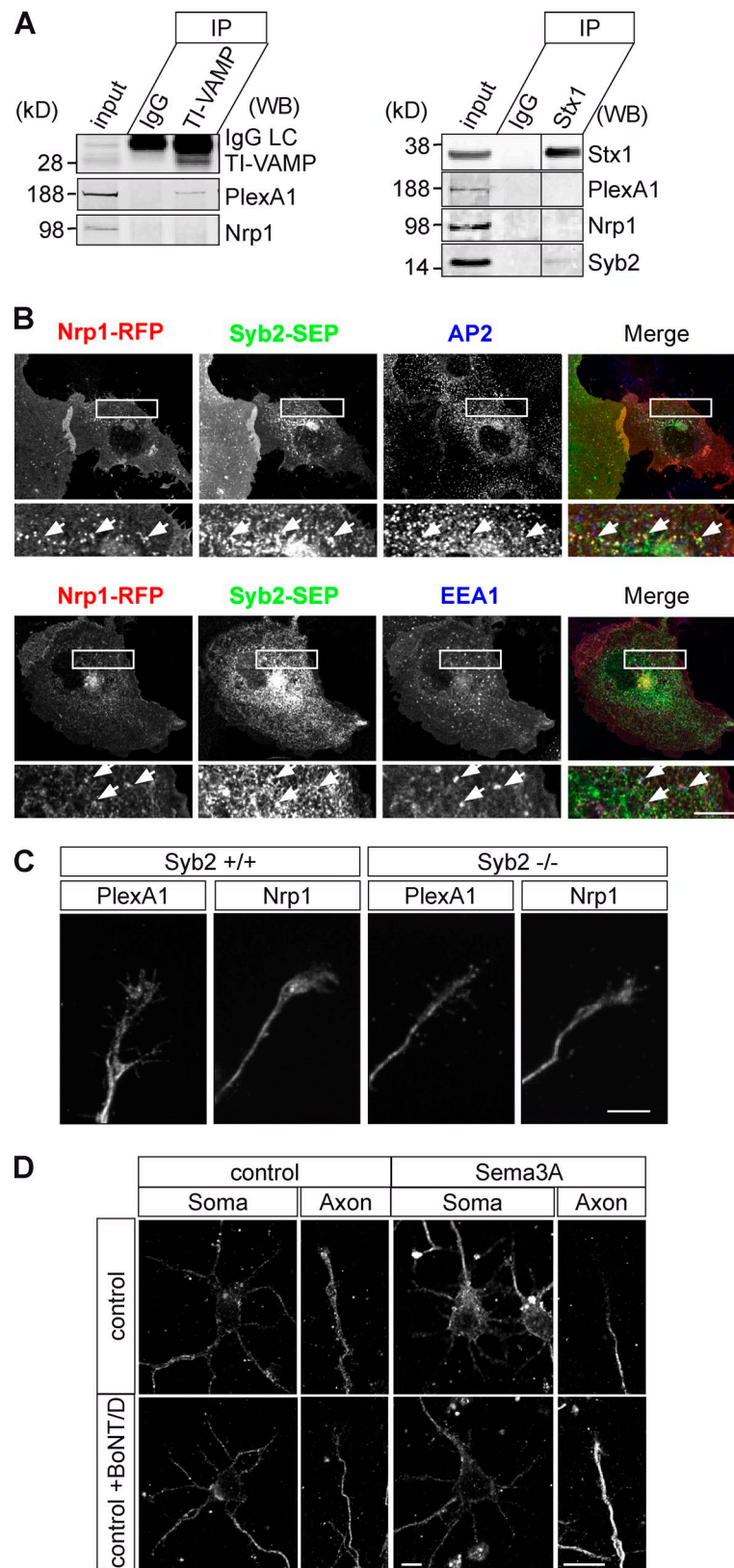
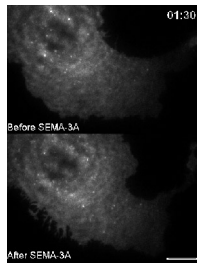


Figure S3. **Colocalization of Syb2 and Sema3A receptor in endocytic membranes.** (A) TI-VAMP, Stx1, and control immunoglobulin immunoprecipitations were performed on E15 brains. Coimmunoprecipitated proteins were identified by Western blotting. Black lines indicate that intervening lanes have been spliced out. (B) Immunofluorescence in Cos7 cells of exogenous Nrp1-mRFP and Syb2-SEP with AP2 or EEA1. Colocalizations are marked with arrows. Bottom panels show enlarged views of the boxed regions. Bar, 10 μ m. (C) Surface staining of PlexA1 and Nrp1 in Syb2^{+/+} and Syb2^{-/-} DIV2 E15 cortical neurons. (D) Immunofluorescence of PlexA1 in growth cones and somas of dissociated cortical neurons treated with or without BoNT/D and/or Sema3A. Bars, 4 μ m.



Video 1. **Sema3A treatment decreases rate of Syb2-SEP exocytosis in Cos7 cells.** Cos7 cells transfected with PlexA1-FLAG, Nrp1-mRFP, and Syb2-SEP were recorded for Syb2-SEP exocytosis events before (top) and after (bottom) treatment with 1 μ g/ml Sema3A-Fc. Images were analyzed by time-lapse microscopy using an inverted epifluorescence microscope (DMI6000B; Leica). Frames were taken every 500 ms for 3 min. Note that exocytic events appear as transient flashes of light. Time is indicated in minutes and seconds. Bar, 10 μ m.