Supplemental data

Myosin5a tail associates directly with Rab3A-containing compartments in neurons

Torsten Wöllert, Anamika Patel, Ying-Lung Lee, D. William Provance, Jr., Valarie E. Vought, Michael S. Cosgrove, John A. Mercer, and George M. Langford

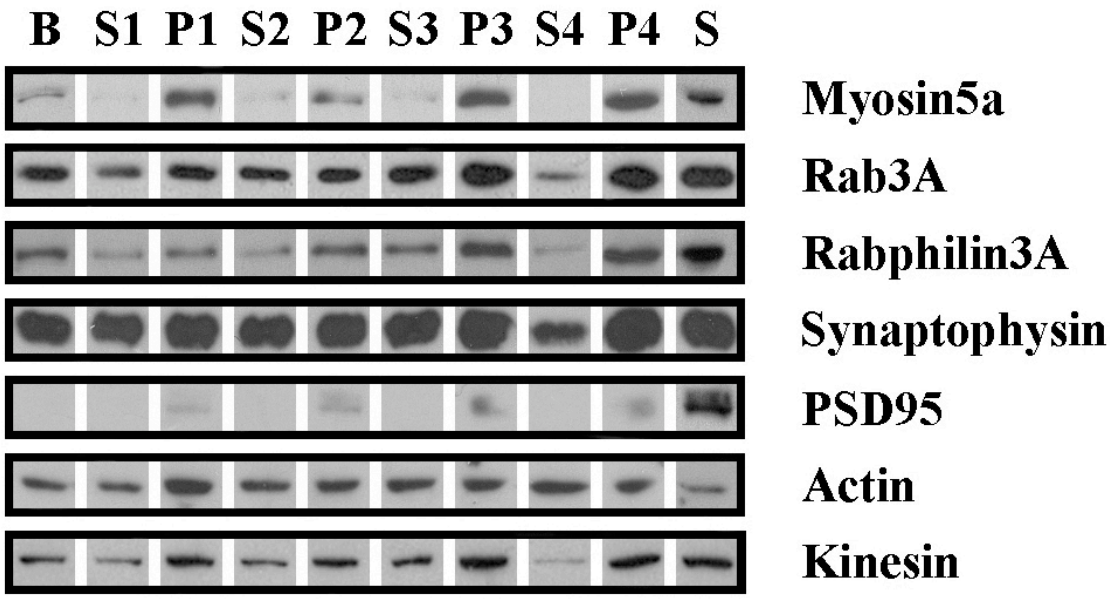
Supplemental Figure 1. Purification of the synaptosomal fraction from adult rat brains by homogenization followed by fractionation using differential centrifugation. Sequential Western blot analysis were performed of brain homogenate (B), crude synaptosomal membranes (P2), crude synaptosomal fraction (P4), and synaptosomal fraction (S) probed for Myo5a, Rab3A, Rabphilin3A, kinesin, actin, and for proteins specific abundant in pre- and postsynaptic elements (synaptophysin and PSD95) as indicated.

Supplemental Figure 2. Rab3A does not cross-link with GST. The synaptosomal fraction (Syn) and bound proteins (BP) eluted from immobilized GST affinity column loaded with 1mg/ml GST were analyzed by Western blots using a specific antibody to Rab3A.

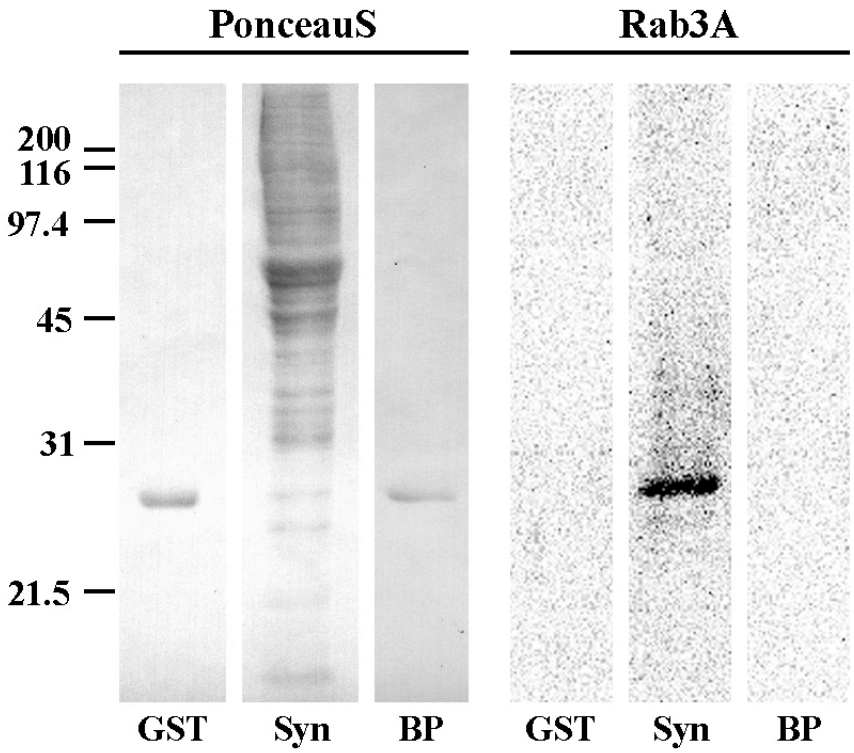
Supplemental Figure 3. Rab3A colocalizes with the SV marker synapsin 1 in mouse neurons. *A-B*, Indirect immunofluorescence localization of Rab3A and synapsin 1 in (*A*) wild-type and (*B*) dilute-lethal (*Myo5a*$^{d\text{-}l/d\text{-}l}$) mouse frontal cortex neurons. Bar, 20 μm

Supplemental Video 1. Movement of axoplasmic organelles on actin filaments in the presence of GST. The actin-based movement of axoplasmic organelles was observed by VEC-DIC microscopy after 15 min of incubation with 20 μM GST. Actin filaments are not detectable by VEC-DIC microscopy but were visualized by fluorescence microscopy after staining with 1 μM rhodamine-phalloidin (see Fig. 1*B*). Images were obtained every 0.1 sec and 192 sequential images (33s) are shown at 10 frames per second. Bar, 5 μm.

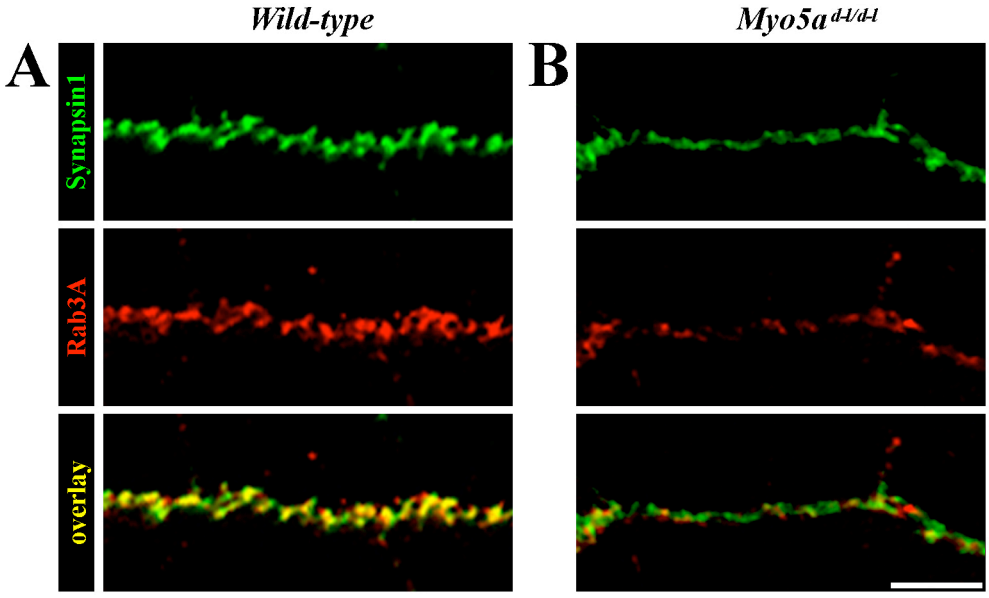
Supplemental Video 2. Inhibition of actin-based movement of axoplasmic organelles after incubation with GST-RabGDI. The movement of axoplasmic organelles on actin filaments was monitored by VEC-DIC microscopy after incubation with 20 μM GST-RabGDI. The presence of actin filaments was verified by fluorescence microscopy after staining with 1 μM rhodamine-phalloidin (see Fig. 1*B*). Images were obtained every 0.1 sec and 173 sequential images (28.9s) are shown at 10 frames per second. Bar, 5 μm.



Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3