

Supplementary Movie 1. Phase contrast time-lapse of wildtype and WAVE-1 null growth cones.

Primary hippocampal neurons were prepared from wildtype (left panel) and WAVE-1 null (right panel) mice and axonal growth cones were imaged at DIV2. Phase contrast images were acquired every second for two minutes duration. Movie, displayed at a 30x playback rate, corresponds to data in Figure 1J.

Supplementary Figure S2. Characterization of the mWAVE mutations.

(A) Schematic of the WAVE-1 signaling complex. (B) Co-transfection of HEK293 cells with either WAVE-1 or mWAVE-1 and other components of the WAVE-1 signaling complex. GST-pulldown of binding partners and analysis by immunoblot of GST-tagged HSPC300, GST-tagged Abi-1, and GST-tagged ArpC4 detected interaction with WAVE-1 (top). Immunoblot detection of extracts show expression levels of WAVE-1 and mWAVE-1 (middle) and each GST tagged protein (bottom). (C) Immunoblot detection of co-immunoprecipitation of Abl with WAVE-1 or mWAVE-1 (top). Expression of WAVE-1 or mWAVE-1 (middle) and Abl (bottom) is shown by immunoblot. (D) Interaction between WAVE-1 or mWAVE-1 and the RII regulatory subunits of PKA is shown by RII overlay (top). Immunoblot detection of the expression levels of WAVE-1 and mWAVE-1 (bottom) show equal loading. (E) Immunoprecipitation of GFP-actin and immunoblot detection of co-immunoprecipitating WAVE-1 or mWAVE-1 (top). Control immunoblots show expression levels of WAVE-1 and mWAVE-1 (middle) and GFP-actin or GFP alone (bottom).

Supplementary Figure S3. WRP associates with Abi-1.

Abi-1 expression enhances WRP association with mWAVE-1. **(A)** Western blot analysis of co-immunoprecipitation of WRP with mWAVE-1 in the absence or presence of co-expressed GST-Abi-1 (top panel). Immunoblot detection of extracts showing expression level of WRP (second from top panel), mWAVE-1 (second from bottom panel), and GST or GST-Abi-1 (bottom panel). **(B)** WRP associates with Abi-1 independent of WAVE-1 expression. Immunoblot detection of WRP associated with GST-Abi versus GST alone (top panel). Control immunoblot showing expression in extracts of WRP (middle panel), GST or GST-Abi (bottom panel).

Supplementary Figure S4. Paired-pulse facilitation in WAVE-1 null and mWAVE-1 hippocampal slices.

Paired-pulse facilitation was normal at 10, 50, and 200 milli-second inter-pulse intervals in **(A)** WAVE-1 null (*wave*^{-/-}) and **(B)** mWAVE-1 knockin (*wave*^{m/m}) mice, suggesting normal presynaptic release probability.