**Supplementary Notes**

**Figure 1** Phospho-RIM1α interacts with 14-3-3.

**Fig. 1A** A: *S413 mutations of GST-RIM1αAP[377-516] abolish 14-3-3 binding.* 1 nmol of recombinant proteins (GST-RIM1αAP and GST, as a negative control) immobilized to glutathione-agarose beads were incubated with 3 mg brain protein lysate in 1 ml final volume for 1 hr at 4 °C, then washed three times to reduce non-specific binding. Proteins were eluted by SDS and subjected to SDS-PAGE, followed by Western blot analysis. Blot was visualized by an ECL reagent. Input shown in lane 1 corresponds to 3% of the material loaded for binding to recombinant proteins shown in lanes 2-9. See also Supplementary Methods on experimental details.

**Fig. 1B.** *Recombinant 14-3-3η associates with phospho-RIM1α.* 1 nmol of GST-14-3-3η or GST were incubated with 3 mg rodent brain lysate protein as described at Fig. 1A. Pharmacological agents were applied to brain lysate prior to addition of detergents (0.1% SDS, 1% Triton-X-100). Equal sample loading was confirmed by Coomassie Blue staining (not shown). Input shown in lane 1 corresponds to 3% of the material loaded for binding to recombinant proteins shown in lanes 2-6.
Figure 2 R56A and R60A mutations of 14-3-3 decrease, while a V415P mutation of RIM1α increases RIM1α/14-3-3 interaction.

A. *R56A,R60A mutations (DN) reduce myc-14-3-3η binding to RIM1α*. 14-3-3 proteins were expressed in 293T cells and extracted by a non-denaturing lyses buffer (M-PER, Pierce, Rockford, IL). Binding of 5 mg lysate protein to 1 nmol GST-RIM1αAP[377-516] was assayed by quantitative Western blotting using a monoclonal myc-antibody and 125I-labelled secondary antibody. (See also Supplementary Methods). B. Myc-14-3-3 and GST-RIM1 signals were quantified by using a 125I-labeled secondary antibody and phosphoimigier. Myc signal was divided by the corresponding GST-RIM1α signal to gain a 14-3-3 binding value independent of sample loads. D. To determine binding values independent of sample loading a 125I-labeled secondary antibody and phosphoimigier were used as described at 2.B. Inputs shown correspond to 2% of the material loaded for binding to recombinant proteins.

Figure 3 *Presynaptic manipulations which interfere with RIM1α/14-3-3 interaction, impair LTP in granule cell-Purkinje cell pairs*. Cultures of embryonic mouse cerebellum were prepared and transfected with plasmids as previously described3,4 (see also Supplementary Methods). Plasmids encoding 14-3-3η proteins plus a separate plasmid encoding EGFP as a marker were delivered using accelerated gold particles on the fourth day in culture and recording were made from EGFP-positive granule cells synaptically connected to EGFP-negative Purkinje cells, 20-50 hours later.