SUPPLEMENTARY MATERIAL FOR

The Janus-Faced Nature of the C_2B Domain Is Fundamental for Synaptotagmin-1 Function

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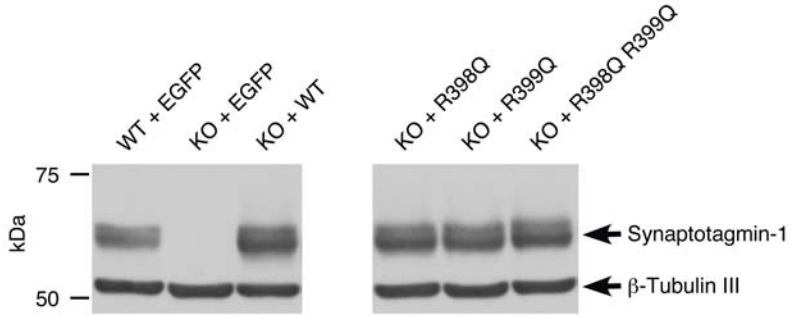
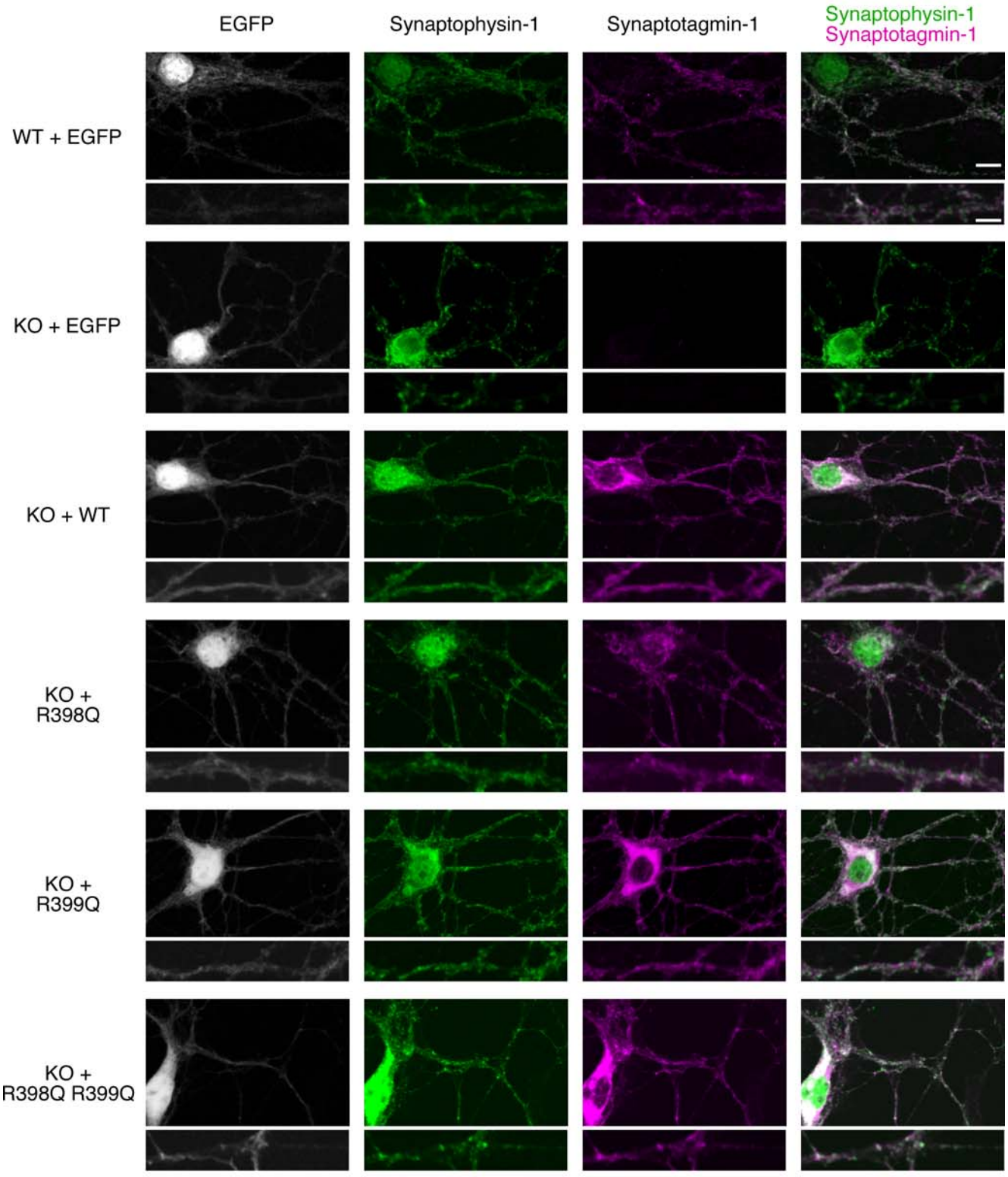


Xue et al.,
Supplementary Figure 1

Supplementary Figure 1. Lentiviral expressions of synaptotagmin-1 in cultured hippocampal neurons. Hippocampal neurons were infected by lentiviruses expressing EGFP alone or synaptotagmin-1 and EGFP together. Representative Western blot shows similar expression levels of WT and mutant synaptotagmin-1 variants in KO neurons. Neuronal specific β-Tubulin III serves as loading control.



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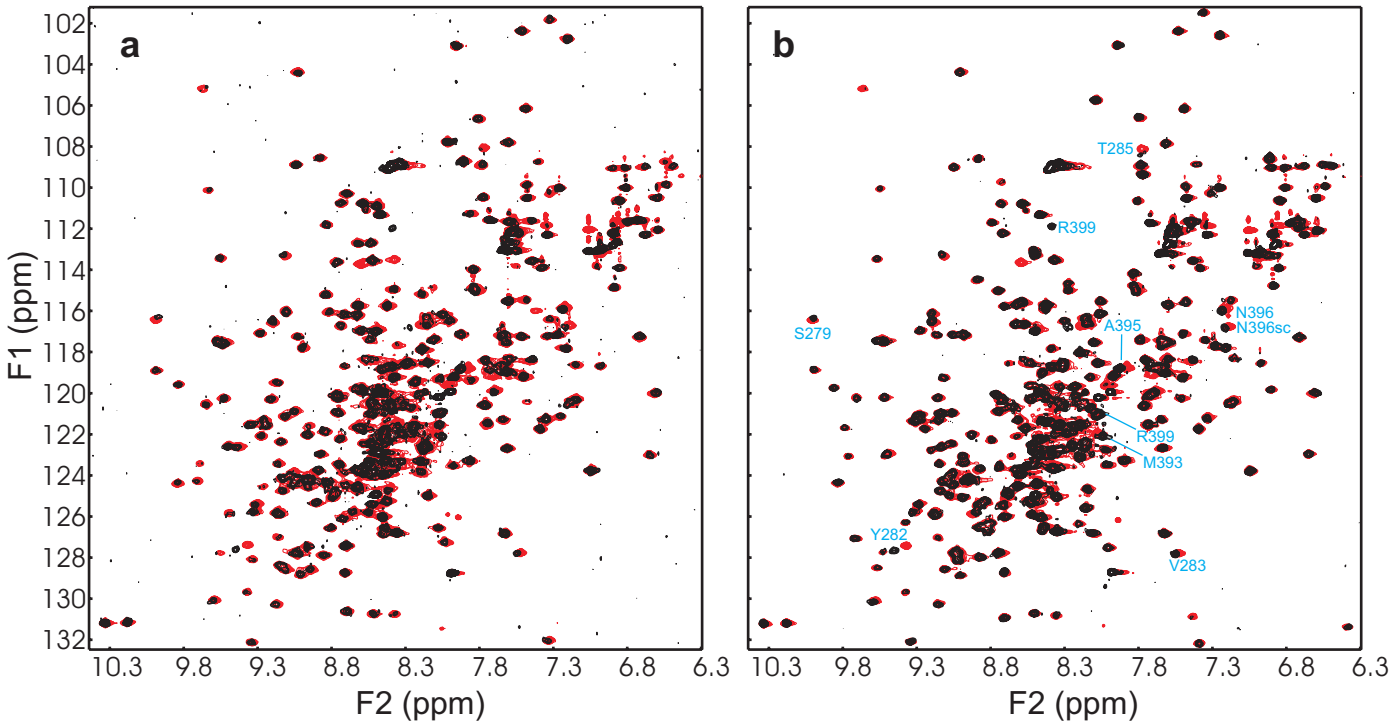
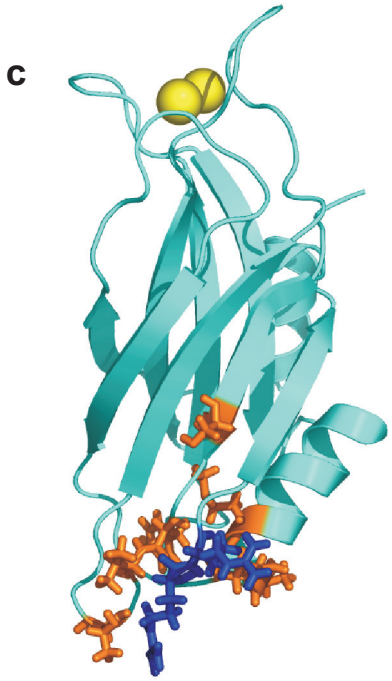
Supplementary Figure 2

Supplementary Figure 2. Presynaptic targeting of WT and mutant synaptotagmin-1 variants. Hippocampal neurons were infected by lentiviruses expressing EGFP alone or synaptotagmin-1 and EGFP together. Representative confocal images show immunostaining of EGFP, synaptophysin-1 and synaptotagmin-1. Merged images show the colocalizations of synaptophysin-1 and synaptotagmin-1. Scale bars indicate 10 μm for low magnification (large images) and 5 μm for high magnification (small images).





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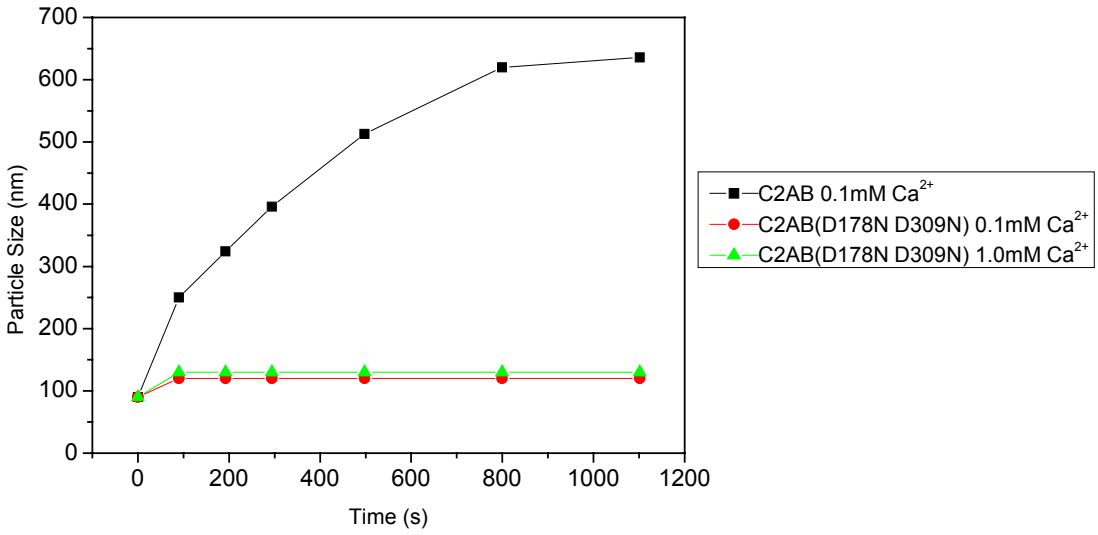
Supplementary Figure 3

Supplementary Figure 3. The R398Q R399Q mutation does not induce allosteric changes in the C_2AB fragment. (**a**,**b**) ^{1}H-^{15}N HSQC spectra of 100 μM WT (black) or R398Q R399Q mutant (red) C_2AB fragment in 1 mM EDTA (**a**) and 20 mM Ca^{2+} (**b**), in a buffer containing 50 mM MES (pH 6.3), 150 mM NaCl and 1 mM DTT. The spectra were acquired at 25 ^{o}C on a Varian INOVA800 NMR spectrometer. (**c**) Ribbon diagram of the synaptotagmin-1 C_2B domain with Ca^{2+} ions shown as yellow spheres. R398 and R399 are shown as blue sticks, and residues exhibiting chemical changes due to the R398Q R399Q mutation are shown as orange sticks. The corresponding cross-peaks are labeled in (**b**). Note that the cross-peak shifts are small and all the corresponding residues are near the sites of the mutation, showing that the mutation does not substantially alter the structure of the C_2AB fragment and does not cause allosteric changes in the Ca^{2+}-binding regions of the C_2 domains. Note also that the Ca^{2+}-induced cross-peak changes are analogous in the WT and R398Q R399Q mutant C_2AB fragments, showing that the mutation does not alter Ca^{2+} binding.



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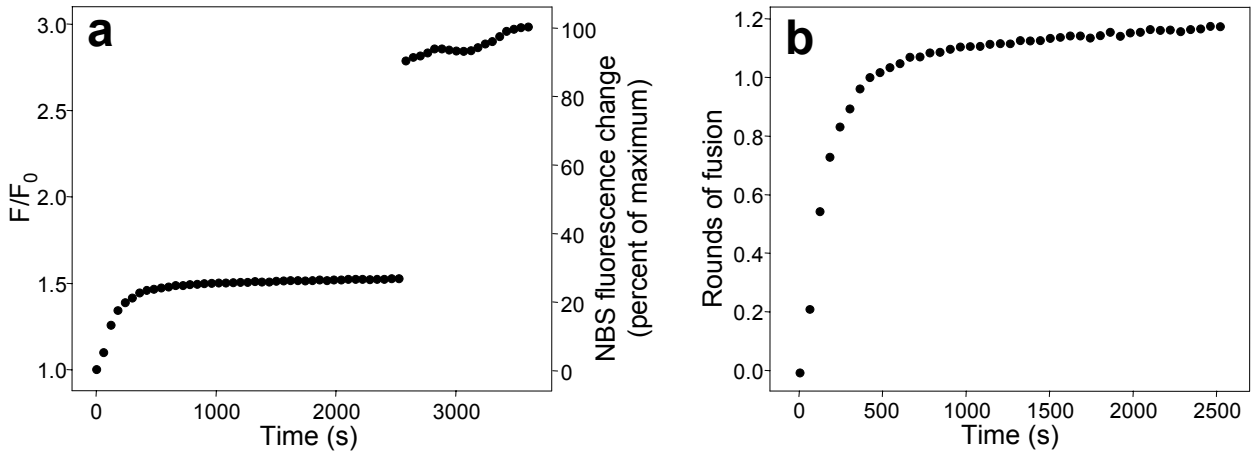
Supplementary Figure 4

Supplementary Figure 4. The vesicle clustering activity of the C_2AB fragment requires Ca^{2+} binding to at least one of its C_2 domains. Vesicle clustering assays monitored by dynamic light scattering were performed as in **Figure 4b** with WT C_2AB fragment in the presence of 100 μM Ca^{2+} (black) or with D178N D309N C_2AB fragment in the presence of 100 μM (red) or 1 mM (green) Ca^{2+}.



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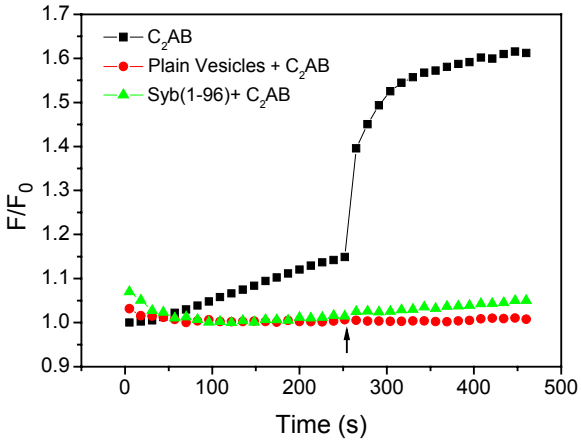
Supplementary Figure 5

Supplementary Figure 5. Lipid mixing between synaptobrevin- and syntaxin-1/SNAP-25-containing proteoliposomes with 1:200 monitored by fluorescence dequenching. In (**a**), the Y axis on the left represents relative fluorescence with respect to the starting value (F/F_0), whereas the right Y axis indicates the percent of increase in fluorescence with respect to the maximum fluorescence observed upon addition of 1% β-octylglucoside (noted from the sharp increase in fluorescence emission), after subtracting the initial fluorescence. In (**b**), the data corresponding to the first 2500 seconds of the reaction shown in (**a**) were converted to rounds of fusion as described [Parlati,F. *et al.* Rapid and efficient fusion of phospholipid vesicles by the alpha-helical core of a SNARE complex in the absence of an N-terminal regulatory domain. *Proc. Natl. Acad. Sci. U. S. A* **96**, 12565-12570 (1999)].



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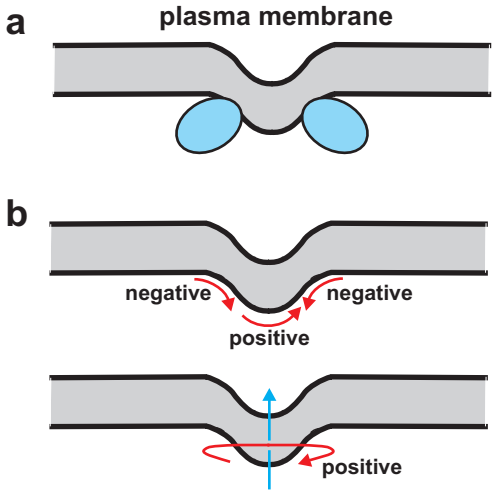
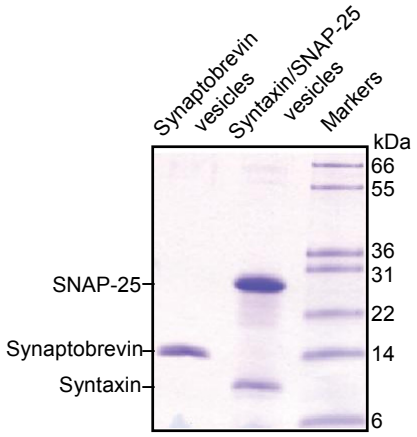
Supplementary Figure 6

Supplementary Figure 6. The Ca^{2+}/C_2AB fragment induced stimulation of lipid mixing is SNARE dependent. Lipid mixing assays were performed as in **Fig. 5c-g** in the presence of 1 μM C_2AB fragment and proteoliposomes lacking (red) or containing SNAREs (1:500 P/L ratio), without (black) or with (green) addition of the synaptobrevin SNARE motif. The arrow indicates the time of addition of 100 μM Ca^{2+}.



Xue et al., Supplementary Figure 7

Supplementary Figure 7. (**a**) Model of how synaptotagmin-1 might induce positive membrane curvature to initiate fusion, adapted from Martens et al. [Martens,S., Kozlov,M.M., & McMahon,H.T. How synaptotagmin promotes membrane fusion. *Science* **316**, 1205-1208 (2007)] but only showing the C_2B domain for simplicity and for an easier comparison with our model presented in **Fig. 7**. In this model, the Ca^{2+}-binding loops of both C_2 domains are proposed to induce positive curvature on the plasma membrane, forming an initial 'nipple' that later evolves to a stalk intermediate and then to a fusion pore. Note that the positive curvature predicted to be induced by synaptagmin-1 in this model is around the vertical axis, i.e. on a horizontal plane perpendicular to the plane of the paper. To help visualize this notion, panel (**b**) shows two membranes with a nipple; the regions of positive and negative membrane curvature in the plane of the paper are indicated by red arrows in the top diagram, whereas the red arrow in the bottom diagram indicates the positive curvature of the nipple around the vertical axis. These diagrams illustrate that formation of the nipple requires both negative curvature, as proposed in our model of **Fig. 7**, as well as positive curvature, as proposed in the model of Martens et al. illustrated here in panel (**a**).



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Supplementary Figure 8

Supplementary Figure 8. SDS-PAGE analysis of synaptobrevin and syntaxin-1/SNAP-25 proteoliposomes (1:500 P/L ratio) used for lipid mixing assays.