Supporting Information

Probing the Diacylglycerol-binding Site of Presynaptic Munc13-1

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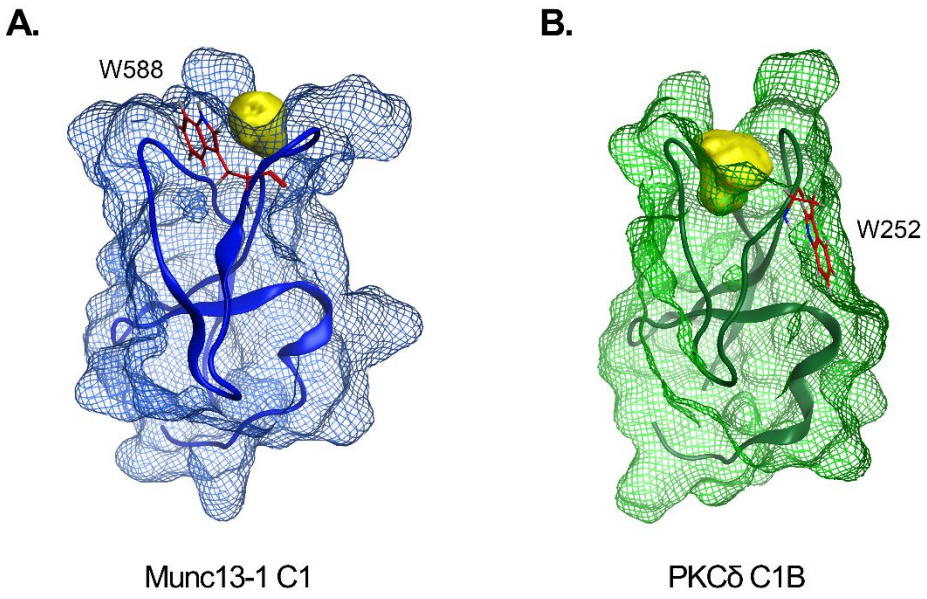

Figure S1. The active site of Munc13-1 C1 and PKCδ C1B. The ligand binding site of Munc13-1 C1 domain (PDB: 1Y8F) (A) and PKCδ C1B domain (PDB: 1PTR) (B). The pocket volumes are presented by yellow solid surface. The active sites of two homologous C1 domains are different because of the different orientation of the Trp-588/252.

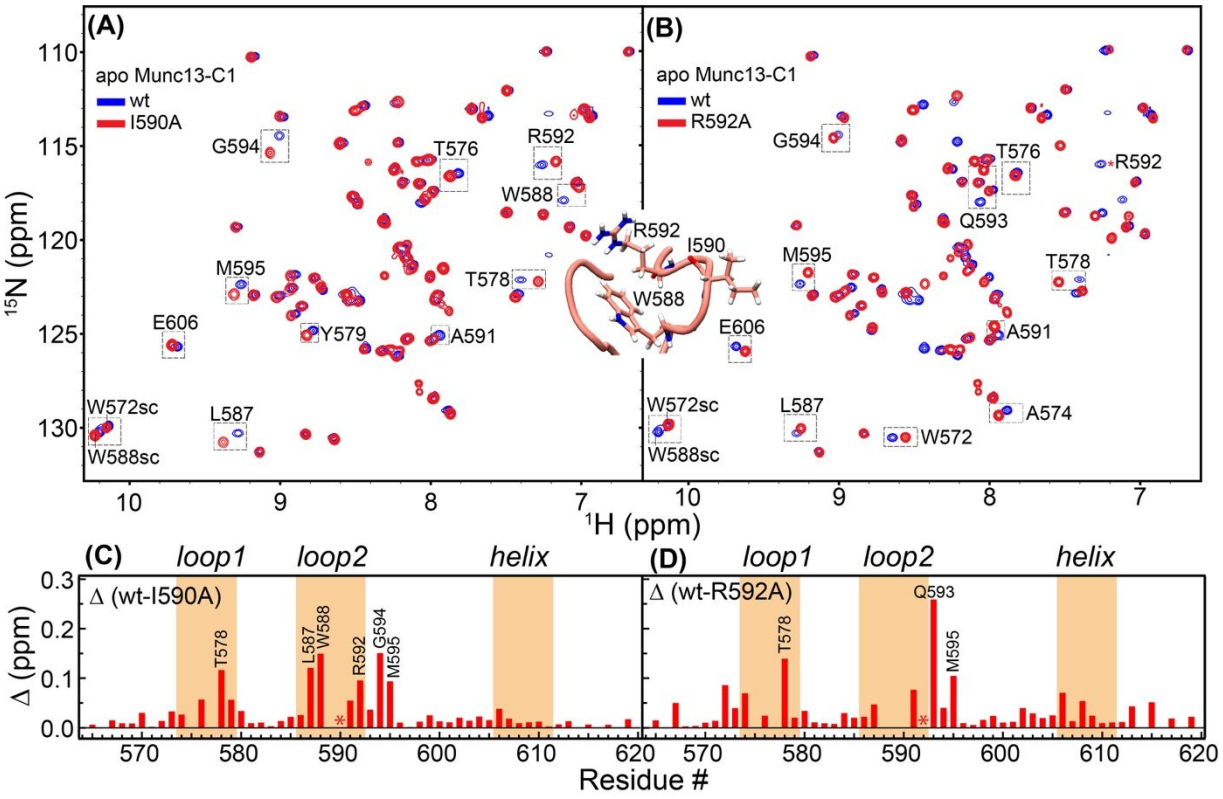

Figure S2. I590A and R592A mutations affect the entire loop region of Munc13-1 C1. Spectral overlays of 100 μM [U-^{15}N]-enriched I590A (A) and R592A (B) Munc13-1 C1 variants with WTC1. The select perturbed residues are highlighted by squares. Inset: The corresponding loop region from the lowest energy NMR conformer (PDB: 1Y8F) showing the locations of these mutation sites with respect to Trp-588. The combined CSPs (referenced to WTC1) due to I590A (C) and R592A (D) mutations are plotted as a function of primary structure. In both variants, the ligand-binding loops and hinge region of the β-sheet following loop 2 are most affected.

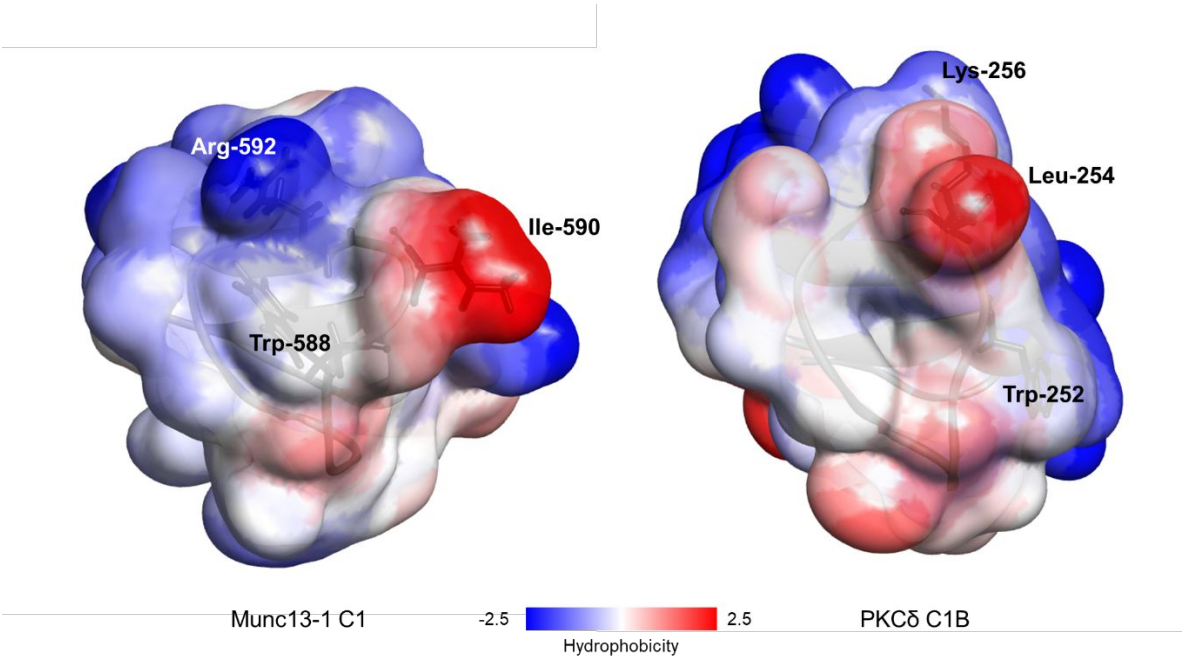

Figure S3. Hydrophobicity of ligand binding loops of Munc13-1 C1. Munc13-1 (left) and PKCδ C1B (right). Trp-588, Ile-590, and Arg-592 of Munc13-1 C1 corresponds to Trp-252, Leu-254, and Lys-256, respectively. Surface structures were colored by hydrophobicity based on the Kyte-Doolittle scale.[1]

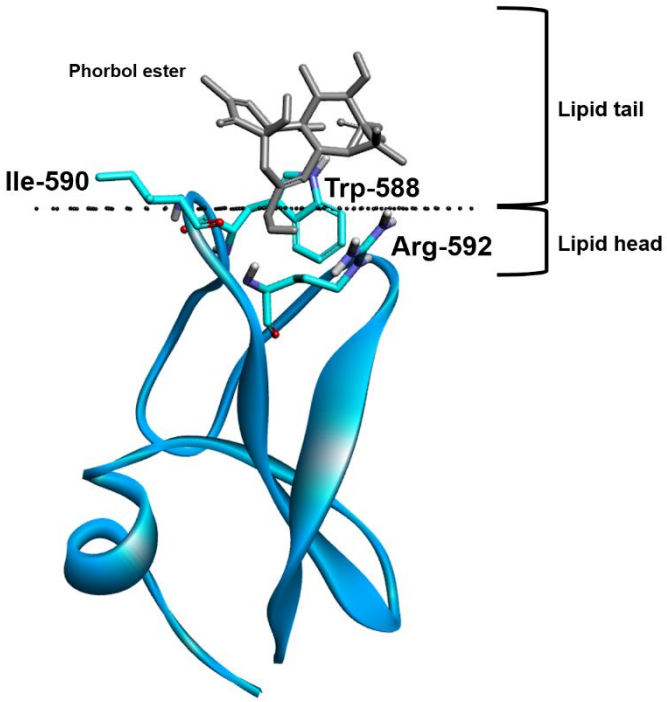

Figure S4. Model of Munc13-1 C1 domain for membrane insertion. The phorbol ester-bound Munc13-1 C1 was embedded into the membrane. The angle and depth of the complex to the membrane were calculated using PPM server.[2]

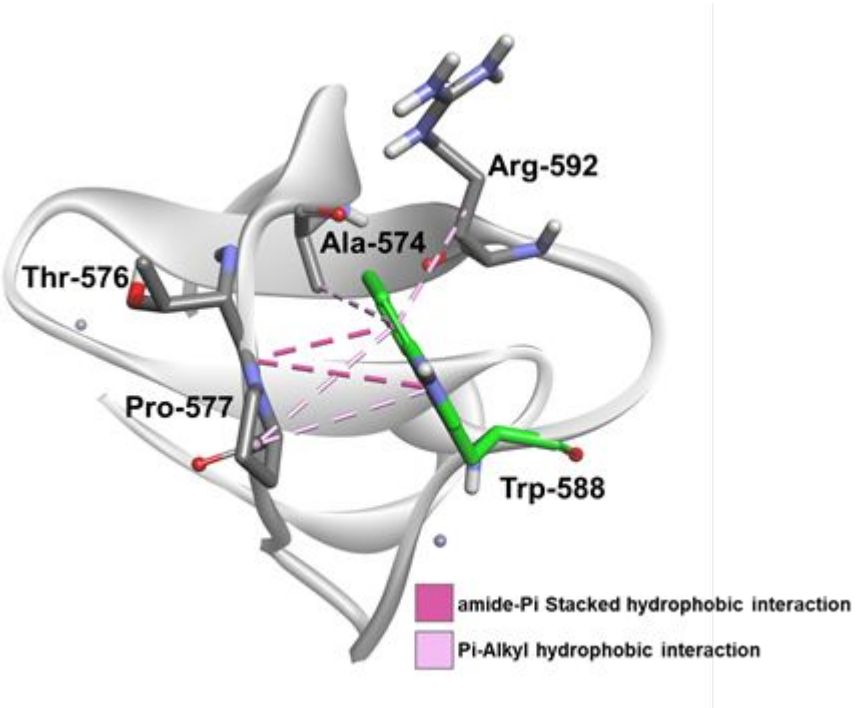

Figure S5. Interaction of Trp-588 with nearby residues at the active site of Munc13-1 C1 (PDB: 1Y8F).

References

1. Kyte, J., and Doolittle, R. F. (1982) A simple method for displaying the hydropathic character of a protein, *J. Mol. Biol. 157*, 105-132.

2. Lomize, M. A., Pogozheva, I. D., Joo, H., Mosberg, H. I., and Lomize, A. L. (2012) OPM database and PPM web server: resources for positioning of proteins in membranes, *Nucleic Acids Res. 40*, D370-376.