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Supplemental Information

Phosphatidylinositol 4,5 Bisphosphate Controls the *cis* and *trans* Interactions of Synaptotagmin 1

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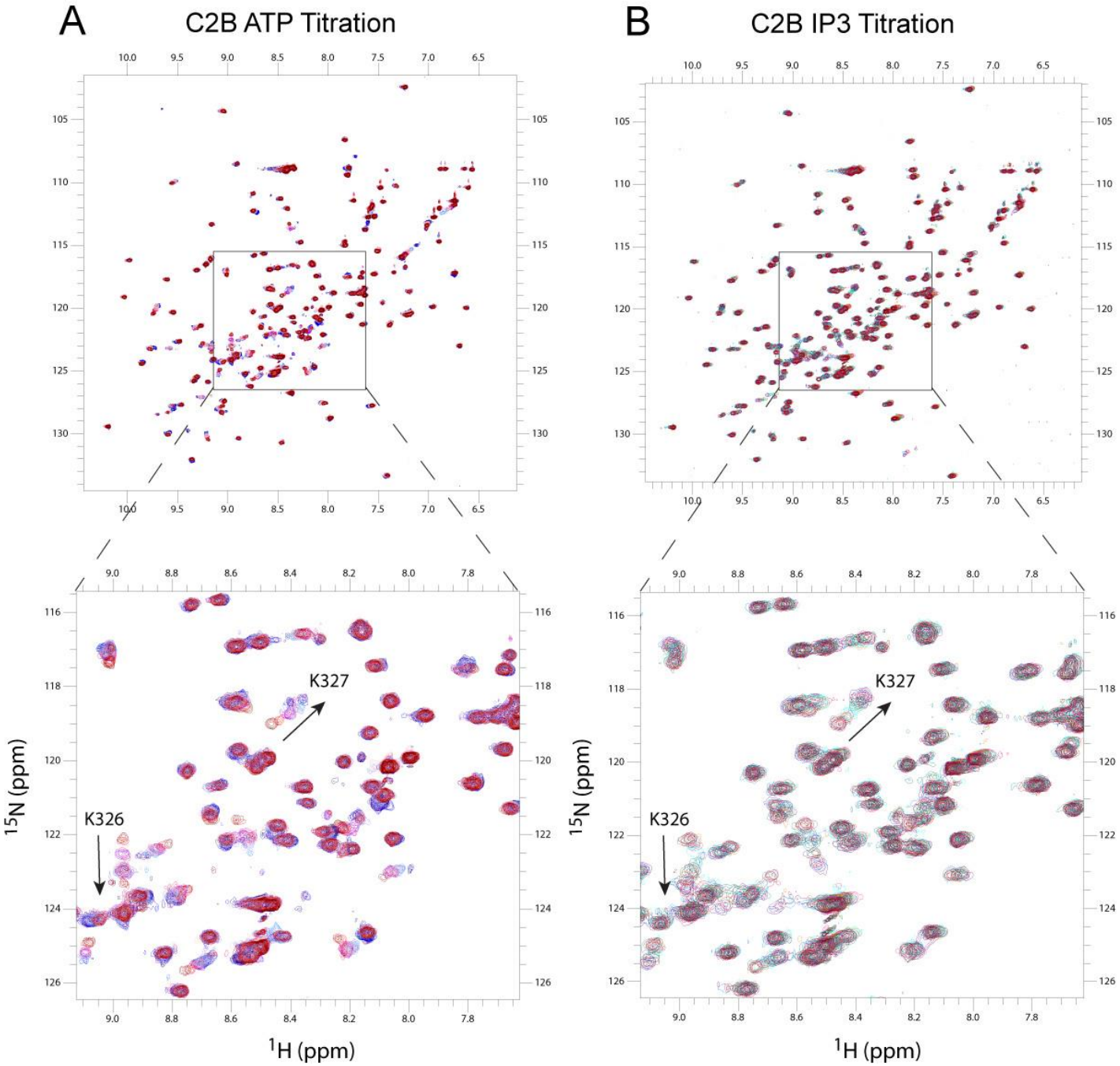


Figure S1. HSQC plots and chemical shift changes with ATP (**A**) or IP_3 (**B**) addition. ATP was titrated at concentrations: 0 mM, 0.2 mM, 0.3 mM, 0.35 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM, 1.5 mM, 2.0 mM, 3 mM, and 5 mM in the presence of C2B. IP_3 was titrated at concentrations: 0 mM, 0.001 mM, 0.0015 mM, 0.002 mM, 0.0025 mM, 0.005 mM, 0.0075 mM, 0.00875 mM, 0.01 mM, 0.02 mM, 0.03 mM, 0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, and 1 mM. The arrows highlight the direction of chemical shifts two lysine residues in the polybasic face at sites 326 and 327.

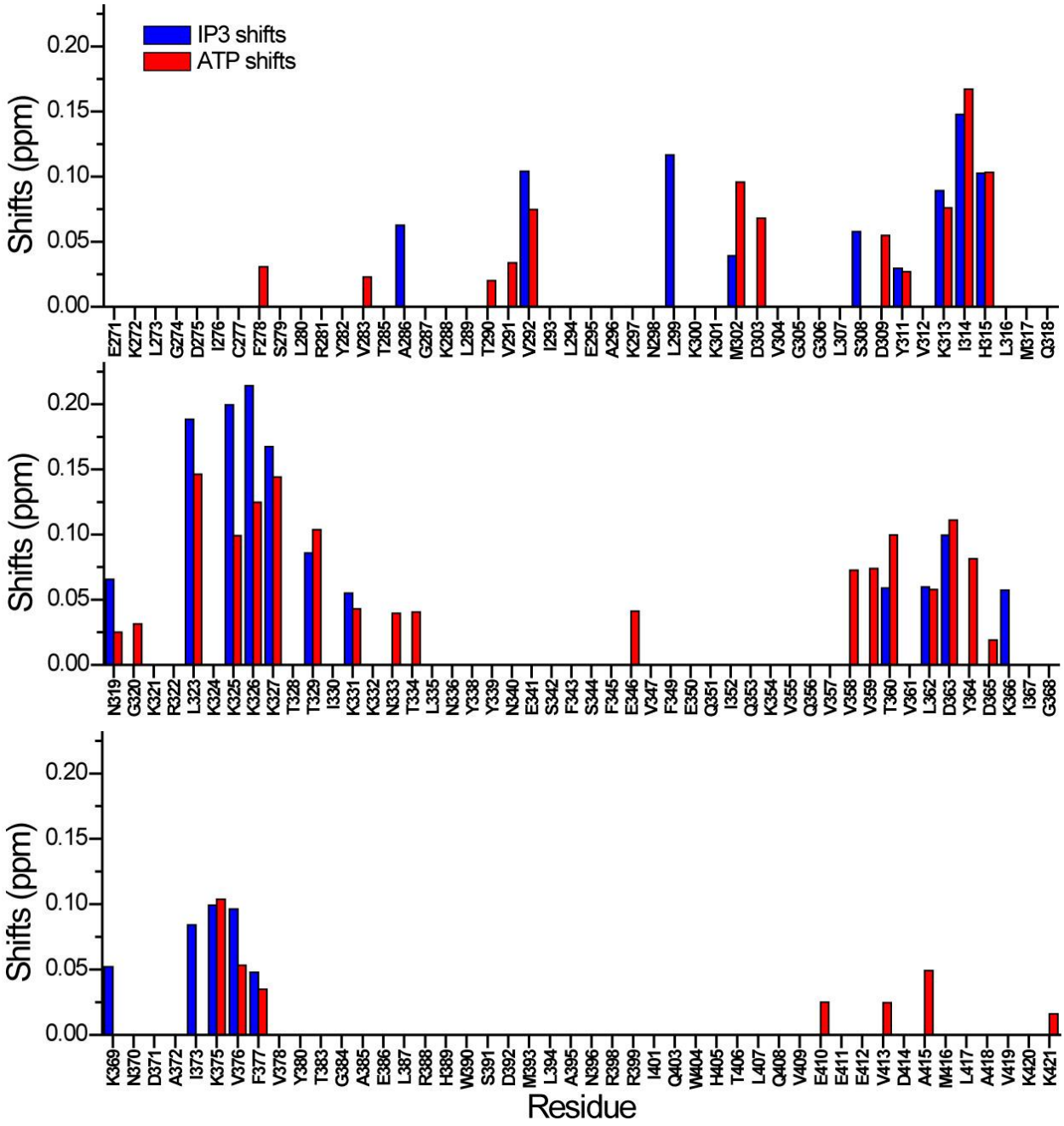


Figure S2. HSQC plots and chemical shift changes with IP_3 or ATP addition. The bar chart shows the average weighted chemical shift changes (ΔHN, N) at each residue position in sC2B with the addition of either 5 mM ATP (red) 1 mM IP_3 (blue).

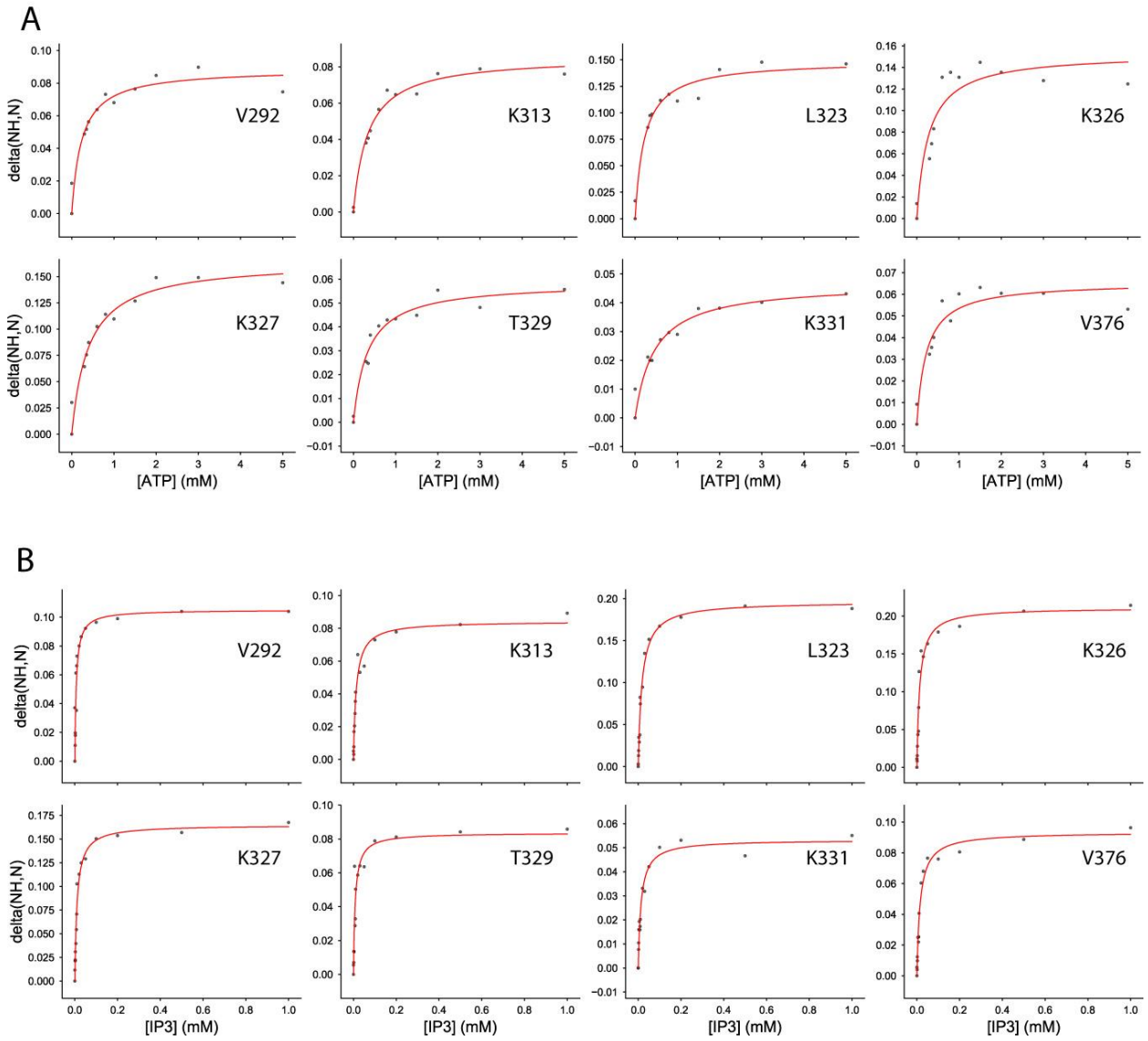


Figure S3. Titration curves and fits for binding affinity determination of IP_3 and ATP. Representative fits to determine the dissociation constants of either ATP (**A**) or IP_3 (**B**) at each residue position. Chemical shifts were fit to the equation: $y = a(bx/(1 + bx)$, y is the chemical shift, x is the ligand concentration, b is the reciprocal molar binding constant and a is a scaling factor.

protein variant	lipid conditions	metal added	depth parameter (Φ)	position from lipid phosphate (Å)
fSyt1 173R1	POPC	Ca^{2+}	-1.9 ± 0.05	aqueous
	POPC:POPS (15%)	Ca^{2+}	1.03 ± 0.05	7.8
sC2AB 173R1	POPC:POPS (20%)	Ca^{2+}	1.2 ± 0.1	8.8
fSyt1 304R1	POPC	Ca^{2+}	-2.0 ± 0.05	aqueous
	POPC:POPS (15%)	Ca^{2+}	-0.54 ± 0.05	3.1
sC2AB 304R1	POPC:POPS (20%)	Ca^{2+}	-0.39 ± 0.2	4.3

Table S1. Power saturation and depth data comparing the C2 domains in fSyt1 and sC2AB. Positive position values represent penetration into the phosphate plate. Measurements of fSyt1 reconstituted into POPC or POPC:Chol in the presence or absence of calcium are also aqueous.

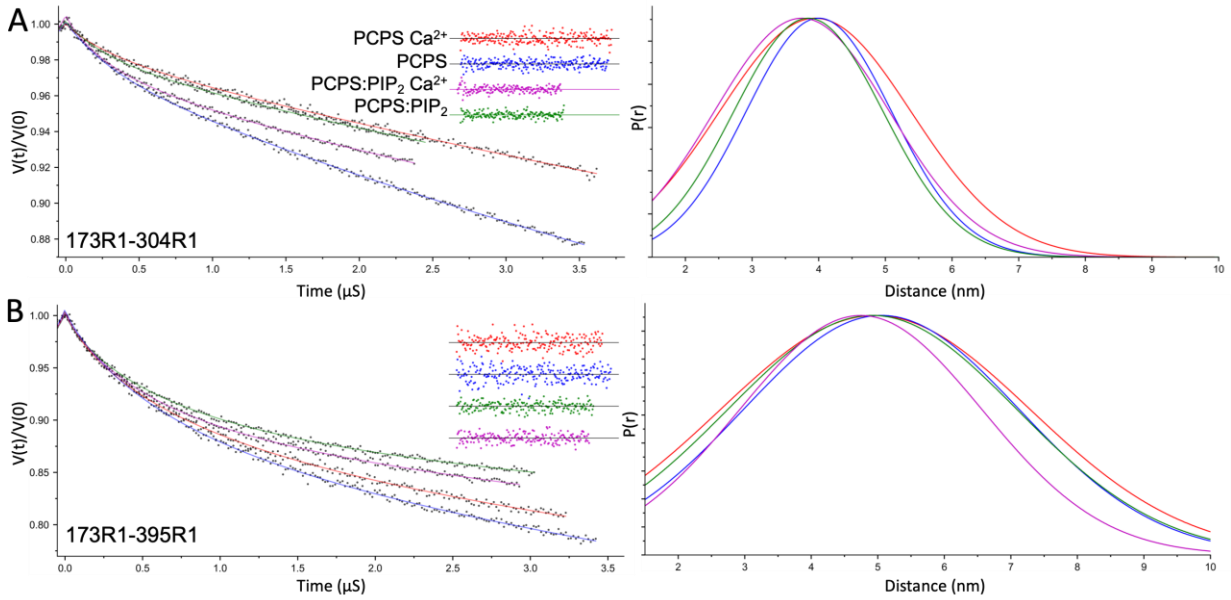


Figure S4. DEER data for 173R1-304R1 and 173R1-395R1 in fSyt1 shows broad distributions. (**A**) Raw DEER data obtained for the 173R1-304R1 spin pair under conditions where fSyt1 is reconstituted into POPC:POPS with bound Ca^{2+} (red trace), without calcium (blue trace), with bound Ca^{2+} and the addition of PIP_2 containing vesicle (magenta trace) and without Ca^{2+} and the addition of PIP_2 containing vesicles (green trace). (**B**) Raw DEER data obtained for the 173R1-395R1 spin pair under the same conditions as in (A). All data fit with broad single Gaussian distributions using DD (1).

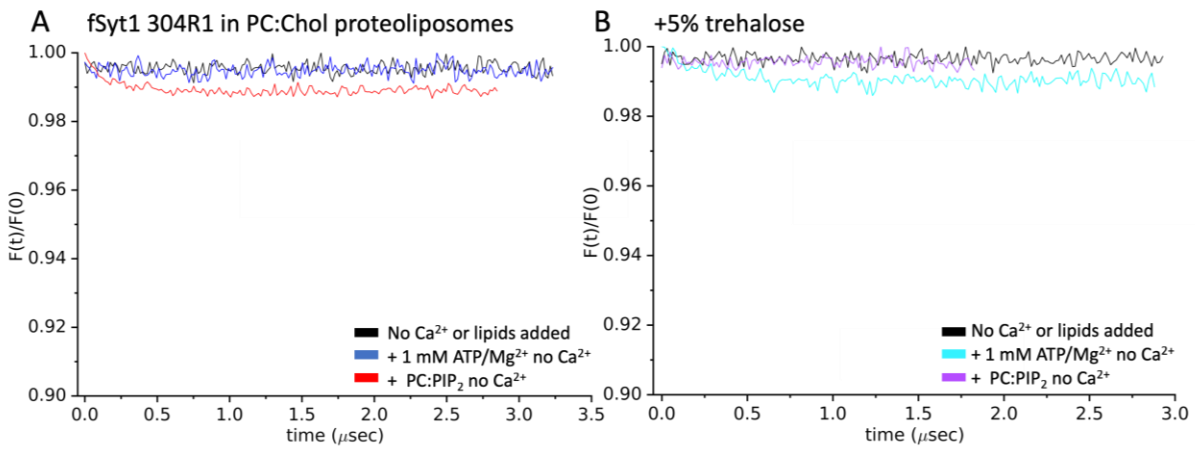
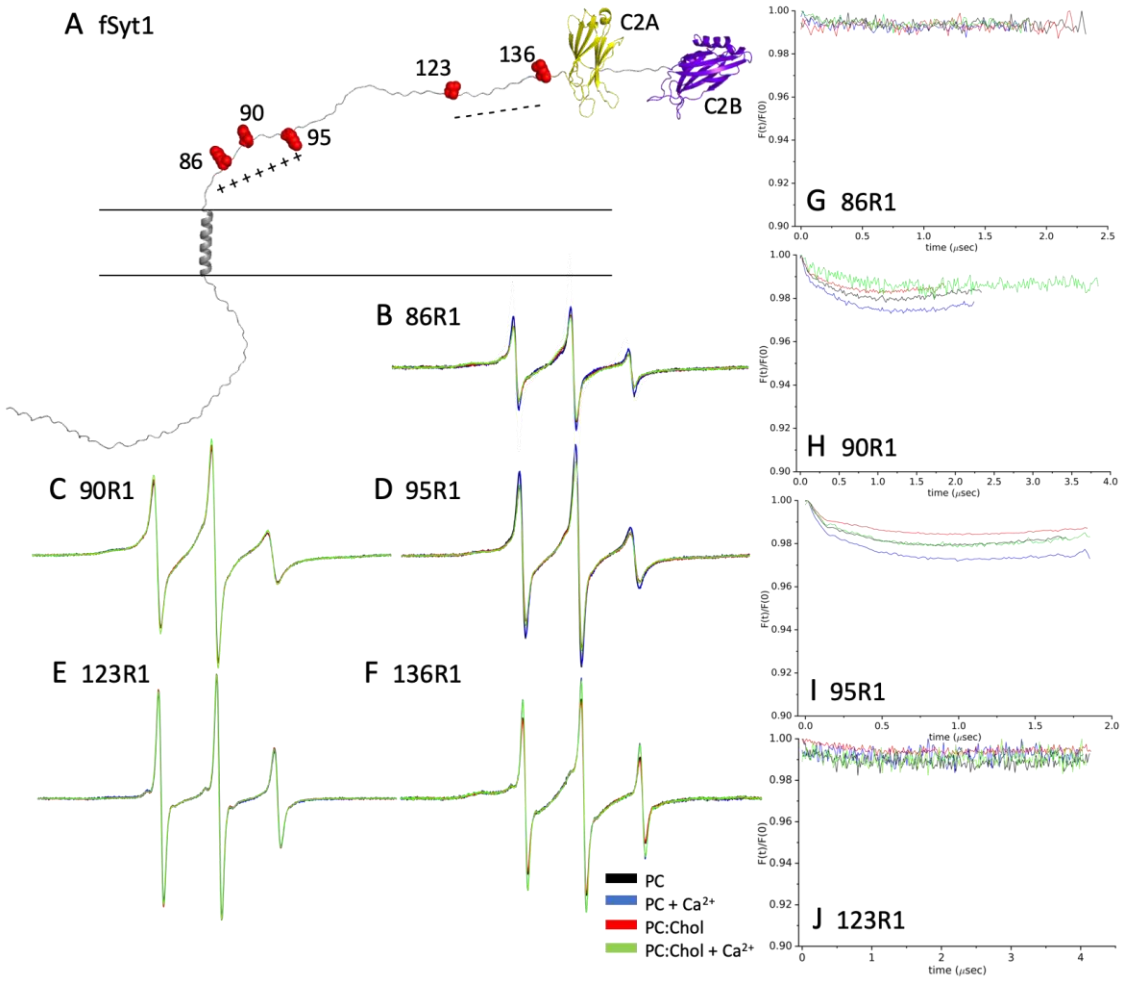


Figure S5. DEER data obtained for site 304R1 which show that there is no significant aggregation of fSyt1 C2 domains under a range of conditions. (**A**) Background corrected DEER data obtained for the single label 304R1 for fSyt1 reconstituted into POPC:Chol (80:20) in the absence of Ca^{2+} (black trace), 1 mM ATP/Mg^{2+} added (blue trace) or with POPC:PIP_2 (95:5) liposomes added (red trace). (**B**) Measurements taken again, but in the presence of 5% trehelose in an attempt to promote oligomerization (ring formation) (2). Here, fSyt1 in POPC:Chol (80:20) with 1 mM ATP/Mg^{2+} added (teal trace) or with POPC:PIP_2 (95:5) liposomes added (purple trace). Modulation depths are 1% or less, which is less than 5% of the signal that would have been obtained under these conditions if all the protein assembled into dimers. In addition, we attempted but did not detect interactions for fSyt1 reconstituted into POPC:POPS (85:15) with added POPC:PIP_2 (95:5) vesicles (with or without Ca^{2+}), or for fSyt1 reconstituted into a mixture designed to mimic the plasma membrane lipid composition (POPC:Chol:POPE:POPS:PIP_2, 36:20:30:15:1) either with or without Ca^{2+}.



Figures S6. EPR CW spectra obtained from sites 86, 90, 95, 123, and 136 in the linker region of fSyt1 near the transmembrane helix show that the linker is largely disordered. (**A**) Label positions are marked in red. (**B**) CW EPR of fSyt1 86R1 is broadened in neutral lipid due to proximity to the membrane, as verified by power saturation (Table S2). Measurements were obtained for fSyt1 reconstituted into POPC in the absence of calcium (black line), in the presence of calcium (blue line), or reconstituted into POPC:Chol in the absence of calcium (red line), and in the presence of calcium (green line). (**C**) fSyt1 90R1, (**D**) fSyt1 95R1, (**E**) fSyt1 123R1, (**F**) fSyt1 136R1 all show mobile EPR lineshapes and are located in the aqueous phase, as verified by power saturation (Table S2). (**G**) Background corrected DEER data obtained for fSyt1 86R1. Measurements were obtained for fSyt1 reconstituted into POPC in the absence of calcium (black trace), in the presence of calcium (blue trace), or reconstituted into POPC:Chol in the absence of calcium (red trace), and in the presence of calcium (green trace). (**H**) Background corrected DEER data obtained for fSyt1 90R1, (**I**) fSyt1 95R1, (**J**) fSyt1 123R1. The modulation depths on these traces range from less than one to between 2 and 3%. A fully dimerized linker would produce a DEER signal of about 20% modulation depth under the conditions of these experiments. Reconstitution of flSyt1 136R1 yielded a limited amount of material and we did not run DEER on this sample.

protein variant	lipid conditions	metal added	depth parameter (Φ)	position from lipid phosphate (Å)
fSyt1 86R1	POPC	Ca^{2+}	-1.81 ± 0.1	-2.59
	POPC	EGTA	-1.76 ± 0.25	-2.19
	POPC:Chol (20%)	Ca^{2+}	-1.80 ± 0.2	-2.51
	POPC:Chol (20%)	EGTA	-1.83 ± 0.1	-2.75
fSyt1 90R1	POPC	Ca^{2+}	-2.4 ± 0.1	aqueous
	POPC	EGTA	-2.4 ± 0.2	aqueous
	POPC:Chol (20%)	Ca^{2+}	-2.4 ± 0.1	aqueous
	POPC:Chol (20%)	EGTA	-2.5 ± 0.2	aqueous
fSyt1 95R1	POPC	Ca^{2+}	-2.3 ± 0.1	aqueous
	POPC	EGTA	-2.4 ± 0.1	aqueous
	POPC:Chol (20%)	Ca^{2+}	-2.7 ± 0.1	aqueous
	POPC:Chol (20%)	EGTA	-2.6 ± 0.05	aqueous
fSyt1 123R1	POPC	Ca^{2+}	-2.5 ± 0.1	aqueous
	POPC	EGTA	-2.4 ± 0.05	aqueous
	POPC:Chol (20%)	Ca^{2+}	-2.5 ± 0.1	aqueous
	POPC:Chol (20%)	EGTA	-2.5 ± 0.1	aqueous
fSyt1 136R1	POPC	Ca^{2+}	-2.4 ± 0.2	aqueous
	POPC	EGTA	-2.5 ± 0.05	aqueous
	POPC:Chol (20%)	Ca^{2+}	-2.5 ± 0.1	aqueous
	POPC:Chol (20%)	EGTA	-2.3 ± 0.2	aqueous

Table S2. Power saturation data for sites 86, 90, 95, 123, and 136 in the linker region show initial bilayer contact at site 86 then aqueous measurements beyond. Negative position values represent positions away from the phosphate plane.

Supporting References

1. Stein, R. A., A. H. Beth, and E. J. Hustedt. 2015. A Straightforward Approach to the Analysis of Double Electron-Electron Resonance Data. Methods Enzymol 563:531-567.
2. Zanetti, M. N., O. D. Bello, J. Wang, J. Coleman, Y. Cai, C. V. Sindelar, J. E. Rothman, and S. S. Krishnakumar. 2016. Ring-like oligomers of Synaptotagmins and related C2 domain proteins. Elife 5.