Triggered Ca^{2+} influx is required for extended-synaptotagmin 1-induced ER-plasma membrane tethering

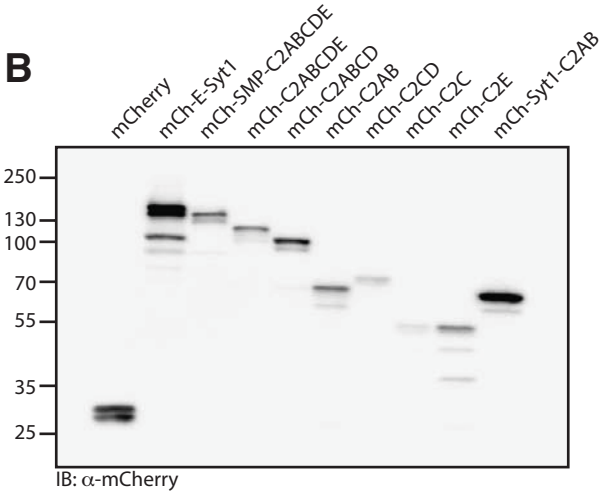
Appendix

Table of Contents

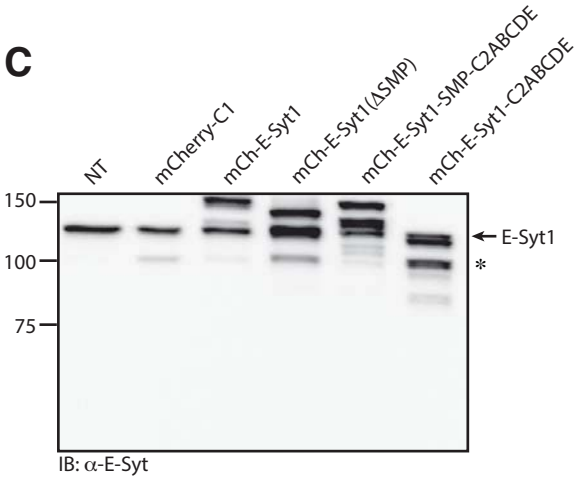
A

Fusion protein	MW
mCh-E-Syt1:	151 kDa
mCh-E-Syt1-SMP-C2ABCDE:	141 kDa
mCh-E-Syt1(ΔSMP):	125 kDa
mCh-E-Syt1-C2ABCDE:	116 kDa
mCh-E-Syt1-C2ABCD:	101 kDa
mCh-E-Syt1-C2AB:	64 kDa
mCh-E-Syt1-C2CD:	73 kDa
mCh-E-Syt1-C2C:	53 kDa
mCh-E-Syt1-C2E:	53 kDa
mCh-Syt1-C2AB:	66 kDa
mCherry:	27 kDa
E-Syt1:	121 kDa

B



C



Appendix Figure S1

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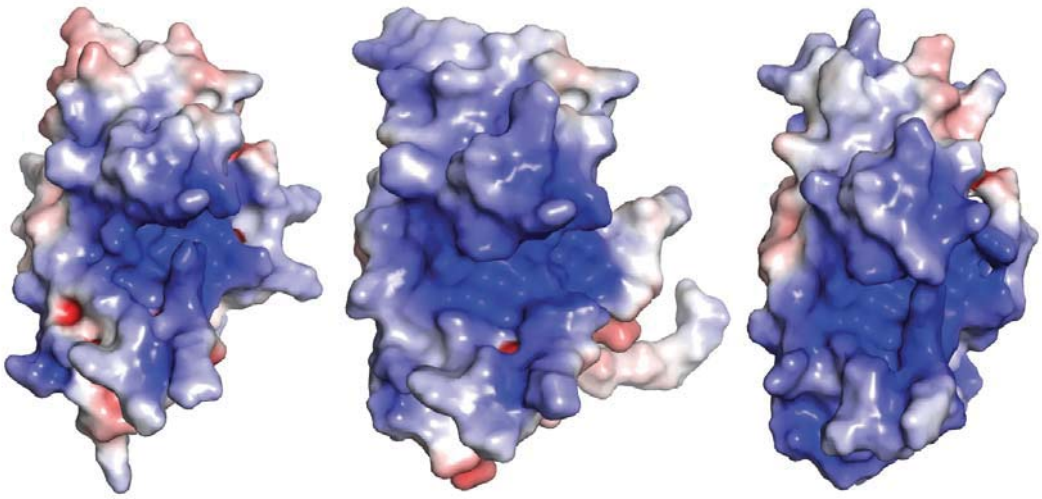

E-Syt1 C2E **E-Syt2 C2C** **E-Syt3 C2C**

Appendix Figure S2

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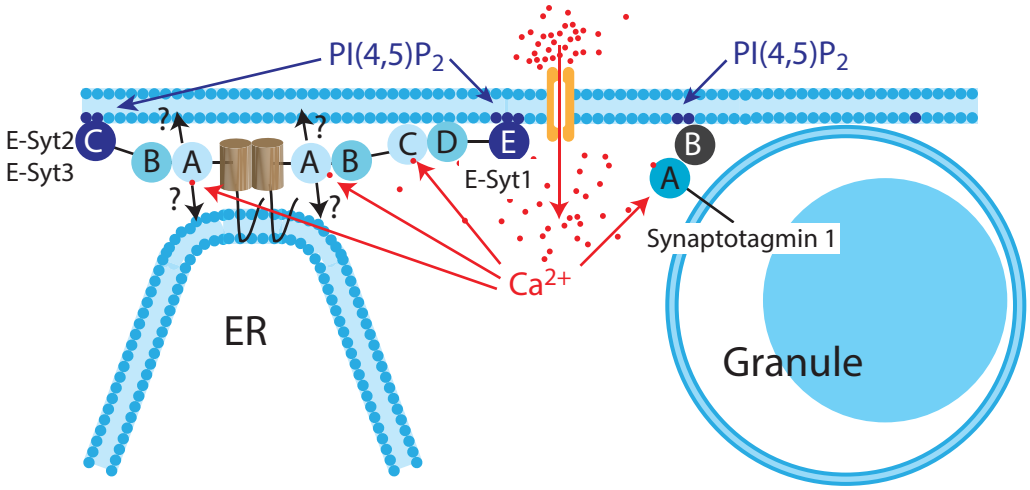

Appendix Figure S3

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Appendix Figure S1 (relates to Figures 1-3). Western blot detection of exogenously expressed proteins in HeLa cells.
A. Predicted molecular weights of mCherry and the mCherry fusion proteins used in the study.
B. Western blot detection of mCherry immunoreativity in HeLa cell lysates.
C. Western blot detection of E-Syt1 immunoreactivity in lysates of HeLa cells expressing the indicated mCherry fusion proteins. NT – non-transfected, * denotes non-specific band.

Appendix Figure S2 (relates to figure 2). Modeling of the C-terminal domains of the E-Syts.
Surface electrostatic potential of human E-Syt1 C2E, E-Syt2 C2C (PDB code 2DMG) and E-Syt3 C2C domains. Positive potential is shown in blue and negative potential in red. Three dimensional models of E-syt1 C2E and E-yt3 C2C domains were generated by Phyre2 (Kelley & Sternberg, 2009).

Appendix Figure S3. Proposed model for Ca^{2+}-induced recruitment of E-Syt1 to the plasma membrane. In excitable cells, Ca^{2+} influx through voltage-regulated Ca^{2+} channels results in exocytosis, triggered by the interaction between secretory vesicle-localized synaptotagmin-1 and plasma membrane $PI(4,5)P_2$. In addition, this stimulus results in E-Syt1/2 recruitment to the plasma membrane. Cooperativity between the Ca^{2+}-binding C2C domain and the $PI(4,5)P_2$-binding C2E domain of E-Syt1 are key to this interaction. The C2A domain of both E-Syt1 and E-Syt2/3 also contains a Ca^{2+} binding site that stimulates binding to lipid bilayers but does not require acidic phospholipids in the bilayer (Xu et al, 2014). Whether this domain binds to the plasma membrane *in trans* or to the ER *in cis* remains unclear ("?").

References

Kelley LA, Sternberg MJ (2009) Protein structure prediction on the Web: a case study using the Phyre server. Nature protocols 4: 363-371

Xu J, Bacaj T, Zhou A, Tomchick DR, Sudhof TC, Rizo J (2014) Structure and Ca(2)(+)-binding properties of the tandem C(2) domains of E-Syt2. Structure 22: 269-280