Supplemental material

Figure S1.  **Syt promotes fusion of both the inner and outer leaflets of SNARE-bearing liposomes.** To determine whether Ca$^{2+}$-syt promoted full fusion or hemifusion, dithionite quenching experiments were performed as previously described (Bhalla et al., 2006). 10 mM dithionite was incubated with Vr-syt to destroy fluorophores on the outer leaflet, thereby revealing the fusion signal for the inner leaflet. Fusion of untreated and treated dithionite vesicles was monitored using the protocol described in Fig. 1 B. Approximately 60% of the fusion signal at all stages of the fusion reaction was quenched by dithionite, demonstrating that Ca$^{2+}$-syt promotes full fusion (i.e., fusion of both the outer and inner leaflets). F.I., fluorescence intensity.

Figure S2.  **Ca$^{2+}$ sensitivity of syt-promoted fusion.** (A) Vr-syt vesicles were incubated with Tr for 20 min followed by injection of Ca$^{2+}$; the final [Ca$^{2+}$] in each reaction is indicated. Membrane fusion was then monitored for 60 min, as described in Fig. 1 B. (B) As a control, fusion reactions were performed in parallel using Vr lacking syt. (C) The final extent of fusion extent from A and B was plotted against the [Ca$^{2+}$]. Circles indicate fusion between Vr-syt and Tr, squares indicate fusion between Vr and Tr, and triangles indicate the difference in the extent of fusion using Vr-syt versus Vr (these data are replotted in Fig. 3 C).

Figure S3.  **C2AB, covalently linked to phospholipids, mimics FL syt during regulated membrane fusion.** (A) Vr bearing C2AB that was conjugated to the head group of PE and incorporated into vesicles was mixed with Tr containing PIP$_2$. Fusion was monitored as described in Fig. 1 B. (B) Samples from A were subjected to SDS-PAGE and stained with Coomassie blue. Mr, molecular mass. (C) The extent of fusion was plotted versus protein copy number. Conjugated C2AB simulated fusion in a manner comparable with FL syt. n = 2; similar results were obtained in each trial.
Figure S4. **C2AB affects FL syt-promoted fusion.** (A) C2AB was incubated with Vr that lacked syt and Tr that harbored 3% PIP2. C2AB alone stimulated fusion; so, in contrast to Fig. 6 (A and B), in the absence of FL syt, inhibition was not observed. (B) The extent of fusion from A was plotted versus [C2AB] (n = 3). (C) syt KO primary chromaffin cells that overexpressed GFP-C2AB were analyzed by immunocytochemistry. Antibodies against GFP and syt were used to confirm the expression of exogenous GFP-C2AB; LDCVs were stained and used as an antibody against chromogranin B (CgB). (D–G) The sizes and rates of the SRP component and sustained release component are plotted for each of the indicated conditions (n ≥ 10); data regarding the RRP are shown in Fig. 6 (D–F). fF, femtofarad. All data shown are represented as mean ± SEM.
Figure S5. **Systematic comparison of FL syt-regulated fusion versus C2AB-regulated fusion.** (A–C) Data (only the Ca\(^{2+}\)-dependent component was analyzed) from Fig. 7 were quantified and plotted \( n = 3 \). All data shown are represented as mean ± SEM. preinc, preincubated.

Reference