

Supplemental Materials

Molecular Biology of the Cell

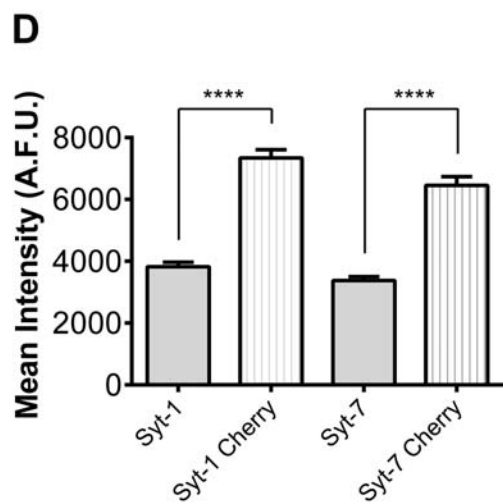
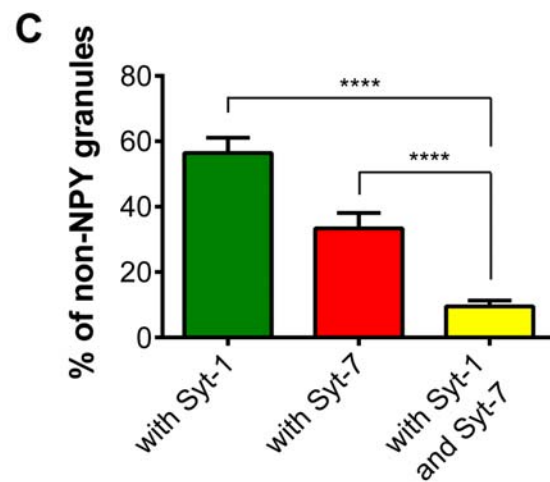
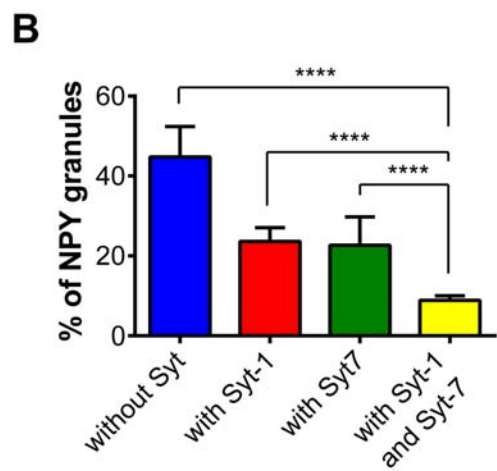
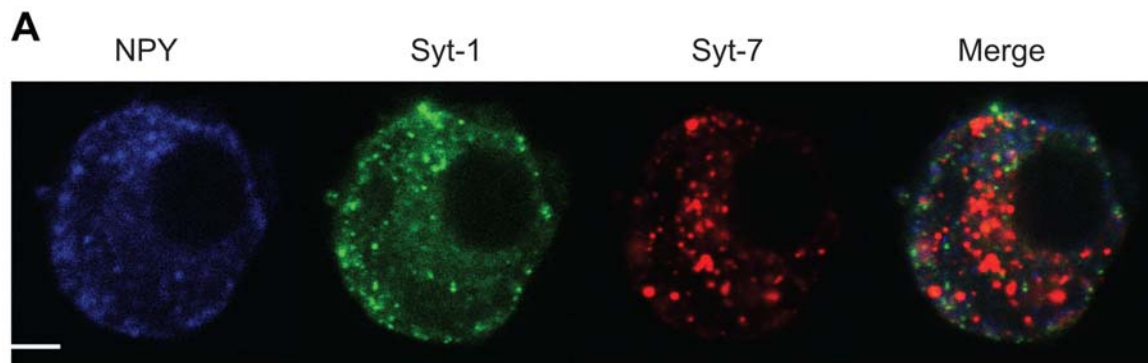
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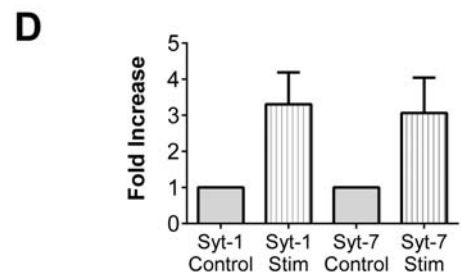
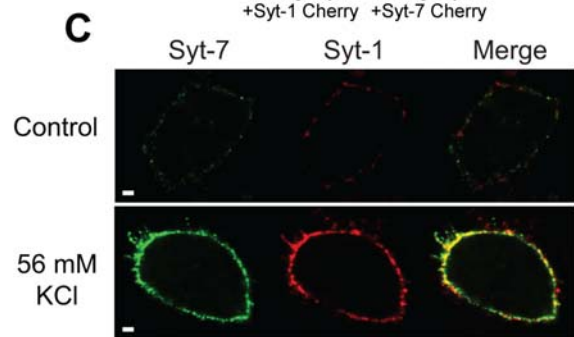
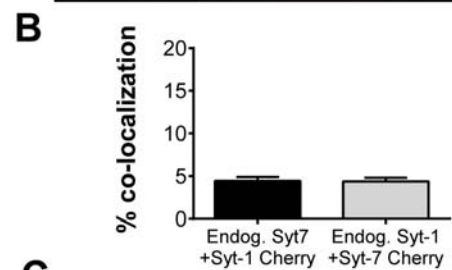
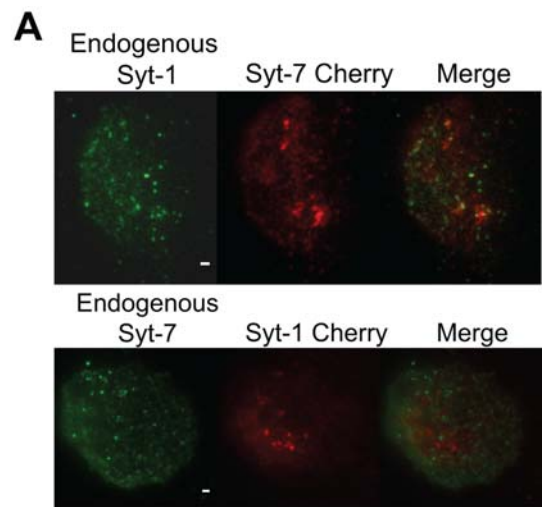
Supplemental Figures.

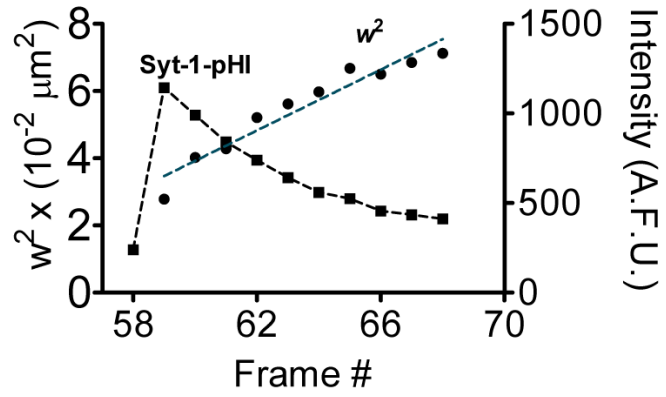
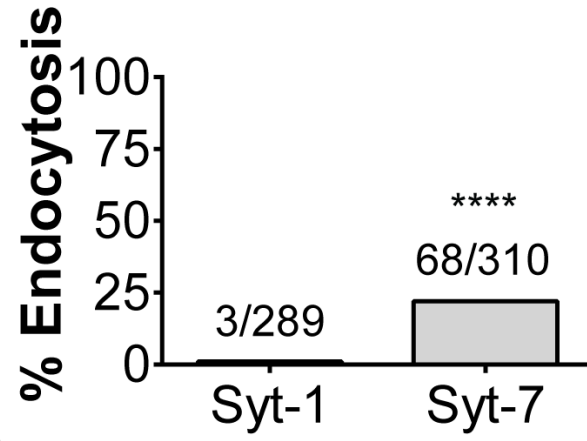
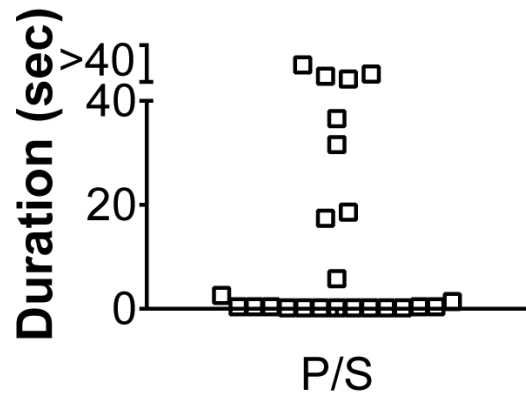
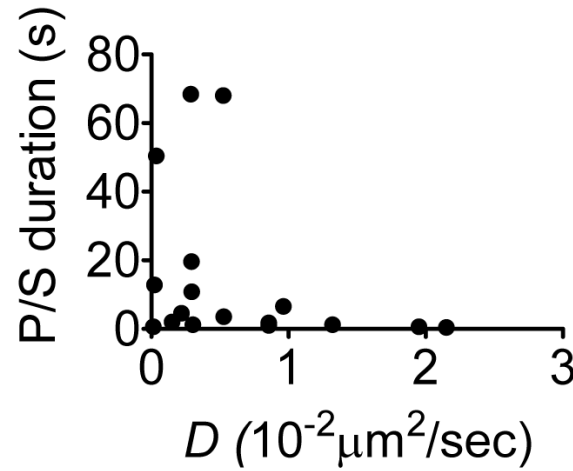
Figure S1. (A) Confocal imaging of overexpressed Syt-1 GFP, Syt-7 Cherry, and a granule lumen protein, NPY-Cer. Scale bar, 3 μ M. (B) Percent co-localization of NPY-Cer granules with Syt-1, Syt-7, or both Syt-1 and Syt-7. Differences between NPY-Cer + Syt-1 + Syt-7 and other groups are significant (**** $p < 0.0001$, Student's t-test). (C) The co-localization of Syt isoforms in granules without NPY is shown. Statistical differences were assessed with the Student's t-test (**** $p < 0.0001$). (D) Expression level of transfected Syt isoforms compared to endogenous protein. Chromaffin cells were transfected with plasmids encoding Syt-1 Cherry or Syt-7 Cherry and stained with the anti-Syt-1 (cytoplasmic domain) or anti-Syt-7 antibody, respectively. The mean pixel intensity for individual granules ($n > 100$ for 6 cells) with Cherry fluorescence (indicating presence of exogenous Syt) was significantly different from that of granules without Cherry fluorescence (indicating only endogenous protein; **** $p < 0.0001$, Student's t test).

Figure S2. (A) Chromaffin cells were stained for endogenous Syt-1 in cells transfected with Syt-7 Cherry or endogenous Syt-7 in cells transfected with Syt-1 Cherry. Scale bar, 1 μ M. (B) The "merge" image of the two channels shows very little co-localization (approximately 4%) between overexpressed Syt-7 and endogenous Syt-1 or overexpressed Syt-1 and endogenous Syt-7. (C) Confocal images of unstimulated (control) or 56 mM KCl PSS. Non-permeabilized cells were exposed to N-terminal luminal domain antibody to Syt-1 or anti-myc antibody to Syt-7 and fluorescent secondary antibodies. The control cell without stimulation shows very little membrane fluorescence while the cell depolarized with 56 mM KCl shows strong fluorescence around the membrane. Scale bar, 4 μ m. (D) The bar graph represents the fold change in membrane intensity of stimulated (Stim) over unstimulated cells (Control). Stim data have been normalized with respect to the mean membrane intensity in the Control cells.

Figure S3. (A) For the event in Figure 5A, the total intensity of Syt-1-pHl and width squared w^2 of the radially averaged Gaussian fit of the intensity profile are plotted. P/S (B) and $P+2S$ (C) lifetimes for individual VMAT-2 fusion events. (D) Endocytic frequency of Syt-1 and Syt-7 pHluorin granules stimulated with 56 mM KCl. Events were tabulated from cells not stained with diD. Syt-7 granules undergo endocytosis with a higher frequency than Syt-1 granules (**** $p < 0.0001$; binomial probability). (E) Scatter plot of curvature duration versus diffusion (D) for individual Syt-1 fusion events.





A**D****B****E****C**