Supporting Information

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SI Text

Whole-Cell Capacitance Measurement. Whole-cell patch clamp experiments were performed 4–6 h after transfection at room temperature. The extracellular solution contained in mM: 145 NaCl, 1 $MgCl_2$, 2.8 KCl, 2 $CaCl_2$, and 10 Hepes pH 7.2, adjusted to 310 mOSM with D-glucose. The whole-cell pipette solution contained in mM: 0.4 fura-4F, 0.4 mag-fura-2 (Invitrogen), 5 o-nitrophenyl-EGTA (NP-EGTA, Synaptic Systems), 100 Cs-glutamate, 0.3 Na-GTP, 2 Mg-ATP, 4 $CaCl_2$ 1 ascorbic acid, and 32 Hepes, pH 7.2. Photolysis of NP-EGTA was achieved by a UV flash lamp (Rapp Optoelektronik). Intracellular calcium measurements were performed by alternating the fura-4F/mag-fura-2 excitation wavelength between 350 nm and 380 nm as described (1). Capacitance changes were measured using an EPC9 using the "sine + dc" technique (2). The amplitude of the exocytotic burst was taken as the capacitance increase 500 ms after the flash. The sustained phase of exocytosis was quantified as the capacitance increase over the next 5 s. Amperometric recordings were performed with homemade 10-μm carbon fiber electrodes fabricated as described (3) using an EPC7 amplifier and filtered at 3 KHz.

1. Voets T (2000) Dissection of three Ca^{2+} dependent steps leading to secretion in chromaffin cells from mouse adrenal slices. *Neuron* 28:537–545.
2. Lindau M, Neher E (1988) Patch-clamp techniques for time-resolved capacitance measurements in single cells. *Pflug Arch Eur J Phy* 411:137–146.
3. Chow RH, Rüden Lv (1995) *Single Channel Recording*, eds Sakmann B, Neher E (Plenum, New York), pp 245–275.

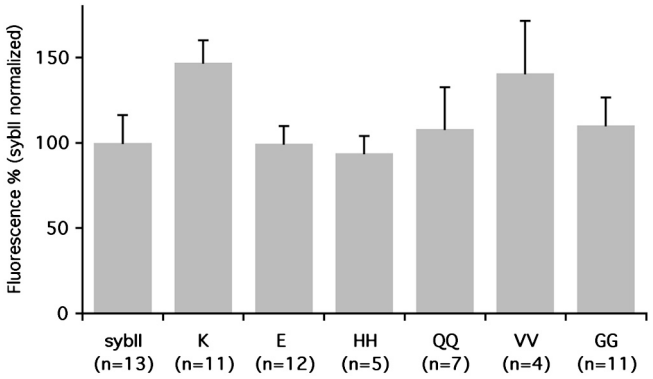
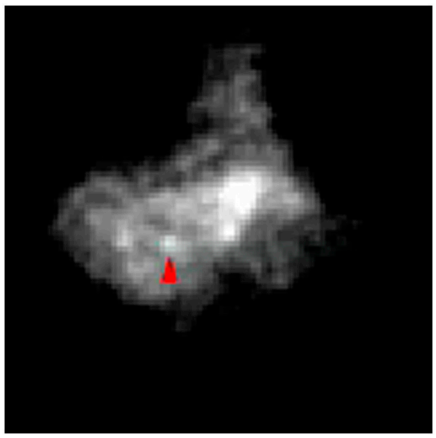


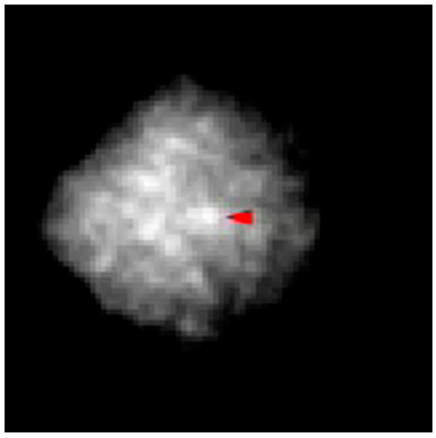
Fig. S1. Analysis of quantitative immunofluorescence staining of sybII and its mutated constructs. Number of cells used to determine mean and SEM of fluorescence intensities for the different constructs are indicated. Cultured chromaffin cells expressing specific constructs were fixed and permeabilized, incubated with mouse anti-sybII and rabbit anti-synaptotagmin-1 antibodies, washed, incubated with secondary antibodies (Alexa Fluor 546-conjugated goat anti-mouse and Alexa Fluor 647-conjugated goat anti-rabbit), washed, and mounted for imaging as described (1). All fluorescence microscope images were acquired by a Sensicam (PCO Imaging) camera using 500-ms exposure time and analyzed using TILLvisION (version 4.0.1; TILL Photonics). Fluorescence intensities were obtained by integrating the intensity within a square region of interest containing the whole cell minus the background intensity determined in another region with the same size. Fluor-546 (sybII) fluorescence data from several cells were averaged and are shown normalized to wild-type sybII overexpression in double knock-out (DKO) cells.

1. Walter AM, Wiederhold K, Bruns D, Fasshauer D, Sorensen JB (2010) Synaptobrevin N-terminally bound to syntaxin-SNAP-25 defines the primed vesicle state in regulated exocytosis. *J Cell Biol* 188:401–413.



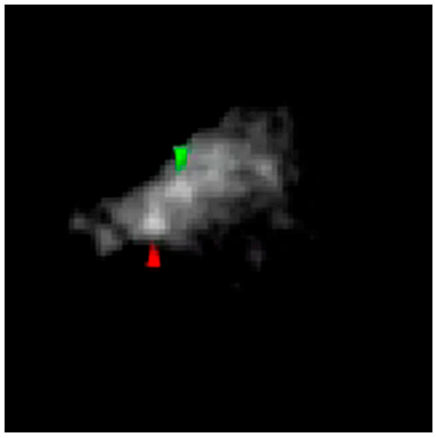
Movie S1. Supporting TIRF movie showing movement of vesicles containing GFP-SybII. Some vesicles appearing in the movie are marked by arrowheads. The movie was created by extracting a series of 501 frames cropped to an 80 × 80 pixel area (13.2 μm × 13.2 μm). The movie plays at approximately twice the actual recording speed.

Movie S1 (MPG)



Movie S2. Supporting TIRF movie showing movement of vesicles containing GFP-SybII-EE. Some vesicles appearing in the movie are marked by arrowheads. The movie was created by extracting a series of 501 frames cropped to an 80 × 80 pixel area (13.2 μm × 13.2 μm). The movie plays at approximately twice the actual recording speed.

Movie S2 (MPG)



Movie S3. Supporting TIRF movie showing movement of vesicles containing GFP-SybII-KK. Some vesicles appearing in the movie are marked by arrowheads. The movie was created by extracting a series of 501 frames cropped to an 80 × 80 pixel area (13.2 μm × 13.2 μm). The movie plays at approximately twice the actual recording speed.

Movie S3 (MPG)

Table S1. Selected whole residue transfer energies from water to membrane interface from ref. 1

Amino acid	ΔG_{wif}, kcal/mol
Gln	0.58 ± 0.08
Glu^{-}	2.02 ± 0.11
Glu^{0}	−0.01 ± 0.15
Gly	0.01 ± 0.05
His^{+}	0.96 ± 0.12
His^{0}	0.17 ± 0.06
Lys^{+}	0.99 ± 0.11
Val	0.07 ± 0.05

1. White SH, Wimley WC (1999) Membrane protein folding and stability: Physical principles. *Annu Rev Biophys Biomol Struct* 28:319–365.