Supplementary Materials and Methods

Capacitance measurements and amperometry

The intracellular solution contained (in mM): 100 Cs-glutamate, 8 NaCl, 4 $CaCl_2$, 32 HEPES, 2 MgATP, 0.3 GTP, 5 nitrophenyl-EGTA (supplied by G. Ellis-Davies, MCP Hahnemann University, Philadelphia, PA), 0.3 Fura-4F (Molecular Probes), 0.3 Magfura-2 (Molecular Probes), pH 7.2. The extracellular bath solution was (in mM): 145 NaCl, 2.8 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES plus 2 mg/ml D-glucose, pH 7.2. Latrunculin A (Calbiochem) was dissolved in the culture medium (1-2 μM for ~12 h) or was applied locally from a separate glass pipette before and during each patch-clamp recording (50μM, ~5 min). Both methods gave similar results and thus the data were pooled. Currents were filtered at 3kHz and sampled at 12 kHz. The kinetics of the capacitative response was analyzed by fitting a sum of exponential functions, as previously described (Nagy et al., 2004). This leads to the determination of the size of the two exocytotic burst components; the fast burst, which represents the fusion of the readily releasable pool of vesicles, RRP, and the slow burst, which represents the fusion of the slowly releasable vesicle pool, SRP (seeVoets, 2000), and their fusion time constant.

Electron microscopy

Chromaffin cells were fixed for 45 min at room temperature with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). In some experiments, *munc18-1* deficient chromaffin cells were incubated for 6 min in 1 mM Latrunculin A (Molecular Probes) in culture medium prior to fixation. After fixation, cells were washed three times 5 min with 0.1 M cacodylate buffer (pH 7.4), post-fixed for 2 hr at room temperature with 1% OsO_4 in bidest, washed and stained with 1% uranyl acetate for 40 min in the dark. Following dehydration through a series of increasing ethanol concentrations, cells were embedded in Epon and polymerized for 24 h at 60°C. After polymerization, the coverslip was removed by alternately dipping in liquid nitrogen and hot water.

TIRF microscopy

For evanescent-wave illumination, a laser beam from an air-cooled argon ion laser (Model 185-F12, 75 mW at 488 nm, Spectra Physics, Stahnsdorf, Germany) was coupled into a 100x 1.45 NA oil-immersion objective on an inverted microscope (Zeiss Axiovert S100 TV, Oberkochen, Germany) via a TIRF condensor (TILL Photonics, Graefelfing, Germany). Footprints of chromaffin cells were imaged in TIRF mode. Images were taken with an intensified CCD camera (PentaMAX, Princeton Instruments, Monmouth Junction, USA) controlled by MetaMorph (Universal Imaging Corporation, Downingtown, USA) or with EMCCD DV-860 (Andor Technology, Belfast, UK) controlled by DaVis 6.2 (LaVision, Goettingen, Germany). Accounting for the CCD pixel size, magnification of the objective and a 1.6x relay lens, the pixel size of resulting images was 141 nm in both X and Y directions.

References:

Nagy, G., Reim, K., Matti, U., Brose, N., Binz, T., Rettig, J., Neher, E. and Sørensen, J.B. (2004) Regulation of Releasable Vesicle Pool Sizes by Protein Kinase A-Dependent Phosphorylation of SNAP-25. *Neuron*, **41**, 417-429.

Voets, T. (2000) Dissection of three Ca2+-dependent steps leading to secretion in chromaffin cells from mouse adrenal slices. *Neuron*, **28**, 537-545.