Deak et al., http://www.jcb.org/cgi/content/full/jcb.200812026/DC1

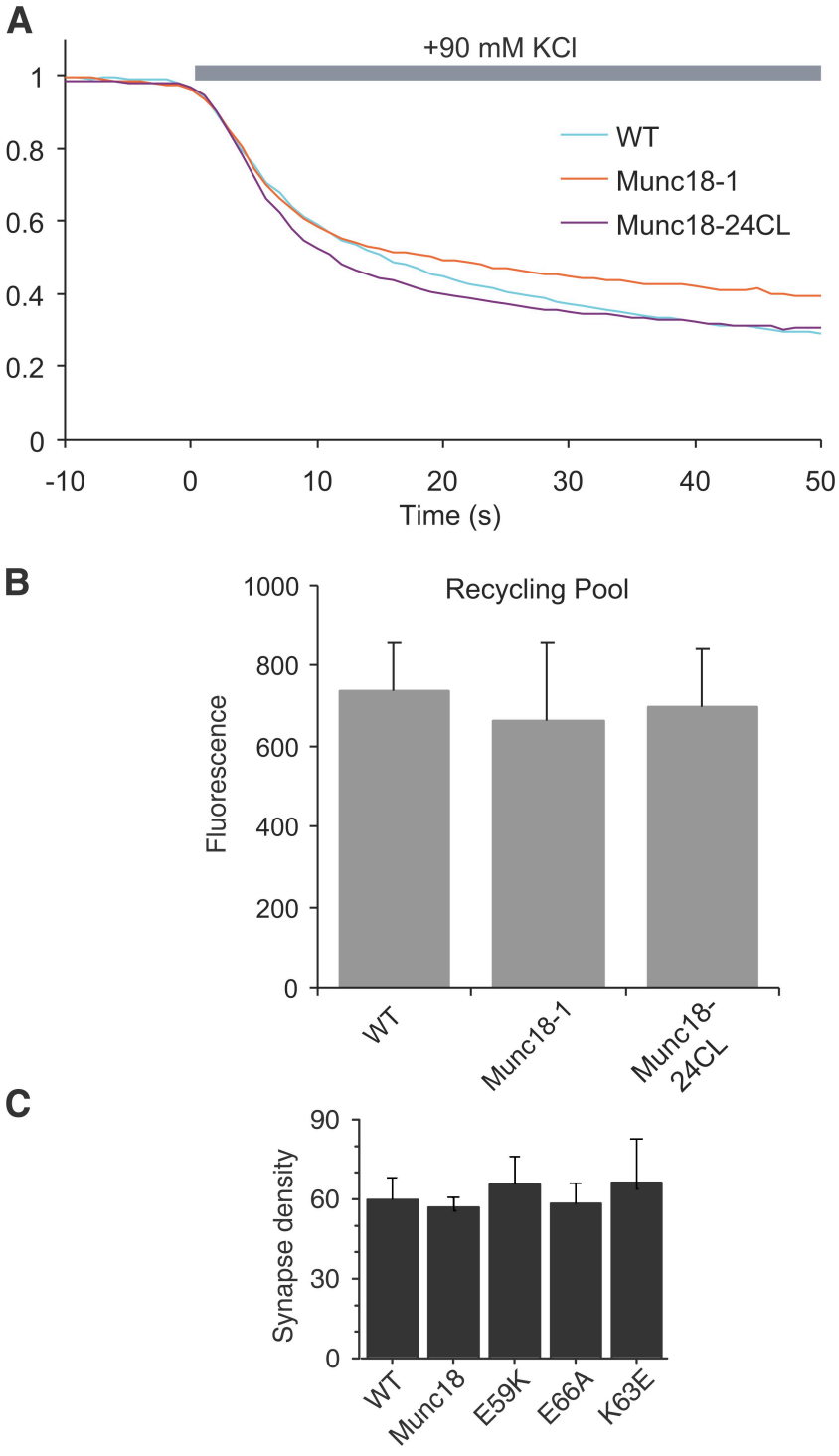

Figure S1. **Comparison of Ca-dependent synaptic release from WT neurons and Munc18-1 KO neurons rescued with WT Munc18-1 or Munc18-1–24-cerulean.** (A) Mean destaining curves during high potassium-induced depolarization. Fluorescence of FM2-10 dye was normalized to the beginning value right before the stimulation (WT control, n = 9 cells; Munc18-1 rescue, n = 3 cells; Munc18-1–24-cerulean rescue, n = 6 cells). An ~310-mosM modified Tyrode bath solution (150 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 10 mM glucose, 10 mM Hepes-NaOH, pH 7.4, 0.01 mM 6-cyano-7-nitroquinoxaline-2,3-dione, 0.05 mM AP-5, and 2 mM $CaCl_2$) was used in all experiments. Synaptic boutons were loaded with 400 µM FM2-10 (Invitrogen) by a 90-s exposure to 47 mM K^+/2 mM Ca^{2+}; full destaining was triggered with a hyperkalemic bath solution containing 90 mM KCl substituted for NaCl. Images were taken after a 10-min wash in a dye-free, nominally Ca^{2+}-free solution to minimize spontaneous release. In all experiments, isolated boutons (~1 μm^2) were selected for analysis. Synaptic vesicle fusion was induced by gravity perfusion of hyperkalemic solution onto the field of interest (1–2 ml/min) for 60 s followed by nominal Ca^{2+}-free Tyrode perfusion for 60 s again. Images were acquired with a cooled charge-coupled device camera (Roper Scientific) during illumination (1 Hz and 200 ms) at 480 ± 20 nm (505-nm dichroic longpass and 534 ± 25–nm bandpass) via an optical switch (Sutter Instrument Co.) and analyzed using Metafluor Software (MDS Analytical Technologies). Background staining levels determined after five consecutive rounds of high K^+ application were subtracted from all fluorescence images. (B) Bar graph depicting the mean size of recycling synaptic pools for WT synapses and synapses rescued with WT Munc18-1 or Munc18-1–24-cerulean. Same experiments as in A. (C) Equal synaptic density in rescued cortical cultures. Synapses were labeled by 200 uM FM2-10 during 47-mM KCl stimulation for 90 s, washed, and recorded. The number of synapses was estimated from 50 × 50–µm sections of fluorescence images offline. Sections (n = 22–50) were analyzed from four independent cultures. Note that no difference is statistically significant. Data are shown as means ± SEMs.

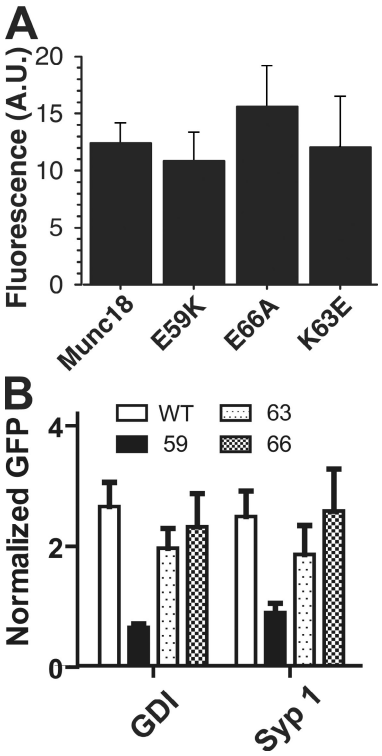

Figure S2. **Expression levels of WT and mutant Munc18-1s.** (A) Quantification by fluorescence microscopy. Fluorescence of cerulean was recorded and compared in synapses infected with WT Munc18-1–24-cerulean (n = 8) or Munc18-1–24-cerulean with the E59K (n = 6), E66A (n = 5), or K63E (n = 5) mutation. Fluorescence intensity acquired on a 200-ms acquisition time is depicted in arbitrary units (A.U.). No difference is statistically significant. (B) Quantification from Western blots. High density hippocampal neurons from Munc18-1 KO mice were infected with WT Munc18-1–24-cerulean or Munc18-1–24-cerulean with the E59K, E66A, or K63E mutation. Cerulean levels were quantified 2–3 wk after infection by phosphoimaging of Western blots performed with GFP antibodies and iodinated secondary antibodies. The data were normalized to the levels of the general marker GDP dissociation inhibitor (GDI) or the neuronal marker Syp1, which were also quantified from Western blots of the same cultures. Data are shown as means ± SEMs.

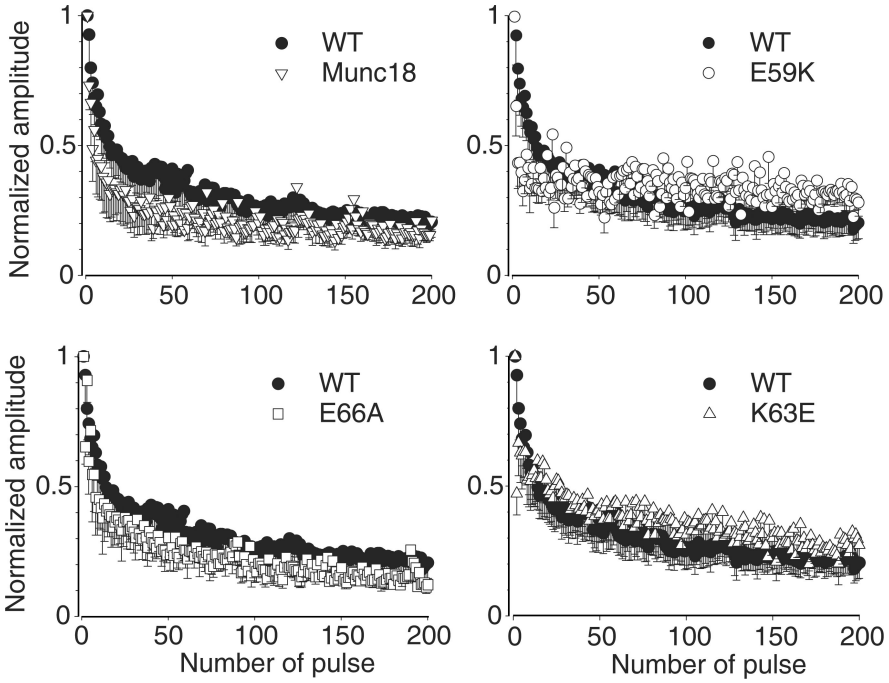

Figure S3. **Synaptic depression with Munc18 mutants.** Relative evoked current amplitudes during 10-Hz field stimulation. Evoked postsynaptic excitatory currents were normalized to the amplitude of the first response in the train (WT, n = 8; Munc18 and E59K, n = 5; E66A, n = 4; and K63E, n = 3). Note the strong synaptic depression for all Munc18-1 variants tested.

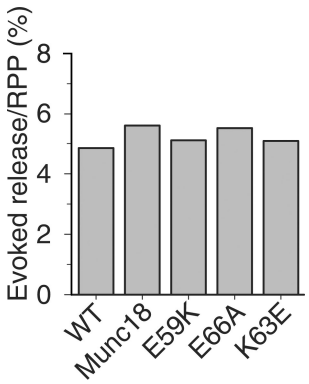

Figure S4. **The Munc18-1 mutations cause parallel decreases in evoked release and RRP.** The bar diagram shows the ratios between the mean synaptic charge transfer with a single action potential (Fig. 5 E) and the mean synaptic charge transfer caused by hypertonic sucrose (Fig. 6 C) in WT neurons and Munc18-1 KO neurons rescued with the WT Munc18-1–24-cerulean or Munc18-1–24-cerulean bearing the E59K, K63E, or E66A mutation.

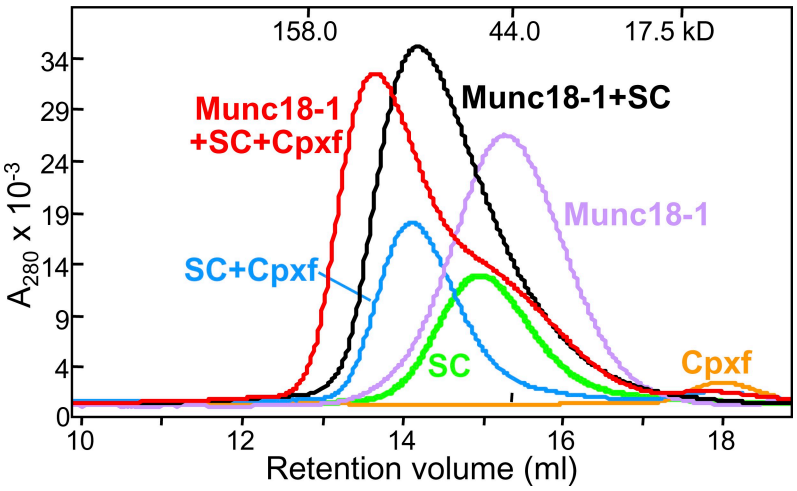

Figure S5. **Gel filtration on a Superdex S200 (10/300GL) column of Munc18-1, complexin-1(26–83), SNARE complex, and mixtures of the SNARE complex with Munc18-1, complexin-1(26–83), or both.** The SNARE complexes used for all of these experiments contained syntaxin-1(2–253), synaptobrevin-2(29–93), SNAP-25(11–82), and SNAP-25(141–203). Cpxf, complexin-1(26–83); SC, SNARE complex.