Figure S1. Total vesicle number for all experimental conditions.

The different expression level of Munc18-1 in null mutants, heterozygotes or wildtype cells does not affect the total number of secretory vesicles in chromaffin cells. The same holds true for SFV infection of null mutant or wildtype cells with *egfp* or *munc18-1*. Although incubation with LatrunculinA (Lat A) increases vesicle docking in *munc18-1* null mutants it does not affect the total number of vesicles. Expression of TeNT increases the total number of vesicles (** indicates $p < 0.01$ compared to wildtype, t-test), whereas BoNT-C does not. The number of vesicles was quantified in the following number of cells (n) and animals (N) for each condition: null: n=23, N=6; null+EGFP: n=19, N=4; null+Lat A: n=20, N=3; null+Munc18: n=2-, N=6; heterozygotes: n=20, N=4; wildtype: n=26, N=6; wildtype+EGFP: n=20, N=4; wildtype+Munc18: n=20, N=4; wildtype+BoNT-C: n=20, N=4; wildtype+TeNT: n=22, N=4.

Figure S2. Reintroduction of Munc18-1 rescues Ca^{2+}-triggered large dense-core vesicle exocytosis in *munc18-1* null mutant cells.

(A-C) Secretory responses in *munc18-1* heterozygote cells (control, n=36 cells, N=8 animals), in *munc18-1* null cells (null, n=21, N=4) and in null cells overexpressing *munc18-1* (null+Munc18-1, n=21, N=5). (A) Secretion was elicited by flash photolysis of caged-Ca^{2+} (at arrow), which resulted in similar intracellular [Ca^{2+}] steps in all experimental groups. Note that also the basal [Ca^{2+}] before the flash was similar for all groups. Secretion was assayed simultaneously with membrane capacitance measurements (B) and amperometry (C), which measures the increase in plasma membrane area and the liberation of catecholamines, respectively, as a result of vesicle fusion. Averaged traces

are shown; the individual amperometric spikes are therefore barely recognizable. The amperometric current was integrated over time to obtain the cumulative charge, which mirrors the capacitance increase except for an additional diffusional delay (C, right ordinate axis). (D-F) A second stimulation by Ca^{2+}-uncaging (2nd flash) 1-2 minute after the 1st flash elicited similar responses in control cells and in null cells overexpressing *munc18-1*, indicating intact vesicle pool refilling after depletion in rescued cells. (G-H) Amplitudes of the different kinetic components of the 1st and 2nd flash responses, respectively. *Left*: the capacitance increase occurring within the first 0.5 s after the flash, and during the following 4.5 s, respectively. *Right:* Following kinetic analysis the amplitudes of the fast burst component (representing the fusion of the readily-releasable vesicle pool) and the slow burst component (representing the fusion of the slowly-releasable vesicle pool) are shown. *** $P<0.001$ (Mann-Whitney). (I-J) Time constants of the fast and slow burst components of the 1st and 2nd flash responses, representing vesicle fusion rates.

Figure S3. Comparison between *munc18-1* heterozygote and wildtype cells.

(A-C) Secretory responses in *munc18-1* heterozygote cells (hetero, n=60, N=5) and in wildtype cells (wildtype, n=58, N=5). For explanation see the legend to Supplementary Figure S2. (D-F) A second stimulation by Ca^{2+}-uncaging (2nd flash) 1-2 minute after the 1st flash elicited similar responses in heterozygote and in wildtype cells, indicating intact vesicle pool refilling after depletion in heterozygote cells. (G-H) *Left:* amplitudes of the fast and the slow burst components for the first and the second stimulation, respectively. *Right:* Sustained rates of secretion, measured between 0.5 and 5 s. after the flash

stimulation. (*) Mann-Whitney test: P<0.001; Two way ANOVA: P=0.07. (I-J) Time constants of the fast and slow burst components of the 1st and 2nd flash responses, representing vesicle fusion rates.

Figure S4. Syntaxin cleavage by BoNT-C does not affect vesicle hit-rate and lifetime distribution

(A) Lifetime distribution of vesicles hitting the plasma membrane during 180 s (600 frames) observations at 3.3 Hz in E18 wildtype chromaffin cells eight hours after infection with SFV particles expressing NPYVenus (WT) and BoNTC_ires_NPYVenus (WT + BoNT-C). No differences were observed in the number of visitors (lifetime <1 sec), short-retained vesicles (1-10 sec) and long-retained vesicles (>10 sec).

(B) Average vesicle abundance at any given time during image acquisition. Total number of vesicles analyzed are 4108 and 2581 for WT (n=32), WT + BoNT-C (n=29 cells).

Chromaffin cells infected with BoNTC_ires_NPYVenus for six hours were secretion deficient upon depolarization with high potassium solution indicating the effectiveness of syntaxin cleavage by BoNTC (not shown). Frequencies are normalized to membrane unit area of the cell's footprint and observation time and logarithmically binned.

Figure S5. Localization of endogenous syntaxin 1 in embryonic chromaffin cells

(A, B) Equatorial confocal sections of immunofluorescence labeling of syntaxin 1 (green) in embryonic chromaffin cells show that syntaxin 1 is present at the plasma membrane. (A) Two adjacent cells at low magnification. (B) Single chromaffin cell. Embryonic

(E18) chromaffin cells were fixed at 2 DIV and stained with polyclonal antibody I378 (Hata et al., 1993) against syntaxin 1. Bars are 2μm.

Reference:

Hata, Y., Slaughter, C.A. and Sudhof, T.C. (1993) Synaptic vesicle fusion complex contains unc-18 homologue bound to syntaxin. *Nature*, **366**, 347-351.