Supporting Information

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Fig. S1. Exocytosis triggered by photolyzing caged Ca\(^{2+}\) at \(-500\) nM basal calcium concentration. (A–C) Averaged intracellular Ca\(^{2+}\) concentration (A), averaged secretion as monitored with membrane capacitance measurements (B) and with amperometry (C) from CPX II\(^{-/-}\) (blue), heterozygous (red) and WT (black) cells. (Inset) \(C_m\) traces were scaled to the same amplitude at 1 sec after flash to compare the kinetics of \(C_m\) increases. The amperometric traces were also integrated to show the cumulative secretion (C). (D) Analysis of the size of burst phase (0–1 sec after flash) and sustained phase (1–5 sec after flash) by capacitance measurements. Number of cells is indicated in A. The burst size in CPX II\(^{-/-}\) cells was significantly (***, \(P < 0.001\), one-way ANOVA with Tukey–Kramer posttest) smaller than both CPX II\(^{+/+}\) and CPX II\(^{+-}\) cells.
Fig. S2. Rescued secretion triggered by photolyzing caged Ca\(^{2+}\). (A–C) Averaged intracellular Ca\(^{2+}\) concentration (A), averaged secretion as monitored with membrane capacitance measurements (B) and with amperometry (C) from WT cells expressing only EGFP (black) and CPX II\(^{+/−}\) cells rescued with CPX II (red). (Inset) \(C_m\) traces were scaled to the same amplitude at 1 s after flash to compare the kinetics of \(C_m\) increases. (D) Analysis of the size of burst phases and sustained rate of secretion by capacitance measurements. Data were averaged from 8 experiments from 6 WT cells expressing EGFP, 3 animals (black, \(n = 8\); 18 experiments from 12 CPX II\(^{+/−}\) cells rescued with CPX II, 4 animals (white, \(n = 18\)). (E) Analysis of the time constants of the fast and slow bursts of secretion (WT, black, \(n = 5\); CPX II KO, white, \(n = 18\)). Data were shown as mean ± SEM.
Fig. S3. Representative cosedimentation assay of WT GST-CPX II, mutant GST-CPX II (D29V, R59H, R63A, K69A/Y70A) fusion proteins and GST alone. GST-CPX II WT and the mutant GST-CPX II D29V show normal SNARE complex binding, while mutants GST-CPX II R59H, GST-CPX II R63A and GST-CPX II K69A/Y70A show reduced binding to the SNARE complex.
Fig. S4. Exocytosis in CPX II−/− cells expressing different CPX mutants. (A and B) Ca^{2+} influxes and ΔCm in response to each of the eight 100-ms depolarizations. (C and D) Total Ca^{2+} influx and total ΔCm (summed over all eight depolarizations). Based on the cosedimentation results (Fig. S3), we picked CPX II D29V as the mutant with normal SNARE binding and CPX II K69A/Y70A as the mutant with reduced SNARE binding for the experiments. Data were averaged from 18 experiments from 13 CPX II−/− cells expressing EGFP (gray, n = 18), 29 experiments from 18 CPX II−/− cells expressing CPX II K69A/Y70A and EGFP (white, n = 29), and from 16 experiments from 9 CPX II−/− cells expressing CPX II D29V and EGFP (black, n = 16). Data are expressed as mean ± SEM. **, P < 0.03.
Fig. S5. Spontaneous release in CPX II−/− and WT cells. (A) Sample traces of CPX II−/− (Upper) and WT (Lower) cells. (Inset) the stand-alone foot (SAF) (in the circle) and spikes after high K⁺ stimulus (in the square frame) are amplified to show detail. Traces were filtered at 100Hz to view SAF. (B) Number of SAF during the 20 min recording from CPX II−/− (CPX II KO, white, n = 9 cells) and WT (WT, black, n = 7 cells) cells.