Expanded View Figures

Figure EV1. Representative slices through tomograms.

A, B  Tomographic slice without (A) and with (B) segmentation of synaptosome with late-fusion events.
C, D  Tomographic slice without (C) and with (D) segmentation of WT SNAP-25 neurons.

Data information: segmentation colors: off-white — cell outline; pink — active zone; blue — synaptic vesicles; green — mitochondria; yellow — connectors and red — tethers. Scale bar, 100 nm.
Figure EV2. 3-D rendered segmented tomograms of neuron synapses.

A–C (A) SNAP-25 WT, (B) SNAP-25-4E, and (C) SNAP-25-4K. (left) Overview, (right) detail. Blue: synaptic vesicles; purple: active-zone plasma membrane; green: endoplasmic reticulum-like organelle; yellow: connectors; and red: tethers. Scale bars: 100 nm.
Figure EV3. Additional SV tethering and connectivity data.

A, B: Histogram of the number of tethers per proximal SV. Statistical test: pairwise $\chi^2$ test between control and each experimental condition in the zero-tether group with Benjamini–Hochberg correction. *$P<0.05$.

C, D: Histogram of connected SV among tethered or non-tethered proximal SVs.

E, F: Histogram of connected SV among proximal non-RRP or RRP SVs.

Data information: (A, C, E) Synapses in mouse cultured neurons. (B, D, F) Rat synaptosomes.
Figure EV4. Tethered connected SVs.
A, B  Tomographic slices showing tethered connected vesicles. Blue arrows highlight the connectors. Scale bar, 50 nm.