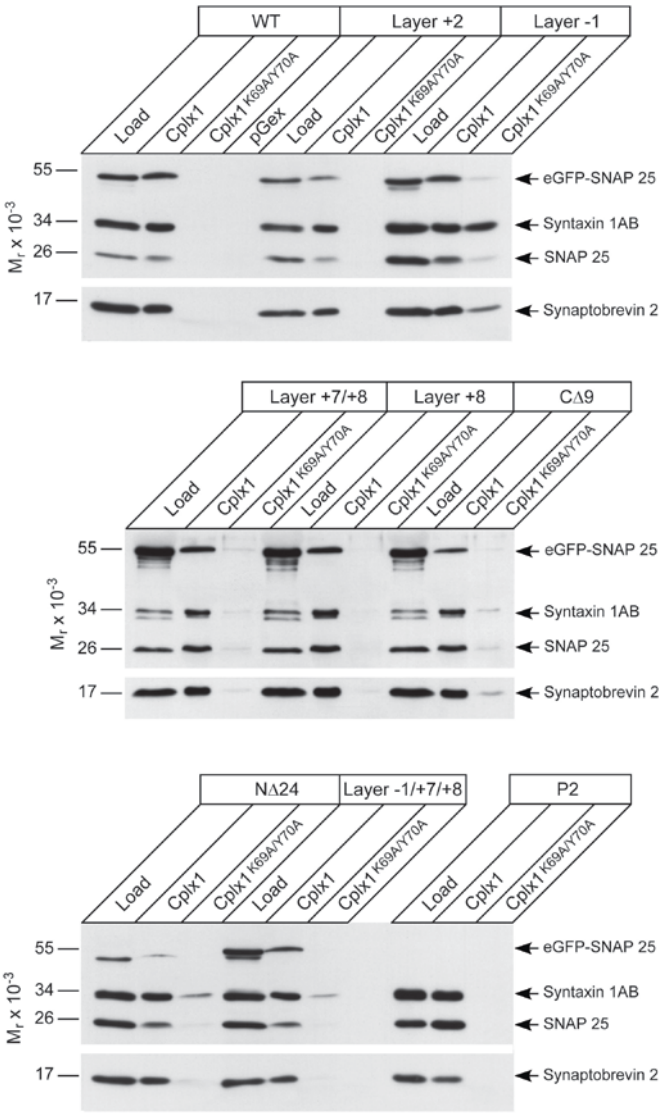
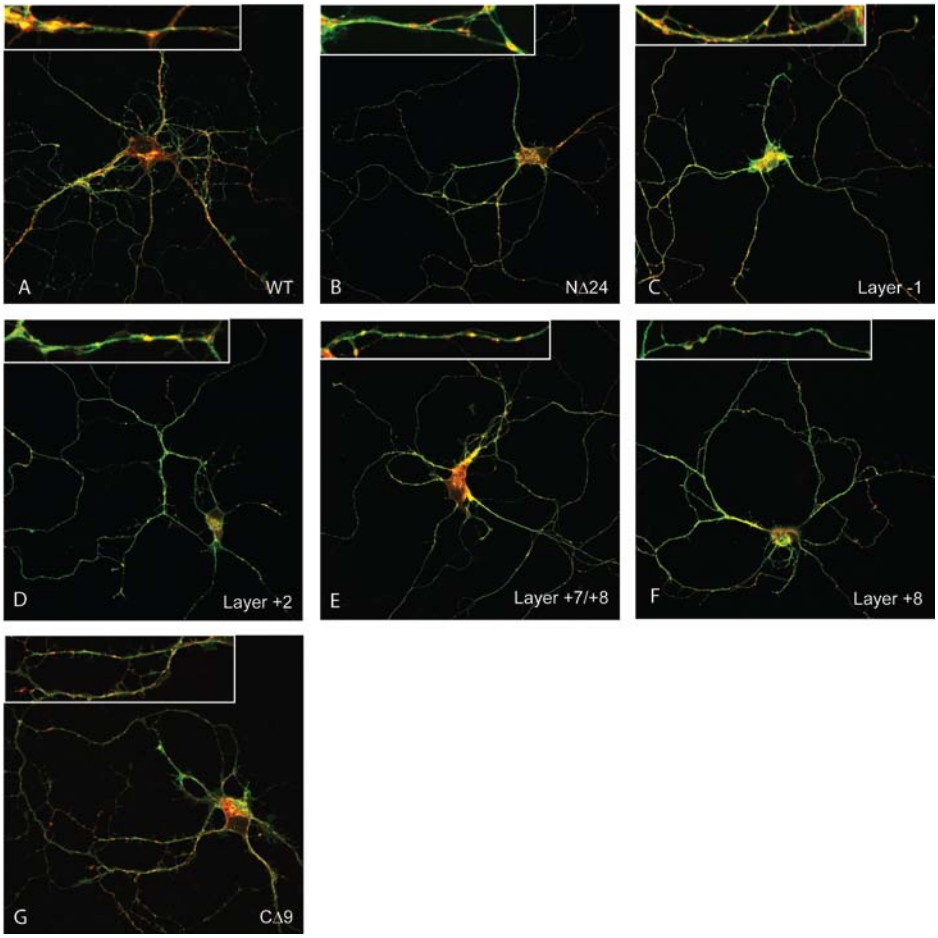
## Supplementary Figures




Weber et al, Suppl. Fig. 1

**Supplementary Figure 1:** Co-sedimentation assay of GST-fused WT or mutant complexin-I with protein extracts from cultured neurons. Complexin I (Cplx-I) and K69A/Y70A mutant were expressed as GST-fusion proteins, immobilized on glutathione agarose, and incubated with solubilized proteins from neurons cell cultures expressing different SNAP-25 constructs fused N-terminally to EGFP. Bound material was analyzed by Western blotting using antibodies to the indicated proteins. Rat brain synaptosomes (P2 fraction, bottom panel right) were used as positive control, and for each construct, complexin K69A/Y70A was used as a negative

control, since it does not bind to SNAREs (Xue et al, 2007). The overexpression level of EGFP-SNAP-25 fusion protein was estimated from the “load” (i.e. on protein extracts before sedimentation) by comparing the intensity of the 53-kDa band to the 25-kDa band, which represents endogeneous SNAP-25. This was 2.1-fold (WT EGFP-SNAP-25), 1.2-fold (Layer +2), 1.0-fold (Layer -1), 3.9-fold (Layer +7/+8), 3.1-fold (Layer +8), 2.3-fold (C-Δ9), 0.4-fold (N-Δ24) and 1.4-fold (Layer -1/+7/+8). All constructs displayed binding to complexin, as shown by comparing the “CplxI” lanes to the “Load” lane. Some unspecific binding (lane “CplxI K69A/Y70A”) was found for some of the mutations, but this was always less than the amount of specific binding.




**Supplementary Figure 2:** Localization of expressed EGFP-SNAP-25 constructs. A-E: co-localization of the expressed EGFP-SNAP-25 constructs (green) and synaptobrevin-2, visualized by antibody staining (red). All SNAP-25 constructs were found distributed broadly along the neurites and overlapping with synaptobrevin-2 staining.

A: EGFP-SNAP-25.

B: EGFP-SNAP-25 NΔ24.

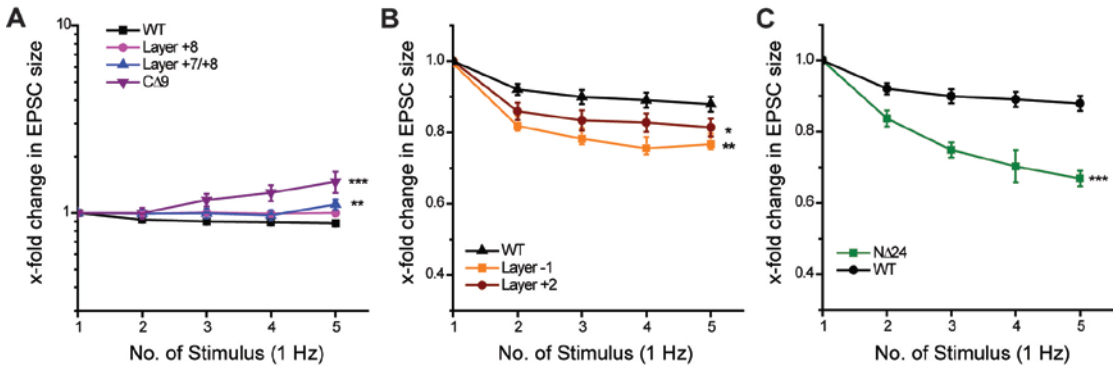
C: EGFP-SNAP-25 Layer-1.

D: EGFP-SNAP-25 Layer+2.

E: EGFP-SNAP-25 Layer +7/+8.

F: EGFP-SNAP-25 Layer +8.

G: EGFP-SNAP-25 CΔ9.



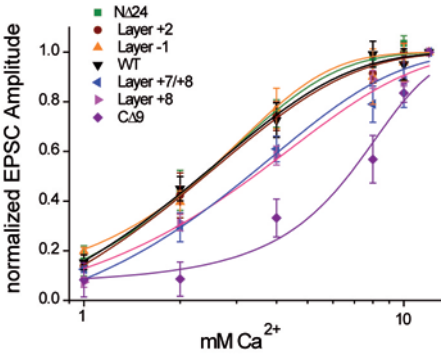

**Supplementary Figure 3:** Short-term synaptic plasticity at 1-Hz frequency.

The neurons were stimulated with five AP, and the EPSCs normalized to the first response.

A: Short-term synaptic plasticity in the presence of C-terminal mutants.

B: Short-term synaptic plasticity in the presence of mutations in the middle of the SNARE-complex.

C: Short-term synaptic plasticity in the presence of a N-terminal deletion. Note that the N-Δ24 mutant displays more depression relative to the WT situation at 1-Hz than at 50-Hz (comp. Fig. 6C). The most likely reason for this is that at 1-Hz EPSC-size is affected by the lower re-priming rate of this mutant.




**Supplementary Figure 4:** Extracellular calcium-dependence of SNAP-25 mutants. Neurons were stimulated at 0.2 Hz in the presence of different extracellular calcium concentrations. For each cell, the responses were normalized to the EPSC size obtained at 12 mM extracellular $Ca^{2+}$. All C-terminal mutants displayed a right-ward shifted calcium-dependence.

**References for Supplementary Figures:**

Xue M, Reim K, Chen X, Chao HT, Deng H, Rizo J, Brose N, Rosenmund C (2007) Distinct domains of complexin I differentially regulate neurotransmitter release. *Nat Struct Mol Biol*