Supplementary information

A coiled-coil nucleation site is essential for rapid binding of synaptobrevin to the SNARE acceptor complex

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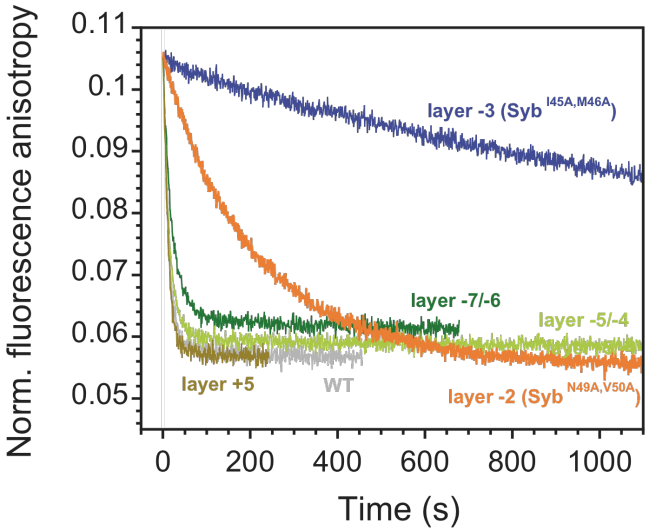

Fig. S1: Comparison of the displacement rates of the Alexa488-labeled synaptobrevin induced by different synaptobrevin layer mutants.

Displacement is visible by a decrease in fluorescence anisotropy. About 100 nM of the ΔN complex was incubated with different synaptobrevin mutants (500 nM). Note that displacement kinetics were very slow for Syb^{I45A,M46A} and Syb^{N49A,V50A}, whereas alanine double mutations in the coiled coil layers upstream of this region (i.e. Syb1-96^{L32A,T35A} and Syb1-96^{V39A,V42A}, corresponding to layers -7 & -6 and -5 & -4, respectively, Walter *et al.*, 2010) had a much less severe effect. The displacement speed induced by a synaptobrevin variant carrying an alanine point mutation in the C-terminal region (Syb1-96^{F77A}, layer +5, Walter *et al.*, 2010) was indistinguishable from wild-type synaptobrevin.

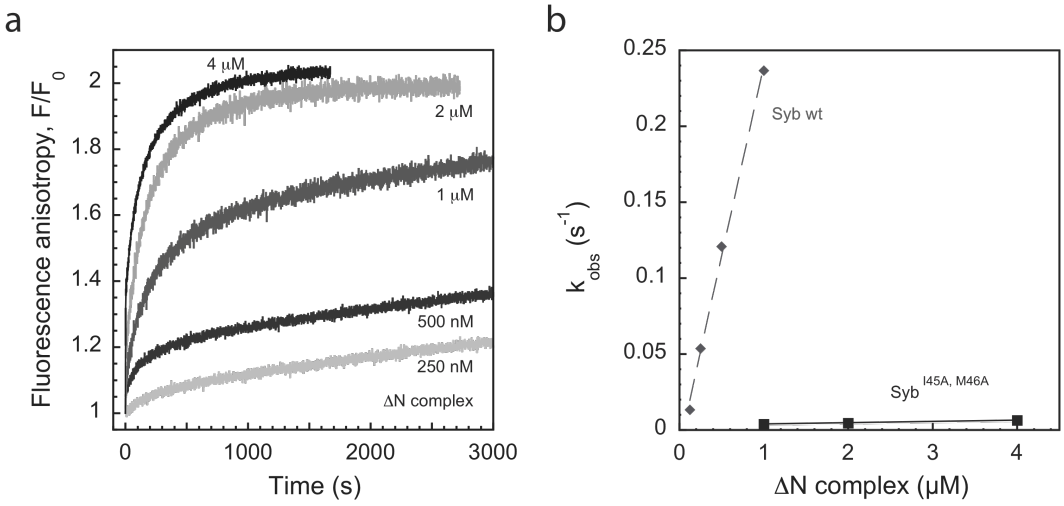

Fig. S2: On-rate of Syb$^{I45A, M46A}$ binding to the ΔN complex.

a) The Alexa488-labeled synaptobrevin mutant Syb$^{I45A, M46A, C28Alexa488}$ binds to the ΔN complex, which is indicated by an increase in fluorescence anisotropy. Syb$^{I45A, M46A, C28Alexa488}$ (100 nM) was mixed with the indicated amounts of purified ΔN complex.

b) The pseudo-first order rate constants obtained from the single exponential fits of the reaction of Syb$^{I45A, M46A, C28Alexa488}$ and of wild-type Syb^{C28Alexa488} (values taken from Walter *et al.*, 2010), with increasing amounts of ΔN complex were plotted against the concentration of ΔN complex. The slope of the linear fit yielded the on-rate of the reaction: ≈ 860 M^{-1} s^{-1} for the double mutant and ≈ 250,000 M^{-1} s^{-1} for wild-type synaptobrevin (Walter *et al.*, 2010). Note, that reactions using 250 nM and 500 nM ΔN complex did not reach saturation and were therefore not included.

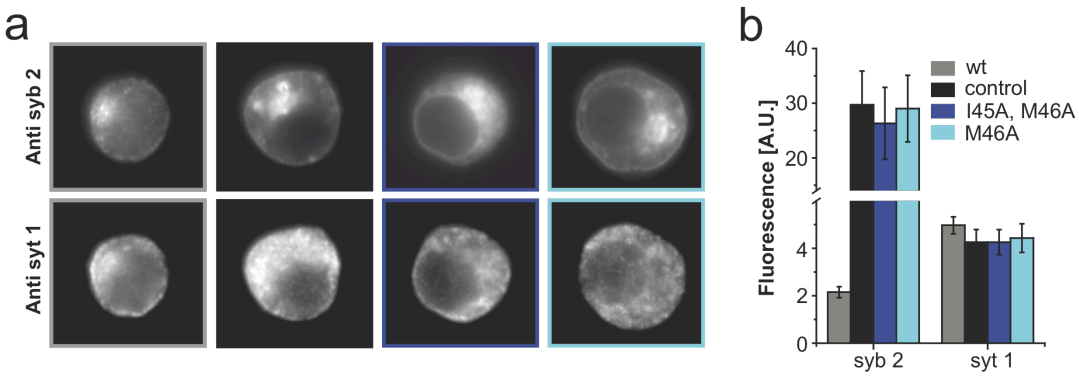

Fig. S3: Immunostainings of overexpressed synaptobrevins.

a) Wide-field fluorescent images of stained and fixed wild-type chromaffin cells, either uninfected (wt) or virally expressing full-length synaptobrevin 2 (control), SybTMR$^{I45A, M46A}$- or SybTMR^{M46A} proteins. Top row: staining for Synaptobrevin 2 (syb 2), indicated by secondary antibody bearing Alexa546. Bottom row: staining for synaptotagmin 1 (syt 1), visualized by secondary antibody bearing Alexa647.
b) Quantification of expression levels by fluorescence intensity (integrated fluorescence over the whole cell).

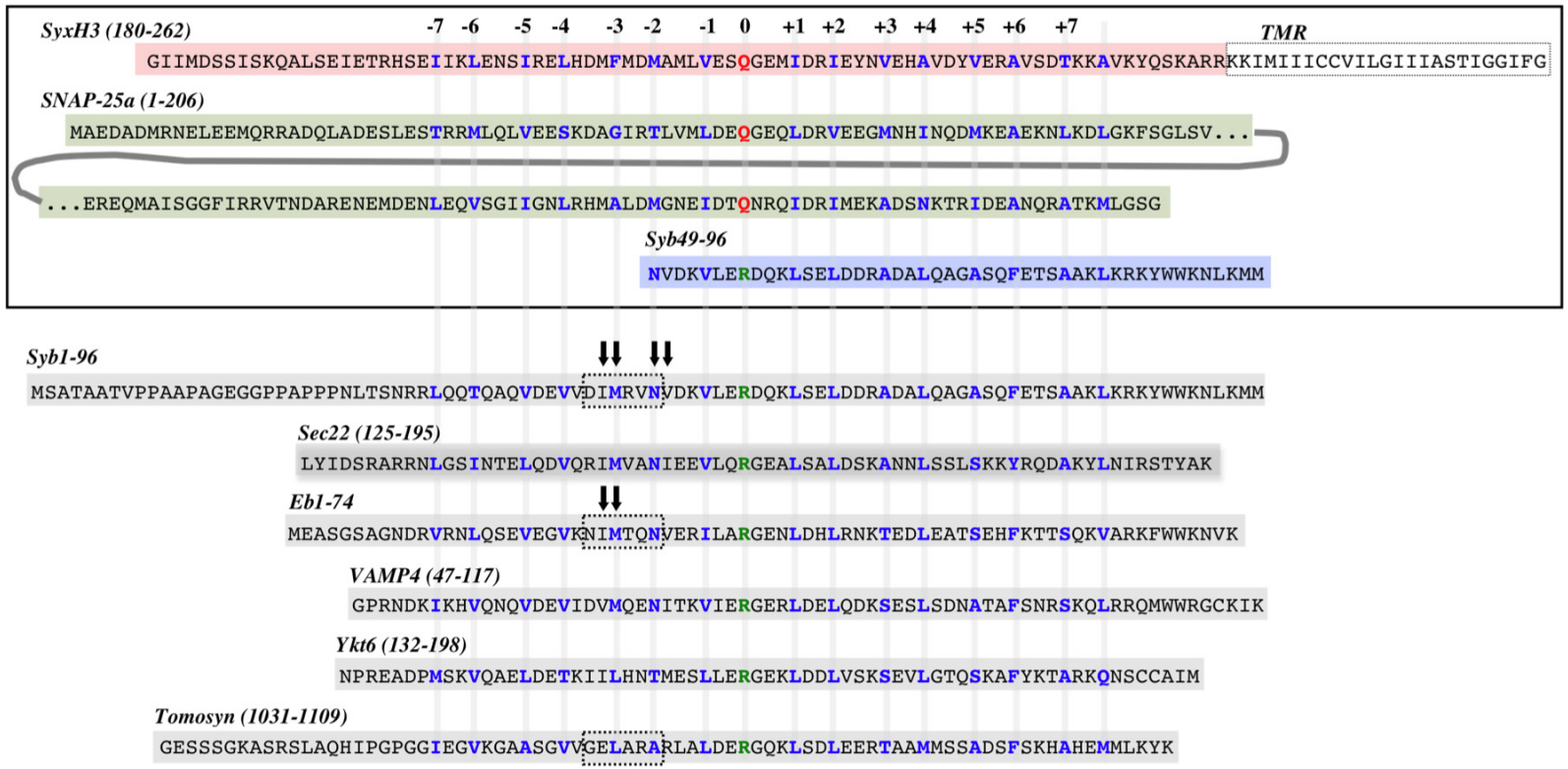

Fig. S4: Schematic representation of the constructs and composition of the ΔN complex used in the study.

All constructs are shown by their amino acid sequence in single letter code. At the top, a box illustrates the composition of the ΔN complex. The interacting coiled-coil layers, numbered from -7 to +8, are indicated. For binding experiments with soluble domains, a ΔN complex was purified that contained the following SNARE protein constructs: SyxH3 (aa 180-262), full-length SNAP-25a (aa 1-206), and Syb49-96. For liposome fusion experiments, the ΔN complex contained the SNARE motif of syntaxin 1a with the transmembrane region (SyxH3TMR, aa 183-288). Below, the constructs of the different R-SNARE homologs used for binding experiments are depicted. Arrows indicate the residues mutated in Syb1-96 and in endobrevin in this study. Dashed boxes indicate the putative trigger sequence region exchanged between tomosyn and synaptobrevin 2, and between tomosyn and endobrevin.

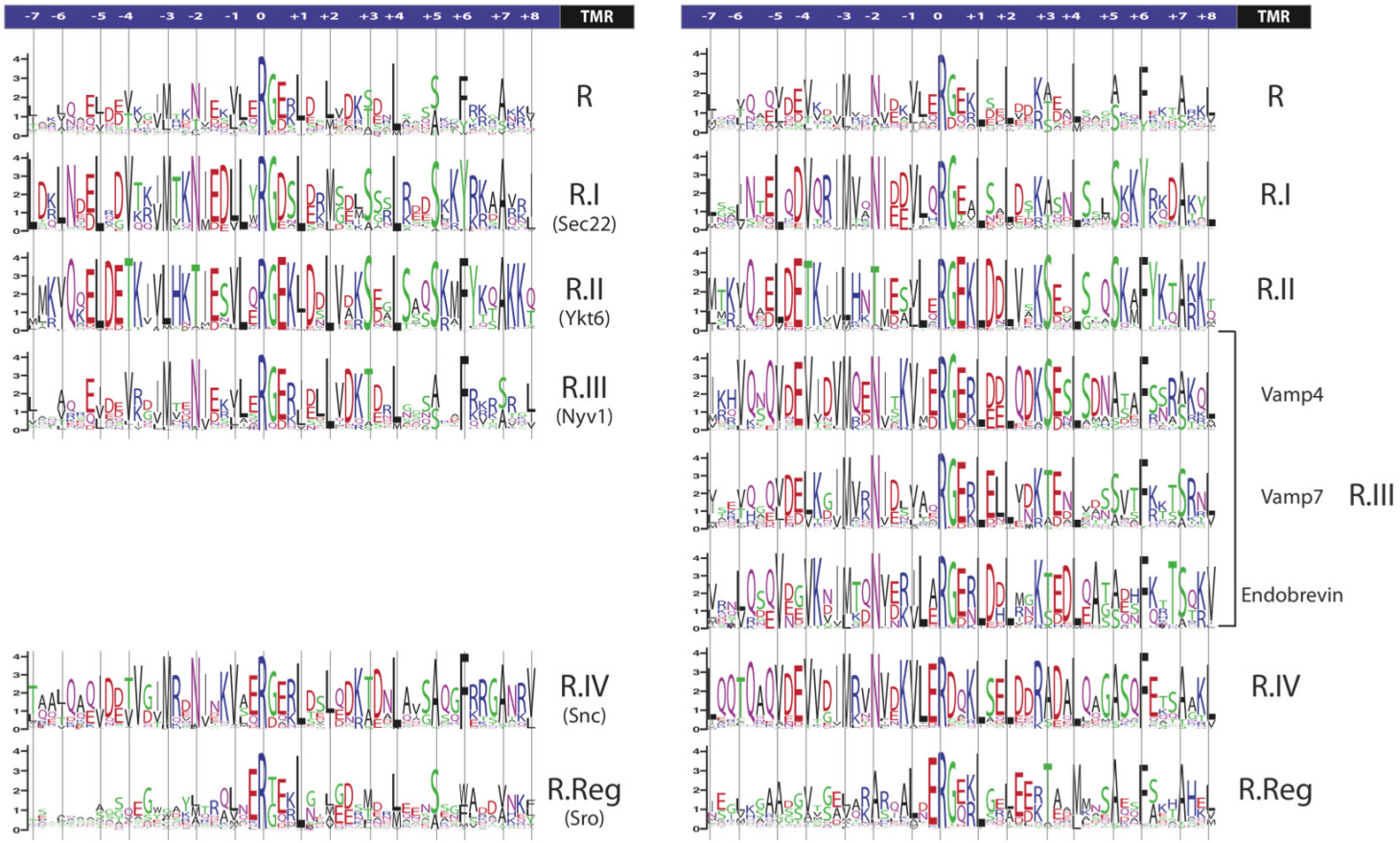

Fig. S5: Conservation of the R-SNARE motifs in fungi and metazoans.

We used the same approach as in Fig. 1 but divided the dataset into two subgroups. On the left hand side we included R-SNARE homologs only from fungi and on the right hand side those from metazoans only. In addition, we have further divided the metazoan R.III subgroup into the three basic groups: Vamp4, Vamp7, and endobrevin.