The first non Clostridial botulinum-like toxin cleaves VAMP within the juxtamembrane domain

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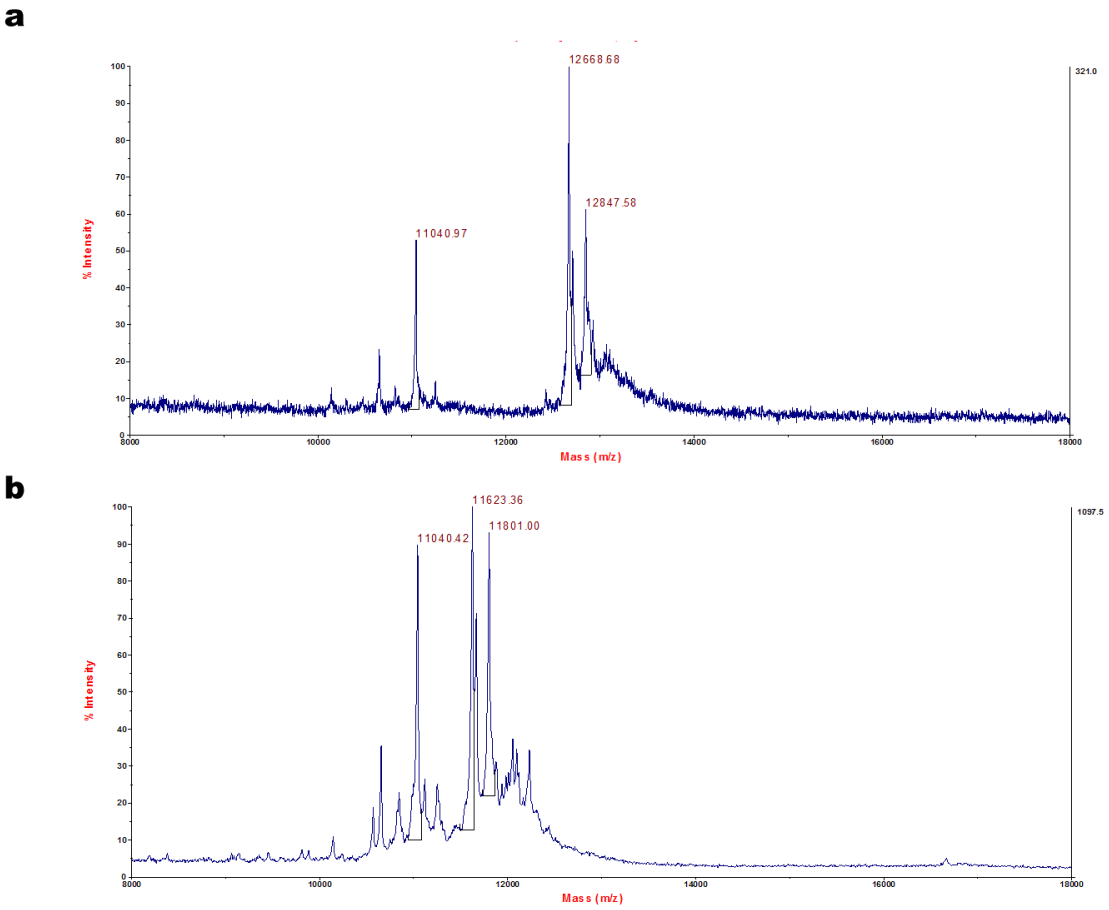

Figure S1. MS spectra of rVAMP2 incubated with Wo-ORF1-LC. MS spectra of rVAMP2 (1-97) acquired in linear mode in the presence (panel a) or absence (panel b) of EDTA. The intact segment 1-97 (m/z 12668.7 Da, panel a) is cleaved by Wo-ORF1-LC with a consequent mass shift at 11623.4 Da (panel b; mass difference of 1045.4 Da). The newly generated peptide (Fig. 4a) was identified with m/z= 1063.69 Da, equivalent to the enzymatic addition of a water molecule to the 1045.4 Da fragment. The peptide sequence was identified by MS/MS as shown in Fig. 4b. The MS spectra analysis was performed twice and representative spectra are shown.

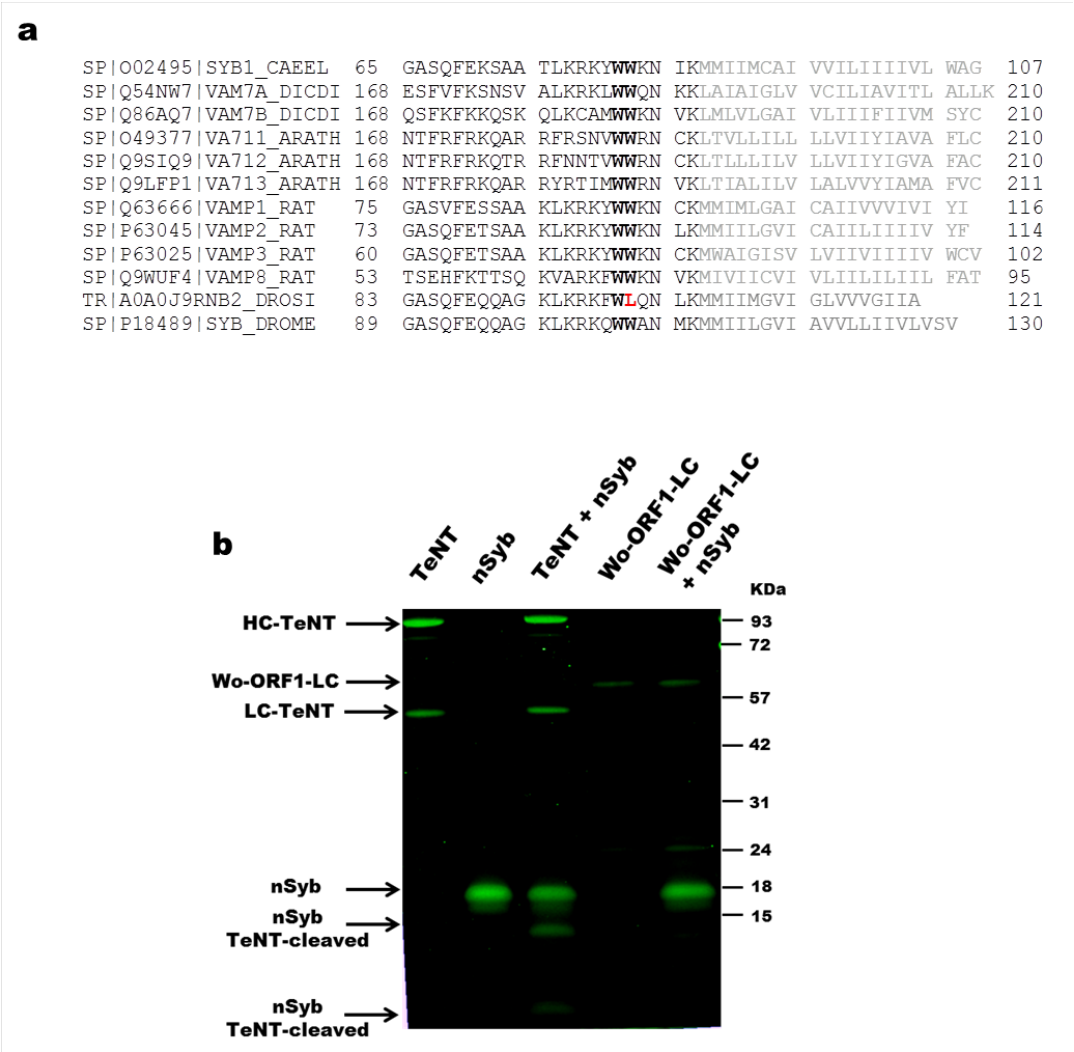

Figure S2. Alignment of the C-terminal part of VAMPs of different organisms and *in vitro* nSyb proteolytic cleavage. For the alignment in (a), we selected the region proximal to the transmembrane, indicated in grey. CAEEL indicates *C. elegans,* DICDI *D. discoideum*, ARATH *A. thaliana*, RAT *R. norvegicus,* DROSI*, D. simulans and* DROME, *D. melanogaster*. (b) 1 µg of nSyb was incubated with 1 µg of Wo-ORF1-LC in 50 mM $NaHPO_4$ buffer supplemented with 0.1% octyl-glucoside, pH 7.4 for 4 hours at 37°C under stirring. SDS-PAGE gel was stained with SimplyBlue™ SafeStain and it was imaged on Odyssey® LI-COR, more sensitive than white-light imaging of Coomassie staining[1]. This image is representative of two independent experiments.

References

1 Luo, S., Wehr, N. B. & Levine, R. L. Quantitation of protein on gels and blots by infrared fluorescence of Coomassie blue and Fast Green. *Anal Biochem* **350**, 233-238 (2006).