

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

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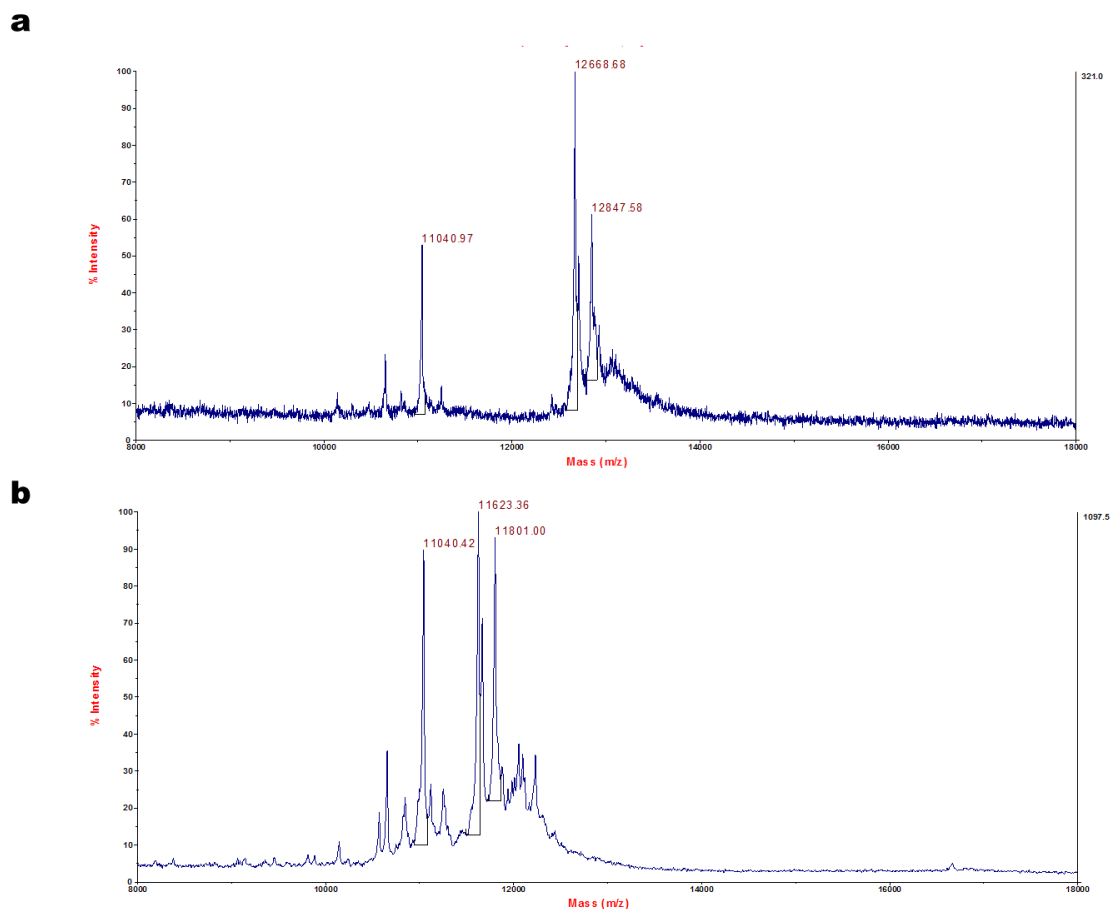


Figure S1. MS spectra of rVAMP2 incubated with Wo-ORF1-LC. MS spectra of rVAMP2 (1-97) acquired in linear mode in the presence (panel a) or absence (panel b) of EDTA. The intact segment 1-97 (m/z 12668.7 Da, panel a) is cleaved by Wo-ORF1-LC with a consequent mass shift at 11623.4 Da (panel b; mass difference 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.

a

SP O02495 SYB1_CAEEL	65	GASQFEKSAA	TLKRKY WW KN	IKMMIIMCAI	VVILIIIIIVL	WAG	107
SP Q54NW7 VAM7A_DICDI	168	ESFVFKSNSV	ALKRKL W QN	KKLAIAIGLV	VCILIAVITL	ALLK	210
SP Q86AQ7 VAM7B_DICDI	168	QSFKFKQSK	QLKCAM W WKN	VKLMLVLGAI	VLIIIFIIVM	SYC	210
SP O49377 VA711_ARATH	168	NTFRFRKQAR	RFRSNV W WRN	CKLTVLLILL	LLVIIYIAVA	FLC	210
SP Q9SIQ9 VA712_ARATH	168	NTFRFRKQTR	RFNNTV W WRN	CKLTLILLIV	LLVIIYIGVA	FAC	210
SP Q9LFP1 VA713_ARATH	168	NTFRFRKQAR	RYRTIM W WRN	VKLTIALILV	LALVVYIAMA	FVC	211
SP Q63666 VAMP1_RAT	75	GASVFESSAA	KLKRKY W WKN	CKMMIMLGAI	CAIIVVVIVI	YI	116
SP P63045 VAMP2_RAT	73	GASQFETSAA	KLKRKY W WKN	LKMMIILGVI	CAIILIIIV	YF	114
SP P63025 VAMP3_RAT	60	GASQFETSAA	KLKRKY W WKN	CKMWAIGISV	LVIIIVIIIV	WCV	102
SP Q9WUF4 VAMP8_RAT	53	TSEHFKTTSQ	KVARK F W W KN	VKMIVIIICVI	VLIIILIIIL	FAT	95
TR A0A0J9RNB2_DROSI	83	GASQFEQQAG	KLKRK F W L QN	LKMMIIMGVI	GLVVVGIIA		121
SP P18489 SYB_DROME	89	GASQFEQQAG	KLKRK Q W W AN	MKMMIILGVI	AVVLLIIVLVS		130

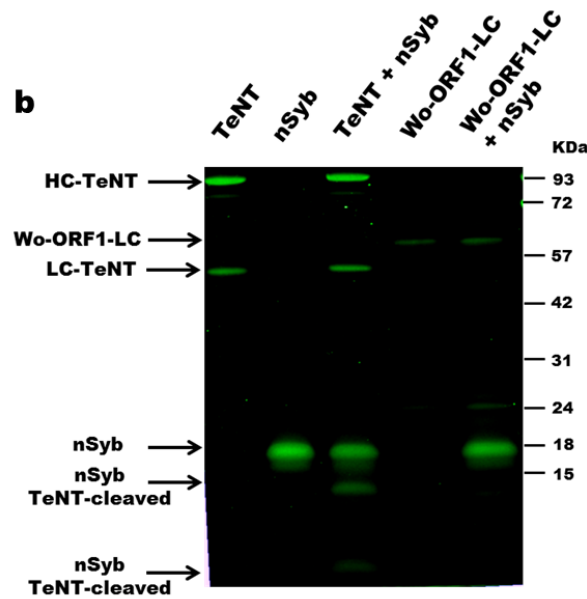


Figure S2. Alignment of the C-terminal part of VAMPs of different organisms and *in vitro* nSyb proteolytic cleavage. For the alignment in (a), we selected the region proximal to the transmembrane, indicated in grey. CAEEL indicates *C. elegans*, DICDI *D. discoideum*, ARATH *A. thaliana*, RAT *R. norvegicus*, DROSI, *D. simulans* and DROME, *D. melanogaster*. (b) 1 μ g of nSyb was incubated with 1 μ g of Wo-ORF1-LC in 50 mM NaHPO₄ buffer supplemented with 0.1% octylglucoside, 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 staining¹. 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).