

## Supplementary Information

### **A camelid single-domain antibody neutralizes botulinum neurotoxin A by blocking host receptor binding**

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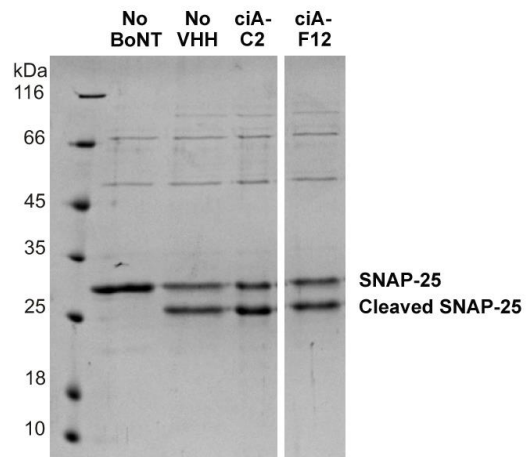
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**Supplementary Figure 1. VHH ciA-C2 and ciA-F12 do not affect the enzymatic activity of BoNT/A1.** *In vitro* SNAP-25 endopeptidase assay where BoNT/A1 (10 nM) was pre-incubated for 15 min at 37 °C with ciA-C2 or ciA-F12 (30 nM), respectively. The BoNT/A1-VHH mixture was then added to recombinant SNAP-25 (3 μM) for 60 min at 37 °C. The lower band shows BoNT/A1-cleaved SNAP-25.

		1064		1112		1146	
HcA1	GCRD	THR	YIWI	...	QYDKP	YYMLNL	...
HcA2	GCRD	PRR	YIMI	...	QYDKP	YYMLNL	...
HcA3	GCRD	PRR	YIMI	...	QYDKP	YYMLNL	...
HcA4	GCRD	PHR	YIVI	...	QYDKS	YYMLNL	...
HcA5	GCRD	PQR	YIWI	...	QYDKP	YYMLNL	...
HcA6	GCRD	PRR	YIMI	...	QYDKP	YYMLNL	...
HcA7	GCRD	PHR	YILI	...	QYDKP	YYMLNL	...
HcA8	GCRD	PRR	YIVI	...	QYDKP	YYMLNL	...
HcHA	GCRD	PHR	YIWI	...	QYDKP	YYMLNL	...
	****	:	*** *		****	*	*****

		1159		1188		1289	1295
HcA1	YRGT	KFI	IKKY	...	NVVVK	NKEY	RL
HcA2	YEGT	KFI	IKKY	...	NVVVK	NKEY	RL
HcA3	YMG	KFI	IKKY	...	NVVVK	NKEY	RL
HcA4	YMG	KFI	IKKY	...	NVVVK	NKEY	RL
HcA5	YMG	KFI	IKKY	...	NVVVK	NKEY	RL
HcA6	YMG	KFI	IKKY	...	NVVVN	NKEY	RL
HcA7	YMG	KFI	IKKH	...	NVLVK	NKEY	RL
HcA8	YMG	KFI	IKKY	...	NVVVK	NKEY	RL
HcHA	YMG	KFI	IKKY	...	NVVVK	NKEY	RL
	*	**	*****	:	**	:	*****

**Supplementary Figure 2: The ciA-C2-interacting residues (indicated by black boxes) are largely conserved among BoNT/A1-A8 and BoNT/HA.** The amino acid sequences are taken from GenBank: AAQ06331.1 (A1), ACO83782.1 (A2), ABA29017.1 (A3), ACQ51417.1 (A4), ACG50065.1 (A5), ACW83608.1 (A6), AFV13854.1 (A7), AJA05787.1 (A8), and KGO15617.1 (HA). The numbering is based on the sequence of BoNT/A1. Sequence alignment was made using Clustal Omega <sup>1</sup>.

**Supplementary Table 1: Hydrogen bonds between ciA-C2 and HcA1**

<b>Residues (ciA-C2, Chain B)</b>	<b>Distance (Å)</b>	<b>Residues (HcA1, Chain A)</b>
Gly 26 [O]	2.71	Asn1188 [ND2]
Gly 28 [N]	2.97	Asn 1188 [OD1]
Trp 30 [NE1]	3.05	Asn 1188 [OD1]
Trp 30 [NE1]	3.37	Asn 1188 [O]
Thr 37 [OG1]	2.69	Asp 1289 [OD1]
Glu 50 [OE1]	2.99	Thr 1146 [N]
Glu 50 [OE1]	2.74	Thr 1146 [OG1]
Leu 54 [O]	2.80	His 1064 [NE2]
Thr 65 [OG1]	2.75	His 1064 [ND1]
Tyr 66 [O]	2.70	Thr 1063 [OG1]
Glu 106 [OE2]	2.53	Tyr 1112 [OH]
Trp 108 [NE1]	3.13	Glu 1293 [O]

**Reference**

- 1 Sievers, F. *et al.* Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* **7**, 539, doi:10.1038/msb.2011.75 (2011).