

Supplementary Materials:

Table S1

ciBoNTA:

	EC ₅₀ (nM)	95% CI (nM)
VNA1-ABE	1.03	0.75 – 1.45
VNA2-ABE	1.11	0.75 – 1.77
VNA-BoNT/A	1.45	1.15 – 1.89

ciBoNTB:

	EC ₅₀ (nM)	95% CI (nM)
VNA1-ABE	1.35	0.99 – 2.04
VNA2-ABE	1.18	0.92 – 1.6
VNA-BoNT/A	0.74	0.47 – 1.4

ciBoNT/E:

	EC ₅₀ (nM)	95% CI (nM)
VNA1-ABE	0.90	0.63 – 1.38
VNA2-ABE	0.86	0.59 – 1.29
VNA-BoNT/A	0.50	0.40 – 0.63

Figure S1. Sequences of the three heterohexamer VNAs employed in this report. The complete encoded amino acid sequences of the three heterohexamer VNAs; VNA1-ABE, VNA2-ABE and VNA3-ABE, are shown. The amino terminal leader peptide sequences that are removed during the secretion process are displayed in lower case. The E-tag sequences are underlined, and the C-terminal mouse albumin binding peptide (only in VNA1-ABE and VNA2-ABE) is italicized. VNA3-ABE contain the following modifications from VNA2-ABE so as to remove unneeded amino acids: 1) the amino terminal six amino acids of each VHH component (determined by degenerate PCR primers used in VHH-display library construction) was replaced by the alpaca consensus FR1 sequence QVQLVE; 2) E-tag was included only at the C-terminus; 3) the mouse albumin binding peptide was removed; 4) only the ‘core’ VHH sequences considered necessary for binding (**Figure 1B**), and; 5): component VHHs were separated only by a GGGGG spacer.

Figure S2. Serum dilution ELISAs used to assess VNA pharmacokinetics following administration of formulated RNA. ELISAs graphically represents the source data used for

Figure 5A, performed as in the Materials and Methods. The EC₅₀ values for each sera were estimated and then serum VNA levels established by comparison to the internal standard. This assay was performed twice for statistical evaluation.

Figure S3. Condition scores of mice following administration of formulated RNA and then BoNT challenge.

Figure S1

Complete heterohexameric VNA sequences

VNA1-ABE
metdtlllwvlllwvpgstgDAAQPARRARRTKLSGAPVPYPDPLEPRAAAGQGVQAQLQLVESGGGLVHPGGSLRLSCAPSASLPSTPFNPFNNMVGWYRQAPGKQREMVASIGLRINYADSVKGRFTISRDNAKNTVDLQMDSLRPEDSATYYCHIEYTHYWKGKTLTVSS
EPKTPKPQSGGGGSGGGGSGGGGSGGGGSGGGGSGQGVQAQLQLVESGGGLVQVGGSLRLSCVVS GSDISGIAMGWYRQAPGKRREM VADIFSGGSTDYAGSVKGRFTISRDN AKKTSYLQMN NVKPEDTG VYYCRLYGSGDYWGQGTQVT VSSAHHSEDP SGGG
GQGVQAQLQLVESGGGLVQAGGSLRLSCAASILT YDL DYYYIGWVRQAPGKEREGVSCISSTDGATYYADSVKGRFTISRNNAKNTVYLQMN NLKPEDTAIYYCAA PLAGRYCPASHEYG YWGQGTQVT VSSAHHSEDP SGGGSGGGGSGGGGSGQGVQAQLQ LVE
SGGGLVQAGGSLRLSCAAEFRAEHFAVGWFRQAPGKEREGVSCVDASGDSTAYADSVKGRFTISRDN NKNVVYLQMD SLEPEDTG DY YCGASYFTVCAKSMRKIEYRYWGQGTQVT VSSEPKTPKPQSGGGGQGVQAQLQLVESGGGLVKPGGSLRLSCVVS GFTFDD
YRMAWVRQAPGKELEWVSSIDSW SINTYYEDSVKGRFTISTDN AKNTLYLQMSS LKPEDTAVYYCAAEDRLGVPTINAHPSKYDYN YWGQGTQVT VSSEPKTPKPQSGGGGSGGGGSGGGGSGQGVQAQLQLVESGGGLVQAGGSLRLSCAASGRTFSSYSMGWFRQAP
GKEREYVA AVNSNGDSTFYADSIKGRFTVSRDAAKNTVYLQMNS LKPEDTALYYCAA VYGRYTYQSPKSYEYWGQGTQVT VSSEPKTPKPQ SARQ GAPVPYPDPLEPRGGGSDICLPRWGCLWED*

VNA2-ABE
metdtlllwvlllwvpgstgDAAQPARRARRTKLSGAPVPYPDPLEPRAAAGQGVQAQLQLVESGGGLVHPGGSLRLSCAPSASLPSTPFNPFNNMVGWYRQAPGKQREMVASIGLRINYADSVKGRFTISRDNAKNTVDLQMDSLRPEDSATYYCHIEYTHYWKGKTLTVSS
EPKTPKPQSGGGGQGVQAQLQLVESGGGLVQAGGSLRLSCAASILT YDL DYYYIGWVRQAPGKEREGVSCISSTDGATYYADSVKGRFTISRNNAKNTVYLQMN NLKPEDTAIYYCAA PLAGRYCPASHEYG YWGQGTQVT VSSAHHSEDP SGGGQGVQAQLQLVES
GGGLVKPGGSLRLSCVVS GFTFDDYRMAWVRQAPGKELEWVSSIDSW SINTYYEDSVKGRFTISTDN AKNTLYLQMSS LKPEDTAVYYCAAEDRLGVPTINAHPSKYDYN YWGQGTQVT VSSEPKTPKPQSGGGGQGVQAQLQLVESGGGLVQVGGSLRLSCVVS GSDIS
GIAMGWYRQAPGKRREM VADIFSGGSTDYAGSVKGRFTISRDN AKKTSYLQMN NVKPEDTG VYYCRLYGSGDYWGQGTQVT VSSAHHSEDP SGGGQGVQAQLQLVESGGGLVQAGGSLRLSCAAEFRAEHFAVGWFRQAPGKEREGVSCVDASGDSTAYADSVK
GRFTISRDN NKNVVYLQMD SLEPEDTG DY YCGASYFTVCAKSMRKIEYRYWGQGTQVT VSSEPKTPKPQSGGGGQGVQAQLQLVESGGGLVQAGGSLRLSCAASGRTFSSYSMGWFRQAPGKEREYVA AVNSNGDSTFYADSIKGRFTVSRDAAKNTVYLQMNS LKPE
DTALYYCAA VYGRYTYQSPKSYEYWGQGTQVT VSSEPKTPKPQ SARQ GAPVPYPDPLEPRGGGSDICLPRWGCLWED*

VNA3-ABE
melglswvllaallqgvqaQLQLVESGGGLVHPGGSLRLSCAPSASLPSTPFNPFNNMVGWYRQAPGKQREMVASIGLRINYADSVKGRFTISRDNAKNTVDLQMDSLRPEDSATYYCHIEYTHYWKGKTLTVSSGGGGGQLQLVESGGGLVQAGGSLRLSCAASILT YDL D
YYIGWVRQAPGKEREGVSCISSTDGATYYADSVKGRFTISRNNAKNTVYLQMN NLKPEDTAIYYCAA PLAGRYCPASHEYG YWGQGTQVT VSSGGGGGQLQLVESGGGLVKPGGSLRLSCVVS GFTFDDYRMAWVRQAPGKELEWVSSIDSW SINTYYEDSVKGRFTIS
TDNAKNTLYLQMSS LKPEDTAVYYCAAEDRLGVPTINAHPSKYDYN YWGQGTQVT VSSGGGGGQLQLVESGGGLVQVGGSLRLSCVVS GSDISGIAMGWYRQAPGKRREM VADIFSGGSTDYAGSVKGRFTISRDN AKKTSYLQMN NVKPEDTG VYYCRLYGSGDYWG
QGTQVT VSSGGGGGQLQLVESGGGLVQAGGSLRLSCAAEFRAEHFAVGWFRQAPGKEREGVSCVDASGDSTAYADSVKGRFTISRDN NKNVVYLQMD SLEPEDTG DY YCGASYFTVCAKSMRKIEYRYWGQGTQVT VSSGGGGGQLQLVESGGGLVQAGGSLRLSCA
ASGRTFSSYSMGWFRQAPGKEREYVA AVNSNGDSTFYADSIKGRFTVSRDAAKNTVYLQMNS LKPEDTALYYCAA VYGRYTYQSPKSYEYWGQGTQVT VSS GAPVPYPDPLEPR*

Figure S2

