# Supplementary Materials: Camelid Single-Domain Antibodies (VHHs) against Crotoxin: A Basis for Developing Modular Building Blocks for the Enhancement of Treatment or Diagnosis of Crotalic Envenoming

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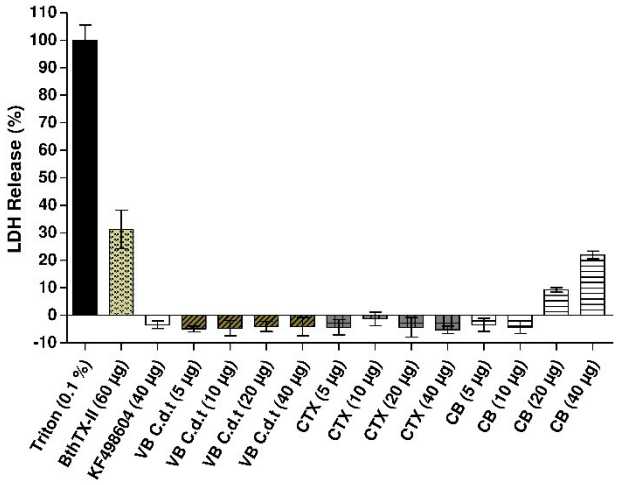


**Figure S1.** Cytotoxic activity evaluation of *Crotalus d. terrificus* venom, CTX and CB in murine C2C12 skeletal muscle myoblasts.

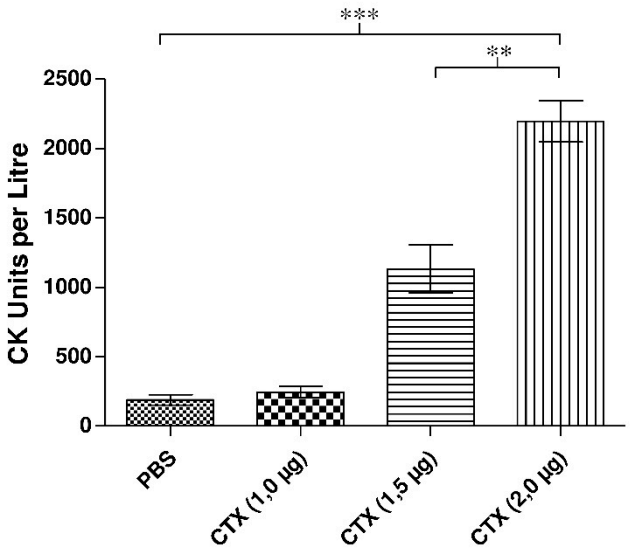


**Figure S2.** Determination of CTX myotoxic activity in mice.

**Table S1.** Interaction analysis by SPR.

| Immobilized antigen | VHH | $k_{on}$ [1/Ms] | $k_{off}$ [1/s] | $R_{max}$[RU] | $K_D$ [M] | $Chi^2$ [RU] |
|---|---|---|---|---|---|---|
| CTX | KF498602 | $2.148x10^{+4}$ | 0.03673 | 67.93 | $1.710x10^{-6}$ | 0.764 |
| | KF498204 | $6.807x10^{+4}$ | 0.005537 | 47.18 | $8.134x10^{-8}$ | 1.10 |

Binding kinetics parameters were measured according to SPR sensorgrams shown in Figure 3 using the 1:1 Langmuir model

**Table S2.** In silico studies' data

| Target (VHH clone) | Template (PDB:chain) | % Identity | Resolution (Å) | RMSD (Å) | Reference Template |
|---|---|---|---|---|---|
| KF498602 | 1xfp:A | 64 | 1.50 | 1.95 | [1] |
| KF498603 | 1xfp:A | 64 | 1.50 | 0.21 | [1] |
| KF498604 | 1kxt:B | 66 | 2.00 | 0.36 | [2] |
| KF498605 | 2vxs:K | 76 | 2.63 | 0.29 | [3] |
| KF498606 | 4b41:A | 78 | 1.19 | 0.27 | [4] |

**Table S3.** Llama immunization schedule with CTX and CA and CB subunits.

| Day | Immunization number | Toxin dose | | | Adjuvant | Serum collection |
|---|---|---|---|---|---|---|
| | | CTX | CA | CB | | |
| -7 | - | - | - | - | - | Yes |
| 0 | 1 | 100 µg | 50 µg | 50 µg | - | Yes |
| 7 | 2 | 100 µg | 50 µg | 50 µg | 50 µL CFA[a] | Yes |
| 14 | 3 | 200 µg | 100 µg | 100 µg | 100 µL IFA[b] | Yes |
| 21 | 4 | 200 µg | 100 µg | 100 µg | 100 µL IFA | Yes |
| 28 | 5 | 200 µg | 100 µg | 100 µg | 100 µL IFA | Yes |
| 31 | - | - | - | - | - | Yes |

[a]CFA: Complete Freund's adjuvant; [b]IFA: Incomplete Freund's adjuvant; The llama was immunized with CTX, CA and CB subunits by subcutaneous route. Blood was collected from the jugular vein three days after final boost.

## References


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