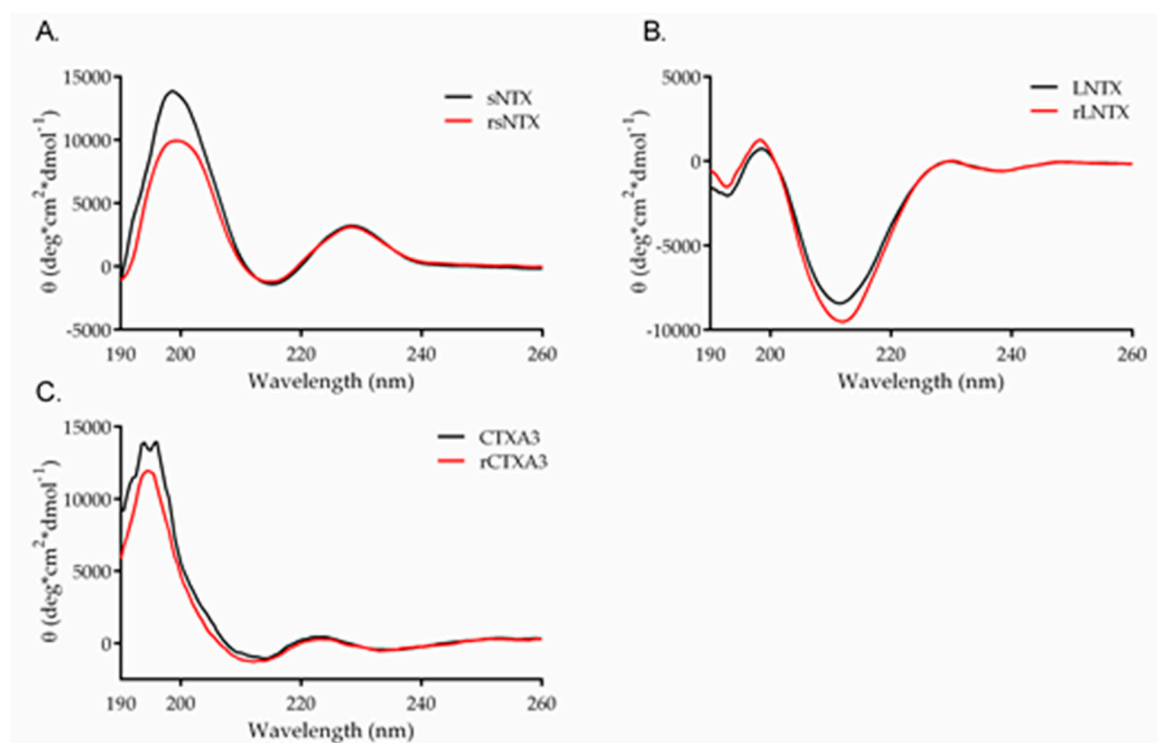
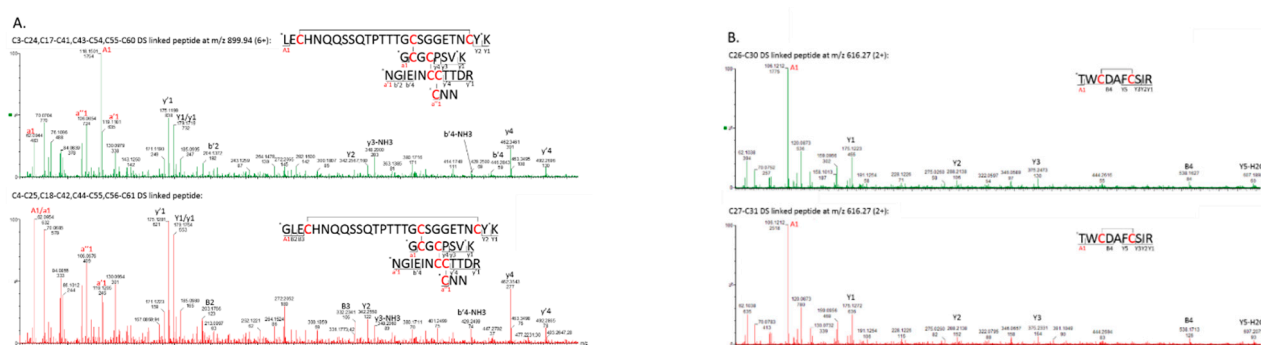


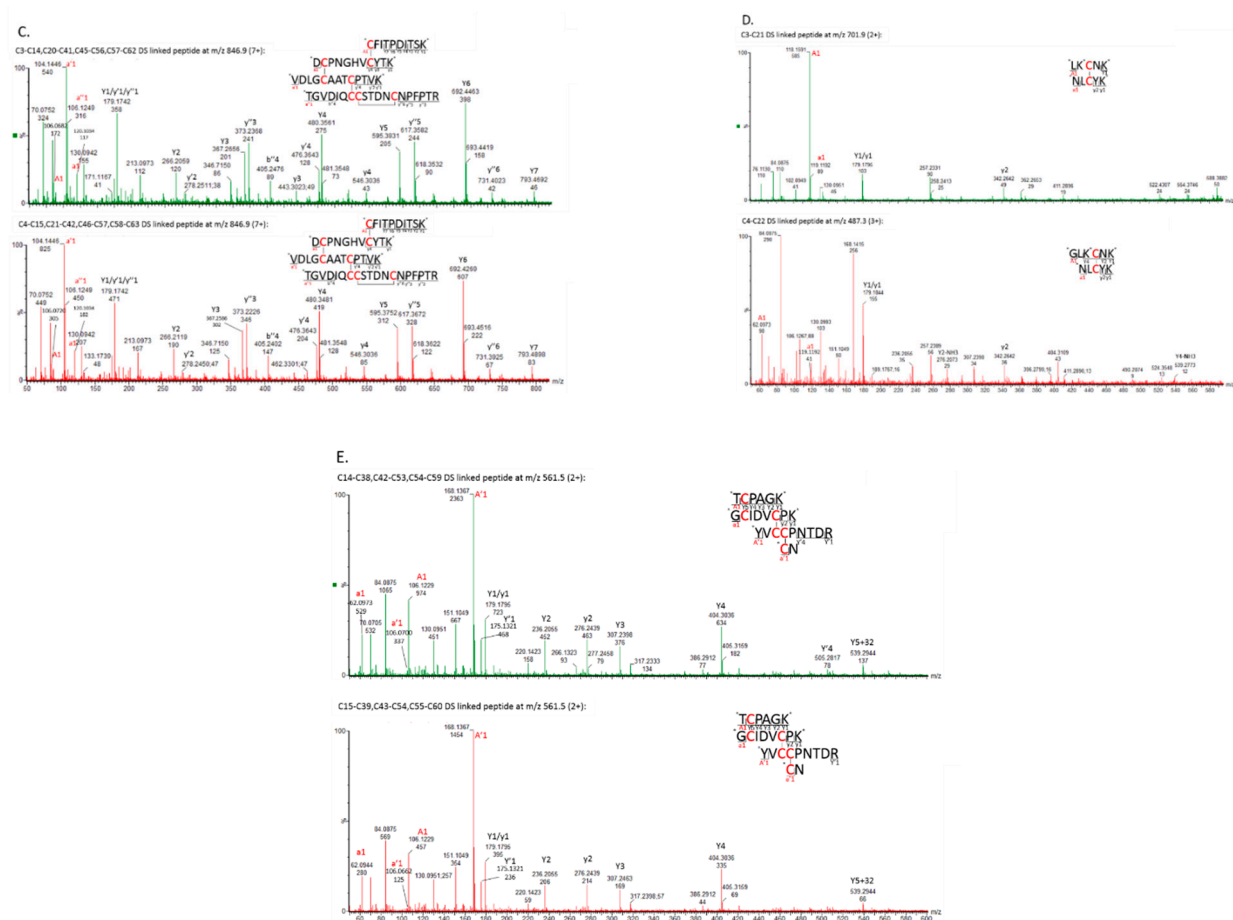
# Supplementary Materials: Development of a Broad-Spectrum Antiserum against Cobra Venoms Using Recombinant Three-Finger Toxins

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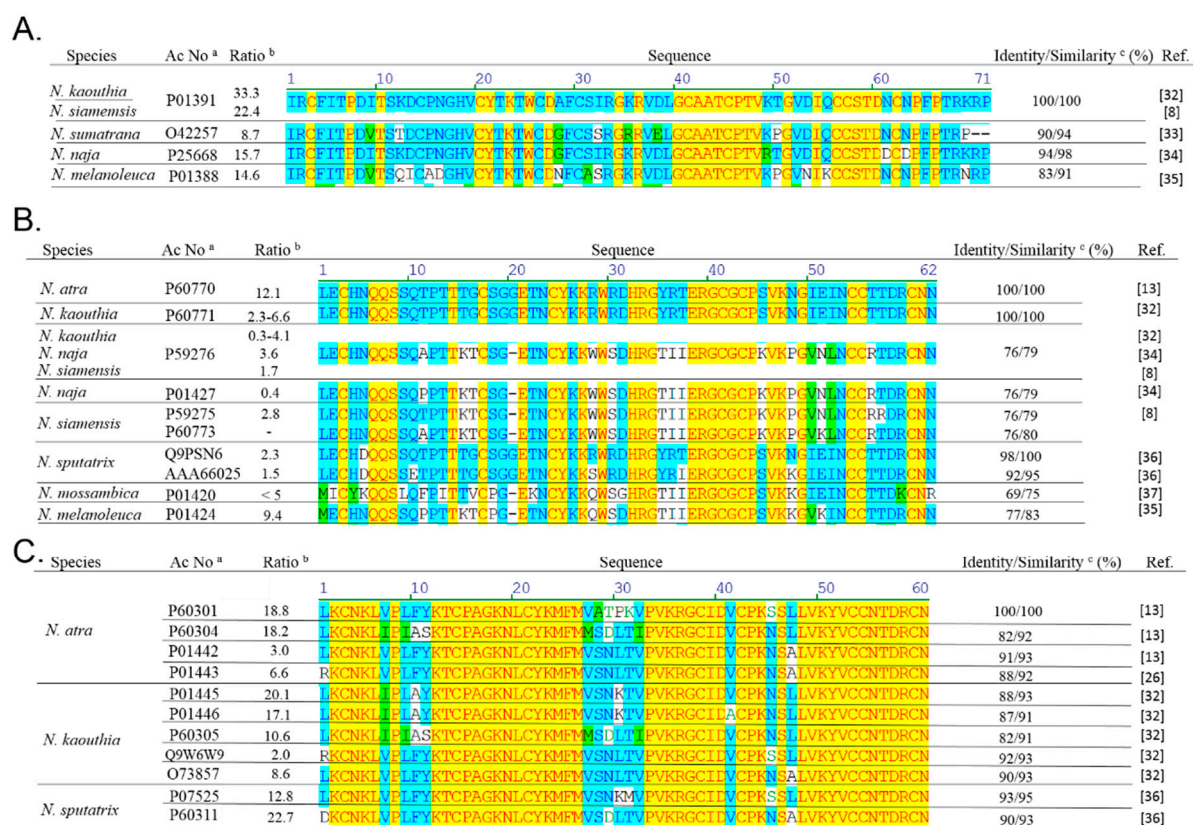


**Figure S1.** The CD spectra of native and recombinant (A) sNTX, (B) LNTX, and (C) CTXA3. The spectra of native toxins purified from venoms are shown in black lines; the spectra of recombinant toxins are shown in red lines.

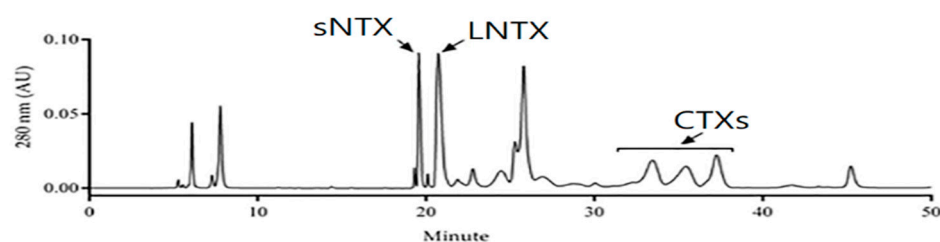




**Figure S2.** Identification of linkage sites of disulfide bonds by MS analysis. Comparative MS/MS spectra of the disulfide bond-containing peptides derived from the tryptic digests of native (green) or recombinant (red) (A) sNTX, (B,C) LNTX, and (D,E) CTXA3. CID fragment ions (a1/a'1/A1/A'1 and y1/y1/Y'/Y' ions) are annotated. Asterisks (\*) indicate the sites of dimethyl (d2) labeling in the peptides.



**Figure S3.** Sequence alignment of the major (A) LNTXs, (B) sNTXs, and (C) CTXs identified in cobra snake venoms. The percentage of amino acids that are identical or similar at the same position compared to the sequence of P01391 (for LNTXs), P60770 (for sNTXs), or P60301 (for CTXs). Conserved amino acids identical in all sequences of the same subfamily are shown in red with bright yellow background; similar amino acids are shown in black with blue or green background. The accession numbers of proteins were from the UniProt database. The percentage (%) of relative abundance in the corresponding venoms was reported in the literatures.



**Figure S4.** RP-HPLC elution chromatogram of the cobra venom mixture for rabbit immunization experiment. The ratio of sNTX:LNTX:CTXs in the venom mixture was estimated to be 1:1:3 based on the peak areas.

**Table S1.** Identification of *N. atra* and *N. kaouthia* venom components in the HPLC fractions using MS.

Peak <sup>1</sup>	Protein Identity	AC no. <sup>2</sup>	Significant Peptide	Observed Ion (m/z)	MS Score	Toxin Family
<b>a</b>	Cobrotoxin	P60770	R.GCGCPSVK.N K.NGIEINCCCTDR.C	432.6748 (2+) 726.7258 (2+)	69	3FTX
<b>b</b>	Acidic phospholipase A2	A4FS04	-.NLYQFK.N K.GGSGTPVDDDLDR.C	406.7119 (2+) 594.7706 (2+)	89	PLA2
<b>c</b>	Cytotoxin 1	P60304	K.MFMMSDLTIPVK.R	706.8398 (2+)	375	3FTX
	Cytotoxin 3	P60301	K.MFMVATPK.V K.LVPLFYK.T	462.7221 (2+) 440.2524 (2+)	101	
	Cytotoxin 5	P07525	K.MVPVKR.G	373.2054 (2+)	46	
<b>d</b>	Cobrotoxin-b	P59275	K.TCSGETNCYK.K K.WWSDHR.G K.VKPGVNLNCCR.R -.LECHNQSSQTPTTK.T -.LECHNQSSQTPTT- KTCSGETNCYK.K	610.2822 (2+) 443.7183 (2+) 439.5856 (3+) 879.9451 (2+) 987.1167 (3+)	197	3FTX
			-.LECHNQSSQAPTTK.T	576.9522 (2+)	149	3FTX
			R.CFITPDITSK.D K.TWCDAFCSIR.G R.VDLGCAATCPTVK.T	591.3514(2+) 658.3432 (2+) 696.3925 (2+)	98	3FTX
			R.GCADCPTVR.K R.EIVECCSTDK.C	518.2423 (2+) 620.8072 (2+)	70	3FTX
	Weak neurotoxin 6	P29180	-.LTCLICEPK.Y	567.3386 (2+)	68	3FTX
<b>e</b>	Alpha-elapitoxin-Nk2a	P01391	R.CFITPDITSK.D K.TWCDAFCSIR.G R.VDLGCAATCPTVK.T	591.3469 (2+) 658.3644 (2+) 696.3752 (2+)	124	3FTX
<b>f</b>	Acidic phospholipase A2 2	P15445	K.ISGCWPYFK.T R.GGSGTPVDDDLDR.C R.SWWDFAFYGCYCGR.G K.NMIKCTVPSR.S <sup>3</sup> R.LAAICFAGAPYNDNNYNIDLK.A	579.3079 (2+) 594.8204 (2+) 921.9120 (2+) 611.3373 (2+) 786.4366 (3+)	309	PLA <sub>2</sub>
			K.NMIQCTVPNR.S R.GGSGTPVDDDLDR.C R.CWPYFK.T	624.8400 (2+) 594.8148 (2+) 450.7263 (2+)	295	PLA <sub>2</sub>
	Acidic phospholipase A2 2	P00597	R.LAAICFAGAPYNNNNYNIDLK.A K.GGSGTPVDDDLDR.C	1178.6711 (2+) 594.8255 (2+)	268	PLA <sub>2</sub>
	Acidic phospholipase A2 DE-II	P00600	R.GGSGTPVDDDLDR.C K.NMIQCTVPNR.S	594.8200 (2+) 624.8298 (2+)	200	PLA <sub>2</sub>
	Venom nerve growth factor 2	Q5YF89	K.TTATDIK.G K.QYFFETK.C K.ALTMEGNQASWR.F K.GNTVTVMENVNLDNK.V	375.2329 (2+) 481.7730 (2+) 682.3720 (2+) 824.4734 (2+)	131	VGF
			R.FDGSPCVLGSFGFR.S K.TVENVGVSQVAPDNPER.F K.ADVTFDSNTAFESLVVSPDK.K	748.4003 (2+) 906.0082 (2+) 1071.5872 (2+)	282	Vespryn
			K.LIPIASK.T R.GCIDVCPK.N K.YVCCNTDR.C K.MFMMSDLTIPVK.R	371.2701 (2+) 474.7489 (2+) 544.2528 (2+) 722.8949 (2+)	236	3FTX
<b>g</b>	Cytotoxin 1	P60305	K.LIPLAYK.T K.MFMVAAPK.V R.GCIDACPK.N	409.2871 (2+) 455.7598 (2+) 460.7300 (2+)	93	3FTX

h	Cytotoxin 4	P60303	K. <u>M</u> FMVATPK.V	478.7623 (2+)	45	3FTX
	Cytotoxin 2	Q9DGH9	K.IF <u>M</u> VATPK.V	461.7617 (2+)	57	3FTX
	Cytotoxin 5	P07525	K.MFMVSNK <u>M</u> VVPVK.R	713.8943 (2+)	57	3FTX
	Cysteine-rich venom protein natrin-1	Q7T1K6	K.LTNCDSLLK.Q	532.2916 (2+)	674	CRISP
			R.VSPTASN <u>M</u> LK.M	532.3129 (2+)		
			K.EIVDLHNSLR.R	399.2381 (3+)		
			R.RVSPTASN <u>M</u> LK.M	610.3337 (2+)		
			K.QSSCQDDWIK.S	633.8123 (2+)		
			K.QKEIVDLHNSLR.R	484.6273 (3+)		
			K. <u>M</u> EWYPEAASNAER.W	785.2921 (2+)		
			R.TWTEIIHLWHDEYK.N	624.3440 (3+)		
			R.VLEGIQCGESIY <u>M</u> SSNAR.T	1015.5442 (2+)		
	Cysteine-rich venom protein annuliferin-a (Fragment)	P0DL14	-.NVDFNSESTR.R	584.8115 (2+)	152	CRISP
	Cysteine-rich venom protein ophanin	Q7ZT98	K.QSSCQDEWIK.S	640.8143 (2+)	119	CRISP
	Cobra venom factor	Q91132	R.IDVPLQIEK.A	527.8409 (2+)	117	CVF

<sup>1</sup> The peak refers to the labeling in Figure 3. <sup>2</sup> AC is the accession number in Uniprot database. <sup>3</sup> The underline of methionine (M) means it was oxidized. Abbreviations: SVMP indicates snake venom metalloproteinase. CRISP is cysteine-rich secretory protein. 3FTX means three finger toxins.