

The Fast and the Furriest: Investigating the Rate of Selection on Mammalian Toxins

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The following supplementary data includes details on the number of sequences used, full FEL, FUBAR and MEME results, all reported PAML results and significance of calculated LRTs, TreeSAAP results and model selection.

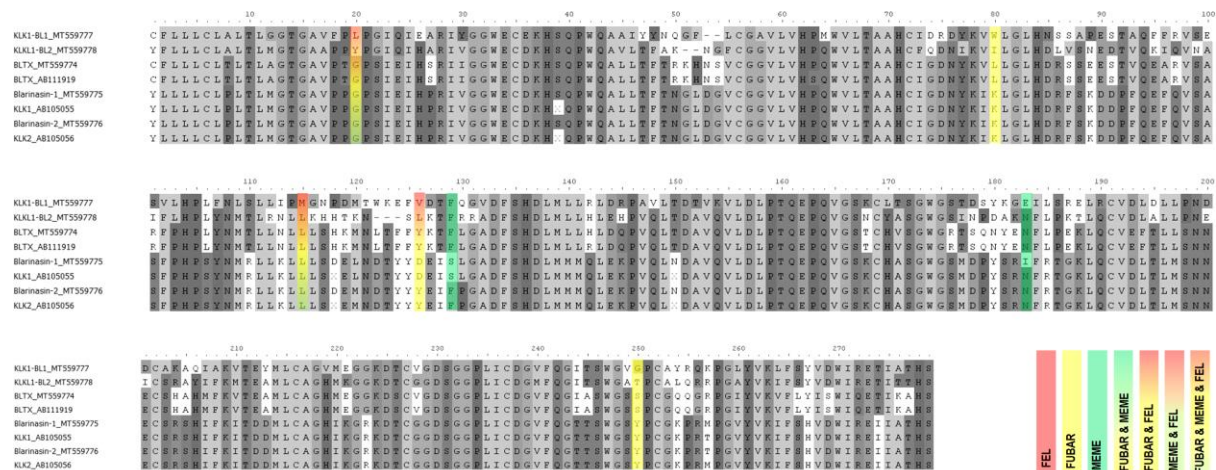


Figure S1. Amino acid alignment of KLK1 *Blarina*, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

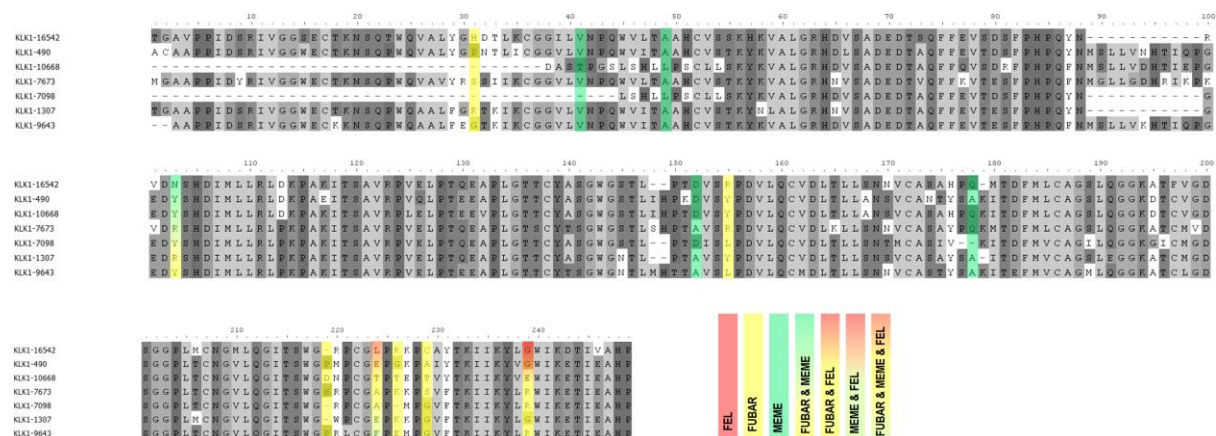


Figure S2. Amino acid alignment of KLK1 *Solenodon*, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

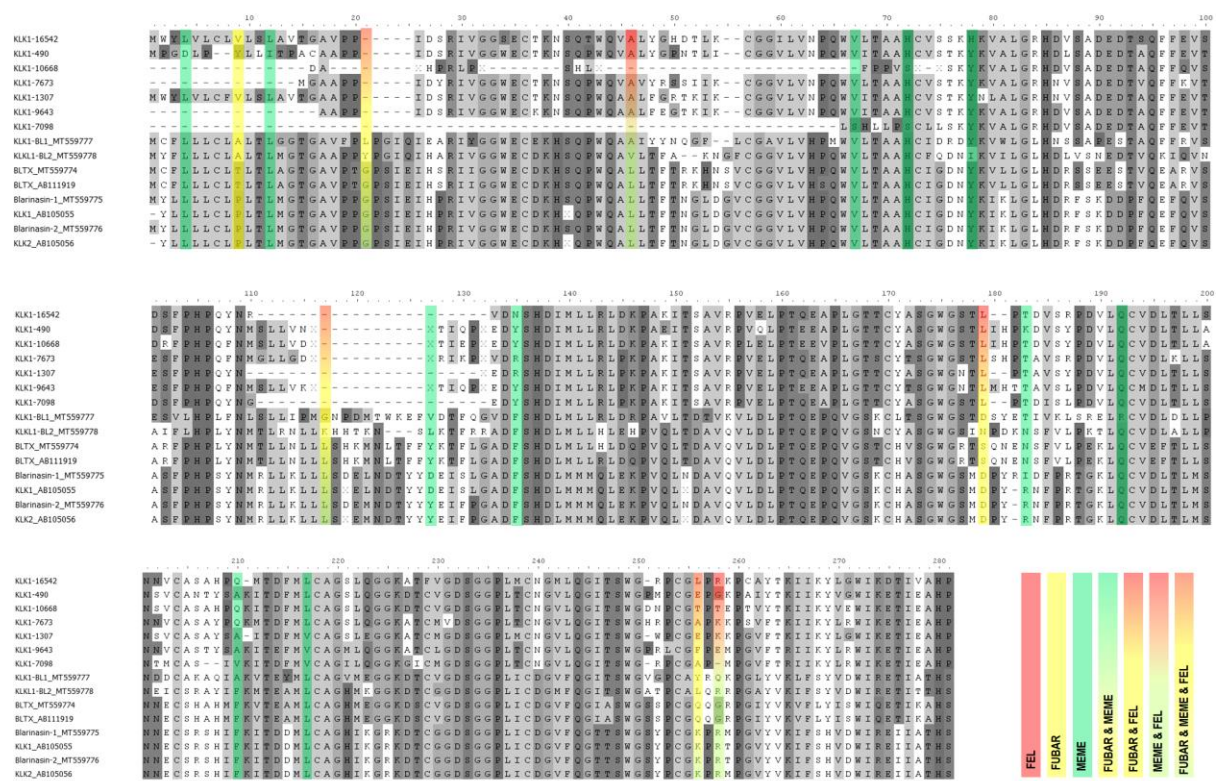


Figure S3. Amino acid alignment of KLK1 Eulipotyphla, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

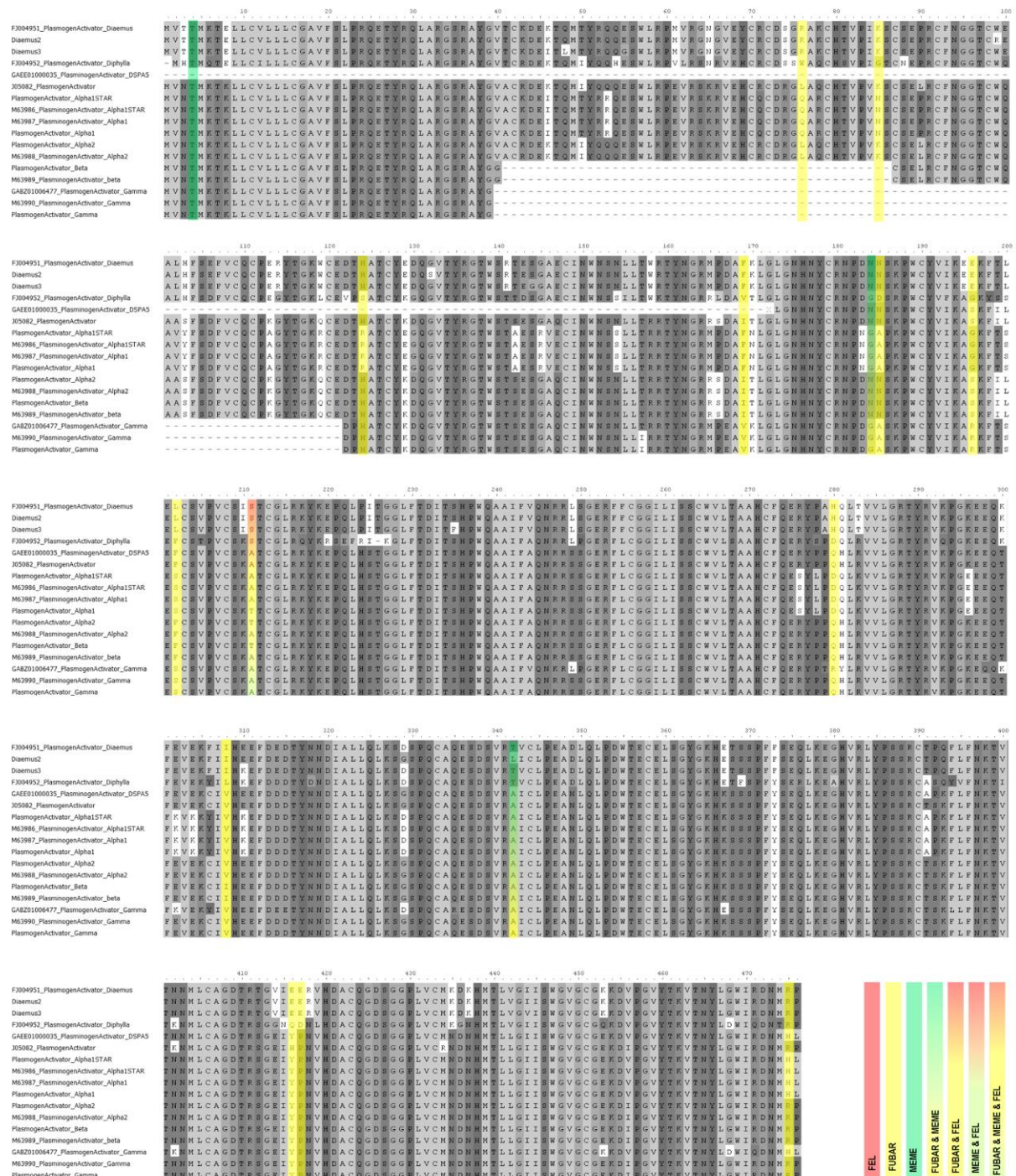


Figure S4. Amino acid alignment of Plasminogen Activator, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

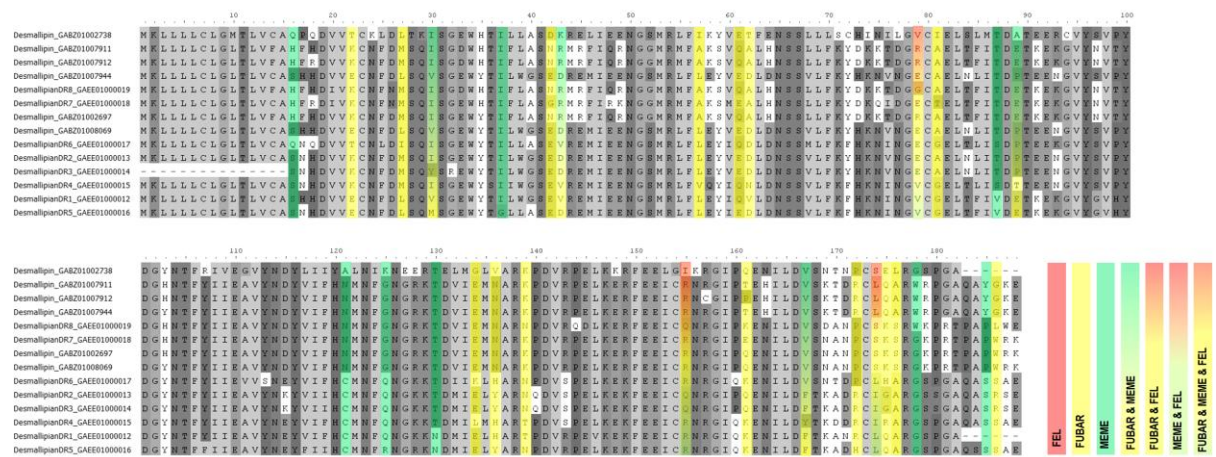


Figure S5. Amino acid alignment of Desmallipins, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

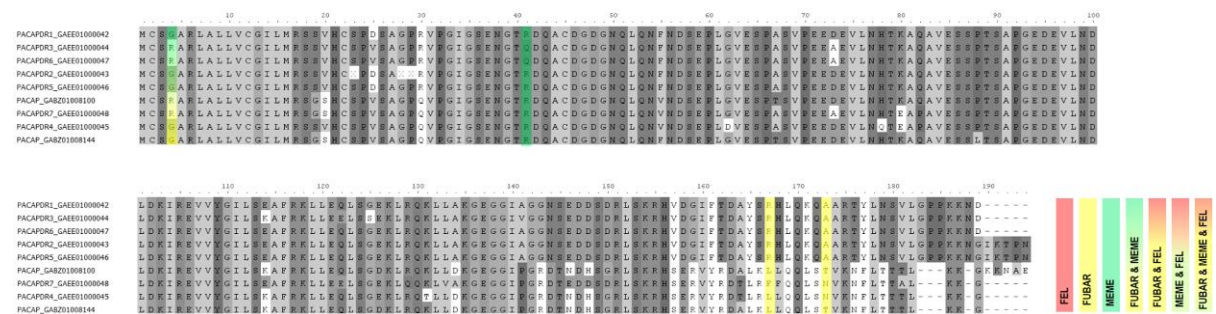


Figure S6. Amino acid alignment of PACAP, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

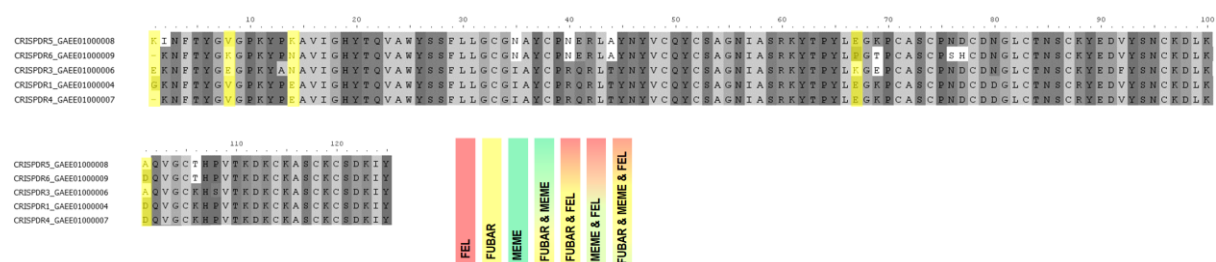


Figure S7. Amino acid alignment of CRiSP *Desmodus*, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

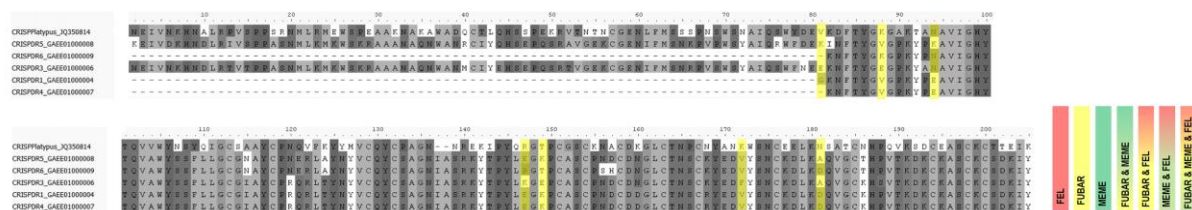


Figure S8. Amino acid alignment of CRiSP *Desmodus* and *Ornithorhynchus*, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

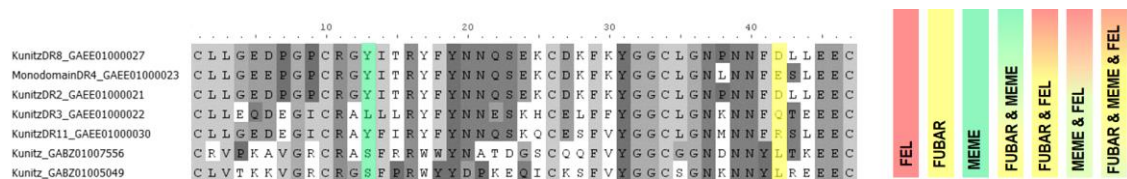


Figure S9. Amino acid alignment of Kunitz Domain One, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

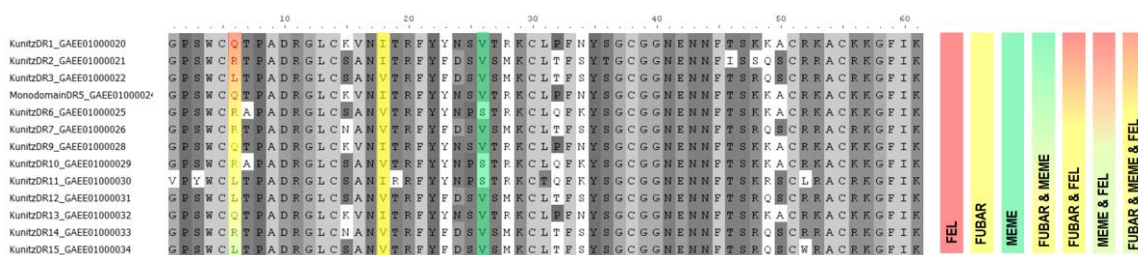


Figure S10. Amino acid alignment of Kunitz Domain Two, with diversifying selection highlighted according to each programme. Red represents FEL, Yellow represents FUBAR and Green represents MEME. Overlapping sites with partial or complete programme agreement are colored as mixed hues.

Table S1: The number of sequences and length of each alignment. Length of alignment does not include STOP codons, which were removed prior to analysis. Each alignment is included within Appendix 1.

Toxin name	Number of sequences	Length of alignment after TrimAI	Species present
Kallikren-1(KLK1) <i>Blarina</i>	8	837	Short tailed shrew (<i>Blarina brevicauda</i>)
Kallikren-1(KLK1) <i>Solenodon</i>	7	747	Hispaniolan solenodon (<i>Solenodon paradoxus</i>)
Kallikrein-1 (KLK1) Eulipotyphla	15	843	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>)
Kallikren-1 (KLK1) All	16	789	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>) Common Vampire Bat (<i>Desmodus rotundus</i>)
Plasminogen Activator	17	1428	Hairy legged vampire bat (<i>Diphylla ecaudata</i>) White winged vampire bat (<i>Diaemus youngi</i>) Common vampire bat (<i>Desmodus rotundus</i>)
Desmallipins (<i>Desmodus</i> allergen-related lipocalins)	14	564	Common Vampire Bat (<i>Desmodus rotundus</i>)
PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide)	9	582	Common Vampire Bat (<i>Desmodus rotundus</i>)
Cysteine-rich secretory proteins (CRiSP) <i>Desmodus</i>	5	375	Common Vampire Bat (<i>Desmodus rotundus</i>)
Cysteine-rich secretory proteins (CRiSP) <i>Desmodus</i> and <i>Ornithorhynchus</i>	6	615	Common Vampire Bat (<i>Desmodus rotundus</i>) Platypus (<i>Ornithorhynchus anatinus</i>)
Kunitz Domain One	7	141	Common Vampire Bat (<i>Desmodus rotundus</i>)
Kunitz Domain Two	13	183	Common Vampire Bat (<i>Desmodus rotundus</i>)

Table S2: FEL results for each alignment including number of sites identified to be under significant diversifying or purifying selection and at which site in each alignment.

Toxin	Species	Sites identified under diversifying selection	Sites number	Sites identified under purifying selection	Sites number
Kallikren-1(KLK1) <i>Blarina</i>	Short tailed shrew (<i>Blarina brevicauda</i>)	3	20, 116, 126	26	13, 14, 23, 29, 38, 39, 83, 94, 104, 136, 146, 150, 151, 160, 173, 175, 191, 199, 202, 209, 211, 216, 224, 233, 238, 267
Kallikren-1(KLK1) <i>Solenodon</i>	Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	2	224, 239	19	16, 21, 52, 53, 75, 76, 86, 104, 118, 139, 144, 154, 166, 186, 192, 196, 204, 223, 228
Kallikrein-1 (KLK1) Eulipotyphla	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	6	21, 46, 117, 179, 256, 258	47	2,6,15,17,24,26, 29,32,35,38,39, 40,61,66,73,85, 95,105,107,134, 136,137,147,15 1,153,161,171,1 74,176,193,198, 201,204,211,21 3,218,224,226,2 28,235,236,239, 241,244,260,27 7,280
Kallikren-1 (KLK1) All	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>) Common Vampire Bat (<i>Desmodus rotundus</i>)	4	83, 179, 238, 240	48	2, 6, 17, 21, 25, 27, 30, 33, 34, 35, 50, 53, 54, 59, 66, 78, 88, 98, 100, 116, 118, 119, 129, 133, 135, 143, 153, 156, 158, 175, 180, 183, 186, 193, 195, 200, 206, 208, 210, 218, 221, 223, 226, 232, 235, 242, 259, 262

Plasminogen Activator	<p>Hairy legged vampire bat (<i>Diphylla ecaudata</i>)</p> <p>White winged vampire bat (<i>Diaemus youngi</i>)</p> <p>Common vampire bat (<i>Desmodus rotundus</i>)</p>	1	211	22	44, 73, 79, 83, 128, 135, 153, 154, 179, 257, 258, 259, 307, 311, 344, 363, 381, 392, 420, 455, 465, 473
Desmallipins (<i>Desmodus</i> allergen-related lipocalins)	Common Vampire Bat (<i>Desmodus rotundus</i>)	3	79, 155, 174	13	19, 41, 64, 65, 73, 83, 88, 105, 118, 151, 158, 173, 180
PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide)	Common Vampire Bat (<i>Desmodus rotundus</i>)	0	N/A	10	66, 81, 92, 99, 144, 163, 186, 187, 189, 193
Cysteine-rich secretory proteins (CRiSP) <i>Desmodus</i>	Common Vampire Bat (<i>Desmodus rotundus</i>)	0	N/A	3	6, 11, 42
Cysteine-rich secretory proteins (CRiSP) <i>Desmodus</i> and <i>Ornithorhynchus</i>	Common Vampire Bat (<i>Desmodus rotundus</i>) Platypus (<i>Ornithorhynchus anatinus</i>)	0	N/A	19	2, 7, 60, 75, 86, 91, 99, 100, 117, 129, 131, 135, 153, 162, 163, 187, 194, 199, 200
Kunitz Domain One	Common Vampire Bat (<i>Desmodus rotundus</i>)	0	N/A	12	16, 19, 29, 31, 33, 34, 37, 39, 40, 45, 46, 47

Kunitz Domain Two	Common Vampire Bat (<i>Desmodus rotundus</i>)	1	6	2	8, 17
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Table S3: FUBAR results for each alignment including number of sites identified to be under significant diversifying or purifying selection and at which site in each alignment.

Toxin	Species	Sites identified under diversifying selection	Sites number	Sites identified under purifying selection	Sites number
Kallikren-1(KLK1) <i>Blarina</i>	Short tailed shrew (<i>Blarina brevicauda</i>)	5	20, 80, 116, 126, 250	23	13, 14, 19, 23, 38, 39, 83, 100, 104, 146, 150, 151, 152, 173, 199, 202, 209, 211, 216, 224, 233, 238, 267
Kallikren-1(KLK1) <i>Solenodon</i>	Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	8	31, 103, 155, 219, 224, 226, 229, 239	16	16, 21, 53, 76, 86, 96, 104, 137, 154, 166, 186, 192, 196, 204, 223, 228
Kallikrein-1 (KLK1) Eulipotyphla	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	5	9, 21, 117, 179, 256	35	6, 15, 17, 24, 32, 35, 39, 40, 61, 73, 85, 95, 105, 107, 134, 136, 137, 147, 151, 153, 171, 174, 176, 198, 201, 204, 211, 213, 218, 224, 226, 228, 236, 244, 260,
Kallikren-1 (KLK1) All	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>) Common Vampire Bat (<i>Desmodus rotundus</i>)	3	169, 179, 238	47	2, 17, 20, 21, 25, 27, 30, 33, 34, 35, 53, 54, 59, 66, 67, 78, 88, 98, 100, 116, 118, 119, 129, 133, 135, 153, 156, 158, 163, 180, 183, 186, 193, 195, 200, 206, 208, 210, 218, 221, 223, 226, 232, 235, 242, 259, 262

Plasminogen Activator	Hairy legged vampire bat (<i>Diphylla ecaudata</i>) White winged vampire bat (<i>Diaemus youngi</i>) Common vampire bat (<i>Desmodus rotundus</i>)	15	76, 85, 124, 169, 184, 185, 196, 202, 211, 280, 308, 342, 416, 417, 475	8	154, 179, 257, 258, 311, 344, 368, 392
Desmallipins (Desmodus allergen-related lipocalins)	Common Vampire Bat (<i>Desmodus rotundus</i>)	22	22, 27, 30, 42, 43, 57, 61, 62, 79, 81, 89, 134, 136, 139, 155, 161, 167, 172, 174, 175, 176, 186	8	19, 64, 73, 88, 105, 118, 170, 184
PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide)	Common Vampire Bat (<i>Desmodus rotundus</i>)	3	4, 167, 173	5	66, 81, 99, 144, 189
Cysteine-rich secretory proteins (CRiSP) <i>Desmodus</i>	Common Vampire Bat (<i>Desmodus rotundus</i>)	5	1, 8, 14, 67, 101	2	6, 11
Cysteine-rich secretory proteins (CRiSP) All	Common Vampire Bat (<i>Desmodus rotundus</i>) Platypus (<i>Ornithorhynchus anatinus</i>)	7	81, 88, 94, 147, 149, 172, 181	2	91, 133

Kunitz Domain One	Common Vampire Bat (<i>Desmodus rotundus</i>)	1	42	7	16, 29, 31, 33, 37, 39, 40
Kunitz Domain Two (Chiroptera)	Common Vampire Bat (<i>Desmodus rotundus</i>)	2	6, 18	1	8

Table S4: MEME results for each alignment including number of sites identified to be under significant diversifying selection and at which site in each alignment.

Toxin	Species	Sites identified under positive selection	Sites number	dN/dS
Kallikren-1(KLK1) <i>Blarina</i>	Short tailed shrew (<i>Blarina brevicauda</i>)	4	20, 116, 129, 183	0.619
Kallikren-1(KLK1) <i>Solenodon</i>	Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	6	41, 49, 103, 152, 178, 224	0.751
Kallikrein-1 (KLK1) Eulipotyphla	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	16	4, 12, 21, 46, 67, 72, 78, 117, 127, 135, 183, 197, 210, 217, 256, 258,	0.593
Kallikren-1 (KLK1) All	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>) Common Vampire Bat (<i>Desmodus rotundus</i>)	21	5, 10, 55, 58, 60, 61, 62, 65, 71, 74, 86, 109, 117, 138, 165, 179, 192, 199, 236, 238, 240	0.513
Plasminogen Activator	Hairy legged vampire bat (<i>Diphylla ecaudata</i>) White winged vampire bat (<i>Diaemus youngi</i>) Common vampire bat (<i>Desmodus rotundus</i>)	4	40, 184, 211, 342	1.04
Desmallipins (Desmodus allergen-related lipocalins)	Common Vampire Bat (<i>Desmodus rotundus</i>)	15	16, 30, 37, 43, 79, 87, 89, 121, 125, 130, 155, 167, 174, 178, 185	1.18

PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide)	Common Vampire Bat (<i>Desmodus rotundus</i>)	2	4, 41	1.04
Cysteine-rich secretory proteins (CRiSP) <i>Desmodus</i>	Common Vampire Bat (<i>Desmodus rotundus</i>)	0	N/A	$\omega = 1.62$
Cysteine-rich secretory proteins (CRiSP) All	Common Vampire Bat (<i>Desmodus rotundus</i>) Platypus (<i>Ornithorhynchus anatinus</i>)	0	N/A	$\omega = 0.482$
Kunitz Domain One	Common Vampire Bat (<i>Desmodus rotundus</i>)	1	13	$\omega = 0.444$
Kunitz Domain Two (Chiroptera)	Common Vampire Bat (<i>Desmodus rotundus</i>)	2	6, 26	$\omega = 2.51$

Table S5: CodeML results for the alignment of KLK1 *Blarina brevicauda*, including results of all models identified in this study.

Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-2742.182956	0.57984	N/A					N/A
M1a (Neutral)	-2700.342314	0.5481	P0: 0.482 ω0: 0.06		P1: 0.518 ω1:1.0			N/A
M2a†	-2694.060310	0.8139	P0: 0.522 ω0: 0.11	P1: 0.324 ω1: 1.0		P2: 0.154 ω2: 2.81		None
M3†	-2693.831198	0.8055	P0: 0.288 ω0: 0.0	P1: 0.484 ω1: 0.51		P2: 0.227 ω2: 2.45		N/A
M7 (beta)	-2701.596702	0.5558	p: 0.10166					N/A
			q: 0.08126					
M8 (beta and ω)†	-2693.907033	0.8075	p0: 0.804	P = 0.380	q = 0.629	P1 = 0.196	ω = 2.58	13 (P>0.95) (12, 20, 49, 84, 90, 111, 113, 117, 136, 172, 240, 244, 247) 3 (P>0.99) (75, 121, 171)
M8a†	-2700.358698	0.5481	p0: 0.482	P = 6.81	q = 99	P1 = 0.517	ω = 1.00	N/A
† Models that allow ω > 1 (checking for diversifying selection) *identified by the Bayes Empirical Bayes (BEB) model								

Table S6: Significance of CodeML results for the alignment of KLK1 *Blarina brevicauda*

Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	96.7	4	P < 0.001 (0.000000000)
M1a	M2a	12.6	2	P < 0.001 (0.001869650)
M7	M8	15.4	2	P < 0.001 (0.000457530)
M8a	M8	12.9	1	P < 0.001 (0.000327998)

Table S7: CodeML results for the alignment of KLK1 *Solenodon paradoxus*, including results of all models identified in this study.

Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-1825.959506	0.58254	N/A					N/A
M1a (Neutral)	-1794.224892	0.4647	P0: 0.555 ω0: 0.04		P1: 0.445 ω1:1.0			N/A
M2a†	-1783.399757	0.7298	P0: 0.483 ω0: 0.005	P1 0.473 ω1: 1.0	P2: 0.044 ω2: 5.85		1 (P>0.95) (48) 2 (P>0.99) (98, 163)	
M3†	-1783.295718	0.7002	P0: 0.462 ω0: 0.000	P1: 0.487 ω1: 0.89	P2: 0.05 ω2: 5.25		N/A	
M7 (beta)	-1794.457015	0.4416	p: 0.01396					N/A
			q: 0.01724					
M8 (beta and ω)†	-1783.338167	0.7073	p0: 0.954	P = 0.04	q = 0.05	P1 = 0.05	ω = 5.55	2 (P>0.95) (119, 167) 4 (P>0.99) (48, 98, 163, 177)
M8a†	-1794.201112	0.4615	p0: 0.565	P = 0.137	q = 1.96	P1 = 0.435	ω = 1.0	N/A

† Models that allow $\omega > 1$ (checking for diversifying selection)

*identified by the Bayes Empirical Bayes (BEB) model

Table S6: Significance of CodeML results for the alignment of KLK1 *Solenodon paradoxus*

Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	85	4	P < 0.001 (0.000000000)
M1a	M2a	21.7	2	P < 0.001 (0.000019893)
M7	M8	22.2	2	P < 0.001 (0.000014830)
M8a	M8	21.7	1	P < 0.001 (0.000003145)

Table S9: CodeML results for the alignment of KLK1 Eulipotyphla, including results of all models identified in this study.								
Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-3254.134397	0.49379	N/A					N/A
M1a (Neutral)	-3181.163308	0.5066	P0: 0.569 ω0: 0.13		P1: 0.430 ω1:1.0			N/A
M2a†	-3167.738986	0.6748	P0: 0.533 ω0: 0.132	P1: 0.436 ω1: 1.0		P2: 0.03 ω2: 5.60		1 (P>0.95) (90) 2 (P>0.99) (111, 155)
M3†	-3167.735906	0.6713	P0: 0.530 ω0: 0.13	P1 0.440 ω1: 0.99		P2:0.03 ω2: 5.53		N/A
M7 (beta)	-3181.985664	0.4700	p: 0.36708					N/A
			q: 0.41393					
M8 (beta and ω)†	-3167.205706	0.6260	p0: 0.963	P = 0.392	q = 0.44	P1 = 0.04	ω = 4.69	3 (P>0.95) (87, 90, 151) 2 (P>0.99) (111, 155)
M8a†	-3179.158058	0.4761	p0: 0.641	P = 0.856	q = 3.60	P1 = 0.36	ω = 1	N/A
† Models that allow ω > 1 (checking for diversifying selection) *identified by the Bayes Empirical Bayes (BEB) model								

Table S10: Significance of CodeML results for the alignment of KLK1 Eulipotyphla				
Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	172.8	4	P < 0.001 (0.000000000)
M1a	M2a	26.9	2	P < 0.001 (0.000001479)
M7	M8	29.6	2	P < 0.001 (0.000000381)
M8a	M8	23.9	1	P < 0.001 (0.000001012)

Table S11: CodeML results for the alignment of KLK1 All, including results of all models identified in this study.								
Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-2982.511043	0.43905	N/A					N/A
M1a (Neutral)	-2910.059147	0.5147	P0: 0.545 ω0: 0.11		P1: 0.456 ω1:1.0			N/A
M2a†	-2904.636186	0.6339	P0: 0.523 ω0: 0.11		P1: 0.436 ω1: 1.0		P2: 0.04 ω2: 3.44	1 (P>0.95) (88)
M3†	-2901.128891	0.5587	P0: 0.222 ω0:0.0	P0: 0.329 ω0:0.22	P1: 0.397 ω1:0.85		P2: 0.052 ω2:2.86	N/A
M7 (beta)	-2908.090763	0.4537	p: 0.32733					N/A
			q: 0.39411					
M8 (beta and ω)†	-2901.550405	0.5592	p0: 0.949	P = 0.364	q = 0.474	P1 = 0.05	ω = 2.87	3 (P>0.95) (103, 108, 148) 1 (P>0.99) (88)
M8a†	-2906.989456	0.4649	p0 = 0.664	P = 0.549	q = 2.13	P1 = 0.336	ω = 1.00	N/A
† Models that allow ω > 1 (checking for diversifying selection)								
*identified by the Bayes Empirical Bayes (BEB) model								

Table S12: Significance of CodeML results for the alignment of KLK1 All				
Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	162.8	6	P < 0.001 (0.000000000)
M1a	M2a	10.8	2	P < 0.01 (0.004414057)
M7	M8	13.1	2	P < 0.001 (0.001443971)
M8a	M8	10.9	1	P < 0.001 (0.000973079)

Table S13: CodeML results for the alignment of Plasminogen Activator, including results of all models identified in this study.								
Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-2402.641004	1.0549	N/A					N/A
M1a (Neutral)	-2376.314343	0.4849	P ₀ : 0.515 ω ₀ : 0.0		P ₁ : 0.485 ω ₁ : 1.0			N/A
M2a†	-2351.136942	1.3046	P ₀ : 0.708 ω ₀ : 0.163	P ₁ : 0.05 ω ₁ : 1.0		P ₂ : 0.24 ω ₂ : 4.74		10 (P>0.95) (13, 24, 30, 133, 169, 196, 219,244, 283, 303) 4 (P>0.99) (12, 39,107, 243)
M3†	-2350.557836	1.3338	P ₀ : 0.711 ω ₀ : 0.139	P ₁ : 0.270 ω ₁ : 3.73		P ₂ : 0.02 ω ₂ : 12.3		N/A
M7 (beta)	-2376.357489	0.5000	p: 0.00500					N/A
			q: 0.00500					
M8 (beta and ω)†	-2351.139833	1.3082	p ₀ : 0.752	P = 23.8	q = 99	P ₁ = 0.248	ω = 4.68	14 (P>0.95) (24, 28, 30, 76, 77, 135, 137, 156, 169, 195, 219, 280, 298, 302) 10 (P>0.99) (12, 13, 39, 107, 133, 218, 243, 244, 283, 303)
M8a†	-2376.363134	0.4849	p ₀ : 0.515	P = 0.005	q = 1.72	P ₁ = 0.49	ω = 1.00	N/A
† Models that allow ω > 1 (checking for diversifying selection) *identified by the Bayes Empirical Bayes (BEB) model								

Table S14: Significance of CodeML results for the alignment of Plasminogen Activator				
Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	104.2	4	P < 0.001 (0.000000000)
M1a	M2a	50.4	2	P < 0.001 (0.000000000)
M7	M8	50.4	2	P < 0.001 (0.000000000)
M8a	M8	125.6	15	P < 0.001 (0.000000000)

Table S15: CodeML results for the alignment of Desmallipins, including results of all models identified in this study.								
Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-2492.789614	1.09464	N/A					N/A
M1a (Neutral)	-2454.891429	0.6265	P0: 0.418 ω0: 0.11		P1: 0.582 ω1:1.0			N/A
M2a†	-2432.882104	1.3216	P0: 0.347 ω0: 0.153	P1: 0.398 ω1: 1.0		P2:0.255 ω2: 3.47		3 (P>0.95) (15, 42, 74) 7 (P>0.99) (1, 28, 64, 110, 146, 159, 160)
M3†	-2432.627521	1.3623	P0: 0.503 ω0: 0.27	P1 0.370 ω1: 1.78		P2:0.126 ω2: 4.51		N/A
M7 (beta)	-2458.205228	0.6304	p: 0.01630					N/A
			q: 0.00896					
M8 (beta and ω)†	-2432.885088	1.3393	p0: 0.725	P = 0.420	q = 0.321	P1 = 0.28	ω = 3.37	17 (P>0.95) (2, 12, 24, 25, 27, 46, 47, 69, 78, 106, 121, 140, 152, 155, 157, 161, 164) 12 (P>0.99) (1, 7, 15, 28, 42, 61, 64, 74, 110, 146, 159, 160)
M8a†	-2454.916520	0.6265	p0: 0.418	P = 11.99	q = 99	P1 = 0.581	ω = 1	
† Models that allow ω > 1 (checking for diversifying selection) *identified by the Bayes Empirical Bayes (BEB) model								

Table S16: Significance of CodeML results for the alignment of Desmallipins				
Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	120.3	4	P < 0.001 (0.000000000)
M1a	M2a	44	2	P < 0.001 (0.000000000)
M7	M8	50.6	2	P < 0.001 (0.000000000)
M8a	M8	44.1	1	P < 0.001 (0.000000000)

Table S17: CodeML results for the alignment of PACAP, including results of all models identified in this study.								
Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-1300.669556	0.90099	N/A					N/A
M1a (Neutral)	-1289.424644	0.5148	P0: 0.485 ω0: 0.0		P1: 0.515 ω1:1.0			N/A
M2a†	-1280.254233	1.3239	P0: 0. 459 ω0: 0.0	P1: 0.389 ω1: 1.0	P2:0.151 ω2: 6.16		4 (P>0.95) (4, 164, 165, 170) 1 (P>0.99) (20)	
M3†	-1280.017379	1.3933	P0: 0. 545 ω0: 0.0	P1: 0.376 ω1: 1.83	P2:0.078 ω2: 8.82		N/A	
M7 (beta)	-1289.446181	0.5000	p: 0.00503					N/A
			q: 0.00504					
M8 (beta and ω)†	-1280.298166	1.3208	p0: 0.840	P = 0.013	q = 0.016	P1 = 0.160	ω = 5.96	14 (P>0.95) (1, 7, 12, 13, 39, 40, 43, 66, 68, 87, 91, 100, 105, 107)
M8a†	-1289.424628	0.5148	p0: 0.485	P = 0.005	q = 1.83	P1 = 0.514	ω = 1.00	N/A
† Models that allow ω > 1 (checking for diversifying selection) *identified by the Bayes Empirical Bayes (BEB) model								

Table S18: Significance of CodeML results for the alignment of PACAP				
Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	41.3	4	P < 0.001 (0.000000023)
M1a	M2a	18.3	2	P < 0.001 (0.000104074)
M7	M8	18.3	2	P < 0.001 (0.000106431)
M8a	M8	18.3	1	P < 0.001 (0.000019343)

Table S19: CodeML results for the alignment of CRiSP <i>Desmodus</i> , including results of all models identified in this study.								
Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-671.081776	1.48836	N/A					N/A
M1a(Neutral)	-668.553921	0.4589	P ₀ : 0.541 ω ₀ : 0.0		P ₁ : 0.459 ω ₁ :1.0			N/A
M2a†	-660.883331	2.0592	P ₀ : 0.905 ω ₀ : 0.58	P ₁ : 0.0 ω ₁ : 1.0		P ₂ :0.1 ω ₂ : 16.125		4 (P>0.95) (7, 13, 39, 66)
M3†	-660.883331	1.4884	P ₀ : 0.760 ω ₀ : 0.58	P ₁ : 0.145 ω ₁ : 0.58		P ₂ :0.1 ω ₂ : 16.125		N/A
M7 (beta)	-668.591488	0.5000	p: 0.00500					N/A
			q: 0.00501					
M8 (beta and ω)†	-660.884052	2.0806	p ₀ : 0.906	P = 99	q = 69.09	P ₁ = 0.094	ω = 16.36	4 (P>0.95) (4, 164, 165, 169) 2 (P>0.99) (20, 170)
M8a†	-668.553916	0.4589	p ₀ : 0.541	P = 0.01	q = 1.1	P ₁ = 0.459	ω = 1	N/A
† Models that allow ω > 1 (checking for diversifying selection) *identified by the Bayes Empirical Bayes (BEB) model								

Table S20: Significance of CodeML results for the alignment of CRiSP <i>Desmodus</i>				
Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	20.4	4	P < 0.001 (0.000416898)
M1a	M2a	15.3	2	P < 0.001 (0.000466343)
M7	M8	15.4	2	P < 0.001 (0.000449472)
M8a	M8	15.3	1	P < 0.001 (0.000089808)

Table S21: CodeML results for the alignment of CRiSP All, including results of all models identified in this study								
Model	Likelihood	dN/dS	Parameters					Number of sites where $\omega > 1^*$
M0 (Null)	-1031.729998	0.34629	N/A					N/A
M1a (Neutral)	-1008.617620	0.4574	P_0 : 0.568 ω_0 : 0.04		P_1 : 0.432 ω_1 : 1.0			N/A
M2a†	-1002.587333	1.3843	P_0 : 0.574 ω_0 : 0.05	P_1 : 0.34 ω_1 : 1.0		P_2 : 0.09 ω_2 : 11.52		3($P > 0.95$) (7, 13, 64)
M3†	-1002.587333	1.4772	P_0 : 0.598 ω_0 : 0.06	P_1 : 0.316 ω_1 : 1.19		P_2 : 0.09 ω_2 : 12.43		N/A
M7 (beta)	-1009.248073	0.4542	p: 0.11168					N/A
			q: 0.13422					
M8 (beta and ω)†	-1002.784971	1.2923	p0: 0.91	P = 0.171	q = 0.3	P1 = 0.09	ω = 10.69	4 ($P > 0.95$) (39, 66, 89, 98) 3 ($P > 0.99$) (7, 13, 64)
M8a†	-1008.623987	0.4567	p0: 0.569	P = 4.78	q = 99	P1 = 0.43	ω = 1.00	
† Models that allow $\omega > 1$ (checking for diversifying selection) *identified by the Bayes Empirical Bayes (BEB) model								

Table S22: Significance of CodeML results for the alignment of CRiSP All				
Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	58.3	4	P < 0.001 (0.000000000)
M1a	M2a	12.1	3	P < 0.01 (0.007178514)
M7	M8	12.9	2	P < 0.01 (0.001559949)
M8a	M8	11.7	1	P < 0.001 (0.000632424)

Table S23: CodeML results for the alignment of Kunitz Domain One, including results of all models identified in this study								
Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-686.243223	0.29323	N/A					N/A
M1a (Neutral)	-650.130441	0.5524	P0: 0.455 ω0: 0.02		P1: 0.545 ω1:1.0			N/A
M2a†	-647.141997	1.4170	P0: 0.444 ω0: 0.015	P1: 0.403 ω1: 1.0		P2:0.15 ω2: 6.56		1(P>0.99) 28
M3†	-645.421643	1.4033	P0: 0.384 ω0: 0.0	P1: 0.304 ω1: 0.33		P2:0.312 ω2: 4.18		
M7 (beta)	-649.398512	0.4602	p: 0.13042					N/A
			q: 0.13042					
M8 (beta and ω)†	-646.307806	1.3985	p0: 0.07	P = 0.18	q = 0.6	P1 = 0.28	ω = 4.49	2 (P>0.95) (42, 43) 2 (P>0.99) (28, 38)
M8a†	-649.149486	0.4661	p0: 0.585	P = 0.148	q = 1.24	P1 = 0.42	ω = 1.00	N/A
† Models that allow ω > 1 (checking for diversifying selection) *identified by the Bayes Empirical Bayes (BEB) model								

Table S24: Significance of CodeML results for the alignment of Kunitz Domain One				
Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	81.6	4	P < 0.001 (0.000000000)
M1a	M2a	6	2	P < 0.05 (0.050365745)
M7	M8	6.2	2	P < 0.05 (0.045469841)
M8a	M8	5.9	1	P < 0.05 (0.017126540)

Table S25: CodeML results for the alignment of Kunitz Domain Two, including results of all models identified in this study								
Model	Likelihood	dN/dS	Parameters					Number of sites where omega > 1*
M0 (Null)	-490.153926	2.28040	N/A					N/A
M1a (Neutral)	-490.985448	0.7345	P0: 0.266 ω0: 0.00		P1: 0.735 ω1:1.0			N/A
M2a†	-484.686490	2.6283	P0: 0.468 ω0: 0.0	P1: 0.0 ω1: 1.0		P2:0.532 ω2: 4.94		3 (P>0.95) (15, 18, 52) 1 (P>0.99) (6)
M3†	-484.686490	2.6283	P0: 0.468 ω0: 0.0	P1: 0.366 ω1: 4.94		P2:0.166 ω2: 4.94		N/A
M7 (beta)	-491.075889	0.8000	p: 0.02008					N/A
			q: 0.00500					
M8 (beta and ω)†	-484.686489	2.6283	p0: 0.468	P = 0.005	q = 12.14	P1 = 0.53	ω = 4.938	4 (P>0.95) (31, 32, 50, 52) 3 (P>0.99) (6, 15, 18)
M8a†	-490.985439	0.7345	p0: 0.266	P = 0.005	q = 1.58	P1 = 0.735	ω = 1.00	N/A
† Models that allow ω > 1 (checking for diversifying selection) *identified by the Bayes Empirical Bayes (BEB) model								

Table S26: Significance of CodeML results for the alignment of Kunitz Domain Two				
Null model vs Alternative model		X2 result	K result	Level of significance
M0	M3	10.9	4	P < 0.05 (0.027305797)
M1a	M2a	12.6	2	P < 0.01 (0.001838219)
M7	M8	12.6	2	P < 0.01 (0.001838217)
M8a	M8	12.6	1	P < 0.001 (0.000386180)

Table S27: TreeSAAP values for each alignment, including magnitude, the value and the site number corresponding to each alignment. Sites with some level of property but at a non-significant value were included as well for completeness.

Toxin	Species	TreeSAAP property	Magnitude	Value	Site number and value	Non-significant sites identified
Kallikren-1(KLK1) <i>Blarina</i>	Short tailed shrew (<i>Blarina brevicauda</i>)	Isoelectric point	8	4.767	181(3.211)	N/A
		Power to be at the C-terminal	7	3.849	88 (3.672), 90(3.398), 118 (3.254) and 200 (4.319)	N/A
Kallikren-1(KLK1) <i>Solenodon</i>	Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	Power to be at the middle of alpha-helix	7	3.598	154 (3.354)	N/A
Kallikrein-1 (KLK1) Eulipotyphla	Short tailed shrew (<i>Blarina brevicauda</i>)	Hydropathy	6	3.212	129(4.122), 185 (4.69) and 208 (3.583)	N/A
	Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	Power to be at the C-terminal	6	5.718	85 (3.101), 114(3.671), 127(3.581), 178(3.128) and 202 (4.449)	10 (magnitude 8), 208 (magnitude 7) and 209 (magnitude 7)
Kallikren-1 (KLK1) All	Short tailed shrew (<i>Blarina brevicauda</i>)	Hydropathy	6	3.182	165(3.915) and 190 (3.7)	171 (magnitude 8)
	Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	Power to be at the C-terminal	6	6.497	78 (3.601), 107 (3.1), 109 (3.718), and 184 (4.202)	190 (magnitude 7)
	Common Vampire Bat (<i>Desmodus rotundus</i>)	Power to be at the middle of alpha-helix	7	3.552	N/A	N/A
Plasminogen Activator	Hairy legged vampire bat (<i>Diphylla ecaudata</i>)	Isoelectric point	8	3.672	N/A	N/A
	White winged vampire bat (<i>Diaemus youngi</i>)					

	Common vampire bat (<i>Desmodus rotundus</i>)					
Desmallipins (Desmodus allergen-related lipocalins)	Common Vampire Bat (<i>Desmodus rotundus</i>)	Isoelectric point	8	4.826	106 (5.322)	42 (Magnitude 7)
PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide)	Common Vampire Bat (<i>Desmodus rotundus</i>)	N/A				
Cysteine-rich secretory proteins (CRiSP) <i>Desmodus</i>	Common Vampire Bat (<i>Desmodus rotundus</i>)	Long-range non-bonded energy	8	3.398	7(3.211)	N/A
		Polar requirement	7	3.939	N/A	N/A
Cysteine-rich secretory proteins (CRiSP) All	Common Vampire Bat (<i>Desmodus rotundus</i>)	Isoelectric point	8	3.8	28 (3.237)	N/A
		Polar requirement	7	4.326	N/A	N/A
	Platypus (<i>Ornithorhynchus anatinus</i>)	Power to be at the C-terminal	6	5.064	N/A	N/A
Kunitz Domain One	Common Vampire Bat (<i>Desmodus rotundus</i>)	Isoelectric point	7	4.711	N/A	N/A
		Partial specific volume	6	3.74	N/A	N/A
Kunitz Domain Two	Common Vampire Bat (<i>Desmodus rotundus</i>)	Normalized consensus hydrophobicity	8	3.253	N/A	N/A
		Power to be at the N-terminal	6	3.64	N/A	N/A

Table S28: Model selection result from MEGA for maximum likelihood tree for each alignment

Toxin	Species	Model
Kallikren-1(KLK1) <i>Blarina</i>	Short tailed shrew (<i>Blarina brevicauda</i>)	T92+I
Kallikren-1(KLK1) <i>Solenodon</i>	Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	T92+G
Kallikrein-1 (KLK1) <i>Eulipotyphla</i>	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>)	T92+G
Kallikren-1 (KLK1) All	Short tailed shrew (<i>Blarina brevicauda</i>) Hispaniolan solenodon (<i>Solenodon paradoxus</i>) Common Vampire Bat (<i>Desmodus rotundus</i>)	T92+G
Plasminogen Activator	Hairy legged vampire bat (<i>Diphylla ecaudata</i>) White winged vampire bat (<i>Diaemus youngi</i>) Common vampire bat (<i>Desmodus rotundus</i>)	K2+G
Desmallipins (<i>Desmodus</i> allergen-related lipocalins)	Common Vampire Bat (<i>Desmodus rotundus</i>)	K2+G
PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide)	Common Vampire Bat (<i>Desmodus rotundus</i>)	HKY+G
Cysteine-rich secretory proteins (CRiSP) <i>Desmodus</i>	Common Vampire Bat (<i>Desmodus rotundus</i>)	T92+G
Cysteine-rich secretory proteins (CRiSP) <i>Desmodus</i> and <i>Ornithorhynchus</i>	Common Vampire Bat (<i>Desmodus rotundus</i>) Platypus (<i>Ornithorhynchus anatinus</i>)	T92
Kunitz Domain One	Common Vampire Bat (<i>Desmodus rotundus</i>)	JC + I
Kunitz Domain Two	Common Vampire Bat (<i>Desmodus rotundus</i>)	JC +G