

Supplementary Materials: Tb II-I, a fraction isolated from *Tityus bahiensis* scorpion venom, alters cytokines level and induces seizures when intrahippocampally injected in rats

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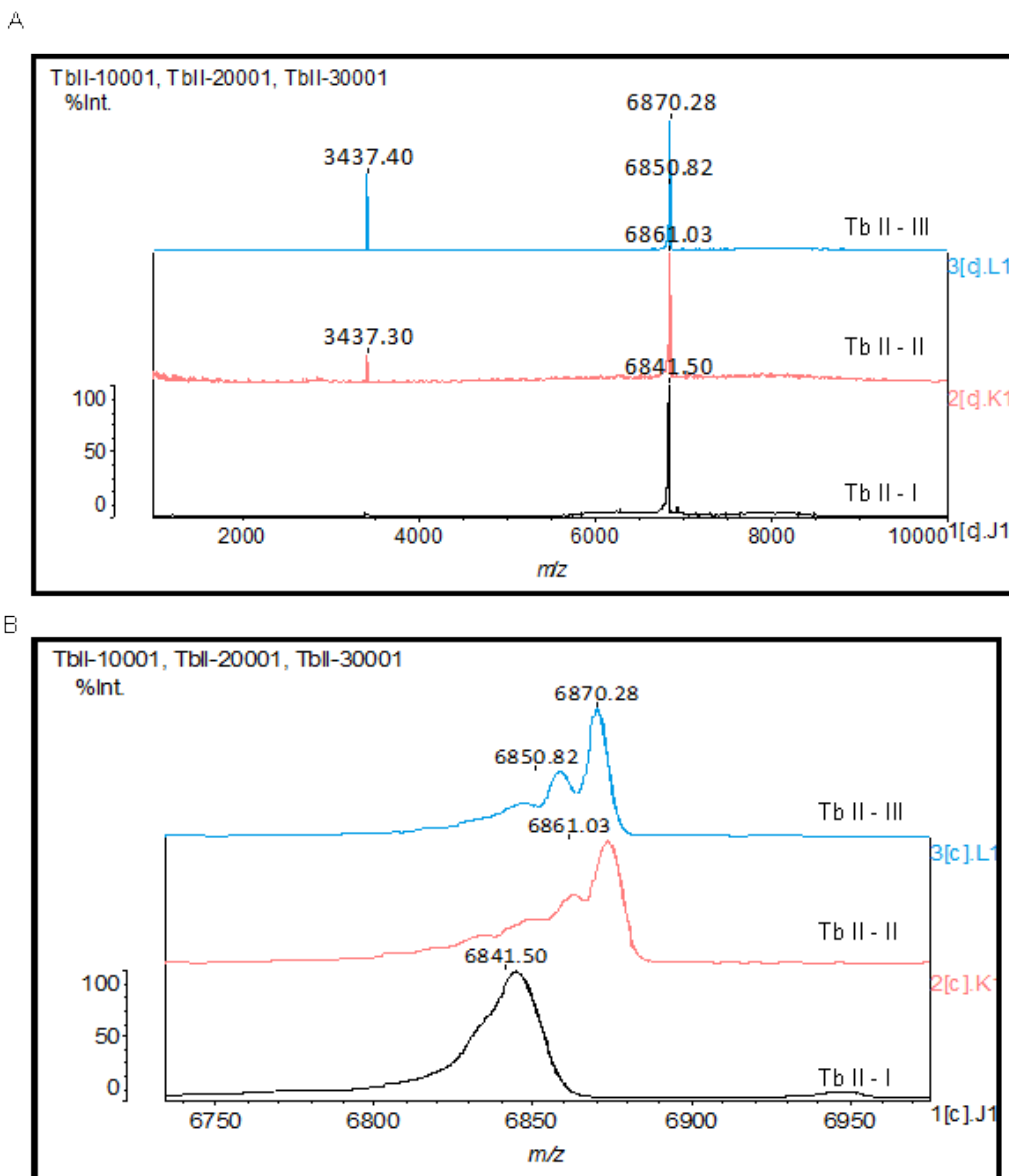
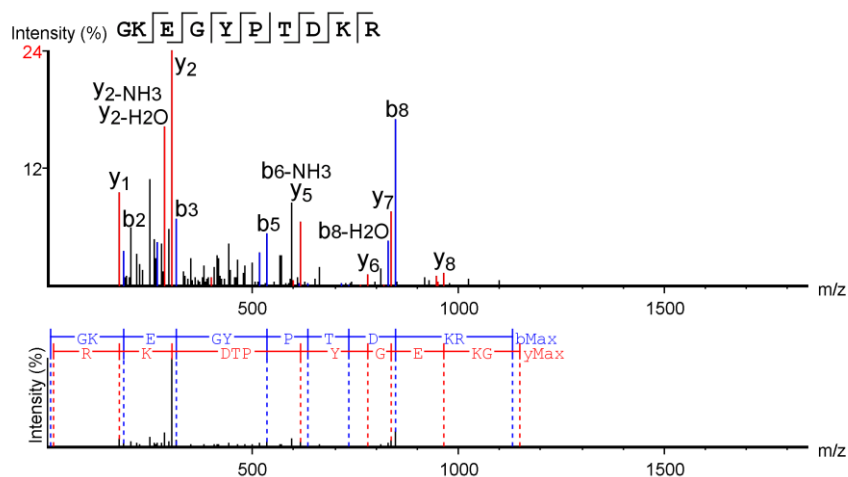


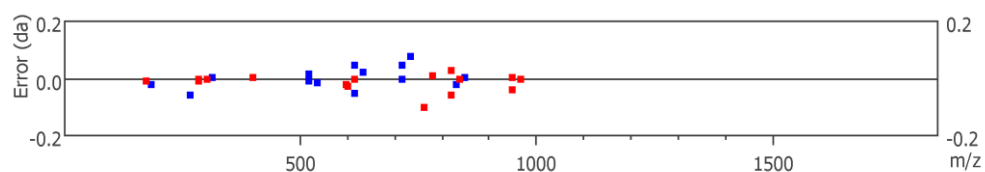
Figure S1. MALDI-TOF/MS profile of Tb II-I, Tb II-II and Tb II-III fractions. (A) It was possible to observe an intense peak around 6800 – 6900 m/z in all three fractions. Besides, the ion 3437 in fraction Tb II-II and Tb II-III is the double charged ion $[M + 2H]^{2+}$ of 6870 m/z. (B) After to limit the mass range between 6750 – 6950 m/z, it was possible to observe the presence of 2 – 3 peaks in Tb II-II and Tb II-III, respectively, with middle to higher intensity. Instead of it, only Tb II-I shows one intensity peak at this mass range.

A

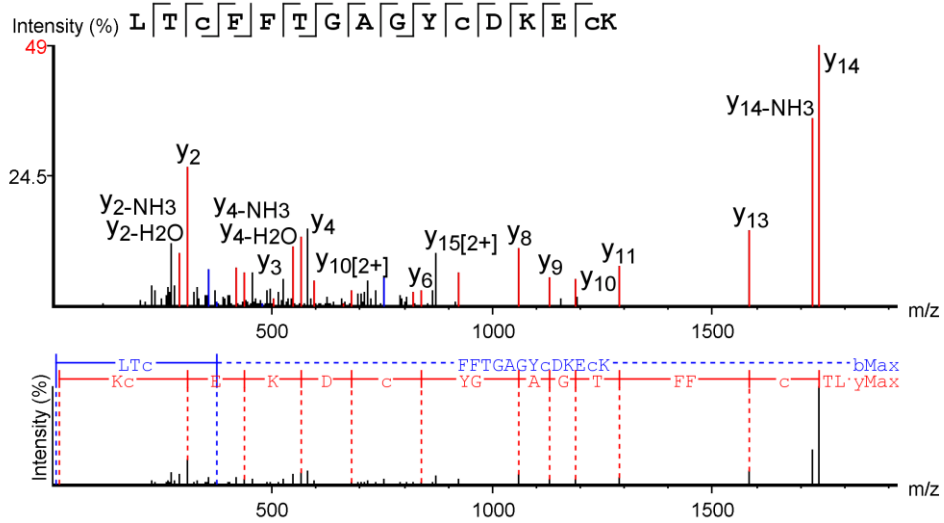


B

#	b	b-H ₂ O	b-NH ₃	b (2+)	Seq	y	y-H ₂ O	y-NH ₃	y (2+)	#
1	58.03	40.02	41.00	29.51	G					10
2	186.14	168.11	169.10	93.56	K	1093.56	1075.55	1076.54	547.28	9
3	315.16	297.16	298.14	158.08	E	965.47	947.50	948.43	483.23	8
4	372.19	354.18	355.16	186.59	G	836.42	818.38	819.46	418.71	7
5	535.27	517.22	518.23	268.19	Y	779.39	761.49	762.38	390.20	6
6	632.28	614.24	615.33	316.65	P	616.34	598.35	599.34	308.67	5
7	733.27	715.34	716.27	367.18	T	519.29	501.28	502.26	260.14	4
8	848.37	830.39	831.35	424.69	D	418.24	400.23	401.20	209.62	3
9	976.47	958.46	959.45	488.74	K	303.22	285.21	286.19	152.11	2
10					R	175.13	157.11	158.09	88.06	1

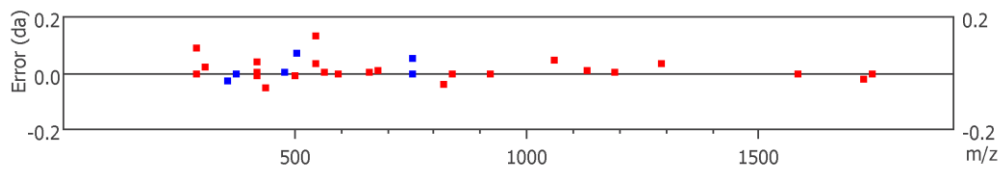


C

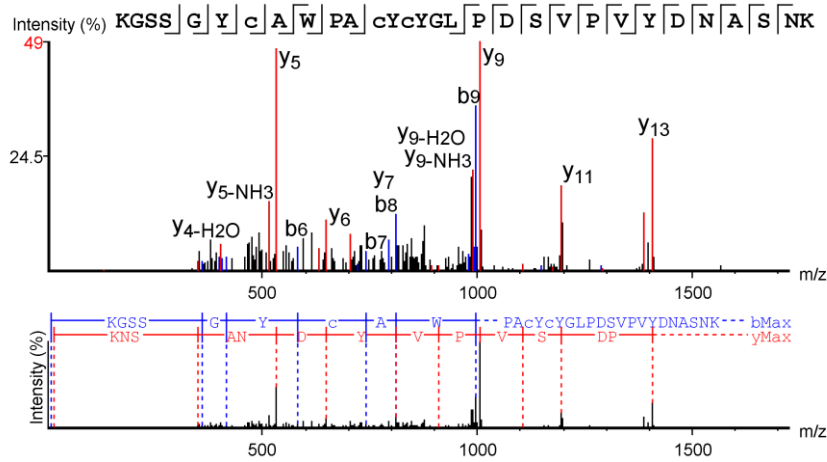


D

#	b	b-H2O	b-NH3	b (2+)	Seq	y	y-H2O	y-NH3	y (2+)	#
1	114.09	96.08	97.06	57.55	L					16
2	215.14	197.13	198.11	108.07	T	1843.74	1825.73	1826.72	922.37	15
3	375.17	357.19	358.14	188.09	C(+57.02)	1742.70	1724.69	1725.69	871.85	14
4	522.24	504.15	505.21	261.62	F	1582.66	1564.66	1565.64	791.83	13
5	669.31	651.30	652.28	335.15	F	1435.60	1417.59	1418.57	718.30	12
6	770.35	752.35	753.27	385.68	T	1288.49	1270.52	1271.50	644.76	11
7	827.38	809.37	810.35	414.19	G	1187.47	1169.47	1170.45	594.24	10
8	898.41	880.40	881.39	449.71	A	1130.44	1112.45	1113.43	565.73	9
9	955.43	937.42	938.41	478.21	G	1059.37	1041.41	1042.40	530.21	8
10	1118.50	1100.49	1101.47	559.75	Y	1002.40	984.39	985.37	501.71	7
11	1278.53	1260.52	1261.50	639.76	C(+57.02)	839.34	821.37	822.31	420.12	6
12	1393.56	1375.55	1376.53	697.28	D	679.29	661.29	662.28	340.15	5
13	1521.65	1503.64	1504.62	761.33	K	564.27	546.23	547.12	282.64	4
14	1650.69	1632.68	1633.67	825.85	E	436.24	418.17	419.17	218.59	3
15	1810.72	1792.71	1793.70	905.86	C(+57.02)	307.12	289.13	290.02	154.07	2
16					K	147.11	129.10	130.09	74.06	1



E



F

#	b	b-H2O	b-NH3	b (2+)	Seq	y	y-H2O	y-NH3	y (2+)	#
1	129.10	111.09	112.08	65.05	K					30
2	186.12	168.11	169.10	93.56	G	3258.36	3240.35	3241.33	1629.68	29
3	273.16	255.15	256.13	137.08	S	3201.34	3183.33	3184.31	1601.17	28
4	360.14	342.18	343.16	180.59	S	3114.31	3096.30	3097.28	1557.65	27
5	417.17	399.20	400.18	209.10	G	3027.27	3009.26	3010.25	1514.14	26
6	580.25	562.26	563.25	290.64	Y	2970.25	2952.24	2953.23	1485.63	25
7	740.33	722.33	723.28	370.65	C(+57.02)	2807.19	2789.18	2790.16	1404.09	24
8	811.35	793.31	794.33	406.16	A	2647.16	2629.15	2630.13	1324.08	23
9	997.42	979.45	980.36	499.21	W	2576.12	2558.11	2559.09	1288.58	22
10	1094.47	1076.46	1077.45	547.74	P	2390.04	2372.03	2373.02	1195.52	21
11	1165.51	1147.46	1148.47	583.26	A	2292.99	2274.98	2275.96	1146.99	20
12	1325.54	1307.53	1308.51	663.27	C(+57.02)	2221.95	2203.94	2204.93	1111.48	19
13	1488.60	1470.59	1471.58	744.80	Y	2061.92	2043.91	2044.90	1031.46	18
14	1648.63	1630.62	1631.61	824.82	C(+57.02)	1898.86	1880.85	1881.83	949.93	17
15	1811.70	1793.69	1794.67	906.35	Y	1738.83	1720.82	1721.80	869.91	16
16	1868.72	1850.71	1851.69	934.86	G	1575.76	1557.75	1558.74	788.38	15
17	1981.80	1963.79	1964.78	991.43	L	1518.74	1500.73	1501.72	759.87	14
18	2078.86	2060.85	2061.83	1039.93	P	1405.66	1387.64	1388.64	703.30	13
19	2193.88	2175.87	2176.86	1097.44	D	1308.61	1290.60	1291.50	654.80	12
20	2280.92	2262.90	2263.89	1140.96	S	1193.60	1175.56	1176.55	597.29	11
21	2379.98	2361.97	2362.96	1190.49	V	1106.47	1088.54	1089.52	553.77	10
22	2477.04	2459.03	2460.01	1239.02	P	1007.47	989.47	990.46	504.24	9
23	2576.10	2558.09	2559.08	1288.58	V	910.37	892.37	893.40	455.71	8
24	2739.17	2721.16	2722.14	1370.08	Y	811.35	793.31	794.33	406.16	7
25	2854.20	2836.18	2837.17	1427.60	D	648.28	630.22	631.28	324.65	6
26	2968.24	2950.23	2951.21	1484.62	N	533.26	515.26	516.23	267.13	5
27	3039.28	3021.26	3022.25	1520.14	A	419.22	401.15	402.20	210.11	4
28	3126.31	3108.30	3109.28	1563.65	S	348.21	330.18	331.16	174.59	3
29	3240.35	3222.34	3223.32	1620.68	N	261.16	243.15	244.13	131.22	2
30					K	147.11	129.10	130.09	74.06	1

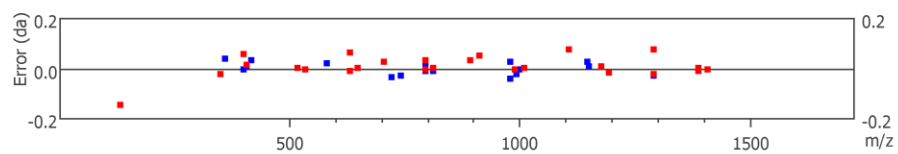
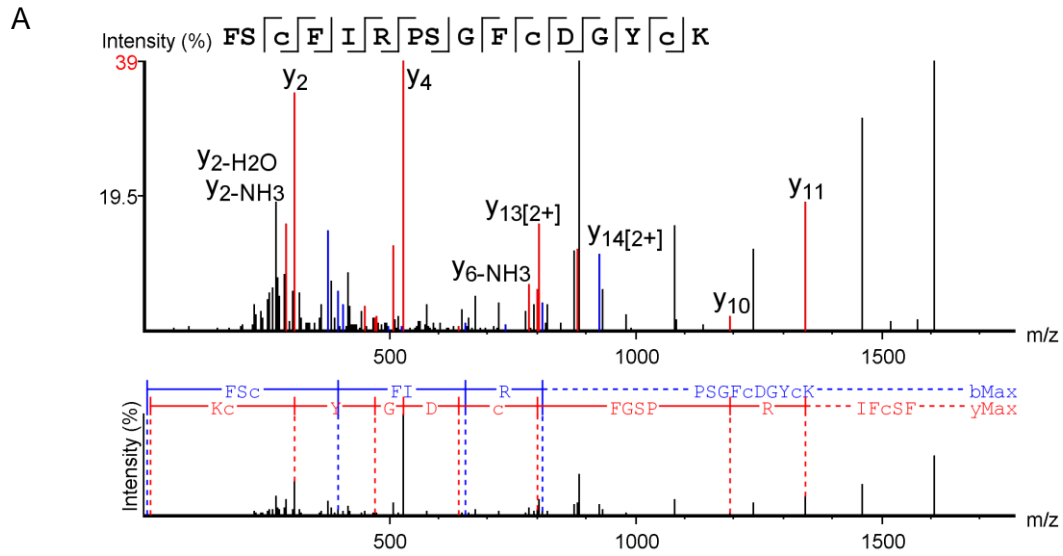
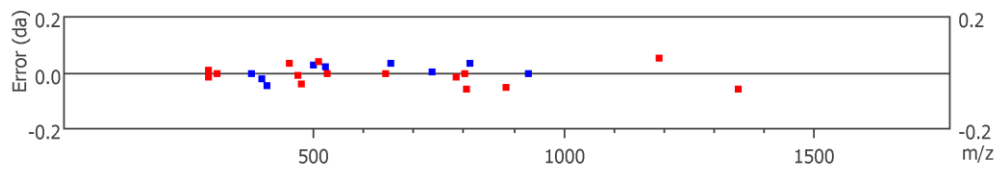


Figure S2. Tryptic peptides identified for Tbah02791 (Toxin Tb4) protein. (A) Annotated spectrum with alignment of the ion 575.77 2+ and its (B) ion table and error map. (C) Annotated spectrum with alignment of the ion 652.89 3+ and its (D) ion table and error map. (E) Annotated spectrum with alignment of the ion 1129.48 3+ and its (F) ion table and error map.

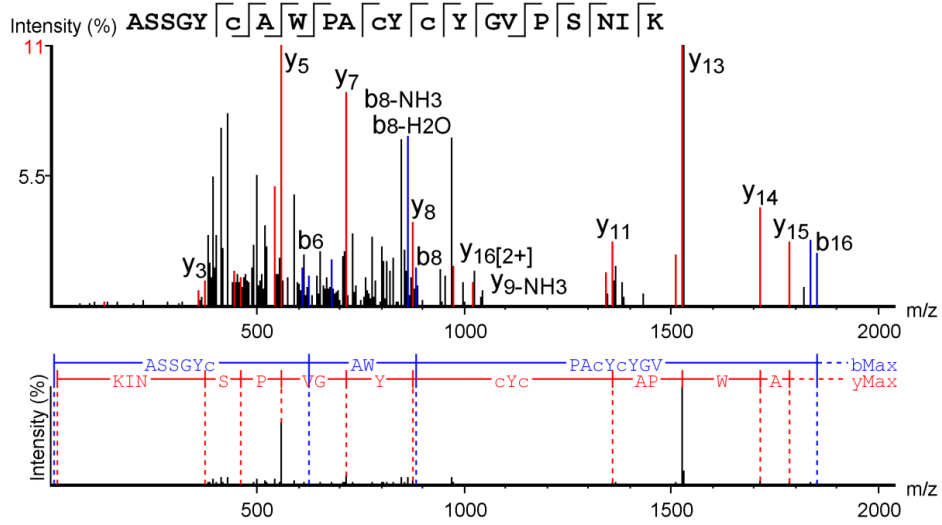


B

#	b	b-H ₂ O	b-NH ₃	b (2+)	Seq	y	y-H ₂ O	y-NH ₃	y (2+)	#
1	148.08	130.07	131.05	74.54	F					16
2	235.11	217.10	218.08	118.05	S	1853.78	1835.77	1836.75	927.39	15
3	395.16	377.13	378.11	198.07	C(+57.02)	1766.74	1748.73	1749.72	883.92	14
4	542.21	524.17	525.18	271.60	F	1606.71	1588.70	1589.69	803.92	13
5	655.25	637.28	638.26	328.15	I	1459.65	1441.64	1442.62	730.32	12
6	811.35	793.38	794.37	406.24	R	1346.62	1328.55	1329.53	673.78	11
7	908.45	890.43	891.42	454.72	P	1190.40	1172.45	1173.43	595.73	10
8	995.48	977.47	978.45	498.21	S	1093.41	1075.40	1076.38	547.20	9
9	1052.50	1034.49	1035.47	526.75	G	1006.38	988.37	989.35	503.69	8
10	1199.57	1181.56	1182.54	600.28	F	949.35	931.34	932.33	475.22	7
11	1359.60	1341.59	1342.57	680.30	C(+57.02)	802.29	784.28	785.27	401.64	6
12	1474.62	1456.61	1457.60	737.81	D	642.25	624.24	625.23	321.63	5
13	1531.65	1513.64	1514.62	766.32	G	527.23	509.17	510.20	264.11	4
14	1694.71	1676.70	1677.68	847.85	Y	470.22	452.15	453.18	235.60	3
15	1854.74	1836.73	1837.71	927.87	C(+57.02)	307.15	289.15	290.10	154.07	2
16					K	147.11	129.10	130.09	74.06	1

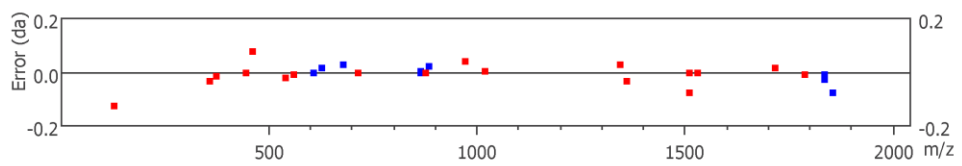


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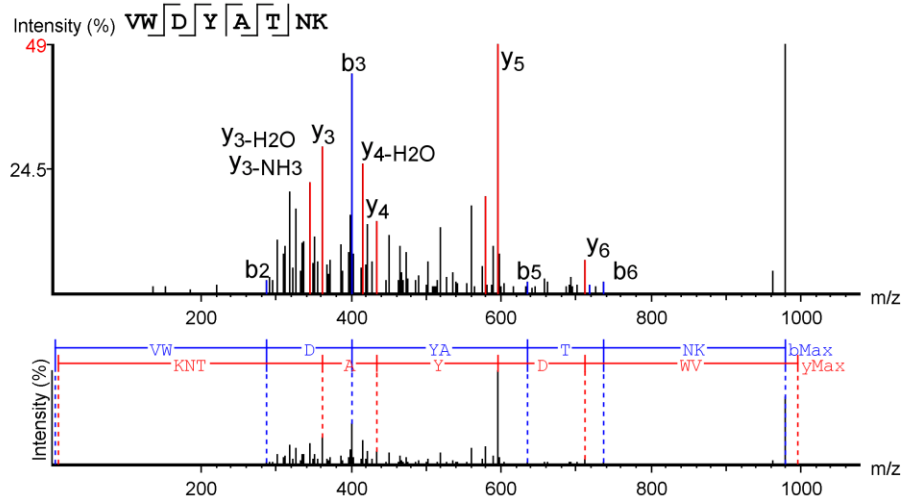


D

#	b	b-H ₂ O	b-NH ₃	b(2+)	Seq	y	y-H ₂ O	y-NH ₃	y(2+)	#
1	72.04	54.03	55.02	36.52	A					21
2	159.08	141.07	142.05	80.04	S	2339.99	2321.98	2322.96	1170.49	20
3	246.11	228.10	229.08	123.55	S	2252.96	2234.95	2235.93	1126.98	19
4	303.13	285.12	286.10	152.07	G	2165.92	2147.91	2148.90	1083.46	18
5	466.19	448.18	449.17	233.60	Y	2108.90	2090.89	2091.88	1054.95	17
6	626.20	608.21	609.20	313.61	C(+57.02)	1945.84	1927.83	1928.81	973.37	16
7	697.26	679.22	680.23	349.13	A	1785.82	1767.80	1768.78	893.40	15
8	883.32	865.32	866.32	442.17	W	1714.75	1696.76	1697.74	857.89	14
9	980.39	962.38	963.37	490.70	P	1528.69	1510.76	1511.67	764.85	13
10	1051.43	1033.42	1034.40	526.22	A	1431.64	1413.63	1414.61	716.32	12
11	1211.46	1193.45	1194.43	606.23	C(+57.02)	1360.63	1342.59	1343.54	680.80	11
12	1374.52	1356.51	1357.50	687.76	Y	1200.57	1182.56	1183.54	600.79	10
13	1534.56	1516.54	1517.53	767.78	C(+57.02)	1037.51	1019.50	1020.47	519.25	9
14	1697.62	1679.61	1680.59	849.31	Y	877.47	859.47	860.45	439.24	8
15	1754.64	1736.63	1737.61	877.82	G	714.41	696.40	697.39	357.71	7
16	1853.79	1835.71	1836.71	927.35	V	657.39	639.38	640.37	329.20	6
17	1950.76	1932.75	1933.73	975.88	P	558.33	540.31	541.32	279.66	5
18	2037.79	2019.78	2020.77	1019.40	S	461.19	443.26	444.24	231.14	4
19	2151.84	2133.83	2134.81	1076.42	N	374.26	356.23	357.25	187.62	3
20	2264.92	2246.91	2247.89	1132.96	I	260.20	242.19	243.17	130.60	2
21					K	147.11	129.10	130.21	74.06	1



E



F

#	b	b-H2O	b-NH3	b (2+)	Seq	y	y-H2O	y-NH3	y (2+)	#
1	100.08	82.07	83.05	50.54	V					8
2	286.14	268.14	269.13	143.58	W	897.41	879.40	880.38	449.21	7
3	401.18	383.17	384.16	201.09	D	711.29	693.32	694.30	356.17	6
4	564.25	546.24	547.22	282.62	Y	596.32	578.29	579.29	298.65	5
5	635.31	617.27	618.26	318.14	A	433.17	415.15	416.21	217.12	4
6	736.30	718.38	719.30	368.67	T	362.16	344.17	345.21	181.60	3
7	850.37	832.36	833.35	425.69	N	261.16	243.15	244.13	131.08	2
8					K	147.11	129.10	130.09	74.06	1

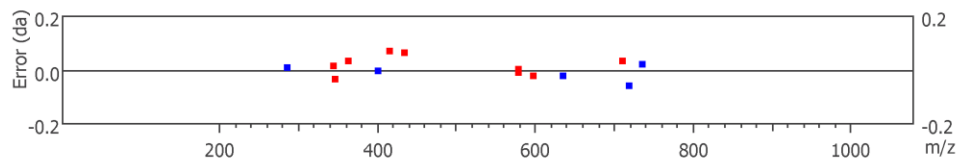
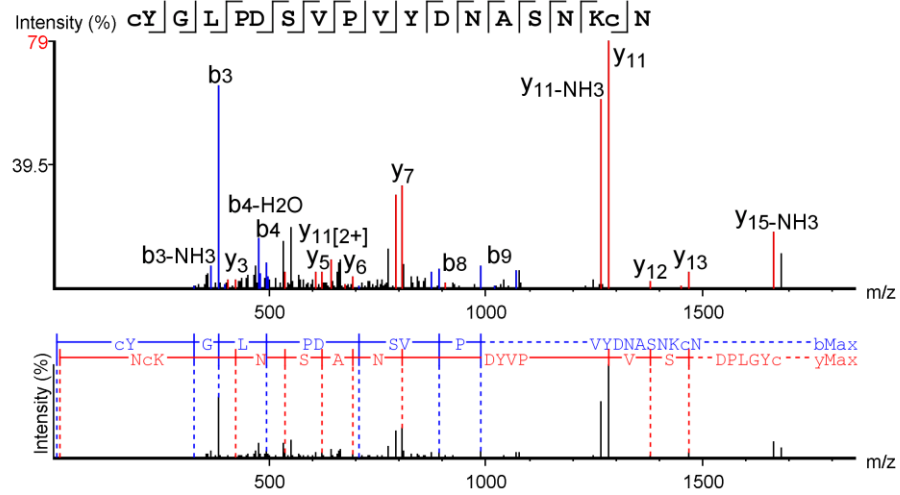


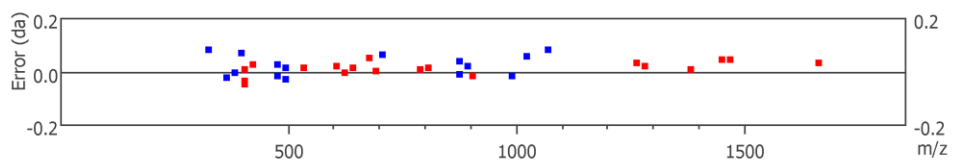
Figure S3. Tryptic peptides identified for Tbah02765 (Toxin Tb2 II) protein. (A) Annotated spectrum with alignment of the ion 667.62 3+ and its (B) ion table and error map. (C) Annotated spectrum with alignment of the ion 1205.935 2+ and its (D) ion table and error map. (E) Annotated spectrum with alignment of the ion 996.509 1+ and its (F) ion table and error map.

A

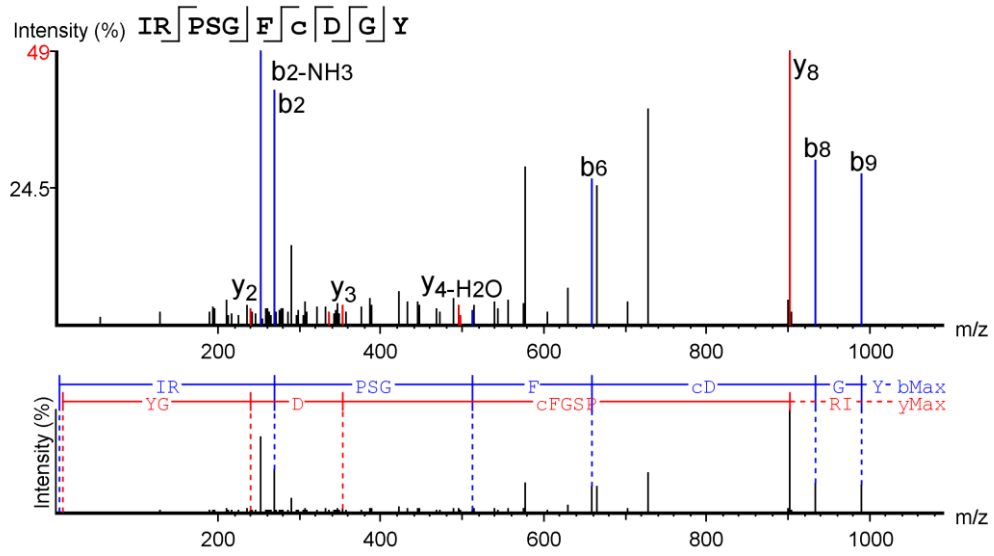


B

#	b	b-H2O	b-NH3	b (2+)	Seq	y	y-H2O	y-NH3	y (2+)	#
1	161.04	143.03	144.01	81.02	C(+57.02)					19
2	324.02	306.09	307.07	162.55	Y	2012.90	1994.89	1995.87	1006.95	18
3	381.12	363.11	364.11	191.06	G	1849.84	1831.83	1832.81	925.42	17
4	494.19	476.21	477.15	247.60	L	1792.82	1774.81	1775.79	896.91	16
5	591.26	573.25	574.23	296.13	P	1679.73	1661.72	1662.67	840.37	15
6	706.21	688.28	689.26	353.64	D	1582.68	1564.67	1565.65	791.84	14
7	793.32	775.31	776.29	397.08	S	1467.60	1449.64	1450.58	734.33	13
8	892.36	874.33	875.37	446.69	V	1380.61	1362.61	1363.59	690.81	12
9	989.45	971.43	972.41	495.25	P	1281.52	1263.54	1264.49	641.25	11
10	1088.51	1070.41	1071.48	544.75	V	1184.50	1166.49	1167.47	592.75	10
11	1251.57	1233.56	1234.54	626.29	Y	1085.43	1067.42	1068.40	543.22	9
12	1366.60	1348.59	1349.57	683.80	D	922.37	904.36	905.36	461.68	8
13	1480.64	1462.63	1463.61	740.82	N	807.32	789.33	790.30	404.20	7
14	1551.68	1533.67	1534.65	776.34	A	693.29	675.29	676.22	347.15	6
15	1638.71	1620.70	1621.68	819.86	S	622.26	604.25	605.21	311.63	5
16	1752.75	1734.74	1735.73	876.88	N	535.21	517.22	518.20	268.11	4
17	1880.85	1862.84	1863.82	940.92	K	421.15	403.16	404.20	211.09	3
18	2040.88	2022.87	2023.85	1020.88	C(+57.02)	293.09	275.08	276.06	147.05	2
19					N	133.06	115.05	116.03	67.03	1

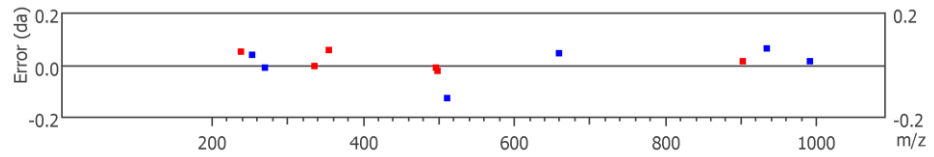


C

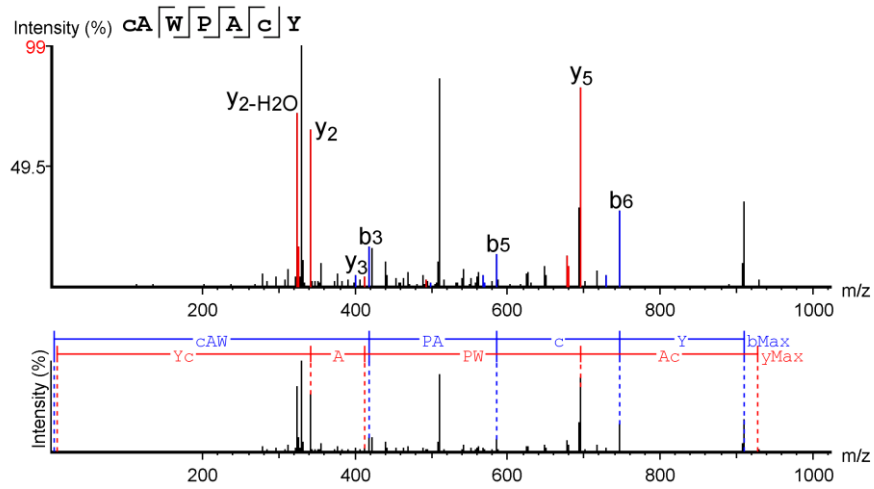


D

#	b	b-H2O	b-NH3	b (2+)	Seq	y	y-H2O	y-NH3	y (2+)	#
1	114.09	96.08	97.06	57.55	I					10
2	270.20	252.18	253.12	135.60	R	1058.44	1040.43	1041.41	529.72	9
3	367.25	349.24	350.22	184.12	P	902.32	884.32	885.31	451.67	8
4	454.28	436.27	437.25	227.64	S	805.28	787.27	788.26	403.14	7
5	511.43	493.29	494.27	256.15	G	718.25	700.24	701.22	359.63	6
6	658.32	640.36	641.34	329.68	F	661.23	643.22	644.20	331.11	5
7	818.40	800.39	801.37	409.70	C(+57.02)	514.16	496.16	497.15	257.58	4
8	933.35	915.41	916.40	467.21	D	354.07	336.12	337.10	177.56	3
9	990.43	972.44	973.42	495.72	G	239.05	221.09	222.08	120.05	2
10					Y	182.08	164.07	165.05	91.54	1



E



F

#	b	b-H2O	b-NH3	b (2+)	Seq	y	y-H2O	y-NH3	y (2+)	#
1	161.04	143.03	144.01	81.02	C(+57.02)					7
2	232.08	214.07	215.05	116.54	A	767.32	749.31	750.29	384.16	6
3	418.09	400.17	401.17	209.58	W	696.25	678.21	679.23	348.64	5
4	515.21	497.20	498.14	258.10	P	510.20	492.11	493.17	255.60	4
5	586.18	568.21	569.23	293.62	A	413.14	395.14	396.12	207.07	3
6	746.24	728.23	729.25	373.64	C(+57.02)	342.07	324.08	325.14	171.56	2
7					Y	182.08	164.07	165.05	91.54	1

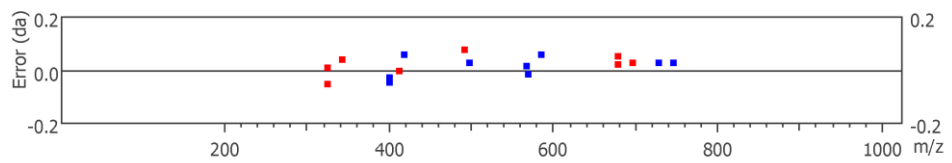
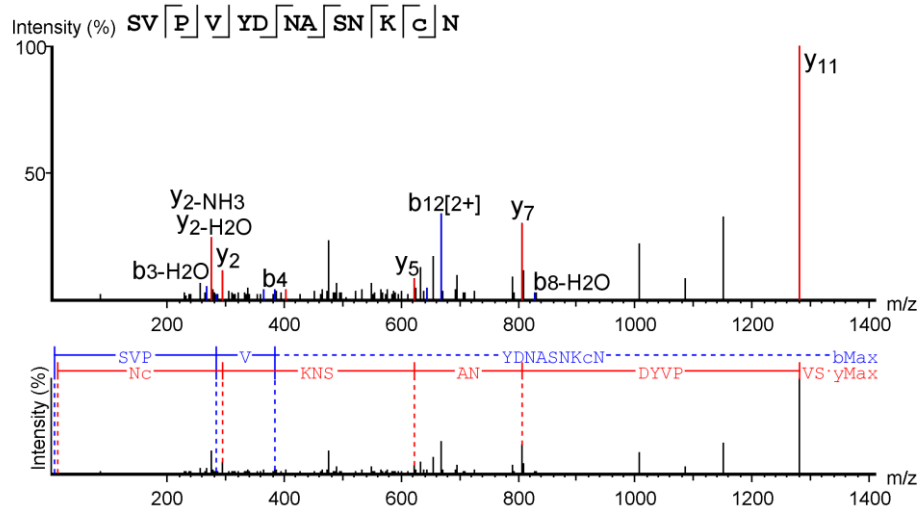


Figure S4. Chymotryptic peptides identified for Tbah02791 (Toxin Tb4) and Tbah02765 (Toxin Tb2 II) proteins. (A) Annotated spectrum with alignment of the ion 586.244 2+ and its (B) ion table and error map identified for Tbah02791. (C) Annotated spectrum with alignment of the ion 1086.938 2+ and its (D) ion table and error map identified for Tbah02765. (E) Annotated spectrum with alignment of the ion 927.30 1+ and its (F) ion table and error map identified in both proteins.

A



B

#	b	b-H2O	b-NH3	b(2+)	Seq	y	y-H2O	y-NH3	y(2+)	#
1	88.04	70.03	71.01	44.52	S					13
2	187.11	169.10	170.08	94.05	V	1380.62	1362.61	1363.59	690.81	12
3	284.11	266.06	267.13	142.58	P	1281.58	1263.54	1264.53	641.28	11
4	383.26	365.23	366.20	192.11	V	1184.50	1166.49	1167.47	592.75	10
5	546.29	528.28	529.27	273.65	Y	1085.43	1067.42	1068.40	543.22	9
6	661.32	643.33	644.29	331.16	D	922.37	904.36	905.34	461.68	8
7	775.36	757.35	758.34	388.18	N	807.35	789.33	790.31	404.17	7
8	846.40	828.32	829.37	423.70	A	693.30	675.29	676.27	347.15	6
9	933.43	915.42	916.40	467.22	S	622.26	604.25	605.23	311.63	5
10	1047.47	1029.46	1030.45	524.24	N	535.23	517.22	518.20	268.11	4
11	1175.57	1157.56	1158.54	588.28	K	421.19	403.19	404.16	211.09	3
12	1335.60	1317.59	1318.57	668.29	C(+57.02)	293.12	275.08	276.12	147.05	2
13					N	133.06	115.05	116.03	67.03	1

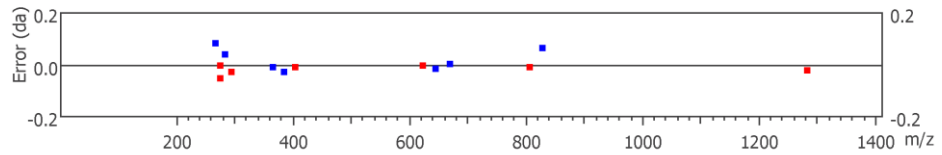


Figure S5. Chemical cleavage peptide identified for Tbah02791 (Toxin Tb4) protein. (A) Annotated spectrum with alignment of the ion 734.303 2+ and its (B) ion table and error map identified.

Table S1. List of peptides identified for Tbah02791 (Toxin Tb4) and Tbah02765 (Toxin Tb2 II) proteins.

Protein Accession	Cleavage	Peptide	-10lgP	Mass	Length	ppm	m/z	z
Tbah02791 (Toxin Tb4)	Trypsin	K.LTC(+57.02)*FFTGAGYC(+57.02)*DK.E	69.51	1538.65	13	-0.7	770.3334	2
		K.LTC(+57.02)*FFTGAGYC(+57.02)*DKEC(+57.02)*K.L	55.62	1955.82	16	-75.8	652.8984	3
		K.KGSSGYC(+57.02)*AWPAC(+57.02)*YC(+57.02)*YGLPDSVPVYDNASNK.C	42.75	3385.44	30	-6.6	1129.4824	3
		G.GKEGYPTDKR.G	37.63	1149.57	10	-37.7	575.7745	2
		K.EGYPTDKR.G	24.92	964.46	8	-36	483.2206	2
	Chymotrypsin Formic acid	G.GKEGYPTDK.R	22.39	993.47	9	-70.5	497.7106	2
		Y.C(+57.02)*AWPAC(+57.02)*Y.C	22.48	926.34	7	-42.8	927.3091	1
		Y.C(+57.02)*YGLPDSVPVYDNASNKC(+57.02)*N.K	16.96	2171.92	19	-29.1	1086.9384	2
		T.SVPVYDNASNKC(+57.02)*N.A	20.32	1466.64	13	-36.1	734.3038	2
		K.VWDYATNK.C	33.92	995.47	8	30.6	996.509	1
Tbah02765 (Toxin Tb2 II)	Trypsin	K.FSC(+57.02)*FIRPSGFC(+57.02)*DGYC(+57.02)*K.T	29.13	1999.83	16	2.2	667.6214	3
		K.ASSGYC(+57.02)*AWPAC(+57.02)*YC(+57.02)*YGVPSNIK.V	21.78	2410.01	21	-66.8	1205.9358	2
	Chymotrypsin	F.IRPSGFC(+57.02)*DGY.C	23.3	1170.51	10	-33	586.2444	2
		Y.C(+57.02)*AWPAC(+57.02)*Y.C	22.48	926.34	7	-42.8	927.3091	1

* +57.02: Carbamidomethylation.