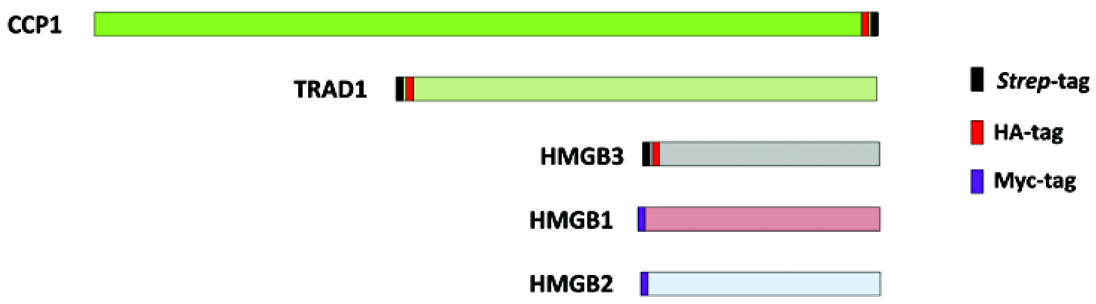
Supplementary Data: Supplementary Figures

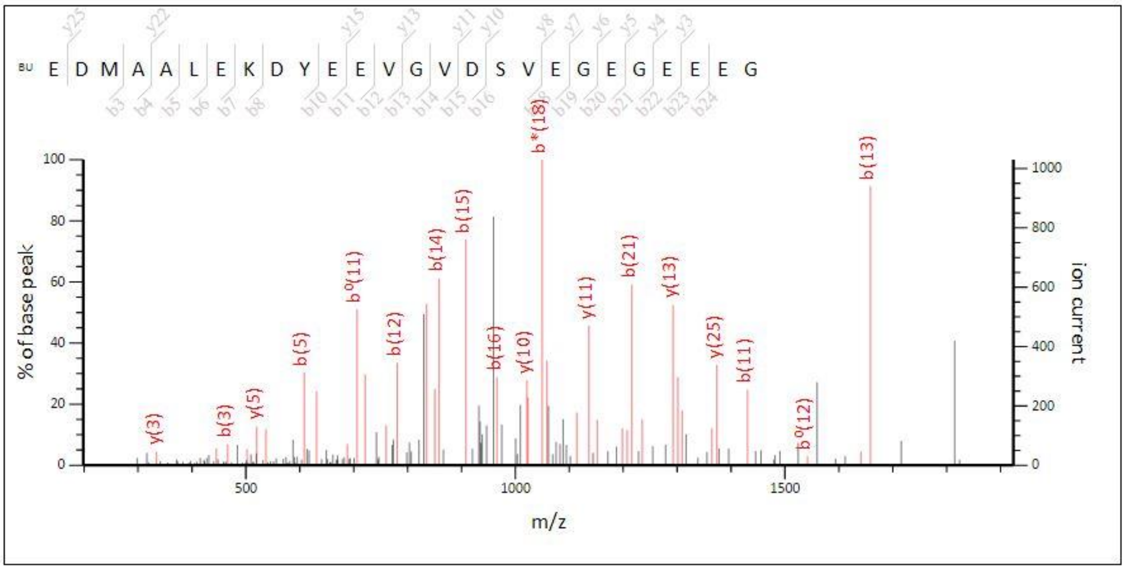
Supplementary Figure S1

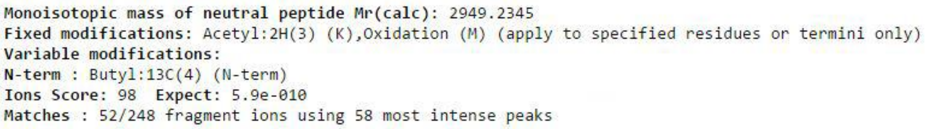


Supplementary Figure S1. Schematic representation of the constructs created for this study.

Supplementary Figure S2 – Annotated spectra corresponding to the identifications presented in Table 1. a) α-Tubulin 1A/1B, EDMAALEKDYEEVGVDSSVEGEGEEEG. **b)** α-Tubulin 1C, EDMAALEKDYEEVGADSADGEDEG. **c)** Eukaryotic translation initiation factor 4H, EEVVQKEQ. **d)** Stathmin, KNKESKDPADETEA. **e)** 40S ribosomal protein S9, KNAKKGQGGAGAGDDE. **f)** 40S ribosomal protein S9, KNAKKGQGGAGAGDD. **g)** TRAF-type zinc finger domain-containing protein, TAKAKPSKQQGAGDA. **h)** High mobility group protein B3, KKVEEEDE. **i)** High mobility group protein B3, KKVEEED.

Suppl. Fig. S2. a) **Protein:** α-Tubulin 1A/1B, Uniprot Q71U36/P68363
Peptide sequence: EDMAALEKDYEEVGVDSVEGEGEEEG
Precursor m/z: 984.4198
Precursor z: +3
Highest Mascot score: 98

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Monoisotopic mass of neutral peptide Mr(calc): 2949.2345
Fixed modifications: Acetyl:2H(3) (K),Oxidation (M) (apply to specified residues or termini only)
Variable modifications:
N-term : Butyl:13C(4) (N-term)
Ions Score: 98  Expect: 5.9e-010
Matches : 52/248 fragment ions using 58 most intense peaks
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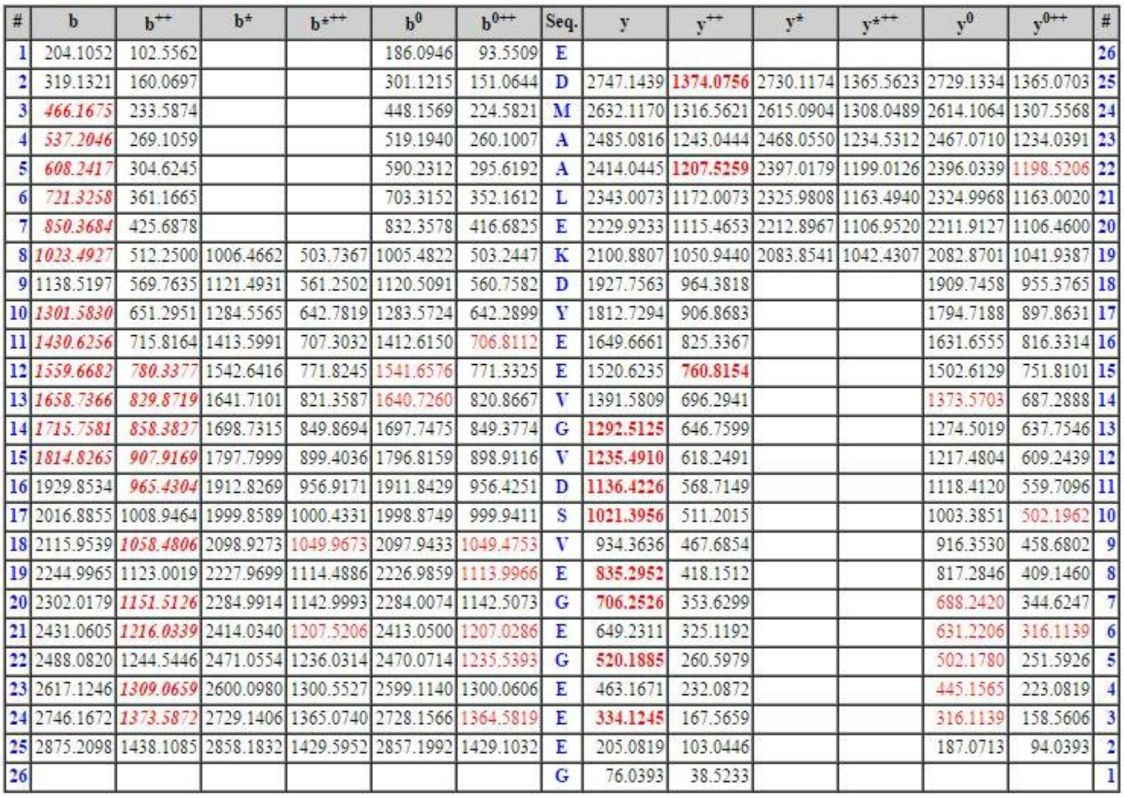
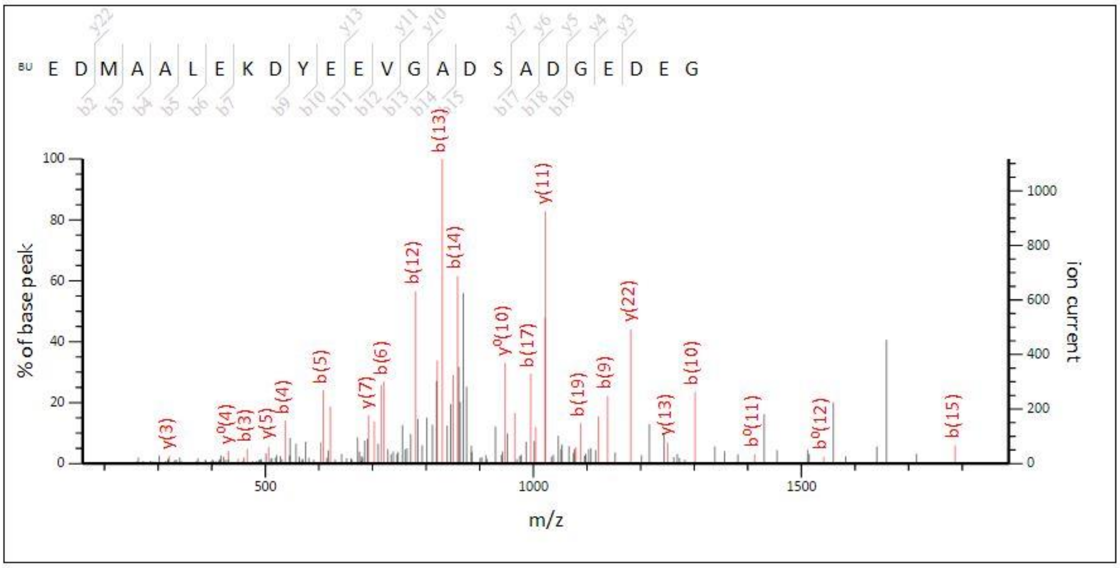
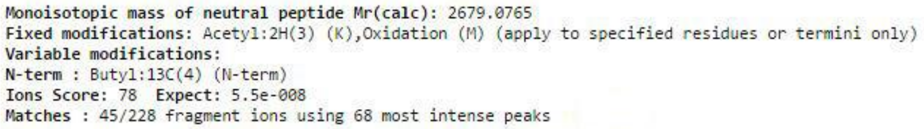


#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	204.1052	102.5562			186.0946	93.5509	E							26
2	319.1321	160.0697			301.1215	151.0644	D	2747.1439	**1374.0756**	2730.1174	1365.5623	2729.1334	1365.0703	25
3	***466.1675***	233.5874			448.1569	224.5821	M	2632.1170	1316.5621	2615.0904	1308.0489	2614.1064	1307.5568	24
4	***537.2046***	269.1059			519.1940	260.1007	A	2485.0816	1243.0444	2468.0550	1234.5312	2467.0710	1234.0391	23
5	***608.2417***	304.6245			590.2312	295.6192	A	2414.0445	**1207.5259**	2397.0179	1199.0126	2396.0339	1198.5206	22
6	***721.3258***	361.1665			703.3152	352.1612	L	2343.0073	1172.0073	2325.9808	1163.4940	2324.9968	1163.0020	21
7	***850.3684***	425.6878			832.3578	416.6825	E	2229.9233	1115.4653	2212.8967	1106.9520	2211.9127	1106.4600	20
8	***1023.4927***	512.2500	1006.4662	503.7367	1005.4822	503.2447	K	2100.8807	1050.9440	2083.8541	1042.4307	2082.8701	1041.9387	19
9	1138.5197	569.7635	1121.4931	561.2502	1120.5091	560.7582	D	1927.7563	964.3818			1909.7458	955.3765	18
10	***1301.5830***	651.2951	1284.5565	642.7819	1283.5724	642.2899	Y	1812.7294	906.8683			1794.7188	897.8631	17
11	***1430.6256***	715.8164	1413.5991	707.3032	1412.6150	706.8112	E	1649.6661	825.3367			1631.6555	816.3314	16
12	***1559.6682***	***780.3377***	1542.6416	771.8245	1541.6576	771.3325	E	1520.6235	**760.8154**			1502.6129	751.8101	15
13	***1658.7366***	***829.8719***	1641.7101	821.3587	1640.7260	820.8667	V	1391.5809	696.2941			1373.5703	687.2888	14
14	***1715.7581***	***858.3827***	1698.7315	849.8694	1697.7475	849.3774	G	**1292.5125**	646.7599			1274.5019	637.7546	13
15	***1814.8265***	***907.9169***	1797.7999	899.4036	1796.8159	898.9116	V	**1235.4910**	618.2491			1217.4804	609.2439	12
16	1929.8534	***965.4304***	1912.8269	956.9171	1911.8429	956.4251	D	**1136.4226**	568.7149			1118.4120	559.7096	11
17	2016.8855	1008.9464	1999.8589	1000.4331	1998.8749	999.9411	S	**1021.3956**	511.2015			1003.3851	502.1962	10
18	2115.9539	***1058.4806***	2098.9273	1049.9673	2097.9433	1049.4753	V	934.3636	467.6854			916.3530	458.6802	9
19	2244.9965	1123.0019	2227.9699	1114.4886	2226.9859	1113.9966	E	**835.2952**	418.1512			817.2846	409.1460	8
20	2302.0179	***1151.5126***	2284.9914	1142.9993	2284.0074	1142.5073	G	**706.2526**	353.6299			688.2420	344.6247	7
21	2431.0605	***1216.0339***	2414.0340	1207.5206	2413.0500	1207.0286	E	649.2311	325.1192			631.2206	316.1139	6
22	2488.0820	1244.5446	2471.0554	1236.0314	2470.0714	1235.5393	G	**520.1885**	260.5979			502.1780	251.5926	5
23	2617.1246	***1309.0659***	2600.0980	1300.5527	2599.1140	1300.0606	E	463.1671	232.0872			445.1565	223.0819	4
24	2746.1672	***1373.5872***	2729.1406	1365.0740	2728.1566	1364.5819	E	**334.1245**	167.5659			316.1139	158.5606	3
25	2875.2098	1438.1085	2858.1832	1429.5952	2857.1992	1429.1032	E	205.0819	103.0446			187.0713	94.0393	2
26							G	76.0393	38.5233					1

Suppl. Fig. S2. b) Protein: α-Tubulin 1C, Uniprot Q9BQE3
Peptide sequence: EDMAALEKDYEEVGADSADGEDEG
Precursor m/z: 894.3645
Precursor z: +3
Highest Mascot score: 78





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Variable modifications:
N-term : Butyl:13C(4) (N-term)
Ions Score: 78  Expect: 5.5e-008
Matches : 45/228 fragment ions using 68 most intense peaks
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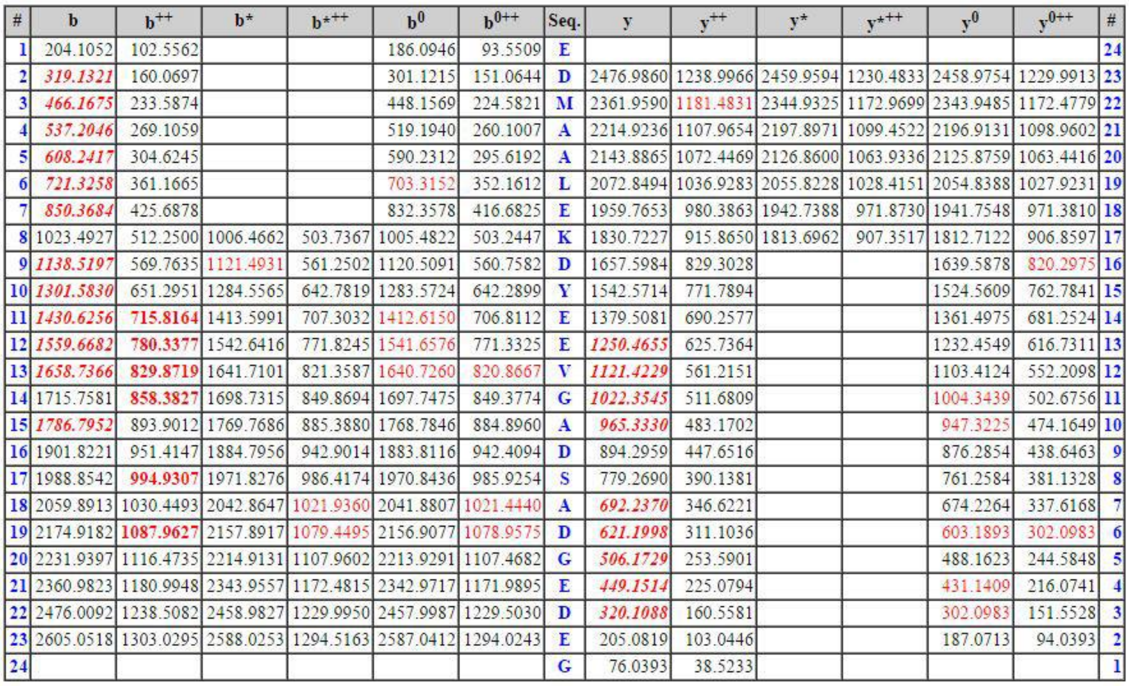
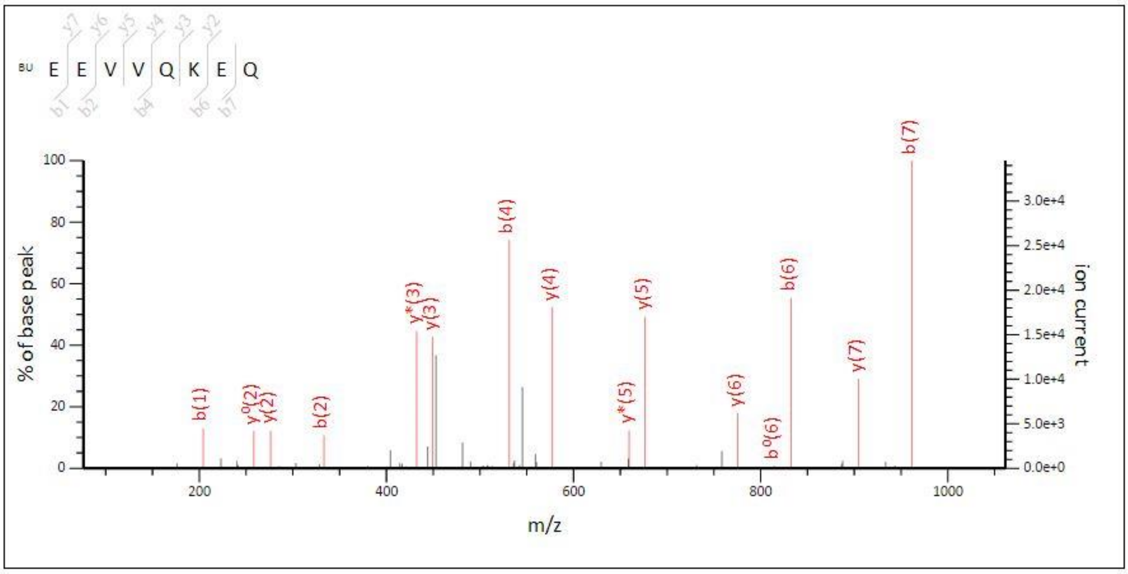
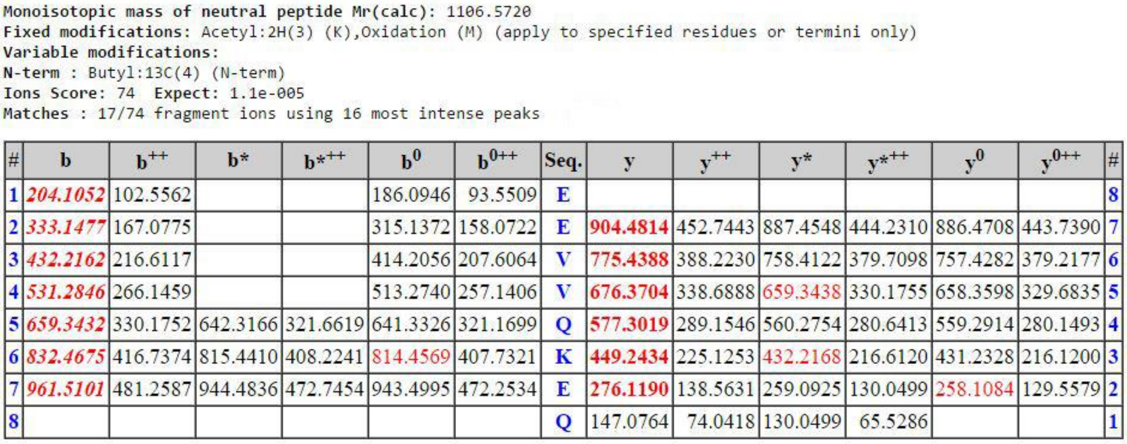


#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	204.1052	102.5562			186.0946	93.5509	E							24
2	***319.1321***	160.0697			301.1215	151.0644	D	2476.9860	1238.9966	2459.9594	1230.4833	2458.9754	1229.9913	23
3	***466.1675***	233.5874			448.1569	224.5821	M	2361.9590	1181.4831	2344.9325	1172.9699	2343.9485	1172.4779	22
4	***537.2046***	269.1059			519.1940	260.1007	A	2214.9236	1107.9654	2197.8971	1099.4522	2196.9131	1098.9602	21
5	***608.2417***	304.6245			590.2312	295.6192	A	2143.8865	1072.4469	2126.8600	1063.9336	2125.8759	1063.4416	20
6	***721.3258***	361.1665			703.3152	352.1612	L	2072.8494	1036.9283	2055.8228	1028.4151	2054.8388	1027.9231	19
7	***850.3684***	425.6878			832.3578	416.6825	E	1959.7653	980.3863	1942.7388	971.8730	1941.7548	971.3810	18
8	1023.4927	512.2500	1006.4662	503.7367	1005.4822	503.2447	K	1830.7227	915.8650	1813.6962	907.3517	1812.7122	906.8597	17
9	***1138.5197***	569.7635	1121.4931	561.2502	1120.5091	560.7582	D	1657.5984	829.3028			1639.5878	820.2975	16
10	***1301.5830***	651.2951	1284.5565	642.7819	1283.5724	642.2899	Y	1542.5714	771.7894			1524.5609	762.7841	15
11	***1430.6256***	**715.8164**	1413.5991	707.3032	1412.6150	706.8112	E	1379.5081	690.2577			1361.4975	681.2524	14
12	***1559.6682***	**780.3377**	1542.6416	771.8245	1541.6576	771.3325	E	***1250.4655***	625.7364			1232.4549	616.7311	13
13	***1658.7366***	**829.8719**	1641.7101	821.3587	1640.7260	820.8667	V	***1121.4229***	561.2151			1103.4124	552.2098	12
14	1715.7581	**858.3827**	1698.7315	849.8694	1697.7475	849.3774	G	***1022.3545***	511.6809			1004.3439	502.6756	11
15	***1786.7952***	893.9012	1769.7686	885.3880	1768.7846	884.8960	A	***965.3330***	483.1702			947.3225	474.1649	10
16	1901.8221	951.4147	1884.7956	942.9014	1883.8116	942.4094	D	894.2959	447.6516			876.2854	438.6463	9
17	1988.8542	**994.9307**	1971.8276	986.4174	1970.8436	985.9254	S	779.2690	390.1381			761.2584	381.1328	8
18	2059.8913	1030.4493	2042.8647	1021.9360	2041.8807	1021.4440	A	***692.2370***	346.6221			674.2264	337.6168	7
19	2174.9182	**1087.9627**	2157.8917	1079.4495	2156.9077	1078.9575	D	***621.1998***	311.1036			603.1893	302.0983	6
20	2231.9397	1116.4735	2214.9131	1107.9602	2213.9291	1107.4682	G	***506.1729***	253.5901			488.1623	244.5848	5
21	2360.9823	1180.9948	2343.9557	1172.4815	2342.9717	1171.9895	E	***449.1514***	225.0794			431.1409	216.0741	4
22	2476.0092	1238.5082	2458.9827	1229.9950	2457.9987	1229.5030	D	***320.1088***	160.5581			302.0983	151.5528	3
23	2605.0518	1303.0295	2588.0253	1294.5163	2587.0412	1294.0243	E	205.0819	103.0446			187.0713	94.0393	2
24							G	76.0393	38.5233					1

Suppl. Fig. S2. c) **Protein:** Eukaryotic translation initiation factor 4H, Uniprot Q15056
Peptide sequence: EEVVQKEQ
Precursor m/z: 554.2928
Precursor z: +2
Highest Mascot score: 74





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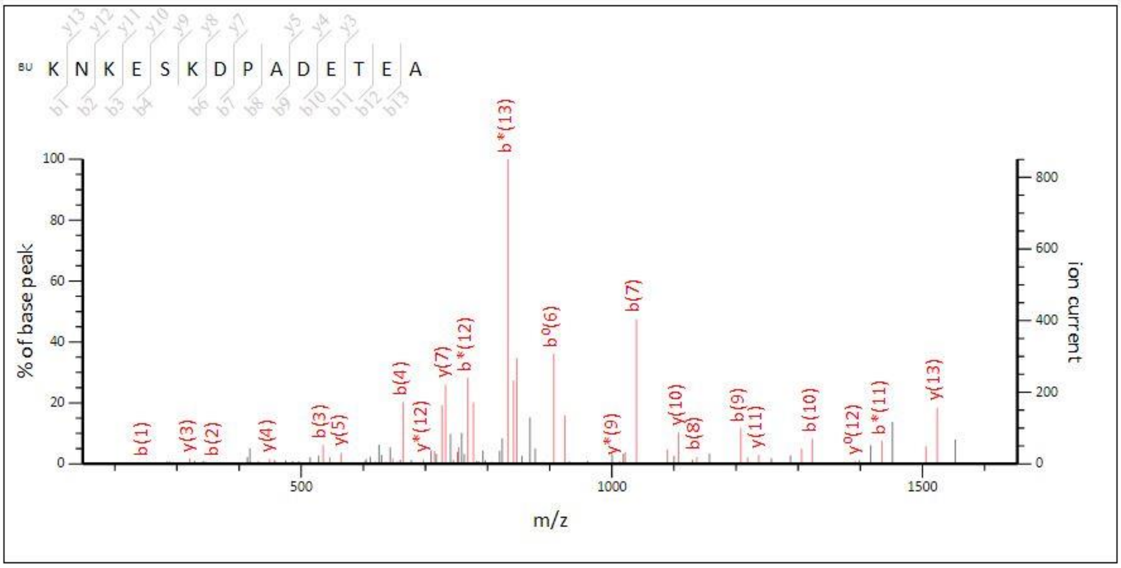
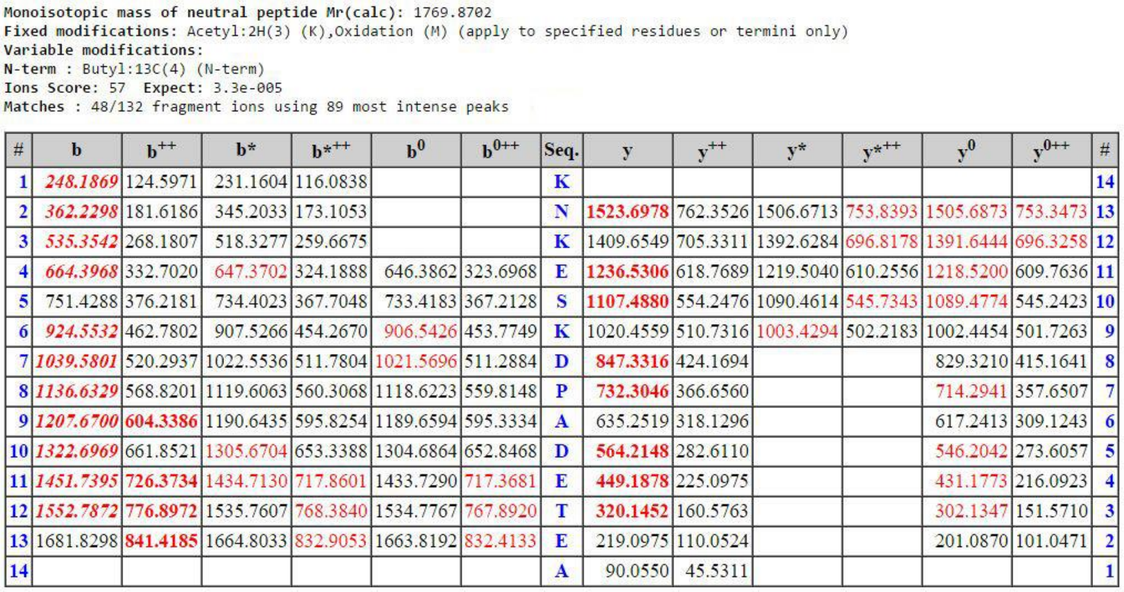
Fixed modifications: Acetyl:2H(3) (K),Oxidation (M) (apply to specified residues or termini only)
Variable modifications:
N-term : Butyl:13C(4) (N-term)
Ions Score: 74  Expect: 1.1e-005
Matches : 17/74 fragment ions using 16 most intense peaks
```

#	b	b⁺⁺	b*	b*⁺⁺	b⁰	b⁰⁺⁺	Seq.	y	y⁺⁺	y*	y*⁺⁺	y⁰	y⁰⁺⁺	#
1	*204.1052*	102.5562			186.0946	93.5509	E							8
2	*333.1477*	167.0775			315.1372	158.0722	E	**904.4814**	452.7443	887.4548	444.2310	886.4708	443.7390	7
3	*432.2162*	216.6117			414.2056	207.6064	V	**775.4388**	388.2230	758.4122	379.7098	757.4282	379.2177	6
4	*531.2846*	266.1459			513.2740	257.1406	V	**676.3704**	338.6888	659.3438	330.1755	658.3598	329.6835	5
5	*659.3432*	330.1752	642.3166	321.6619	641.3326	321.1699	Q	**577.3019**	289.1546	560.2754	280.6413	559.2914	280.1493	4
6	*832.4675*	416.7374	815.4410	408.2241	814.4569	407.7321	K	**449.2434**	225.1253	432.2168	216.6120	431.2328	216.1200	3
7	*961.5101*	481.2587	944.4836	472.7454	943.4995	472.2534	E	**276.1190**	138.5631	259.0925	130.0499	258.1084	129.5579	2
8							Q	147.0764	74.0418	130.0499	65.5286			1

Suppl. Fig. S2. d) Protein: Stathmin, Uniprot P16949
Peptide sequence: KNKESKDPADETEA
Precursor m/z: 885.9427
Precursor z: +2
Highest Mascot score: 57





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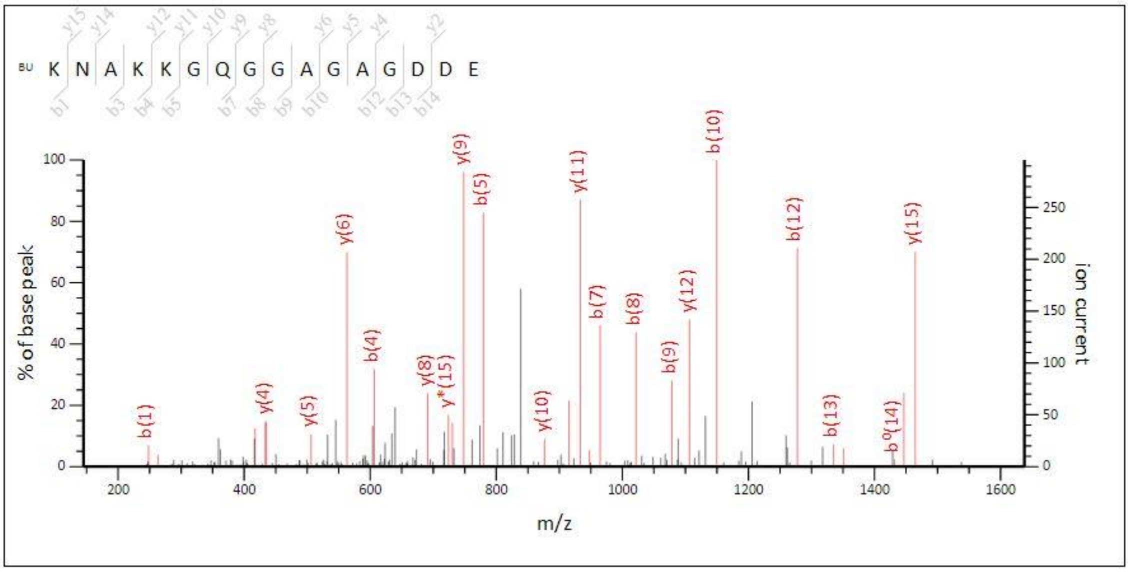
Fixed modifications: Acetyl:2H(3) (K),Oxidation (M) (apply to specified residues or termini only)
Variable modifications:
N-term : Butyl:13C(4) (N-term)
Ions Score: 57  Expect: 3.3e-005
Matches : 48/132 fragment ions using 89 most intense peaks
```

#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	***248.1869***	124.5971	231.1604	116.0838			K							14
2	***362.2298***	181.6186	345.2033	173.1053			N	**1523.6978**	762.3526	1506.6713	753.8393	1505.6873	753.3473	13
3	***535.3542***	268.1807	518.3277	259.6675			K	1409.6549	705.3311	1392.6284	696.8178	1391.6444	696.3258	12
4	***664.3968***	332.7020	647.3702	324.1888	646.3862	323.6968	E	**1236.5306**	618.7689	1219.5040	610.2556	1218.5200	609.7636	11
5	751.4288	376.2181	734.4023	367.7048	733.4183	367.2128	S	**1107.4880**	554.2476	1090.4614	545.7343	1089.4774	545.2423	10
6	***924.5532***	462.7802	907.5266	454.2670	906.5426	453.7749	K	1020.4559	510.7316	1003.4294	502.2183	1002.4454	501.7263	9
7	***1039.5801***	520.2937	1022.5536	511.7804	1021.5696	511.2884	D	**847.3316**	424.1694			829.3210	415.1641	8
8	***1136.6329***	568.8201	1119.6063	560.3068	1118.6223	559.8148	P	**732.3046**	366.6560			714.2941	357.6507	7
9	***1207.6700***	**604.3386**	1190.6435	595.8254	1189.6594	595.3334	A	635.2519	318.1296			617.2413	309.1243	6
10	***1322.6969***	661.8521	1305.6704	653.3388	1304.6864	652.8468	D	**564.2148**	282.6110			546.2042	273.6057	5
11	***1451.7395***	**726.3734**	1434.7130	717.8601	1433.7290	717.3681	E	**449.1878**	225.0975			431.1773	216.0923	4
12	***1552.7872***	**776.8972**	1535.7607	768.3840	1534.7767	767.8920	T	**320.1452**	160.5763			302.1347	151.5710	3
13	1681.8298	**841.4185**	1664.8033	832.9053	1663.8192	832.4133	E	219.0975	110.0524			201.0870	101.0471	2
14							A	90.0550	45.5311					1

Suppl. Fig. S2. e) **Protein:** 40S ribosomal protein S9, Uniprot P46781
Peptide sequence: KNAKKGQGGAGAGDDE
Precursor m/z: 856.4344
Precursor z: +2
Highest Mascot score: 102



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Fixed modifications: Acetyl:2H(3) (K),Oxidation (M) (apply to specified residues or termini only)
Variable modifications:
N-term : Butyl:13C(4) (N-term)
Ions Score: 102  Expect: 3.2e-009
Matches : 30/136 fragment ions using 39 most intense peaks
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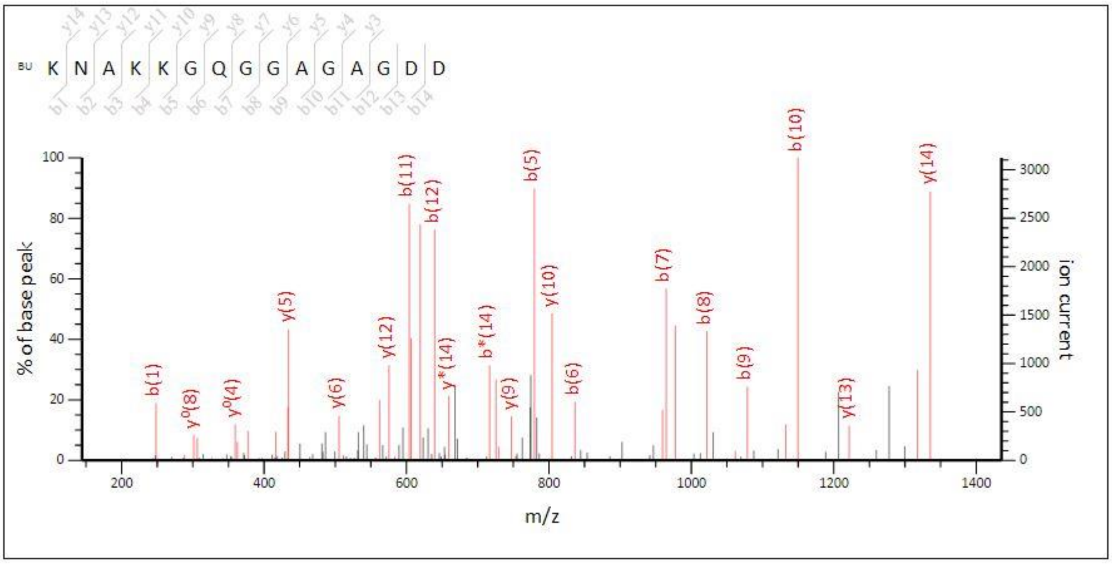
#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	***248.1869***	124.5971	231.1604	116.0838			K							16
2	362.2298	181.6186	345.2033	173.1053			N	***1464.6832***	732.8452	1447.6567	724.3320	1446.6726	723.8400	15
3	***433.2670***	217.1371	416.2404	208.6238			A	***1350.6403***	675.8238	1333.6137	667.3105	1332.6297	666.8185	14
4	***606.3913***	303.6993	589.3648	295.1860			K	1279.6032	640.3052	1262.5766	631.7919	1261.5926	631.2999	13
5	***779.5157***	390.2615	762.4891	381.7482			K	***1106.4788***	553.7430	1089.4523	545.2298	1088.4682	544.7378	12
6	836.5371	418.7722	819.5106	410.2589			G	***933.3544***	467.1809	916.3279	458.6676	915.3439	458.1756	11
7	***964.5957***	482.8015	947.5692	474.2882			Q	***876.3330***	438.6701	859.3064	430.1569	858.3224	429.6648	10
8	***1021.6172***	511.3122	1004.5906	502.7990			G	***748.2744***	374.6408			730.2638	365.6356	9
9	***1078.6386***	539.8230	1061.6121	531.3097			G	***691.2529***	346.1301			673.2424	337.1248	8
10	***1149.6758***	575.3415	1132.6492	566.8282			A	634.2315	317.6194			616.2209	308.6141	7
11	1206.6972	603.8522	1189.6707	595.3390			G	***563.1944***	282.1008			545.1838	273.0955	6
12	***1277.7343***	639.3708	1260.7078	630.8575			A	***506.1729***	253.5901			488.1623	244.5848	5
13	***1334.7558***	667.8815	1317.7293	659.3683			G	***435.1358***	218.0715			417.1252	209.0662	4
14	1449.7827	725.3950	1432.7562	716.8817	1431.7722	716.3897	D	378.1143	189.5608			360.1038	180.5555	3
15	1564.8097	782.9085	1547.7831	774.3952	1546.7991	773.9032	D	***263.0874***	132.0473			245.0768	123.0420	2
16							E	148.0604	74.5339			130.0499	65.5286	1

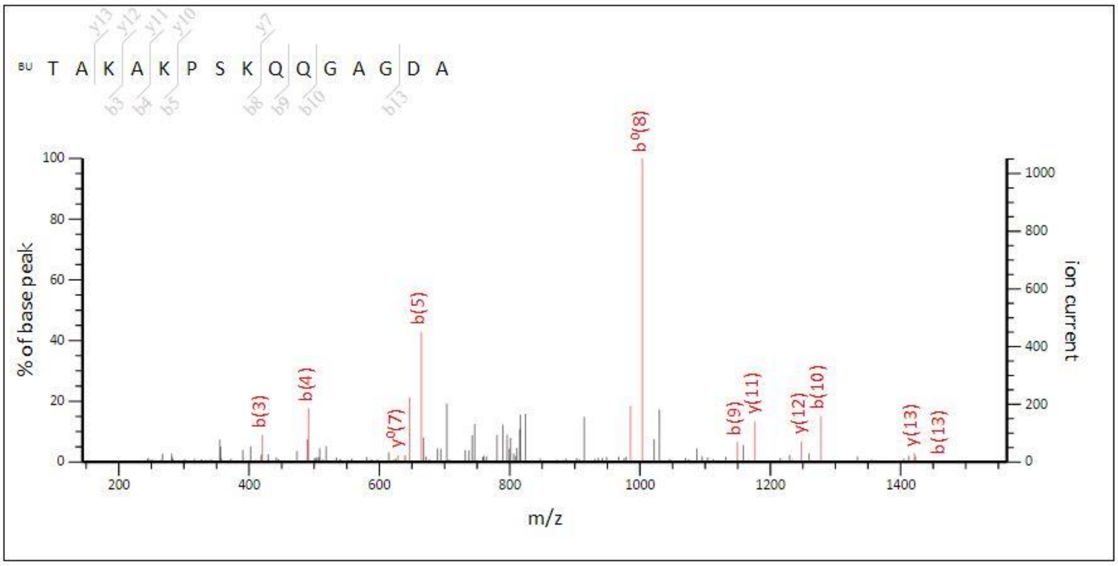
Suppl. Fig. S2. f) **Protein:** 40S ribosomal protein S9, Uniprot P46781
Peptide sequence: KNAKKGQGGAGAGDD
Precursor m/z: 791.9128
Precursor z: +2
Highest Mascot score: 92

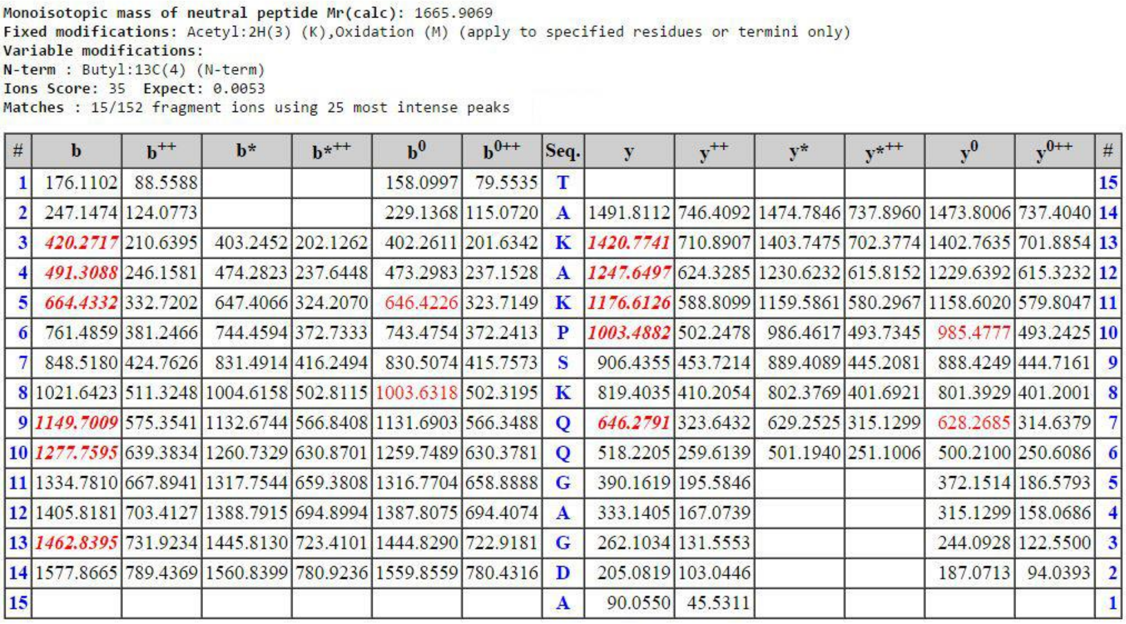


```
Monoisotopic mass of neutral peptide Mr(calc): 1581.8130
Fixed modifications: Acetyl:2H(3) (K),Oxidation (M) (apply to specified residues or termini only)
Variable modifications:
N-term : Butyl:13C(4) (N-term)
Ions Score: 92  Expect: 4.8e-008
Matches : 48/126 fragment ions using 62 most intense peaks
```

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	***248.1869***	124.5971	231.1604	116.0838			K							15
2	***362.2298***	181.6186	345.2033	173.1053			N	**1335.6406**	668.3239	1318.6141	659.8107	1317.6300	659.3187	14
3	***433.2670***	217.1371	416.2404	208.6238			A	**1221.5977**	611.3025	1204.5711	602.7892	1203.5871	602.2972	13
4	***606.3913***	303.6993	589.3648	295.1860			K	1150.5606	575.7839	1133.5340	567.2706	1132.5500	566.7786	12
5	***779.5157***	390.2615	762.4891	381.7482			K	**977.4362**	489.2217	960.4097	480.7085	959.4256	480.2165	11
6	***836.5371***	418.7722	819.5106	410.2589			G	**804.3119**	402.6596	787.2853	394.1463	786.3013	393.6543	10
7	***964.5957***	482.8015	947.5692	474.2882			Q	**747.2904**	374.1488	730.2638	365.6356	729.2798	365.1436	9
8	***1021.6172***	511.3122	1004.5906	502.7990			G	**619.2318**	310.1195			601.2212	301.1143	8
9	***1078.6386***	**539.8230**	1061.6121	531.3097			G	**562.2103**	281.6088			544.1998	272.6035	7
10	***1149.6758***	**575.3415**	1132.6492	566.8282			A	**505.1889**	253.0981			487.1783	244.0928	6
11	***1206.6972***	**603.8522**	1189.6707	595.3390			G	**434.1518**	217.5795			416.1412	208.5742	5
12	***1277.7343***	**639.3708**	1260.7078	630.8575			A	**377.1303**	189.0688			359.1197	180.0635	4
13	1334.7558	**667.8815**	1317.7293	659.3683			G	**306.0932**	153.5502			288.0826	144.5450	3
14	1449.7827	**725.3950**	1432.7562	716.8817	1431.7722	716.3897	D	249.0717	125.0395			231.0612	116.0342	2
15							D	134.0448	67.5260			116.0342	58.5207	1

Suppl. Fig. S2. g) **Protein:** TRAF-type zinc finger domain-containing protein, Uniprot O14545
Peptide sequence: TAKAKPSKQQGAGDA
Precursor m/z: 833.9609
Precursor z: +2
Highest Mascot score: 35





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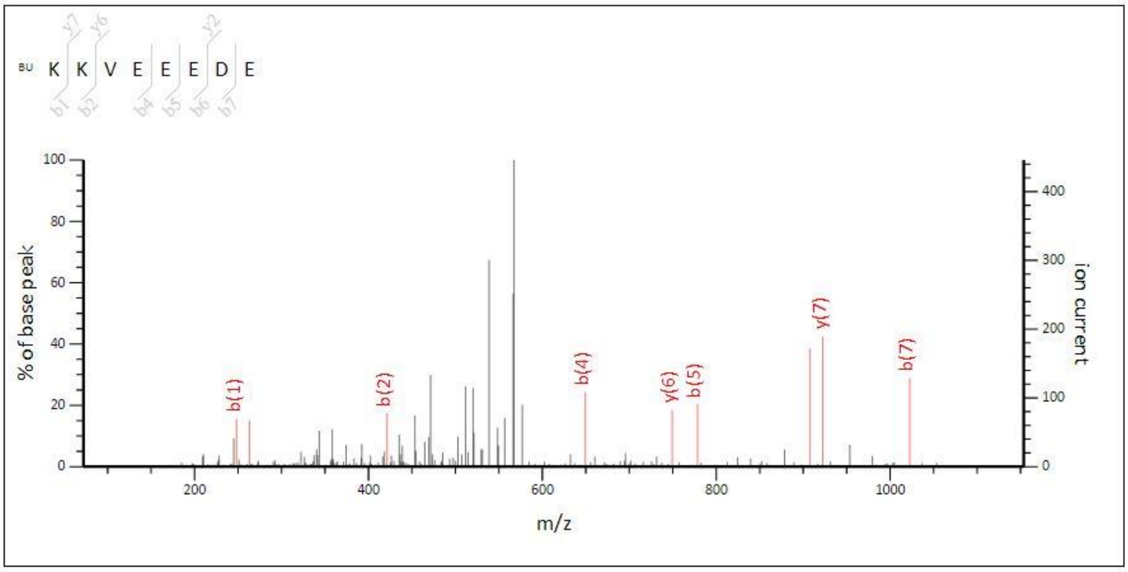
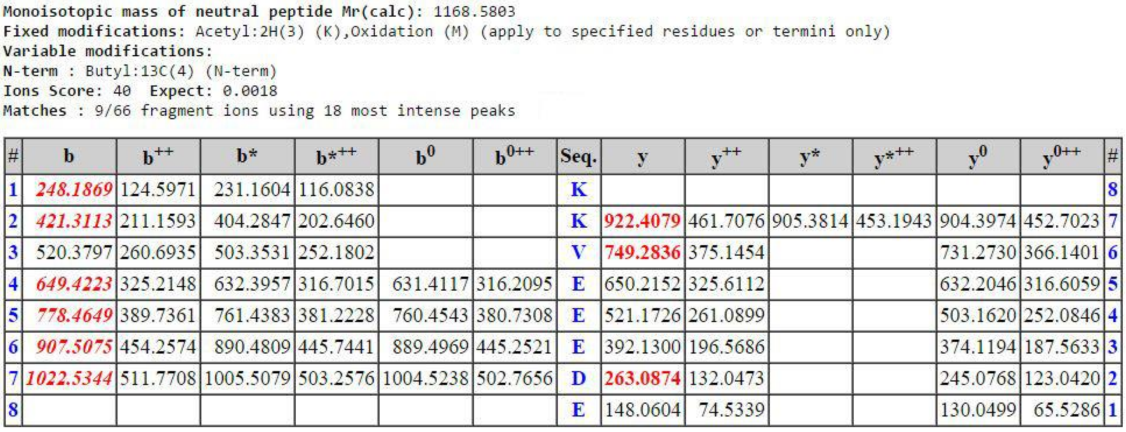
Monoisotopic mass of neutral peptide Mr(calc): 1665.9069
Fixed modifications: Acetyl:2H(3) (K),Oxidation (M) (apply to specified residues or termini only)
Variable modifications:
N-term : Butyl:13C(4) (N-term)
Ions Score: 35  Expect: 0.0053
Matches : 15/152 fragment ions using 25 most intense peaks
```

#	b	b^{++}	b*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^0	y^{0++}	#
1	176.1102	88.5588			158.0997	79.5535	T							15
2	247.1474	124.0773			229.1368	115.0720	A	1491.8112	746.4092	1474.7846	737.8960	1473.8006	737.4040	14
3	***420.2717***	210.6395	403.2452	202.1262	402.2611	201.6342	K	***1420.7741***	710.8907	1403.7475	702.3774	1402.7635	701.8854	13
4	***491.3088***	246.1581	474.2823	237.6448	473.2983	237.1528	A	***1247.6497***	624.3285	1230.6232	615.8152	1229.6392	615.3232	12
5	***664.4332***	332.7202	647.4066	324.2070	646.4226	323.7149	K	***1176.6126***	588.8099	1159.5861	580.2967	1158.6020	579.8047	11
6	761.4859	381.2466	744.4594	372.7333	743.4754	372.2413	P	***1003.4882***	502.2478	986.4617	493.7345	985.4777	493.2425	10
7	848.5180	424.7626	831.4914	416.2494	830.5074	415.7573	S	906.4355	453.7214	889.4089	445.2081	888.4249	444.7161	9
8	1021.6423	511.3248	1004.6158	502.8115	1003.6318	502.3195	K	819.4035	410.2054	802.3769	401.6921	801.3929	401.2001	8
9	***1149.7009***	575.3541	1132.6744	566.8408	1131.6903	566.3488	Q	***646.2791***	323.6432	629.2525	315.1299	628.2685	314.6379	7
10	***1277.7595***	639.3834	1260.7329	630.8701	1259.7489	630.3781	Q	518.2205	259.6139	501.1940	251.1006	500.2100	250.6086	6
11	1334.7810	667.8941	1317.7544	659.3808	1316.7704	658.8888	G	390.1619	195.5846			372.1514	186.5793	5
12	1405.8181	703.4127	1388.7915	694.8994	1387.8075	694.4074	A	333.1405	167.0739			315.1299	158.0686	4
13	***1462.8395***	731.9234	1445.8130	723.4101	1444.8290	722.9181	G	262.1034	131.5553			244.0928	122.5500	3
14	1577.8665	789.4369	1560.8399	780.9236	1559.8559	780.4316	D	205.0819	103.0446			187.0713	94.0393	2
15							A	90.0550	45.5311					1

Suppl. Fig. S2. h) **Protein:** High mobility group protein B3, Uniprot O15347
Peptide sequence: KKVEEEDE
Precursor m/z: 585.2972
Precursor z: +2
Highest Mascot score: 40





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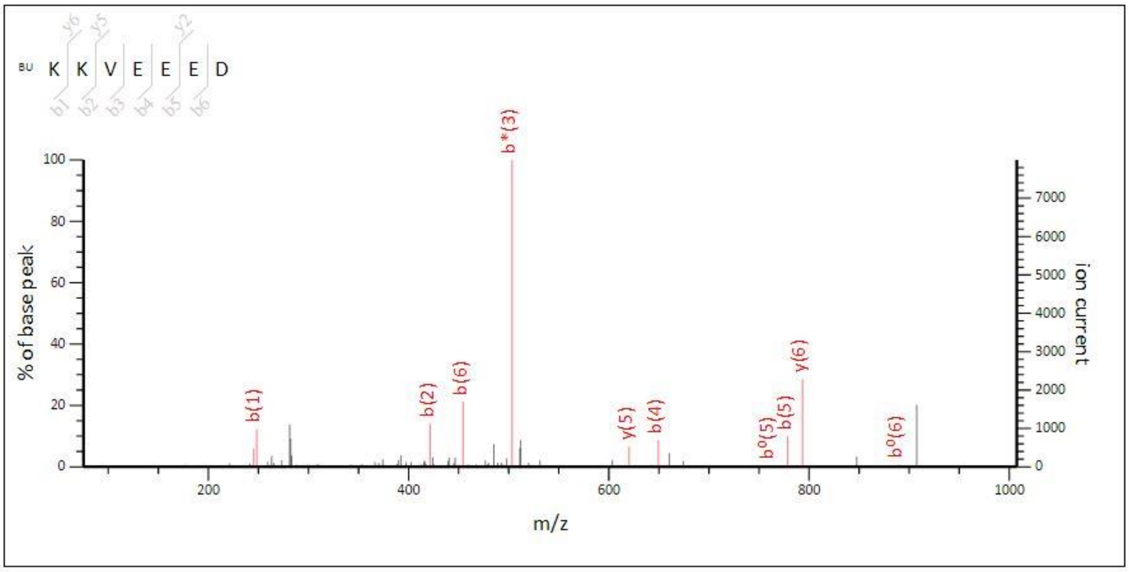
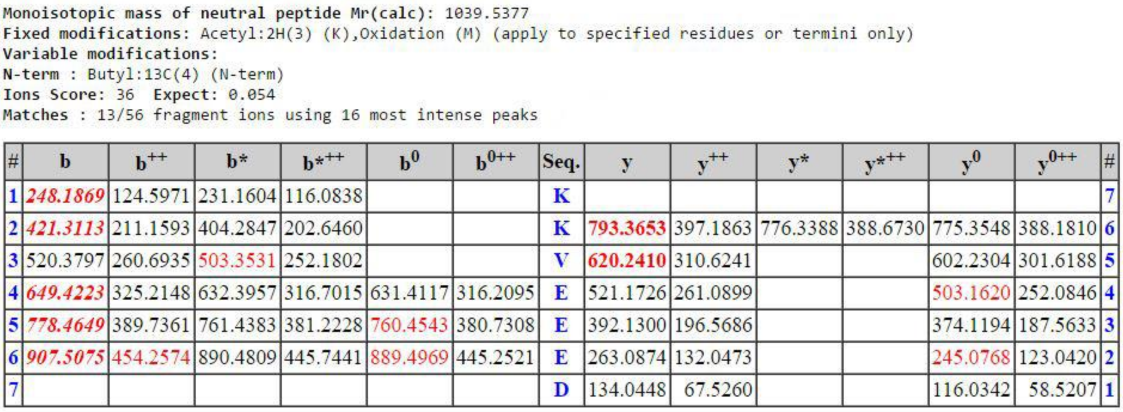
Monoisotopic mass of neutral peptide Mr(calc): 1168.5803
Fixed modifications: Acetyl:2H(3) (K),Oxidation (M) (apply to specified residues or termini only)
Variable modifications:
N-term : Butyl:13C(4) (N-term)
Ions Score: 40  Expect: 0.0018
Matches : 9/66 fragment ions using 18 most intense peaks
```

#	b	b^{++}	b^{*}	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	***248.1869***	124.5971	231.1604	116.0838			K							8
2	***421.3113***	211.1593	404.2847	202.6460			K	**922.4079**	461.7076	905.3814	453.1943	904.3974	452.7023	7
3	520.3797	260.6935	503.3531	252.1802			V	**749.2836**	375.1454			731.2730	366.1401	6
4	***649.4223***	325.2148	632.3957	316.7015	631.4117	316.2095	E	650.2152	325.6112			632.2046	316.6059	5
5	***778.4649***	389.7361	761.4383	381.2228	760.4543	380.7308	E	521.1726	261.0899			503.1620	252.0846	4
6	***907.5075***	454.2574	890.4809	445.7441	889.4969	445.2521	E	392.1300	196.5686			374.1194	187.5633	3
7	***1022.5344***	511.7708	1005.5079	503.2576	1004.5238	502.7656	D	**263.0874**	132.0473			245.0768	123.0420	2
8							E	148.0604	74.5339			130.0499	65.5286	1

Suppl. Fig. S2. i) **Protein:** High mobility group protein B3, Uniprot O15347
Peptide sequence: KKVEEED
Precursor m/z: 520.7759
Precursor z: +2
Highest Mascot score: 36





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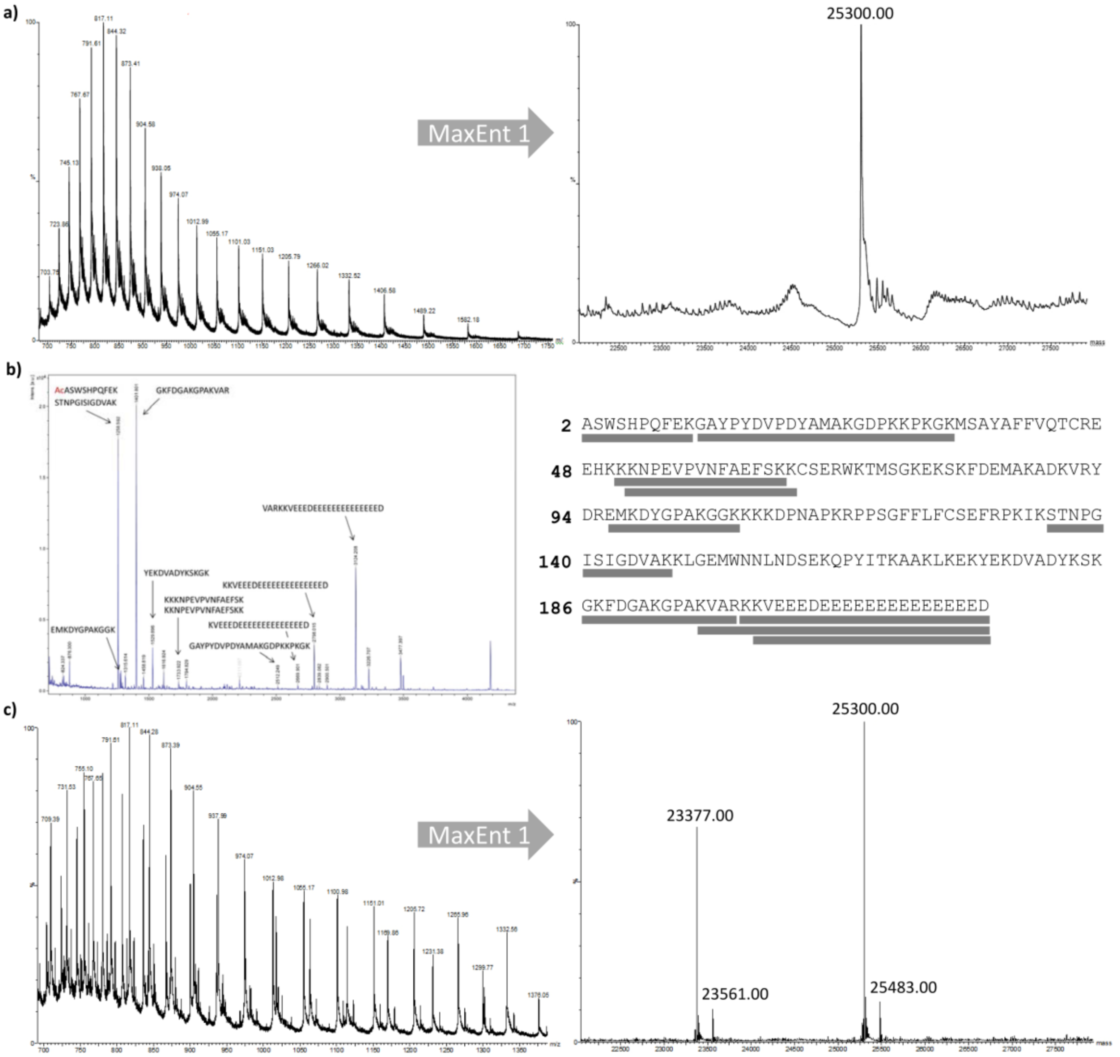
Monoisotopic mass of neutral peptide Mr(calc): 1039.5377
Fixed modifications: Acetyl:2H(3) (K),Oxidation (M) (apply to specified residues or termini only)
Variable modifications:
N-term : Butyl:13C(4) (N-term)
Ions Score: 36  Expect: 0.054
Matches : 13/56 fragment ions using 16 most intense peaks
```

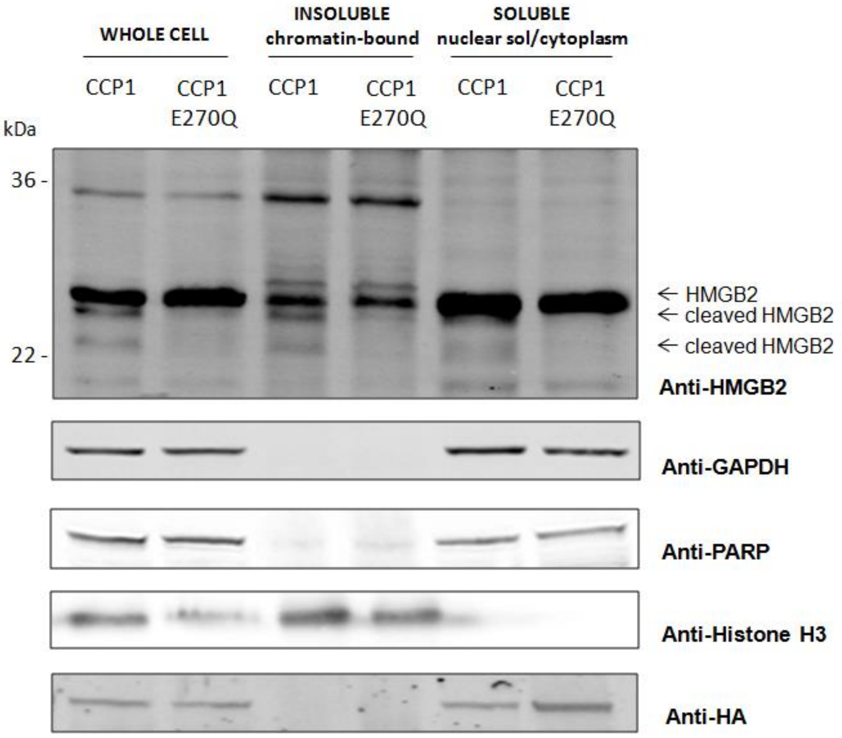
#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	***248.1869***	124.5971	231.1604	116.0838			K							7
2	***421.3113***	211.1593	404.2847	202.6460			K	**793.3653**	397.1863	776.3388	388.6730	775.3548	388.1810	6
3	520.3797	260.6935	503.3531	252.1802			V	**620.2410**	310.6241			602.2304	301.6188	5
4	***649.4223***	325.2148	632.3957	316.7015	631.4117	316.2095	E	521.1726	261.0899			503.1620	252.0846	4
5	***778.4649***	389.7361	761.4383	381.2228	760.4543	380.7308	E	392.1300	196.5686			374.1194	187.5633	3
6	***907.5075***	454.2574	890.4809	445.7441	889.4969	445.2521	E	263.0874	132.0473			245.0768	123.0420	2
7							D	134.0448	67.5260			116.0342	58.5207	1

Supplementary Figure S3



Supplementary Figure S3. Mass-spectrometric analysis of purified recombinant HMGB3 and its cleavage products. a) Q-TOF spectrum (left) of purified recombinant HMGB3 and data deconvolution spectrum revealing a molecular mass of 25,300 Da. **b)** Protein characterization by peptide mass fingerprinting allowed for 50% sequence coverage of recombinant purified HMGB3. The distributions of matching peptides in the complete protein sequence are marked with underlying grey boxes. No matching masses were found when considering the unprocessed N- and C-termini of HMGB3. The calculated and observed *m*/*z* values for matching peptides are listed in Supplementary Table S3. The complete peak list is shown in Supplementary Table S4. **c)** Q-TOF spectrum (left) of the purified recombinant HMGB3 after CCP1 treatment. Data deconvolution spectrum reveals the presence of a cleavage product with a molecular mass of 23,377 Da.

Supplementary Figure S4



Supplementary Figure S4. Processing of endogenous HMGB2 upon CCP1 overexpression. HEK293T cells were transfected with active or inactive CCP1 (CCP1 E270Q). Western blotting using an anti-HMGB2 antibody shows the appearance of processed forms of native HMGB2 when active CCP1 is over-expressed (lanes 1, 3 and 5). Whole cell protein lysates were fractionated into soluble fractions (containing cytosolic and nuclear soluble proteins) and chromatin-bound insoluble fractions (lanes 3-6). Anti-GAPDH (cytosolic marker), anti-PARP (nuclear soluble fraction marker) and anti-histone H3 (chromatin-bound/insoluble nuclear marker) were used to demonstrate the purity of the fractions. The anti-HA-tag antibody shows equal expression levels of both CCP1 variants.

Supplementary Data: Supplementary Tables

Supplementary Table S1: List of the identified butyrylated peptides, corresponding to database annotated and processed C-termini. In all cases the corresponding Swiss-Prot accession number, protein description, sequence identified, modifications observed, start position of the peptide, *m/z* and z of the highest scoring spectra are indicated. The light over heavy ratios (control sample over CCP1 overexpressing sample) of the ion intensities observed in MS for the highest scoring peptide, is also shown (L/H ratio HS). In case of the absence of light peptide ions, the peptide was indicated as heayy singleton. Similarly, light singleton was used in the absence of the heavy ions. In addition, the ratios significantly altered using a stringent p-value threshold of 0.0001 are indicated. Peptides are classified (column peptide type) as database annotated C-termini and processed C-termini, and for the latter the residues missing from the C-terminus are indicated (C-terminal residues in protein sequence). Additional information related to the observed mass error (Da), possible protein isoforms holding the same peptide, number of identified spectra (# spectra), the maximum Mascot ion score, minimum threshold score and delta threshold (= maximum score – minimum threshold) are additionally indicated. **** = p-value <0.0001. Abbreviations: But, $^{12}C_4$-butyrylation of peptide N-termini; But4C13, $^{13}C_4$-butyrylation of peptide N-termini; AcD3K, D3-acetylation at lysines; Mox, oxidized methionine.

Supplementary Table S2. Intact C-termini of the identified putative CCP1 substrates

Protein	Identified C-terminal peptide	L/H ratio	Z-score
α-tubulin 1A/1B	EDMAALEKDYEEVGVDSVEGEGEEEGEEY	0.86	-0.55
α-tubulin 1C	EDMAALEKDYEEVGADSADGEDEGEEY	0.20	-4.79
Eukaryotic translation initiation factor 4H	EEVVQKEQE	0.95	-0.28
Stathmin	KNKESKDPADETEAD	0.56	-1.81
40S ribosomal protein S9	KNAKKGQGGAGAGDDEEED	0.83	-0.69

Supplementary Table S3. Calculated and observed masses for tryptic peptides of purified recombinant HMGB3.

Peak	Intensity	Amino acid sequence		[M+H]+		Missed Cleavages	Sequence	Modified sequence
		From [a]	To	Calculated	Observed			
				m/z				
4	16399.156	1	10	1258.585	1258.592	0	ASWSHPQFEK	**Ac**-ASWSHPQFEK
4	16399.156	134	146	1258.663	1258.592	0	STNPGISIGDVAK	STNPGISIGDVAK
6	1315.728	95	106	1280.630	1280.573	2	EMKDYGPAKGGK	EMKDYGPAKGGK
8	18940.384	185	198	1401.796	1401.801	3	GKFDGAKGPAKVAR	GKFDGAKGPAKVAR
10	3103.273	174	186	1530.780	1529.896	3	YEKDVADYKSKGK	YEKDVADYKSKGK
12	1733.922	50	64	1733.922	1733.922	2	KKNPEVPVNFAEFSK	KKNPEVPVNFAEFSK
12	1733.922	51	65	1733.922	1733.922	2	KNPEVPVNFAEFSKK	KNPEVPVNFAEFSKK
15	186.000	11	33	2512.254	2512.249	3	GAYPYDVPDYAMAKGDPKKPKGK	GAYPYDVPDYAM**<Mox*>**AKGDPKKPKGK
16	259.000	200	220	2669.959	2669.901	1	KVEEEDEEEEEEEEEEEEED	KVEEEDEEEEEEEEEEEEED
18	2032.654	199	220	2798.054	2798.015	2	KKVEEEDEEEEEEEEEEEEED	KKVEEEDEEEEEEEEEEEEED
21	4425.123	196	220	3124.261	3124.208	3	VARKKVEEEDEEEEEEEEEEEEED	VARKKVEEEDEEEEEEEEEEEEED

Abbreviations: Ac, acetyl; Mox, oxidized methionine.

[a] Residues are numbered considering an HMGB3 form lacking the N-terminal initiator methionine.

Supplementary Table S4 - Peak list generated by MALDI-TOF analysis of a tryptic digest of purified recombinant HMGB3.

Peak	m/z	Time	Intensity	Signal/Noise ratio	Quality Factor	Resolution	Area
1	824.337	45500.89	875.668	6.970	392.441	8805.339	134.872
2	832.342	45719.15	941.832	7.585	297.453	8424.265	151.887
3	876.300	46899.39	1750.101	15.081	525.985	7435.218	355.768
4	1258.592	56114.12	16399.156	217.190	18328.231	14154.026	3305.032
5	1274.587	56466.48	1179.179	15.335	1966.674	14269.501	229.097
6	1280.573	56597.80	1315.728	17.222	3133.839	13562.994	275.144
7	1315.614	57360.31	990.743	13.095	1694.030	14525.900	199.083
8	1401.801	59193.53	18940.384	276.900	23777.888	14670.167	4469.781
9	1458.819	60375.39	885.523	12.373	3085.337	15548.733	198.282
10	1529.896	61816.65	3103.273	46.345	15679.582	16631.633	754.291
11	1616.924	63536.37	1048.364	15.380	4074.548	14744.940	305.465
12	1733.922	65776.72	477.462	6.698	1549.114	17323.771	129.940
13	1794.829	66912.99	521.084	7.434	1178.839	12730.754	205.746
14	2211.097	74210.00	564.000	8.095		16216.774	82.952
15	2512.249	79066.41	186.000	2.267		10779.765	39.754
16	2669.901	81492.30	259.000	3.758		15420.364	46.824
17	2783.212	83191.80	236.000	3.431		12862.457	56.125
18	2798.015	83411.24	2032.654	55.798	12446.153	17981.107	1179.762
19	2839.082	84016.99	184.000	2.564		15689.759	24.586
20	2900.501	84914.80	226.000	3.509		12411.483	45.378
21	3124.208	88106.87	4425.123	152.159	10333.571	18328.942	3105.778
22	3226.707	89531.07	794.478	28.088	3920.512	15759.491	647.241
23	3477.397	92921.58	1031.823	44.047	3284.136	16922.644	903.032
24	3496.864	93179.64	501.346	21.123	3444.219	15235.753	473.585

Supplementary Table S5 – List of human proteins containing a stretch of at least 3 consecutive acidic residues in their gene-encoded C-terminus

Number	Name	UniProt	C-terminus
1	**High mobility group protein B1**	P09429	SKKKK**EEEEDEEDEEDEEEEEDEEDEDEEEDDDDE**
2	**Putative high mobility group protein B1-like 1**	B2RPK0	SKKKK**EEEEDEEDEEDEEEEDEEDEEDDDDE**
3	**High mobility group protein B2**	P26583	KKNEP**EDEEEEEEEEDEDEEEEDEDEE**
4	**High mobility group protein B3** [a]	O15347	ARKKV**EEEDEEEEEEEEEEEEEEDE**
5	**Homeobox protein Hox-A7**	P31268	AADKA**DEEDDDEEEEDEEE**
6	**Cytosolic purine 5'-nucleotidase**	P49902	ITHCH**DEDDDEEEEEEEE**
7	**Translocation protein SEC63 homolog**	Q9UGP8	FEDSF**EEEEEEEEDDD**
8	**mTERF domain-containing protein 2**	Q7Z6M4	EAEDN**DEDEDDDEEE**
9	**60S ribosomal protein L22**	P35268	FQINQ**DEEEEEDED**
10	**Calsequestrin-1**	P31415	GEINT**EDDDDDDDD**
11	**Ubiquitin thioesterase ZRANB1**	Q9UGI0	SLSDG**EEDEDDEDE**
12	**Calcium-dependent secretion activator 1**	Q9ULU8	SMKDS**DEEDEEDD**
13	**Putative high mobility group protein B3-like**	P0C6E5	AQKKV**EEEDEDEE**
14	**RNA polymerase-associated protein LEO1**	Q8WVC0	KYVIS**DEEEEDDD**
15	**LIM domain and actin-binding protein 1**	Q9UHB6	RNRYY**DEDEDEE**
16	**Essential MCU regulator, mitochondrial**	Q9H4I9	DIFVP**EDDDDDD**
17	**Calsequestrin-2**	O14958	DNDDS**DDDDDE**
18	**Cell cycle control protein 50B**	Q3MIR4	RYQDQ**DDDDEE**
19	**Dipeptidyl aminopeptidase-like protein 6**	P42658	TVTAK**EDEEED**
20	**Homeobox protein Hox-B7**	P09629	DRAEA**EEEEEE**
21	**Metaxin-1**	Q13505	LGMA**EEDEEE**
22	**40S ribosomal protein S9** [a]	P46781	GAGAG**DDEEED**
23	**Protein timeless homolog**	Q9UNS1	KRYQI**EDDEDD**
24	**TRAF-type zinc finger domain-containing protein 1** [a]	O14545	GAGDA**EEEEEE**
25	**Acidic leucine-rich nuclear phosphoprotein 32 family member E**	Q9BTT0	AEDDG**EEEDD**
26	**Interferon alpha-inducible protein 6**	P09912	KYLDS**EEDEE**
27	**Myosin light chain kinase, smooth muscle** [a]	Q15746	GEGEG**EEEEE**
28	**Choline-phosphate cytidylyltransferase A**	P49585	AYDIS**EDEED**
39	**Protein SSX1**	Q16384	EISDP**EEDDE**
30	**Protein SSX2**	Q16385	EISDP**EEDDE**
31	**Protein SSX3**	Q99909	EISDP**EEDDE**
32	**Protein SSX4**	O60224	EISDP**EEDDE**
33	**Protein SSX7**	Q7RTT5	EISDP**EEDDE**
34	**Protein SSX8**	Q7RTT4	EIRDP**EEDDE**
35	**Protein SSX9**	Q7RTT3	EISDP**EEDDE**
36	**Transmembrane protein 190**	Q8WZ59	EETEG**EEEED**
37	**Acidic leucine-rich nuclear phosphoprotein 32 family member A**	P39687	PEDEG**EDDD**
38	**Complement C1r subcomponent**	P00736	IKKEM**EEED**
39	**Mitotic interactor and substrate of PLK1**	Q8IVT2	RIYAS**EEDD**
40	**Doublesex- and mab-3-related transcription factor 1**	Q9Y5R6	VTPVI**EEDE**

41	**Inactive dipeptidyl peptidase 10**	Q8N608	LPQEP**EEDE**
42	**Inositol monophosphatase 1**	P29218	IPLQR**DDED**
43	**mRNA turnover protein 4 homolog**	Q9UKD2	EESDS**EDDD**
44	**NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 2, mitochondrial**	O95178	LGIPP**DDED**
45	**Ornithine decarboxylase antizyme 1**	P54368	RESSG**EEEE**
46	**cGMP-inhibited 3',5'-cyclic phosphodiesterase B**	Q13370	VIEEA**DEEE**
47	**Protein SSX5**	O60225	ISDPQ**EDDE**
48	**Transcription factor Dp-1**	Q14186	DFNEN**DEDD**
49	**Transcription factor Dp family member 3**	Q5H9I0	DLSEN**DEDD**
50	**Guanine nucleotide exchange factor VAV3**	Q9UKW4	PSTYV**EEDE**
51	**DDB1- and CUL4-associated factor 6**	Q58WW2	QENEN**EDEE**
52	**Prothymosin alpha**	P06454	KKQKT**DEDD**
53	**Acidic leucine-rich nuclear phosphoprotein 32 family member B**	Q92688	TDDEG**EDD**
54	**Ankyrin repeat domain-containing protein 35**	Q8N283	QILSM**EEE**
55	**Annexin A5**	P08758	LLLCG**EDD**
56	**Complement C1q tumor necrosis factor-related protein 6**	Q9BXI9	HLIKA**EDD**
57	**Cadherin-1**	P12830	MYGGG**EDD**
58	**Cadherin-3**	P22223	MYGGG**EDD**
59	**Cadherin-4**	P55283	MYGGG**EDD**
60	**Cell division cycle protein 123 homolog**	O75794	KRNQQ**EDD**
61	**Phosphatidate cytidylyltransferase 2**	O95674	LTSTT**EDE**
62	**Uncharacterized protein C11orf57**	Q6ZUT1	SAESS**EDD**
63	**Cytochrome c oxidase subunit 6A1, mitochondrial**	P12074	LPTGY**EDE**
64	**EGF-like repeat and discoidin I-like domain-containing protein 3**	O43854	LLGCT**EEE**
65	**EF-hand domain-containing family member C2**	Q5JST6	DAFGL**EEE**
66	**Translation initiation factor eIF-2B subunit epsilon**	Q13144	EEESS**EDD**
67	**Protein FAM47A**	Q5JRC9	IVEAS**EED**
68	**Putative protein FAM47C**	Q5HY64	VTDAS**EED**
69	**Guanylate cyclase soluble subunit beta-1**	Q02153	EETKQ**DDD**
70	**G patch domain and KOW motifs-containing protein**	Q92917	GPSDT**DDD**
71	**Homeobox-containing protein 1**	Q6NT76	KTEAL**DDD**
72	**High mobility group protein HMGI-C**	P52926	SQESA**EED**
73	**Putative eukaryotic translation initiation factor 2 subunit 3-like protein**	Q2VIR3	IKPTV**DDD**
74	**Eukaryotic translation initiation factor 2 subunit 3**	P41091	IKPTV**DDD**
75	**Interferon-induced helicase C domain-containing protein 1**	Q9BYX4	CCLFS**DED**
76	**Interleukin-1 receptor antagonist protein**	P18510	KFYFQ**EDE**
77	**Mitochondrial inner membrane protease subunit 2**	Q96T52	LPVQR**EEE**
78	**Leucine-rich repeat-containing protein 8E**	Q6NSJ5	VRDKM**EEE**
79	**Mitogen-activated protein kinase kinase kinase 4**	Q9Y6R4	VKVCT**DEE**
80	**Mitotic interactor and substrate of PLK1**	Q8IVT2	IYASE**EDD**
81	**Monocarboxylate transporter 13**	Q7RTY0	PKEGL**EED**

82	**Myosin-6**	P13533	KQKMH**DEE**
83	**NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial**	Q9NX14	KIQLP**EDE**
84	**Nucleolar complex protein 2 homolog**	Q9Y3T9	DLQLS**EDD**
85	**Nuclear speckle splicing regulatory protein 1**	Q9H0G5	TYIEK**EDD**
86	**Ornithine decarboxylase antizyme 2**	O95190	DQNLS**DED**
87	**Inositol polyphosphate 5-phosphatase OCRL-1**	Q01968	FLLGS**EED**
88	**Serine/threonine-protein phosphatase 2A regulatory subunit B'' subunit alpha**	Q06190	KLQSV**DEE**
89	**Protein pelota homolog**	Q9BRX2	GDSSS**EED**
90	**Histone-lysine N-methyltransferase PRDM9**	Q9NQV7	PYVCR**EDE**
91	**PTPN13-like protein, Y-linked**	O14603	CGHVH**EED**
92	**Transcriptional activator protein Pur-alpha.**	Q00577	GEEEG**EED**
93	**Transcriptional activator protein Pur-beta**	Q96QR8	EGEEV**DED**
94	**UV excision repair protein RAD23 homolog A**	P54725	LSQNF**DDE**
95	**UV excision repair protein RAD23 homolog B**	P54727	LQQNF**DED**
96	**Rho GTPase-activating protein 15**	Q53QZ3	KIFGS**EED**
97	**RWD domain-containing protein 4**	Q6NW29	KTGSK**DDE**
98	**Protein SET**	Q01105	EEDEG**EDD**
99	**Probable global transcription activator SNF2L2**	P51531	EGSGT**DDE**
100	**Transcription activator BRG1**	P51532	SGSGS**EED**
101	**Small integral membrane protein 4**	Q8WVI0	YQRRL**EDE**
102	**Small integral membrane protein 6**	P0DI80	QCEAG**EEE**
103	**Sorting nexin-16**	P57768	VAYDA**EED**
104	**TLD domain-containing protein 1**	Q6P9B6	LREVP**DDE**
105	**Transmembrane protein 109**	Q9BVC6	GARSV**EEE**
106	**Transmembrane protein 45B**	Q96B21	LLSGS**DEE**
107	**Transmembrane protein 44**	Q2T9K0	TAHLS**DDD**
108	**Triple QxxK/R motif-containing protein**	Q629K1	PDLDQ**DED**
109	**Teashirt homolog 2**	Q9NRE2	FVTDV**DEE**
110	**Thioredoxin domain-containing protein 9**	O14530	YDSDS**DDD**
111	**WD repeat-containing protein 43**	Q15061	PENES**EEE**

[a] These proteins have been identified as CCP1 substrates in this or previous studies.