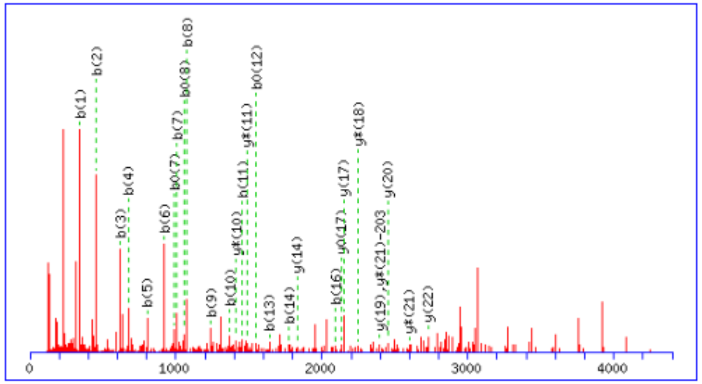
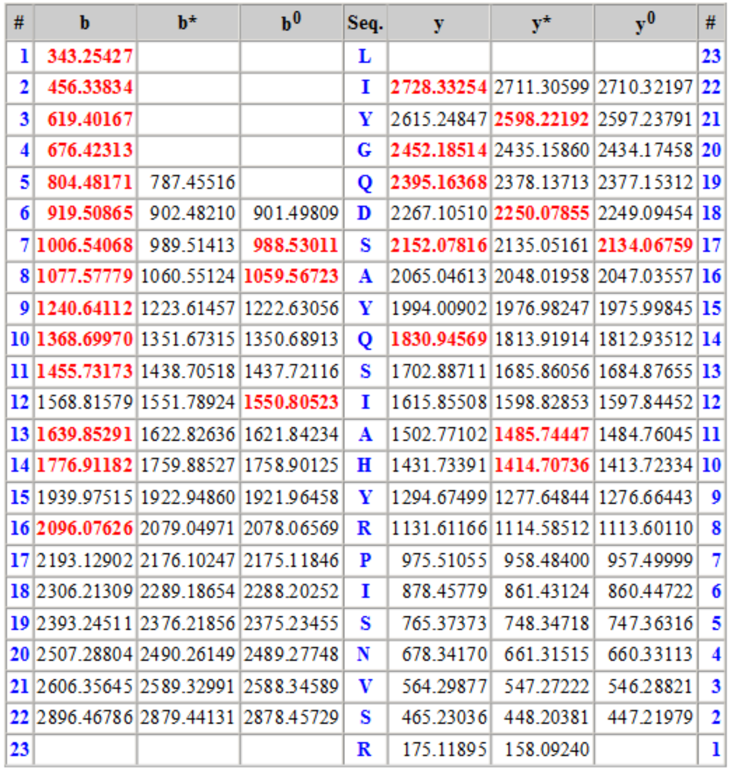
a LIYGQDSAYQSIAHYRPISNVSR (Emd) + HexNAc2.Hex10

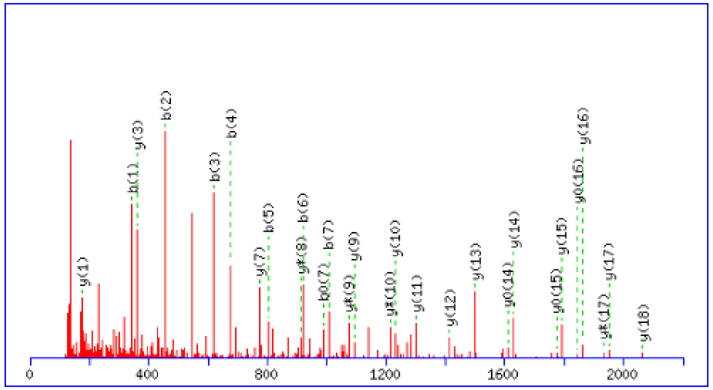


```
Monoisotopic mass of neutral peptide Mr(calc): 3069.57222
Fixed modifications: Methylthio (C)
Variable modifications:
N-term : TMT6plex (N-term)
S22    : HexNAc(NL) (NST), with neutral losses 0.00000(shown in table), 203.07937
Ions Score: 65  Expect: 0.0013
Matches (Bold Red): 28/188 fragment ions using 71 most intense peaks
```

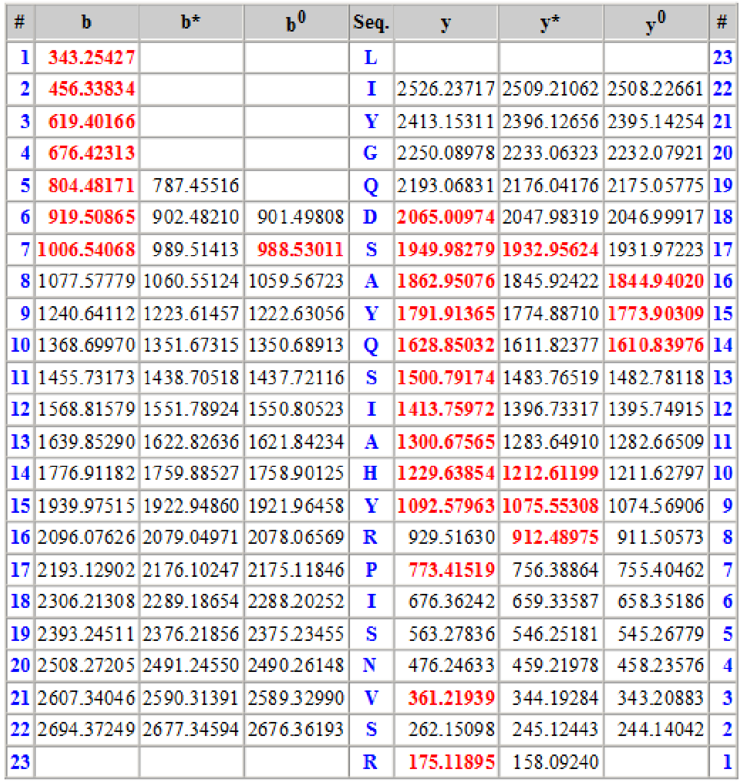


#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**343.25427**			L				23
2	**456.33834**			I	**2728.33254**	2711.30599	2710.32197	22
3	**619.40167**			Y	2615.24847	**2598.22192**	2597.23791	21
4	**676.42313**			G	**2452.18514**	2435.15860	2434.17458	20
5	**804.48171**	787.45516		Q	**2395.16368**	2378.13713	2377.15312	19
6	**919.50865**	902.48210	901.49809	D	2267.10510	**2250.07855**	2249.09454	18
7	**1006.54068**	989.51413	**988.53011**	S	**2152.07816**	2135.05161	**2134.06759**	17
8	**1077.57779**	1060.55124	**1059.56723**	A	2065.04613	2048.01958	2047.03557	16
9	**1240.64112**	1223.61457	1222.63056	Y	1994.00902	1976.98247	1975.99845	15
10	**1368.69970**	1351.67315	1350.68913	Q	**1830.94569**	1813.91914	1812.93512	14
11	**1455.73173**	1438.70518	1437.72116	S	1702.88711	1685.86056	1684.87655	13
12	1568.81579	1551.78924	**1550.80523**	I	1615.85508	1598.82853	1597.84452	12
13	**1639.85291**	1622.82636	1621.84234	A	1502.77102	**1485.74447**	1484.76045	11
14	**1776.91182**	1759.88527	1758.90125	H	1431.73391	**1414.70736**	1413.72334	10
15	1939.97515	1922.94860	1921.96458	Y	1294.67499	1277.64844	1276.66443	9
16	**2096.07626**	2079.04971	2078.06569	R	1131.61166	1114.58512	1113.60110	8
17	2193.12902	2176.10247	2175.11846	P	975.51055	958.48400	957.49999	7
18	2306.21309	2289.18654	2288.20252	I	878.45779	861.43124	860.44722	6
19	2393.24511	2376.21856	2375.23455	S	765.37373	748.34718	747.36316	5
20	2507.28804	2490.26149	2489.27748	N	678.34170	661.31515	660.33113	4
21	2606.35645	2589.32991	2588.34589	V	564.29877	547.27222	546.28821	3
22	2896.46786	2879.44131	2878.45729	S	465.23036	448.20381	447.21979	2
23				R	175.11895	158.09240		1

b LIYGQDSAYQSIAHYRPISNVSR (Emd), deamidated



```
Monoisotopic mass of neutral peptide Mr(calc): 2867.47685
Fixed modifications: Methylthio (C),TMT6plex (N-term)
Variable modifications:
N20    : Deamidated (N)
Ions Score: 94  Expect: 6e-08
Matches (Bold Red): 28/122 fragment ions using 41 most intense peaks
```



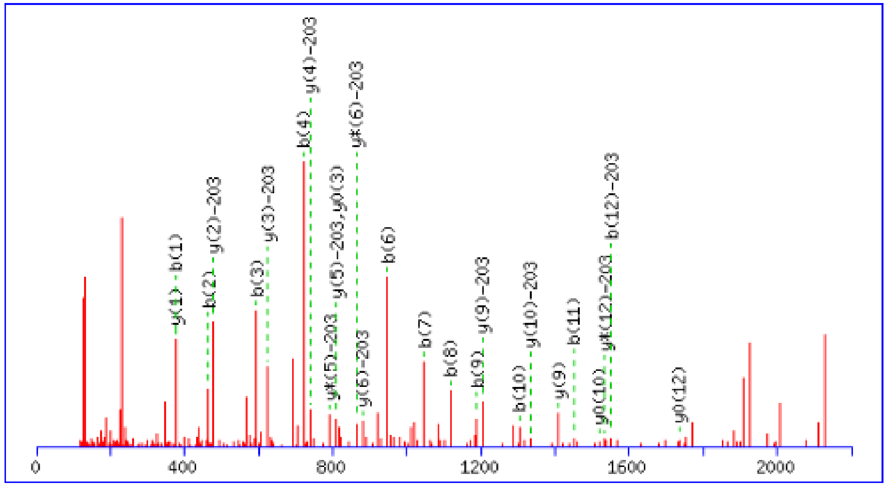
#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**343.25427**			L				23
2	**456.33834**			I	2526.23717	2509.21062	2508.22661	22
3	**619.40166**			Y	2413.15311	2396.12656	2395.14254	21
4	**676.42313**			G	2250.08978	2233.06323	2232.07921	20
5	**804.48171**	787.45516		Q	2193.06831	2176.04176	2175.05775	19
6	**919.50865**	902.48210	901.49808	D	**2065.00974**	2047.98319	2046.99917	18
7	**1006.54068**	989.51413	**988.53011**	S	**1949.98279**	**1932.95624**	1931.97223	17
8	1077.57779	1060.55124	1059.56723	A	**1862.95076**	1845.92422	**1844.94020**	16
9	1240.64112	1223.61457	1222.63056	Y	**1791.91365**	1774.88710	**1773.90309**	15
10	1368.69970	1351.67315	1350.68913	Q	**1628.85032**	1611.82377	**1610.83976**	14
11	1455.73173	1438.70518	1437.72116	S	**1500.79174**	1483.76519	1482.78118	13
12	1568.81579	1551.78924	1550.80523	I	**1413.75972**	1396.73317	1395.74915	12
13	1639.85290	1622.82636	1621.84234	A	**1300.67565**	1283.64910	1282.66509	11
14	1776.91182	1759.88527	1758.90125	H	**1229.63854**	**1212.61199**	1211.62797	10
15	1939.97515	1922.94860	1921.96458	Y	**1092.57963**	**1075.55308**	1074.56906	9
16	2096.07626	2079.04971	2078.06569	R	929.51630	**912.48975**	911.50573	8
17	2193.12902	2176.10247	2175.11846	P	**773.41519**	756.38864	755.40462	7
18	2306.21308	2289.18654	2288.20252	I	676.36242	659.33587	658.35186	6
19	2393.24511	2376.21856	2375.23455	S	563.27836	546.25181	545.26779	5
20	2508.27205	2491.24550	2490.26148	N	476.24633	459.21978	458.23576	4
21	2607.34046	2590.31391	2589.32990	V	**361.21939**	344.19284	343.20883	3
22	2694.37249	2677.34594	2676.36193	S	262.15098	245.12443	244.14042	2
23				R	**175.11895**	158.09240		1

Supplementary Figure 1.

Supplementary Figure 1. Identification of Emerin (Emd) as N-glycoprotein.

(**a**) N-Glycopeptide LIYGQDSAYQSIAHYRPISNVSR (Emd) with the glycan composition HexNAc2.Hex10 was identified with high confidence via MS/MS. (**b**) Analysis of the corresponding PNGaseF-treated HILIC fraction identified the non-glycosylated peptide LIYGQDSAYQSIAHYRPISNVSR bearing the enzymatically induced deamidation of formerly N-glycosylated asparagine residues at position 20. Of note, in the context of the amino-acid sequence, asparagine 20 is part of a potential N-glycosylation sequence-motive.

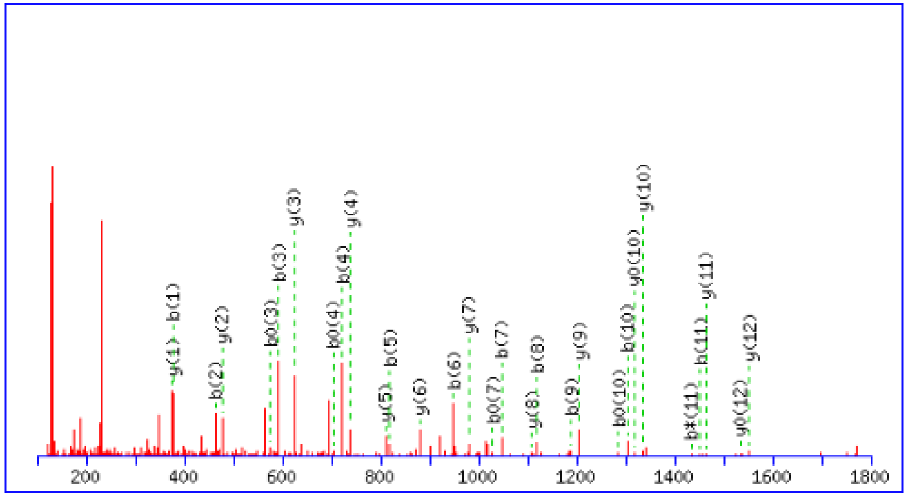
a **FSEEPEVAANFTK (Nop56) + HexNAc2.Hex6**



```
Monoisotopic mass of neutral peptide Mr(calc): 2129.09340
Fixed modifications: Methylthio (C)
Variable modifications:
N-term : TMT6plex (N-term)
T12    : HexNAc(NL) (NST), with neutral losses 203.07937(shown in table), 0.00000
K13    : TMT6plex (K)
Ions Score: 67  Expect: 0.0003
Matches (Bold Red): 26/97 fragment ions using 46 most intense peaks
```

#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**377.23862**			F				13
2	**464.27065**		446.26009	S	1550.78997	**1533.76342**	1532.77941	12
3	**593.31324**		575.30268	E	1463.75795	1446.73140	1445.74738	11
4	**722.35584**		704.34527	E	**1334.71535**	1317.68880	1316.70479	10
5	819.40860		801.39804	P	**1205.67276**	1188.64621	1187.66219	9
6	**948.45119**		930.44063	E	1108.62000	1091.59345	1090.60943	8
7	**1047.51961**		1029.50904	V	979.57740	962.55085	961.56684	7
8	**1118.55672**		1100.54616	A	**880.50899**	**863.48244**	862.49842	6
9	**1189.59384**		1171.58327	A	**809.47187**	**792.44533**	791.46131	5
10	**1303.63676**	1286.61021	1285.62620	N	**738.43476**	721.40821	720.42420	4
11	**1450.70518**	1433.67863	1432.69461	F	**624.39183**	607.36528	606.38127	3
12	**1551.75286**	1534.72631	1533.74229	T	**477.32342**	460.29687	459.31285	2
13				K	**376.27574**	359.24919		1

b **FSEEPEVAANFTK (Nop56), deamidated**



```
Monoisotopic mass of neutral peptide Mr(calc): 1926.99803
Fixed modifications: Methylthio (C),TMT6plex (N-term)
Variable modifications:
N10    : Deamidated (N)
K13    : TMT6plex (K)
Ions Score: 65  Expect: 3.6e-05
Matches (Bold Red): 30/61 fragment ions using 68 most intense peaks
```

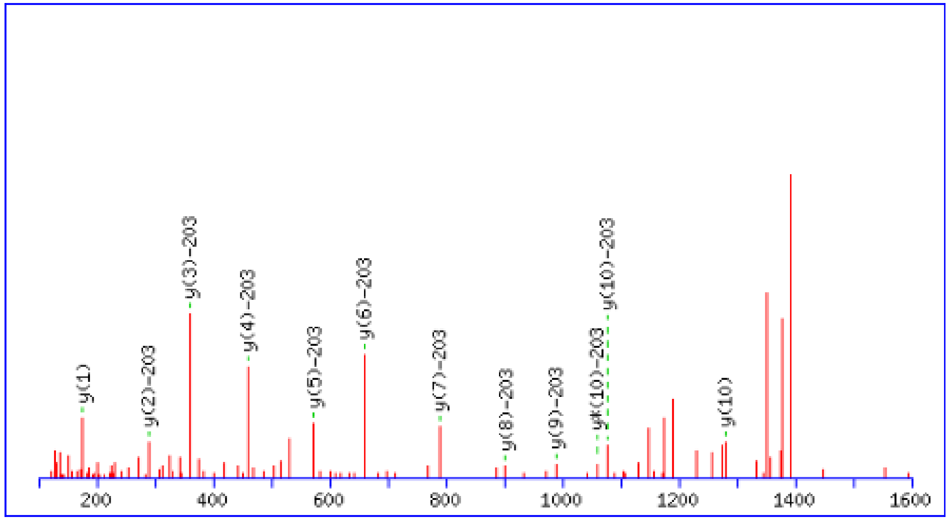
#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**377.23862**			F				13
2	**464.27065**		446.26009	S	**1551.77398**	1534.74743	**1533.76341**	12
3	**593.31324**		**575.30268**	E	**1464.74195**	1447.71540	1446.73139	11
4	**722.35584**		**704.34527**	E	**1335.69936**	1318.67281	**1317.68879**	10
5	**819.40860**		801.39803	P	**1206.65677**	1189.63022	1188.64620	9
6	**948.45119**		930.44063	E	**1109.60400**	1092.57745	1091.59344	8
7	**1047.51961**		**1029.50904**	V	**980.56141**	963.53486	962.55084	7
8	**1118.55672**		1100.54616	A	**881.49299**	864.46645	863.48243	6
9	**1189.59384**		1171.58327	A	**810.45588**	793.42933	792.44532	5
10	**1304.62077**	1287.59422	**1286.61021**	N	**739.41877**	722.39222	721.40820	4
11	**1451.68919**	**1434.66264**	1433.67862	F	**624.39183**	607.36528	606.38127	3
12	1552.73686	1535.71032	1534.72630	T	**477.32342**	460.29687	459.31285	2
13				K	**376.27574**	359.24919		1

Supplementary Figure 2.

Supplementary Figure 2. Identification of Nuclear protein 56 (Nop56) as N-glycoprotein.

(**a**) N-Glycopeptide FSEEPEVAANFTK (Nop56) with the glycan composition HexNAc2.Hex6 was identified with high confidence via MS/MS. (**b**) MS/MS data analysis of the corresponding PNGaseF-treated HILIC fraction identified the non-glycosylated peptide FSEEPEVAANFTK bearing the enzymatically induced deamidation of formerly N-glycosylated asparagine residues at position 10. Of note, in the context of the amino-acid sequence, asparagine 10 is part of a potential N-glycosylation sequence-motive.

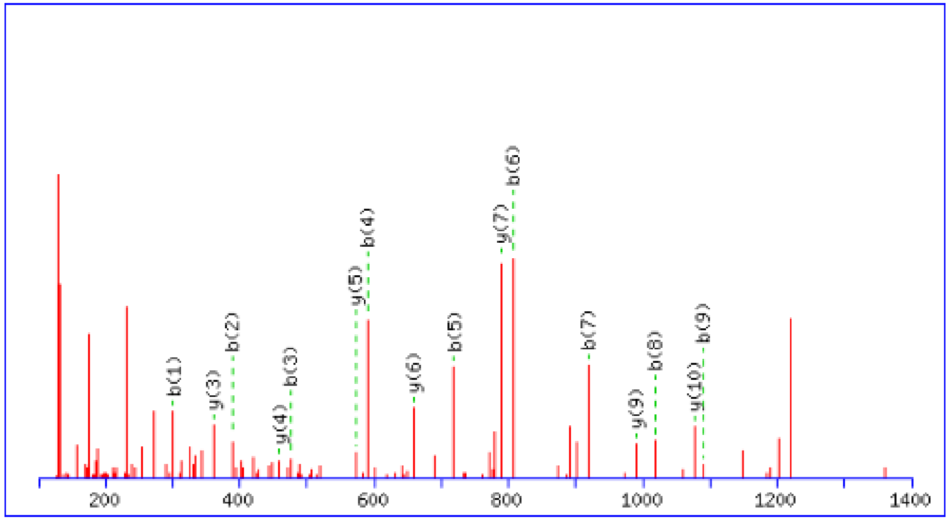
a **ASSNESLVANR (Tmpo) + HexNAc2.Hex11**



```
Monoisotopic mass of neutral peptide Mr(calc): 1349.64226
Fixed modifications: Methylthio (C)
Variable modifications:
N10     : HexNAc(NL) (NST), with neutral losses 203.07937(shown in table), 0.00000
Ions Score: 102  Expect: 2.3e-08
Matches (Bold Red): 12/77 fragment ions using 21 most intense peaks
```

#	b	b*	b^0	Seq.	y	y*	y^0	#
1	72.04439			A				11
2	159.07642		141.06585	S	**1076.53308**	**1059.50653**	1058.52251	10
3	246.10845		228.09788	S	**989.50105**	972.47450	971.49048	9
4	360.15137	343.12482	342.14081	N	**902.46902**	885.44247	884.45846	8
5	489.19397	472.16742	471.18340	E	**788.42609**	771.39955	770.41553	7
6	576.22599	559.19944	558.21543	S	**659.38350**	642.35695	641.37294	6
7	689.31006	672.28351	671.29949	L	**572.35147**	555.32492		5
8	788.37847	771.35192	770.36791	V	**459.26741**	442.24086		4
9	859.41559	842.38904	841.40502	A	**360.19900**	343.17245		3
10	973.45852	956.43197	955.44795	N	**289.16188**	272.13533		2
11				R	**175.11895**	158.09240		1

b **ASSNESLVANR (Tmpo), deamidated**



```
Monoisotopic mass of neutral peptide Mr(calc): 1376.70982
Fixed modifications: Methylthio (C),TMT6plex (N-term)
Variable modifications:
N4      : Deamidated (N)
Ions Score: 75  Expect: 2.1e-06
Matches (Bold Red): 16/51 fragment ions using 26 most intense peaks
```

#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**301.20732**			A				11
2	**388.23935**		370.22879	S	**1077.51708**	1060.49054	1059.50652	10
3	**475.27138**		457.26081	S	**990.48506**	973.45851	972.47449	9
4	**590.29831**	573.27176	572.28775	N	903.45303	886.42648	885.44246	8
5	**719.34091**	702.31436	701.33034	E	**788.42609**	771.39954	770.41553	7
6	**806.37293**	789.34639	788.36237	S	**659.38350**	642.35695	641.37293	6
7	**919.45700**	902.43045	901.44643	L	**572.35147**	555.32492		5
8	**1018.52541**	1001.49886	1000.51485	V	**459.26741**	442.24086		4
9	**1089.56253**	1072.53598	1071.55196	A	**360.19899**	343.17244		3
10	1203.60545	1186.57891	1185.59489	N	289.16188	272.13533		2
11				R	175.11895	158.09240		1

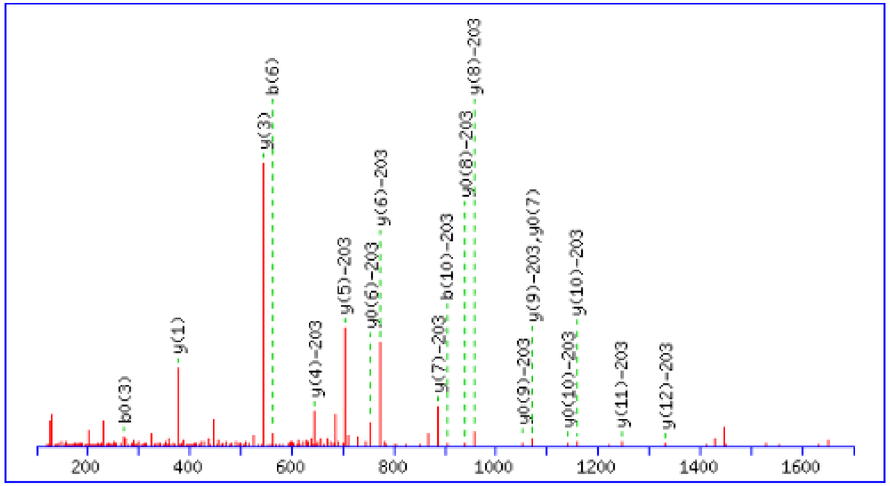
Supplementary Figure 3.

Supplementary Figure 3. Identification of Lamina-associated polypeptide 2 (Tmpo) as N-glycoprotein.

(**a**) N-Glycopeptide ASSNESLVANR (Tmpo) with the glycan composition HexNAc2.Hex11 was identified with high confidence via MS/MS. (**b**) MS/MS data analysis of the corresponding PNGaseF-treated HILIC fraction identified the non-glycosylated peptide ASSNESLVANR bearing the enzymatically induced deamidation of formerly N-glycosylated asparagine residues at position 4. Of note, in the context of the amino-acid sequence, asparagine 4 is part of a potential N-glycosylation sequence-motive.

a

NSSSNALAGTPAK (Rad51ap1) + HexNAc2.Hex12

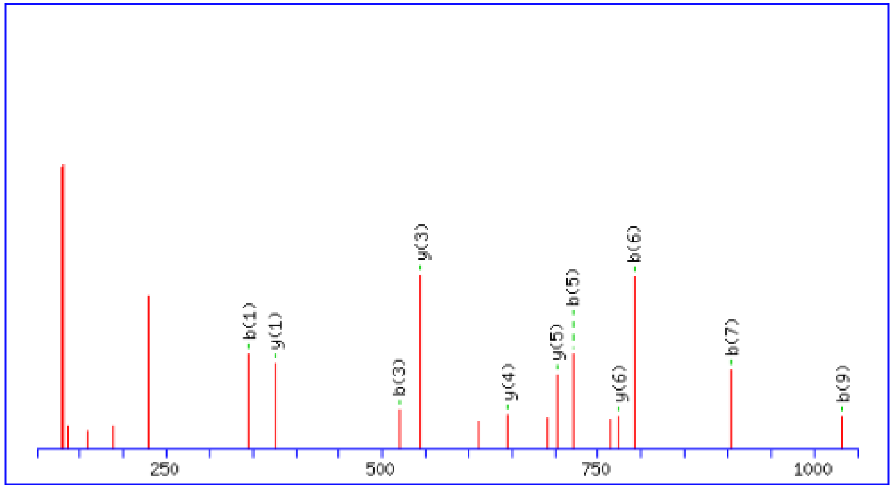


```
Monoisotopic mass of neutral peptide Mr(calc): 1648.84706
Fixed modifications: Methylthio (C)
Variable modifications:
T10     : HexNAc(NL) (NST), with neutral losses 203.07937(shown in table), 0.00000
K13     : TMT6plex (K)
Ions Score: 90  Expect: 8.2e-07
Matches (Bold Red): 19/104 fragment ions using 33 most intense peaks
```

#	b	b*	b^0	Seq.	y	y*	y^0	#
1	115.05020	98.02365		N				13
2	202.08223	185.05568	184.07167	S	**1332.73206**	1315.70552	1314.72150	12
3	289.11426	272.08771	**271.10369**	S	**1245.70004**	1228.67349	1227.68947	11
4	376.14629	359.11974	358.13572	S	**1158.66801**	1141.64146	**1140.65744**	10
5	490.18921	473.16267	472.17865	N	**1071.63598**	1054.60943	**1053.62542**	9
6	**561.22633**	544.19978	543.21576	A	**957.59305**	940.56650	**939.58249**	8
7	674.31039	657.28384	656.29983	L	**886.55594**	869.52939	868.54537	7
8	745.34751	728.32096	727.33694	A	**773.47188**	756.44533	**755.46131**	6
9	802.36897	785.34242	784.35841	G	**702.43476**	685.40821	684.42420	5
10	**903.41665**	886.39010	885.40609	T	**645.41330**	628.38675	627.40273	4
11	1000.46942	983.44287	982.45885	P	**544.36562**	527.33907		3
12	1071.50653	1054.47998	1053.49597	A	447.31285	430.28630		2
13				K	**376.27574**	359.24919		1

b

NSSSNALAGTPAK (Rad51ap1), deamidated



```
Monoisotopic mass of neutral peptide Mr(calc): 1675.91463
Fixed modifications: Methylthio (C),TMT6plex (N-term)
Variable modifications:
N1      : Deamidated (N)
K13     : TMT6plex (K)
Ions Score: 48  Expect: 0.0019
Matches (Bold Red): 11/68 fragment ions using 16 most intense peaks
```

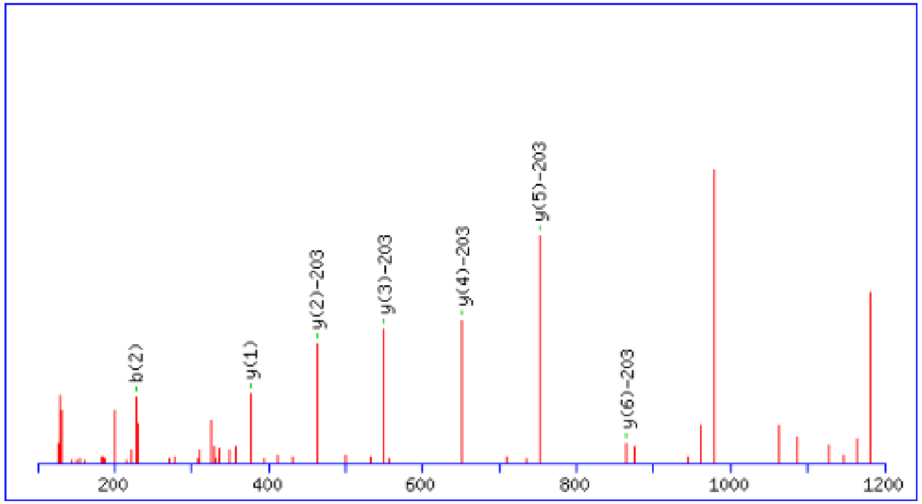
#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**345.19714**	328.17059		N				13
2	432.22917	415.20262	414.21861	S	1332.73206	1315.70551	1314.72150	12
3	**519.26120**	502.23465	501.25064	S	1245.70003	1228.67348	1227.68947	11
4	606.29323	589.26668	588.28266	S	1158.66801	1141.64146	1140.65744	10
5	**720.33616**	703.30961	702.32559	N	1071.63598	1054.60943	1053.62541	9
6	**791.37327**	774.34672	773.36270	A	957.59305	940.56650	939.58249	8
7	**904.45733**	887.43078	886.44677	L	886.55594	869.52939	868.54537	7
8	975.49445	958.46790	957.48388	A	**773.47187**	756.44532	755.46131	6
9	**1032.51591**	1015.48936	1014.50535	G	**702.43476**	685.40821	684.42419	5
10	1133.56359	1116.53704	1115.55303	T	**645.41329**	628.38675	627.40273	4
11	1230.61635	1213.58981	1212.60579	P	**544.36562**	527.33907		3
12	1301.65347	1284.62692	1283.64290	A	447.31285	430.28630		2
13				K	**376.27574**	359.24919		1

Supplementary Figure 4.

Supplementary Figure 4. Identification of RAD51-associated protein 1 (Rad51ap1) as N-glycoprotein.

(**a**) N-Glycopeptide NSSSNASLAGTPAK (Rad51ap1) with the glycan composition HexNAc2.Hex11 was identified with high confidence via MS/MS. (**b**) MS/MS data analysis of the corresponding PNGaseF-treated HILIC fraction identified the non-glycosylated peptide NSSSNASLAGTPAK bearing the enzymatically induced deamidation of formerly N-glycosylated asparagine residues at position 1. Of note, in the context of the amino-acid sequence, asparagine 1 is part of a potential N-glycosylation sequence-motive.

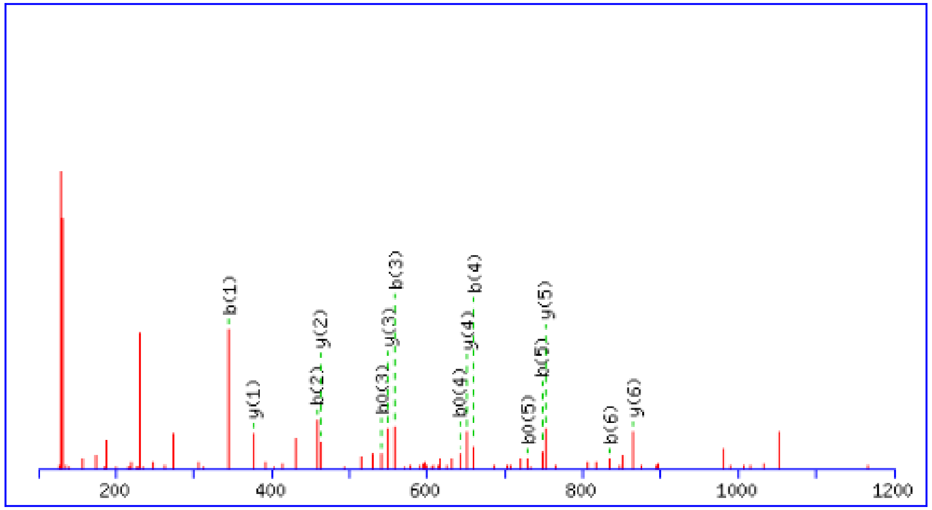
a NLTTSSK (Smg7) + HexNAc2.Hex12



```
Monoisotopic mass of neutral peptide Mr(calc): 1181.63423
Fixed modifications: Methylthio (C)
Variable modifications:
S6      : HexNAc(NL) (NST), with neutral losses 203.07937(shown in table), 0.00000
K7      : TMT6plex (K)
Ions Score: 54  Expect: 0.0017
Matches (Bold Red): 7/51 fragment ions using 15 most intense peaks
```

#	b	b*	b^0	Seq.	y	y*	y^0	#
1	115.05020	98.02365		N				7
2	**228.13427**	211.10772		L	**865.51922**	848.49267	847.50865	6
3	329.18195	312.15540	311.17138	T	**752.43515**	735.40861	734.42459	5
4	430.22962	413.20308	412.21906	T	**651.38748**	634.36093	633.37691	4
5	517.26165	500.23510	499.25109	S	**550.33980**	533.31325	532.32923	3
6	604.29368	587.26713	586.28312	S	**463.30777**	446.28122	445.29720	2
7				K	**376.27574**	359.24919		1

b NLTTSSK (Smg7), deamidated



```
Monoisotopic mass of neutral peptide Mr(calc): 1208.70180
Fixed modifications: Methylthio (C),TMT6plex (N-term)
Variable modifications:
N1      : Deamidated (N)
K7      : TMT6plex (K)
Ions Score: 30  Expect: 0.061
Matches (Bold Red): 15/33 fragment ions using 30 most intense peaks
```

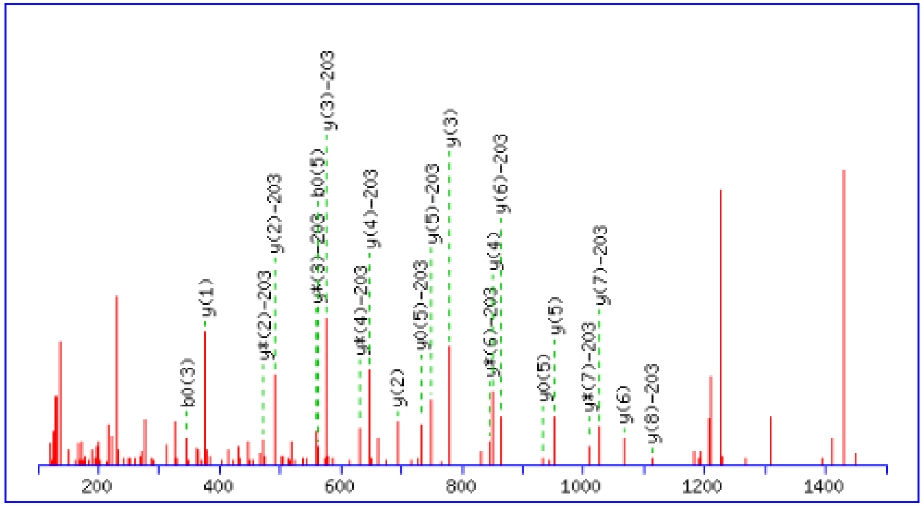
#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**345.19714**	328.17059		N				7
2	**458.28121**	441.25466		L	**865.51922**	848.49267	847.50865	6
3	**559.32889**	542.30234	**541.31832**	T	**752.43515**	735.40860	734.42459	5
4	**660.37657**	643.35002	**642.36600**	T	**651.38747**	634.36092	633.37691	4
5	**747.40859**	730.38204	**729.39803**	S	**550.33979**	533.31324	532.32923	3
6	**834.44062**	817.41407	816.43006	S	**463.30776**	446.28122	445.29720	2
7				K	**376.27574**	359.24919		1

Supplementary Figure 5.

Supplementary Figure 5. Identification of Smg7 as N-glycoprotein.

(**a**) N-Glycopeptide NLTTSSK (Smg7) with the glycan composition HexNAc2.Hex12 was identified with high confidence via MS/MS. (**b**) MS/MS data analysis of the corresponding PNGaseF-treated HILIC fraction identified the non-glycosylated peptide NLTTSSK bearing the enzymatically induced deamidation of formerly N-glycosylated asparagine residues at position 1. Of note, in the context of the amino-acid sequence, asparagine 1 is part of a potential N-glycosylation sequence-motive.

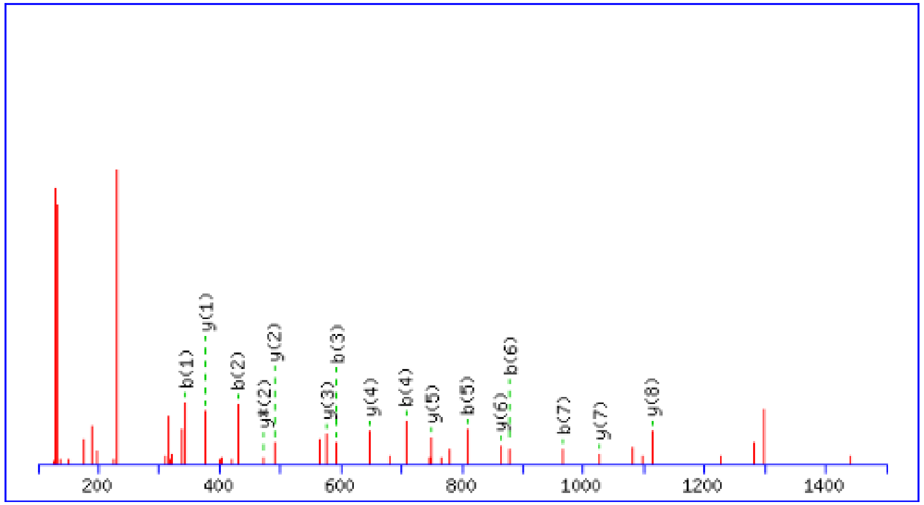
a LSYNTASNK (Rpl34) + HexNAc2.Hex12



Monoisotopic mass of neutral peptide Mr(calc): 1428.72990
Fixed modifications: Methylthio (C)
Variable modifications:
N8 : HexNAc(NL) (NST), with neutral losses 203.07937(shown in table), 0.00000
K9 : TMT6plex (K)
Ions Score: 50 **Expect:** 0.0046
Matches (**Bold Red**)**:** 22/65 fragment ions using 40 most intense peaks

#	b	b*	b^0	Seq.	y	y*	y^0	#
1	114.09134			L				9
2	201.12337		183.11280	S	**1113.57377**	1096.54722	1095.56321	8
3	364.18670		**346.17613**	Y	**1026.54174**	**1009.51520**	1008.53118	7
4	478.22962	461.20308	460.21906	N	**863.47841**	**846.45187**	845.46785	6
5	579.27730	562.25075	**561.26674**	T	**749.43549**	732.40894	**731.42492**	5
6	650.31442	633.28787	632.30385	A	**648.38781**	**631.36126**	630.37724	4
7	737.34645	720.31990	719.33588	S	**577.35070**	**560.32415**	559.34013	3
8	851.38937	834.36283	833.37881	N	**490.31867**	**473.29212**		2
9				K	**376.27574**	359.24919		1

b LSYNTASNK (Rpl34), deamidated



Monoisotopic mass of neutral peptide Mr(calc): 1454.81346
Fixed modifications: Methylthio (C),TMT6plex (N-term)
Variable modifications:
K9 : TMT6plex (K)
Ions Score: 43 **Expect:** 0.0042
Matches (**Bold Red**)**:** 16/42 fragment ions using 34 most intense peaks

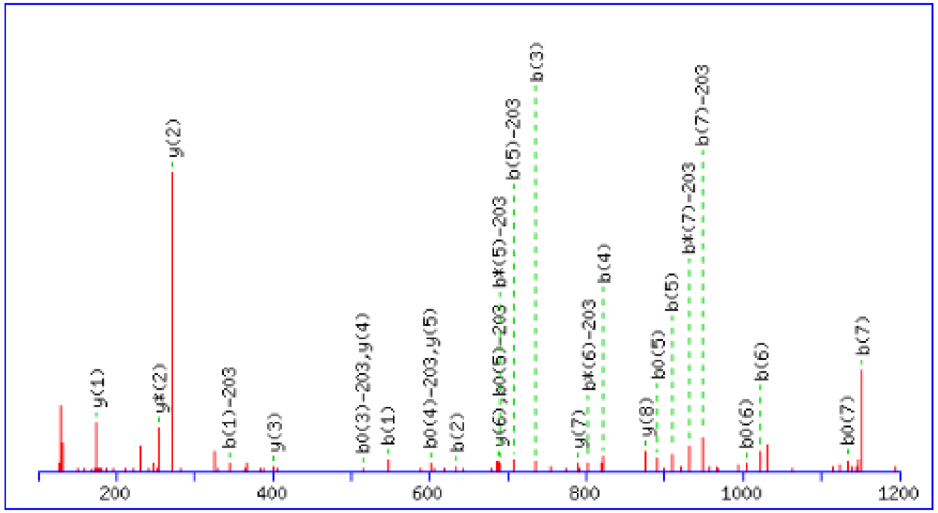
#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**343.25427**			L				9
2	**430.28630**		412.27573	S	**1113.57377**	1096.54722	1095.56320	8
3	**593.34963**		575.33906	Y	**1026.54174**	1009.51519	1008.53118	7
4	**707.39256**	690.36601	689.38199	N	**863.47841**	846.45186	845.46785	6
5	**808.44024**	791.41369	790.42967	T	**749.43548**	732.40894	731.42492	5
6	**879.47735**	862.45080	861.46678	A	**648.38781**	631.36126	630.37724	4
7	**966.50938**	949.48283	948.49881	S	**577.35069**	560.32414	559.34013	3
8	1080.55230	1063.52576	1062.54174	N	**490.31866**	**473.29211**		2
9				K	**376.27574**	359.24919		1

Supplementary Figure 6.

Supplementary Figure 6. Identification of the 60S ribosomal protein L34 (Rpl34) as N-glycoprotein.

(**a**) N-Glycopeptide LSYNTASNK (Rpl34) with the glycan composition HexNAc2.Hex12 was identified with high confidence via MS/MS. (**b**) MS/MS data analysis of the corresponding PNGaseF-treated HILIC fraction identified the non-glycosylated peptide LSYNTASNK bearing the enzymatically induced deamidation of formerly N-glycosylated asparagine residues at position 8. Of note, in the context of the amino-acid sequence, asparagine 8 is part of a potential N-glycosylation sequence-motive.

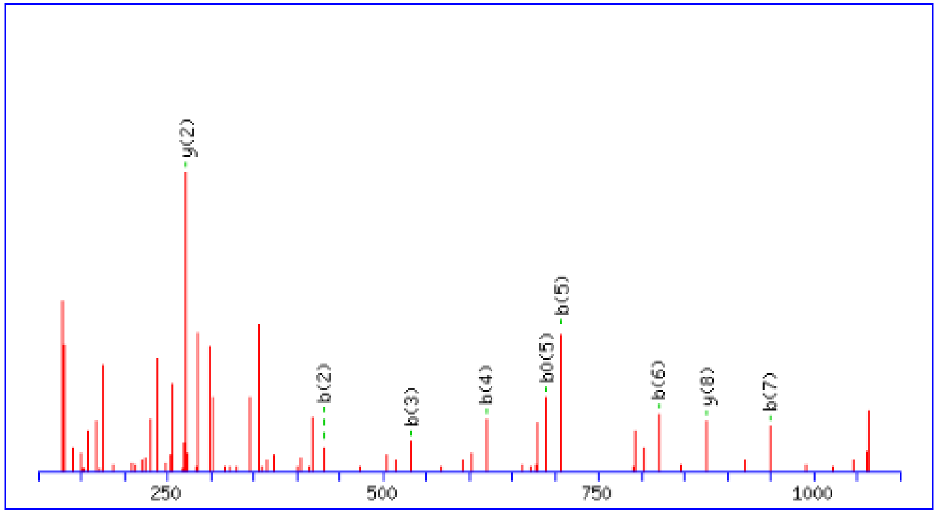
a NSTSSIEPR (Map4k4) + HexNAc2.Hex12



```
Monoisotopic mass of neutral peptide Mr(calc): 1421.72008
Fixed modifications: Methylthio (C)
Variable modifications:
N-term : TMT6plex (N-term)
N1     : HexNAc(NL) (NST), with neutral losses 203.07937(shown in table), 0.00000
Ions Score: 41  Expect: 0.051
Matches (Bold Red): 28/68 fragment ions using 52 most intense peaks
```

#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**344.21314**	327.18659		N				9
2	431.24517	414.21862	413.23460	S	**876.44214**	859.41559	858.43157	8
3	532.29285	515.26630	**514.28228**	T	**789.41011**	772.38356	771.39954	7
4	619.32487	602.29832	**601.31431**	S	**688.36243**	671.33588	670.35186	6
5	**706.35690**	**689.33035**	**688.34634**	S	**601.33040**	584.30385	583.31984	5
6	819.44097	**802.41442**	801.43040	I	**514.29837**	497.27182	496.28781	4
7	**948.48356**	**931.45701**	930.47299	E	**401.21431**	384.18776	383.20374	3
8	1045.53632	1028.50977	1027.52576	P	**272.17172**	**255.14517**		2
9				R	**175.11895**	158.09240		1

b NSTSSIEPR (Map4k4), deamidated



```
Monoisotopic mass of neutral peptide Mr(calc): 1219.62471
Fixed modifications: Methylthio (C),TMT6plex (N-term)
Variable modifications:
N1     : Deamidated (N)
Ions Score: 34  Expect: 0.02
Matches (Bold Red): 9/45 fragment ions using 13 most intense peaks
```

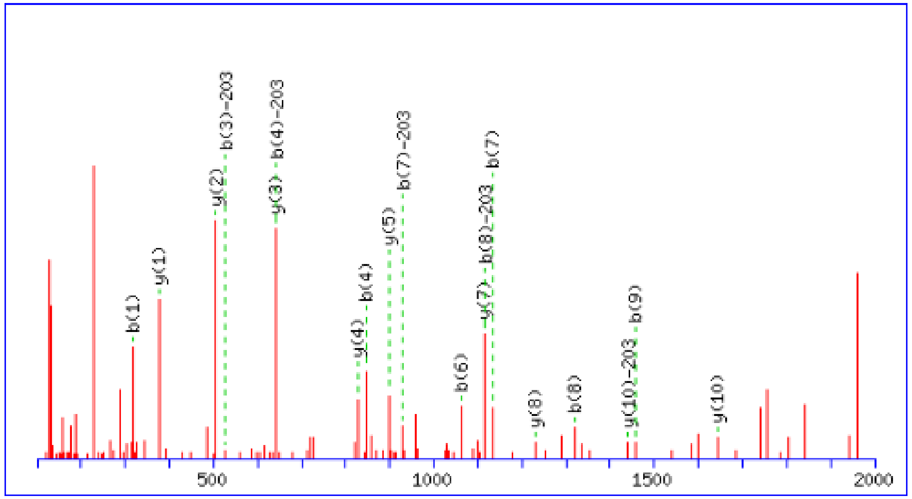
#	b	b*	b^0	Seq.	y	y*	y^0	#
1	345.19714	328.17059		N				9
2	**432.22917**	415.20262	414.21861	S	**876.44214**	859.41559	858.43157	8
3	**533.27685**	516.25030	515.26629	T	789.41011	772.38356	771.39954	7
4	**620.30888**	603.28233	602.29831	S	688.36243	671.33588	670.35186	6
5	**707.34091**	690.31436	**689.33034**	S	601.33040	584.30385	583.31984	5
6	**820.42497**	803.39842	802.41441	I	514.29837	497.27182	496.28781	4
7	**949.46756**	932.44101	931.45700	E	401.21431	384.18776	383.20374	3
8	1046.52033	1029.49378	1028.50976	P	**272.17172**	255.14517		2
9				R	175.11895	158.09240		1

Supplementary Figure 7.

Supplementary Figure 7. Identification of Mitogen-activated protein kinase kinase kinase kinase 4 (M4K4) as N-glycoprotein.

(**a**) The N-Glycopeptide NSTSSIEPR (M4K4) with the glycan composition HexNAc2.Hex12 was identified with high confidence via MS/MS. (**b**) Analysis of the corresponding PNGaseF-treated HILIC fraction identified the non-glycosylated peptide NSTSSIEPR bearing the enzymatically induced deamidation of formerly N-glycosylated asparagine residues at position 1. Of note, in the context of the amino-acid sequence, asparagine 1 is part of a potential N-glycosylation sequence-motive.

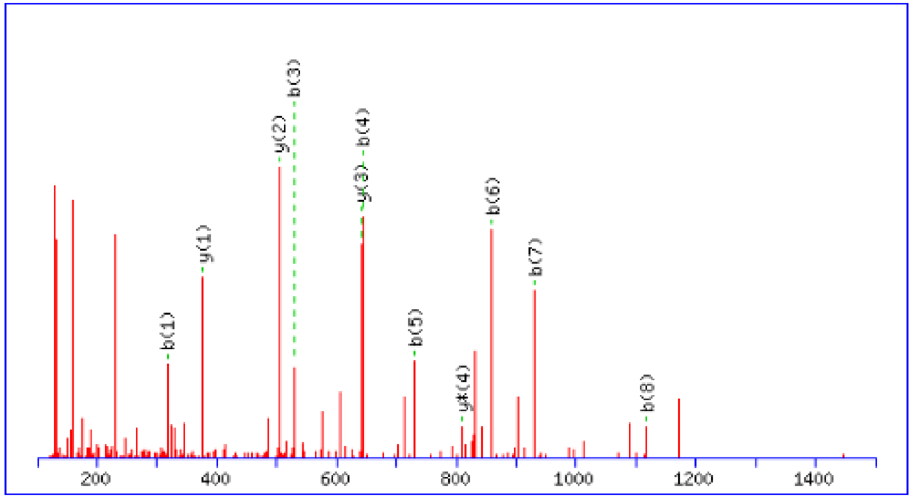
a SPNDSEAWHQK (Pbxip1) + HexNAc2.Hex8



```
Monoisotopic mass of neutral peptide Mr(calc): 1958.97395
Fixed modifications: Methylthio (C)
Variable modifications:
N-term : TMT6plex (N-term)
N3     : HexNAc(NL) (NST), with neutral losses 203.07937(shown in table), 0.00000
K11    : TMT6plex (K)
Ions Score: 31  Expect: 0.87
Matches (Bold Red): 19/83 fragment ions using 50 most intense peaks
```

#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**317.20224**		299.19167	S				11
2	414.25500		396.24444	P	**1440.70691**	1423.68036	1422.69635	10
3	**528.29793**	511.27138	510.28737	N	1343.65415	1326.62760	1325.64358	9
4	**643.32487**	626.29832	625.31431	D	**1229.61122**	1212.58467	1211.60065	8
5	730.35690	713.33035	712.34634	S	**1114.58427**	1097.55773	1096.57371	7
6	859.39950	842.37295	841.38893	E	1027.55225	1010.52570	1009.54168	6
7	**930.43661**	913.41006	912.42604	A	**898.50965**	881.48311		5
8	**1116.51592**	1099.48937	1098.50536	W	**827.47254**	810.44599		4
9	1253.57483	1236.54829	1235.56427	H	**641.39323**	624.36668		3
10	1381.63341	1364.60686	1363.62285	Q	**504.33431**	487.30777		2
11				K	**376.27574**	359.24919		1

b SPNDSEAWHQK (Pbxip1), deamidated



```
Monoisotopic mass of neutral peptide Mr(calc): 1756.87859
Fixed modifications: Methylthio (C),TMT6plex (N-term)
Variable modifications:
N3     : Deamidated (N)
K11    : TMT6plex (K)
Ions Score: 37  Expect: 0.014
Matches (Bold Red): 11/53 fragment ions using 22 most intense peaks
```

#	b	b*	b^0	Seq.	y	y*	y^0	#
1	**317.20224**		299.19167	S				11
2	414.25500		396.24443	P	1441.69092	1424.66437	1423.68035	10
3	**529.28194**	512.25539	511.27137	N	1344.63815	1327.61161	1326.62759	9
4	**644.30888**	627.28233	626.29831	D	1229.61122	1212.58467	1211.60065	8
5	**731.34091**	714.31436	713.33034	S	1114.58427	1097.55773	1096.57371	7
6	**860.38350**	843.35695	842.37293	E	1027.55225	1010.52570	1009.54168	6
7	**931.42061**	914.39406	913.41005	A	898.50965	881.48311		5
8	**1117.49993**	1100.47338	1099.48936	W	827.47254	**810.44599**		4
9	1254.55884	1237.53229	1236.54827	H	**641.39323**	624.36668		3
10	1382.61742	1365.59087	1364.60685	Q	**504.33431**	487.30777		2
11				K	**376.27574**	359.24919		1

Supplementary Figure 8.

Supplementary Figure 8. Identification of pre-B-cell leukemia transcription factor-interacting protein 1 (Pbxip1) as N-glycoprotein.

(**a**) N-Glycopeptide SPNDSEAWHQK (Pbxip1) with the glycan composition HexNAc2.Hex8 was identified with high confidence via MS/MS. (**b**) MS/MS data analysis of the corresponding PNGaseF-treated HILIC fraction identified the non-glycosylated peptide SPNDSEAWHQK bearing the enzymatically induced deamidation of formerly N-glycosylated asparagine residues at position 3. Of note, asparagine 3 is part of a potential N-glycosylation sequence-motive.