Supporting Information:

Precision Mapping of O-Linked N-Acetylglucosamine Sites in Proteins using Ultraviolet Photodissociation Mass Spectrometry

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Section S1 Methods

Preparation of O-GlcNAc modified peptides

Glycopeptides used in this study were generated chemoenzymatically using recombinantly-expressed O-GlcNAc Transferase (OGT). All unmodified peptides were obtained commercially (Biomatik Corporation, Cambridge, ON). Generally, 1-2 mg oligopeptide at a final concentration of 1 mM were incubated with UDP-GlcNAc (5 mM), OGT (1 µM, prepared as previously described[1]) and 2U shrimp alkaline phosphatase (New England Biolabs, Whitby, ON) in PBS at pH 7.2 containing 12.5 mM $MgCl_2$. Reactions were incubated at 37 °C for 4 h or overnight. Prior to purification, the reactions were terminated by heating at 95 °C for 10 min and then centrifuged at 13,000 x *g* for 2 minutes. The supernatants were recovered and were purified on an Agilent 1200 series HPLC equipped with an Agilent XDB-C18 Eclipse reversed-phase column (9.4 × 250 mm, 5 µ particle size). Glycopeptides were eluted using a mobile phase consisting of H_2O and CH_3CN with 0.1% trifluoroacetic acid over a gradient of 10 to 50% acetonitrile as appropriate. Fractions containing the product were lyophilized to yield up to 1 mg glycopeptide as white powders. All glycopeptides were purified to >95% purity and were analyzed by high-resolution mass spectrometry performed using a Bruker maXis Impact UltraHigh-Resolution Quadrupole

Time-of-Flight (UHR-QTOF) mass spectrometer (mobile phase water:acetonitrile 1:1 + 0.1% formic acid, flow rate of 0.3 ml/min). Ions were sprayed in positive mode with a voltage of 4200 V. Nitrogen drying gas was 180 °C at a flow rate of 8 L/min. Data was acquired over a mass range of 300 - 2500 atomic mass units.

TAB1-O-GlcNAc: Biotin-NH-PVSVPY**S(GlcNAc)**SAQSTS-OH (**X**):

Prepared according to above method. Yield 1.2 mg (55%), mobile phase gradient 20 – 25% CH_3CN over 20 min, 2 mL/min flow rate, RT = 15.3 min, HRMS (ESI^+) Calculated 869.8956, found 869.8870 $[M+2H]^{2+}$.

CKII-O-GlcNAc: NH_2-YPGGSTPV**S(GlcNAc)**SANMM-OH (**X**):

Prepared according to general method. Yield 0.85 mg (68%), mobile phase gradient 20 – 30% CH_3CN over 20 min, 2 mL/min flow rate, RT = 9.6 min, LRMS (ESI^+) Calculated 801.34, found 801.36 $[M+2H]^{2+}$.

HCF-1-O-GlcNAc: NH_2-YVRVCSNPPC**S(GlcNAc)**THETGTTNTATTATS-OH (**X**):

Prepared according to general method. Yield 0.5 mg (42%), mobile phase gradient 15-30% % CH_3CN over 30 min, 2 mL/min flow rate, RT = 14.2 min, HRMS (ESI^+) Calculated 968.0991, found 968.0948 $[M+3H]^{3+}$.

<u>Annotation and Labeling of UVPD mass spectra</u>:

Several of the characteristic fragment ions produced by UVPD, including *a, x, y* and *z* ions, display contributions from both even and odd electron forms, resulting in mass shifts of <u>+</u>1.0078 Da corresponding to one hydrogen atom. The high density, information-rich nature of the UVPD mass spectra precludes inclusion of these hydrogen shifts in the ion labels, but they are accounted for in the supporting tables.

Section S2 Supplementary Figures

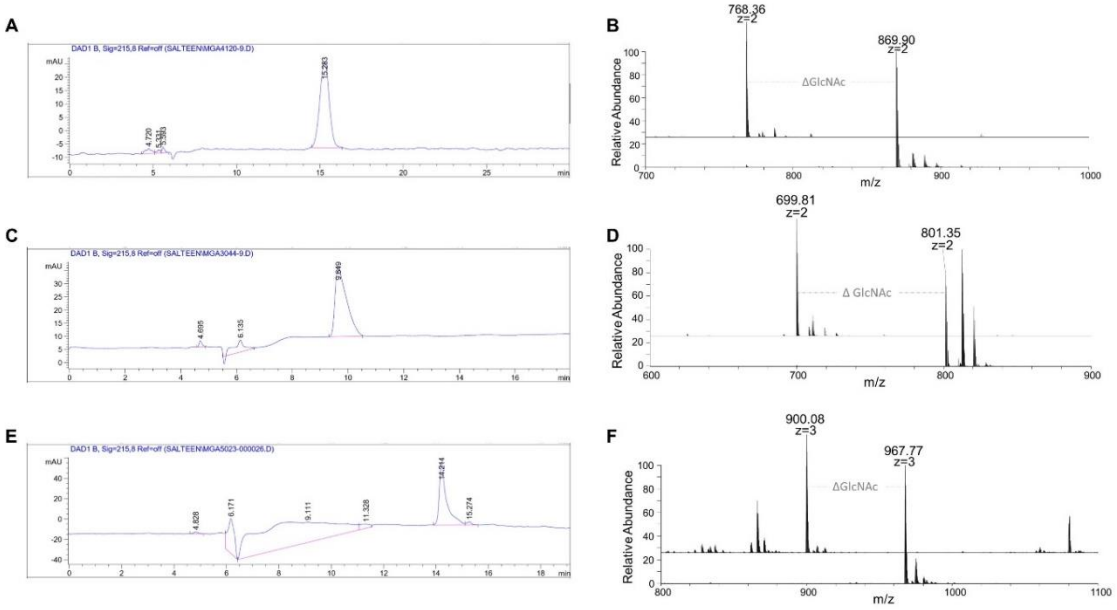

Figure S1: HPLC and MS1 spectra for O-GlcNAcylated peptides. A, C, and E: HPLC traces for O-GlcNAcylated peptides A) TAB-1, C) CKII, and E) HCF-1 peptides. HPLC parameters for each peptide are outlined in the above methods section. B, D, and F: Overlay of MS^1 spectra of the unmodified and O-GlcNAc modified forms of (**A**) TAB1, (**B**) CKII, and (**C**) HCF-1 peptides. The mass difference between the pairs of featured ions corresponds to GlcNAc.

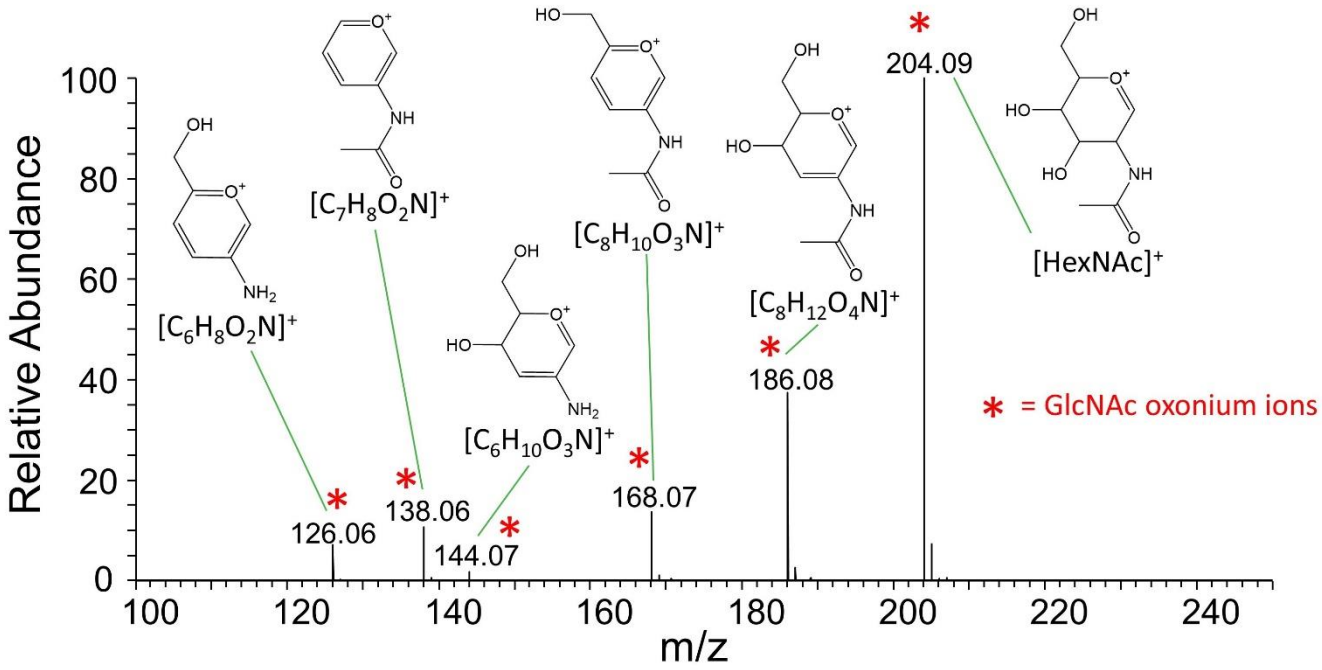

Figure S2: HCD-produced oxocarbenium ions from TAB1-O-GlcNAc, all arising from cleavage of the glycosidic bond.

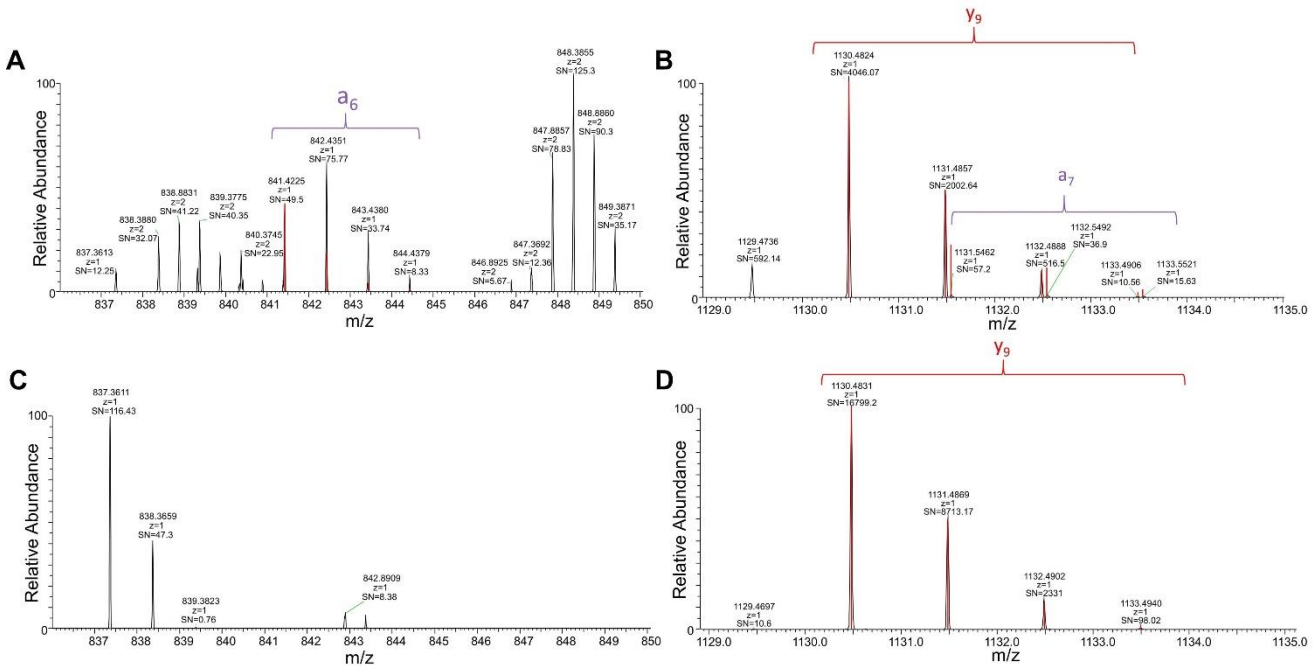

Figure S3: Expansions of MS/MS spectra for the TAB1-O-GlcNAc glycopeptide (biotin-PVSVPYS$_{(g)}$SAQSTS, 2+, *m/z* 869.90) corresponding to the spectra in **Figure 2A,B**. (**A**) Expansion of *m/z* 836-850 (UVPD) showing fragment ion a_6. (**B**) Expansion of *m/z* 1129-1135 (UVPD) showing the fragment ion assignment of a_7 resolved from the adjacent y_9 ion. (**C**) Expansion of *m/z* 836-850 (HCD) showing absence of a_6 fragment ion, (**D**) Expansion of *m/z* 1129-1135 (HCD) showing a y_9 fragment ion with no a_7 ion.

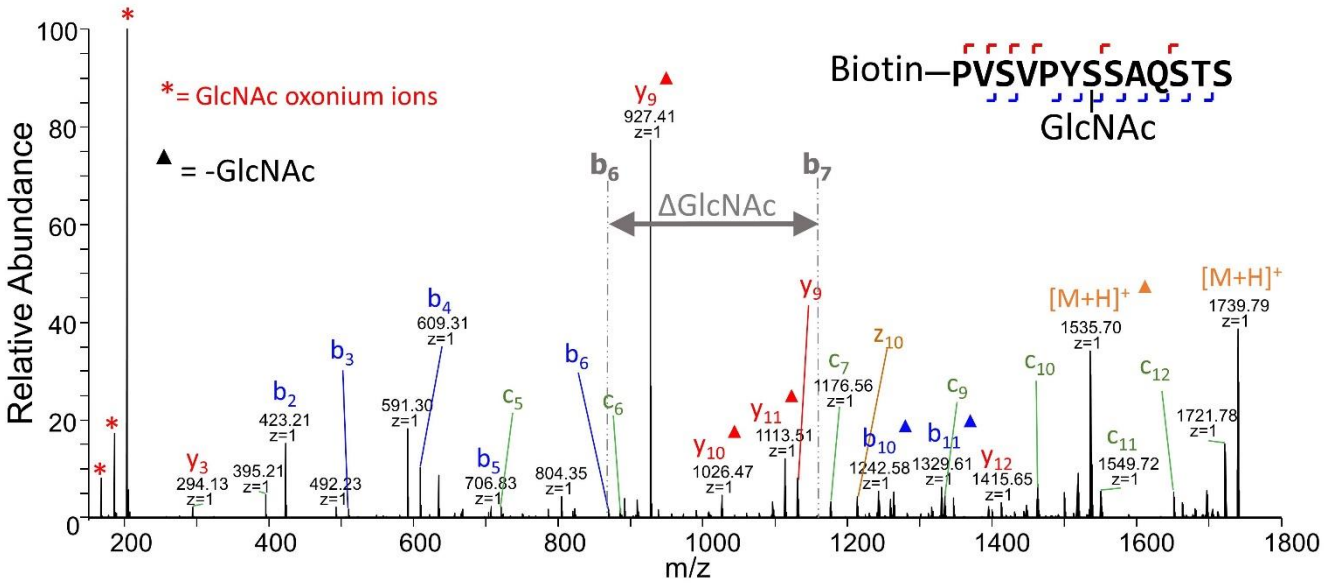

Figure S4: HCD oxocarbenium ion triggered EThcD mass spectrum of TAB1-O-GlcNAc. EThcD was run with a 50 ms activation time and 33% supplemental energy, generating the spectrum above for the TAB1-O-GlcNAc peptide (Biotin-PVSVPYS$_{(g)}$SAQSTS, 2+, *m/z* 869.90)); PCS score: 3.1e-36. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S4**.

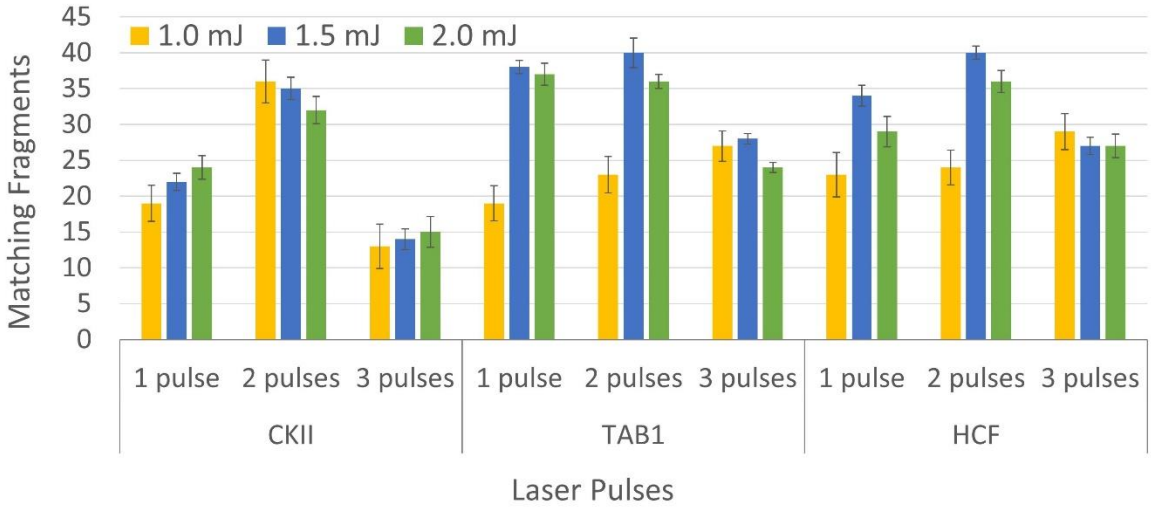

Figure S5: Optimization of pulse number and laser energy for 193 nm UVPD based on the number of assigned fragment ions. Experiments were performed using CKII-O-GlcNAc (YPGGSTPVS(g)SANMM; 2+, *m/z* 801.30), TAB1-O-GlcNAc (biotin-PVSVPYS(g)SAQSTS; 2+, *m/z* 869.9), and HCF-O-GlcNAc (YVRVCSNPPCS(g)THETGTTNTATTATS, 3+, *m/z* 967.76). Error bars correspond to standard deviations from technical triplicates.

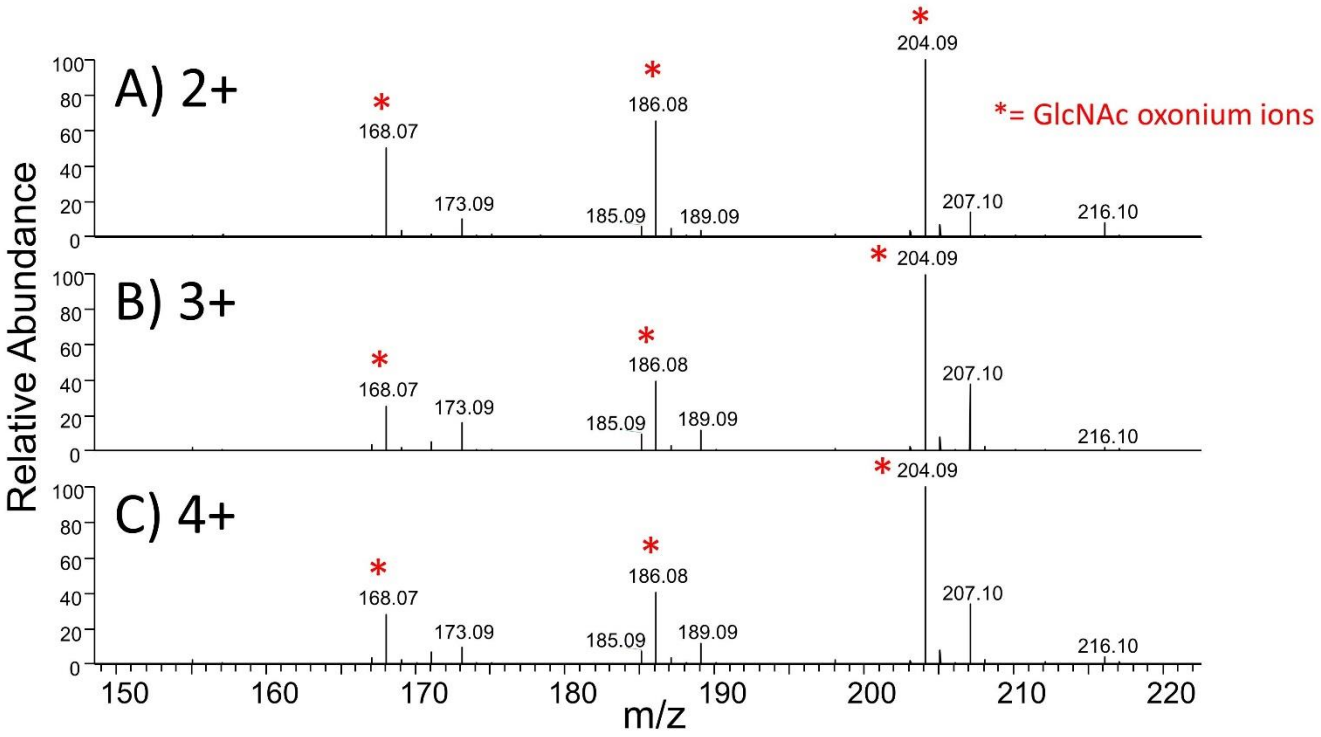

Figure S6: HCD induces robust production of oxocarbenium ions across multiple precursor charge states upon activation of the HCF-1-O-GlcNAc peptide. Spectra in A-C represent the oxocarbenium ions produced upon HCD of 2+, 3+, and 4+ charge states of the peptide, respectively. Fragmentation of the HCF-1-O-GlcNAc peptide (YVRVCSNPPCS(g)THETGTTNTATTATS) was induced using a normalized collision energy of 28.

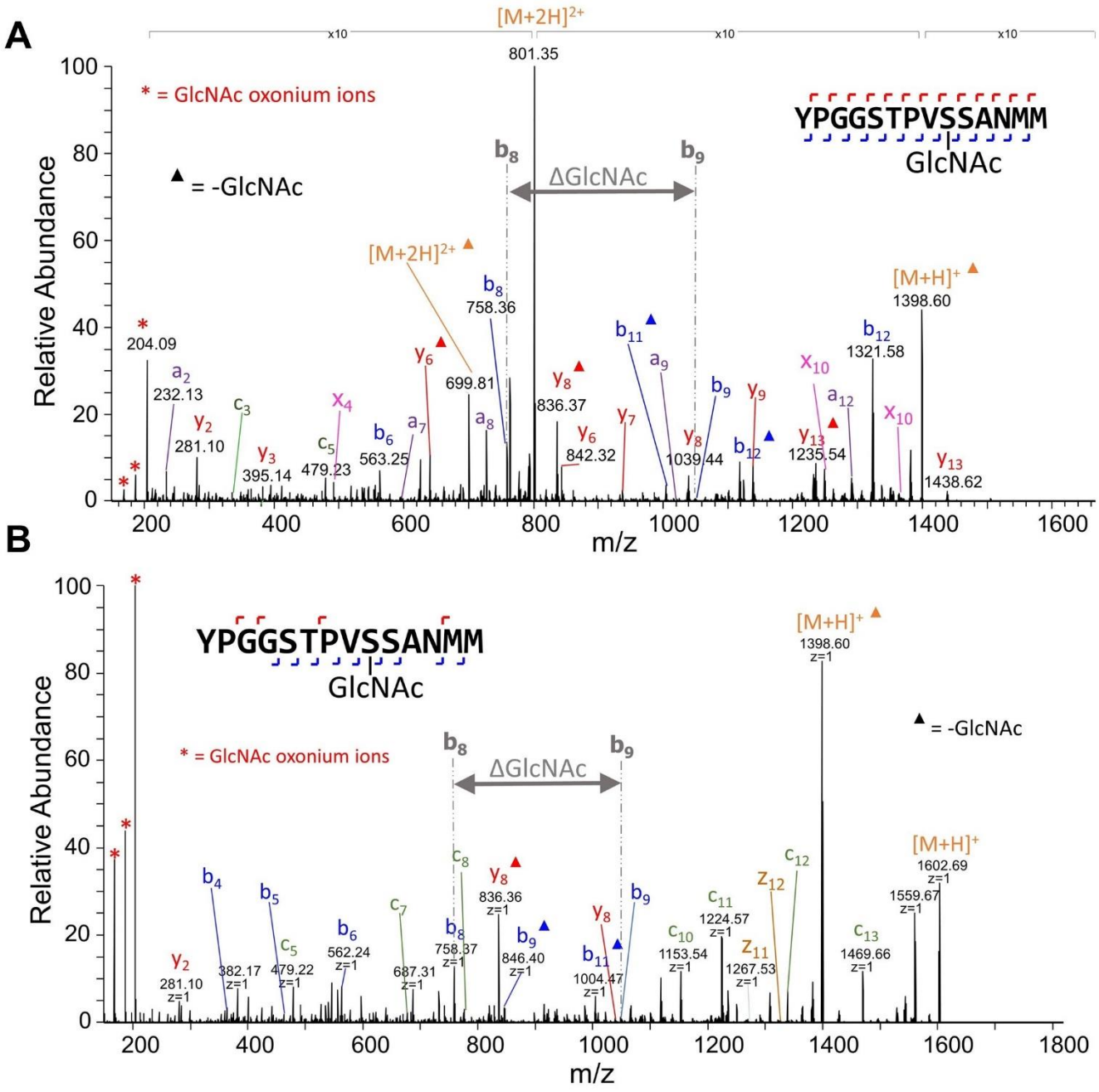

Figure S7: MS/MS comparison of CKII-O-GlcNAc standard glycopeptide. The double-headed arrows and ΔGlcNAc indicate the mass shifts representing loss of the O-GlcNAc-modified amino acid between fragment ions (labelled in grey at each end of the double headed arrow) that bracket the site of the modification. (**A**) 193 nm UVPD mass spectrum of CKII-O-GlcNAc peptide. Fragmentation of the CKII-O-GlcNAc peptide (YPGGSTPVSSANMM, 2+, m/z 801.3) was induced using two laser pulses at 1.5 mJ laser energy per pulse. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S6**. (**B**) HCD oxocarbenium ion triggered EThcD mass spectrum of CKII-O-GlcNAc. EThcD was run with a 50 ms activation time and 25% supplemental energy, generating the spectrum above for the CKII-O-GlcNAc peptide (YPGGSTPVS(g)SANMM; 2+, *m/z* 801.30). A sequence map is shown in the upper left, and the complete list of identified fragment ions is shown in **Table S8**.

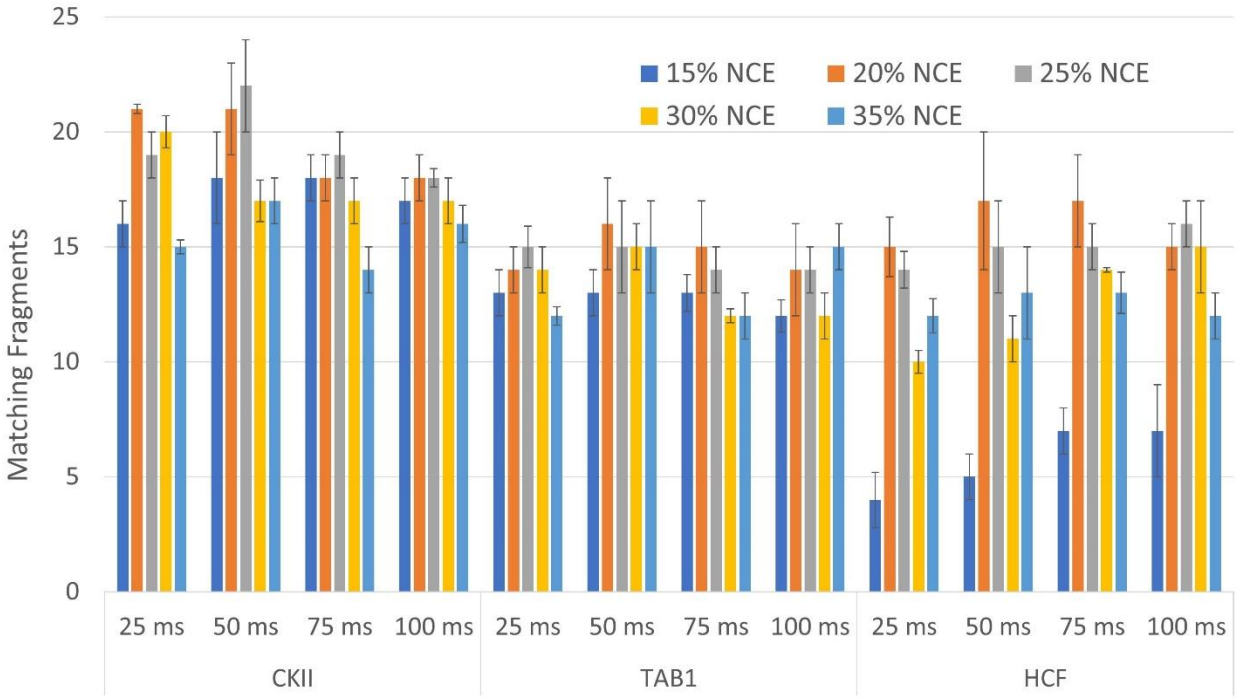

Figure S8: Optimization of electron activation period and supplemental collision energy for EThcD based on the number of assigned fragment ions. Experiments were performed using CKII-O-GlcNAc (YPGGSTPVS(g)SANMM; 2+, *m/z* 801.30), TAB1-O-GlcNAc (biotin-PVSVPYS(g)SAQSTS; 2+, *m/z* 869.9), and HCF-O-GlcNAc (YVRVCSNPPCS(g)THETGTTNTATTATS, 3+, *m/z* 967.76). Error bars correspond to standard deviations from technical triplicates.

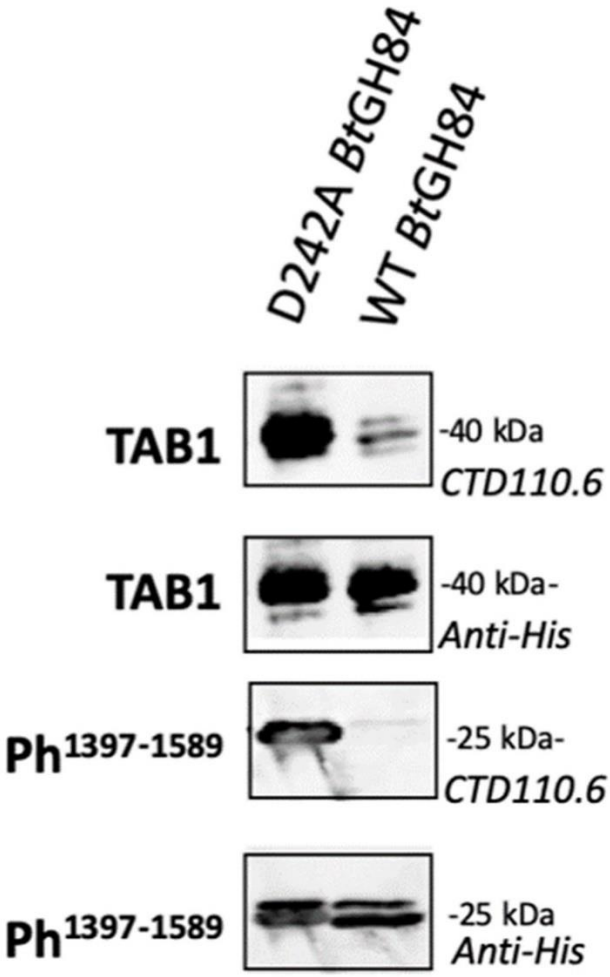

Figure S9: Anti-O-GlcNAc Immunoblots confirm O-GlcNAcylation of TAB-1 and Ph1397-1589. Recombinant protein (10μM) was incubated in reaction buffer (20mM sodium phosphate pH 7.2, 150 mM NaCl) in the presence of either 1 μM WT or D242A BtGH84 overnight. Following reaction, 0.25 μg total protein was loaded per well for immunoblotting.

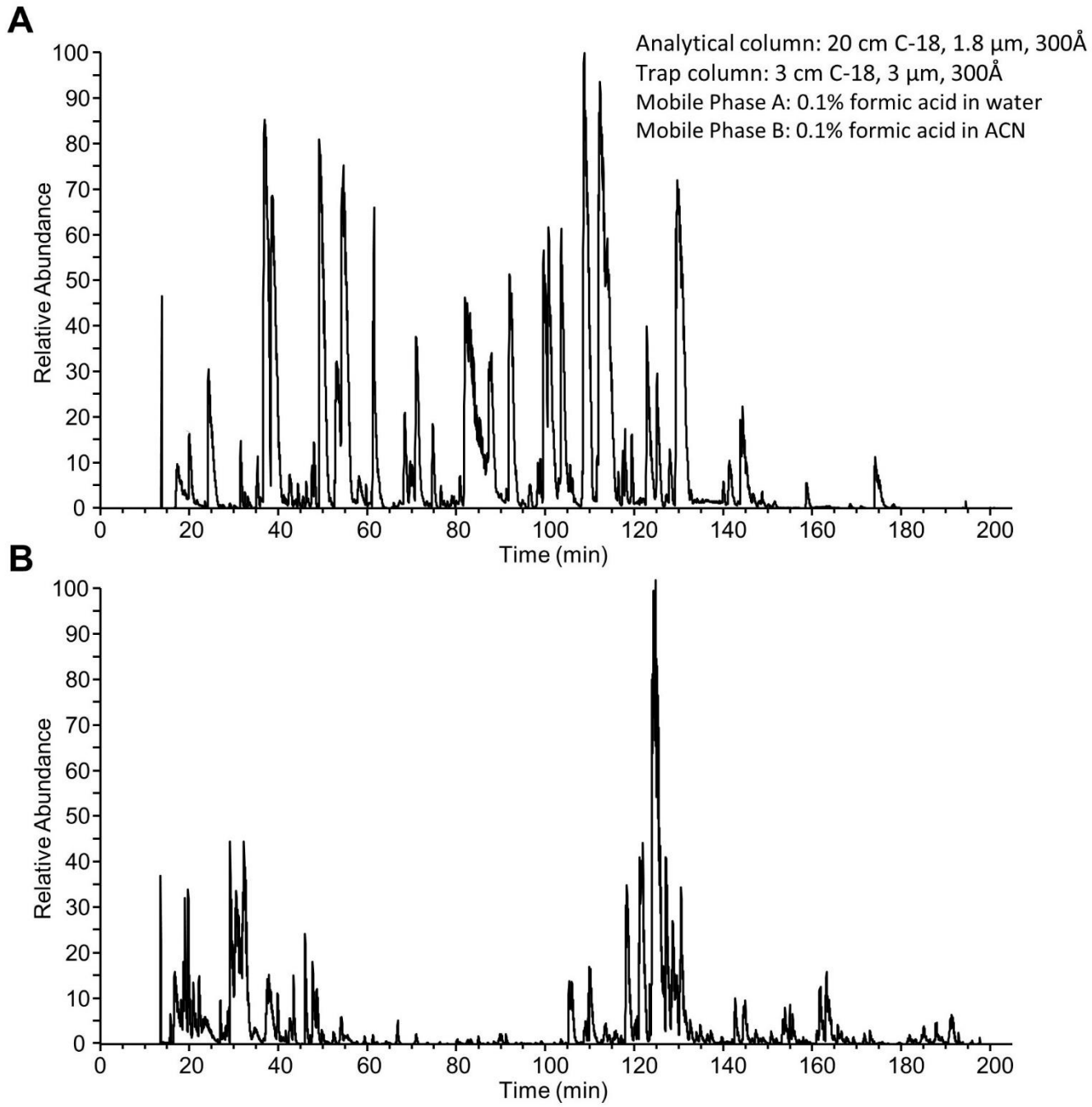

Figure S10: Typical liquid chromatographic traces of a (**A**) trypsin digest of TAB1-O-GlcNAc modified protein, and (**B**) trypsin digest of $Ph^{1397-1589}$-O-GlcNAc modified protein.

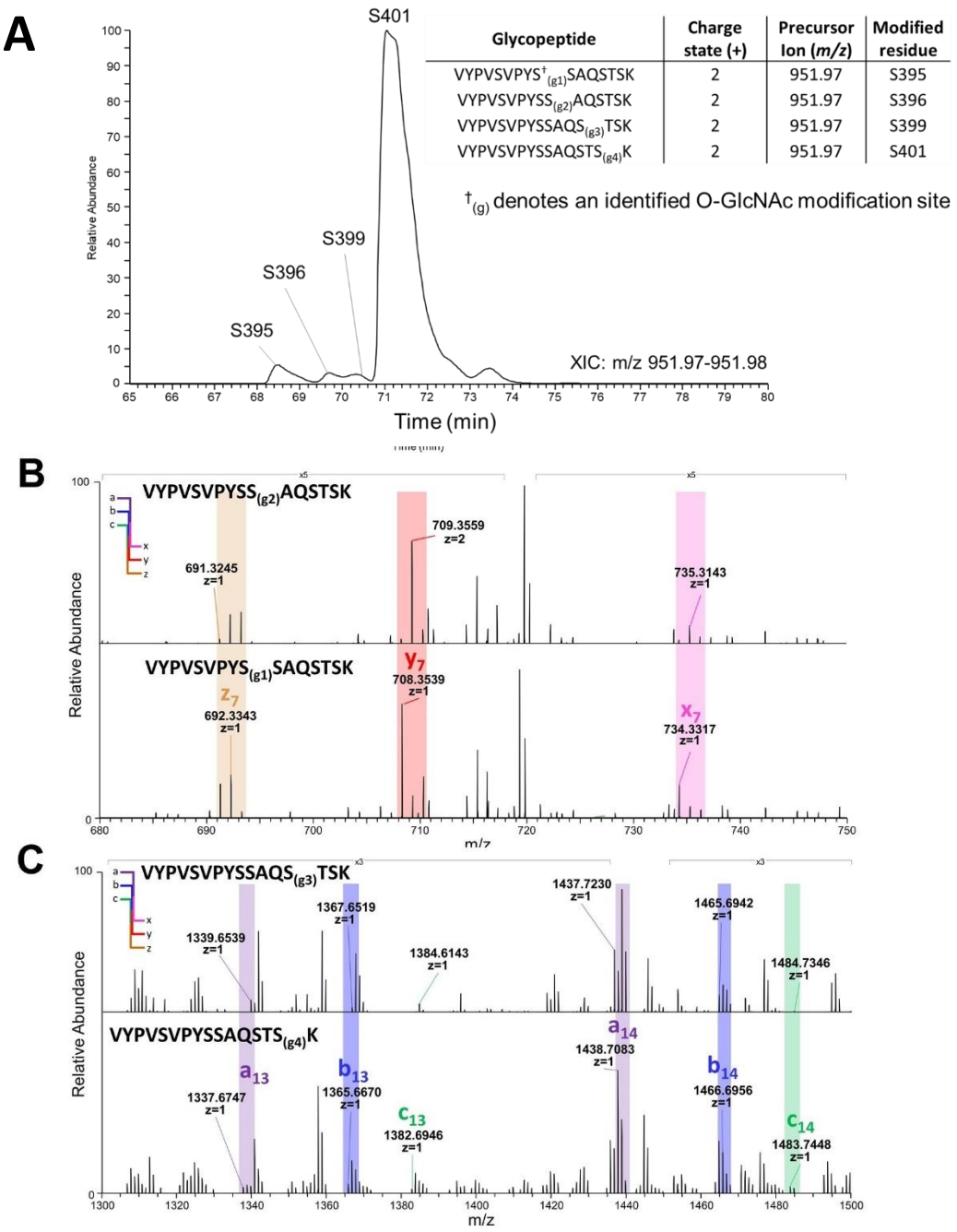

Figure S11: LC-MS/MS analysis of TAB1-O- GlcNAc protein VYPVSVPS395(g1)S396(g2)AQS399(g3)TS401(g4)K glycopeptide. (**A**) Extracted ion chromatogram of *m/z* 951.97-951.98 from LC-MS of the tryptic digest of TAB1-O-GlcNAc modified protein showing each confidently identified positional isomer of the VYPVSVPS395($_{g1}$)S396($_{g2}$)AQS399($_{g3}$)TS401($_{g4}$)K glycopeptide. (**B**) Comparison of expanded *m/z* regions of the UVPD spectra obtained for the S395 and S396 glycopeptide isomers showing distinctive C-terminal fragment ions differentiating the two isomers. The unmodified x_7, y_7, and z_7 fragment ions were found only for the S396 glycoisomer and not for the S395 glycoisomer. (**C**) Comparison of expanded *m/z* regions of the UVPD spectra obtained for the S399 and S401 glycopeptide isomers showing distinctive N-terminal fragment ions differentiating the two isomers. The unmodified a_{13}, a_{14}, b_{13}, b_{14}, c_{13}, and c_{14} fragment ions were found only for the S401 glycoisomer and not for the S399 glycoisomer.

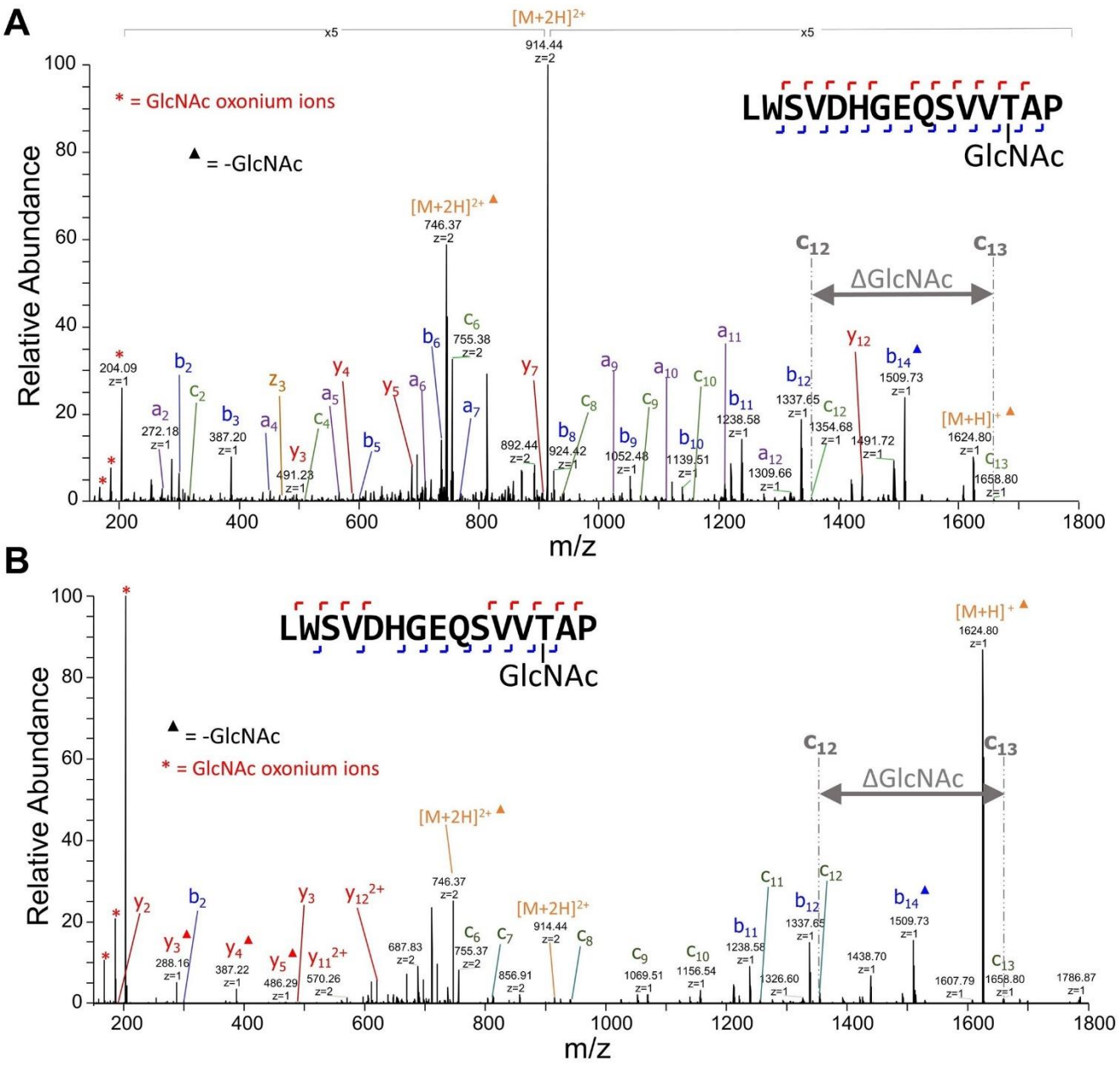

Figure S12: MS/MS comparison of LWSVDHGEQSVVT[+203]AP glycopeptide. The double-headed arrows and ΔGlcNAc indicate the mass shifts representing loss of the O-GlcNAc-modified amino acid between fragment ions (labelled in grey at each end of the double headed arrow) that bracket the site of the modification. (**A**) HCD oxocarbenium ion-triggered 193 nm UVPD mass spectrum of glycosylated peptide LWSVDHGEQSVVT[+203]AP (2+, m/z 914.43) using two pulses of 1.5 mJ. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S13**. (**B**) HCD oxocarbenium ion-triggered EThcD mass spectrum of glycosylated peptide LWSVDHGEQSVVT[+203]AP (2+, *m/z* 914.43) using 50 ms activation time and 25% supplemental energy. A sequence map is shown in the upper right and the complete list of identified fragment ions is shown in **Table S19**.

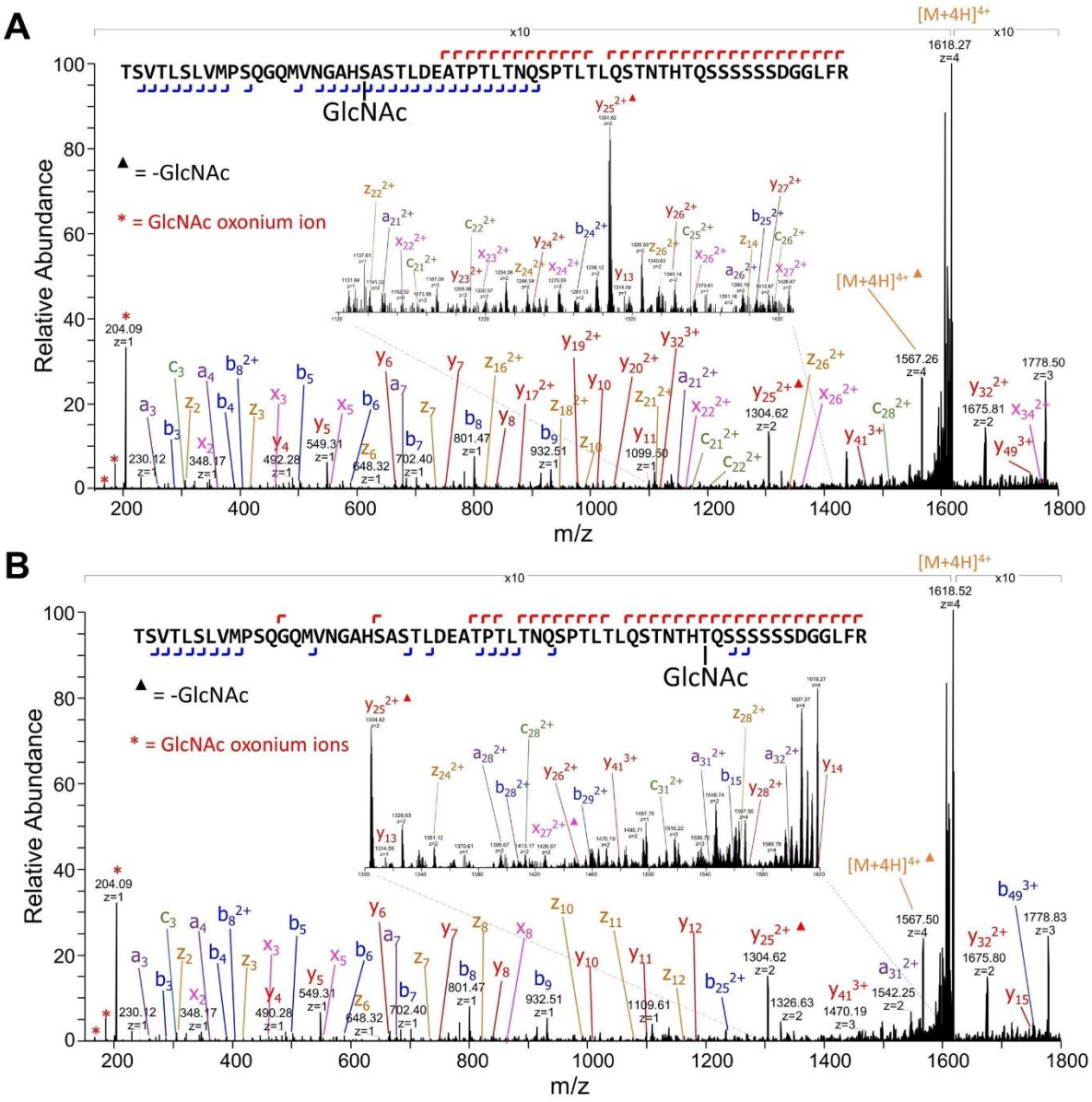

Figure S13: UVPD analysis of TSVTLSLVMPSQGQMVNGAHSASTLDEATPTLTNQSPTLTLQSTNTHTQSSSSSSDGGLFR glycopeptide. (**A**) HCD oxocarbenium ion-triggered UVPD mass spectra of an O-GlcNAc-modified isoform TSVTLSLVMPSQGQMVNGAHS[+203]ASTLDEATPTLTNQSPTLTLQSTNTHTQSSSSSSDGGLFR (4+, *m/z* 1617.52) using two pulses of 1.5 mJ. A sequence map is shown above the spectrum. The inset shows an expansion of the region from *m/z* 1100-1400 to illustrate the level of fragmentation detail that prevents every fragment ion from being labelled in the UVPD spectra. The complete list of identified fragment ions is shown in **Table S14**. (**B**) HCD oxocarbenium ion-triggered UVPD mass spectra of of TSVTLSLVMPSQGQMVNGAHSASTLDEATPTLTNQSPTLTLQSTNTHT[+203]QSSSSSSDGGLFR (4+, *m/z* 1617.52) using two pulses of 1.5 mJ. A sequence map is shown above the spectrum, and the complete list of identified fragment ions is shown in **Table S15**.

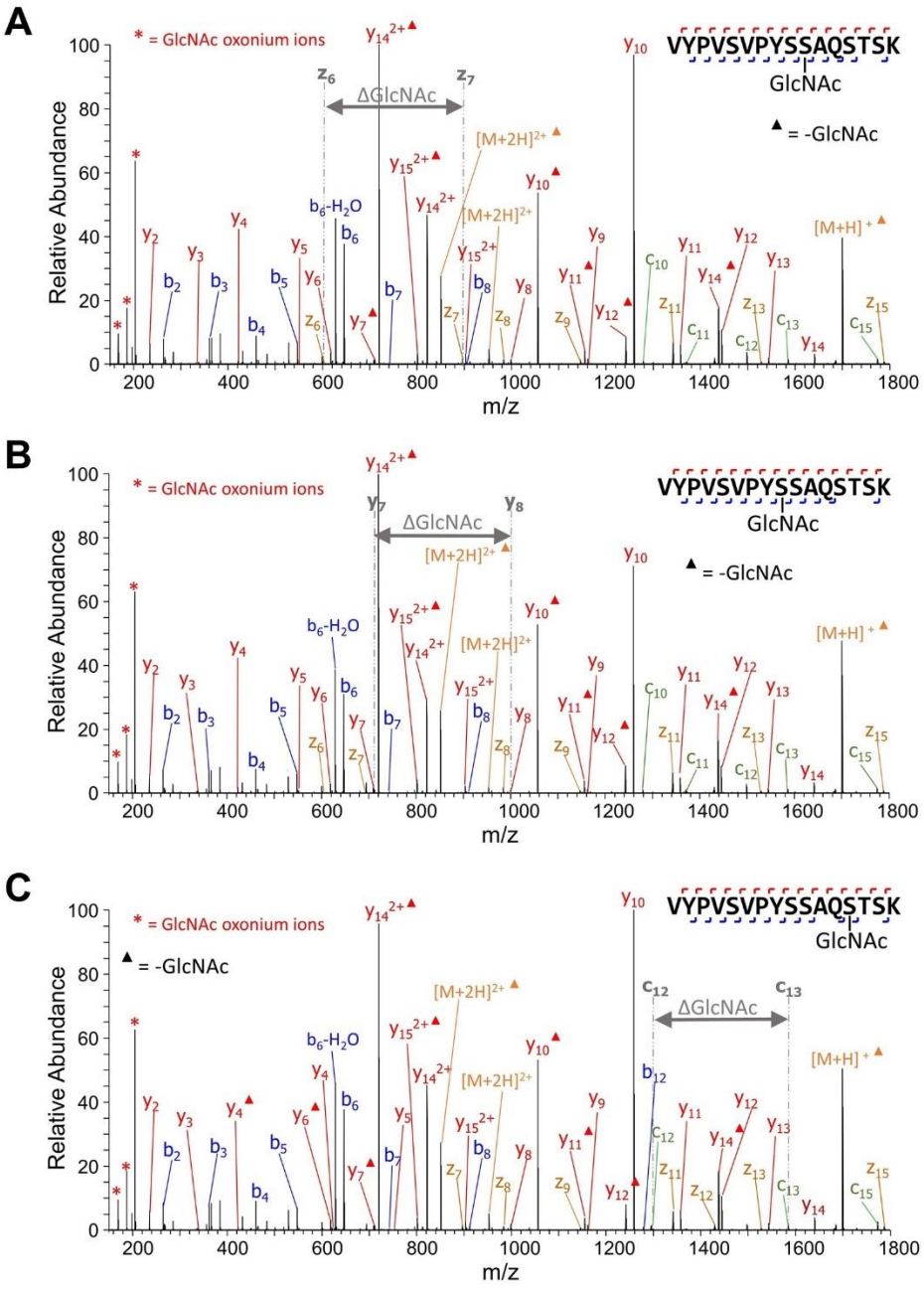

Figure S14: EThcD analysis of VYPVSVPS395(g1)S396(g2)AQS399(g3)TSK glycopeptide using 50 ms activation time and 25% supplemental energy. (**A**) HCD oxocarbenium ion-triggered EThcD mass spectrum of glycosylated peptide VYPVSVPYSS[+203]AQSTSK (2+, m/z 951.97) from TAB-1-O-GlcNAc tryptic. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S16**. (**B**) HCD oxocarbenium ion-triggered EThcD mass spectrum of glycosylated peptide VYPVSVPYS[+203]SAQSTSK (2+, m/z 951.97). A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S17**. (**C**) HCD oxocarbenium ion-triggered EThcD mass spectrum of glycosylated peptide VYPVSVPYSSAQS[+203]TSK (2+, m/z 951.97) from TAB-1-O-GlcNAc tryptic digest. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S18**.

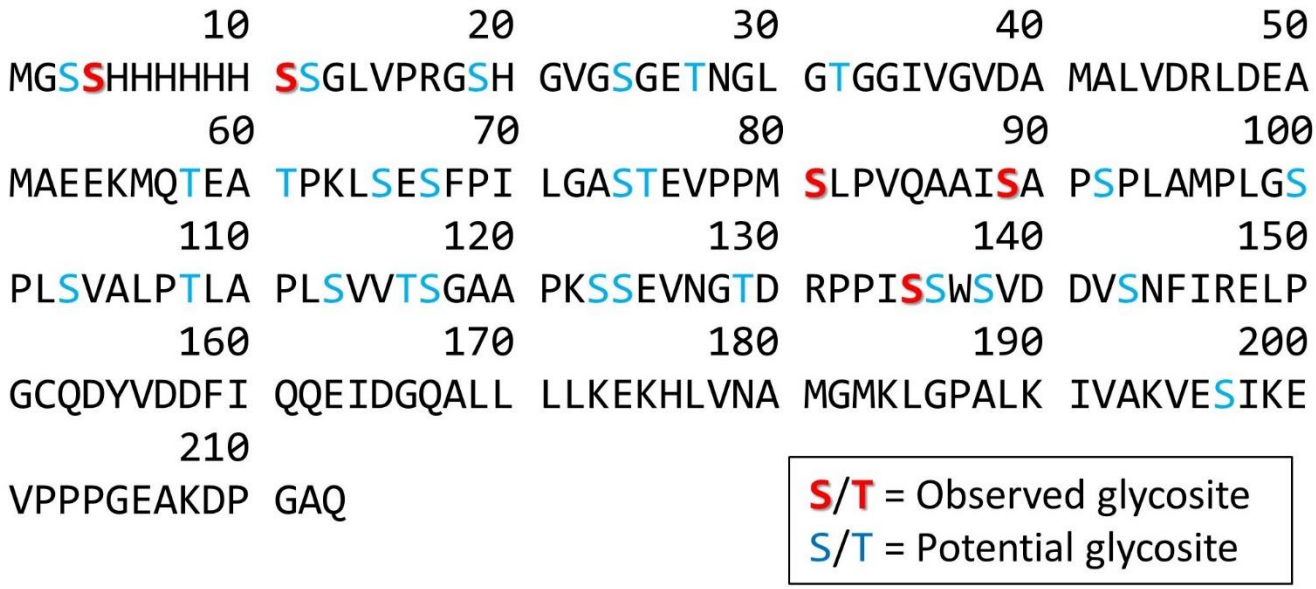

Figure S15: Ph1397-1589 construct sequence with identified O-GlcNAc sites highlighted.

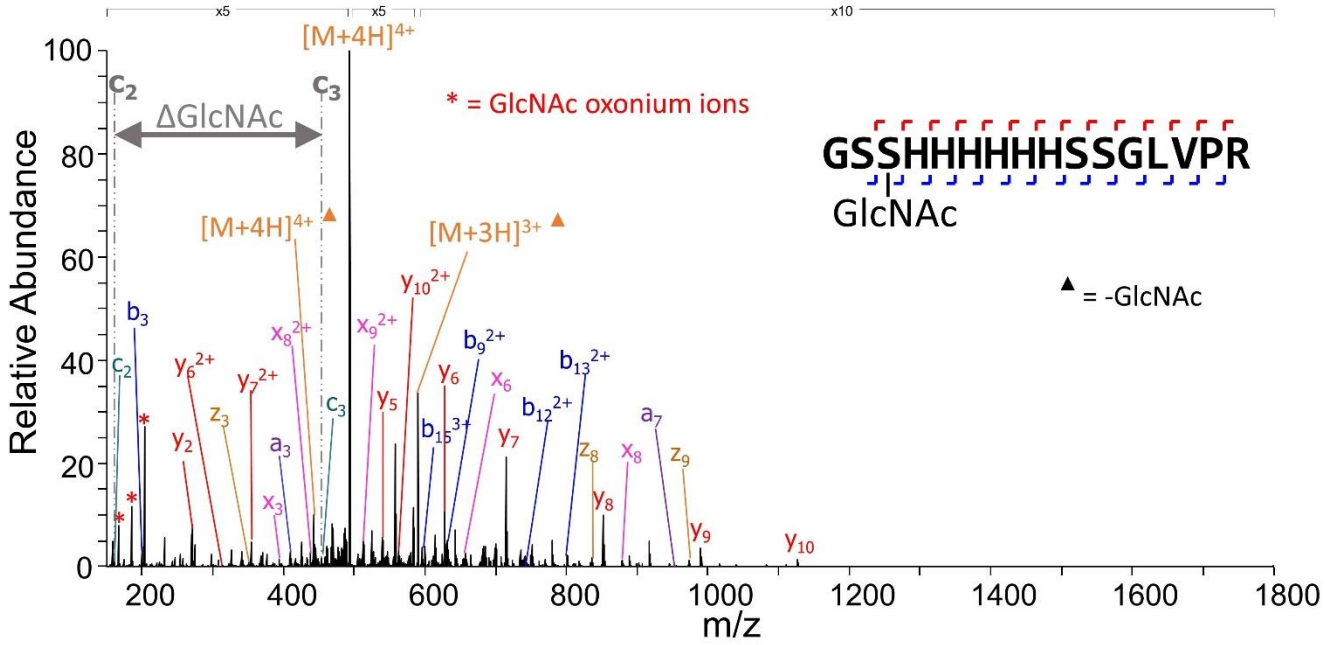

Figure S16: HCD oxocarbenium ion-triggered 193 nm UVPD mass spectrum of glycosylated peptide GSS[+203]HHHHHHSSGLVPR (4+, m/z 493.73) from Ph1397-1589-O-GlcNAc tryptic digest using two pulses of 1.5 mJ. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S20**.

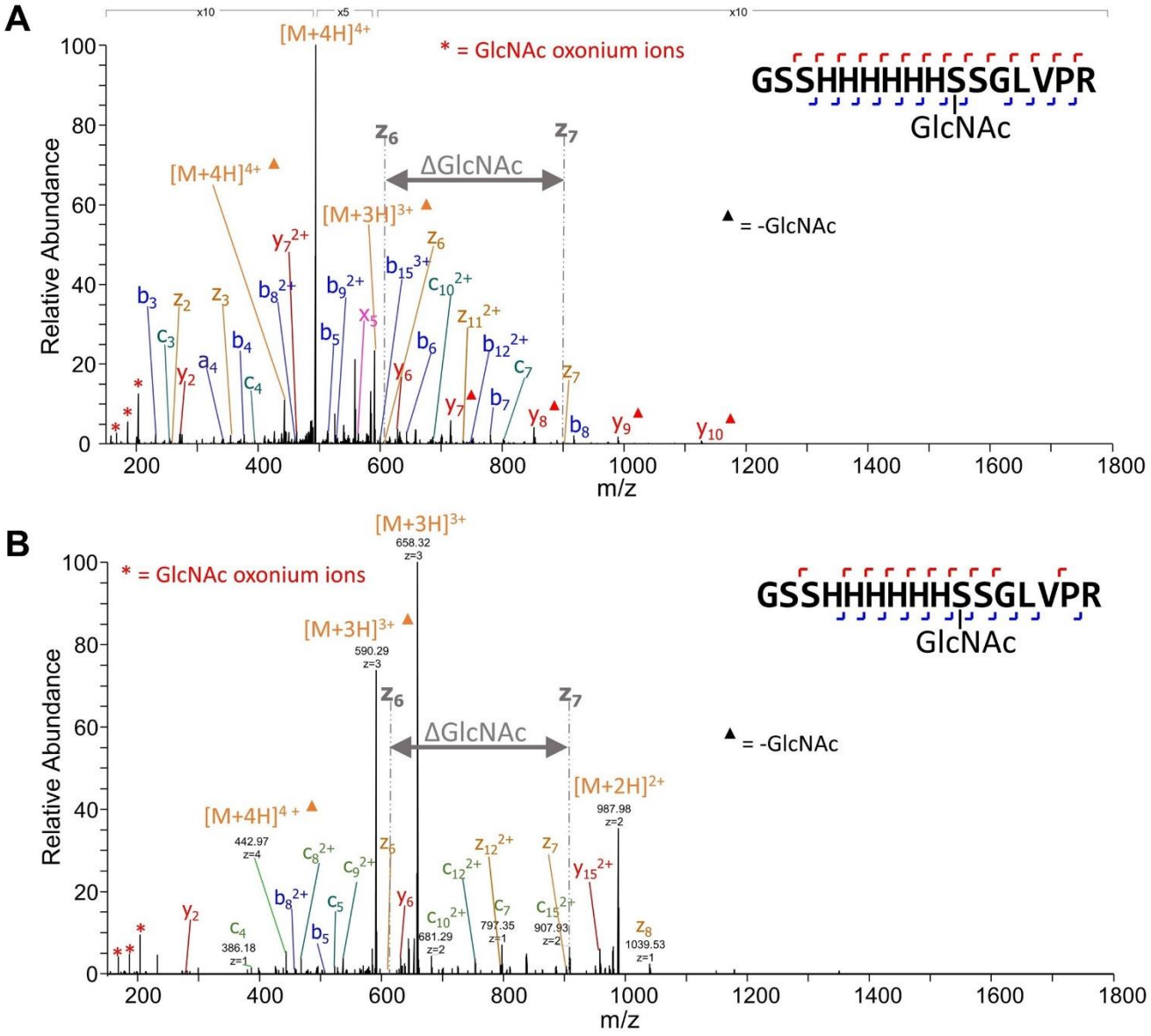

Figure S17: MS/MS comparison of GSSHHHHHHS[+203]SGLVPR glycopeptide. The double-headed arrows and ΔGlcNAc indicate the mass shifts representing loss of the O-GlcNAc-modified amino acid between fragment ions (labelled in grey at each end of the double headed arrow) that bracket the site of the modification. (**A**) HCD oxocarbenium ion-triggered 193 nm UVPD mass spectrum of glycosylated peptide GSSHHHHHHS[+203]SGLVPR (4+, m/z 493.73) from Ph1397-1589-O-GlcNAc tryptic digest using two pulses of 1.5 mJ. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S21**. (**B**) HCD oxocarbenium ion-triggered EThcD mass spectrum of glycosylated peptide GSSHHHHHHS[+203]SGLVPR (4+, m/z 493.73) using 50 ms activation time and 25% supplemental energy. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S25**.

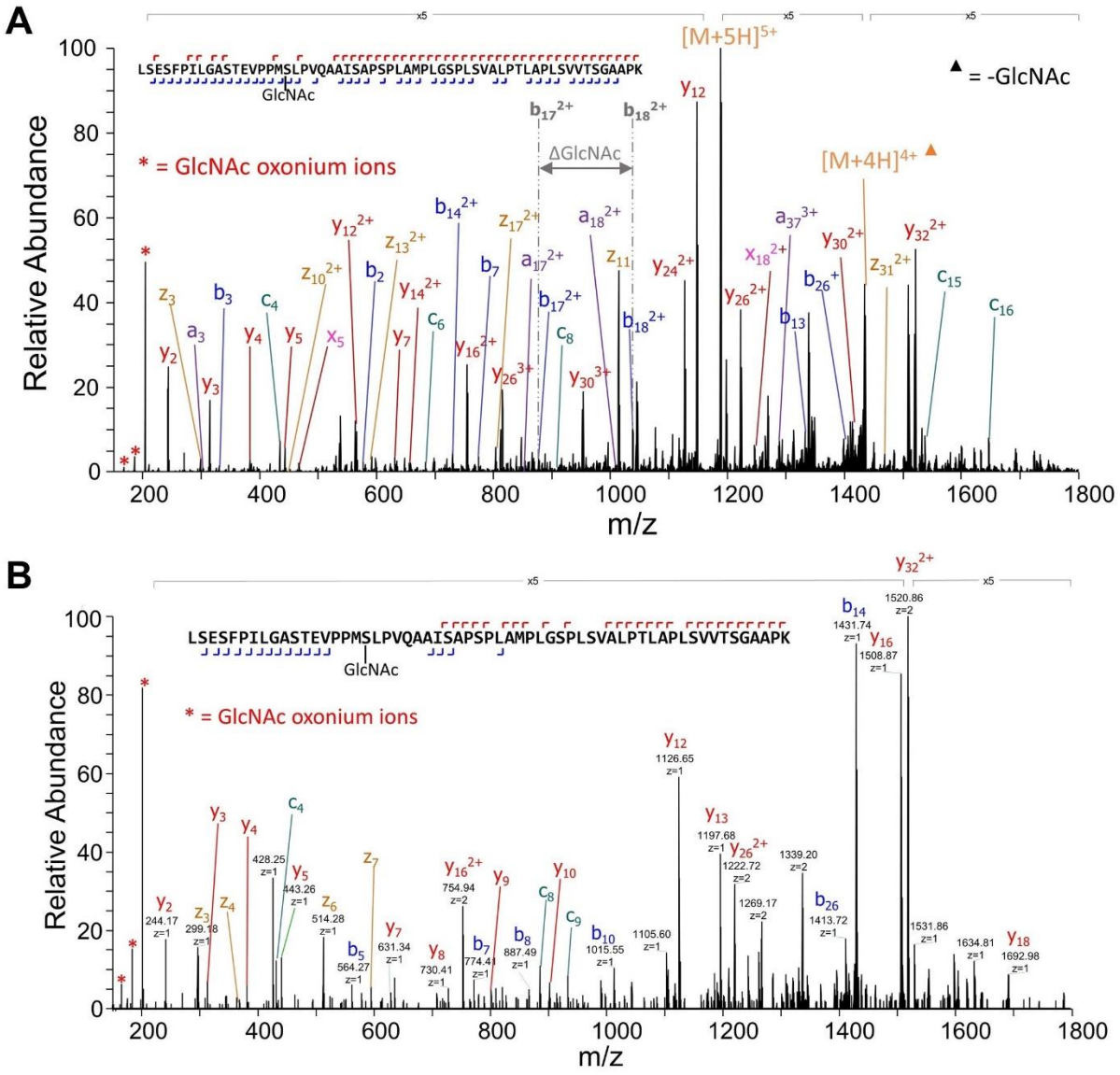

Figure S18: MS/MS comparison of LSESFPILGASTEVPPMS[+203]LPVQAAISAPSPLAMPLGSPLSVALPTLAPLSVVTSGAAPK glycopeptide. (**A**) HCD oxocarbenium ion-triggered UVPD mass spectra of an O-GlcNAc-modified isoform of peptide originating from a Ph1397-1589-O-GlcNAc tryptic digest. 193 nm UVPD of LSESFPILGASTEVPPMS[+203]LPVQAAISAPSPLAMPLGSPLSVALPTLAPLSVVTSGAAPK (5+, m/z 1188.24). Two laser pulses of 1.5 mJ were used. The double-headed arrows and ΔGlcNAc indicate the mass shifts representing loss of the O-GlcNAc-modified amino acid between fragment ions (labelled in grey at each end of the double headed arrow) that bracket the site of the modification. A sequence map is shown above the spectrum, and the complete list of identified fragment ions is shown in **Table S22**. (**B**) HCD oxocarbenium ion-triggered EThcD mass spectra of LSESFPILGASTEVPPMS[+203]LPVQAAISAP SPLAMPLGSPLSVALPTLAPLSVVTSGAAPK (5+, m/z 1188.24) using 50 ms activation time and 25% supplemental energy were used for EThcD. A sequence map is shown above the spectrum, and the complete list of identified fragment ions is shown in **Table S26**.

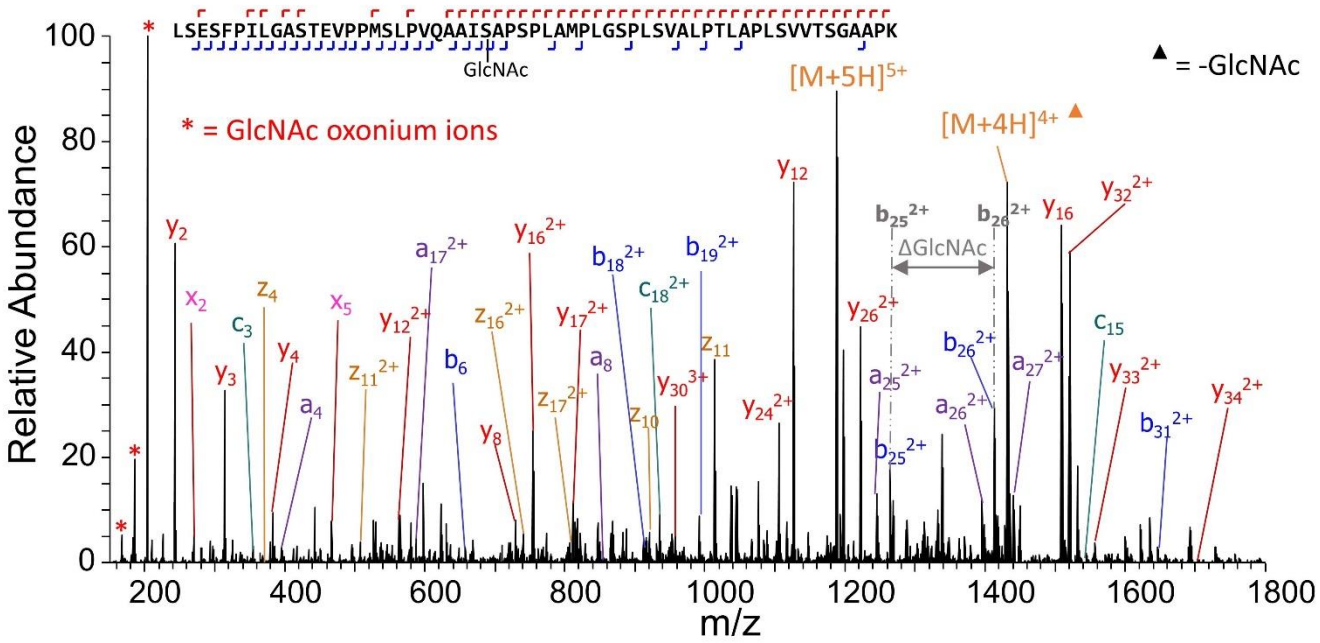

Figure S19: HCD oxocarbenium ion-triggered UVPD mass spectra of an O-GlcNAc-modified isoform of peptide LSESFPILGASTEVPPMSLPVQAAISAPSPLAMPLGSPLSVALPTLAPLSVVTSGAAPK glycopeptide originating from a Ph1397-1589-O-GlcNAc tryptic digest. 193 nm UVPD of LSESFPILGASTEVPPMSLPVQ AAIS[+203]APSPLAMPLGSPLSVALPTLAPLSVVTSGAAPK (5+, m/z 1188.24). The double-headed arrows and ΔGlcNAc indicate the mass shifts representing loss of the O-GlcNAc-modified amino acid between fragment ions (labelled in grey at each end of the double headed arrow) that bracket the site of the modification. Two laser pulses of 1.5 mJ were used. A sequence map is shown above the spectrum, and the complete list of identified fragment ions is shown in **Table S23**.

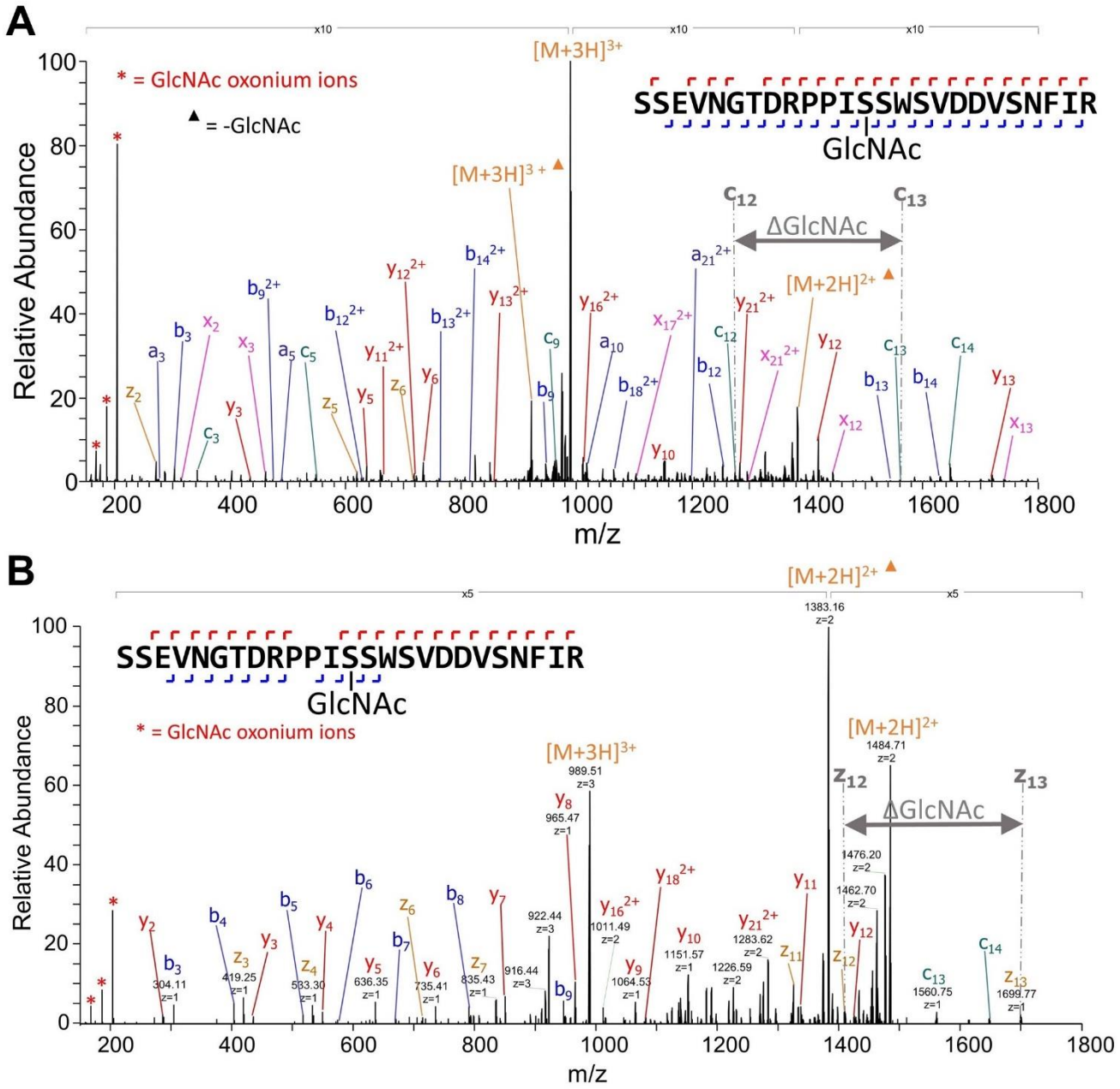

Figure S20: MS/MS comparison of SSEVNGTDRPPIS[+203]SWSVDDVSNFIR glycopeptide. The double-headed arrows and ΔGlcNAc indicate the mass shifts representing loss of the O-GlcNAc-modified amino acid between fragment ions (labelled in grey at each end of the double headed arrow) that bracket the site of the modification. (**A**) HCD oxocarbenium ion-triggered 193 nm UVPD mass spectrum of glycosylated peptide SSEVNGTDRPPIS[+203]SWSVDDVSNFIR (3+, m/z 989.80) from Ph1397-1589-O-GlcNAc tryptic digest using two pulses of 1.5 mJ. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S24**. (**B**) HCD oxocarbenium ion-triggered EThcD mass spectrum of glycosylated peptide SSEVNGTDRPPIS[+203]SWSVDDVSNFIR (3+, m/z 989.80) using 50 ms activation time and 25% supplemental energy. A sequence map is shown in the upper right, and the complete list of identified fragment ions is shown in **Table S27**.

Section S3 Supplementary Tables

Table S1: Summary of O-GlcNAc-containing peptides identified using HCD-triggered-EThcD for analysis of tryptic digests of O-GlcNAc-modified TAB1 and Ph proteins.

Protein	Glycopeptide	Charge state (+)	Detected Ion (*m/z*)	Peptide Mass (Da)	Mass error (ppm)	PEP 2D‡	Delta Mod Score	Sequence Coverage	Modified res$^{\zeta}$/known site?
TAB1	VYPVSVPYS†$_{(g1)}$SAQSTSK	2	951.97	1902.93	0.2	2.2e-14	901	100%	S395 / Y[26]
TAB1	VYPVSVPYSS$_{(g2)}$AQSTSK	2	951.97	1902.93	0.9	7.5e-15	934	100%	S396 / Y[27]
TAB1	VYPVSVPYSSAQS$_{(g3)}$TSK	2	951.97	1902.93	2.1	7.4e-14	898	100%	S399 / N
TAB1	LWSVDHGEQSVVT$_{(g7)}$AP	2	914.44	1826.87	1.0	2.7e-8	650	93%	T502 / N
Ph[1397-1589]	LSESFPILGASTEVPPMS$_{(g2)}$LP VQAAISAPSPLAMPLGSPLSV ALPTLAPLSVVTSGAAPK	5	118.24	5937.19	-0.3	2.9e-18	1039	72%	S1457 / N
Ph[1397-1589]	SSEVNGTDRPPIS$_{(g4)}$S WSVDDVSNFIR	3	989.80	2967.40	0.5	9.1e-15	773	88%	S1511 / N

†$_{(g)}$ denotes an identified O-GlcNAc modification site

‡Scoring criteria used for assessing quality of PTM site localization based previously established two-dimensional target decoy strategy (PEP 2D scores < 10^{-5} & Delta Mod scores > 40)[42] using Byonic

$^{\zeta}$ Modified amino acid position in the full-length protein.

*Data are based on triplicate runs

Table S2: Complete list of ions generated by HCD of TAB1-O-GlcNAc peptide using an NCE of 25 to activate the TAB1-O-GlcNAc peptide (biotin-PVSVPYS$_{(g)}$SAQSTS, 2+, m/z 869.9). Fragment ions that undergo loss of water or ammonia are not listed in this table.

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b_2	422.2012	422.1991	-5.0
b_3	509.2332	509.2316	-3.1
b_4	608.3016	608.2999	-2.9
b_5	705.3544	705.3493	-7.2
b_7▲	955.4497	955.4484	-1.4
b_8▲	1042.4818	1042.4820	0.2
b_{10}▲	1241.5775	1241.5767	-0.6
b_{11}▲	1328.6095	1328.6096	0.1
b_{12}▲	1429.6572	1429.6569	-0.2
y_2	206.0903	206.0904	0.7
y_3	293.1223	293.1225	0.7
y_4	421.1809	421.1813	1.1
y_5	492.2180	492.2188	1.7
y_6	579.2500	579.2508	1.3
y_7▲	666.2821	666.2824	0.6
y_9▲	926.3981	926.3987	0.6
y_{10}▲	1025.4666	1025.4693	2.7
y_{11}▲	1112.4986	1112.4999	1.1

Table S3: Complete list of ions generated by UVPD of TAB1-O-GlcNAc peptide using two pulse of 1.5 mJ laser energy to activate the TAB1-O-GlcNAc peptide (biotin-PVSVPYS$_{(g)}$SAQSTS, 2+, m/z 869.9). Fragment ions that undergo loss of water or ammonia are not listed in this table. Ions identified as a_n + 1 have an extra hydrogen atom. The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
a_1+1	296.1433	296.1429	-1.2
a_2	394.2039	394.2041	0.5
a_3	481.2359	481.2363	0.9
a_4+1	581.3121	581.3121	0.0
a_5+1	678.3649	678.3655	0.9
a_6+1	841.4277	841.4225	-6.2
a_7	1131.5391	1131.5462	6.3
a_8	1217.5638	1217.5636	-0.1
a_9+1	1289.6088	1289.6039	-3.8
a_{10}	1416.6595	1416.6621	1.8
a_{10}+1	1417.6674	1417.6662	-0.8
a_{11}	1503.6916	1503.6816	-6.6
b_1	323.1304	323.1306	0.6
b_2	422.1988	422.1989	0.4
b_3	509.2308	509.2313	0.9
b_4	608.2992	608.2996	0.7
b_5	705.3520	705.3528	1.2
b_7	1158.5267	1158.5297	-1.1
b_8	1245.5587	1245.5574	2.5
b_9	1316.5959	1316.5992	2.5
b_{10}	1444.6545	1444.6537	-0.5
b_{10}▲	1241.5751	1241.5753	0.2
b_{11}▲	1328.6071	1328.6081	0.7
b_{12}	1632.7341	1632.7298	-2.6
b_{12}▲	1429.6548	1429.6548	0.0
c_2	439.2251	439.2258	1.6
c_3	526.2571	526.2577	1.2
c_5	722.3783	722.3789	0.9
c_6	885.4416	885.4427	1.2
c_8	1262.5850	1262.5795	-4.4
c_9	1333.6221	1333.6205	-1.2
x_2+1	233.0774	233.0774	0.0
x_3+1	320.1094	320.1088	-1.9
x_8+1	1059.4119	1059.4122	0.3
x_{11}+1	1342.5651	1342.5667	1.2
x_{12}	1440.6257	1440.6289	2.2
y_3	293.1223	293.1224	0.2
y_6	579.2500	579.2504	0.6
y_8	1032.4248	1032.4228	-1.9

y_9	1129.4775	1129.4771	-0.4
y_9▲	926.3981	926.3960	-2.3
y_{10}	1228.5460	1228.5448	-0.9
y_{10}▲	1025.4666	1025.4644	-2.1
y_{11}	1315.5780	1315.5792	0.9
y_{11}▲	1112.4986	1112.4961	-2.3
z_{12}	1398.6276	1398.6257	-1.4
z_5	476.1992	476.1999	1.3
z_6	563.2313	563.2301	-2.0
z_7	853.3427	853.3429	0.2

Table S4: Complete list of ions generated by EThcD of TAB1-O-GlcNAc peptide using 50 ms of ETD activation energy and 25% supplemental energy to activate the TAB1-O-GlcNAc peptide (biotin-PVSVPYS$_{(g)}$SAQSTS, 2+, m/z 869.9). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b_2	422.1988	422.1986	-0.5
b_3	509.2308	509.2305	-0.5
b_6	868.4153	868.4161	0.9
b_{10}▲	1241.5751	1241.5751	0.0
b_{11}▲	1328.6071	1328.6077	0.5
b_{12}	1632.7342	1632.7404	3.8
b_{12}▲	1429.6548	1429.6551	0.2
c_5	722.3783	722.3790	1.1
c_6	885.4416	885.4409	-0.8
c_7	1175.5530	1175.5516	-1.2
c_7▲	972.4736	972.4750	1.4
c_8	1262.5851	1262.5861	0.8
c_8▲	1059.5057	1059.5067	1.0
c_9	1333.6222	1333.6216	-0.4
c_{10}	1461.6808	1461.6815	0.5
c_{10}▲	1258.6014	1258.6017	0.3
c_{11}	1548.7128	1548.7151	1.5
c_{11}▲	1345.6334	1345.6342	0.6
c_{12}	1649.7605	1649.7611	0.4
c_{12}▲	1446.6811	1446.6815	0.3
y_3	293.1223	293.1223	0.0
y_6	579.2500	579.2495	-0.9
y_9	1129.4775	1129.4775	0.0
y_9▲	926.3981	926.3979	-0.2
y_{10}	1228.5460	1228.5468	0.7
y_{11}	1315.5780	1315.5818	2.9
y_{11}▲	1112.4986	1112.4983	-0.3
z_{10}	1212.5272	1212.5284	1.0
z_{10}▲	1009.4478	1009.4486	0.8
z_{11}	1299.5592	1299.5612	1.5
z_{12}	1398.6276	1398.6289	0.9
z_{12}▲	1195.5482	1195.5417	-5.5

Table S5: Complete list of ions generated by UVPD of HCF-OGlcNAc peptide using two pulse of 1.5 mJ laser energy to activate the HCF-OGlcNAc peptide (YVRVCSNPPCS$_{(g)}$THETGTTNTATTATS, 3+, m/z 967.76). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
a_2	234.1368	234.1373	1.9
a_3+1	391.2458	391.2456	-0.4
a_4	489.3063	489.3066	0.6
a_4+1	490.3142	490.3129	-2.7
a_6+1	680.3554	680.3521	-4.9
a_7	793.3905	793.3961	7.1
a_{10}+1	1091.5052	1091.4982	-6.3
a_{11}+1	1381.6245	1381.6145	-7.2
a_{12}	1481.6643	1481.6545	-6.6
a_{18}+1	2108.9381	2108.9274	-5.1
a_{19}	2221.9732	2221.9703	-1.3
a_{23}+1	2597.1612	2597.1415	-7.6
a_{24}	2667.1905	2667.1824	-3.0
b_2	262.1317	262.1323	2.3
b_3	418.2329	418.2335	1.6
b_4	517.3013	517.3021	1.6
b_7	821.3854	821.3896	5.1
b_8	918.4382	918.4394	1.3
b_{10}	1118.5001	1118.4894	-9.6
b_{13}▲	1443.6387	1443.6389	0.1
b_{14}▲	1572.6813	1572.6906	5.9
b_{15}▲	1673.7290	1673.7280	-0.6
b_{17}	2034.8776	2034.8970	9.5
b_{24}▲	2492.1060	2492.1015	-1.8
b_{25}	2796.2331	2796.2333	0.1
b_{25}▲	2593.1537	2593.1421	-4.5
c_4	534.3276	534.3288	2.3
c_{19}	2266.9945	2266.9772	-7.6
x_{12}	1151.4942	1151.5037	8.2
y_3	277.1274	277.1278	1.7
y_4	378.1751	378.1758	1.8
y_5	479.2227	479.2240	2.6
y_6	550.2599	550.2612	2.5
y_8	765.3505	765.3534	3.9
y_{11}	1024.4673	1024.4733	5.9
y_{14}	1391.6165	1391.6204	2.8
y_{15}	1492.6641	1492.6729	5.8
y_{16}	1782.7756	1782.7713	-2.4
y_{20}	2193.9332	2193.9411	3.6
y_{21}	2280.9652	2280.9743	4.0
y_{24}-1	2638.1361	2638.1440	3.0
z_9	850.3794	850.3761	-3.9
z_{18}	1966.8188	1966.8319	6.7
z_{24}	2623.1252	2623.1344	3.5

Table S6: Complete list of ions generated by UVPD of CKII-OGlcNAc peptide using two pulse of 1.5 mJ laser energy to activate the CKII-OGlcNAc peptide (YPGGSTPVS$_{(g)}$SANMM, 2+, m/z 801.3). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
a_2	232.1212	232.1216	2.0
a_5	433.1961	433.1975	3.1
a_6	534.2438	534.2439	0.2
a_9	1020.4764	1020.4834	6.8
a_{13}+1	1424.6368	1424.6390	1.5
a_4+1	347.1719	347.1726	2.0
a_7+1	632.3044	632.3035	-1.4
a_8+1	731.3728	731.3723	-0.7
a_{10}	1107.5084	1107.5179	8.5
a_{11}	1178.5455	1178.5482	2.2
b_4	374.1590	374.1596	1.6
b_5	461.1910	461.1922	2.6
b_6	562.2387	562.2402	2.7
b_8	758.3599	758.3613	1.9
b_9	1048.4713	1048.4766	5.0
b_9▲	845.3919	845.3925	0.7
b_{10}	1135.5034	1135.5096	5.5
b_{10}▲	932.4240	932.4240	0.0
b_{11}	1206.5405	1206.5422	1.5
b_{11}▲	1003.4611	1003.4625	1.5
b_{12}	1320.5834	1320.5873	2.9
b_{12}▲	1117.5040	1117.5036	-0.3
b_{13}	1451.6239	1451.6267	2.0
b_{13}▲	1248.5445	1248.5465	1.6
c_2	277.1424	277.1432	2.8
c_3	334.1639	334.1647	2.5
c_4	391.1853	391.1858	1.3
c_7	676.3178	676.3230	7.7
c_8	775.3862	775.3882	2.6
c_{10}	1152.5297	1152.5307	0.9
c_{11}	1223.5668	1223.5725	4.7
c_{12}▲	1134.5303	1134.5253	-4.4
c_{13}▲	1265.5708	1265.5671	-2.9
x_6+1	869.3021	869.3017	-0.6
x_7+1	968.3705	968.3709	0.4
x_8+1	1065.4233	1065.4315	7.7
y_1	149.0511	149.0515	3.0
y_2	280.0915	280.0921	1.8
y_3	394.1345	394.1353	2.0
y_4	465.1716	465.1726	2.3

y_5	552.2036	552.2051	2.7
y_6	842.3150	842.3166	1.9
$y_6^{\blacktriangle}$	639.2356	639.2353	-0.5
y_8	1038.4362	1038.4373	1.0
$y_8^{\blacktriangle}$	835.3568	835.3558	-1.2
$y_9^{\blacktriangle}$	936.4045	936.4057	1.3
$y_{12}^{\blacktriangle}$	1137.4794	1137.4786	-0.8
$y_{13}^{\blacktriangle}$	1234.5322	1234.5319	-0.2
y_{10}	1226.5159	1226.5163	0.3
y_{11}	1283.5374	1283.5411	2.9
y_{11}-1	1282.5296	1282.5279	-1.3
y_{12}	1340.5588	1340.5583	-0.4
y_{13}	1437.6116	1437.6136	1.4
z_9	1123.4651	1123.4629	-2.0

Table S7: Complete list of ions generated by EThcD of HCF-OGlcNAc peptide using 50 ms of ETD activation energy and 25% supplemental energy to activate the HCF-OGlcNAc peptide (YVRVCSNPPCS$_{(g)}$THETGTTNTATTATS, 3+, m/z 967.76). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
b_{21} ▲	2218.9805	2218.9735	-3.2
b_{24}	2695.1760	2695.1807	1.7
b_{24} ▲	2492.1060	2492.1055	-0.2
b_{25} ▲	2593.1537	2593.1521	-0.6
c_4	534.3276	534.3276	0.2
c_{19}	2266.9850	2266.9900	2.1
y_2	206.0903	206.0902	-0.2
y_3	277.1274	277.1274	0.1
y_4	378.1751	378.1748	-0.6
y_5	479.2227	479.2227	0.0
y_6	550.2599	550.2597	-0.3
y_8	765.3505	765.3491	-1.7
y_{11}	1024.4670	1024.4670	-0.3
y_{13}	1254.5580	1254.5550	-2.4
y_{14}	1391.6170	1391.6180	0.8
y_{15}	1492.6640	1492.6670	1.6
y_{24}	2639.1350	2639.1600	9.5
z_8	749.3317	749.3317	0.0
z_{14}	1375.5980	1375.6030	3.6
z_{15}	1476.6450	1476.6440	-1.2

Table S8: Complete list of ions generated by EThcD of CKII-OGlcNAc peptide using 50 ms of ETD activation energy and 25% supplemental energy to activate the CKII-OGlcNAc peptide (YPGGSTPVS$_{(g)}$SANMM, 2+, m/z 801.3). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b_4	374.1590	374.1585	-1.2
b_5	461.1910	461.1906	-0.9
b_6	562.2387	562.2385	-0.4
b_8	758.3599	758.3597	-0.2
b_9	1048.4713	1048.4656	-5.4
b_9▲	845.3919	845.3925	0.7
b_{10}▲	932.4240	932.4240	0.0
b_{11}▲	1003.4611	1003.4625	1.5
b_{12}▲	1117.5040	1117.5036	-0.3
b_{13}▲	1248.5445	1248.5465	1.6
c_5	478.2173	478.2170	-0.7
c_7	676.3178	676.3183	0.8
c_8	775.3862	775.3846	-2.1
c_{10}	1152.5297	1152.5285	-1.0
c_{10}▲	949.4503	949.4444	-6.1
c_{11}▲	1020.4874	1020.4902	2.8
c_{12}	1337.6097	1337.6080	-1.3
c_{12}▲	1134.5303	1134.5253	-4.4
c_{13}	1468.6502	1468.6479	-1.5
c_{13}▲	1265.5708	1265.5671	-2.9
y_2	280.0915	280.0915	-0.1
y_8	1038.4362	1038.4355	-0.7
y_8▲	835.3568	835.3558	-1.2
y_9▲	936.4045	936.4057	1.3
y_{12}▲	1137.4794	1137.4786	-0.8
y_{13}▲	1234.5322	1234.5319	-0.2
z_{11}	1267.5186	1267.5296	8.6
z_{12}	1324.5401	1324.5485	6.4

Table S9: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (VYPVSVPYSS$_{(g)}$AQSTSK, 2+, m/z 951.97). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_2	235.1441	235.1443	0.9
a_3	332.1969	332.1974	1.5
a_4	431.2653	431.2657	0.9
a_5	518.2973	518.2982	1.7
a_6	617.3657	617.3668	1.8
a_7	714.4185	714.4211	3.6
a_8	877.4818	877.4841	2.6
a_9	964.5138	964.5183	4.7
a_{10}	1254.6252	1254.6274	1.8
a_{11}	1325.6623	1325.6588	-2.6
a_{12}	1453.7209	1453.7260	3.5
a_{13}	1540.7530	1540.7603	4.7
a_{15}	1728.8327	1728.8295	-1.9
b_2	263.1390	263.1393	1.1
b_3	360.1918	360.1922	1.1
b_4	459.2602	459.2608	1.3
b_5	546.2922	546.2932	1.8
b_6	645.3606	645.3617	1.7
b_7	742.4134	742.4123	-1.5
b_8	905.4767	905.4822	6.1
b_9	992.5087	992.5151	6.4
b_{11}	1353.6573	1353.6592	1.4
b_{14}	1669.7955	1669.7964	0.5
c_3	377.2183	377.2187	1.1
c_4	476.2867	476.2873	1.3
c_5	563.3188	563.3196	1.4
c_7	759.4399	759.4406	0.9
c_8	922.5033	922.5055	2.4
c_{13}	1585.7744	1585.7734	-0.6
c_{14}	1686.8221	1686.8252	1.8
c_{15}	1773.8541	1773.8438	-5.8
x_1	173.0921	173.0924	1.7
x_2	260.1241	260.1245	1.5
x_3	361.1718	361.1726	2.2
x_4	448.2038	448.2044	1.3
x_5	576.2624	576.2640	2.8
x_6	647.2995	647.3011	2.5
x_7	937.4109	937.4147	4.1
x_8	1024.4429	1024.4454	2.4
x_9	1187.5063	1187.5084	1.8
x_{10}	1284.5590	1284.5569	-1.6

x_{11}	1383.6274	1383.6179	-6.9
x_{12}	1470.6595	1470.6633	2.6
x_{13}	1569.7279	1569.7321	2.7
x_{14}	1666.7806	1666.7867	3.7
y_2	234.1448	234.1451	1.3
y_3	335.1925	335.1928	0.9
y_4	422.2245	422.2250	1.2
y_5	550.2831	550.2840	1.6
y_6	621.3202	621.3217	2.4
y_7	911.4316	911.4340	2.6
y_7▲	708.3523	708.3538	2.1
y_8	998.4637	998.4648	1.1
y_8▲	795.3843	795.3853	1.3
y_9	1161.5270	1161.5309	3.4
y_{10}	1258.5798	1258.5823	2.0
y_{10}▲	1055.5004	1055.5005	0.1
y_{11}	1357.6482	1357.6516	2.5
y_{11}▲	1154.5688	1154.5697	0.8
y_{12}	1444.6802	1444.6840	2.6
y_{12}▲	1241.6008	1241.6022	1.1
y_{13}	1543.7486	1543.7529	2.8
y_{13}▲	1340.6692	1340.6719	2.0
y_{14}	1640.8014	1640.8013	-0.1
y_{14}▲	1437.7220	1437.7240	1.4
y_{15}	902.4360	902.4366	0.7
y_{10}^{2+}▲	528.2538	528.2540	0.4
y_{14}^{2+}▲	719.3646	719.3646	0.0
z_2	218.1261	218.1263	0.9
z_3	319.1738	319.1743	1.6
z_4	406.2058	406.2063	1.2
z_5	534.2644	534.2652	1.5
z_6	605.3015	605.3024	1.5
z_7	895.4129	895.4158	3.2
z_8	982.4449	982.4473	2.4
z_9	1145.5083	1145.5094	1.0
z_{12}	1428.6615	1428.6638	1.6
z_{13}	1527.7299	1527.7352	3.5
z_{14}	1624.7826	1624.7925	6.1
z_{15}	1787.8460	1787.8484	1.3

Table S10: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (VYPVSVPYS$_{(g)}$SAQSTSK, 2+, m/z 951.97). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_2	235.1441	235.1445	1.7
a_3	332.1969	332.1974	1.5
a_4	431.2653	431.2659	1.4
a_5	518.2973	518.2984	2.1
a_6	617.3657	617.3671	2.3
a_7	714.4185	714.4208	3.2
a_8	877.4818	877.4847	3.3
a_9	1167.5932	1167.6032	8.6
a_{10}	1254.6252	1254.6211	-3.3
a_{11}	1325.6623	1325.6631	0.6
a_{12}	1453.7209	1453.7235	1.8
a_{13}	1540.7530	1540.7572	2.7
a_{15}	1728.8327	1728.8345	1.0
b_2	263.1390	263.1395	1.9
b_3	360.1918	360.1923	1.4
b_4	459.2602	459.2610	1.7
b_5	546.2922	546.2933	2.0
b_6	645.3606	645.3619	2.0
b_7	742.4134	742.4135	0.1
b_8	905.4767	905.4821	6.0
b_9	1195.5881	1195.5811	-5.9
b_{10}	1282.6201	1282.6222	1.6
b_{11}	1353.6573	1353.6619	3.4
b_{12}	1481.7158	1481.7180	1.5
b_{14}	1669.7955	1669.8009	3.2
c_3	377.2183	377.2190	1.9
c_4	476.2867	476.2876	1.9
c_5	563.3188	563.3201	2.3
c_7	759.4399	759.4416	2.2
c_8	922.5033	922.5061	3.0
c_{11}	1370.6838	1370.6893	4.0
c_{13}	1585.7744	1585.7745	0.1
c_{14}	1686.8221	1686.8324	6.1
c_{15}	1773.8541	1773.8557	0.9
x_1	173.0921	173.0925	2.3
x_2	260.1241	260.1245	1.5
x_3	361.1718	361.1729	3.0
x_4	448.2038	448.2046	1.8
x_5	576.2624	576.2635	1.9
x_6	647.2995	647.3016	3.2
x_7	734.3315	734.3312	-0.4

x_8	1024.4429	1024.4448	1.9
x_9	1187.5063	1187.5099	3.0
x_{10}	1284.5590	1284.5594	0.3
x_{11}	1383.6274	1383.6245	-2.1
x_{12}	1470.6595	1470.6643	3.3
x_{13}	1569.7279	1569.7332	3.4
x_{14}	1666.7806	1666.7876	4.2
x_{15}	902.4360	902.4374	1.6
y_2	234.1448	234.1452	1.7
y_3	335.1925	335.1930	1.5
y_4	422.2245	422.2252	1.7
y_5	550.2831	550.2843	2.2
y_6	621.3202	621.3221	3.1
y_7	708.3523	708.3540	2.4
y_8	998.4637	998.4652	1.5
$y_8^{\blacktriangle}$	795.3843	795.3853	1.3
y_9	1161.5270	1161.5306	3.1
$y_9^{\blacktriangle}$	958.4476	958.4480	0.4
y_{10}	1258.5798	1258.5830	2.5
y_{11}	1357.6482	1357.6523	3.0
$y_{11}^{\blacktriangle}$	1154.5688	1154.5697	0.8
y_{12}	1444.6802	1444.6848	3.2
$y_{12}^{\blacktriangle}$	1241.6008	1241.6022	1.1
y_{13}	1543.7486	1543.7521	2.3
$y_{13}^{\blacktriangle}$	1340.6692	1340.6719	2.0
y_{14}	1640.8014	1640.8027	0.8
$y_{14}^{\blacktriangle}$	1437.7220	1437.7240	1.4
$y_{15}^{\blacktriangle}$	1600.7853	1600.7859	0.4
$y_{10}^{2+\blacktriangle}$	528.2538	528.2539	0.2
$y_{14}^{2+\blacktriangle}$	719.3646	719.3642	-0.6
$y_{15}^{2+\blacktriangle}$	800.8963	800.8975	1.5
z_2	218.1261	218.1265	1.8
z_3	319.1738	319.1744	1.9
z_4	406.2058	406.2065	1.7
z_5	534.2644	534.2654	1.9
z_6	605.3015	605.3026	1.8
z_7	692.3335	692.3334	-0.1
z_8	982.4449	982.4475	2.6
z_9	1145.5083	1145.5103	1.7
z_{12}	1428.6615	1428.6649	2.4
z_{13}	1527.7299	1527.7357	3.8
z_{14}	1624.7826	1624.7918	5.7
z_{15}	1787.8460	1787.8507	2.6

Table S11: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (VYPVSVPYSSAQS$_{(g)}$TSK, 2+, m/z 951.97). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_2	235.1441	235.1445	1.7
a_3	332.1969	332.1975	1.8
a_4	431.2653	431.2660	1.6
a_5	518.2973	518.2985	2.3
a_6	617.3657	617.3672	2.4
a_7	714.4185	714.4211	3.6
a_8	877.4818	877.4844	3.0
a_9	964.5138	964.5201	6.5
a_{10}	1051.5459	1051.5529	6.7
a_{11}	1122.5830	1122.5906	6.8
a_{12}	1250.6416	1250.6532	9.3
a_{13}	1540.7530	1540.7573	2.8
a_{15}	1728.8327	1728.8348	1.2
b_2	263.1390	263.1395	1.9
b_3	360.1918	360.1924	1.7
b_4	459.2602	459.2611	2.0
b_5	546.2922	546.2935	2.4
b_6	645.3606	645.3622	2.5
b_7	742.4134	742.4135	0.1
b_8	905.4767	905.4831	7.1
b_9	992.5087	992.5156	7.0
b_{10}	1079.5408	1079.5428	1.9
b_{12}	1278.6365	1278.6444	6.2
b_{14}	1669.7955	1669.7991	2.2
b_{15}	1756.8276	1756.8192	-4.8
c_3	377.2183	377.2190	1.9
c_4	476.2867	476.2877	2.1
c_5	563.3188	563.3200	2.1
c_7	759.4399	759.4426	3.6
c_8	922.5033	922.5058	2.7
c_{11}	1167.6044	1167.6100	4.8
c_{13}	1585.7744	1585.7750	0.4
c_{14}	1686.8221	1686.8304	4.9
c_{15}	1773.8541	1773.8492	-2.8
x_1	173.0921	173.0925	2.3
x_2	260.1241	260.1246	1.9
x_3	361.1718	361.1733	4.2
x_4	651.2832	651.2872	6.1
x_7	937.4109	937.4161	5.5
x_8	1024.4429	1024.4458	2.8
x_9	1187.5063	1187.5099	3.0

x_{10}	1284.5590	1284.5605	1.2
x_{11}	1383.6274	1383.6241	-2.4
x_{12}	1470.6595	1470.6649	3.7
x_{13}	1569.7279	1569.7333	3.4
x_{14}	1666.7806	1666.7903	5.8
y_2	234.1448	234.1452	1.7
y_3	335.1925	335.1931	1.8
y_4	625.3039	625.3054	2.4
y_4▲	422.2245	422.2250	1.2
y_5	753.3625	753.3649	3.2
y_5▲	550.2831	550.2836	0.9
y_6	824.3996	824.4039	5.2
y_7	911.4316	911.4349	3.6
y_7▲	708.3523	708.3538	2.1
y_8	998.4637	998.4654	1.7
y_8▲	795.3843	795.3853	1.3
y_9	1161.5270	1161.5313	3.7
y_{10}	1258.5798	1258.5835	2.9
y_{10}▲	1055.5004	1055.5005	0.1
y_{11}	1357.6482	1357.6526	3.2
y_{11}▲	1154.5688	1154.5697	0.8
y_{12}	1444.6802	1444.6853	3.5
y_{12}▲	1241.6008	1241.6022	1.1
y_{13}	1543.7486	1543.7537	3.3
y_{13}▲	1340.6692	1340.6719	2.0
y_{14}	1640.8014	1640.8033	1.2
y_{14}▲	1437.7220	1437.7240	1.4
y_{15}	902.4360	902.4376	1.8
y_{14}^{2+}▲	719.3646	719.3646	0.0
z_2	218.1261	218.1266	2.3
z_3	319.1738	319.1746	2.5
z_4	609.2852	609.2864	2.0
z_5	737.3438	737.3461	3.1
z_6	808.3809	808.3778	-3.8
z_7	895.4129	895.4186	6.4
z_8	982.4449	982.4477	2.9
z_9	1145.5083	1145.5115	2.8
z_{11}	1341.6294	1341.6309	1.1
z_{12}	1428.6615	1428.6663	3.4
z_{13}	1527.7299	1527.7362	4.1
z_{14}	1624.7826	1624.7982	9.6
z_{15}	1787.8460	1787.8501	2.3

Table S12: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (VYPVSVPYSSAQSTS$_{(g)}$K, 2+, m/z 951.97). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_2	235.1441	235.1445	1.7
a_3	332.1969	332.1974	1.5
a_4	431.2653	431.2660	1.6
a_5	518.2973	518.2985	2.3
a_6	617.3657	617.3672	2.4
a_7	714.4185	714.4202	2.4
a_8	877.4818	877.4850	3.6
a_9	964.5138	964.5187	5.1
a_{10}	1051.5459	1051.5494	3.3
a_{11}	1122.5830	1122.5913	7.4
a_{13}	1337.6736	1337.6647	-6.7
a_{14}	1438.7213	1438.7299	6.0
a_{15}	1728.8327	1728.8347	1.2
b_2	263.1390	263.1396	2.3
b_3	360.1918	360.1924	1.7
b_4	459.2602	459.2611	2.0
b_5	546.2922	546.2935	2.4
b_6	645.3606	645.3622	2.5
b_7	742.4134	742.4150	2.2
b_8	905.4767	905.4810	4.7
b_9	992.5087	992.5151	6.4
b_{10}	1079.5408	1079.5469	5.7
b_{12}	1278.6365	1278.6454	7.0
b_{13}	1365.6685	1365.6754	5.1
b_{14}	1466.7162	1466.7214	3.5
b_{15}	1756.8276	1756.8242	-1.9
c_3	377.2183	377.2192	2.4
c_4	476.2867	476.2878	2.3
c_5	563.3188	563.3201	2.3
c_7	759.4399	759.4427	3.7
c_8	922.5033	922.5065	3.5
c_{11}	1167.6044	1167.6145	8.7
c_{12}	1295.6630	1295.6661	2.4
c_{14}	1483.7427	1483.7472	3.0
c_{15}	1773.8541	1773.8499	-2.4
x_1	173.0921	173.0924	1.7
x_5	779.3418	779.3431	1.7
x_7	937.4109	937.4172	6.7
x_8	1024.4429	1024.4445	1.6
x_9	1187.5063	1187.5104	3.5
x_{10}	1284.5590	1284.5620	2.3

x_{11}	1383.6274	1383.6262	-0.9
x_{12}	1470.6595	1470.6650	3.7
x_{13}	1569.7279	1569.7327	3.1
x_{14}	1666.7806	1666.7876	4.2
$y_3^{\blacktriangle}$	335.1925	335.1924	-0.3
$y_4^{\blacktriangle}$	422.2245	422.2250	1.2
$y_5^{\blacktriangle}$	550.2831	550.2836	0.9
y_6	824.3996	824.3967	-3.5
y_7	911.4316	911.4360	4.8
$y_7^{\blacktriangle}$	708.3523	708.3538	2.1
y_8	998.4637	998.4660	2.3
$y_8^{\blacktriangle}$	795.3843	795.3853	1.3
y_9	1161.5270	1161.5313	3.7
y_{10}	1258.5798	1258.5837	3.1
$y_{10}^{\blacktriangle}$	1055.5004	1055.5005	0.1
y_{11}	1357.6482	1357.6523	3.0
y_{12}	1444.6802	1444.6857	3.8
y_{13}	1543.7486	1543.7534	3.1
y_{14}	1640.8014	1640.8043	1.8
$y_{15}^{\blacktriangle}$	1600.7853	1600.7840	-0.8
$y_{14}^{2+\blacktriangle}$	719.3646	719.3646	0.0
z_2	421.2055	421.2081	6.2
z_3	808.3809	808.3802	-0.9
z_4	895.4129	895.4171	4.7
z_5	982.4449	982.4475	2.6
z_6	1145.5083	1145.5121	3.3
z_7	1428.6615	1428.6676	4.3
z_8	1527.7299	1527.7355	3.7
z_9	1624.7826	1624.7843	1.0
z_{10}	1787.8460	1787.8496	2.0

Table S13: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (LWSVDHGEQSVVT$_{(g)}$AP, 2+, *m/z* 914.43). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_2	272.1757	272.1757	0.0
a_3	359.2078	359.2076	-0.6
a_4	458.2762	458.2765	0.7
a_5	573.3031	573.3025	-1.0
a_6	710.3620	710.3655	4.9
a_7	767.3835	767.3790	-5.9
a_9	1024.4847	1024.4816	-3.0
a_{10}	1111.5167	1111.5126	-3.7
a_{11}	1210.5851	1210.5841	-0.8
a_{12}	1309.6535	1309.6570	2.7
b_2	300.1706	300.1704	-0.7
b_3	387.2027	387.2025	-0.5
b_4	486.2711	486.2712	0.2
b_5	601.2980	601.2972	-1.3
b_6	738.3569	738.3547	-3.0
b_7	795.3784	795.3757	-3.4
b_8	924.4210	924.4193	-1.8
b_9	1052.4796	1052.4800	0.4
b_{10}	1139.5116	1139.5131	1.3
b_{11}	1238.5800	1238.5807	0.6
b_{12}	1337.6484	1337.6486	0.1
b_{13}	1641.7755	1641.7672	-5.1
b_{13}▲	1438.6961	1438.6962	0.0
b_{14}▲	1509.7332	1509.7334	0.1
c_2	317.1972	317.1969	-0.9
c_3	404.2292	404.2293	0.2
c_4	503.2976	503.2983	1.4
c_5	618.3246	618.3244	-0.3
c_6	755.3835	755.3800	-4.6
c_7	812.4049	812.3983	-8.1
c_8	941.4475	941.4472	-0.3
c_9	1069.5061	1069.5028	-3.1
c_{10}	1156.5381	1156.5431	4.3
c_{11}	1255.6066	1255.6046	-1.6
c_{12}	1354.6750	1354.6802	3.8
c_{13}	1658.8020	1658.8044	1.4
x_3	517.2140	517.2180	7.7
x_5	715.3508	715.3579	9.9
x_6	802.3829	802.3799	-3.7
x_{10}	1253.5644	1253.5714	5.6

x_{11}	1368.5914	1368.5956	3.1
x_{12}	1467.6598	1467.6666	4.6
y_2	187.1077	187.1077	0.0
y_3	491.2348	491.2344	-0.8
y_3▲	288.1554	288.1551	-1.0
y_4	590.3032	590.3036	0.7
y_4▲	387.2238	387.2238	-0.1
y_5	689.3716	689.3663	-7.7
y_5▲	486.2922	486.2925	0.7
y_6	776.4036	776.4042	0.8
y_6▲	573.3243	573.3253	1.7
y_7	904.4622	904.4609	-1.4
y_7▲	701.3828	701.3830	0.3
y_{10}	1227.5852	1227.5775	-6.3
y_{11}	1342.6121	1342.6188	5.0
y_{12}	1441.6805	1441.6860	3.8
y_{13}	1528.7125	1528.7130	0.3
y_{13}▲	1325.6332	1325.6295	-2.8
y_{14}	857.8996	857.8916	-9.3
z_3	475.2160	475.2160	0.0

Table S14: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (TSVTLSLVMPSQGQMVNGAHS$_{(g)}$ASTLDEATP TLTNQSPTLTLQSTNTHTQSSSSSSDGGLFR, 4+, *m/z* 1617.52). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_3	260.1605	260.1607	0.8
a_4	361.2082	361.2086	1.1
a_5	474.2922	474.2927	1.1
a_6	561.3243	561.3254	2.0
a_7	674.4083	674.4100	2.5
a_8	773.4767	773.4773	0.8
a_9	904.5172	904.5195	2.5
a_{11}	1088.6020	1088.5989	-2.8
a_9^{2+}	452.2586	452.2576	-2.2
a_{21}^{2+}	1150.5607	1150.5565	-3.7
a_{26}^{2+}	1394.1746	1394.1774	2.0
a_{27}^{2+}	1458.6959	1458.6949	-0.7
a_{28}^{2+}	1494.2144	1494.2272	8.6
a_{29}^{2+}	1544.7383	1544.7318	-4.2
a_{30}^{2+}	1593.2646	1593.2621	-1.6
a_{31}^{2+}	1643.7885	1643.7771	-6.9
a_{32}^{2+}	1700.3305	1700.3246	-3.5
b_2	189.0870	189.0871	0.5
b_3	288.1554	288.1556	0.7
b_4	389.2031	389.2033	0.5
b_5	502.2871	502.2872	0.2
b_6	589.3192	589.3196	0.7
b_7	702.4032	702.4035	0.4
b_8	801.4716	801.4722	0.7
b_9	932.5121	932.5130	1.0
b_{15}	1560.7760	1560.7788	1.8
b_8^{2+}	401.2395	401.2396	0.2
b_{17}^{2+}	887.4473	887.4393	-9.0
b_{19}^{2+}	951.4766	951.4690	-8.0
b_{24}^{2+}	1294.6202	1294.6328	9.7
b_{25}^{2+}	1351.1622	1351.1571	-3.8
b_{26}^{2+}	1408.6757	1408.6799	3.0
b_{27}^{2+}	1473.1970	1473.2104	9.1
b_{28}^{2+}	1508.7155	1508.7216	4.0
b_{29}^{2+}	1559.2394	1559.2487	6.0
b_{30}^{2+}	1607.7657	1607.7727	4.4
b_{31}^{2+}	1658.2896	1658.2921	1.5

b_{32}^{2+}	1714.8316	1714.8273	-2.5
b_{50}^{3+}	1790.1980	1790.1943	-2.1
c_{3}	305.1819	305.1821	0.7
c_{5}	519.3137	519.3145	1.5
c_{8}	818.4982	818.4993	1.3
c_{20}^{2+}	1028.5198	1028.5133	-5.8
c_{21}^{2+}	1173.5750	1173.5739	-0.9
c_{22}^{2+}	1209.0936	1209.0929	-0.6
c_{23}^{2+}	1252.6096	1252.6129	2.6
c_{24}^{2+}	1303.1334	1303.1295	-3.0
c_{25}^{2+}	1359.6755	1359.6735	-1.5
c_{26}^{2+}	1417.1889	1417.1835	-3.8
c_{28}^{2+}	1517.2288	1517.2291	0.2
c_{29}^{2+}	1567.7526	1567.7563	2.4
c_{30}^{2+}	1616.2790	1616.2788	-0.1
c_{31}^{2+}	1666.8028	1666.7987	-2.5
c_{32}^{2+}	1723.3449	1723.3409	-2.3
x_{1}	201.0982	201.0983	0.5
x_{2}	348.1666	348.1667	0.3
x_{3}	461.2507	461.2512	1.1
x_{4}	518.2721	518.2718	-0.6
x_{5}	575.2936	575.2939	0.5
x_{7}	777.3526	777.3539	1.7
x_{8}	864.3846	864.3864	2.1
x_{9}	951.4166	951.4172	0.6
x_{10}	1038.4487	1038.4502	1.4
x_{12}	1212.5127	1212.5122	-0.4
x_{15}^{2+}	789.8426	789.8439	1.6
x_{17}^{2+}	897.3879	897.3911	3.6
x_{18}^{2+}	947.9117	947.9127	1.1
x_{19}^{2+}	991.4277	991.4286	0.9
x_{20}^{2+}	1055.4570	1055.4615	4.3
x_{21}^{2+}	1111.9991	1111.9979	-1.1
x_{22}^{2+}	1162.5229	1162.5203	-2.2
x_{23}^{2+}	1219.0649	1219.0668	1.6
x_{24}^{2+}	1269.5888	1269.5880	-0.6
x_{25}^{2+}	1318.1152	1318.1272	9.1
x_{26}^{2+}	1361.6312	1361.6270	-3.1
x_{27}^{2+}	1425.6605	1425.6648	3.0
x_{28}^{2+}	1482.6819	1482.6910	6.1
x_{29}^{2+}	1533.2058	1533.2173	7.5
x_{30}^{2+}	1589.7478	1589.7584	6.7

x_{31}^{2+}	1640.2716	1640.2734	1.1
x_{32}^{2+}	1688.7980	1688.7920	-3.6
x_{33}^{2+}	1739.3219	1739.3363	8.3
x_{34}^{2+}	1774.8404	1774.8545	7.9
y_2	322.1874	322.1875	0.3
y_3	435.2714	435.2718	0.9
y_4	492.2929	492.2930	0.2
y_5	549.3143	549.3147	0.7
y_6	664.3413	664.3417	0.6
y_7	751.3733	751.3746	1.7
y_8	838.4053	838.4059	0.7
y_9	925.4374	925.4383	1.0
y_{10}	1012.4694	1012.4702	0.8
y_{11}	1099.5014	1099.5026	1.1
y_{13}	1314.5920	1314.5935	1.1
y_{14}	1415.6397	1415.6354	-3.0
y_{16}^{2+}	827.3768	827.3771	0.4
y_{17}^{2+}	884.3983	884.3974	-1.0
y_{18}^{2+}	934.9221	934.9235	1.5
y_{19}^{2+}	978.4381	978.4391	1.0
y_{20}^{2+}	1042.4674	1042.4681	0.7
y_{23}^{2+}	1206.0753	1206.0762	0.7
y_{24}^{2+}	1256.5991	1256.5997	0.5
y_{25}^{2+}	1305.1255	1305.1238	-1.3
y_{25}^{2+}▲	1305.1255	1305.1256	0.1
y_{26}^{2+}	1348.6415	1348.6432	1.3
y_{27}^{2+}	1412.6708	1412.6721	0.9
y_{28}^{2+}	1469.6923	1469.6917	-0.4
y_{29}^{2+}	1520.2161	1520.2178	1.1
y_{30}^{2+}	1576.7582	1576.7584	0.1
y_{31}^{2+}	1627.2820	1627.2797	-1.4
y_{32}^{2+}	1675.8084	1675.8069	-0.9
y_{33}^{2+}	1726.3322	1726.3324	0.1
y_{34}^{2+}	1761.8508	1761.8506	-0.1
y_{32}^{3+}	1117.5413	1117.5469	5.0
y_{41}^{3+}	1477.0302	1477.0354	3.5
y_{49}^{3+}	1741.8133	1741.8134	0.1
z_2	306.1686	306.1689	1.0
z_3	419.2527	419.2528	0.2
z_4	476.2742	476.2745	0.6
z_5	533.2956	533.2956	0.0
z_6	648.3226	648.3231	0.8
z_7	735.3546	735.3532	-1.9

z_8	822.3866	822.3924	7.1
z_9	909.4186	909.4188	0.2
z_{10}	996.4507	996.4511	0.4
z_{11}	1083.4827	1083.4808	-1.8
z_{13}	1298.5733	1298.5696	-2.8
z_{14}	1399.6210	1399.6171	-2.8
z_{16}	1637.7276	1637.7262	-0.9
z_{15}^{2+}	768.8436	768.8498	8.1
z_{16}^{2+}	819.3674	819.3654	-2.4
z_{18}^{2+}	926.9127	926.9117	-1.1
z_{20}^{2+}	1034.4580	1034.4567	-1.3
z_{22}^{2+}	1141.5239	1141.5182	-5.0
z_{24}^{2+}	1248.5898	1248.5898	0.0
z_{25}^{2+}	1297.1162	1297.1196	2.6
z_{26}^{2+}	1340.6322	1340.6364	3.1
z_{27}^{2+}	1404.6615	1404.6642	1.9
z_{28}^{2+}	1461.6829	1461.6843	1.0
z_{29}^{2+}	1512.2068	1512.2067	-0.1
z_{31}^{2+}	1619.2726	1619.2648	-4.8
z_{33}^{2+}	1718.3229	1718.3218	-0.6

Table S15: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (TSVTLSLVMPSQGQMVNGAHSASTLDEATP-TLTNQSPTLTLQSTNTHT$_{(g)}$QSSSSSSDGGLFR, 4+, *m/z* 1617.52). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_3	260.1605	260.1606	0.4
a_4	361.2082	361.2076	-1.7
a_5	474.2922	474.2922	0.0
a_7	674.4083	674.4089	0.9
a_8	773.4767	773.4775	1.0
a_9	904.5172	904.5170	-0.2
a_9^{2+}	452.2586	452.2582	-0.9
a_{31}^{2+}	1542.2488	1542.2433	-3.6
a_{32}^{2+}	1598.7908	1598.7877	-1.9
b_2	189.0870	189.0870	0.0
b_3	288.1554	288.1553	-0.3
b_4	389.2031	389.2024	-1.8
b_5	502.2871	502.2870	-0.2
b_6	589.3192	589.3190	-0.3
b_7	702.4032	702.4033	0.1
b_8	801.4716	801.4717	0.1
b_9	932.5121	932.5126	0.5
b_{15}	1560.7760	1560.7613	-9.4
b_{25}^{2+}	1249.6225	1249.6123	-8.2
b_{29}^{2+}	1457.6997	1457.6875	-8.4
b_{32}^{2+}	1613.2919	1613.2977	3.6
b_{35}^{2+}	1784.8665	1784.8520	-8.1
b_{49}^{3+}	1761.1873	1761.2014	8.0
b_{50}^{3+}	1790.1980	1790.1940	-2.2
c_3	305.1819	305.1817	-0.7
c_4	406.2296	406.2286	-2.5
c_5	519.3137	519.3134	-0.6
c_8	818.4982	818.5009	3.3
c_9	949.5387	949.5302	-9.0
c_{24}^{2+}	1151.0699	1151.0688	-1.0
c_{31}^{2+}	1514.7393	1514.7309	-5.5
c_{32}^{2+}	1565.2632	1565.2507	-8.0
x_1	201.0982	201.0982	0.0
x_2	348.1666	348.1664	-0.6
x_3	461.2507	461.2504	-0.7
x_4	518.2721	518.2719	-0.4
x_5	575.2936	575.2929	-1.2
x_6	690.3205	690.3217	1.7

x_7	777.3526	777.3541	1.9
x_8	864.3846	864.3821	-2.9
x_9	951.4166	951.4167	0.1
x_{10}	1038.4487	1038.4462	-2.4
x_{11}	1125.4807	1125.4807	0.0
x_{12}	1212.5127	1212.5127	0.0
x_7^{2+}	389.1799	389.1832	8.5
y_2	322.1874	322.1873	-0.3
y_3	435.2714	435.2716	0.5
y_4	492.2929	492.2933	0.8
y_5	549.3143	549.3144	0.2
y_6	664.3413	664.3412	-0.2
y_7	751.3733	751.3740	0.9
y_8	838.4053	838.4049	-0.5
y_9	925.4374	925.4384	1.1
y_{10}	1012.4694	1012.4688	-0.6
y_{11}	1099.5014	1099.5028	1.3
y_{12}	1186.5335	1186.5298	-3.1
y_{13}	1314.5920	1314.5923	0.2
y_{14}	1618.7191	1618.7260	4.3
y_{15}	1755.7780	1755.7890	6.3
y_{26}^{2+}	1450.1812	1450.1879	4.6
y_{25}^{2+}▲	1305.1255	1305.1256	0.1
y_{28}^{2+}	1571.2320	1571.2399	5.0
y_{29}^{2+}	1621.7558	1621.7544	-0.9
y_{41}^{3+}	1477.0302	1477.0210	-6.2
y_{49}^{3+}	1741.8133	1741.8300	9.6
z_2	306.1686	306.1688	0.7
z_3	419.2527	419.2524	-0.7
z_4	476.2742	476.2738	-0.8
z_5	533.2956	533.2957	0.2
z_6	648.3226	648.3225	-0.2
z_7	735.3546	735.3535	-1.5
z_8	822.3866	822.3843	-2.8
z_9	909.4186	909.4196	1.1
z_{10}	996.4507	996.4516	0.9
z_{11}	1083.4827	1083.4838	1.0
z_{12}	1170.5147	1170.5128	-1.6
z_{13}	1298.5733	1298.5709	-1.8
z_{28}^{2+}	1563.2226	1563.2202	-1.5

Table S16: Complete list of ions generated by EThcD of a tryptic peptide from TAB1 glycoprotein using 50 ms ETD and 25% supplemental energy to activate the peptide (VYPVSVPYSS$_{(g)}$AQSTSK, 2+, m/z 951.97). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_2	263.1390	263.1389	-0.4
a_3	360.1918	360.1918	0.0
a_4	459.2602	459.2602	0.0
a_5	546.2922	546.2921	-0.2
a_6	645.3606	645.3607	0.2
a_7	742.4134	742.4117	-2.3
a_8	905.4767	905.4761	-0.7
a_9	992.5087	992.5121	3.4
c_{10}	1299.6467	1299.6447	-1.5
c_{11}	1370.6838	1370.6864	1.9
c_{12}	1498.7424	1498.7385	-2.6
c_{13}	1585.7744	1585.7721	-1.5
c_{15}	1773.8541	1773.8472	-3.9
y_1	147.1128	147.1127	-0.7
y_2	234.1448	234.1450	0.9
y_3	335.1925	335.1926	0.3
y_4	422.2245	422.2239	-1.4
y_5	550.2831	550.2816	-2.7
y_6	621.3202	621.3191	-1.8
y_7	911.4316	911.4345	3.2
y_7▲	708.3523	708.3540	2.4
y_8	998.4637	998.4646	0.9
y_8▲	795.3843	795.3842	-0.1
y_9	1161.5270	1161.5265	-0.4
y_{10}	1258.5798	1258.5798	0.0
y_{10}▲	1055.5004	1055.5010	0.6
y_{11}	1357.6482	1357.6492	0.7
y_{11}▲	1154.5688	1154.5697	0.8
y_{12}	1444.6802	1444.6799	-0.2
y_{12}▲	1241.6008	1241.6022	1.1
y_{13}	1543.7486	1543.7520	2.2
y_{14}	1640.8014	1640.8004	-0.6
y_{14}▲	1437.7220	1437.7222	0.1
y_{15}▲	1600.7853	1600.7850	-0.2
y_{10}^{2+}	629.7935	629.7957	3.5
y_{11}^{2+}	679.3277	679.3250	-4.0
y_{14}^{2+}▲	719.3646	719.3646	0.0
y_{15}^{2+}▲	800.8963	800.8969	0.7
z_6	605.3015	605.3008	-1.2

z_7	895.4129	895.4066	-7.0
z_8	982.4449	982.4484	3.6
z_9	1145.5083	1145.5103	1.7
z_{11}	1341.6294	1341.6312	1.3
z_{12}	1428.6615	1428.6582	-2.3
z_{13}	1527.7299	1527.7295	-0.3
z_{15}	1787.8460	1787.8423	-2.1

Table S17: Complete list of ions generated by EThcD of a tryptic peptide from TAB1 glycoprotein using 50 ms ETD and 25% supplemental energy to activate the peptide (VYPVSVPYS$_{(g)}$SAQSTSK, 2+, m/z 951.97). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
b_2	263.1390	263.1390	0.0
b_3	360.1918	360.1917	-0.3
b_4	459.2602	459.2602	0.0
b_5	546.2922	546.2923	0.2
b_6	645.3606	645.3607	0.2
b_7	742.4134	742.4092	-5.7
b_8	905.4767	905.4782	1.7
c_9	1212.6147	1212.6122	-2.1
c_{10}	1299.6467	1299.6456	-0.8
c_{11}	1370.6838	1370.6874	2.6
c_{12}	1498.7424	1498.7385	-2.6
c_{15}	1773.8541	1773.8507	-1.9
y_1	147.1128	147.1128	0.0
y_2	234.1448	234.1446	-0.9
y_3	335.1925	335.1924	-0.3
y_4	422.2245	422.2238	-1.7
y_5	550.2831	550.2828	-0.5
y_6	621.3202	621.3186	-2.6
y_7	708.3523	708.3528	0.7
$y_7^{\blacktriangle}$	708.3523	708.3538	2.1
y_8	998.4637	998.4664	2.7
$y_8^{\blacktriangle}$	795.3843	795.3853	1.3
y_9	1161.5270	1161.5270	0.0
y_{10}	1258.5798	1258.5801	0.2
$y_{10}^{\blacktriangle}$	1055.5004	1055.5005	0.1
y_{11}	1357.6482	1357.6494	0.9
$y_{11}^{\blacktriangle}$	1154.5688	1154.5697	0.8
y_{12}	1444.6802	1444.6805	0.2
$y_{12}^{\blacktriangle}$	1241.6008	1241.6022	1.1
y_{13}	1543.7486	1543.7490	0.3
$y_{14}^{\blacktriangle}$	1437.7220	1437.7240	1.4
y_{14}	1640.8014	1640.8007	-0.4
y_{10}^{2+}	629.7935	629.7947	1.9
y_{11}^{2+}	679.3277	679.3300	3.4
y_{14}^{2+}	820.9043	820.9044	0.1
$y_{14}^{2+\blacktriangle}$	719.3646	719.3646	0.0
y_{15}^{2+}	902.4360	902.4369	1.0
$y_{15}^{2+\blacktriangle}$	800.8963	800.8969	0.7

z_7	692.3335	692.3344	1.3
z_9	1145.5083	1145.5133	4.4
z_{11}	1341.6294	1341.6324	2.2
z_{12}	1428.6615	1428.6699	5.9
z_{13}	1527.7299	1527.7269	-2.0
z_{15}	1787.8460	1787.8439	-1.2

Table S18: Complete list of ions generated by EThcD of a tryptic peptide from TAB1 glycoprotein using 50 ms ETD and 25% supplemental energy to activate the peptide (VYPVSVPYSSAQS$_{(g)}$TSK, 2+, m/z 951.97). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
b_2	263.1390	263.1389	-0.4
b_3	360.1918	360.1917	-0.3
b_4	459.2602	459.2599	-0.7
b_5	546.2922	546.2920	-0.4
b_6	645.3606	645.3605	-0.2
b_7	742.4134	742.4145	1.5
b_8	905.4767	905.4750	-1.9
b_9	992.5087	992.5079	-0.8
b_{12}	1278.6365	1278.6403	3.0
c_{12}	1295.6630	1295.6610	-1.5
c_{13}	1585.7744	1585.7743	-0.1
c_{15}	1773.8541	1773.8518	-1.3
y_1	147.1128	147.1126	-1.4
y_2	234.1448	234.1446	-0.9
y_3	335.1925	335.1927	0.6
y_4	625.3039	625.3065	4.2
y_4▲	422.2245	422.2250	1.2
y_5	753.3625	753.3661	4.8
y_6	824.3996	824.4019	2.8
y_6▲	621.3202	621.3210	1.3
y_7	911.4316	911.4299	-1.9
y_7▲	708.3523	708.3538	2.1
y_8	998.4637	998.4656	1.9
y_9	1161.5270	1161.5299	2.5
y_{10}	1258.5798	1258.5797	-0.1
y_{10}▲	1055.5004	1055.5005	0.1
y_{11}	1357.6482	1357.6481	-0.1
y_{11}▲	1154.5688	1154.5697	0.8
y_{12}	1444.6802	1444.6801	-0.1
y_{12}▲	1241.6008	1241.6022	1.1
y_{13}	1543.7486	1543.7502	1.0
y_{14}	1640.8014	1640.8046	2.0
y_{14}▲	1437.7220	1437.7240	1.4
y_{15}▲	1600.7853	1600.7840	-0.8
y_{10}^{2+}	629.7935	629.7933	-0.3
y_{11}^{2+}	679.3277	679.3251	-3.8
y_{14}^{2+}	820.9043	820.9042	-0.1
y_{14}^{2+}▲	719.3646	719.3646	0.0

y_{15}^{2+}	902.4360	902.4358	-0.2
$y_{15}^{2+▲}$	800.8963	800.8969	0.7
z_5	737.3438	737.3425	-1.8
z_6	808.3809	808.3851	5.2
z_7	895.4129	895.4124	-0.6
z_8	982.4449	982.4487	3.9
z_9	1145.5083	1145.5057	-2.3
z_{11}	1341.6294	1341.6299	0.4
z_{12}	1428.6615	1428.6534	-5.7
z_{13}	1527.7299	1527.7290	-0.6
z_{15}	1787.8460	1787.8477	1.0

Table S19: Complete list of ions generated by EThcD of a tryptic peptide from TAB1 glycoprotein using 50 ms ETD and 25% supplemental energy to activate the peptide (LWSVDHGEQSVVT$_{(g)}$AP, 2+, *m/z* 914.43). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
b_2	300.1706	300.1707	0.3
b_6	738.3569	738.3556	-1.8
b_8	924.4210	924.4221	1.2
b_9	1052.4796	1052.4814	1.7
b_{10}	1139.5116	1139.5183	5.9
b_{11}	1238.5800	1238.5812	1.0
b_{12}	1337.6484	1337.6488	0.3
b_{14}▲	1509.7332	1509.7334	0.1
c_6	755.3835	755.3799	-4.8
c_7	812.4049	812.4052	0.4
c_8	941.4475	941.4412	-6.7
c_9	1069.5061	1069.5055	-0.6
c_{10}	1156.5381	1156.5386	0.4
c_{11}	1255.6066	1255.6151	6.8
c_{12}	1354.6750	1354.6748	-0.1
c_{13}	1658.8020	1658.7994	-1.6
y_1	116.0706	116.0707	0.9
y_2	187.1077	187.1078	0.5
y_3	491.2348	491.2333	-3.1
y_3▲	288.1554	288.1555	0.2
y_4▲	387.2238	387.2239	0.1
y_5▲	486.2922	486.2942	4.0
y_{11}	1342.6121	1342.6097	-1.8
y_{11}^{2+}▲	570.2700	570.2700	-0.1
y_{12}^{2+}▲	619.8042	619.7997	-7.3
y_{13}	1528.7125	1528.7189	4.2
z_{11}	1326.5934	1326.5957	1.7
z_{12}	1425.6618	1425.6713	6.7
z_{13}	1512.6938	1512.6932	-0.4
z_{14}	1698.7731	1698.7721	-0.6

Table S20: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide ($GSS_{(g)}$HHHHHHSSGLVPR, 4+, *m/z* 493.73). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_3	407.1773	407.1763	-2.5
a_4	544.2362	544.2322	-7.3
a_5	681.2951	681.2953	0.3
a_6	818.3540	818.3542	0.2
a_7	955.4129	955.4185	5.9
a_6^{2+}	409.1770	409.1774	1.0
a_7^{2+}	477.7064	477.7083	4.0
a_8^{2+}	546.2359	546.2378	3.5
a_9^{2+}	614.7654	614.7598	-9.1
b_4▲	369.1517	369.1510	-1.9
b_5▲	506.2106	506.2105	-0.1
b_6▲	643.2695	643.2671	-3.7
b_7▲	780.3284	780.3276	-1.0
b_8▲	917.3874	917.3854	-2.1
b_3^{2+}	218.0897	218.0901	1.8
b_8^{2+}	560.7370	560.7370	0.0
b_9^{2+}	629.2665	629.2726	9.7
b_{12}^{2+}	744.8092	744.8125	4.4
b_{13}^{2+}	801.3512	801.3527	1.9
b_{14}^{2+}	850.8854	850.8862	0.9
b_{15}^{3+}	599.9436	599.9446	1.7
c_2	162.0873	162.0875	1.2
c_3	452.1987	452.2015	6.2
c_{13}^{2+}	809.8645	809.8692	5.8
x_1	201.0982	201.0983	0.5
x_3	397.2194	397.2187	-1.8
x_4	510.3034	510.3056	4.3
x_5	567.3249	567.3256	1.2
x_6	654.3569	654.3579	1.5
x_7	741.3890	741.3914	3.2
x_8	878.4479	878.4503	2.7
x_8^{2+}	439.7276	439.7288	2.7
x_9^{2+}	508.2570	508.2571	0.2
x_{10}^{2+}	576.7865	576.7892	4.7
x_{11}^{2+}	645.3159	645.3174	2.3
y_1	175.1190	175.1191	0.6
y_2	272.1717	272.1721	1.5
y_3	371.2401	371.2402	0.3

y_4	484.3242	484.3237	-1.0
y_5	541.3456	541.3462	1.1
y_6	628.3777	628.3785	1.3
y_7	715.4097	715.4105	1.1
y_8	852.4686	852.4694	0.9
y_9	989.5275	989.5294	1.9
y_{10}	1126.5864	1126.5869	0.4
y_6^{2+}	314.6925	314.6919	-1.9
y_7^{2+}	358.2085	358.2079	-1.7
y_8^{2+}	426.7379	426.7383	0.9
y_9^{2+}	495.2674	495.2644	-6.1
y_{10}^{2+}	563.7969	563.7969	0.0
y_{11}^{2+}	632.3263	632.3264	0.2
y_{12}^{2+}	700.8558	700.8565	1.0
y_{13}^{2+}	769.3852	769.3868	2.1
y_{14}^{2+}	914.4409	914.4399	-1.1
z_1	159.1002	159.1004	1.3
z_2	256.1530	256.1538	3.1
z_3	355.2214	355.2215	0.3
z_4	468.3055	468.3070	3.2
z_5	525.3269	525.3280	2.1
z_6	612.3589	612.3578	-1.8
z_7	699.3910	699.3900	-1.4
z_8	836.4499	836.4489	-1.2
z_9	973.5088	973.5038	-5.1
z_8^{2+}	418.7286	418.7259	-6.4
z_9^{2+}	487.2580	487.2592	2.5
z_{10}^{2+}	555.7875	555.7879	0.7
z_{11}^{2+}	624.3169	624.3182	2.1
z_{12}^{2+}	692.8464	692.8455	-1.3

Table S21: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (GSSHHHHHHS$_{(g)}$SGLVPR, 4+, *m/z* 493.73). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
z_4	341.1568	341.1585	5.0
z_5	478.2157	478.2175	3.8
z_6	615.2746	615.2782	5.9
z_7	752.3335	752.3380	6.0
z_8	889.3924	889.3973	5.5
b_3	232.0928	232.0945	7.3
b_4	369.1517	369.1534	4.6
b_5	506.2106	506.2119	2.6
b_6	643.2695	643.2731	5.6
b_7	780.3284	780.3334	6.4
b_8	917.3873	917.3928	6.0
b_6^{2+}	322.1384	322.1399	4.7
b_7^{2+}	390.6679	390.6681	0.5
b_8^{2+}	459.1973	459.1995	4.8
b_9^{2+}	527.7268	527.7297	5.5
b_{12}^{2+}	744.8092	744.8111	2.6
b_{13}^{2+}	801.3512	801.3561	6.1
b_{14}^{2+}	850.8854	850.8901	5.5
b_{15}^{3+}	599.9436	599.9461	4.2
c_3	249.1193	249.1196	1.2
c_4	386.1783	386.1801	4.7
c_6	660.2961	660.2983	3.3
c_7	797.3550	797.3581	3.9
c_6^{2+}	330.6517	330.6528	3.3
c_7^{2+}	399.1811	399.1860	12.3
c_8^{2+}	467.7106	467.7093	-2.8
c_9^{2+}	536.2400	536.2419	3.5
c_{10}^{2+}	681.2957	681.2999	6.2
c_{13}^{2+}	809.8645	809.8698	6.5
x_1	201.0982	201.0990	4.0
x_3	397.2194	397.2222	7.0
x_4	510.3034	510.3035	0.2
x_5	567.3249	567.3287	6.7
x_6	654.3569	654.3594	3.8
x_9^{2+}	609.7967	609.8019	8.5
x_{10}^{2+}	678.3262	678.3271	1.3
y_1	175.1190	175.1197	4.0
y_2	272.1717	272.1731	5.1

y_3	371.2401	371.2422	5.7
y_4	484.3242	484.3267	5.2
y_5	541.3456	541.3477	3.9
y_6	628.3777	628.3809	5.1
y_7	918.4891	918.4933	4.6
$y_7^{\blacktriangle}$	715.4097	715.4141	6.1
$y_8^{\blacktriangle}$	852.4686	852.4741	6.5
$y_9^{\blacktriangle}$	989.5275	989.5335	6.1
$y_{10}^{\blacktriangle}$	1126.5826	1126.5925	8.8
y_6^{2+}	314.6925	314.6943	5.7
y_9^{2+}	596.8071	596.8091	3.4
$y_{11}^{2+\blacktriangle}$	632.3263	632.3305	6.7
$y_{12}^{2+\blacktriangle}$	700.8558	700.8596	5.4
z_1	159.1002	159.1010	5.0
z_2	256.1530	256.1507	-9.0
z_3	355.2214	355.2231	4.8
z_4	468.3055	468.3071	3.4
z_5	525.3269	525.3303	6.5
z_6	612.3589	612.3626	6.0
z_7	902.4703	902.4740	4.1
z_8	1039.5293	1039.5345	5.0
z_8^{2+}	520.2683	520.2722	7.5
z_9^{2+}	588.7977	588.7988	1.9
z_{10}^{2+}	657.3272	657.3303	4.7
z_{11}^{2+}	725.8566	725.8628	8.5

Table S22: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (LSESFPILGASTEVPPMS$_{(g)}$LPVQAAISAPSP-LAMPLGSPLSVALPTLAPLSVVTSGAAPK, 5+, *m/z* 1188.24). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_2	173.1285	173.1286	0.6
a_3	302.1710	302.1712	0.7
a_4	389.2031	389.2032	0.3
a_5	536.2715	536.2720	0.9
a_6	633.3242	633.3235	-1.1
a_7	746.4083	746.4067	-2.1
a_8	859.4924	859.4941	2.0
a_{10}	987.5509	987.5492	-1.7
a_{11}	1074.5830	1074.5873	4.0
a_{12}	1175.6306	1175.6359	4.5
a_{13}	1304.6732	1304.6765	2.5
a_{14}	1403.7416	1403.7434	1.3
a_{15}	1500.7944	1500.8040	6.4
a_{16}	1597.8472	1597.8594	7.6
a_{17}	1728.8876	1728.9029	8.8
a_7^{2+}	373.2042	373.2054	3.2
a_{17}^{2+}	864.4438	864.4491	6.1
a_{24}^{2+}	1299.1685	1299.1811	9.7
a_{25}^{2+}	1355.7106	1355.7218	8.3
a_{31}^{3+}	1088.9114	1088.9159	4.1
a_{35}^{3+}	1226.3162	1226.3181	1.5
a_{36}^{3+}	1245.3234	1245.3320	6.9
a_{38}^{3+}	1306.6850	1306.6853	0.2
a_{39}^{3+}	1344.3797	1344.3806	0.7
a_{43}^{3+}	1467.7869	1467.7858	-0.7
a_{46}^{3+}	1571.5150	1571.5120	-1.9
a_{47}^{3+}	1595.1941	1595.1934	-0.4
a_{48}^{3+}	1627.5450	1627.5548	6.0
b_2	201.1234	201.1236	1.0
b_3	330.1660	330.1661	0.3
b_4	417.1980	417.1981	0.2
b_5	564.2664	564.2670	1.1
b_6	661.3192	661.3195	0.5
b_7	774.4032	774.4040	1.0
b_8	887.4873	887.4888	1.7
b_9	944.5087	944.4995	-9.7
b_{10}	1015.5458	1015.5410	-4.7
b_{11}	1102.5779	1102.5861	7.4

b_{13}	1332.6681	1332.6674	-0.5
b_{14}	1431.7366	1431.7400	2.4
b_{15}	1528.7893	1528.8032	9.1
b_{17}	1756.8826	1756.8800	-1.5
b_{18}	2046.9940	2046.9943	0.1
b_{19}	2160.0781	2160.0776	-0.2
b_{13}^{2+}	666.8377	666.8400	3.4
b_{14}^{2+}	716.3719	716.3727	1.1
b_{15}^{2+}	764.8983	764.8980	-0.4
b_{17}^{2+}	878.9449	878.9419	-3.4
b_{24}^{2+}	1313.6696	1313.6801	8.0
b_{26}^{2+}	1413.7277	1413.7278	0.1
b_{27}^{2+}	1449.2462	1449.2454	-0.6
b_{31}^{2+}	1646.3570	1646.3612	2.6
b_{33}^{2+}	1747.3958	1747.3976	1.0
b_{27}^{3+}	966.4999	966.5000	0.1
b_{31}^{3+}	1097.9071	1097.9133	5.6
b_{33}^{3+}	1165.2663	1165.2710	4.0
b_{35}^{3+}	1235.3119	1235.3191	5.8
b_{36}^{3+}	1254.3191	1254.3250	4.7
b_{37}^{3+}	1283.3297	1283.3337	3.1
b_{42}^{3+}	1439.0879	1439.0799	-5.6
b_{43}^{3+}	1476.7826	1476.7811	-1.0
b_{46}^{3+}	1580.5107	1580.5129	1.4
b_{47}^{3+}	1604.1898	1604.2002	6.5
b_{47}^{4+}	1203.3941	1203.3834	-8.9
b_{49}^{4+}	1255.9283	1255.9383	8.0
b_{51}^{4+}	1302.4535	1302.4447	-6.8
b_{52}^{4+}	1327.2206	1327.2335	9.7
b_{53}^{4+}	1352.4825	1352.4911	6.4
b_{54}^{4+}	1374.2405	1374.2537	9.6
b_{55}^{4+}	1388.4959	1388.4821	-9.9
b_{56}^{4+}	1406.2551	1406.2518	-2.4
c_2	218.1499	218.1502	1.4
c_3	347.1925	347.1927	0.6
c_4	434.2245	434.2249	0.9
c_6	678.3457	678.3464	1.0
c_7	791.4298	791.4298	0.0
c_8	904.5138	904.5126	-1.3
c_9	961.5353	961.5359	0.6
c_{10}	1032.5724	1032.5810	8.3
c_{11}	1119.6044	1119.6071	2.4

c_{13}	1349.6947	1349.7010	4.7
c_{15}	1545.8159	1545.8308	9.6
c_{16}	1642.8686	1642.8708	1.3
x_{1}	173.0921	173.0923	1.2
x_{3}	341.1819	341.1820	0.3
x_{4}	412.2190	412.2182	-1.9
x_{5}	469.2405	469.2387	-3.8
x_{6}	556.2725	556.2752	4.9
x_{8}	756.3886	756.3885	-0.1
x_{10}	942.4891	942.4977	9.1
x_{11}	1055.5731	1055.5651	-7.6
x_{12}	1152.6259	1152.6350	7.9
x_{14}	1336.7471	1336.7428	-3.2
x_{16}	1534.8475	1534.8620	9.4
x_{11}^{2+}	528.2902	528.2852	-9.5
x_{13}^{2+}	612.3351	612.3369	2.9
x_{15}^{2+}	719.4010	719.3974	-5.0
x_{16}^{2+}	767.9274	767.9350	9.9
x_{17}^{2+}	824.4694	824.4738	5.3
x_{18}^{2+}	859.9880	859.9898	2.1
x_{19}^{2+}	909.5222	909.5237	1.6
x_{21}^{2+}	1009.5802	1009.5804	0.2
x_{29}^{2+}	1392.7826	1392.7760	-4.7
x_{32}^{2+}	1533.3514	1533.3423	-5.9
x_{35}^{2+}	1668.9280	1668.9292	0.7
y_{2}	244.1656	244.1657	0.4
y_{3}	315.2027	315.2029	0.6
y_{4}	386.2398	386.2398	0.0
y_{5}	443.2612	443.2617	1.1
y_{6}	530.2933	530.2939	1.1
y_{7}	631.3410	631.3420	1.6
y_{8}	730.4094	730.4102	1.1
y_{9}	829.4778	829.4799	2.5
y_{10}	916.5098	916.5114	1.7
y_{11}	1029.5939	1029.6013	7.2
y_{12}	1126.6466	1126.6495	2.6
y_{13}	1197.6837	1197.6866	2.4
y_{14}	1310.7678	1310.7751	5.6
y_{16}	1508.8682	1508.8724	2.8
y_{17}	1621.9523	1621.9584	3.8
y_{18}	1692.9894	1692.9963	4.1
y_{19}	1792.0578	1792.0530	-2.7
y_{12}^{2+}	563.8269	563.8265	-0.7
y_{13}^{2+}	599.3455	599.3452	-0.5
y_{14}^{2+}	655.8875	655.8860	-2.3

y_{15}^{2+}	706.4114	706.4107	-1.0
y_{16}^{2+}	754.9378	754.9382	0.5
y_{17}^{2+}	811.4798	811.4810	1.5
y_{18}^{2+}	846.9983	846.9995	1.4
y_{19}^{2+}	896.5325	896.5341	1.8
y_{20}^{2+}	940.0486	940.0494	0.9
y_{21}^{2+}	996.5906	996.5960	5.4
y_{22}^{2+}	1045.1170	1045.1173	0.3
y_{23}^{2+}	1088.6330	1088.6360	2.8
y_{24}^{2+}	1117.1437	1117.1459	2.0
y_{26}^{2+}	1222.2121	1222.2144	1.9
y_{27}^{2+}	1287.7324	1287.7372	3.7
y_{28}^{2+}	1323.2509	1323.2594	6.4
y_{30}^{2+}	1428.3193	1428.3134	-4.1
y_{31}^{2+}	1471.8353	1471.8455	6.9
y_{32}^{2+}	1520.3617	1520.3689	4.7
y_{33}^{2+}	1555.8803	1555.8871	4.4
y_{34}^{2+}	1599.3963	1599.4034	4.4
y_{35}^{2+}	1655.9383	1655.9492	6.6
y_{36}^{2+}	1691.4569	1691.4501	-4.0
y_{22}^{3+}	697.0804	697.0795	-1.3
y_{23}^{3+}	726.0911	726.0924	1.8
y_{24}^{3+}	745.0982	745.0974	-1.1
y_{26}^{3+}	815.1438	815.1429	-1.1
y_{28}^{3+}	882.5030	882.5063	3.7
y_{29}^{3+}	920.1977	920.1969	-0.9
y_{30}^{3+}	952.5486	952.5484	-0.2
y_{31}^{3+}	981.5593	981.5632	4.0
y_{34}^{3+}	1066.6000	1066.6025	2.3
y_{35}^{3+}	1104.2946	1104.2988	3.8
y_{36}^{3+}	1127.9737	1127.9654	-7.4
y_{40}^{3+}	1259.7126	1259.7192	5.2
y_{45}^{3+}	1502.4931	1502.5030	6.6
y_{40}^{4+}	945.0363	945.0372	1.0
y_{43}^{4+}	1078.5953	1078.5879	-6.9
y_{49}^{4+}	1231.1693	1231.1653	-3.2
y_{50}^{4+}	1248.9286	1248.9260	-2.1
y_{52}^{4+}	1291.4550	1291.4600	3.9
y_{53}^{4+}	1319.7260	1319.7250	-0.8
y_{57}^{4+}	1434.7750	1434.7640	-7.7

z_3	299.1839	299.1842	1.0
z_4	370.2211	370.2213	0.5
z_5	427.2425	427.2431	1.4
z_6	514.2745	514.2751	1.2
z_7	615.3222	615.3226	0.7
z_8	714.3906	714.3907	0.1
z_9	813.4590	813.4580	-1.2
z_{10}	900.4911	900.4889	-2.4
z_{11}	1013.5751	1013.5754	0.3
z_{13}	1181.6650	1181.6555	-8.0
z_{10}^{2+}	450.7492	450.7490	-0.4
z_{11}^{2+}	507.2912	507.2911	-0.2
z_{13}^{2+}	591.3361	591.3364	0.5
z_{14}^{2+}	647.8782	647.8788	0.9
z_{17}^{2+}	803.4704	803.4714	1.2
z_{18}^{2+}	838.9890	838.9901	1.3
z_{19}^{2+}	888.5232	888.5313	9.1
z_{20}^{2+}	932.0392	932.0414	2.4
z_{21}^{2+}	988.5812	988.5760	-5.3
z_{22}^{2+}	1037.1076	1037.1077	0.1
z_{25}^{2+}	1165.6764	1165.6782	1.5
z_{28}^{2+}	1315.2416	1315.2292	-9.4
z_{30}^{2+}	1420.3100	1420.2990	-7.7
z_{31}^{2+}	1463.8260	1463.8142	-8.1
z_{32}^{2+}	1512.3524	1512.3625	6.7
z_{34}^{2+}	1591.3869	1591.3914	2.8

Table S23: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (LSESFPILGASTEVPPMSLPVQAAIS$_{(g)}$APSP-LAMPLGSPLSVALPTLAPLSVVTSGAAPK, 5+, m/z 1188.24). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_2	173.1285	173.1284	-0.6
a_3	302.1710	302.1707	-1.0
a_4	389.2031	389.2021	-2.6
a_5	536.2715	536.2697	-3.4
a_7	746.4083	746.4058	-3.3
a_8	859.4924	859.4971	5.5
a_{10}	987.5509	987.5500	-0.9
a_{13}	1304.6732	1304.6812	6.1
a_{14}	1403.7416	1403.7388	-2.0
a_{15}	1500.7944	1500.7982	2.5
a_{17}	1728.8876	1728.8846	-1.7
a_7^{2+}	373.2042	373.2078	9.6
a_{12}^{2+}	587.8153	587.8176	3.9
a_{17}^{2+}	864.4438	864.4445	0.8
a_{43}^{3+}	1467.7869	1467.7894	1.7
b_2	201.1234	201.1233	-0.5
b_3	330.1660	330.1657	-0.9
b_4	417.1980	417.1978	-0.5
b_5	564.2664	564.2660	-0.7
b_7	774.4032	774.4025	-0.9
b_8	887.4873	887.4873	0.0
b_9	944.5087	944.5046	-4.3
b_{10}	1015.5458	1015.5406	-5.1
b_{11}	1102.5779	1102.5780	0.1
b_{12}	1203.6256	1203.6189	-5.6
b_{13}	1332.6681	1332.6660	-1.6
b_{14}	1431.7366	1431.7366	0.0
b_{15}	1528.7893	1528.7870	-1.5
b_{17}	1756.8826	1756.8792	-1.9
b_6^{2+}	331.1632	331.1661	8.8
b_{13}^{2+}	666.8377	666.8378	0.1
b_{14}^{2+}	716.3719	716.3705	-2.0
b_{15}^{2+}	764.8983	764.9037	7.1
b_{19}^{2+}	979.0030	979.0051	2.1
b_{23}^{2+}	1176.6114	1176.6038	-6.5
b_{24}^{2+}	1212.1299	1212.1295	-0.3
b_{25}^{2+}	1268.6720	1268.6687	-2.6
b_{26}^{2+}	1413.7277	1413.7269	-0.6

b_{27}^{2+}	1449.2462	1449.2455	-0.5
b_{31}^{2+}	1646.3570	1646.3560	-0.6
b_{33}^{2+}	1747.3958	1747.3933	-1.4
b_{33}^{3+}	1165.2663	1165.2571	-7.9
b_{37}^{3+}	1283.3297	1283.3235	-4.8
b_{41}^{3+}	1415.4088	1415.4190	7.2
b_{43}^{3+}	1476.7826	1476.7703	-8.3
b_{46}^{3+}	1580.5107	1580.4990	-7.4
b_{56}^{4+}	1406.2551	1406.2515	-2.6
c_2	218.1499	218.1499	0.0
c_3	347.1925	347.1922	-0.9
c_4	434.2245	434.2239	-1.4
c_8	904.5138	904.5212	8.2
c_{10}	1032.5724	1032.5804	7.7
c_{11}	1119.6044	1119.6082	3.4
c_{13}	1349.6947	1349.7020	5.4
c_{15}	1545.8159	1545.8146	-0.8
c_7^{2+}	396.2185	396.2222	9.3
c_{14}^{2+}	724.8852	724.8831	-2.9
c_{18}^{2+}	930.9742	930.9804	6.7
c_{20}^{2+}	1036.0426	1036.0503	7.4
x_1	173.0921	173.0921	0.0
x_2	270.1448	270.1447	-0.4
x_3	341.1819	341.1813	-1.8
x_4	412.2190	412.2199	2.2
x_5	469.2405	469.2404	-0.2
x_6	556.2725	556.2749	4.3
x_8	756.3886	756.3837	-6.5
x_9	855.4570	855.4510	-7.0
x_{11}	1055.5731	1055.5685	-4.4
x_{14}	1336.7471	1336.7351	-9.0
x_{15}	1437.7947	1437.7878	-4.8
x_{16}	1534.8475	1534.8614	9.1
x_{13}^{2+}	612.3351	612.3365	2.3
x_{15}^{2+}	719.4010	719.3943	-9.3
x_{21}^{2+}	1009.5802	1009.5767	-3.5
y_2	244.1656	244.1653	-1.2
y_3	315.2027	315.2023	-1.3
y_4	386.2398	386.2395	-0.8
y_5	443.2612	443.2610	-0.5
y_6	530.2933	530.2932	-0.2
y_7	631.3410	631.3406	-0.6
y_8	730.4094	730.4091	-0.4
y_9	829.4778	829.4816	4.6

y_{10}	916.5098	916.5115	1.9
y_{11}	1029.5939	1029.5975	3.5
y_{12}	1126.6466	1126.6462	-0.4
y_{13}	1197.6837	1197.6860	1.9
y_{14}	1310.7678	1310.7698	1.5
y_{15}	1411.8155	1411.8184	2.1
y_{16}	1508.8682	1508.8688	0.4
y_{17}	1621.9523	1621.9541	1.1
y_{18}	1692.9894	1692.9904	0.6
y_{19}	1792.0578	1792.0706	7.1
y_{12}^{2+}	563.8269	563.8263	-1.1
y_{13}^{2+}	599.3455	599.3422	-5.5
y_{14}^{2+}	655.8875	655.8853	-3.4
y_{15}^{2+}	706.4114	706.4102	-1.7
y_{16}^{2+}	754.9378	754.9374	-0.5
y_{17}^{2+}	811.4798	811.4797	-0.1
y_{18}^{2+}	846.9983	846.9983	0.0
y_{19}^{2+}	896.5325	896.5338	1.5
y_{20}^{2+}	940.0486	940.0466	-2.1
y_{21}^{2+}	996.5906	996.5842	-6.4
y_{22}^{2+}	1045.1170	1045.1163	-0.7
y_{23}^{2+}	1088.6330	1088.6371	3.8
y_{24}^{2+}	1117.1437	1117.1443	0.5
y_{25}^{2+}	1173.6857	1173.6941	7.2
y_{26}^{2+}	1222.2121	1222.2129	0.7
y_{27}^{2+}	1287.7324	1287.7338	1.1
y_{28}^{2+}	1323.2509	1323.2544	2.6
y_{30}^{2+}	1428.3193	1428.3226	2.3
y_{31}^{2+}	1471.8353	1471.8447	6.4
y_{32}^{2+}	1520.3617	1520.3630	0.9
y_{33}^{2+}	1555.8803	1555.8866	4.0
y_{22}^{3+}	697.0804	697.0797	-1.0
y_{26}^{3+}	815.1438	815.1423	-1.8
y_{30}^{3+}	952.5486	952.5470	-1.7
y_{31}^{3+}	981.5593	981.5654	6.2
y_{33}^{3+}	1037.5893	1037.5894	0.1
y_{34}^{3+}	1134.2931	1134.2943	1.1
y_{35}^{3+}	1171.9878	1171.9781	-8.3
y_{57}^{4+}	1434.7750	1434.7818	4.7
z_3	299.1839	299.1841	0.7
z_4	370.2211	370.2209	-0.5
z_5	427.2425	427.2417	-1.9

z_6	514.2745	514.2740	-1.0
z_7	615.3222	615.3193	-4.7
z_8	714.3906	714.3903	-0.4
z_9	813.4590	813.4521	-8.5
z_{10}	900.4911	900.4858	-5.9
z_{11}	1013.5751	1013.5757	0.6
z_{13}	1181.6650	1181.6600	-4.2
z_7^{2+}	308.1648	308.1651	1.0
z_{10}^{2+}	450.7492	450.7477	-3.3
z_{11}^{2+}	507.2912	507.2917	1.0
z_{13}^{2+}	591.3361	591.3341	-3.4
z_{14}^{2+}	647.8782	647.8779	-0.5
z_{15}^{2+}	698.4020	698.3998	-3.2
z_{17}^{2+}	803.4704	803.4717	1.6
z_{18}^{2+}	838.9890	838.9891	0.1
z_{21}^{2+}	988.5812	988.5867	5.6

Table S24: Complete list of ions generated by UVPD of a tryptic peptide from TAB1 glycoprotein using two pulse of 1.5 mJ laser energy to activate the peptide (SSEVNGTDRPPIS$_{(g)}$SWSVDDVSNFIR, 3+, m/z 989.80). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
a_3	276.1190	276.1191	0.4
a_4	375.1874	375.1872	-0.5
a_5	489.2304	489.2309	1.0
a_8	762.3264	762.3265	0.1
a_9	918.4275	918.4253	-2.4
a_{10}	1015.4803	1015.4777	-2.6
a_{11}	1112.5331	1112.5260	-6.4
a_{12}	1225.6171	1225.6254	6.8
a_6^{2+}	273.1259	273.1264	1.8
b_2	175.0713	175.0712	-0.6
b_3	304.1139	304.1135	-1.3
b_4	403.1823	403.1819	-1.0
b_5	517.2253	517.2254	0.2
b_6	574.2467	574.2477	1.7
b_7	675.2944	675.2928	-2.4
b_8	790.3213	790.3232	2.4
b_9	946.4225	946.4194	-3.3
b_{12}	1253.6120	1253.6173	4.2
b_{13}	1543.7234	1543.7310	4.9
b_{14}	1630.7555	1630.7710	9.5
b_9^{2+}	473.7149	473.7148	-0.2
b_{10}^{2+}	522.2412	522.2418	1.1
b_{12}^{2+}	627.3097	627.3082	-2.4
b_{24}^{2+}	1397.1461	1397.1532	5.1
c_2	192.0979	192.0976	-1.6
c_3	321.1405	321.1397	-2.5
c_4	420.2089	420.2090	0.2
c_5	534.2518	534.2526	1.5
c_6	591.2733	591.2723	-1.7
c_7	692.3210	692.3201	-1.3
c_8	807.3479	807.3432	-5.8
c_9	963.4490	963.4566	7.9
c_{12}	1270.6386	1270.6400	1.1
c_{13}	1560.7500	1560.7491	-0.6
c_{14}	1647.7820	1647.7827	0.4
x_1	201.0982	201.0981	-0.5
x_2	314.1823	314.1817	-1.9
x_3	461.2507	461.2506	-0.2
x_4	575.2936	575.2935	-0.2
x_5	662.3256	662.3259	0.5

x_6	761.3941	761.3940	-0.1
x_7	876.4210	876.4251	4.7
x_8	991.4479	991.4454	-2.5
x_{10}	1177.5484	1177.5498	1.2
x_{12}	1450.6597	1450.6720	8.5
x_{13}	1740.7711	1740.7800	5.1
x_{17}^{2+}	1102.5345	1102.5316	-2.6
x_{19}^{2+}	1210.5719	1210.5729	0.8
x_{20}^{2+}	1239.0826	1239.0807	-1.5
x_{21}^{2+}	1296.1040	1296.1071	2.4
x_{22}^{2+}	1345.6383	1345.6353	-2.2
x_{23}^{2+}	1410.1595	1410.1670	5.3
y_1	175.1190	175.1188	-1.1
y_2	288.2030	288.2027	-1.0
y_3	435.2714	435.2708	-1.4
y_4	549.3144	549.3137	-1.3
y_5	636.3464	636.3457	-1.1
y_6	735.4148	735.4142	-0.8
y_7	850.4417	850.4413	-0.5
y_8	965.4687	965.4680	-0.7
y_9	1064.5371	1064.5355	-1.5
y_{11}	1337.6484	1337.6487	0.2
y_{12}	1424.6805	1424.6775	-2.1
y_{13}	1714.7919	1714.7876	-2.5
$y_{13}^{▲}$	1511.7125	1511.7133	0.5
y_{11}^{2+}	669.3278	669.3319	6.1
y_{13}^{2+}	857.8996	857.8997	0.1
y_{16}^{2+}	1011.4944	1011.4911	-3.3
y_{17}^{2+}	1089.5449	1089.5427	-2.0
$y_{17}^{2+▲}$	988.0052	988.0082	3.0
y_{18}^{2+}	1147.0584	1147.0538	-4.0
$y_{18}^{2+▲}$	1045.5187	1045.5215	2.7
$y_{19}^{2+▲}$	1096.0425	1096.0392	-3.0
$y_{20}^{2+▲}$	1124.5533	1124.5481	-4.6
$y_{21}^{2+▲}$	1181.5747	1181.5858	9.4
y_{21}^{2+}	1283.1144	1283.1165	1.6
y_{22}^{2+}	1332.6486	1332.6423	-4.7
$y_{22}^{2+▲}$	1231.1090	1231.1139	4.0
z_2	272.1843	272.1840	-1.1
z_3	419.2527	419.2520	-1.7
z_4	533.2956	533.2942	-2.6
z_5	620.3277	620.3262	-2.4
z_6	719.3961	719.3939	-3.1

z_7	834.4230	834.4173	-6.8
z_8	949.4499	949.4485	-1.5
z_{10}	1135.5504	1135.5460	-3.9
z_{11}	1321.6297	1321.6205	-7.0
z_{12}	1408.6617	1408.6622	0.4

Table S25: Complete list of ions generated by EThcD of a tryptic peptide from Ph1 glycoprotein using 50 ms ETD and 25% supplemental energy to activate the peptide (GSSHHHHHHS$_{(g)}$SGLVPR, 4+, m/z 493.73). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical Mass	Observed Mass	Mass Error (ppm)
b_5	506.2106	506.2112	1.2
b_7	780.3284	780.3329	5.8
b_8^{2+}	459.1973	459.1996	5.0
b_9^{2+}	527.7268	527.7278	1.9
c_4	386.1783	386.1778	-1.3
c_5	523.2372	523.2363	-1.7
c_6	660.2961	660.2950	-1.7
c_7	797.3550	797.3523	-3.4
c_8^{2+}	467.7106	467.7113	1.5
c_9^{2+}	536.2400	536.2391	-1.7
c_{10}^{2+}	681.2957	681.2936	-3.1
c_{11}^{2+}	724.8118	724.8126	1.1
c_{12}^{2+}	753.3225	753.3207	-2.4
c_{13}^{2+}	809.8645	809.8663	2.2
c_{15}^{2+}	907.9251	907.9266	1.7
y_2	272.1717	272.1713	-1.5
y_5	541.3456	541.3416	-7.4
y_6	628.3777	628.3768	-1.4
$y_7^{▲}$	715.4097	715.4075	-3.1
$y_8^{▲}$	852.4686	852.4691	0.5
$y_8^{2+▲}$	426.7380	426.7377	-0.8
$y_9^{2+▲}$	495.2674	495.2686	2.3
$y_{10}^{2+▲}$	563.7969	563.7963	-1.1
$y_{11}^{2+▲}$	632.3263	632.3290	4.3
y_{15}^{2+}	957.9569	957.9496	-7.6
z_6	612.3589	612.3563	-4.2
z_7	902.4703	902.4720	1.9
z_8	1039.5293	1039.5297	0.4
z_{10}^{2+}	657.3272	657.3287	2.3
z_{12}^{2+}	794.3861	794.3862	0.1
z_{13}^{2+}	862.9155	862.9128	-3.1

Table S26: Complete list of ions generated by EThcD of a tryptic peptide from Ph1 glycoprotein using 50 ms ETD and 25% supplemental energy to activate the peptide (LSESFPILGASTEVPPMS$_{(g)}$LPVQ AAISAPSPLAMPLGSPLSVALPTLAPLSVVTSGAAPK, 5+, *m/z* 1188.24). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
b_2	201.1234	201.1236	1.0
b_4	417.1980	417.1990	2.4
b_5	564.2664	564.2670	1.1
b_7	774.4032	774.4053	2.7
b_8	887.4873	887.4870	-0.3
b_9	944.5087	944.5119	3.4
b_{10}	1015.5458	1015.5461	0.3
b_{12}	1203.6256	1203.6387	10.9
b_{13}	1332.6681	1332.6656	-1.9
b_{14}	1431.7366	1431.7362	-0.3
b_{24}^{2+}	1313.6696	1313.6777	6.2
b_{25}^{2+}	1370.2117	1370.2126	0.7
b_{26}^{2+}	1413.7277	1413.7212	-4.6
b_{31}^{2+}	1646.3570	1646.3518	-3.2
c_3	347.1925	347.1926	0.3
c_4	434.2245	434.2238	-1.6
c_6	678.3457	678.3471	2.1
c_7	791.4298	791.4313	1.9
c_8	904.5138	904.5111	-3.0
c_9	961.5353	961.5326	-2.8
c_{10}	1032.5724	1032.5743	1.8
c_{11}	1119.6044	1119.6035	-0.8
c_{12}	1220.6521	1220.6475	-3.8
y_1	147.1128	147.1126	-1.4
y_2	244.1656	244.1654	-0.8
y_3	315.2027	315.2024	-1.0
y_4	386.2398	386.2357	-10.6
y_5	443.2612	443.2608	-0.9
y_6	530.2933	530.2952	3.6
y_7	631.3410	631.3420	1.6
y_8	730.4094	730.4095	0.1
y_9	829.4778	829.4715	-7.6
y_{10}	916.5098	916.5112	1.5
y_{12}	1126.6466	1126.6462	-0.4
y_{13}	1197.6837	1197.6819	-1.5
y_{14}	1310.7678	1310.7688	0.8
y_{15}	1411.8155	1411.8010	-10.3
y_{16}	1508.8682	1508.8679	-0.2
y_{17}	1621.9523	1621.9426	-6.0

y_{18}	1692.9894	1692.9816	-4.6
y_{12}^{2+}	563.8269	563.8272	0.5
y_{16}^{2+}	754.9378	754.9376	-0.3
y_{17}^{2+}	811.4798	811.4783	-1.8
y_{18}^{2+}	846.9983	846.9935	-5.7
y_{22}^{2+}	1045.1170	1045.1129	-3.9
y_{24}^{2+}	1117.1437	1117.1415	-2.0
y_{26}^{2+}	1222.2121	1222.2117	-0.3
y_{27}^{2+}	1287.7324	1287.7374	3.9
y_{28}^{2+}	1323.2509	1323.2511	0.2
y_{30}^{2+}	1428.3193	1428.3143	-3.5
y_{32}^{2+}	1520.3617	1520.3646	1.9
y_{33}^{2+}	1555.8803	1555.8899	6.2
y_{34}^{2+}	1599.3963	1599.3969	0.4
z_3	299.1839	299.1835	-1.3
z_4	370.2211	370.2210	-0.3
z_6	514.2745	514.2732	-2.5
z_7	615.3222	615.3186	-5.9
z_{17}	1605.9336	1605.9177	-9.9
z_{17}^{2+}	803.4704	803.4708	0.5
z_{31}^{2+}	1463.8260	1463.8190	-4.8
z_{34}^{2+}	1591.3869	1591.3899	1.9

Table S27: Complete list of ions generated by EThcD of a tryptic peptide from Ph1 glycoprotein using 50 ms ETD and 25% supplemental energy to activate the peptide (SSEVNGTDRPPIS$_{(g)}$SWSVDDVSNFIR, 3+, m/z 989.80). The fragment ions that are critical for localization of GlcNAc sites are listed in blue font.

Identified Ions	Theoretical m/z	Observed m/z	Mass Error (ppm)
b_2	175.0713	175.0717	2.3
b_3	304.1139	304.1138	-0.3
b_4	403.1823	403.1824	0.2
b_5	517.2253	517.2246	-1.4
b_6	574.2467	574.248	2.3
b_7	675.2944	675.2939	-0.7
b_9	946.4225	946.4227	0.2
b_{12}	1253.612	1253.6106	-1.1
c_6	591.2733	591.2773	6.8
c_7	692.321	692.3225	2.2
c_{11}	1157.5545	1157.5541	-0.3
c_{12}	1270.6386	1270.6384	-0.2
c_{13}	1560.75	1560.7474	-1.7
c_{14}	1647.782	1647.7845	1.5
y_1	175.119	175.1187	-1.7
y_2	288.203	288.2032	0.7
y_3	435.2714	435.2709	-1.1
y_4	549.3144	549.3159	2.7
y_5	636.3464	636.3458	-0.9
y_6	735.4148	735.4141	-1
y_7	850.4417	850.4415	-0.2
y_8	965.4687	965.4685	-0.2
y_9	1064.5371	1064.5372	0.1
y_{10}	1151.5691	1151.569	-0.1
y_{11}	1337.6484	1337.651	1.9
y_{12}	1424.6805	1424.682	1.1
$y_{13}^{▲}$	1511.7125	1511.7133	0.5
y_{11}^{2+}	669.3278	669.3291	1.9
y_{16}^{2+}	1011.4944	1011.494	-0.4
y_{17}^{2+}	1089.5449	1089.5424	-2.3
$y_{17}^{2+▲}$	988.0052	988.0082	3
y_{18}^{2+}	1147.0584	1147.0551	-2.9
$y_{18}^{2+▲}$	1045.5187	1045.5215	2.7
y_{19}^{2+}	1197.5822	1197.5818	-0.3
$y_{20}^{2+▲}$	1124.5533	1124.5481	-4.6
y_{21}^{2+}	1283.1144	1283.1151	0.5
$y_{21}^{2+▲}$	1181.5747	1181.5858	9.4
y_{22}^{2+}	1332.6486	1332.6501	1.1

y_{22}^{2+}▲	1231.109	1231.1139	4
y_{23}^{2+}	1397.1699	1397.1722	1.6
z_1	159.1002	159.1001	-0.6
z_2	272.1843	272.1845	0.7
z_4	533.2956	533.2958	0.4
z_5	620.3277	620.3283	1
z_6	719.3961	719.397	1.3
z_7	834.423	834.4247	2
z_8	949.4499	949.4532	3.5
z_{10}	1135.5504	1135.5532	2.5
z_{11}	1321.6297	1321.6234	-4.8
z_{12}	1408.6617	1408.6635	1.3
z_{13}	1698.7731	1698.7736	0.3

Section S4 Supplementary References

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