Supplemental Information

S. cerevisiae Cell Culture and Protein Expression

Two to three single colonies of yeast containing GST-tagged isoforms of the protein of interest were inoculated into 15 ml of minimal medium (-ura, +2% raffinose), shaking at 300 rpm and 30°C until OD_{600} was about 2.5-3.0.

Culture was diluted with minimal medium (-ura, +2% raffinose) to OD_{600}=0.02, shaking at 30°C (300 rpm) for about 24 hours until OD_{600}=1.0.

One hundred ml of 40% galactose (final concentration is 4%) was added to induce protein expression, shaking at 300 rpm and 30°C O/N.

Cells were pelleted at 3000 x g for 10 min and washed with 200 ml of cold PBS. Cells were resuspended in 10 ml of glass bead disruption buffer (7 M urea, 2 M thio-urea, 4% CHAPS, 50 mM Tris-Cl, pH 8.0, 10 mM DTT and protease inhibitors). The suspension was divided into two 50 ml tubes and 2 volumes of pre-cooled glass beads were added. Vigorously vortexed for 20 cycles (1 min vortex/1 min on ice). Decanted and saved the supernatant. Added 2 ml of glass bead disruption buffer to wash the glass beads and pooled with previous supernatant.

Pooled supernatants were centrifuged at 12,000 rpm for 10 min at 4 ºC. Supernatants were transferred to fresh tubes and centrifuged at 100,000 × g for 20 min at 4ºC.

Supernatants were transferred to dialysis membranes and dialyzed against dialysis buffer (20 mM Tris-Cl pH 7.3, 20% glycerol, 200 μM EDTA, 100 mM KCl, 1 mM DTT, 1 mM PMSF) at 4°C O/N.

Dialyzed sample was centrifuged at 100,000 x g and 4°C for 20 minutes.

Purification of GST-tagged Yeast Proteins

GST-beads were washed twice in 10 volumes PBS, sedimenting the GST-beads by centrifugation at 500 ×g for 0.5 min. PBS was added to the beads to make a 50% slurry. Five hundred μl of the GST-bead slurry was added to the dialyzed crude protein extract and incubated with gentle agitation at 4 ºC for 1 hour. GST-beads were sedimented by centrifugation at 500 ×g for 0.5 min and the supernatant decanted. Beads were washed 3 times with 10 ml of RIPA buffer and once with PBS. Bound protein was eluted twice with 200 μl elution buffer (50 mM Tris-Cl pH 8.0, 10 mM reduced glutathione, 1 mM DTT, 100 mM KCl), incubating at room temperature for 10 min with gentle agitation.

Supplemental Figure S1:

Spectra comparisons of *in vivo* peptides with their corresponding synthetic peptides or *in vivo* unmodified peptides.

(A) (i) MS/MS spectrum of peptide "VQALEEANND$_{Me}$LENK" identified *in vivo* from HeLa samples, (ii) MS/MS spectrum of the synthetic peptide with the same sequence and modification site, (iii) MS/MS spectrum of unmodified peptide "VQALEEANNDLENK" identified *in vivo*.

(B) (i) MS/MS spectrum of peptide "ISM$_{Ox}$PDVD$_{Me}$LHLK" identified *in vivo* from HeLa samples, (ii) MS/MS spectrum of the synthetic peptide with the same sequence and modification site, (iii) MS/MS spectrum of unmodified peptide "ISM$_{Ox}$PDVDLHLK" identified *in vivo*.

(C) (i) MS/MS spectrum of peptide "LLLIGD$_{Me}$SGVGK" identified *in vivo* from HeLa samples, (ii) MS/MS spectrum of the synthetic peptide with the same sequence and modification site, (iii) MS/MS spectrum of unmodified peptide "LLLIGDSGVGK" identified *in vivo*.

(D) (i) MS/MS spectrum of peptide "VINDAFGIE$_{Me}$E$_{Me}$GLMTTVHSLTATQK" identified *in vivo* from *S. cerevisiae* samples, (ii) MS/MS spectrum of the synthetic peptide with the same sequence and modification site.

(E) (i) MS/MS spectrum of peptide "TTTGHLIYK$_{Me}$" identified *in vivo*.

(F) (i) MS/MS spectrum of a putative D-methylated peptide "VDID$_{Me}$APDVSIEGPDAK" identified *in vivo* from HeLa samples, (ii) MS/MS spectrum of the synthetic peptide with the same sequence and modification site.

(G) (i) MS/MS spectrum of a putative D-methylated peptide "ETENDD$_{Me}$VTNVIQK" identified *in vivo* from HeLa samples, (ii) MS/MS spectrum of the synthetic peptide with the same sequence and modification site.

(H) (i) MS/MS spectrum of a putative D-methylated peptide "AADD$_{Me}$TWEPFASGK" identified *in vivo* from HeLa samples, (ii) MS/MS spectrum of the synthetic peptide with the same sequence and modification site.

(I) (i) MS/MS spectrum of a putative D-methylated peptide "VLGTAYD$_{Me}$K" identified *in vivo* from *S. cerevisiae* samples, (ii) MS/MS spectrum of the synthetic peptide with the same sequence and modification site.

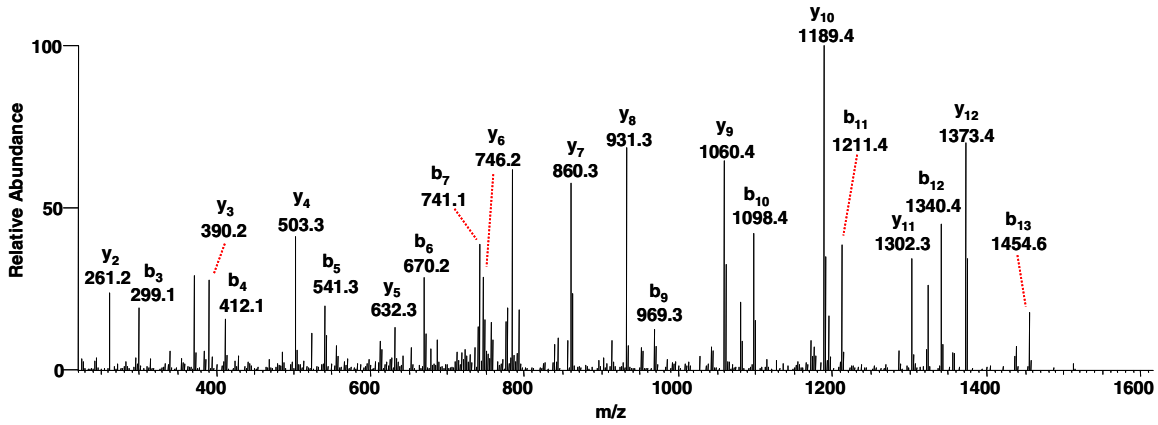
(J) (i) MS/MS spectrum of a putative D-methylated peptide "LIQD$_{Me}$ESTK" identified *in vivo* from *S. cerevisiae* samples, (ii) MS/MS spectrum of the synthetic peptide with the same sequence and modification site.

Supplemental Table T1

A list of the number of identified proteins, peptides and unique peptides for each Hela and Yeast sample in this study

Supplemental Figure S1 (A)

(i)



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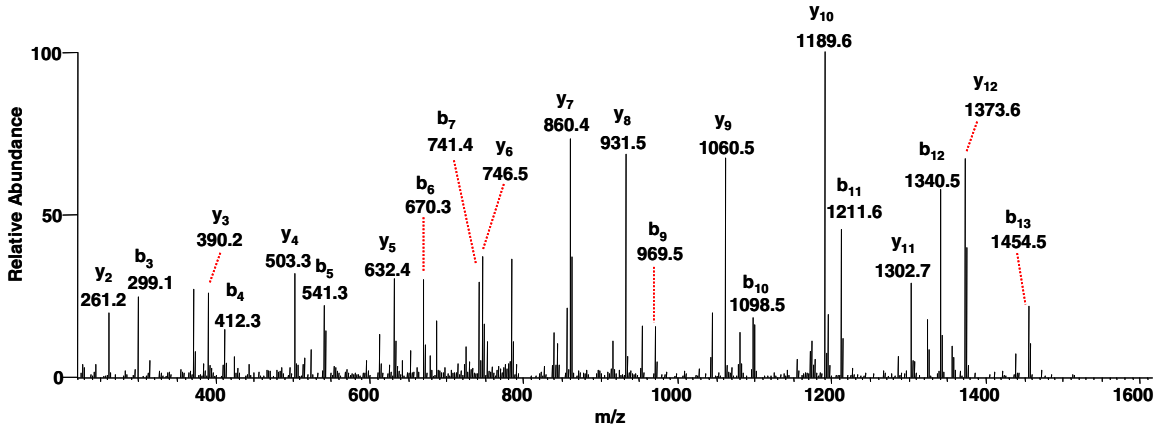

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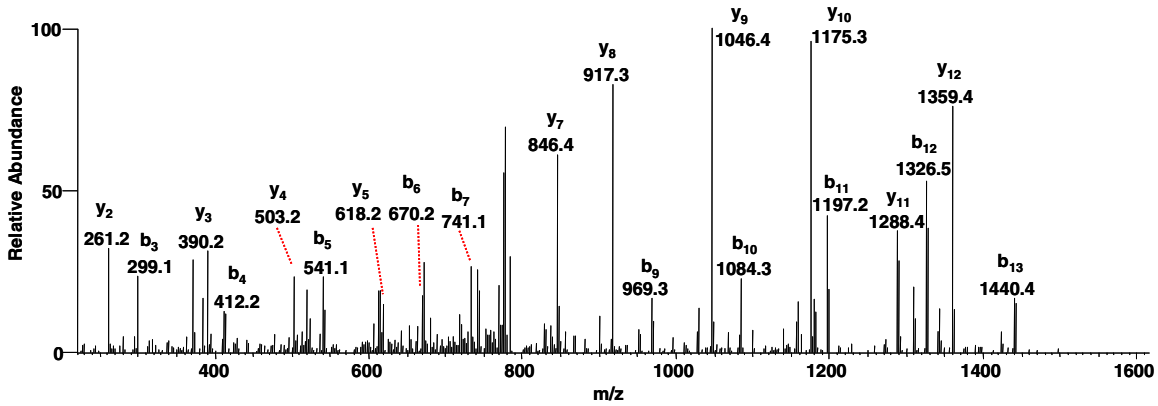

Supplemental Figure S1 (B)

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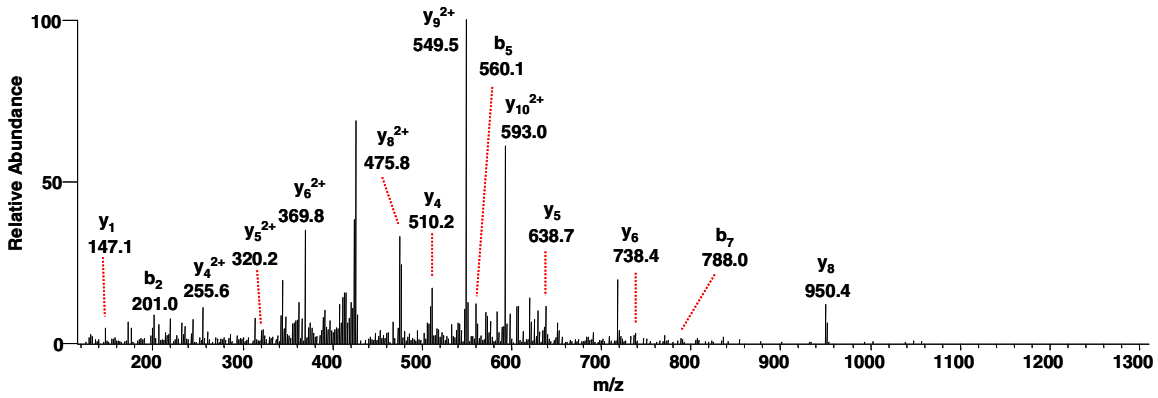

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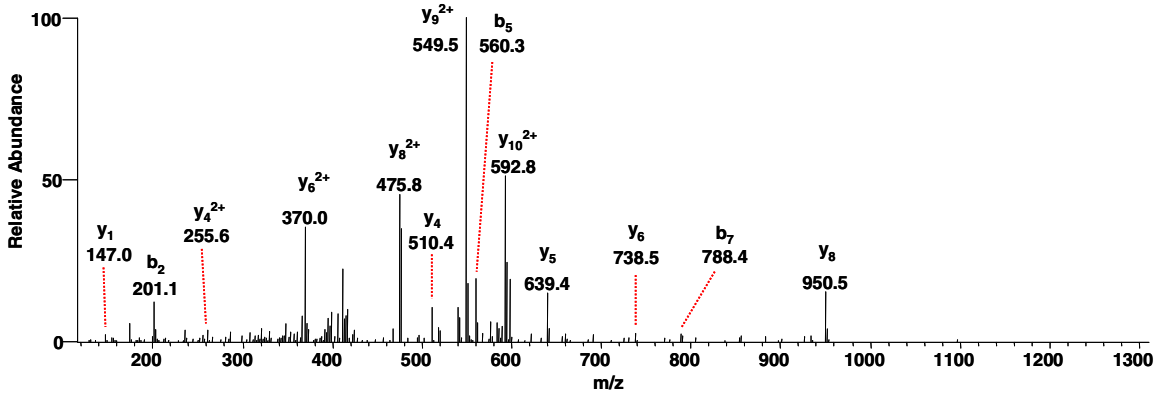

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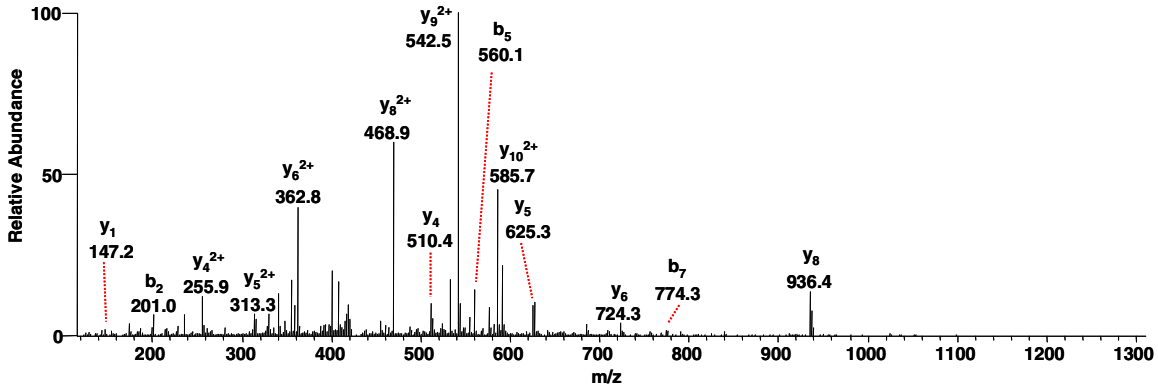

Supplemental Figure S1 (C)

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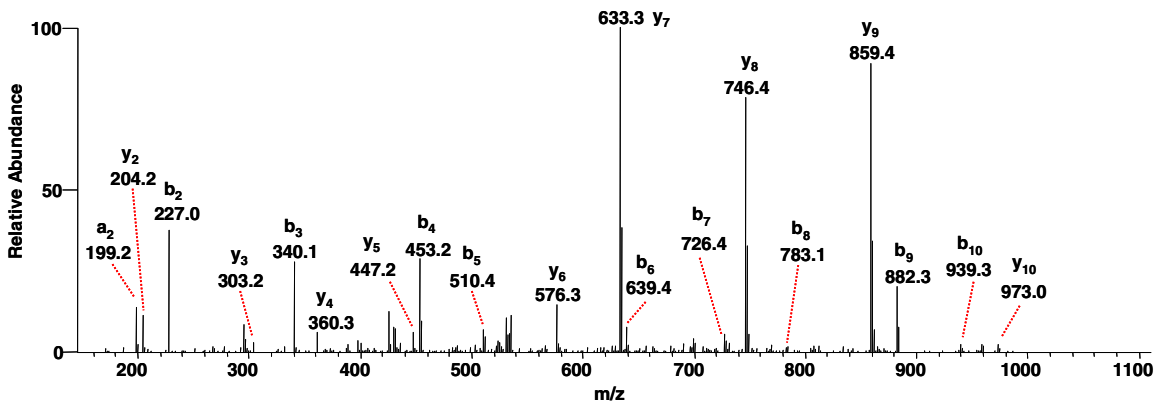

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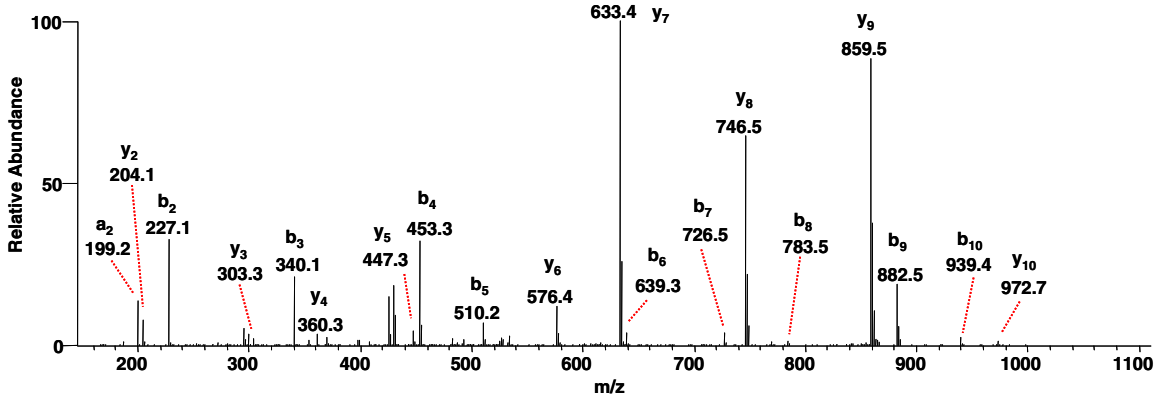

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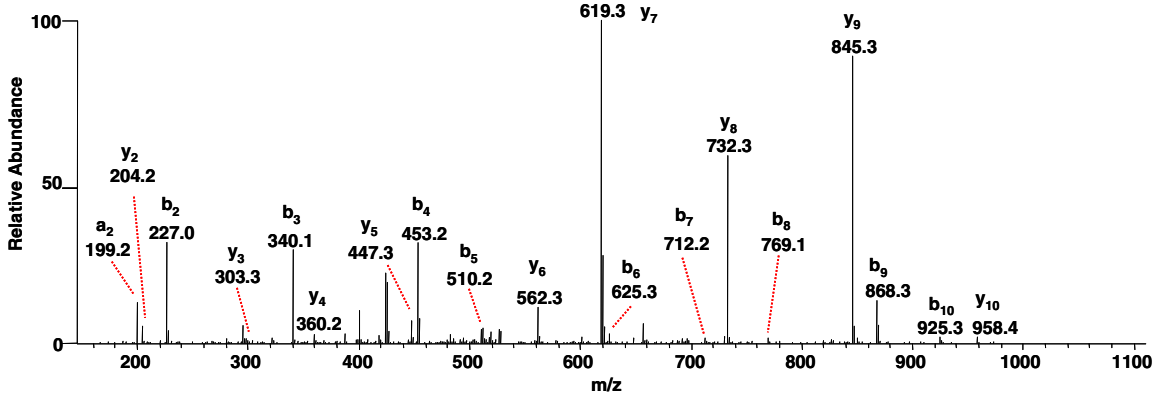

Supplemental Figure S1 (D)

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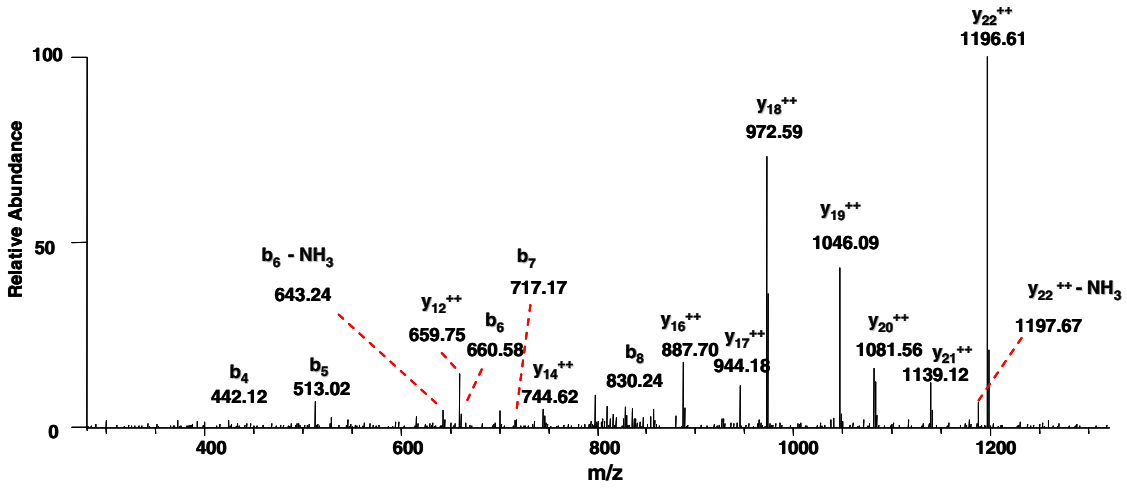

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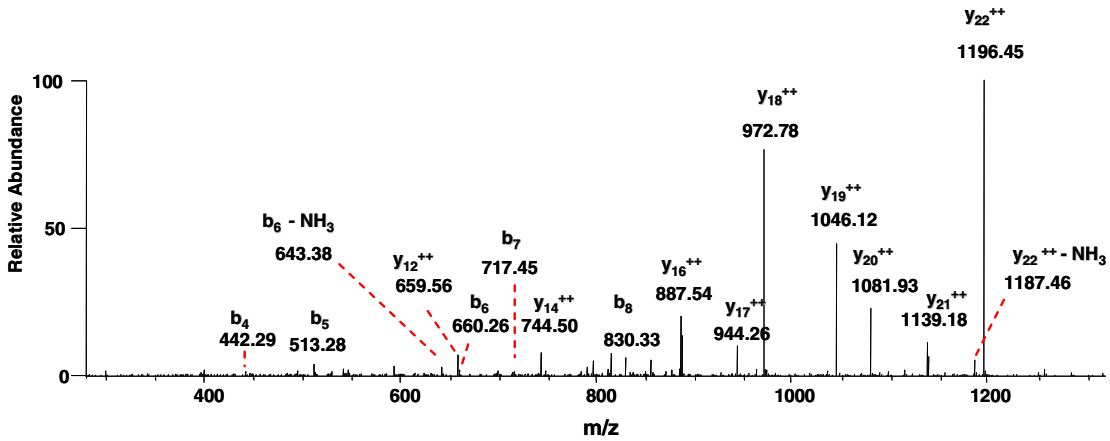
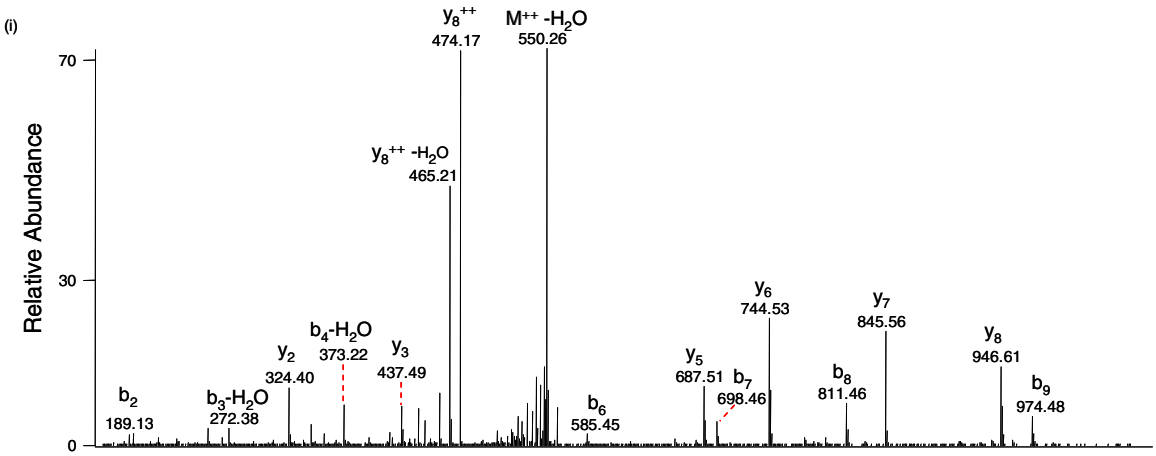


Supplemental Figure S1 (E)



Supplemental Figure S1 (F)

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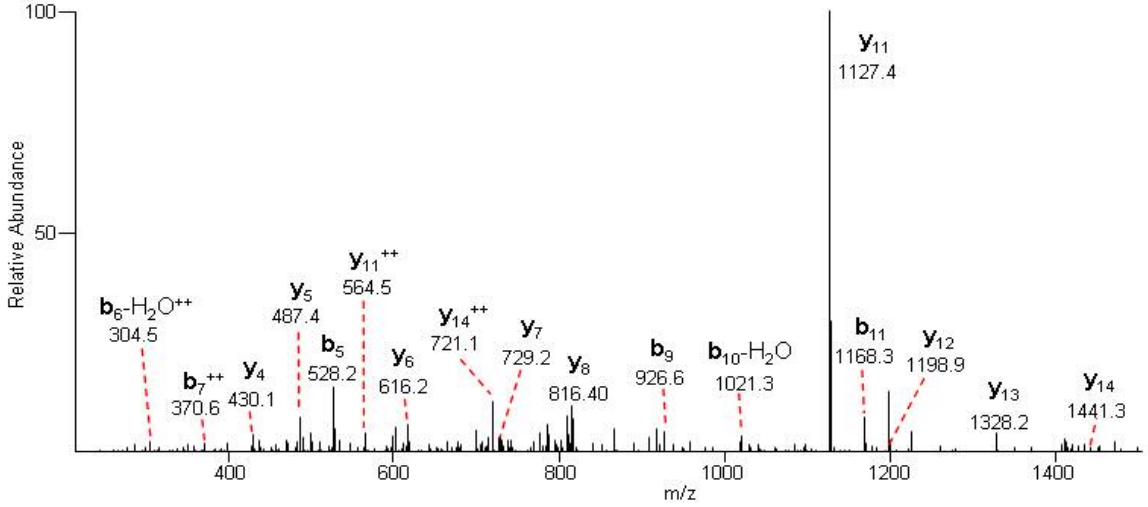

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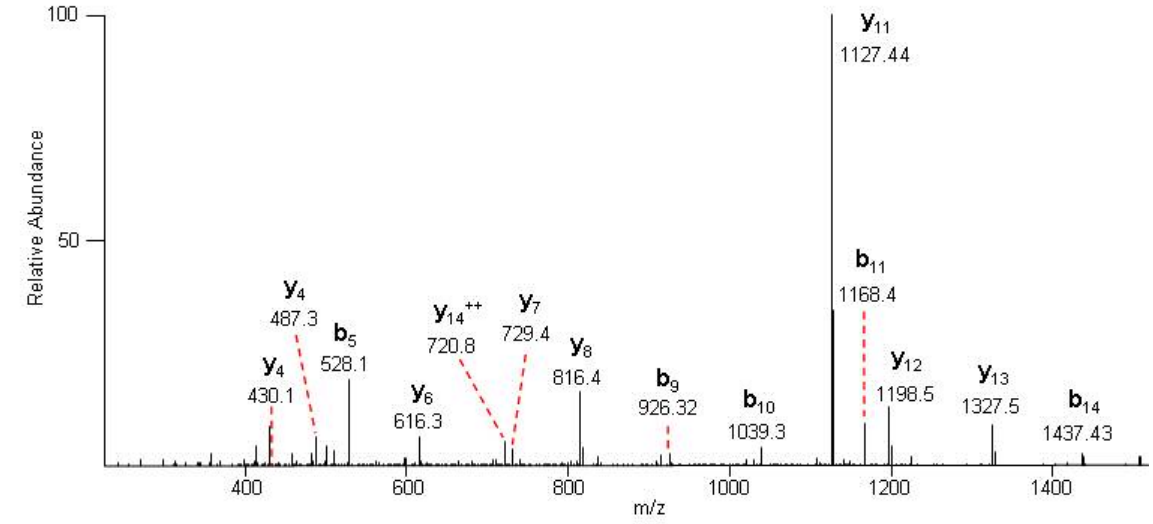

Supplemental Figure S1 (G)

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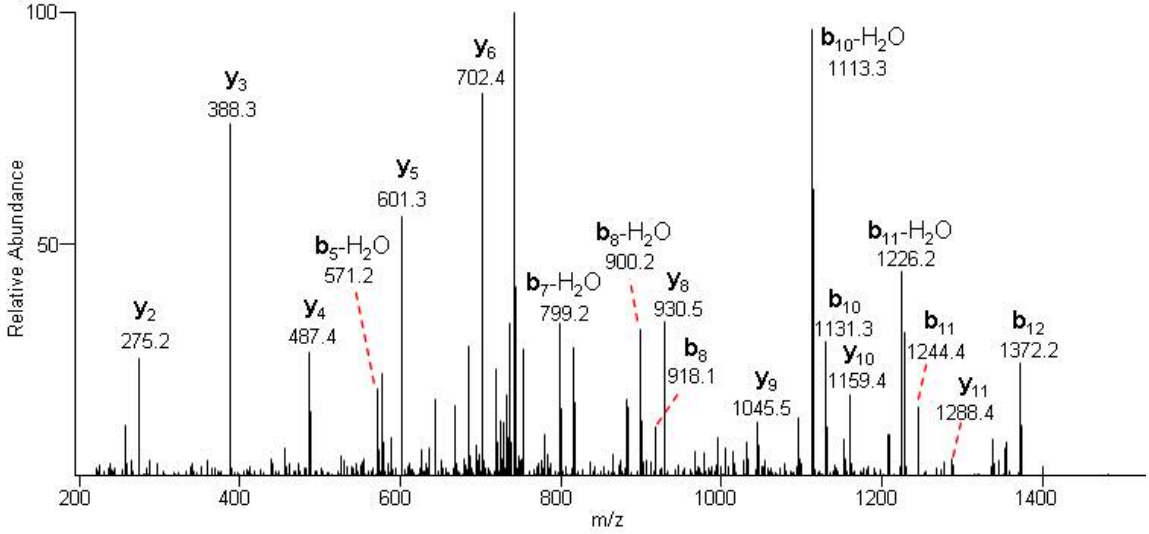

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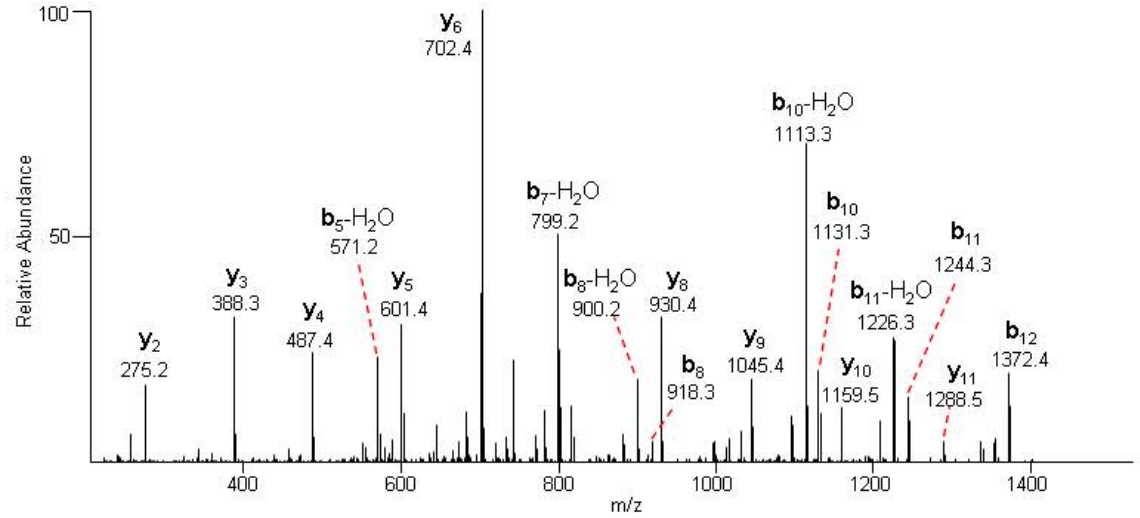

Supplemental Figure S1 (H)

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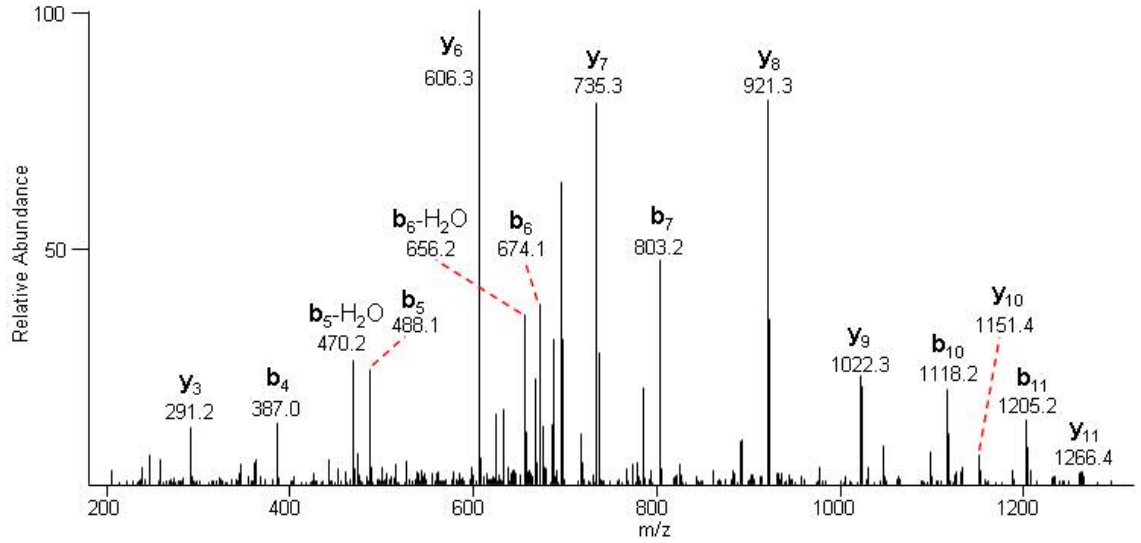

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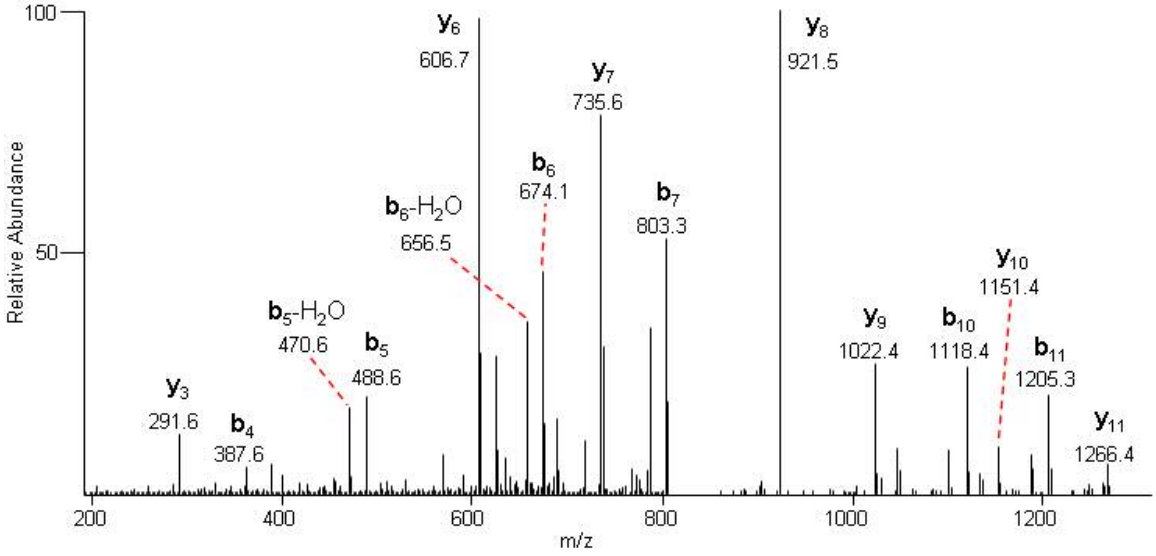

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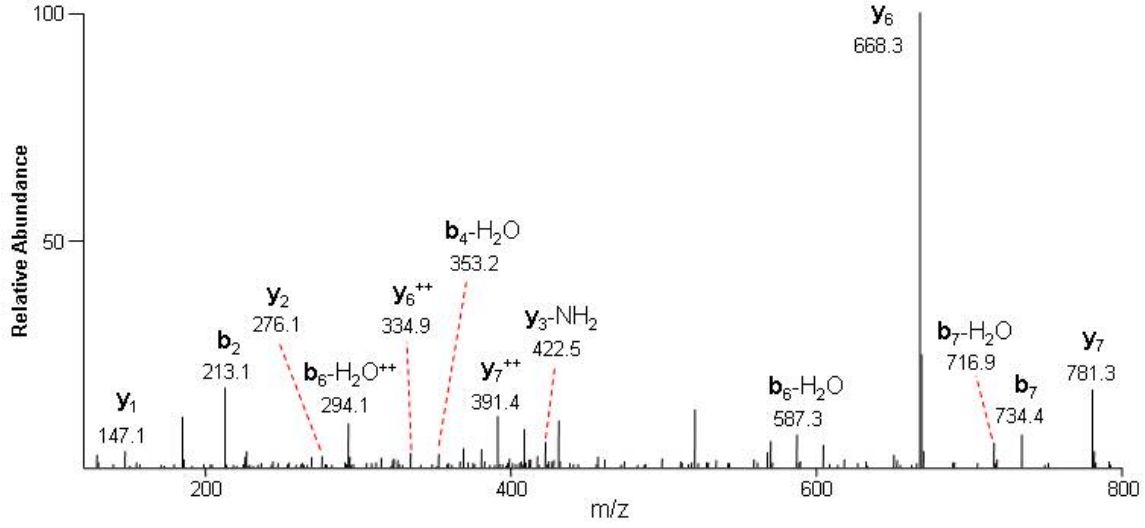
(i)



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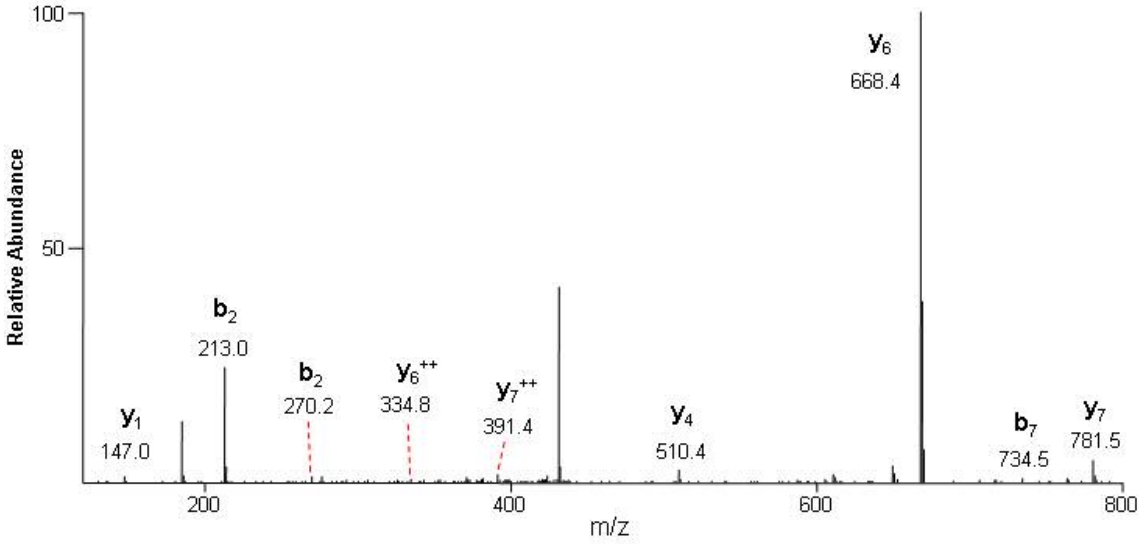

Supplemental Figure S1 (J)

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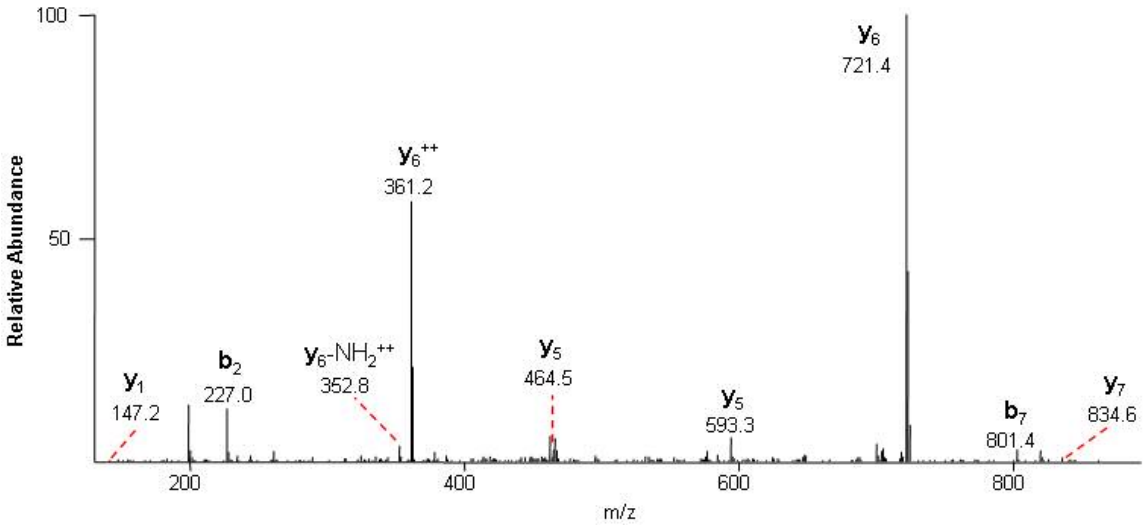

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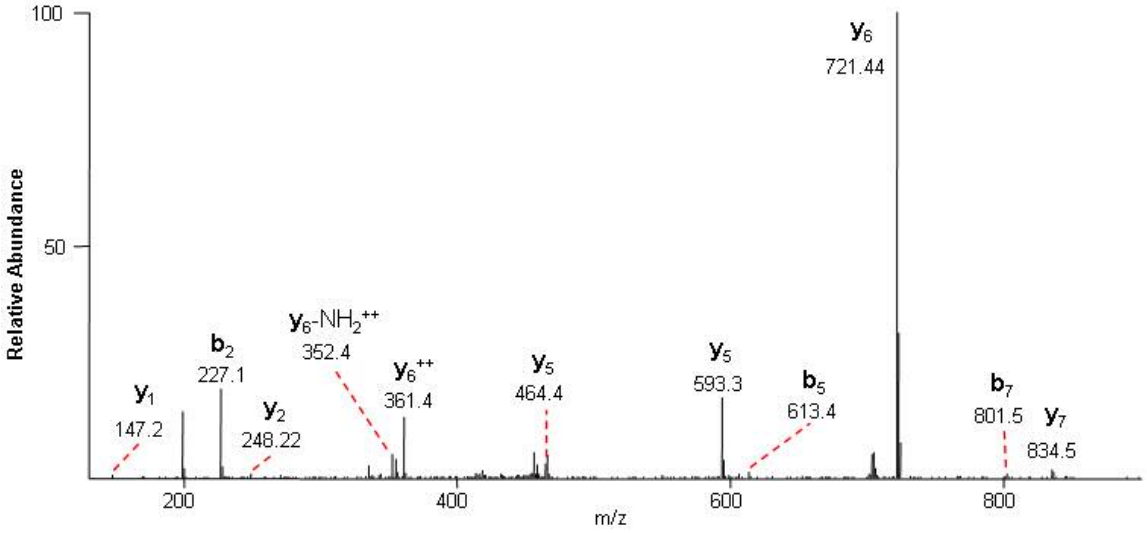

Supplemental Table T1

SampleName	Protein#	Peptide#	UniqPeptide#
h01	166	829	366
h02	217	1242	513
h03	210	1547	548
h04	230	1531	584
h05	230	1369	642
h06	248	1922	833
h07	284	2338	966
h08	210	1741	826
h09	220	1724	787
h10	200	1845	773
h11	204	2106	983
h12	190	3605	993
h13	237	2366	1252
h14	189	1903	953
h15	211	2420	1257
h16	231	2807	1641
h17	201	2477	1538
h18	154	2396	1380
h19	170	2401	1356
h20	191	1374	738
y01	156	704	327
y02	219	1202	508
y03	190	1340	522
y04	172	1375	501
y05	215	1537	586
y06	231	1956	725
y07	224	2178	834
y08	250	2366	945
y09	176	2301	768
y10	237	3317	1175
y11	150	2256	753
y12	183	2590	894
y13	172	2551	995
y14	176	2731	1071
y15	158	2502	935
y16	174	2255	976
y17	163	2312	1164
y18	208	3196	1537
y19	152	3182	1362
y20	271	2075	1134