

**S-Glutathionylation of Estrogen Receptor Alpha Regulates Dendritic Cell Metabolic Function**

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**Table S1. Mouse Estrogen receptor signaling (SAB Target List) M96, predesigned 96-well panel for use with SYBR® Green.**

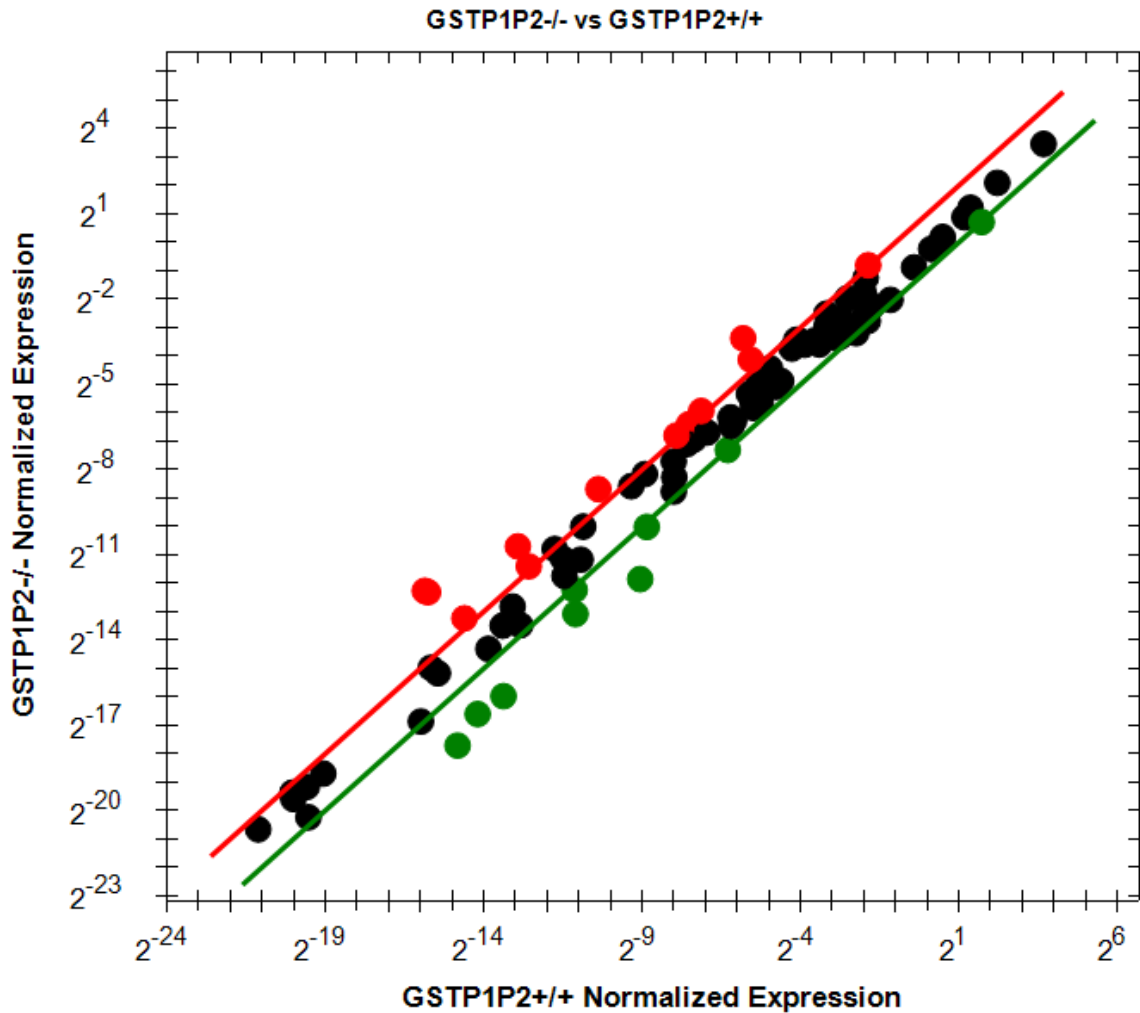
**FIGURE S1.** mRNAs from WT or *Gstp1/p2<sup>-/-</sup>* BMDDC were extracted and quantified by estrogen receptor signaling PCR array (Bio-Rad). Quantitative analyses of each gene were applied by Bio-Rad PrimePCR Analysis software (v.1.0.030.1023), and relative gene expression is shown in red for up-regulation and in green for down-regulation in *Gstp1/p2<sup>-/-</sup>* mice. This plot was created from the data in Table 1.

**FIGURE S2.** Characteristic fragmentation patterns of S-glutathionylated peptides by CID and ETD.

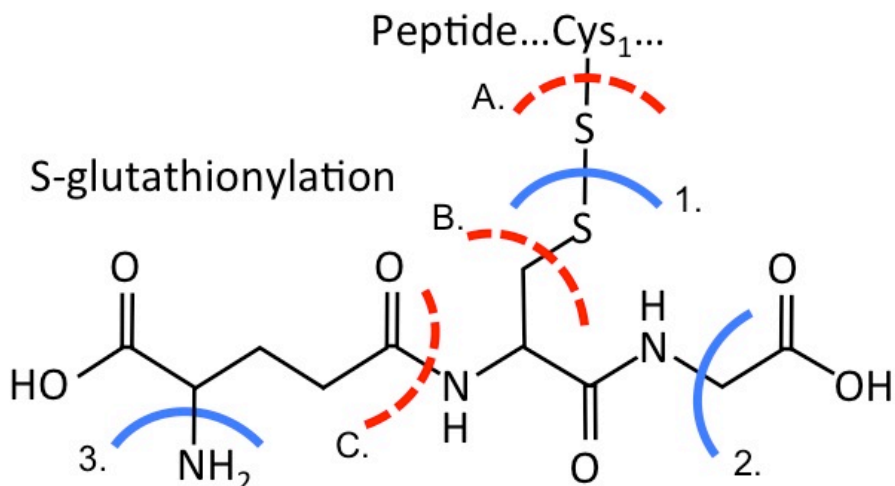
**FIGURE S3.** Raw data for proteomic identification of modified cysteine residues.

**Supporting Information**
**Table S1. Mouse Estrogen receptor signaling (SAB Target List) M96, predesigned 96-well panel for use with SYBR® Green.**

Well	Gene Name	Symbol	Well	Gene Name	Symbol
A1	Actin, beta	Actb	E1	Amyloid beta (A4) precursor protein-binding, family B1	Apbb1
A2	Brain derived neurotrophic factor	Bdnf	E2	Complement component 3	C3
A3	Cbp/p300-interacting transactivator	Cited2	E3	Cytochrome P450, family 19, subfamily a, polypeptide 1	Cyp19a1
A4	Early growth response 3	Egr3	E4	Estrogen receptor 2 (beta)	Esr2
A5	Glucose-6-phosphate dehydrogenase X-linked	G6pdx	E5	Insulin-like growth factor 1	Igf1
A6	Jun-B oncogene	Junb	E6	Lipoprotein lipase	Lpl
A7	Matrix metalloproteinase 9	Mmp9	E7	Nuclear receptor coactivator 1	Ncoa1
A8	Nephroblastoma overexpressed gene	Nov	E8	Nuclear receptor subfamily 3, group C, member 1	Nr3c1
A9	PDZ domain containing 1	Pdzk1	E9	Patched homolog 1	Ptch1
A10	S100 calcium binding protein A6 (calyculin)	S100a6	E10	Secreted phosphoprotein 1	Spp1
A11	Thrombospondin 1	Thbs1	E11	Wingless-related MMTV integration site 4	Wnt4
A12	Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	E12	PrimePCR RNA Quality Assay	RQ1
B1	Adenosine A1 receptor	Adora1	F1	Beta-2 microglobulin	B2m
B2	Bone morphogenetic protein 4	Bmp4	F2	Caveolin 1, caveolae protein	Cav1
B3	Creatine kinase, brain	Ckb	F3	Cytochrome P450, family 1, subfamily a, polypeptide 1	Cyp1a1
B4	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2	ErbB2	F4	FBJ osteosarcoma oncogene	Fos
B5	Glucuronidase, beta	Gusb	F5	Insulin-like growth factor binding protein 4	Igfbp4
B6	Kallikrein B, plasma 1	Klkb1	F6	Latent transforming growth factor beta binding protein 1	Ltbp1
B7	Metastasis associated 1	Mta1	F7	Nuclear receptor coactivator 2	Ncoa2
B8	Nuclear receptor subfamily 0, group B, member 1	Nr0b1	F8	Nuclear receptor subfamily 5, group A, member 2	Nr5a2
B9	Proline, glutamic acid and leucine rich protein 1	Pelp1	F9	Prostaglandin-endoperoxide synthase 2	Ptgs2
B10	Scaffold attachment factor B	Safb	F10	Trefoil factor 1	Tff1
B11	Vitamin D receptor	Vdr	F11	Wingless-related MMTV integration site 5A	Wnt5a
B12	Hypoxanthine guanine phosphoribosyl transferase	Hprt	F12	PrimePCR RNA Quality Assay	RQ2
C1	Aryl-hydrocarbon receptor	Ahr	G1	Breast cancer anti-estrogen resistance 1	Bcar1
C2	Bone morphogenetic protein 7	Bmp7	G2	Chemokine (C-C motif) ligand 12	Ccl12
C3	Connective tissue growth factor	Ctgf	G3	Estrogen receptor-binding fragment-associated gene 9	Ebag9
C4	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3	ErbB3	G4	Forkhead box A1	Foxa1
C5	Heat shock protein 90, alpha (cytosolic), class A member 1	Hsp90aa1	G5	Insulin-like growth factor binding protein 5	Igfbp5
C6	L1 cell adhesion molecule	L1cam	G6	V-maf musculoaponeurotic fibrosarcoma oncogene family	Maff
C7	Myelocytomatosis oncogene	Myc	G7	Nuclear receptor coactivator 3	Ncoa3
C8	Nuclear receptor subfamily 0, group B, member 2	Nr0b2	G8	Nuclear receptor interacting protein 1	Nrip1
C9	Progesterone receptor	Pgr	G9	V-ral simian leukemia viral oncogene homolog A	Rala
C10	Snail homolog 1 (Drosophila)	Snai1	G10	Transforming growth factor alpha	Tgfa
C11	Vascular endothelial growth factor A	Vegfa	G11	X-box binding protein 1	Xbp1
C12	PrimePCR DNA Contamination Control Assay	gDNA	G12	PrimePCR Reverse Transcription Control Assay	RT
D1	A kinase (PRKA) anchor protein 1	Akap1	H1	BCL2-like 1	Bcl2l1
D2	Breast cancer 1	Brca1	H2	Cyclin D1	Ccnd1
D3	Cathepsin D	Ctsd	H3	Ephrin A5	Efna5
D4	Estrogen receptor 1 (alpha)	Esr1	H4	Follistatin	Fst
D5	Heat shock protein 90 alpha (cytosolic), class B member 1	Hsp90ab1	H5	Insulin receptor substrate 1	Irs1
D6	Lectin, galactose binding, soluble 1	Lgals1	H6	Mediator complex subunit 1	Med1
D7	Ngfi-A binding protein 2	Nab2	H7	Nuclear receptor co-repressor 2	Ncor2
D8	Nuclear receptor subfamily 2, group F, member 6	Nr2f6	H8	Neuropilin 1	Nrp1
D9	Prohibitin 2	Phb2	H9	Retinoic acid receptor, alpha	Rara
D10	Suppressor of cytokine signaling 3	Socs3	H10	Transforming growth factor, beta 3	Tgfb3
D11	WNT1 inducible signaling pathway protein 2	Wisp2	H11	TATA box binding protein	Tbp
D12	PrimePCR Positive Control Assay	PCR	H12	Blank	Blank



**FIGURE S1.** mRNAs from WT or *Gstp1/p2*<sup>-/-</sup> BMDDC were extracted and quantified by estrogen receptor signaling PCR array (Bio-Rad). Quantitative analyses of each gene were applied by Bio-Rad PrimePCR Analysis software (v.1.0.030.1023), and relative gene expression is shown in red for up-regulation and in green for down-regulation in *Gstp1/p2*<sup>-/-</sup> mice. This plot was created from the data in Table 1.



Signature Fragments of S-glutathionylated Peptides:

Fragmentation by ETD MS/MS (blue)

1.  $\Delta$  mass = Glutathionylated peptide (MH<sup>+</sup>) -305.07 Da; Neutral loss of glutathione
2.  $\Delta$  mass = Glutathionylated peptide (MH<sup>+</sup>) -58 Da; (Cys +246)
3.  $\Delta$  mass = Glutathionylated peptide (MH<sup>+</sup>) -15 Da; (Cys +290)

Glutathione ions: 308, 290, 246

Fragmentation by CID MS/MS (red)

- A.  $\Delta$  mass = Glutathionylated peptide (MH<sup>+</sup>) -338 Da; (Cys -33.99)
- B.  $\Delta$  mass = Glutathionylated peptide (MH<sup>+</sup>) -273 Da; (Cys +31.97)
- C.  $\Delta$  mass = Glutathionylated peptide (MH<sup>+</sup>) -129 Da; Neutral loss  $\gamma$ glutamic acid

**FIGURE S2.** Characteristic fragmentation patterns of S-glutathionylated peptides by CID and ETD.

A

011917\_SCW\_V\_97\_DrZhang\_ERAlpha\_Trypsin#18082 RT: 102.32 AV: 1 NL: 9.05E2  
T: ITMS + c NSI.d Full.ms2 1288.48@cid35.00 [340.00-2000.00]

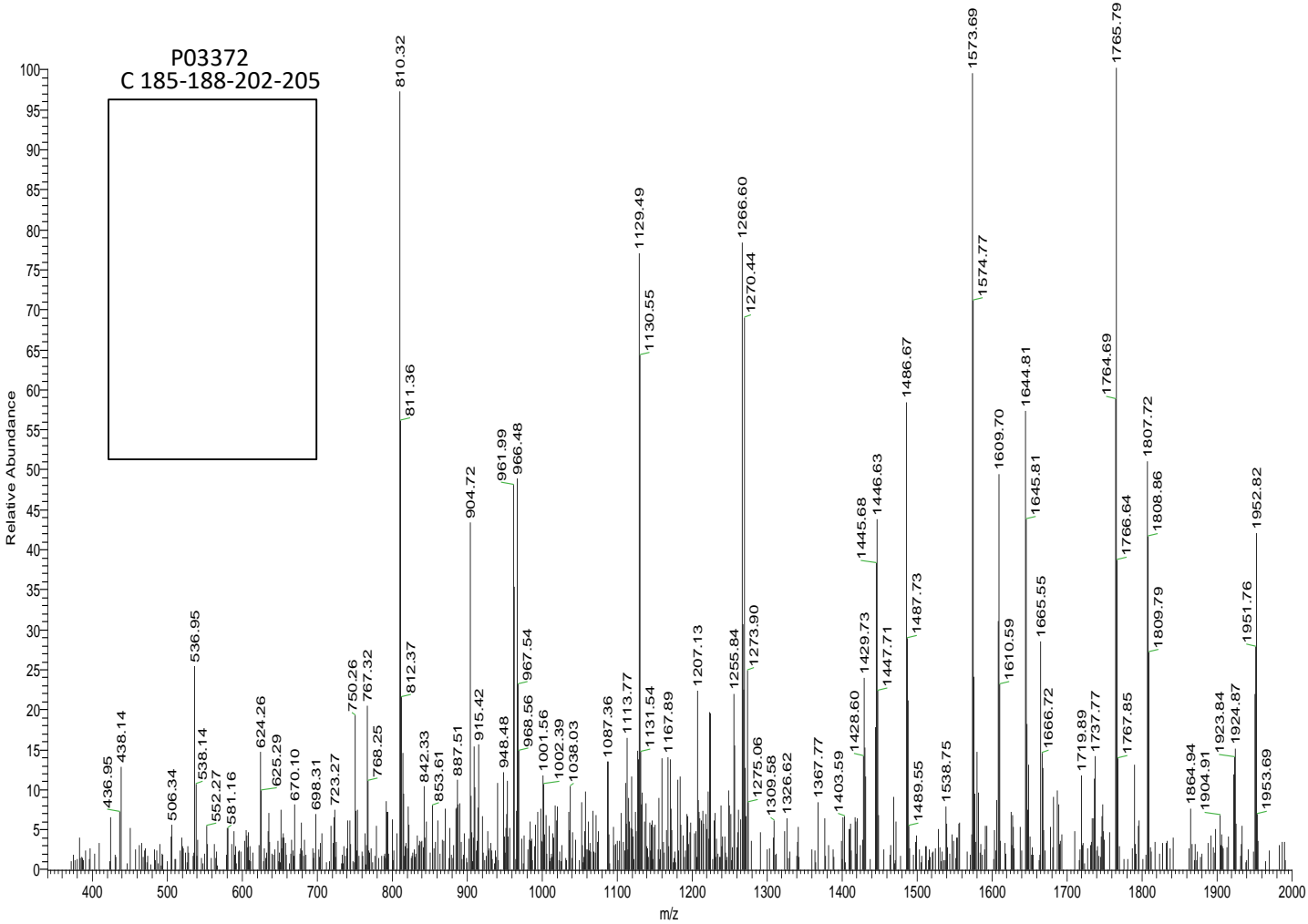


FIGURE S3. Raw data for proteomic identification of modified cysteine residues.

B

011917\_SCW\_V\_97\_DrZhang\_ERAalpha\_LysC\_AspN#11066 RT: 73.14 AV: 1 NL: 6.73E3  
F: FTMS + c NSI d sa Full ms2 641.59@etd100.00 [50.00-1935.00]

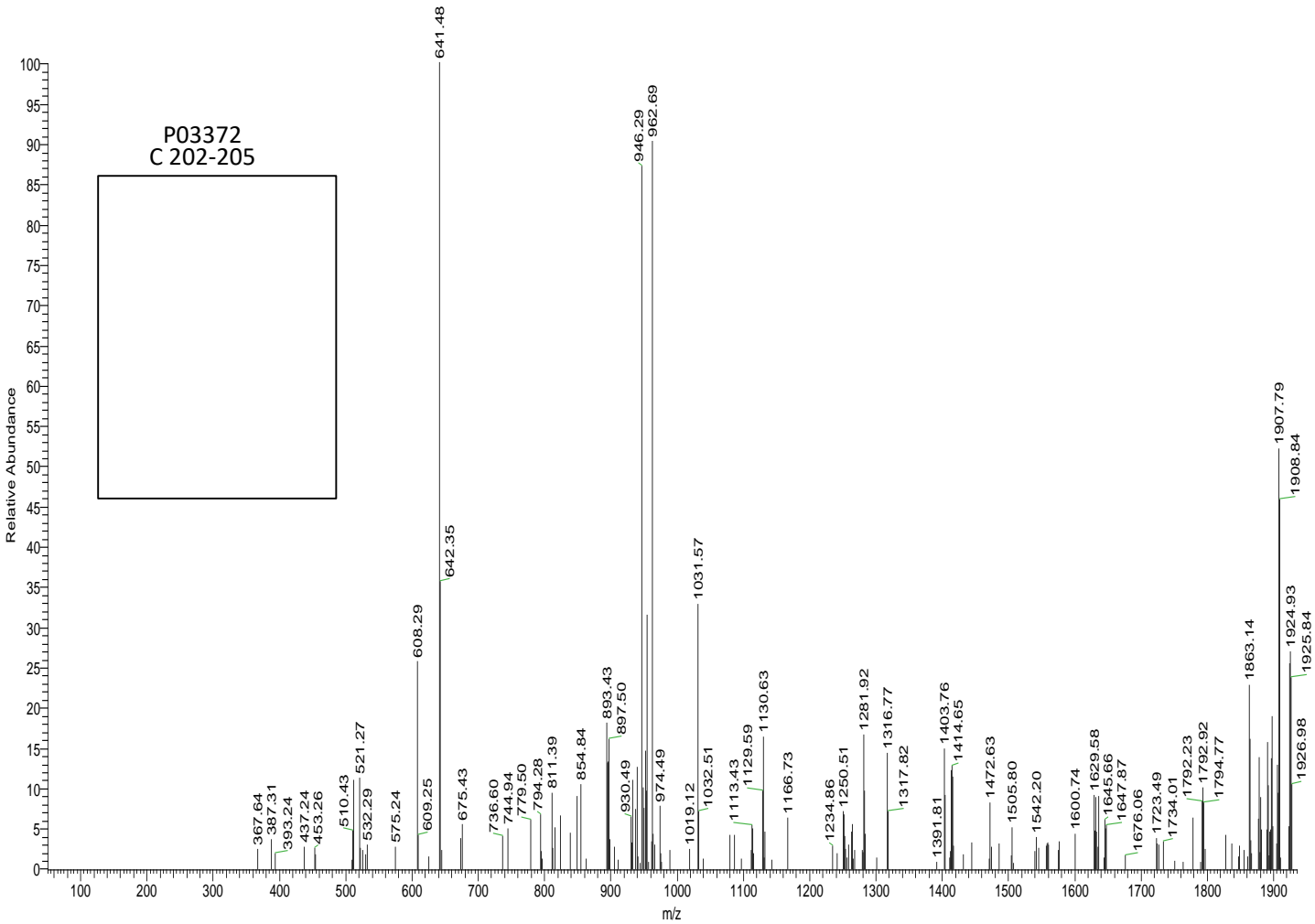
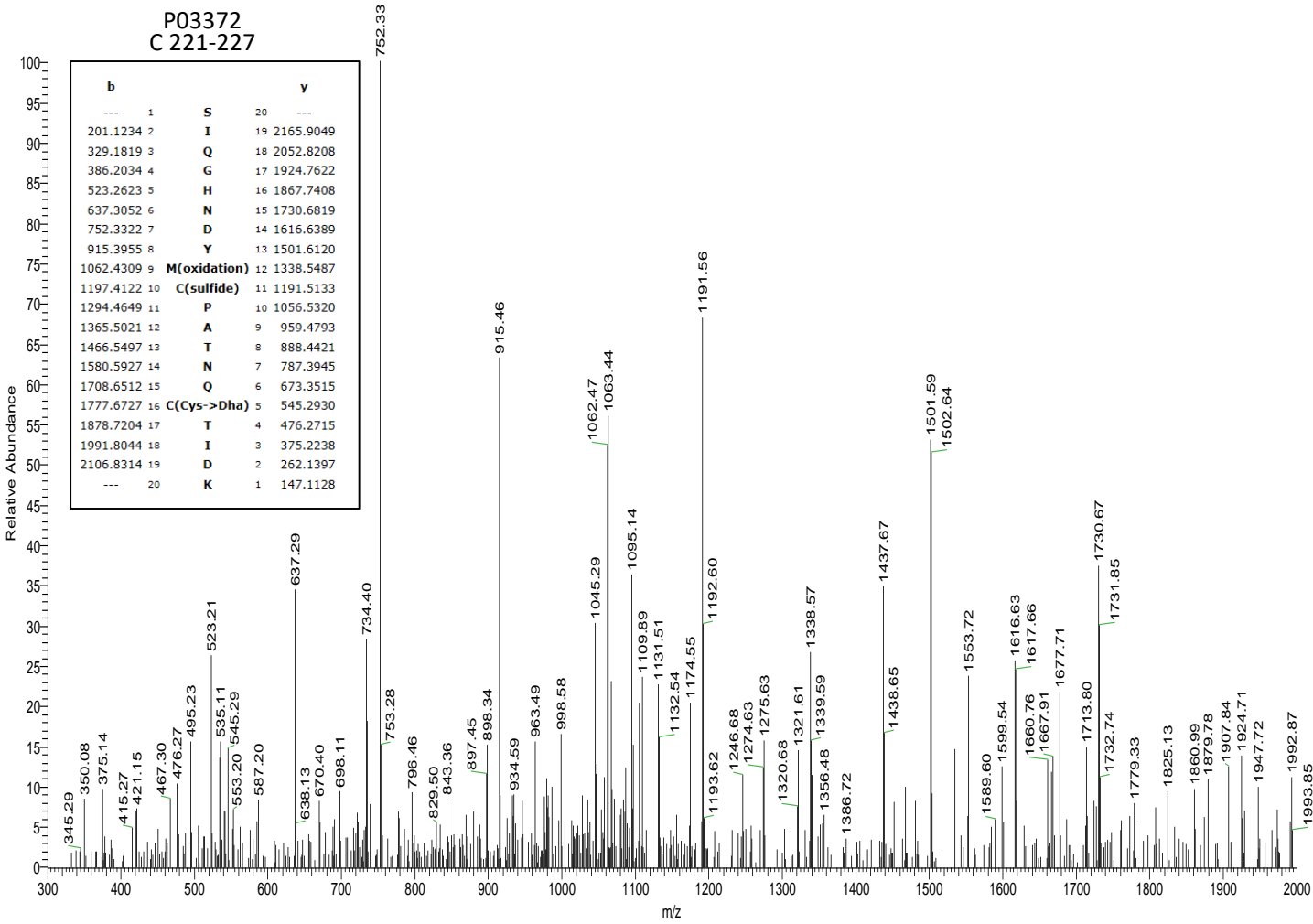


FIGURE S3. Raw data for proteomic identification of modified cysteine residues.

C

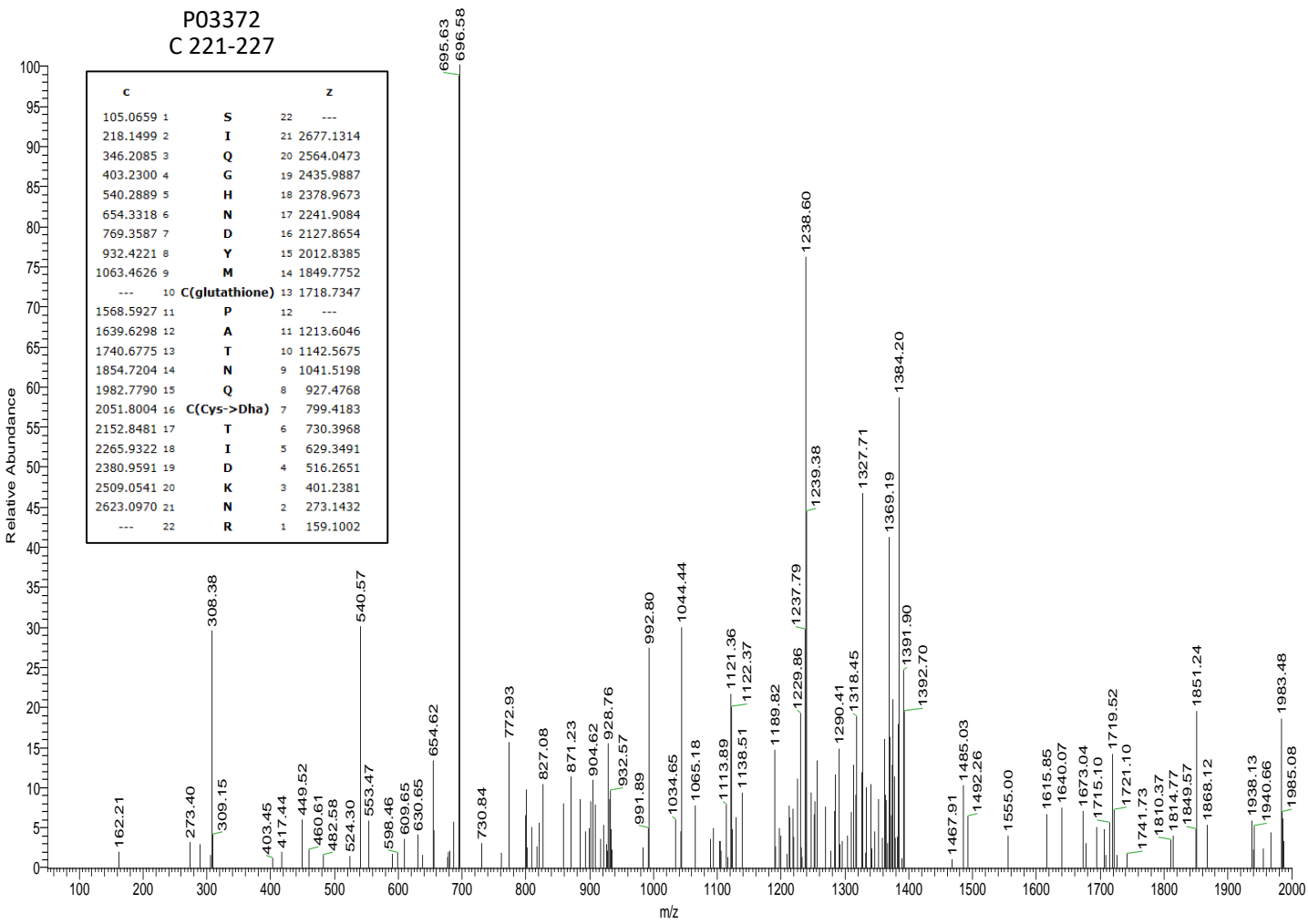
021417\_SCW\_V\_97\_ER\_Trypsin\_Rerun#7742 RT: 58.20 AV: 1 NL: 4.64E2  
 T: TMS+ c NSI.d Full.ms2 1127.47@cid35.00 [300.00-2000.00]

P03372  
 C 221-227



D

011917\_SCW\_V\_97\_DrZhang\_ERAlpha\_Trypsin#7334 RT: 49.94 AV: 1 NL: 4.30E3  
 F: ITMS +c NSI d sa Full ms2 696.05@etd75.00 [50.00-2000.00]



**FIGURE S3.** Raw data for proteomic identification of modified cysteine residues.



E

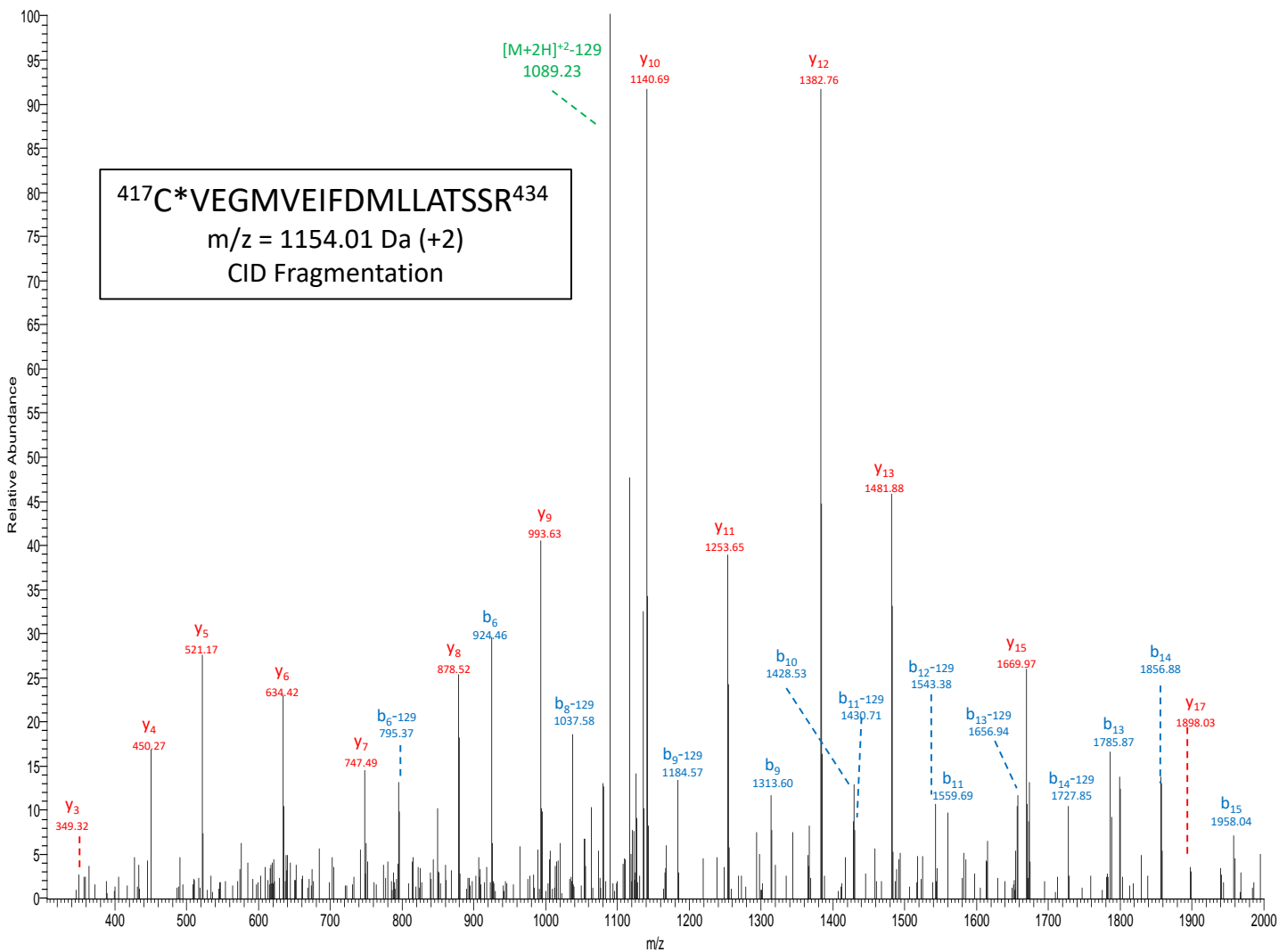


FIGURE S3. Raw data for proteomic identification of modified cysteine residues.

F

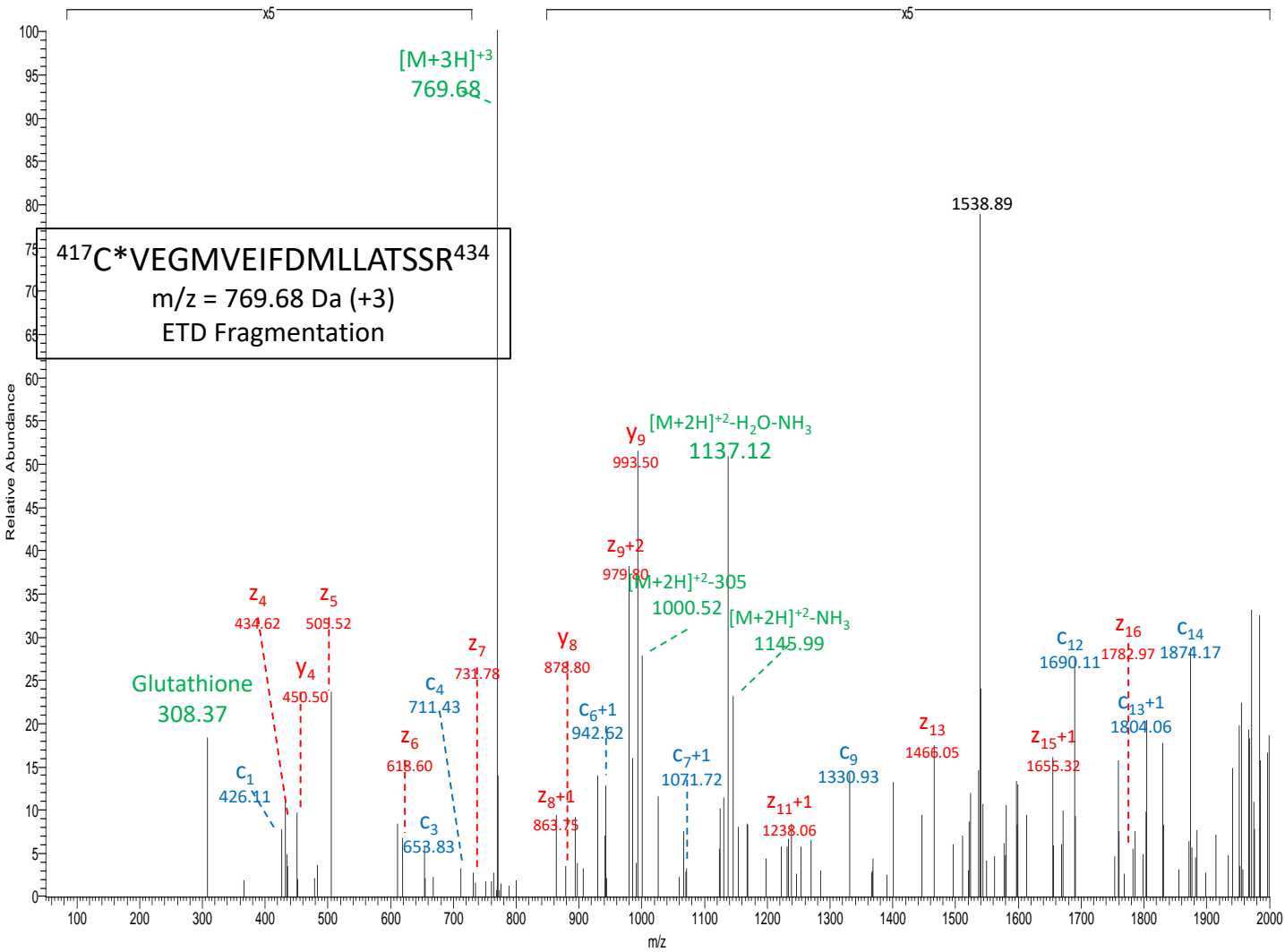


FIGURE S3. Raw data for proteomic identification of modified cysteine residues.

G

011917\_SCW\_V\_97\_DrZhang\_ERAlpha\_Trypsin#2490 RT: 23.71 AV: 1 NL: 8.27E2  
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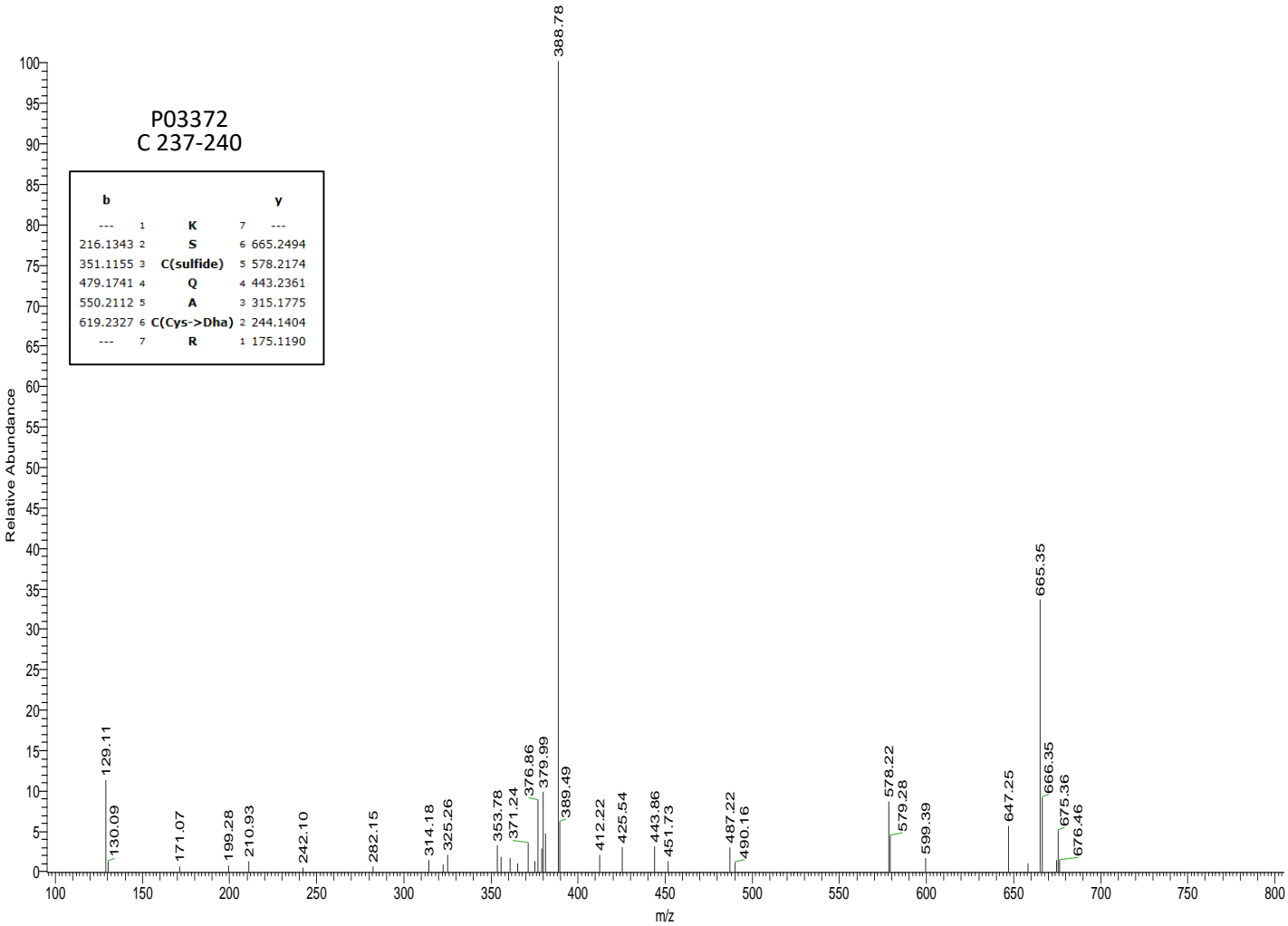


FIGURE S3. Raw data for proteomic identification of modified cysteine residues.

H

011917\_SCW\_V\_97\_DrZhang\_ERAlpha\_Trypsin#7532 RT: 50.82 AV: 1 NL: 2.43E4  
T: TMS + c NSI d sa Full ms2 422.49@etd100.00 [50.00-1280.00]

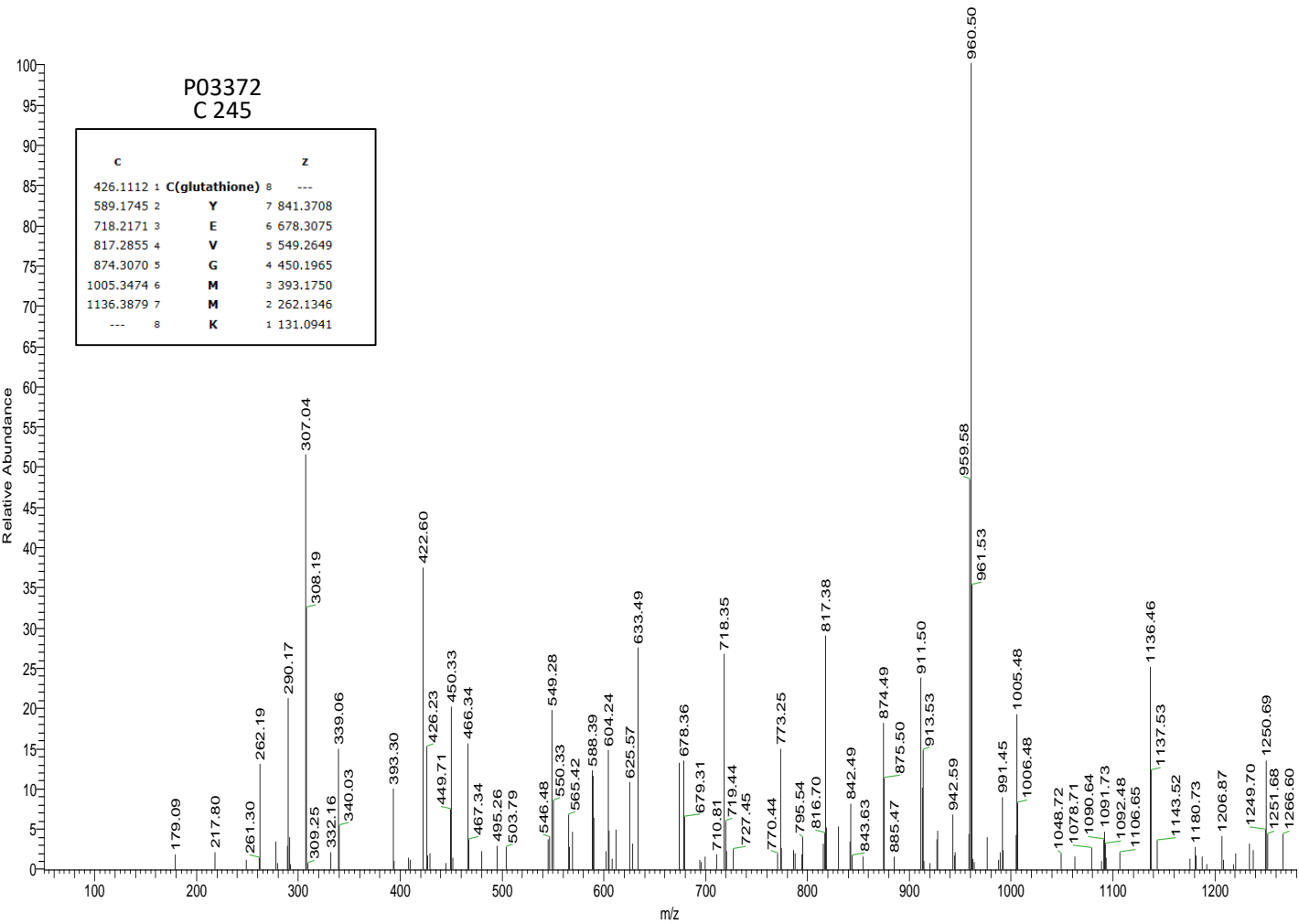
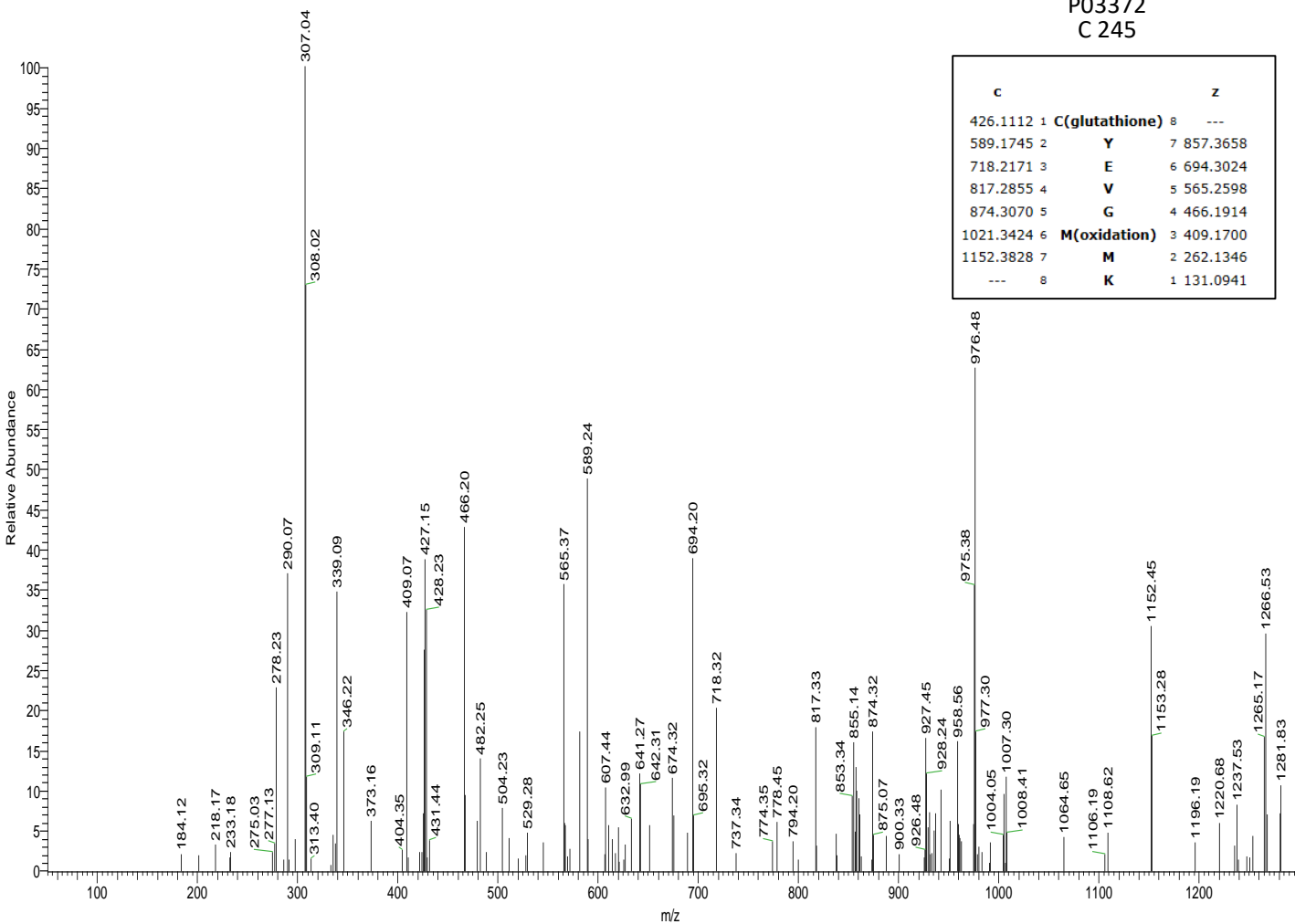


FIGURE S3. Raw data for proteomic identification of modified cysteine residues.

021417\_SCW\_V\_97\_ER\_Trypsin\_Rerun#5897 RT: 46.88 AV: 1 NL: 1.29E3  
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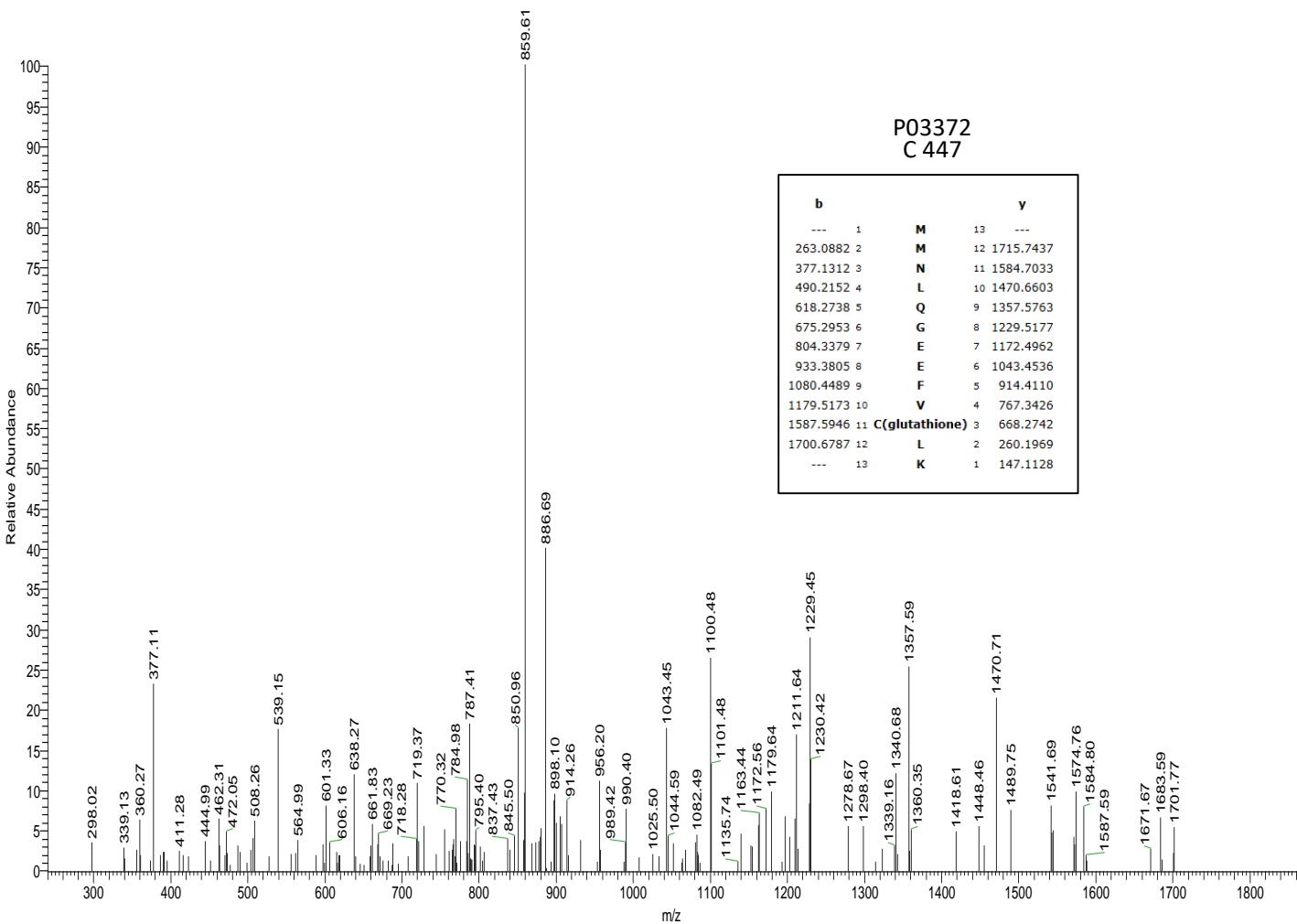
P03372  
 C 245

c	z
426.1112 1	C(glutathione) 8 ---
589.1745 2	Y 7 857.3658
718.2171 3	E 6 694.3024
817.2855 4	V 5 565.2598
874.3070 5	G 4 466.1914
1021.3424 6	M(oxidation) 3 409.1700
1152.3828 7	M 2 262.1346
---	K 1 131.0941

FIGURE S3. Raw data for proteomic identification of modified cysteine residues.

J

011917\_SCW\_V\_97\_DrZhang\_ERAlpha\_Trypsin#14816 RT: 86.10 AV: 1 NL: 3.67E4  
 T: TMS+ c NSI d Full ms2 923.89@cid35.00 [240.00-1860.00]



**FIGURE S3.** Raw data for proteomic identification of modified cysteine residues.

K

011917\_SCW\_V\_97\_DrZhang\_ERAalpha\_Trypsin#12809 RT: 76.25 AV: 1 NL: 6.03E4  
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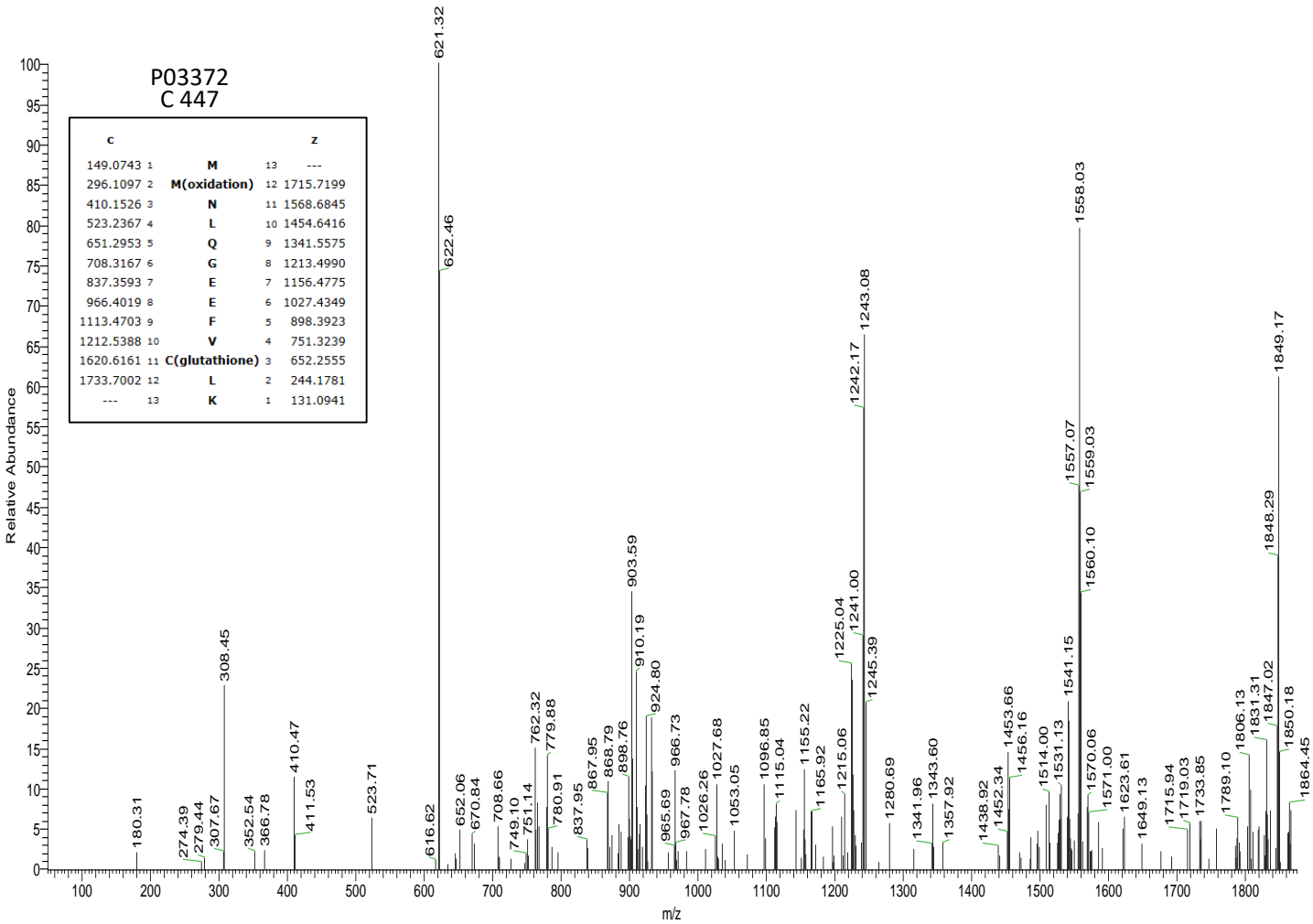


FIGURE S3. Raw data for proteomic identification of modified cysteine residues.

L

110916\_SCW\_V\_85\_Lys\_C\_Trypsin\_First\_ZT#19506 RT: 114.32 AV: 1 NL: 5.11E3  
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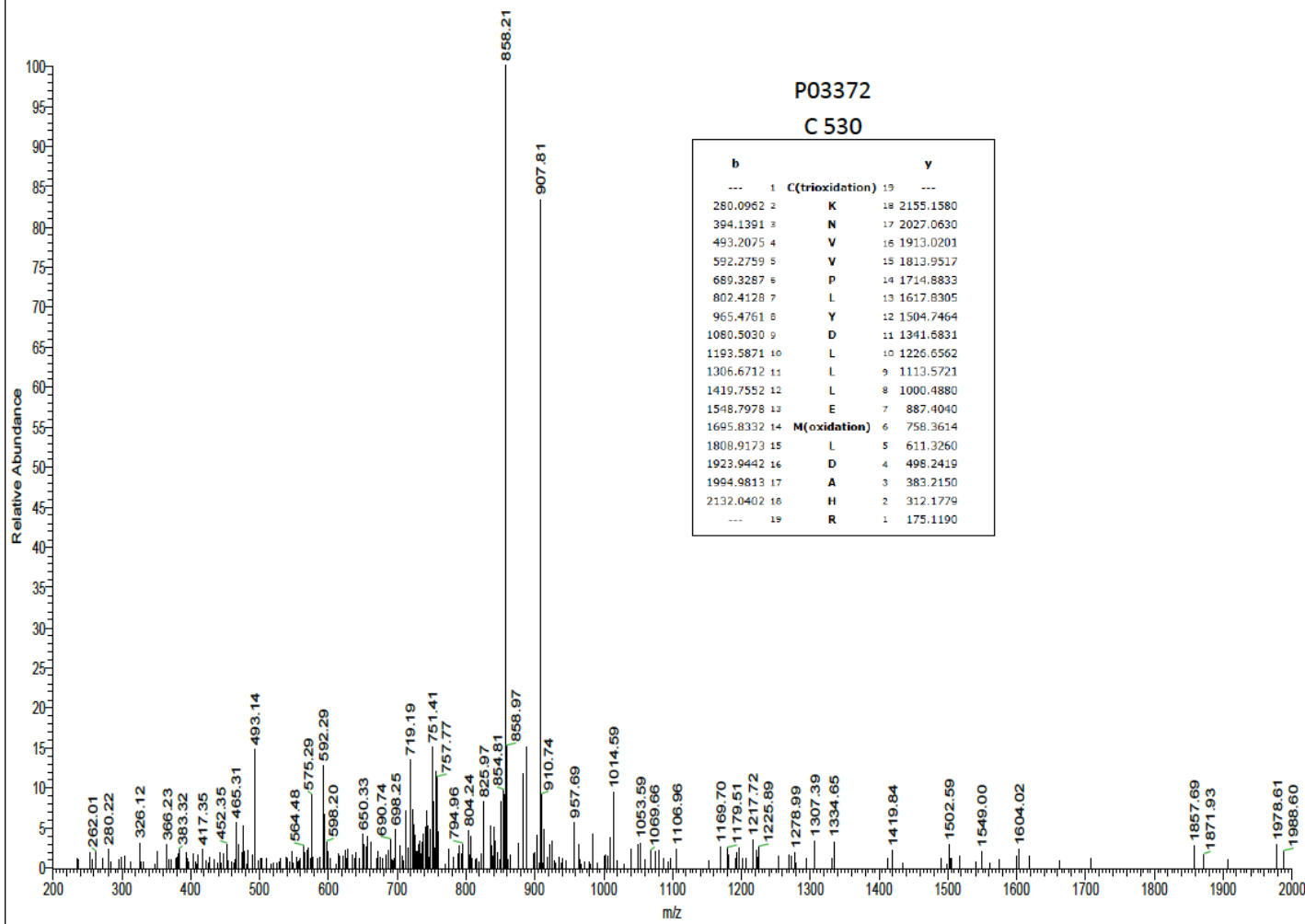


FIGURE S3. Raw data for proteomic identification of modified cysteine residues.