Figure S1

A. S-Acylation

	-6	-5	-4	-3	-2	-1	C	1	2	3	4	5	6	Average
A	-26.4%	45.8%	6.9%	4.4%	33.8%	-17.6%		87.8%	31.3%	43.9%	34.2%	-0.3%	31.7%	23.0%
C	-62.1%	-46.2%	-73.3%	-82.9%	-91.5%	-45.0%		-57.3%	-88.8%	-71.0%	-81.7%	-78.6%	-75.2%	-71.1%
D	-18.4%	-29.1%	-19.9%	-39.9%	-49.3%	-26.3%		17.8%	-27.2%	-3.0%	-12.8%	-14.5%	-13.3%	-19.7%
E	-5.8%	-42.4%	11.4%	-7.2%	-18.9%	-39.8%		6.3%	-48.7%	6.3%	-5.1%	-10.8%	38.4%	-9.7%
F	48.5%	-39.9%	21.1%	57.4%	18.4%	14.0%		35.0%	95.7%	22.7%	28.2%	-55.9%	38.9%	23.7%
G	-10.9%	38.9%	10.6%	12.2%	12.2%	31.1%		-2.4%	20.7%	-13.2%	22.7%	-35.0%	8.1%	7.9%
H	57.8%	-3.8%	41.7%	19.1%	-9.5%	92.3%		18.0%	71.0%	-18.4%	53.9%	45.3%	-6.0%	30.1%
I	28.2%	54.6%	-0.3%	-19.9%	103.0%	-8.4%		10.5%	34.1%	-1.4%	12.6%	52.0%	14.5%	23.3%
K	45.9%	68.0%	-10.9%	5.2%	77.3%	56.3%		-17.4%	-19.0%	43.3%	20.5%	31.7%	-1.8%	24.9%
L	-22.3%	28.2%	-15.6%	8.2%	30.7%	30.5%		8.9%	8.6%	-2.2%	10.2%	49.2%	13.1%	12.3%
M	11.1%	-84.9%	-19.9%	-54.2%	-70.4%	-17.6%		-14.5%	-72.5%	-14.5%	-81.7%	28.2%	-23.1%	-34.5%
N	13.4%	-10.8%	38.5%	0.2%	8.5%	40.4%		-10.8%	-20.4%	23.5%	1.7%	-29.7%	88.6%	11.9%
P	-43.9%	-24.0%	43.0%	18.4%	-21.3%	4.9%		-37.0%	-3.1%	1.4%	-12.6%	9.9%	-21.9%	-7.2%
Q	-10.6%	9.2%	-17.0%	-22.4%	-34.2%	-29.3%		1.9%	15.8%	-29.5%	58.8%	-1.7%	-27.2%	-7.2%
R	73.3%	45.6%	54.6%	58.4%	33.8%	77.9%		17.5%	12.5%	148.5%	35.4%	24.9%	81.0%	55.3%
S	5.2%	-33.5%	-27.6%	-10.5%	-30.8%	-28.3%		1.2%	4.9%	-38.3%	-30.1%	-1.9%	-17.2%	-17.2%
T	10.9%	-23.8%	-24.9%	16.0%	41.5%	-30.5%		-34.7%	2.0%	-6.2%	-14.5%	-26.0%	-22.4%	-9.4%
V	42.1%	37.2%	48.5%	48.5%	-6.3%	-22.2%		15.1%	16.6%	-3.8%	18.6%	75.9%	-30.1%	20.0%
Y	58.4%	-32.8%	-28.2%	138.1%	-35.9%	33.6%		6.9%	48.0%	62.0%	-30.1%	85.2%	-16.4%	24.1%
W	-46.6%	-76.7%	-89.4%	-90.1%	-74.4%	-78.6%		-100.0%	-100.0%	-100.0%	-78.6%	-63.4%	-67.9%	-80.5%

B. S-Nitrosylation

	-6	-5	-4	-3	-2	-1	C	1	2	3	4	5	6	Average
A	-6.4%	41.9%	12.5%	46.9%	32.3%	-15.9%		105.1%	23.0%	59.7%	62.9%	63.3%	33.5%	38.2%
C	-76.5%	-59.2%	-61.7%	-61.7%	-84.7%	-75.4%		-80.9%	-75.0%	-59.2%	-90.2%	-65.5%	-88.9%	-73.3%
D	-23.4%	-24.4%	-54.1%	-6.7%	-57.3%	9.1%		11.7%	-0.7%	-30.0%	47.0%	-31.1%	14.8%	-12.1%
E	3.1%	-20.3%	-3.3%	-2.3%	-6.2%	-34.4%		1.7%	-29.3%	26.0%	-19.6%	9.9%	2.8%	-6.0%
F	20.9%	-6.7%	-33.0%	25.3%	23.7%	2.1%		45.1%	63.2%	9.9%	-6.0%	-35.4%	10.1%	9.9%
G	9.0%	17.2%	21.6%	37.4%	12.5%	35.3%		44.8%	26.1%	-8.1%	19.8%	-30.8%	17.1%	16.8%
H	103.2%	110.5%	87.4%	23.0%	82.4%	108.2%		70.0%	122.0%	9.6%	106.7%	60.8%	22.5%	75.5%
I	23.7%	28.4%	48.9%	32.8%	62.7%	-7.0%		30.7%	103.6%	-14.6%	-2.0%	40.4%	-5.7%	28.5%
K	34.6%	58.4%	62.7%	26.6%	25.0%	47.1%		-3.0%	-0.3%	28.4%	11.4%	39.7%	27.1%	29.8%
L	9.6%	-1.6%	1.8%	-12.1%	28.1%	0.7%		-7.2%	-7.5%	1.2%	-3.3%	10.2%	-18.9%	0.1%
M	-0.5%	-59.5%	36.4%	-1.6%	-47.0%	47.7%		78.6%	-1.6%	30.2%	55.9%	104.2%	49.3%	24.3%
N	-33.7%	24.8%	28.6%	-17.5%	-33.7%	58.6%		-0.1%	-60.4%	6.3%	-5.0%	-14.8%	89.2%	3.5%
P	-13.9%	-38.3%	1.6%	-14.6%	0.7%	-26.9%		-43.6%	7.2%	-19.9%	-29.5%	-28.9%	-30.1%	-19.7%
Q	0.9%	19.1%	-25.7%	-51.6%	-25.5%	-20.8%		-26.4%	-14.8%	-39.7%	14.8%	-31.1%	-25.5%	-18.9%
R	-6.9%	-16.2%	18.2%	-5.4%	-17.6%	74.1%		-13.9%	-43.7%	36.4%	-17.1%	3.1%	8.1%	1.6%
S	8.0%	-12.8%	-50.0%	-24.2%	-28.8%	-20.2%		-33.5%	-0.8%	-9.4%	-14.7%	-14.4%	10.1%	-15.9%
T	14.8%	-6.9%	-24.4%	-4.3%	26.7%	-23.4%		-30.7%	7.0%	0.8%	-9.8%	-16.1%	5.8%	-5.0%
V	36.6%	44.2%	51.1%	23.9%	30.3%	-26.6%		3.1%	20.1%	10.1%	20.6%	60.8%	-10.2%	22.0%
Y	14.8%	-34.4%	-35.7%	277.3%	3.4%	19.6%		34.0%	14.8%	51.1%	9.6%	116.9%	-15.1%	38.0%
W	-80.9%	-47.8%	-90.5%	-100.0%	-100.0%	-100.0%		-100.0%	-100.0%	-83.6%	-71.3%	-83.6%	-56.9%	-84.5%

C. S-sulfenylation

	-6	-5	-4	-3	-2	-1	C	1	2	3	4	5	6	Average
A	-19.9%	1.2%	-23.7%	8.4%	-16.8%	-7.9%		12.9%	18.9%	-10.7%	8.4%	3.6%	39.4%	1.1%
C	-5.4%	-3.4%	-16.8%	25.7%	-44.5%	54.5%		11.0%	-42.1%	50.3%	-33.4%	-0.1%	-30.2%	-2.9%
D	5.9%	18.3%	-37.6%	4.0%	-14.8%	-29.3%		-10.0%	30.4%	25.8%	13.2%	-44.5%	12.6%	-2.2%
E	-15.1%	-25.3%	18.3%	-15.0%	-21.9%	-11.7%		33.1%	-48.8%	-6.6%	-13.5%	1.3%	22.6%	-6.9%
F	-21.2%	-48.0%	-49.1%	21.0%	40.8%	-44.5%		5.1%	13.9%	8.5%	-39.5%	-42.8%	-23.7%	-15.0%
G	-6.9%	-9.9%	-34.7%	-49.5%	-34.1%	-18.6%		52.0%	-21.7%	-23.2%	33.9%	-31.6%	4.4%	-11.7%
H	2.4%	121.9%	-21.2%	-28.7%	-41.3%	14.4%		-20.1%	22.0%	-69.7%	-33.4%	-0.1%	-61.2%	-9.6%
I	34.4%	-36.4%	-1.4%	-1.2%	11.0%	-0.9%		-2.4%	36.2%	-27.5%	-14.8%	4.8%	-10.8%	-0.8%
K	55.0%	95.1%	98.8%	28.0%	32.2%	92.4%		11.0%	40.2%	51.7%	41.2%	106.9%	16.9%	55.8%
L	31.1%	33.7%	-7.3%	1.4%	47.2%	34.3%		-11.1%	4.0%	24.1%	51.8%	66.4%	32.2%	25.7%
M	-44.5%	27.3%	4.0%	30.8%	2.4%	-16.8%		84.9%	78.3%	88.6%	-16.8%	29.4%	33.1%	25.1%
N	-23.2%	-5.9%	53.1%	-58.4%	8.8%	42.7%		15.8%	-8.2%	11.0%	-13.9%	-51.7%	105.6%	6.3%
P	-13.3%	-41.4%	34.4%	32.3%	-12.4%	-24.3%		-21.2%	18.4%	-11.0%	2.1%	18.9%	-9.5%	-2.3%
Q	11.0%	17.1%	-21.7%	-38.7%	-37.0%	3.3%		-48.8%	-3.4%	-45.9%	34.7%	-0.1%	-5.5%	-11.3%
R	39.4%	25.9%	32.2%	22.4%	59.2%	34.2%		47.9%	-18.5%	118.4%	29.4%	19.5%	-11.9%	33.2%
S	19.8%	4.8%	-30.2%	3.6%	3.2%	-4.1%		8.0%	24.8%	-26.4%	-14.3%	-5.4%	24.8%	0.7%
T	-55.0%	-14.5%	-2.6%	-12.8%	37.7%	-9.9%		-27.8%	-39.5%	-6.6%	-40.6%	-32.8%	-29.9%	-19.5%
V	-1.0%	-3.2%	101.5%	48.9%	-4.0%	-26.3%		-25.3%	-1.7%	-2.9%	-4.3%	-4.9%	-33.4%	3.6%
Y	7.7%	-44.5%	-53.4%	114.0%	-25.1%	-16.8%		-35.3%	53.6%	-21.2%	-24.3%	47.9%	-56.6%	-4.5%
W	-72.3%	-39.5%	-81.7%	-61.6%	-33.4%	11.0%		24.8%	-76.2%	-28.7%	-16.8%	-52.4%	-37.6%	-38.7%

D. S-gluathionylation

	-6	-5	-4	-3	-2	-1	C	1	2	3	4	5	6	Average
A	-23.0%	-8.7%	-22.1%	2.4%	4.8%	-27.3%		75.1%	38.5%	21.6%	17.9%	-4.0%	7.8%	6.9%
C	-64.1%	-35.7%	-58.4%	-64.9%	-83.4%	-70.3%		-76.9%	-89.2%	-46.4%	-59.6%	-58.4%	-62.5%	-64.1%
D	25.9%	-12.5%	-23.1%	9.1%	-38.1%	-23.1%		-5.7%	-12.4%	-18.9%	16.4%	-16.9%	-14.4%	-9.5%
E	0.1%	-10.1%	31.2%	-6.3%	-30.5%	-35.5%		-2.6%	-46.3%	-6.7%	-18.5%	10.2%	31.2%	-7.0%
F	26.9%	-48.1%	-30.7%	20.9%	47.1%	1.6%		-3.8%	44.4%	33.7%	-6.8%	-19.5%	7.4%	6.1%
G	5.7%	14.3%	15.7%	9.8%	17.7%	9.0%		13.8%	4.3%	-2.8%	3.0%	-26.0%	9.2%	6.1%
H	40.7%	73.2%	66.2%	119.7%	32.0%	190.9%		76.2%	110.6%	24.7%	71.8%	33.0%	10.8%	70.8%
I	18.3%	14.9%	20.1%	-9.1%	59.3%	-26.8%		-5.4%	20.9%	-19.0%	-8.8%	47.8%	-2.0%	9.2%
K	54.8%	103.5%	10.8%	13.0%	61.3%	37.7%		-16.9%	-1.6%	44.2%	15.9%	28.0%	-1.0%	29.1%
L	-16.9%	-0.6%	-28.5%	-11.7%	21.5%	23.9%		-15.7%	-19.2%	15.5%	2.1%	40.8%	2.7%	1.2%
M	-39.0%	-51.1%	-48.1%	-58.4%	-68.0%	-28.8%		-44.6%	-64.4%	-44.6%	-34.7%	10.8%	-41.8%	-42.7%
N	2.3%	-16.9%	39.6%	1.3%	8.7%	30.6%		33.7%	6.0%	10.8%	-16.9%	-38.3%	61.3%	10.2%
P	-23.8%	-33.8%	31.1%	15.1%	6.4%	5.8%		19.6%	-7.6%	16.0%	30.3%	-22.8%	-6.0%	2.5%
Q	-11.8%	26.2%	0.2%	-43.1%	-37.1%	-8.3%		6.6%	23.3%	-19.0%	54.4%	-28.0%	-5.7%	-3.5%
R	41.5%	41.5%	76.0%	51.6%	13.8%	71.6%		17.7%	17.0%	196.1%	29.3%	38.5%	68.7%	55.3%
S	8.1%	-26.1%	-26.3%	-10.6%	-33.5%	2.8%		-0.8%	26.6%	-23.3%	-23.2%	-2.2%	-6.5%	-9.6%
T	-3.4%	-7.9%	-39.2%	-9.0%	31.8%	-18.6%		-38.8%	-1.8%	-29.0%	-9.0%	-13.7%	-5.9%	-12.0%
V	14.6%	35.3%	66.2%	29.0%	-8.9%	-34.6%		-10.1%	5.8%	-44.6%	12.2%	37.7%	-35.0%	5.6%
Y	71.1%	6.9%	-26.9%	185.0%	41.3%	21.2%		-12.3%	72.6%	44.4%	5.8%	75.5%	15.6%	41.7%
W	-51.5%	-9.3%	-79.4%	-55.2%	-83.4%	24.7%		-48.1%	-88.1%	-28.8%	-58.4%	18.7%	-58.4%	-43.1%

Figure S1, related to Figure 3. Average amino acid composition of linear sequences. The composition of amino acids within a 12 amino acid window was examined for S-acylation (A), S-nitrosylation (B), S-sulfenylation (C), and S-glutathionylation (D). The occurrence of each of the 20 amino acids was calculated as a relative change (in %) of observed amino acid abundance around Cys-PTM, as compared to expectation based on random peptides.

Figure S2.

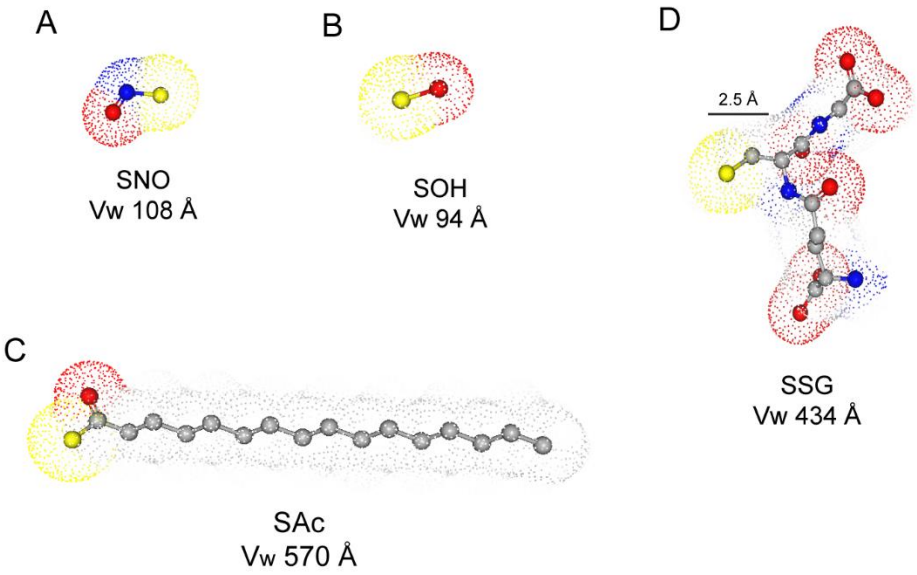
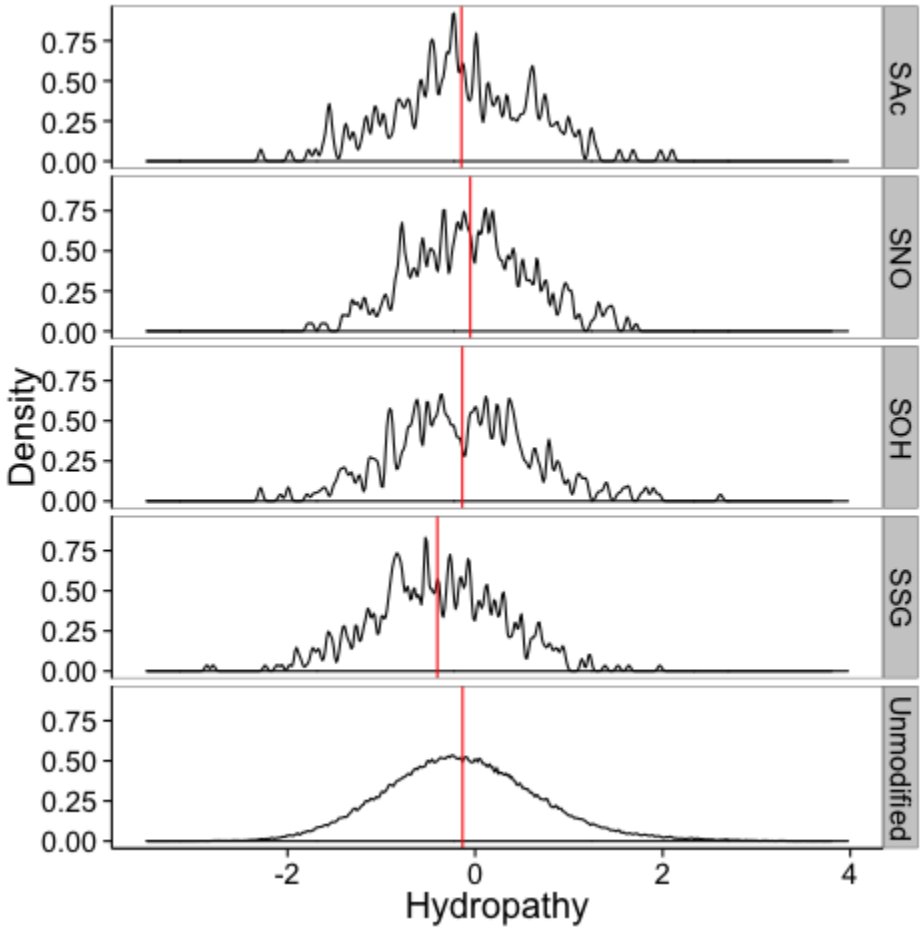


Figure S2, related to figure 3C. Comparison of molecular sizes of modifying agents. SNO (A) and SOH (B) have similar sizes and thus similar secondary structure distribution. SAc (C), which is denoted by the most common species palmitate exhibits the largest predicted molecular volume. Owing the location of the thiol in glutathione, the SSG modification (D) is predicted to adopt a bent configuration, potentially limiting binding to flexible protein regions. V_w, van der Waals volume. Yellow denotes sulfur atoms, blue is nitrogen, red is oxygen and grey are carbon atoms.

Figure S3



	Average	Count
SAc	-0.12652	581
SNO	-0.04352	682
SOH	-0.13295	431
SSG	-0.32022	875
UnMod	-0.15924	20977

Figure S3, related to Figure 4A. Distribution of Kyte-Doolittle hydropathy index values. Hydropathy was calculated for a 13 amino acid window surrounding the cysteine residue of interest. Residues from the reference proteome that did not have annotated disulfide status or modification were used for the unmodified comparison group. Red line represents the average for each dataset, with corresponding values and the count of residues analyzed for each group indicated in table.

Supplemental Experimental Procedures

Protein extraction- Organs were perfused with PBS through the left ventricle, whole organs were collected and snap frozen in liquid nitrogen. Tissue from an n=6 biological replicates were maintained at -80°C until homogenization. Liver tissue was homogenized in HEN buffer (250 mM HEPES, 1 mM DTPA, 0.1 mM Neocuporine, pH 7.7) with 1% Triton X-100, 20 mM NEM, 1x EDTA-free complete mini protease inhibitors (Roche) added. For preparation of SOH proteomes, 10 mM 5,5-Dimethyl-1,3-cyclohexanedione (dimedone) (Sigma) and 200 U/ml catalase were added to the homogenization buffer. Tissues were homogenized into 2 mL chilled lysis buffer using a Teflon pestle and a Jumbo Stirrer. The lysates were cleared of debris by centrifugation for 10 min at 14,000 rpm. Protein concentrations of cell lysates were determined by standard BCA assay (Pierce).

Detailed Preparation of modified cysteine proteomes-

S-Nitrosylation- Soluble proteins suspensions are treated with 35 mM MMTS for 35 min at 50°C with occasional vortexing. Following acetone precipitation and re-suspension in loading buffer (250 mM MES, 1 mM DTPA, pH 6.0, 1% SDS), the soluble proteins are loaded on the mercury resin columns. Proteins are extensively washed and then treated with MS-grade trypsin (Promega) overnight. Following trypsinization, the resulting peptides are extensively washed to remove non-cysteine containing peptides. Peptides are then eluted with 1% performic acid in MS-grade ultrapure water (Fisher). Peptide solution is frozen at -80°C and lyophilized. Following lyophilization, peptides are re-suspended in 0.1% performic acid/0.1% trifluoroacetic acid (v/v) in MS-grade ultrapure water. The volume of the peptide solution is then adjusted to 20 µL and desalted with C18 StageTips (Thermo) prior to LC-MS/MS analysis.

S-Glutathionylation- Proteins are first exposed to UV in HEN buffer with 20 mM NEM and 0.1M mannitol to remove endogenous cysteine bound nitric oxide. Following precipitation, proteins are re-suspended and alkylated in HEN buffer containing 5% SDS and 100 mM NEM for 60 min at 50°C with frequent vortexing. Following acetone precipitation, proteins are solubilized in HEN buffer with 0.2% SDS. Protein suspension is then subjected to buffer exchange using Sephadex-G25 packed PD-10 columns, preconditioned with PBS. The protein suspension is then treated with 13.5 ug/mL human recombinant Glutaredoxin (Grx) 1 (Cayman), 4 mM GSH, and 2 mM NADPH for 60 min at 37°C to selectively reduce S-glutathionylated cysteine residues (Lind, et al., 2002). Negative controls are generated following all steps with Grx omitted from the reaction mixture. Following incubation another round of acetone precipitation is preformed and proteins are re-suspended in loading buffer and loaded onto mercury columns. All subsequent steps follow the same protocol previously described (Doulias, et al., 2013).

*S-Acylation-*After incubation with hydroxylamine, proteins are desalted using Sephadex G-25 packed PD-10 columns preconditioned with HEN buffer. The resulting protein suspension is then precipitated in acetone, re-suspended in loading buffer and loaded on mercury columns. All subsequent steps follow the same methodology (Doulias, et al., 2013).

Mass Spectrometry- Tryptic digests were analyzed by LC-MS/MS on a hybrid LTQ Orbitrap Elite mass spectrometer (Thermo fisher Scientific San Jose, CA) coupled with a nanoLC Ultra (Eksigent). Peptides were separated by reverse phase (RP)-HPLC on a nanocapillary column, 75 µm id × 15 cm Reprosil-pur 3uM, 120A (Dr. Maisch, Germany) in a Nanoflex chip system (Eksigent). Mobile phase A consisted of 1% methanol/0.1% formic acid and mobile phase B of 1% methanol/0.1% formic acid/80% acetonitrile. Peptides were eluted into the MS at 300 nL/min with each RP-LC run comprising a 90 minute gradient from 10 to 25 % B in 65 min, 25-40%B in 25 min. The mass spectrometer was set to repetitively scan m/z from 300 to 1800 (R = 240,000 for LTQ-Orbitrap Elite) followed by data-dependent MS/MS scans on the twenty most abundant ions, with a minimum signal of 1500, dynamic exclusion with a repeat count of 1, repeat duration of 30s, exclusion size of 500 and duration of 60s, isolation width of 2.0, normalized collision energy

of 33, and waveform injection and dynamic exclusion enabled. FTMS full scan AGC target value was 1e6, while MSn AGC was 1e4, respectively. FTMS full scan maximum fill time was 500 ms, while ion trap MSn fill time was 50 ms; microscans were set at one. FT preview mode; charge state screening, and monoisotopic precursor selection were all enabled with rejection of unassigned charge states.

Peptide Identification- Protein identification from a total of six biological replicates was carried out using SEQUEST algorithm (Sorcerer2 4.3.0, Sage-N Research Inc or Bioworks 3.2 package, Thermo Fisher Scientific), allowing differential modifications (Met oxidation +15.99 Da, Cys modification depending on the reagent used, +125.05 for NEM-adducts, and +138.08 Da for DMD-adducts or +48.99 for sulfonic acid), two missed cleavages, and mass tolerance of 2 and 1.2 amu for precursor and fragment ions, respectively for SOH proteomes and 10 ppm for all others. MS/MS raw files were searched against the Mouse Swissprot database containing porcine trypsin. The raw files were also searched against inverted databases constructed from the corresponding target databases. SEQUEST results were analyzed using the probability ratio method and false discovery rates (FDR) of peptide identifications were calculated from the search results against the inverted databases. The identified modified peptides were manually validated (Tabb, et al., 2006). After validation all sites that appeared in negative controls were removed from further analysis. For label-free quantification, peptides were restricted to detection in at least two out of three biological replicates. For equivalent peptides, total ion current was averaged among biological replicates and compared between genotypes.

Bioinformatics- For the generation of a reference proteome published studies contained within PaxDB, an online database of protein abundance across organisms, were utilized (Huttlin, et al., 2010; Kislinger, et al., 2006; Kruger, et al., 2008). Proteins were restricted to mouse liver and quantification was provided by spectral counts. Any non-cysteine containing proteins were filtered out. The resulting protein dataset originating from the literature was combined with our experimentally derived datasets to produce a comprehensive reference proteome of mouse liver proteins detected by mass spectrometry. Protein sequence information, molecular weight, secondary structure, protein domains, and annotated disulfides were obtained from UniProt. Van der Waals surface area for each of the modifications was calculated using MarvinSpace version 14.9.15.0. Kyte-Doolittle hydropathy was calculated based on linear sequence for a 13 amino acid window surrounding the modified cysteine. Structure based analysis was performed using PDB structures with at least 80% Blastp homology with the added criterion of 100% homology within 5 amino acids of the cysteine residue. Structure based disulfide prediction was based on SG-SG distances ≤ 2.5Å using PROPKa 3.0. Solvent accessibility was measured using DSSP, pKa was analyzed using PROPKa 1.0, and neighboring charges were analyzed from PDB structures.

Gene Ontology analysis- Gene Ontology (GO) terms that were associated with one or more modifications were retrieved from Uniprot for uniquely modified proteins. Each term's ancestor associations were condensed using "is a" and "part of" relationships. For each GO term and modification, the fraction of modified proteins annotated with the term was determined. GO terms were evaluated in each of the three GO categories: cellular location, biological process, and molecular function. Proteins without GO associations for a category were not included when calculating fractions. Subsets of GO terms were selected as features that could be used to classify modified proteins. These GO terms had 1) at least one modification with 10% of proteins associated with the term, 2) at least one modification with more than 20% of proteins not associated with the term, and 3) a significant association with modification status, as indicated by a Chi-square test Benjamini-Hochberg adjusted p-value < 0.001, 4) an entropy below 1.97 across the distribution of modified fractions. Given the redundancy of GO term associations, we first clustered the distinguishing GO terms into seven groups using k-means clustering. Each GO term

was represented by a feature vector of the fraction of annotated proteins for each modification. The vector was normalized by the sum of each annotated fraction for a term. As an evaluation of how well these GO terms were associated with different modifications, we constructed a random forest classifier to predict modifications and rank the importance of each cluster in the correct assignment of modification labels according to its mean decrease impurity. The importance measure represents the decrease in accuracy when the protein assignments for a cluster's terms have been permuted to erase the association between modification and term.

Supplemental References

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