Supporting Information

Thioredoxin cross-linking by nitrogen mustard in lung epithelial cells: formation of multimeric thioredoxin/thioredoxin reductase complexes and inhibition of disulfide reduction

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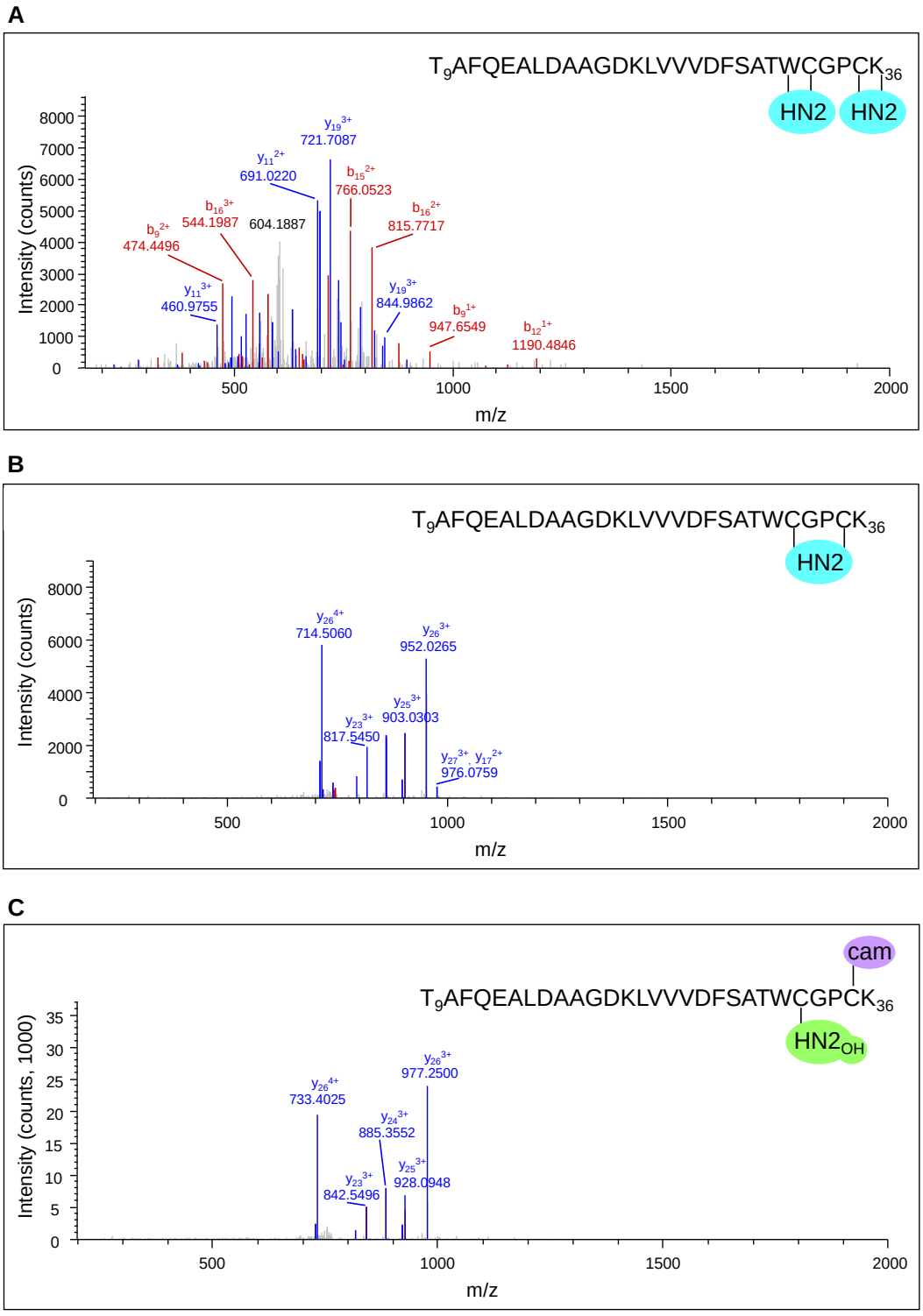
Figure S1


A
T9AFQEALDAAGDKLVVVDFSATWCGPCK36
HN2
HN2
Intensity (counts)
m/z
y19 3+ 721.7087
y11 2+ 691.0220
b15 2+ 766.0523
b16 3+ 544.1987
604.1887
b16 2+ 815.7717
b9 2+ 474.4496
y11 3+ 460.9755
y19 3+ 844.9862
b9 1+ 947.6549
b12 1+ 1190.4846
B
T9AFQEALDAAGDKLVVVDFSATWCGPCK36
HN2
Intensity (counts)
m/z
y26 4+ 714.5060
y26 3+ 952.0265
y25 3+ 903.0303
y23 3+ 817.5450
y27 3+ . y17 2+ 976.0759
C
cam
T9AFQEALDAAGDKLVVVDFSATWCGPCK36
HN2OH
Intensity (counts, 1000)
m/z
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y26 4+ 733.4025
y24 3+ 885.3552
y25 3+ 928.0948
y23 3+ 842.5496

Figure S1

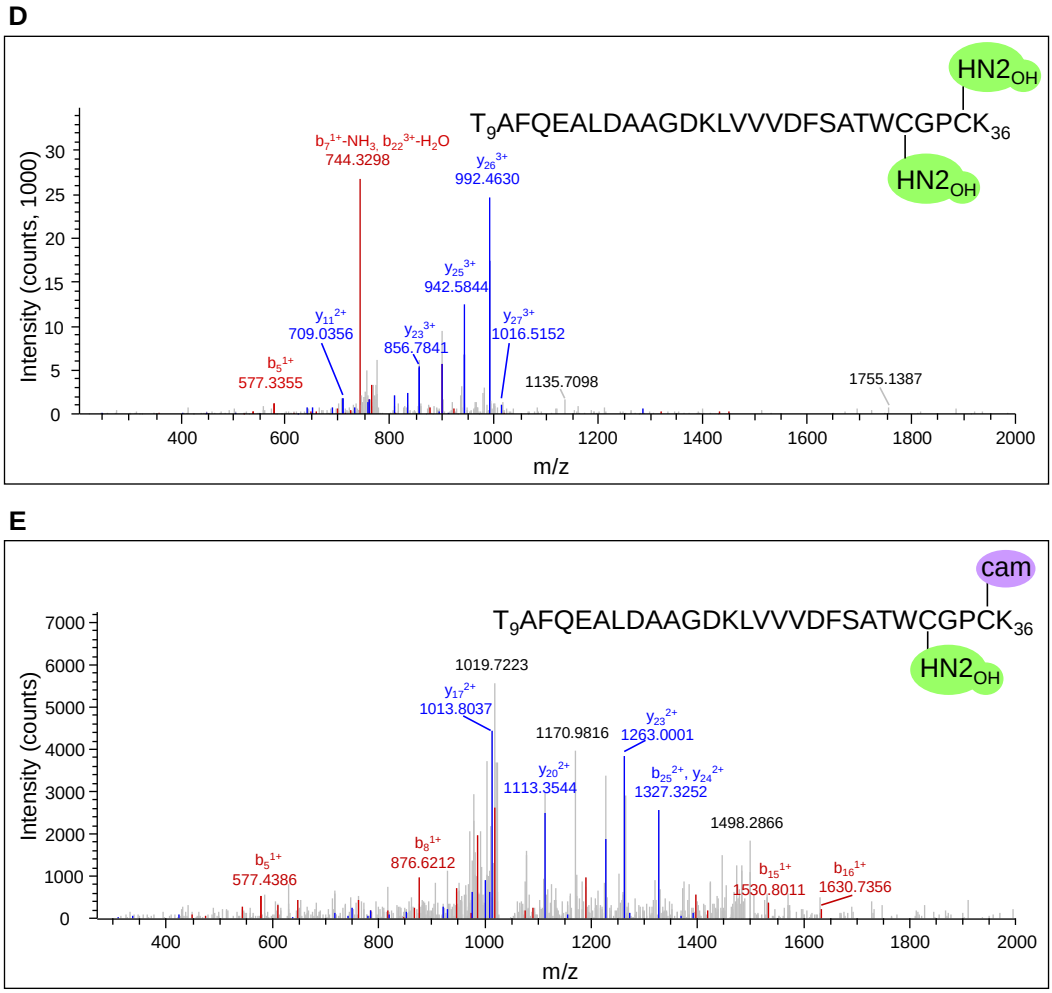


Figure S1. HN2- modified TAFQEALDAAGDKLVVVDFSATWCGPCK peptide in human Trx1. (A) MS/MS spectrum of a quintuply charged ion at *m/z* 622.52. The mass of this ion corresponds to the mass of peptide 9-36 from human Trx1 plus two HN2 modifications. The fragments in the spectra indicate that one HN2 cross-links residues tryptophan 31 and cysteine 32 on Trx1 and the other HN2 cross-links residues cysteine 35 and lysine 36. Matched b (shown in red) and y (shown in blue) fragments are marked. (B) MS/MS spectrum of a quadruply charged ion at *m/z* 757.13. The mass of this ion corresponds to peptide 9-36 with one HN2 modification in which HN2 linked to cysteine 32 and cysteine 35 in human Trx1. (C, E) MS/MS spectra of a quadruply charged ion at *m/z* 775.89 (C) and a triply charged ion at *m/z* 1034.18 (E). The masses of these ions correspond to peptide 9-36 from human Trx1 modified with one HN2 monoadduct and one carbamidomethyl adduct. The fragment ions indicate that the HN2 monoadduct is bound to cysteine 32 with a carbamidomethyl adduct bound to cysteine 35. (D) MS/MS spectrum of a quadruply charged ion at *m/z* 786.90. The mass of this ion corresponds to peptide 9-36 with two HN2 monoadducts, one bound to cysteine 32 and another on cysteine 35 in human Trx1.

Figure S2

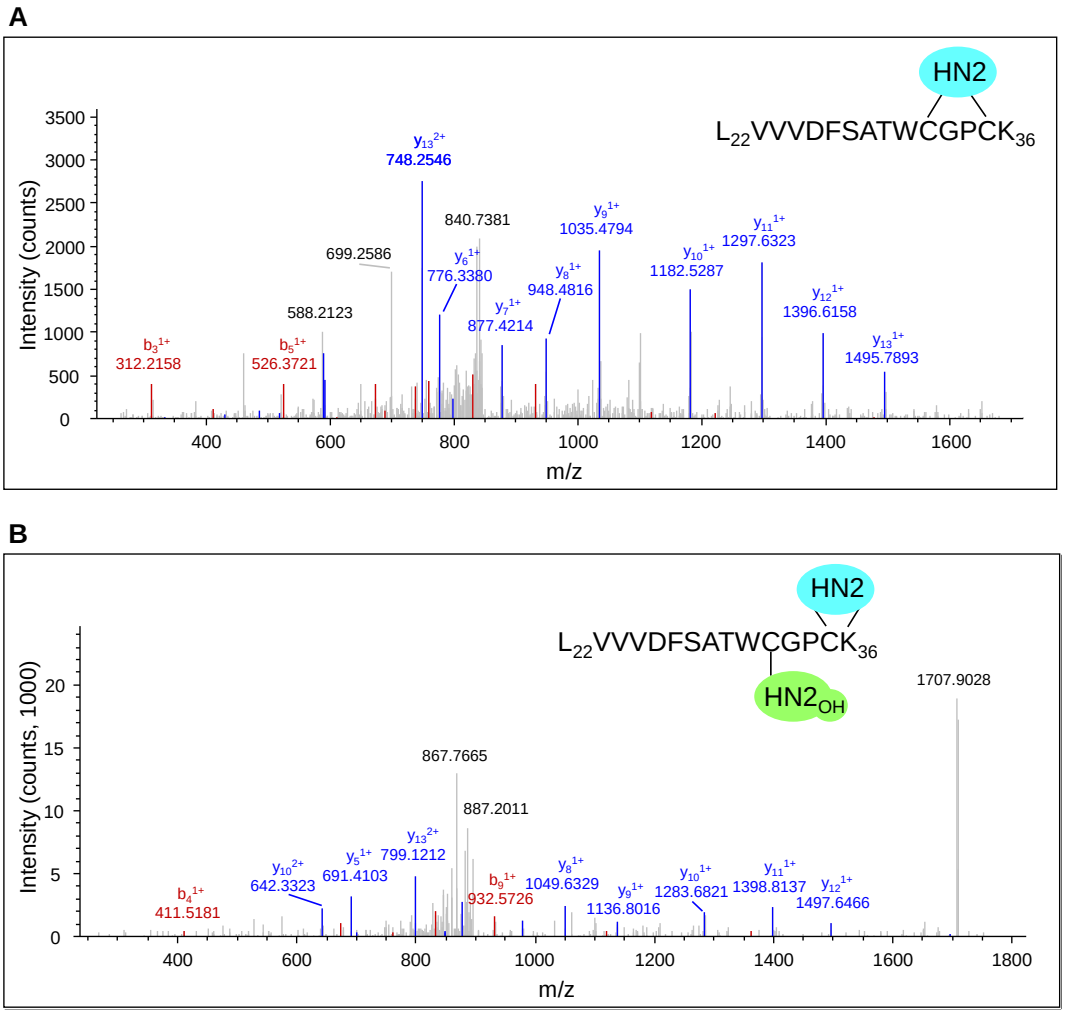
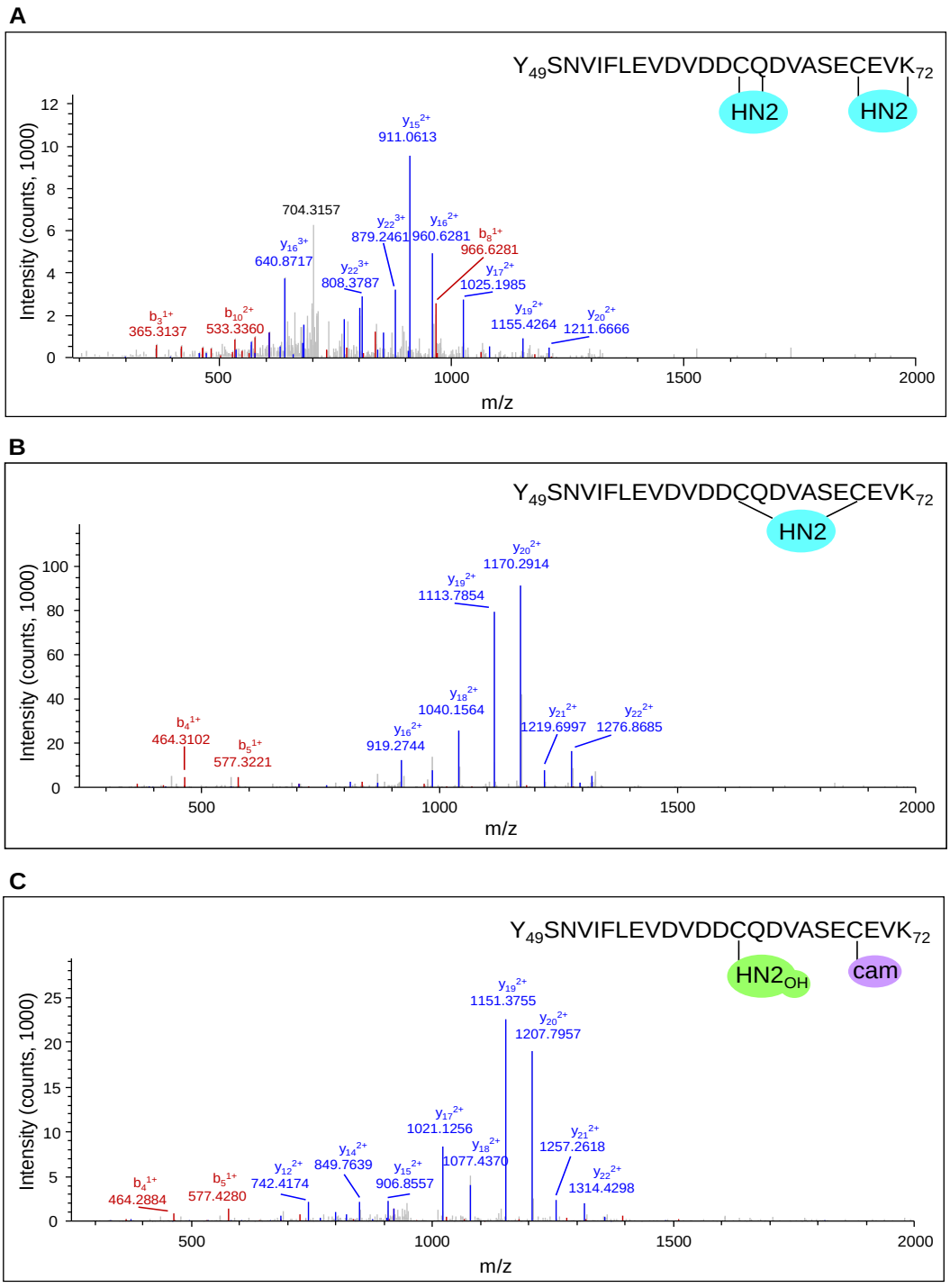


Figure S2. HN2- modified LVVVDFSATWCGPCK peptide in human Trx1. (A) MS/MS spectrum of a doubly charged ion at *m/z* 854.44. The mass of this ion corresponds to the peptide 22-36 from human Trx1 with one HN2 modification in which HN2 links to cysteine 32 and cysteine 35 on the protein. Matched b (shown in red) and y (shown in blue) fragments are marked. (B) MS/MS spectrum of a doubly charged ion at *m/z* 904.98. The mass of this ion corresponds to peptide 22-36 from human Trx1 modified with one HN2 cross-link and one HN2 monoadduct. The fragment ions indicate that HN2 cross-links cysteine 35 and lysine 36 and form a monoadduct with cysteine 32 on the protein.

Figure S3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Y49SNVIFLEVDVDDCQDVASECEVK72
HN2
HN2
Intensity (counts, 1000)
m/z
y15 2+ 911.0613
704.3157
y22 3+ 879.2461
y16 2+ 960.6281
b8 1+ 966.6281
y16 3+ 640.8717
y17 2+ 1025.1985
y22 3+ 808.3787
y19 2+ 1155.4264
y20 2+ 1211.6666
b3 1+ 365.3137
b10 2+ 533.3360
B
Y49SNVIFLEVDVDDCQDVASECEVK72
HN2
Intensity (counts, 1000)
m/z
y20 2+ 1170.2914
y19 2+ 1113.7854
y18 2+ 1040.1564
y21 2+ 1219.6997
y22 2+ 1276.8685
y16 2+ 919.2744
b4 1+ 464.3102
b5 1+ 577.3221
C
Y49SNVIFLEVDVDDCQDVASECEVK72
HN2OH
cam
Intensity (counts, 1000)
m/z
y19 2+ 1151.3755
y20 2+ 1207.7957
y17 2+ 1021.1256
y18 2+ 1077.4370
y21 2+ 1257.2618
y14 2+ 849.7639
y12 2+ 742.4174
y15 2+ 906.8557
y22 2+ 1314.4298
b4 1+ 464.2884
b5 1+ 577.4280

Figure S3

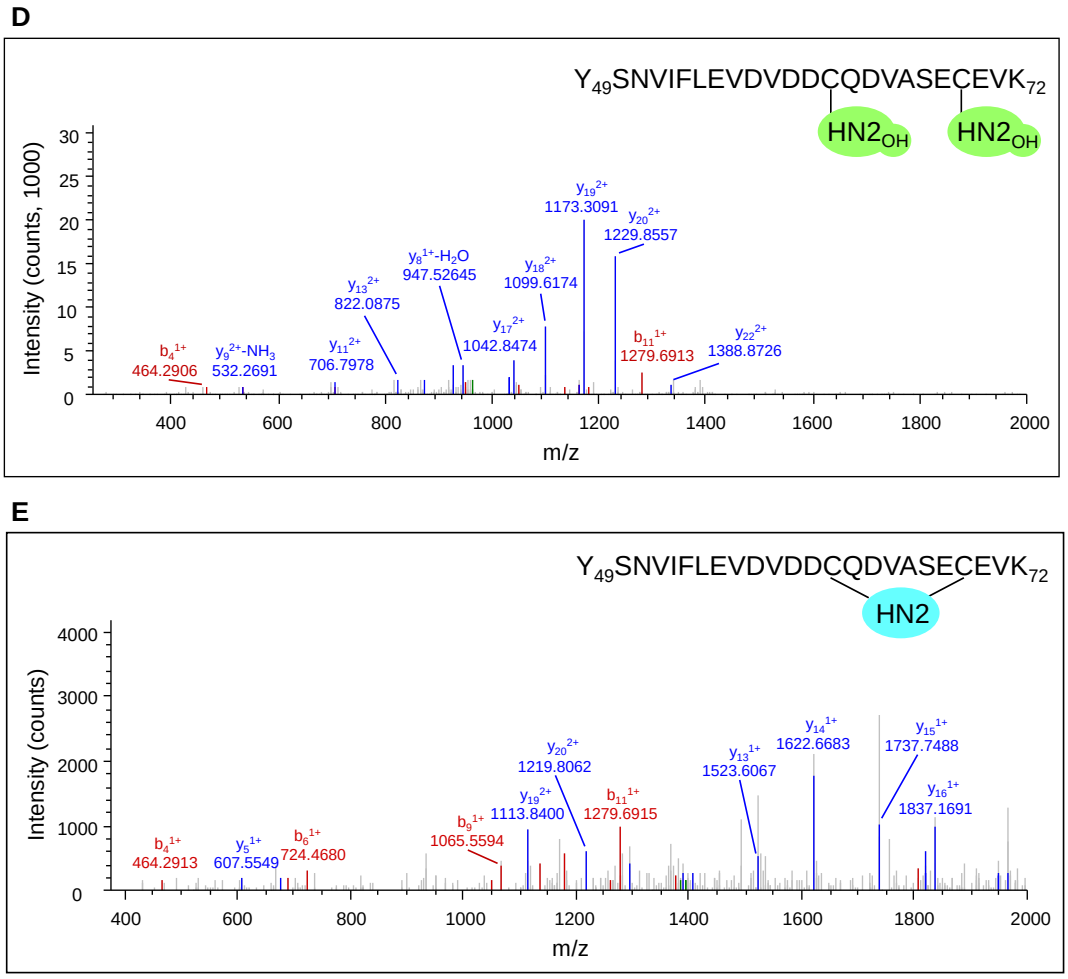

Figure S3. HN2-modified YSNVIFLEVDVDDCQDVASECEVK peptide in human Trx1. (A) MS/MS spectrum of a quadruply charged ion at *m/z* 722.10. The peptide sequence was identified as YSNVIFLEVDVDDCQDVASECEVK (residues 49-72 in human Trx1) with two HN2 modifications. One HN2 forms an intrapeptide cross-link with cysteine 62 and glutamine 63 and the other HN2 forms an intrapeptide cross-link with cysteine 69 and lysine 72. Matched b (shown in red) and y (shown in blue) fragments are marked. (B) MS/MS spectrum of a triply charged ion at *m/z* 934.77. The mass of this ion corresponds to peptide 49-72 with one HN2 cross-link. The fragments of the spectra indicate that HN2 forms an intrapeptide cross-link between cysteine 62 and cysteine 69 in human Trx1. (C) MS/MS spectrum of a triply charged ion at *m/z* 959.78. The peptide sequence was identified as YSNVIFLEVDVDDCQDVASECEVK with an HN2 monoadduct bound to cysteine 62 and a carbamidomethyl adduct bound to cysteine 69 on human Trx1. (D) MS/MS spectrum of a triply charged ion at *m/z* 974.47. The peptide sequence was identified as YSNVIFLEVDVDDCQDVASECEVK with two HN2 monoadducts; one bound to cysteine 62 and the other bound to cysteine 69 on human Trx1. (E) MS/MS spectrum of a doubly charged ion at *m/z* 1401.65. The peptide sequence was identified as YSNVIFLEVDVDDCQDVASECEVK with one HN2 crosslinking cysteine 62 and cysteine 69 on human Trx1.

Figure S4

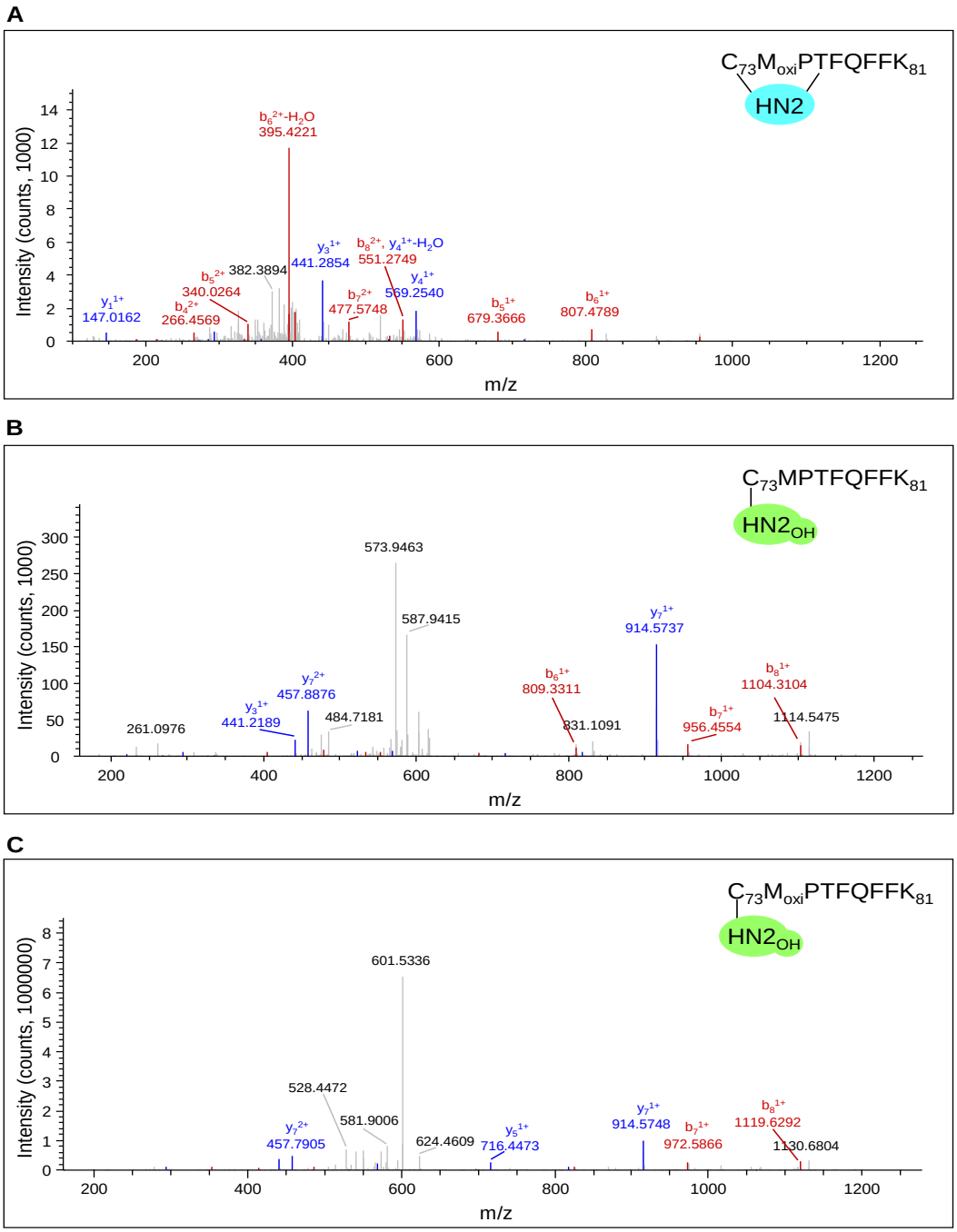

Figure S4. HN2-modified CMPTFQFFK peptide in human Trx1. (A) MS/MS spectrum of a triply charged ion at *m/z* 416.54. The mass of this ion corresponds to peptide 73-81 from human Trx1 with one HN2 modification and oxidation at methionine. HN2 was found to cross-link cysteine 73 and threonine 76 on the protein. Matched b (shown in red) and y (shown in blue) fragments are marked. (B) MS/MS spectrum of a doubly charged ion at *m/z* 625.31. The mass of this ion matches peptide 73-81 modified with one HN2 monoadduct. Hydroxyl HN2 was bound to cysteine 71. (C) MS/MS spectrum of a doubly charged ion at *m/z* 633.31. The mass of

this ion corresponds to the mass of peptide 73-81 plus one HN2 monoadduct and one oxidation. Modifications include HN2 monoalkylated on cysteine 73 and oxidation on methionine 74.

Figure S5

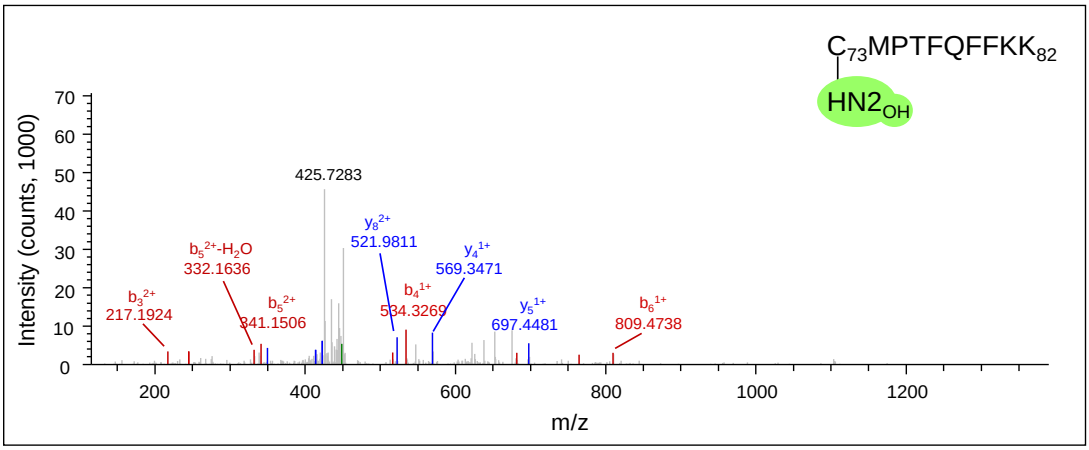

Figure S5. HN2-modified CMPTFQFFKK peptide in human Trx1 at *m/z* 459.91, *z* = 3. The mass of this ion corresponds to the mass of peptide 73-82 plus one HN2 monoaddcut. Tandem mass spectra indicated that an HN2 monoadduct was bound to cysteine 73 on the protein.

Figure S6

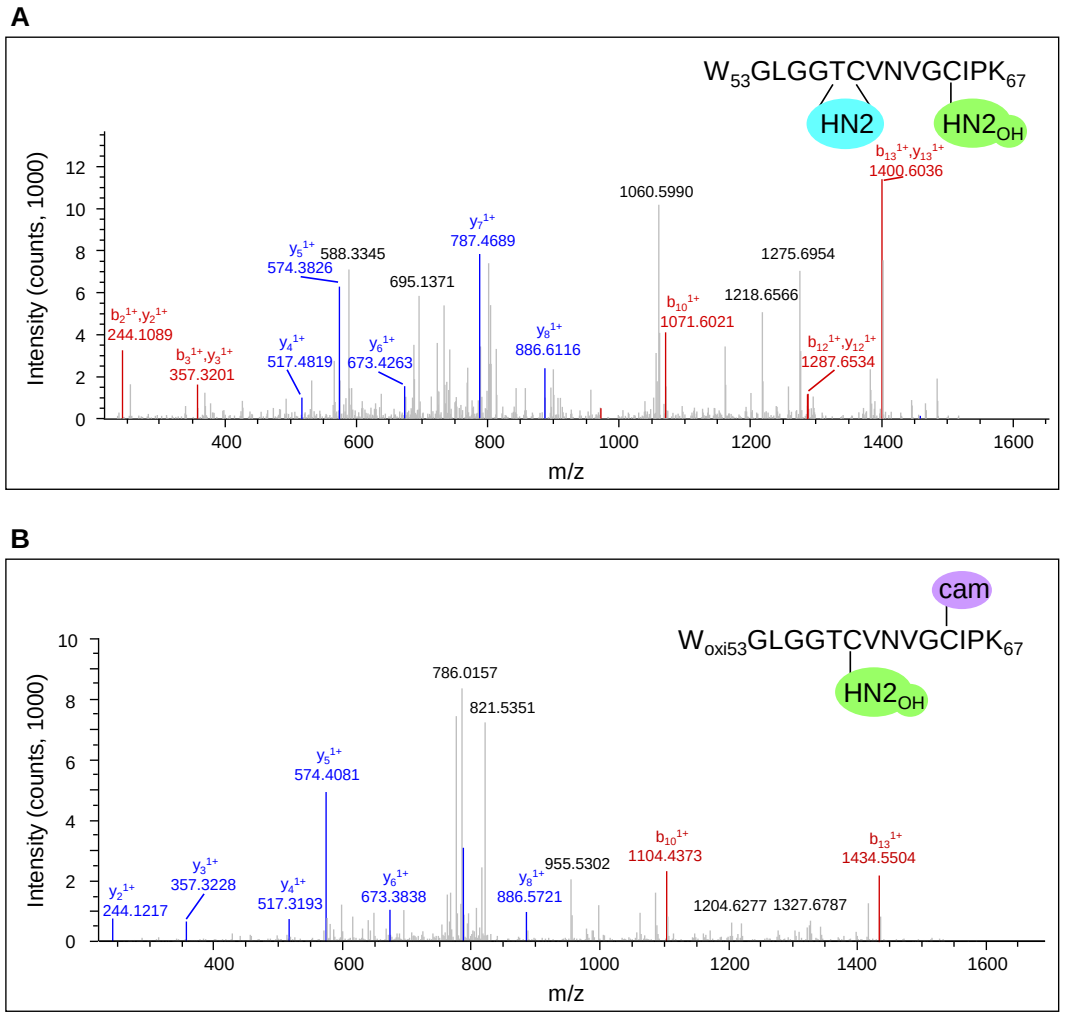

Figure S6. HN2-modified rat TrxR1 WGLGGTCVNVGCIPK peptide. (A) MS/MS spectrum of a doubly charge ion at m/z 822.40. The mass of this ion matches the mass of WGLGGTCVNVGCIPK peptide plus one HN2 cross-link and one HN2 monoadduct. Fragment ions revealed that HN2 cross-linked between threonine 58 and cysteine 59 and HN2 monoadduct was alkylated to cysteine 64. (B) MS/MS spectrum of a doubly charge ion at m/z 839.39. The mass of this precursor ion corresponds to the mass of WGLGGTCVNVGCIPK peptide with one HN2 monoadduct, one carbamidomethyl modification, and one oxidation. Fragment ions showed an HN2 monoadduct on cysteine 59, carbamidomethylation on cysteine 64, and oxidation on tryptophan 53.

Figure S7

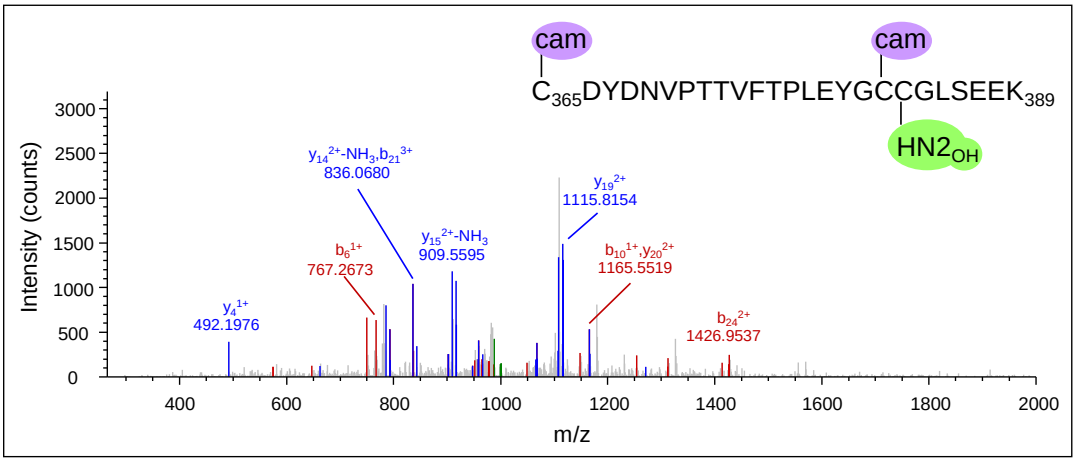

Figure S7. HN2-modified rat TrxR1 CDYDNVPTTVFTPLEYGCCGLSEEK peptide (residue 365-389 on rTrxR1) at m/z 1000.09, z = 3. The mass of precursor ion matched the mass of peptide 365-389 from rat TrxR1 plus one HN2 monoadduct and two carbamidomethyl modifications. MS/MS spectra shows an HN2 monoadduct on cysteine 383 and carbamidomethyl modifications on cysteine 365 and cysteine 382.

Figure S8

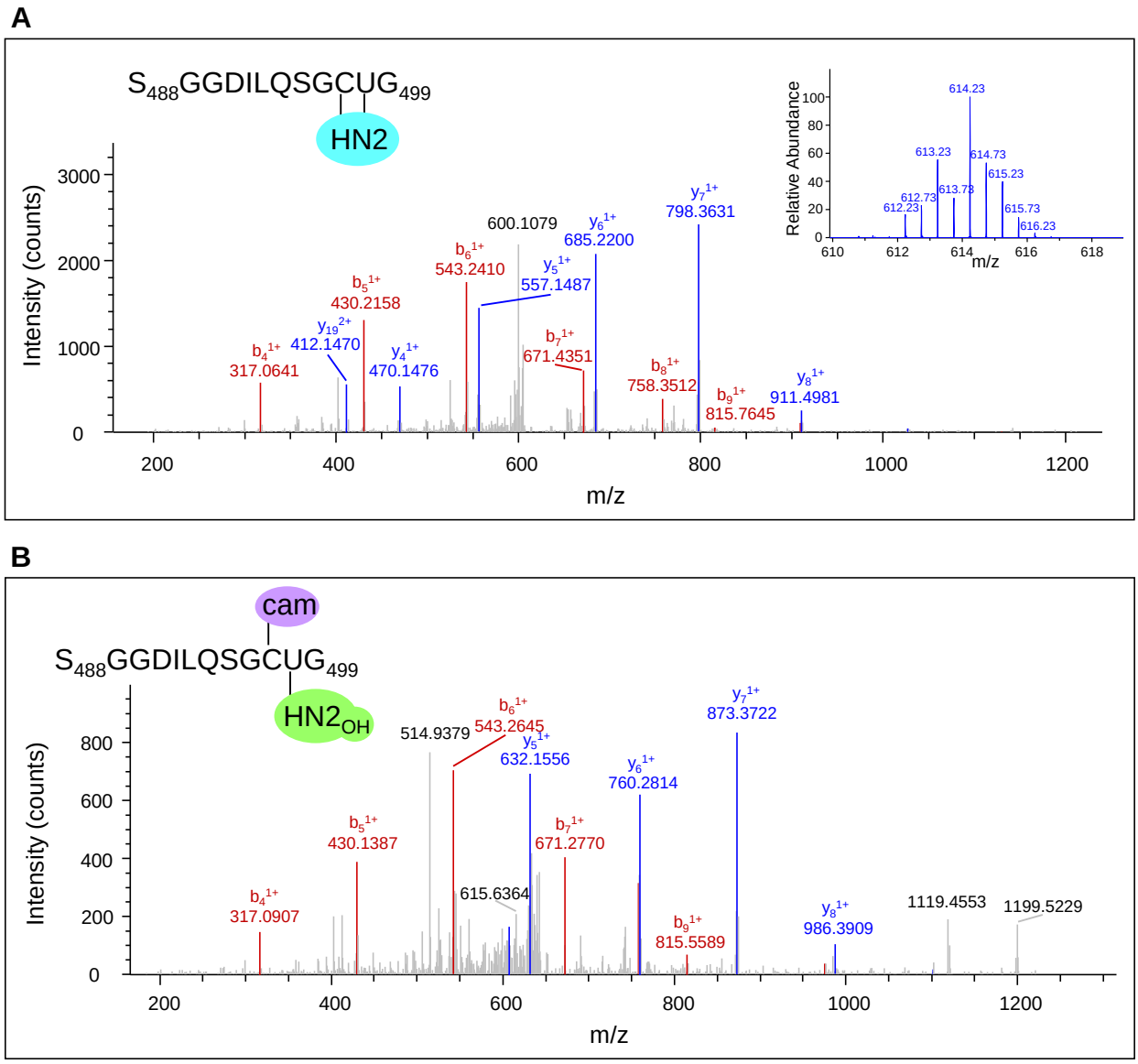

Figure S8. HN2-modified rat TrxR1 SGGDILQSGCUG peptide. (A) MS/MS spectrum of a doubly charged ion at m/z 614.23. The mass of this ion corresponds to the peptide 488-499 from rat TrxR1 with one HN2 cross-linked cysteine 497 and selenocysteine 498 on the protein. Inset shows the isotopic spectrum of the selenocysteine containing parent ion. Matched b (shown in red) and y (shown in blue) fragments are marked. (B) MS/MS spectrum of a doubly charged ion at m/z 651.73. The mass of this ion corresponds to the peptide 488-499 from rat TrxR1 with one carbamidomethyl modification on cysteine 497 and one HN2 monoadduct on selenocysteine 498.