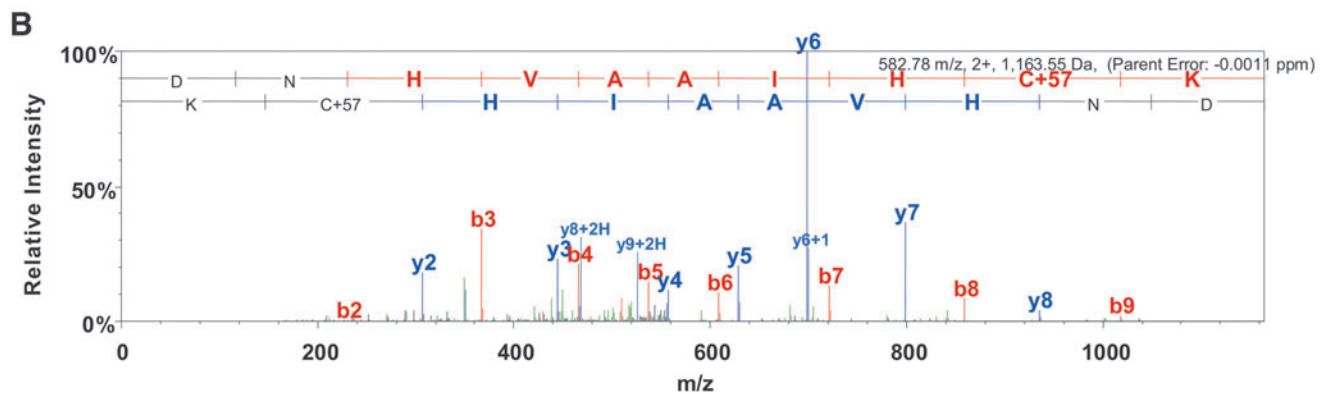


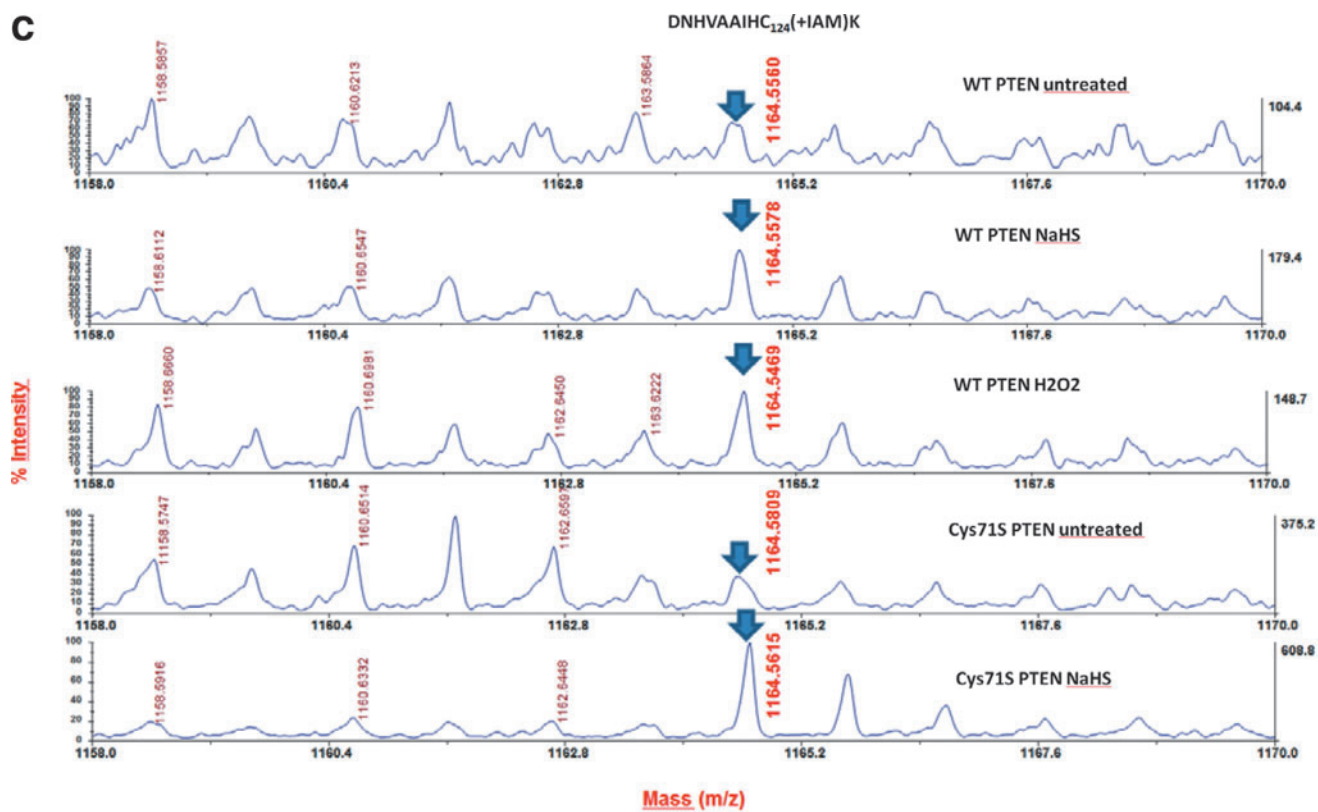
Sample	WT untreated	WT + NaHS	WT + H2O2	C71S + NaHS
Sequence	(D)DNHVAAIHcK(A)	(D)DNHVAAIHcK(A)	(D)DNHVAAIHcK(A)	(D)DNHVAAIHcK(A)
Prob	95%	95%	95%	95%
SEQUEST XCorr	3,2965	3,4398	3,5264	3,4063
SEQUEST Δ Cn	0,6091	0,5861	0,6456	0,6111
Modifications	IAM (+57)	IAM (+57)	IAM (+57)	IAM (+57)
Observed precurs or ion mass	582,7825	582,7824	582,7826	582,7827
Neutral molecular peptide mass	1163,5505	1163,5503	1163,5506	1163,5508
Charge	2	2	2	2
Δ AMU	-0,000124	-0,000368	-0,000001	0,000121
Δ PPM	-0,106700	-0,316300	-0,001118	0,103600



B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	116.0			98.0	D	1,164.6	582.8	1,147.5	1,146.5	10
2	230.1		213.1	212.1	N	1,049.5	525.3	1,032.5		9
3	367.1	184.1	350.1	349.1	H	935.5	468.2	918.5		8
4	466.2	233.6	449.2	448.2	V	798.4	399.7	781.4		7
5	537.2	269.1	520.2	519.2	A	699.4	350.2	682.3		6
6	608.3	304.6	591.3	590.3	A	628.3	314.7	611.3		5
7	721.4	361.2	704.3	703.4	I	557.3	279.1	540.3		4
8	858.4	429.7	841.4	840.4	H	444.2	222.6	427.2		3
9	1,018.5	509.7	1,001.4	1,000.4	C+57	307.1		290.1		2
10	1,164.6	582.8	1,147.5	1,146.5	K	147.1		130.1		1

SUPPLEMENTARY FIG. S9. Mass spectrometric analysis of differentially alkylated PTEN-WT or -C71S reveals NaHS-induced oxidation of Cys-124. PTEN-WT or -C71S was treated with NaHS or H₂O₂ and reduced thiols blocked with NEM. Oxidized thiols (disulfides and persulfides) were reduced with DTT, following alkylation with IAM as described in Materials and Methods. Differentially alkylated PTEN was digested with AspN and trypsin. The resulting peptides were subjected to LTQ Orbitrap Velos mass spectrometry as described in Materials and Methods, and analyzed using the Scaffold software. **(A)** Xcorr, deltaCn scores, and both actual and observed masses of the Cys124-IAM-alkylated peptide DNHVAAIHCK. **(B)** CID MS/MS spectra and fragment ion table of a representative Cys124-IAM peptide with the b- and y-fragment ion series labeled in red and blue, respectively. **(C)** The Cys124-IAM peptides were also semiquantitatively assessed by MALDI-TOF MS/MS, and their mass peaks are shown for the specified samples. IAM, iodoacetamide.

C



SUPPLEMENTARY FIG. S9. (Continued).