A

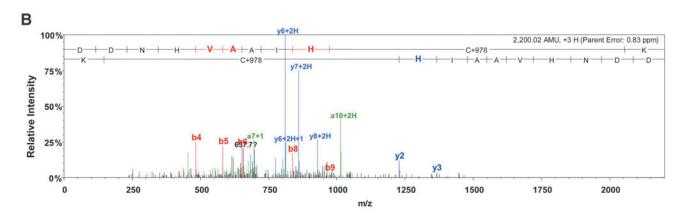
Sample WT + NaHS

Sequence (E)DDNHVAAIHc124(+978)K(A)

Prob95%SEQUEST XCorr2,3416SEQUEST ΔCn0,1887Modifications\$ (+978)Observed precursor ion mass734,3462Neutral molecular peptide mass2200,0167

Charge 3

Delta AMU 0,0018
Delta PPM 0,8341



| В | B Ions | B+2H | B-NH3 | B-H2O | AA | Y Ions | Y+2H | Y-NH3 | Y-H2O | Υ |
|----|---------|---------|---------|---------|-------|---------|---------|---------|---------|----|
| 1 | 116.0 | | | 98.0 | D | 2,201.0 | 1,101.0 | 2,184.0 | 2,183.0 | 11 |
| 2 | 231.1 | | | 213.1 | D | 2,086.0 | 1,043.5 | 2,069.0 | 2,068.0 | 10 |
| 3 | 345.1 | | 328.1 | 327.1 | N | 1,971.0 | 986.0 | 1,953.9 | | 9 |
| 4 | 482.2 | 241.6 | 465.1 | 464.2 | Н | 1,856.9 | 929.0 | 1,839.9 | | 8 |
| 5 | 581.2 | 291.1 | 564.2 | 563.2 | V | 1,719.9 | 860.4 | 1,702.8 | | 7 |
| 6 | 652.3 | 326.6 | 635.2 | 634.3 | Α | 1,620.8 | 810.9 | 1,603.8 | | 6 |
| 7 | 723.3 | 362.2 | 706.3 | 705.3 | Α | 1,549.8 | 775.4 | 1,532.7 | | 5 |
| 8 | 836.4 | 418.7 | 819.4 | 818.4 | I | 1,478.7 | 739.9 | 1,461.7 | | 4 |
| 9 | 973.4 | 487.2 | 956.4 | 955.4 | Н | 1,365.6 | 683.3 | 1,348.6 | | 3 |
| 10 | 2,054.9 | 1,028.0 | 2,037.9 | 2,036.9 | C+978 | 1,228.6 | | 1,211.6 | | 2 |
| 11 | 2,201.0 | 1,101.0 | 2,184.0 | 2,183.0 | K | 147.1 | | 130.1 | | 1 |

SUPPLEMENTARY FIG. S8. Mass spectrometric analysis of NaHS-treated PTEN-WT reveals disulfide bond formation between Cys-124 and Cys-71. Prereduced PTEN-WT was treated with NaHS, free thiols were then blocked with NEM, and the protein was digested with AspN and trypsin. The resulting peptides were analyzed using LTQ Orbitrap Velos mass spectrometry as described in the Methods section and analyzed using the Scaffold software for disulfides between Cys-124 and Cys-71. (A) The table shows Xcorr and deltaCn scores, and both actual and observed masses of the Cys71-SS-Cys124 disulfide-linked peptide (E)DDNHVAAIHc124(+978)K(A)-(IYNLc71AER). (B) CID MS/MS spectra and the fragment ion table of the disulfide-linked peptides are shown with the b- and y-fragment ion series labeled in red and blue, respectively.