

SUPPLEMENTARY TABLE S1. H<sub>2</sub>S IS RELEASED BY POLYSULFIDE-TREATED PTEN-WT, -C71A, OR -C124S ONLY UPON REDUCTION WITH DTT

| PTEN  | Prereduction | No. of repeat | Measured sulfide ( $\mu$ M) |      | Protein conc. ( $\mu$ M) | Stoichiometry (sulfide/protein) | Average ( $\pm$ SD) stoichiometry |
|-------|--------------|---------------|-----------------------------|------|--------------------------|---------------------------------|-----------------------------------|
|       |              |               | -DTT                        | +DTT |                          |                                 |                                   |
| WT    | -            | 1             | 0                           | 1    | 20                       | -                               | n.a.                              |
|       |              | 1             | 4                           | 12   | 17                       | 0.7                             |                                   |
|       | +            | 2             | 2                           | 28   | 15                       | 1.9                             | 1.6 $\pm$ 0.67                    |
|       |              | 3             | 4                           | 23   | 14                       | 1.6                             |                                   |
|       |              | 4             | 3                           | 30   | 13                       | 2.3                             |                                   |
| C71A  | +            | 1             | 2                           | 17   | 20                       | 0.9                             | 0.9 $\pm$ 0.10                    |
|       |              | 2             |                             | 7    | 7                        | 1.0                             |                                   |
| C124S | +            | 1             | 2.5                         | 11.5 | 18                       | 0.6                             | 1.0 $\pm$ 0.58                    |
|       |              | 2             | 2                           | 22   | 15                       | 1.5                             |                                   |

Recombinant PTEN (WT/C71A/C124S) was incubated with or without 1 mM DTT for prereduction before the assay. After treatment with an excess of polysulfides for 30 min, samples were subjected to buffer exchange and concentration, before they were split into two aliquots. One aliquot was left untreated (-DTT), and the other exposed to 1 mM DTT for 30 min (+DTT) to liberate protein-bound sulfane sulfur, which was detected by monobromobimane derivatization and HPLC. Concentrations of released sulfide are background corrected using a protein-free polysulfide-treated control.

DTT, dithiothreitol; H<sub>2</sub>S, hydrogen sulfide; PTEN, phosphatase and tensin homolog; WT, wild type; n.a., not applicable.