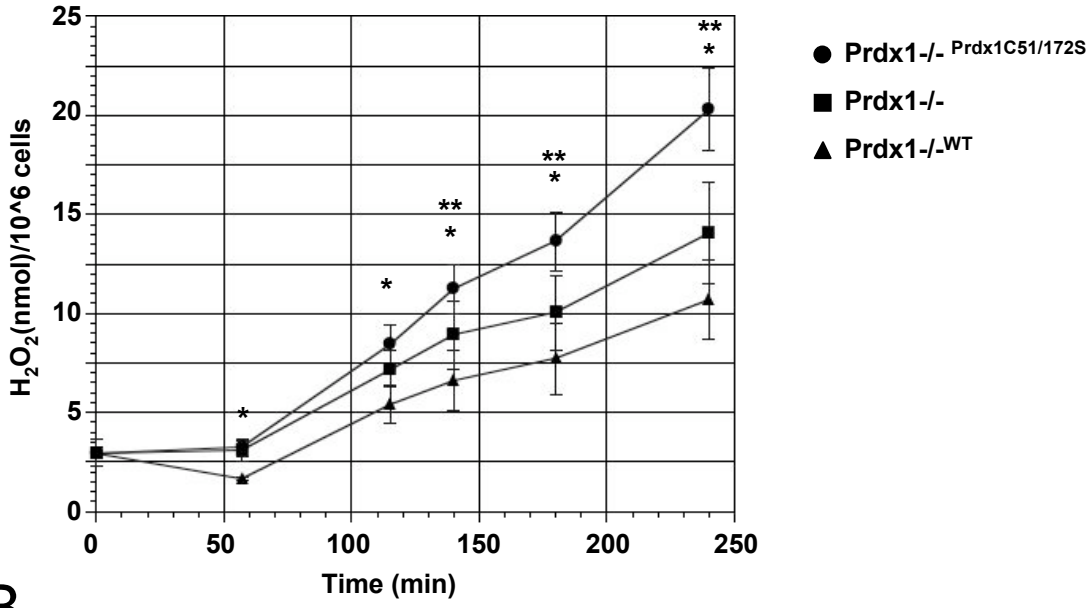
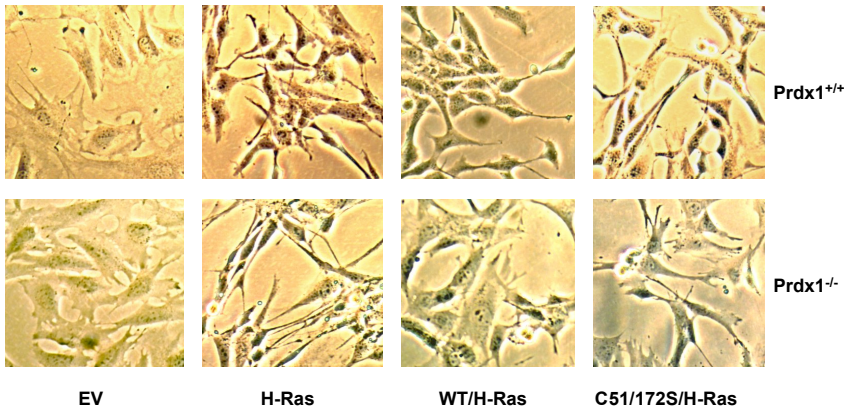
A



B

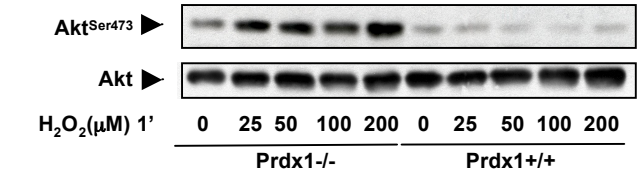
H_2O_2 release of Prdx1$^{-/-}$MEFs (filled square), Prdx1$^{-/-}$MEFs reconstituted with the *Prdx1* wild-type gene (*Prdx1WT*) (filled triangle) and Prdx1$^{-/-}$MEFs expressing Prdx1$^{C51/172S}$ (filled circle) were measured as described in detail in Materials and methods, by using Amplex Red reagent, which via oxidation, becomes fluorescent resorufin. The y-Axis presents H_2O_2 release accumulated over time per 1x10^6 cells. For each clone, 6 wells were plated and analyzed. The experiment shown here is representative of 5 independent studies from 3 different sets of MEF clones from Prdx1-littermates. P values were calculated using an unpaired Student's t-Test. *P<0.05. P-values for Prdx1$^{-/-}$MEFs compared with Prdx1$^{-/-Prdx1Cys51/172S}$: **P<0.008;



MEFs used in (B) were analyzed for morphological changes induced by *Prdx1*^{WT}, *Prdx1*$^{C51/172S}$, and *H-Ras* by using a Nikon Eclipse TE2000-S microscope. Images were captured with Micropublisher 3-3RTV.

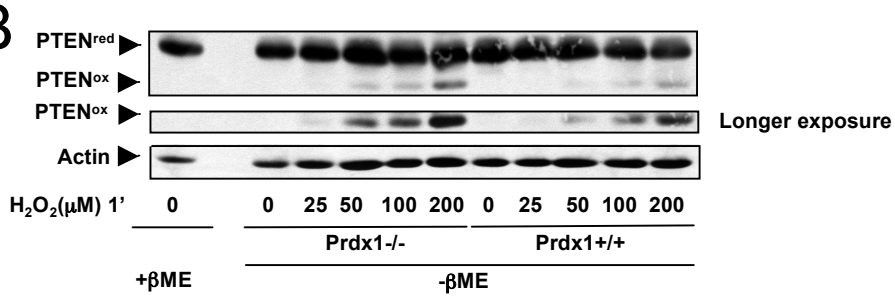
J. Cao et al., Supp. Figure 1

A



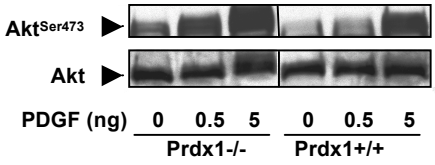
Prdx1$^{-/-}$MEFs and Prdx1$^{+/+}$MEFs were serum-starved for 48 hours and stimulated for 1 minute with H_2O_2 at the indicated concentrations. Protein lysates were collected under argonized conditions by scrapping cells into degassed lysis buffer (Material and methods) and analyzed under non-reducing conditions on SDS-PAGE. Akt phosphorylation was detected on Ser473. Akt protein as loading control

B



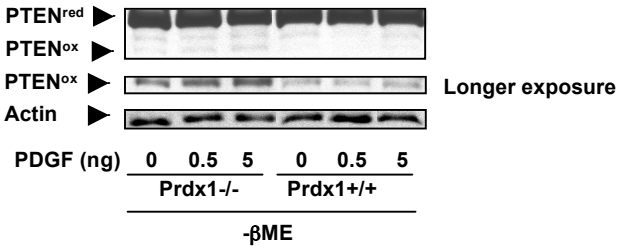
Prdx1$^{-/-}$MEFs and Prdx1$^{+/+}$MEFs protein lysates were treated as described under (A) and analyzed for oxidized PTEN proteins.

C



Prdx1$^{-/-}$MEFs and Prdx1$^{+/+}$MEFs were serum-starved for 48 hours and stimulated 1 minute with different PDGF concentrations as indicated. Protein lysates were collected and analyzed as described under (A).

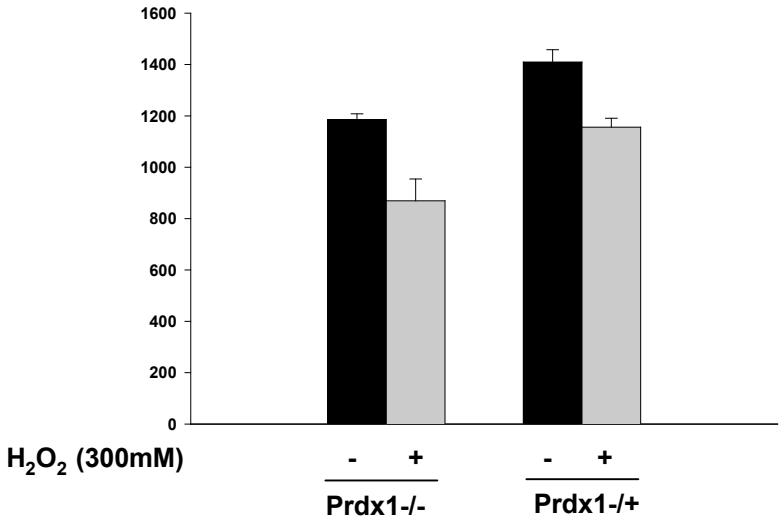
D



Prdx1$^{-/-}$MEFs and Prdx1$^{+/+}$MEFs protein lysates were treated as described under (G) and analyzed for oxidized PTEN proteins. Actin as loading control.

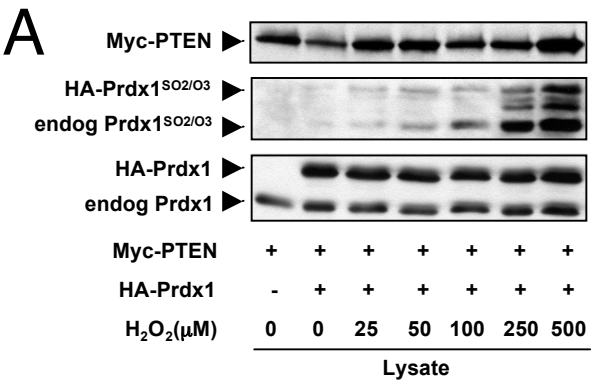
J. Cao et al., Supp. Figure 2

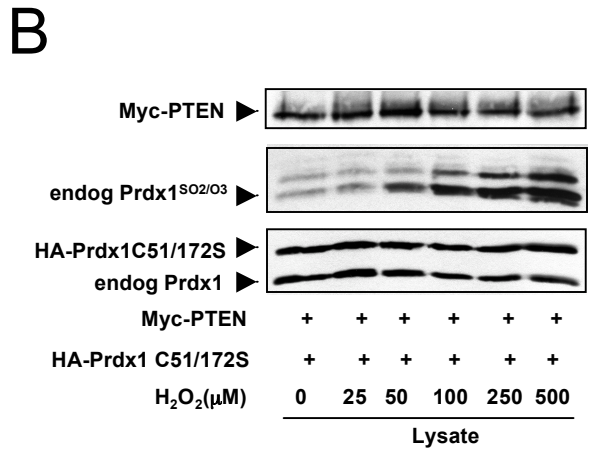
E



Endogenous PP2A activity is higher in Prdx1 $^{+/+}$ MEFs compared to Prdx1 $^{-/-}$ MEFs. Cells were starved for 48 h in DMEM with 0.25% FBS and then treated with 300 μM H_2O_2 for 30 min. The cell lysates were used for IP-PP2A phosphatase assay. The results are presented as mean of released phosphate levels from three independent experiments with SD as indicated.

J. Cao et al., Supp. Figure 2

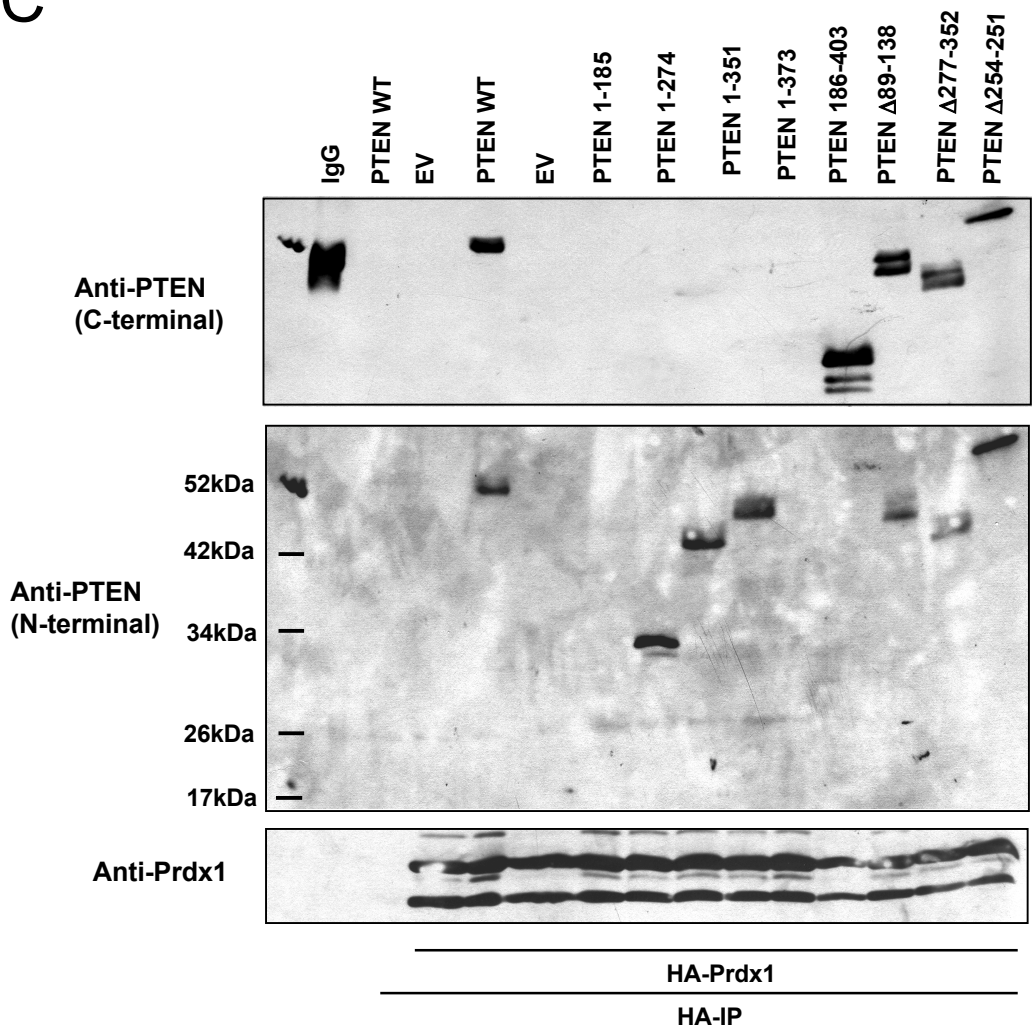




Part of the protein lysates from Figure 3B and C were collected immediately and analyzed by Western blotting as in parallel to the IPs. Proteins were detected through staining of membranes with anti-PTEN, anti-Prdx1-SO_2/-SO_3 and anti-Prdx1 antibodies. Epitope-tagged Prdx1 migrates slower electrophoretically than endogenous Prdx1, labeled as HA-Prdx1. Epitope-tagged PTEN is labeled as Myc-PTEN. Anti- Prdx1-SO_2/-SO_3 can cross-react with Prdx2-4 and does not bind Prdx1$^{C51/172S}$.

J. Cao et al., Supp. Figure 3

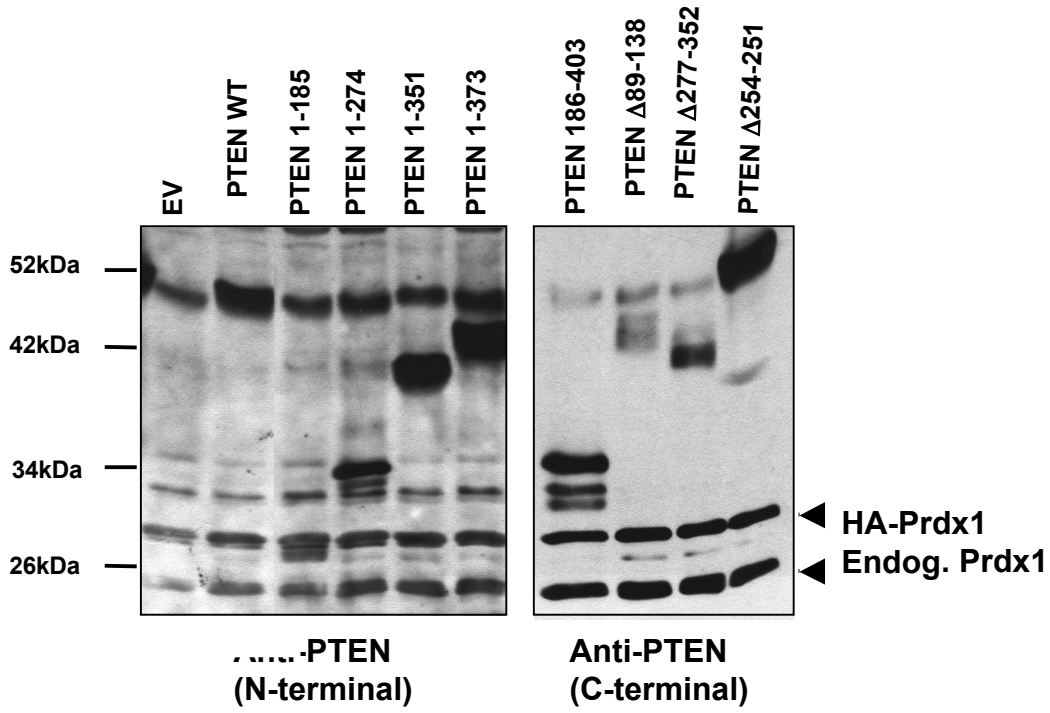
C



Epitope-tagged Myc-PTEN deletion mutants were co-expressed with epitope-tagged Prdx1 in 293T cells. Cell lysates were prepared under anaerobic conditions, and precipitated over night using HA-conjugated agarose beads (Roche). IPs were washed four times with degassed lysis buffer and analyzed by Western blotting. Proteins were detected through staining of membranes with Anti-PTEN antibodies recognizing either PTEN N-terminus (Santa Cruz) or C-Terminus (Cell Signaling), respectively.

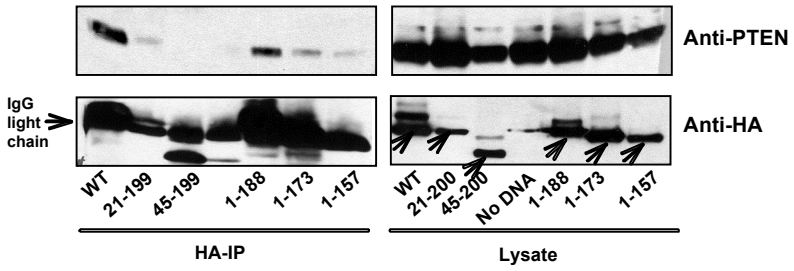
J. Cao et al., Supp. Figure 3

D



Protein samples of epitope-tagged Myc-PTEN mutants from (F) were analyzed by SDS-PAGE for expression of deletions mutants with Anti-PTEN antibodies recognizing either PTEN N-terminus (Santa Cruz) or C-Terminus (Cell Signaling), respectively; *: PTEN 1-185.

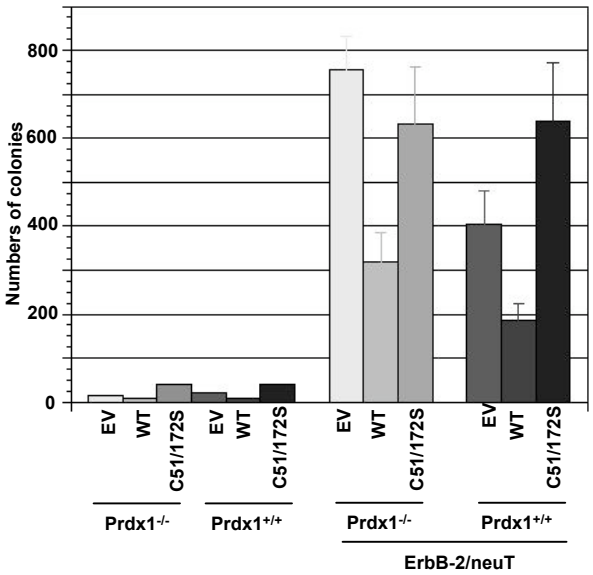
E



HA-Prdx1 truncation mutants are tested to pull down Myc-PTEN wild type protein. Epitope-tagged Myc-PTEN and HA-Prdx1 various N- and C-terminal truncation mutants were co-expressed In 293T cells. HA-Ips were processed as decribed under Figure 3A and B). Left side shows co-immuneprecipitations, right side shows expression of PTEN and Prdx1 wild type and mutants. Arrows indicate expression of Prdx1 wild type and truncation mutants in protein lysate.

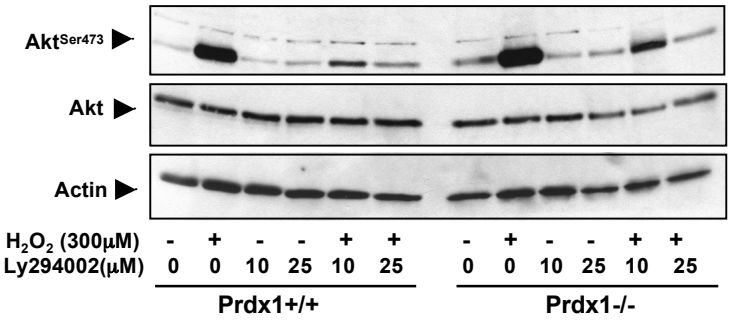
J. Cao et al., Supp. Figure 3

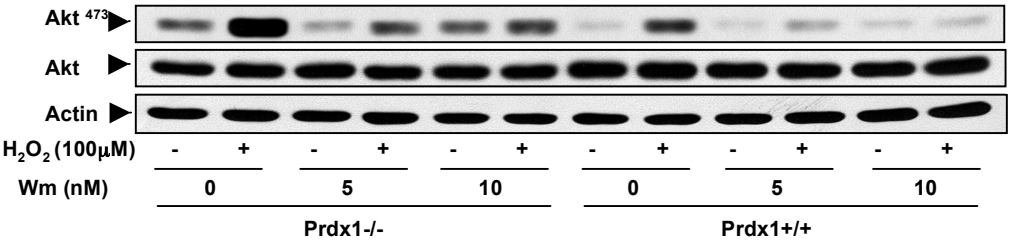
A



Prdx1$^{-/-}$MEFs and Prdx1$^{+/+}$MEFs were infected with retroviral constructs carrying genes for *Prdx1^{WT}* or *Prdx1$^{C51/172S}$* or pQXCIP expressing the puromycin gene only (empty vector=EV) and selected for 10 days in 2μg/ml puromycin. Before plating in soft agar, MEFs were infected with a retrovirus carrying ErbB-2/neu, selected in 5μg/ml puromycin for 4 days and seeded in soft agar and analyzed as described under (Figure 1C). Expression levels for ErbB-2 and Prdx1 proteins was assessed by Western blotting.

B





Prdx1$^{-/-}$MEFs and Prdx1$^{+/+}$MEFs were serum-starved for 48 hours and incubated with either H_2O_2 for 20 minutes or Ly294002 (**B,** upper panel) or Wortmannin (**B,** lower panel) for one hour as indicated. Protein lysates were obtained anaerobically as described in Materials and methods and analyzed by SDS-PAGE.

J. Cao et al., Supp. Figure 5

Material and Methods Supplementary Figures:

Measurement of PP2A Activity

The activity of PP2A was measured with the PP2A immunoprecipitation phosphatase assay kit (Millipore, Bedford, MA).

Threonine phosphopeptide (K-R-Pt-I-R-R) was used as PP2A substrate. Briefly, after starvation (DMEM with 0.25% FBS) for 48 h, Prdx1-/- and Prdx1+/+ MEFs were treated with 300 μM H_2O_2 for 30 min in serum free medium. The cells were lysed in phosphate free buffer (20 mM immidazole (pH 7.0), 2 mM EDTA, 2 mM EGTA, 1% Triton X-100 with 1 mM PMSF and 1 μ g/ml aprotinin). 500 μg of the cell lysate was incubated with 4 μg of anti-PP2A [C subunit, clone 1D6] and protein A agarose at 4−C for 2 h with constant rocking. The immunoprecipitates were then washed twice with Tris-buffered saline and once with Ser/Thr reaction buffer followed by the addition of diluted phosphopeptide (final concentration 750 μM). The reactions were incubated for 10 min at 30ºC with gentle shaking and 25 μl of supernatants were used for PP2A activity. PP2A activities were determined by adding Malachite green phosphate detection solution and measuring the absorbance at 650 nm.