1A

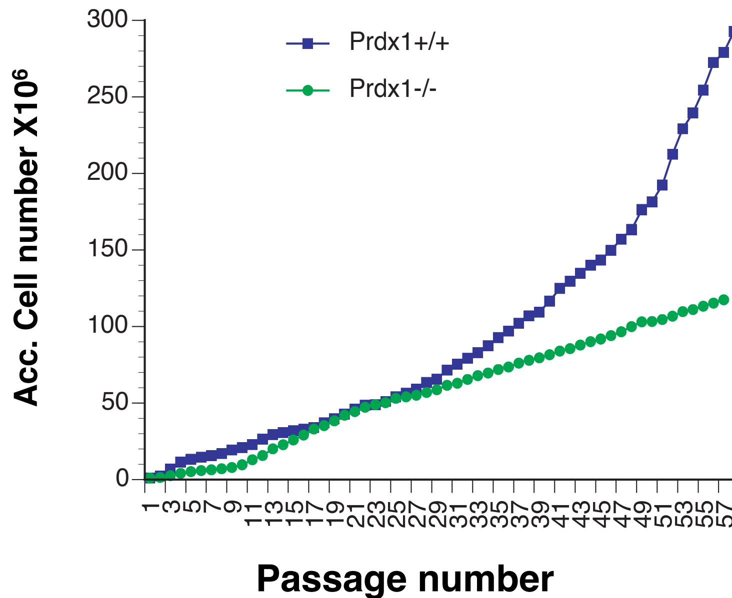
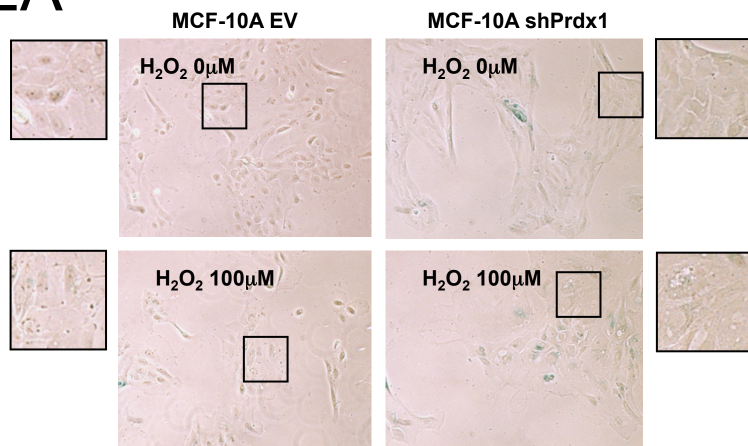
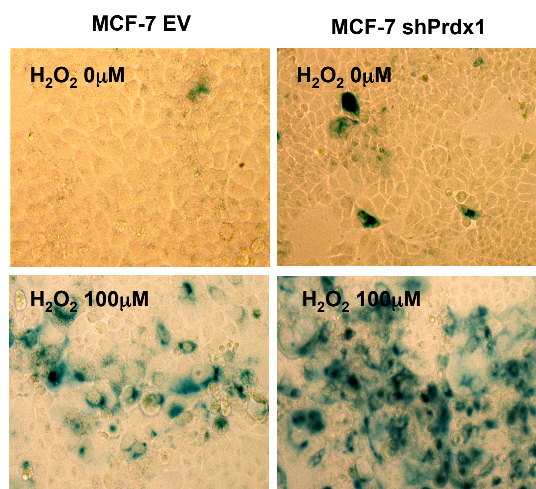
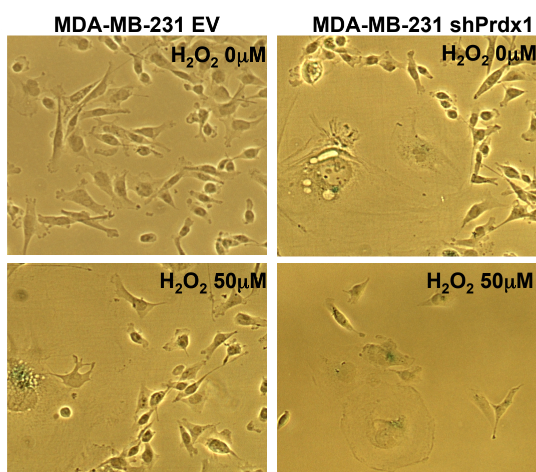


Fig S1: Prdx1$^{-/-}$ primary MEFs enter crisis before Prdx1$^{+/+}$ cells. **1A.** Prdx1$^{-/-}$ and Prdx1$^{+/+}$ MEFs were immortalized following a standard 3T3 protocol. Prdx1 deficient MEFs do not exhibit exponential growth in the first several months, compared to MEFs expressing Prdx1.


2A
MCF-10A EV
MCF-10A shPrdx1
H_2O_2 0μM
H_2O_2 0μM
H_2O_2 100μM
H_2O_2 100μM


2B
MCF-7 EV
MCF-7 shPrdx1
H_2O_2 0μM
H_2O_2 0μM
H_2O_2 100μM
H_2O_2 100μM


2C
MDA-MB-231 EV
MDA-MB-231 shPrdx1
H_2O_2 0μM
H_2O_2 0μM
H_2O_2 50μM
H_2O_2 50μM

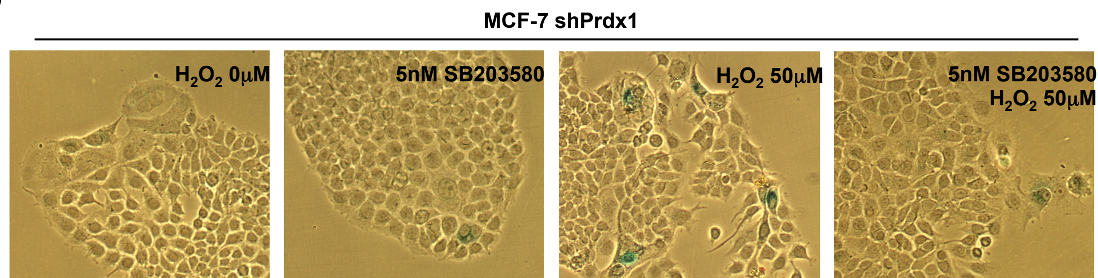
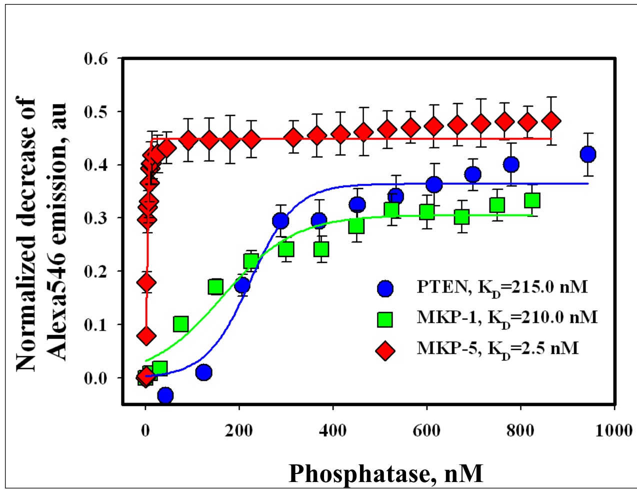
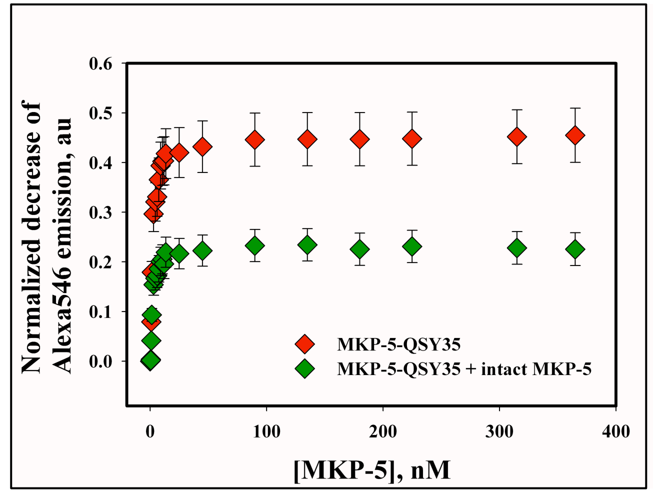

2D
MCF-7 shPrdx1
H_2O_2 0μM
5nM SB203580
H_2O_2 50μM
5nM SB203580
H_2O_2 50μM

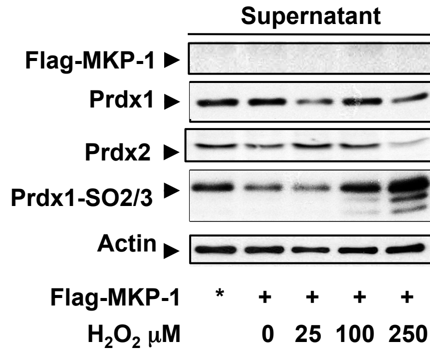
Fig S2: A-C. Prdx1 expression was decreased in various human breast epithelial cells using lentiviral shRNA. MCF-10A, MCF-7 and MDA-MB-231 cells expressing vector control (empty vector = EV) or shPrdx1 were plated at 35,000 cells/well in 6-well plates overnight. Cells were treated with H_2O_2 in DMEM containing 10% FBS for 4 days. Following treatment, cells were washed with sterile 1X PBS and incubated in fresh medium for 24 h, and sub-cultured at low confluency for 10 days. Plates were stained for SA-bgal activity as previously described (53). Up to 6000 cells per treatment and genotype were quantified. D. MCF-$7^{shPrdx1}$ cells were plated overnight at 35,000 cells/well in 6-well plates, and treated as in C with the addition of the p38MAPK inhibitor SB203580 every other day.

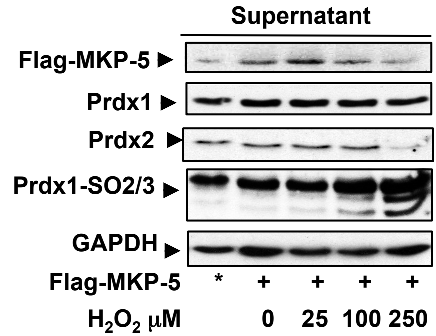
3A



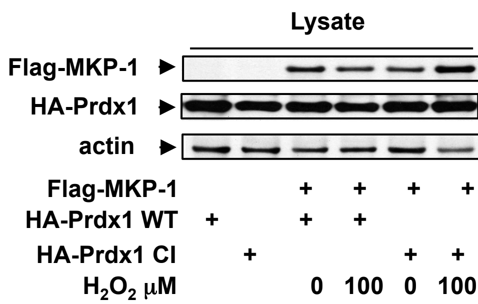


3B





3C



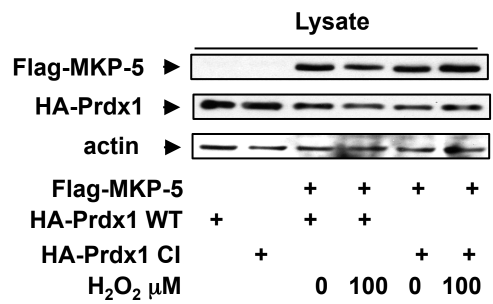
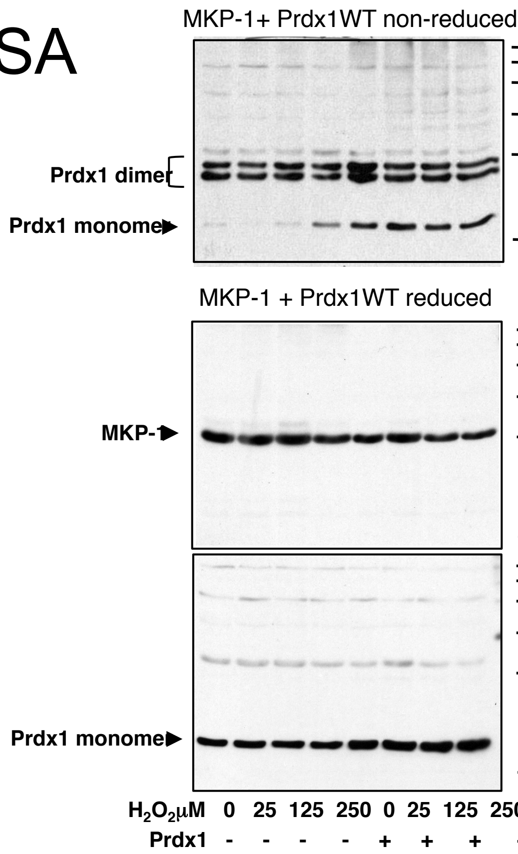


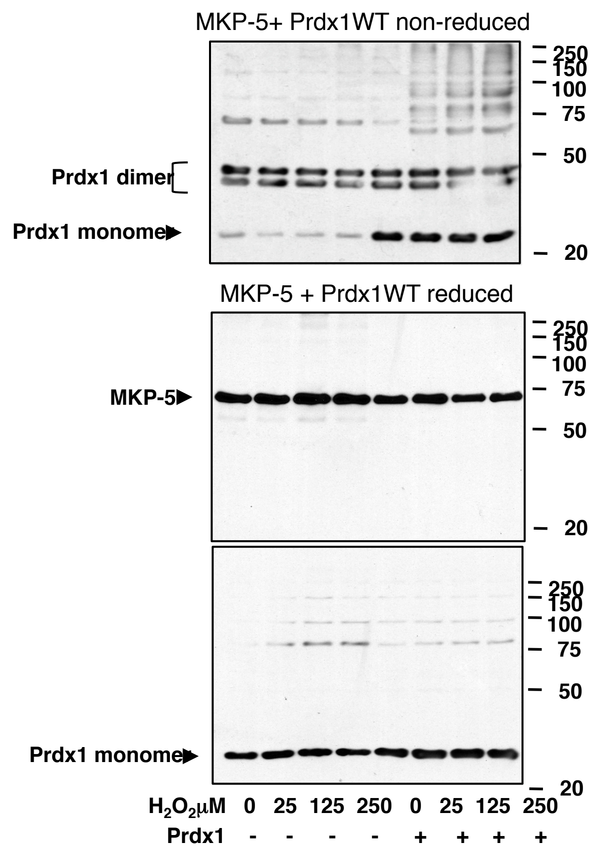
Fig S3A, left panel: purified Prdx1 protein (~1 μM) labeled with Alexa Fluor® 546 was titrated with indicated amount of the purified various phosphatase proteins labeled with QSY® 35. Alexa546 fluorescence decrease (normalized to initial Alexa546 emission) was recorded and plotted against the final concentrations of added phosphatase proteins. Data represent mean±SD for 3 independent experiments. The Kd is concentration of MKP or PTEN corresponding with 50% of Alexa 546 emission decrease. The experimental data of Prx1 titration with MKPs were fit ($R^2 \geq 0.96$) with sigmoid curve (3 parameters) (SigmaPlot 10.0, Systat, MA) according to eq.:**Y=a/(1+exp(-(x-x0)/b))**, where: **Y**-is the normalized decrease of Alexa®546 fluorescence; **x** - is a concentration of QSY®35-labeled phosphatases; $\mathbf{x_0}$ – is a constant corresponding to specific phosphatase; **a, b** - are coefficients corresponding to individual phosphatases. The K_D value was derived from particular equation as a concentration of phosphatase corresponding to 50% of maximal normalized decrease of Alexa®546 fluorescence. **Right panel:** as left panel with the exception of showing dynamic reversibility of protein binding since equal amounts of unlabeled intact MKP-5 was added to MKP-5 QSY®35-labeled proteins to several points of the titration curve shown in Fig. 3B (50, 100, 150 and 200 nM).

Fig S3B: Supernatants for co-immuneprecipitations from Figure 3C and 3D: 293T HEK cells were transfected with Flag-MKP-1 or Flag-MKP-5, and treated with increasing amounts of H_20_2 for 30 min in serum free medium. Lysate and supernatant samples were run under reducing conditions and analyzed by western blotting for MKP-1 and MKP-5 expression, oxidation of Prdx1/2, and Prdx1 protein levels. Supernatant samples refer to unbound protein collected from the co-IP following the first centrifugation step.

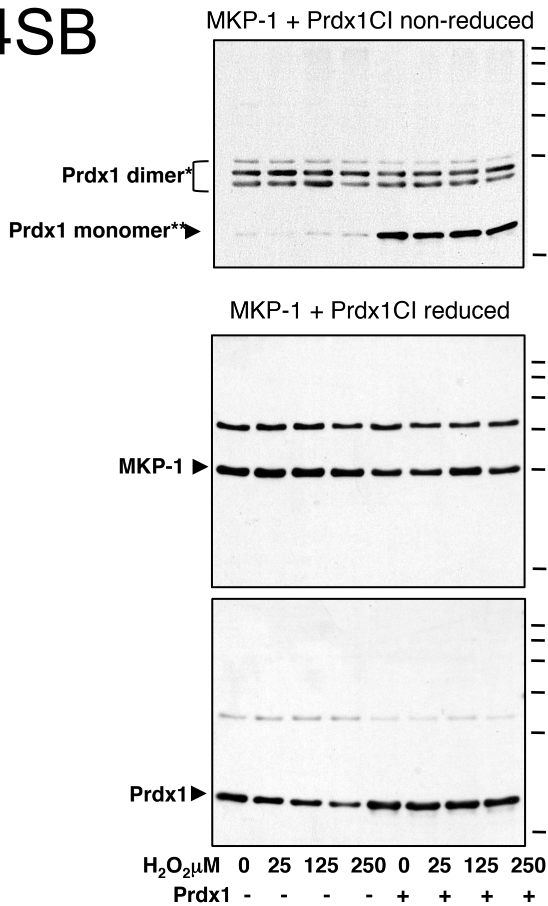
Fig S3C: Lysates for co-immunoprecipitations from Figure 3E and 3F. 293T HEK cells were co-transfected with Flag-MKP-1 or Flag-MKP-5 either with HA-Prdx1-WT or HA-Prdx1-CI, and treated with increasing amounts of H_20_2 for 30 min in serum free medium. Lysates were run under reducing conditions and analyzed by western blotting for MKP expression levels, HA-Prdx1-WT and HA-Prdx1-CI protein levels, and actin.

4SA





4SB



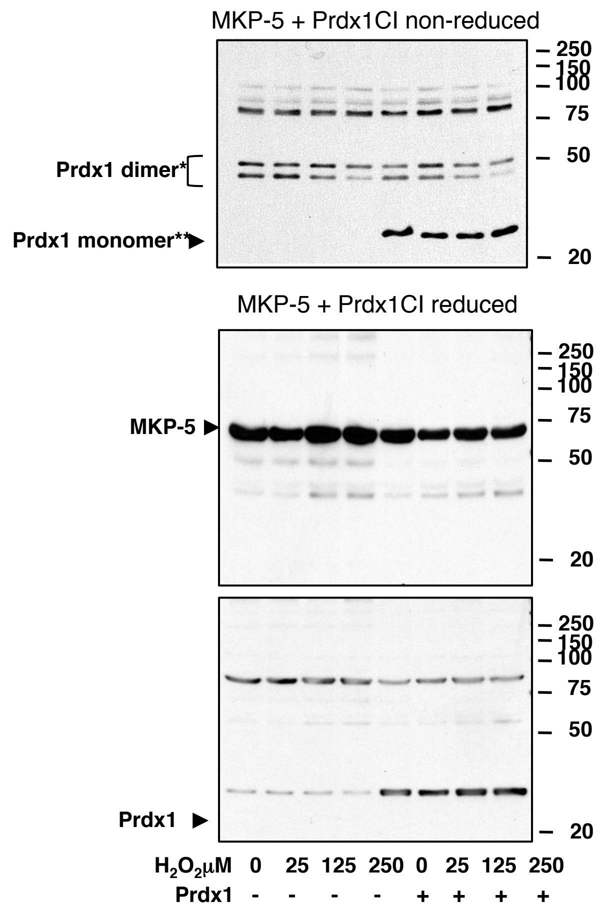


Fig S4A: 293T HEK cells were co-transfected with Flag-MKP-1 or Flag-MKP5 with Prdx1-WT, and treated with increasing amounts of H_20_2 for 30 min in serum free medium. Lysates were run under non-reducing or reducing conditions, with the addition of β-ME in the sample buffer, and analyzed by Western blotting for expression of MKPs and Prdx1. These lysates represent controls for oligomeric structures of MKP-1 and MKP-5 presented in Figure 4C and 4D, respectively.

Fig S4B: 293T HEK cells were co-transfected with Flag-MKP-1 or Flag-MKP5 with Prdx1-CI, and treated with increasing amounts of H_20_2 for 30 min in serum free medium. Lysates were run under reducing conditions, as stated above, and analyzed by Western blotting for expression of MKP and Prdx1. These lysates represent controls for oligomeric structures of MKP-1 and MKP-5 presented in Figure 4C and 4D, respectively.

Note: * indicates Prdx1 dimer formation of endogenous Prdx1. ** indicates Prdx1 monomers: endogenous Prdx1 and exogenous Prdx1CI.