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Supplemental Material to:

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Wild-type p53-induced phosphatase 1 (Wip1) forestalls cellular premature senescence at physiological oxygen levels by regulating DNA damage response signaling during DNA replication

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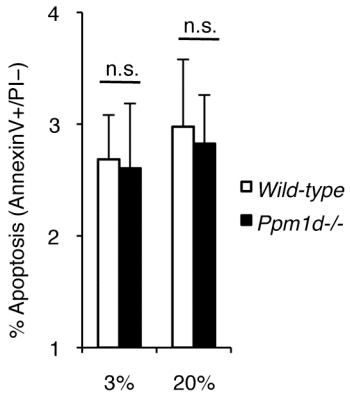
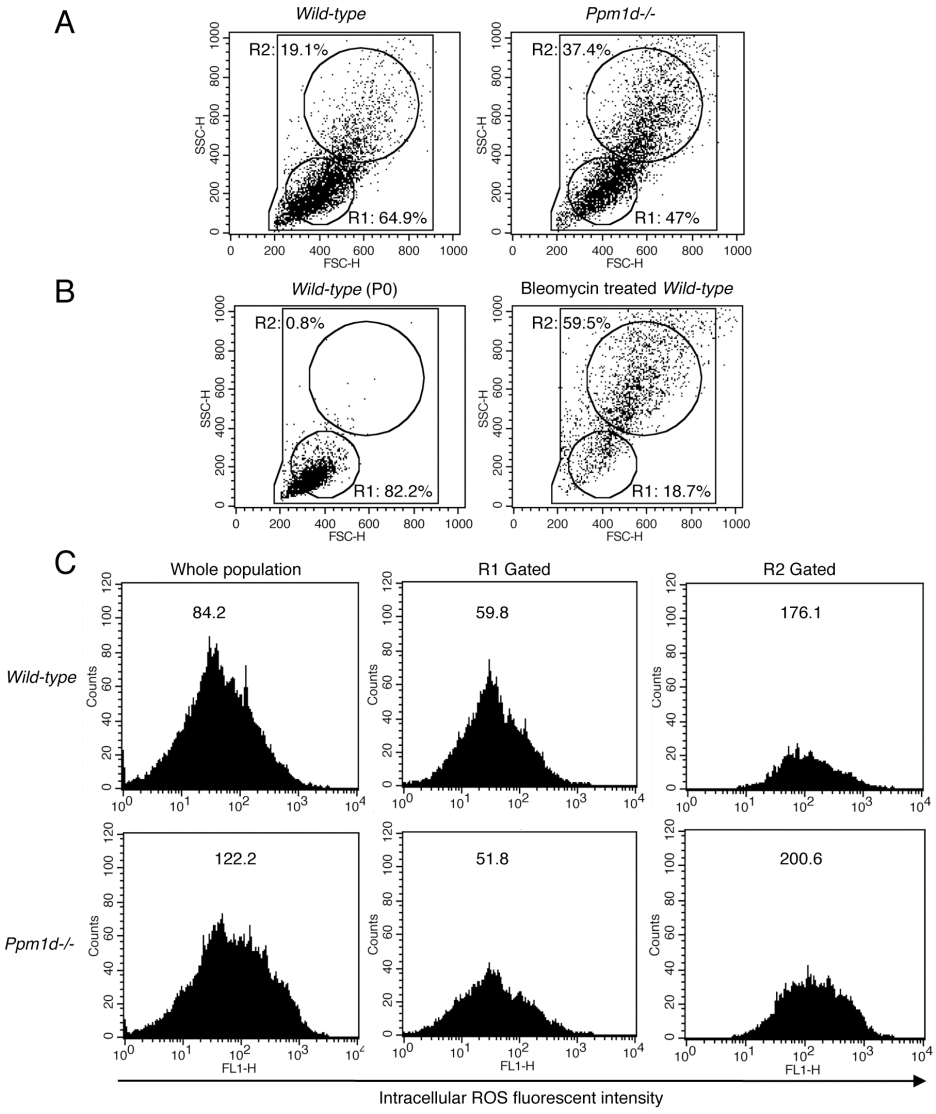
Sakai *et al*. Supplementary Figure 1



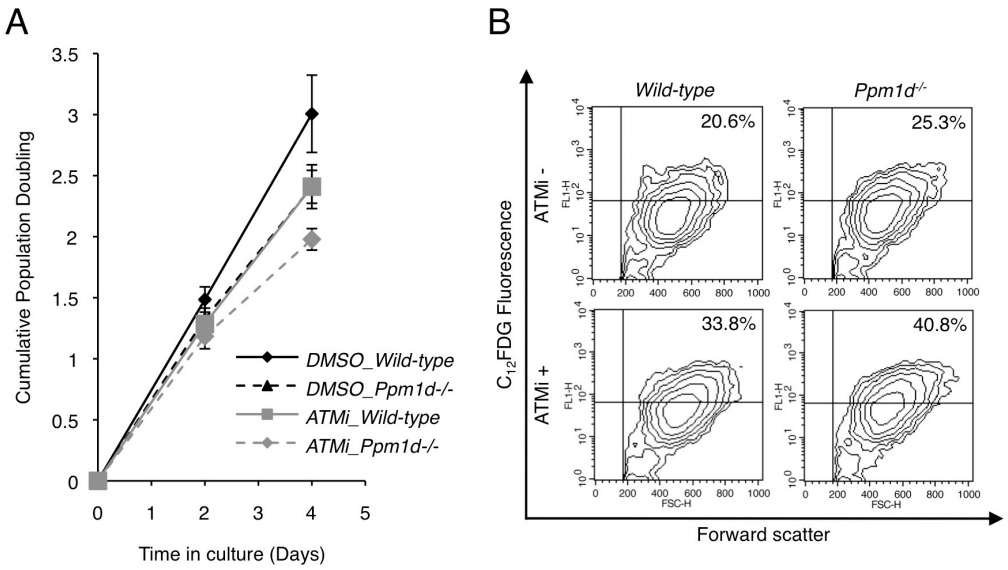
Supplementary Figure 1. Apoptosis assay in Passage 2 Wt and *Ppm1d*$^{-/-}$ MEFs. Cells were stained with annexin-V-FITC and PI then analyzed by flow cytometry. The percentage of apoptotic cells (annexin-V-positive and PI negative) is shown. The averages of three independent experiments with SD are shown. n.s.: non significant.

Sakai *et al*. Supplementary Figure 2



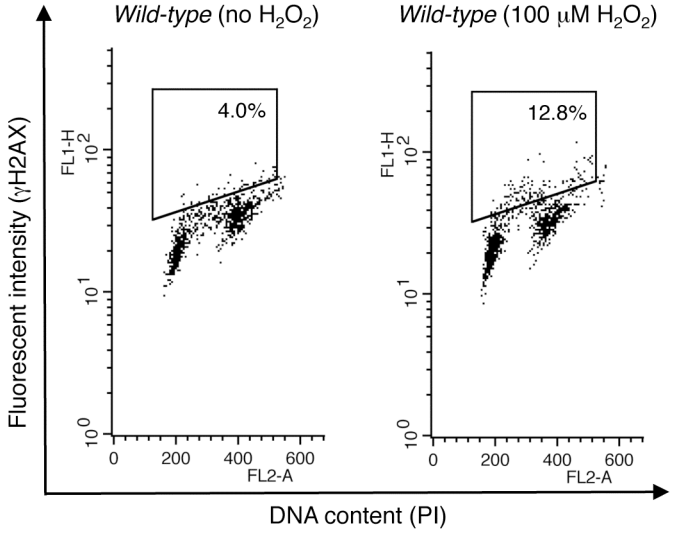
Supplementary Figure 2. Increased intracellular ROS levels observed in *Ppm1d*$^{-/-}$ MEFs cultured in 20% O_2 result from increased numbers of senescent cells. (A) Cell distributions (passage 2) Wt and *Ppm1d*$^{-/-}$ MEFs were determined by flow cytometry. The percentages of cells in R1 gate (low FSC and low SSC) or R2 gate (high FSC and high SSC) were determined by CELLQuest software (Becton Dickinson). (B) Distributions in Wt MEFs (passage 0, senescence negative control) and bleomycin-treated Wt MEFs (senescence positive control) were determined similarly. Wt MEFs were treated with 0.05 U/ml of bleomycin for 18 h and then cultured in fresh medium for 5 days. (C) ROS level distributions of all cells, R1-gated cells, and R2-gated cells in Wt or *Ppm1d*$^{-/-}$ MEFs (passage 2) are represented as histograms. The mean fluorescent intensity is indicated. Data are representative of three independent experiments.

Sakai *et al.* Supplementary Figure 3



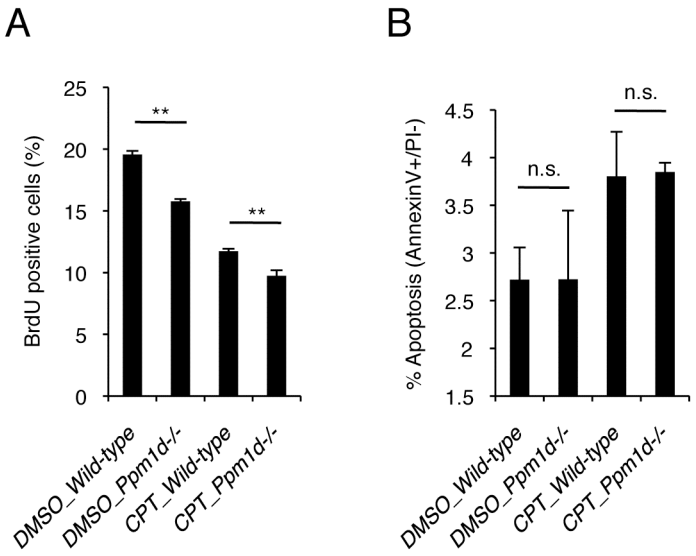
Supplementary Figure 3. Prolonged treatment with ATM kinase inhibitor induced cellular senescence in both Wt and *Ppm1d*$^{-/-}$ cells in 3% O_2 conditions. (A) Cells were cultured in 3% O_2 in medium with10 μM ATM kinase inhibitor KU55933 (ATMi) or 0.1% DMSO and passaged at 5 x 10^5 cells per 75 cm^2 flask every 2 days. Cell numbers were determined and cumulative population doubling levels were calculated at each passage. The averages of three independent cultures with SD are shown. (B) Flow cytometric detection of SA-β-Gal activity in cells at passage 3, cultured as described in (A). The percentage of SA-β-Gal positive cells is shown in the upper right of each diagram. The data are representative of three independent experiments.

Sakai *et al.* Supplementary Figure 4



Supplementary Figure 4. H_2O_2 induced H2AX phosphorylation during S phase in Wt MEFs. Passage 2 Wt MEFs cultured in 3% O_2 were treated with or without 100 μM H_2O_2 for 15 min. Cells were washed with 1 × PBS and incubated in fresh completed medium for 30 min. The collected cells were stained with anti-γ-H2AX antibody and PI and then analyzed by dual parameter flow cytometry. γH2AX intensity is represented on the Y-axis and PI staining (DNA content) is plotted along the X-axis. The percentage of γH2AX positive cells is indicated in the upper right of each diagram. The gating parameters for selecting γH2AX-positive cells were determined by using *H2AX*$^{-/-}$ MEFs treated with the same primary antibody. The data are representative of three independent experiments.

Sakai *et al.* Supplementary Figure 5



Supplementary Figure 5. The number of S phase cells is reduced in *Ppm1d*$^{-/-}$ MEFs, both with and without camptothecin-treatment, without an increase in apoptosis. Passage 2 MEFs were cultured in 3% O_2 in medium with 0.1% DMSO or 20 nM camptothecin (CPT) for four days. Medium was replaced every two days. (A) Cell cycle analysis of Wt and *Ppm1d*$^{-/-}$ MEFs. The averages of three independent experiments with SD are shown (∗∗$p < 0.01$, Student's t-test). (B) Apoptosis assay of Wt and *Ppm1d*$^{-/-}$ MEFs. Cells were stained with annexin-V-FITC and PI then analyzed by flow cytometry. The percentage of apoptotic cells (annexin-V-positive and PI negative) is shown. The averages of three independent experiments with SD are shown. n.s.: non significant.