

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. **p53 is not necessary for p21-induced apoptosis** (A) Representative immunoblot analysis of p21 and p53 and protein levels in lysates of HT1080p21-9 stably expressing shRNA against luciferase (C) or p53 (p53), uninduced (Control) or induced for 5 days (5d). (B) FACS analysis of PI-stained HT1080p21-9 cells stably expressing shRNA against p53 (shp53) or luciferase (shControl), uninduced (Control) or 5 days after p21 induction. As expected, no upregulation of p53 or its targets is observed. (C) Representative immunoblot analysis of p53 and some of its target genes in lysates of 5d induced HT1080p21-9, treated or not with 20 μ M Pifithrin (PFT) (D) Representative FACS analysis of PI-stained HT1080p21-9 cells 5 days after p21 induction in the presence or absence of 10-20 μ M PFT. Numbers indicate percentage of events in SubG₁ phase.

Supplementary Figure 2. **Measures of intracellular ROS levels and cell death in HT1080p21-9 treated with antioxidants.** (A) FACS analysis of HT1080p21-9, uninduced (Control) or after 5 days of p21 induction, unstained or stained with DCF. Plot represents fold increases in mean fluorescence intensity in the FL1 channel, compared to unstained control cells. “Subtracted (S-U)” column reflects mean fluorescence intensity of DCF-stained cells (S) minus that of corresponding unstained cells (U), in other words, DCF fluorescence minus autofluorescence. (B) Representative FACS analysis of cells in (A). FSC represents cell size; DCF marks fluorescence intensity in FL1 channel. Black cells are DCF negative; green cells are DCF positive; red cells have low DCF intake and are likely to be dead. (C) Representative FACS analysis of Annexin V stained HT1080p21-9 cells, uninduced (Control) or after 5 days of p21 expression (5d). Percentages show Annexinpositive/PI-positive cells (late apoptotic/necrotic, top right quadrant) and Annexinpositive/PI-negative cells (early apoptotic, bottom right quadrant). (D) Representative immunoblot analysis of p21 protein levels and PARP cleavage in lysates of HT1080p21-9 uninduced (Control) or induced for 5 days (5d). (E) FACS analysis of Annexin/PI-stained HT1080p21-9 cells uninduced, 5 days after p21 induction in the presence or absence of 10 mM NAC. Percentages of live cells (Annexin negative and PI negative) are plotted. (F) Immunoblot analysis of p21 protein levels in lysates of HT1080p21-9 treated with 10 mM NAC.

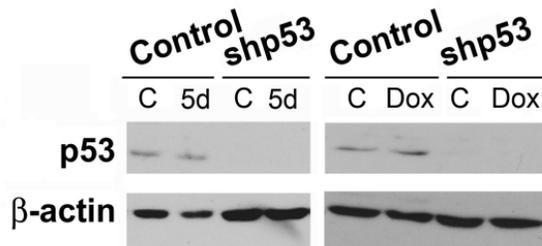
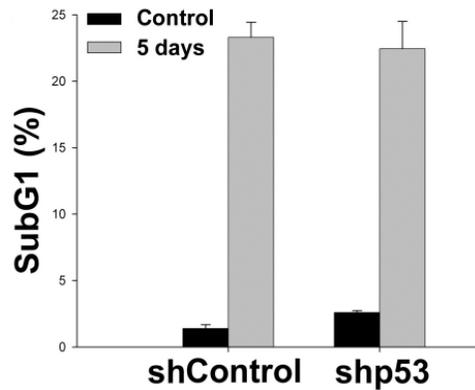
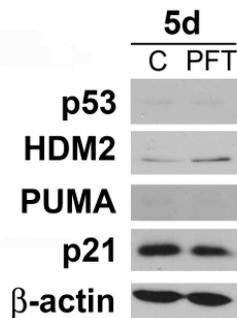
Supplementary Figure 3. **U2OS osteosarcoma cells also undergo cell death after p21 expression.** (A) Immunoblot analysis of p21 protein levels in lysates of U2OSp53^{-/-} cells one week after infection with a retrovirus containing p21. (B) Representative FACS analysis of the same cells stained with PI (left column) or DCF (right column). Numbers indicate percentage of events in the SubG₁ phase of the cell cycle. Vertical white bars indicate mean fluorescence intensity of DCF-stained control cells. (C) and (D) Same as (B). Plots represent averages of three independent experiments. Error bars represent standard deviation. (E) Representative FACS analysis of U2OSp53^{-/-} cells stained with PI. Cells were infected with a retrovirus containing p21 as described above and treated with 10mM NAC from the moment of infection (fresh media with NAC was added after two days). Numbers indicate percentage of events in the SubG₁.

Supplementary Figure 4. **p21 induces cell cycle arrest in the absence of significant apoptosis in EJp21 and PC3p21 cells.** (A) FACS analysis of PI-stained EJp21 uninduced (Control) or 5 days after p21 induction (5d). Bars show percentages of cells in each phase of the cell cycle. (B) Representative FACS analysis of Annexin V stained EJp21 cells, uninduced (Control) or after 5 days of p21 expression (5d). Percentages show Annexin-positive/PI-positive cells (late apoptotic/necrotic, top right quadrant) and Annexin-positive/PI-negative cells (early apoptotic, bottom right quadrant). (C) FACS analysis of PI-stained PC3p21 cells uninduced (Control) or 5 days after p21 induction by tetracycline removal (5d). Bars show percentages of cells in each phase of the cell cycle.

Supplementary Figure 5. **Effects of p21 expression in different cell lines.** (A) Representative immunoblot analysis of ZEB1 (expressed in mesenchymal carcinoma lines), vimentin (mesenchymal

marker) and ecadherin (epithelial marker) protein levels in lysates of EJp21 and HT1080p21-9 (uninduced and 5 days after p21 induction), compared to those in HOS (osteosarcoma, mesenchymal control) and A431 (squamous carcinoma, epithelial control) cells. **(B)** Representative FACS analysis of HA847 and MDA-MB-175 cells stained with PI. Cells were infected with a retrovirus containing p21, as above. Numbers indicate percentage of events in the SubG₁ phase of the cell cycle. **(C)** PI-stained uninduced PC3p21 cells, 24 hours after exposure to different concentrations of tBH. Percentages of live cells (not SubG₁ events) are plotted.

Supplementary Figure 6. **Sensitivity of HT1080p21-9 and EJp21 to oxidative stress.** **(A)** FACS analysis of Annexin/PI-stained uninduced EJp21 and HT1080p21-9, infected for 48 hours with an adenovirus expressing Lac Z (Control) or p53 (Adp53). Graph shows percentage of live cells (Annexin and PI negative). **(B)** Immunoblot analysis of p53 protein levels in lysates of HT1080p21-9 (uninduced and induced for 5 days) and EJp21, infected with Adp53 for 24 hours. **(C)** FACS analysis of PI-stained EJp21 uninduced (+tet) or 5 days after p21 induction (-tet), infected with AdLacZ or AdCat. Media was changed after 3 days in culture and new viruses were added. Bars show percentages of cells in each phase of the cell cycle. **(D)** Immunoblot analysis of catalase protein levels in lysates of EJp21, infected with AdLacZ or AdCat for 24 hours. **(E)** Immunoblots of EJp21 uninduced (-) or induced to express p21 for three days (+), in the absence of shRNA (-), or the presence of a shRNA against catalase (Cat) or an unrelated target (Un). Untreated U2OSp53^{-/-} cells are provided as control. **(F)** Representative FACS analysis of EJp21 cells stained with PI. Cells were induced to express p21 for three days in the presence of a shRNA construct against luciferase (Control) or catalase (Cat RNAi). Numbers indicate percentage of events in the SubG₁ phase of the cell cycle.

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