Figure S1. All DNA damage modalities induce p53 but manifest different p21 expression levels. (A) Immunoblot of p53 or caspase-3 in A549 or Panc-1 cells following chromium (Cr(VI)), gemcitabine (Gem), doxorubicin (Doxo) or γ-irradiation (γ-IR) exposure. β-actin serves as loading control for this study. (B) FACS time course in A549 or Panc-1 cells to quantify the sub-G1, G1 and G2 cell populations post DNA damage. Average data from three independent experiments performed in triplicate (20,000 total events counted per sample). (C) Fold change in p21 mRNA levels in A549 or Panc-1 cells 12 h post DNA damage. Expression level was normalized to GAPDH and is represented as fold increase or decrease over untreated cells. Error bars represent the standard deviation obtained from three independent experiments. (D) Chromatin immunoprecipitations (ChIP) for total p53 from A549 or Panc-1 cells 6 h post Cr(VI), Gem, Doxo, or γ-IR treatment. PCR was performed for the distal (-2.2 kb) p21 or the proximal (-1.3 kb) p21 promoter regions. Errors represent standard deviation obtained from three independent experiments.
Figure S2. p53 binds to p21 promoters under both pro-apoptotic and pro-arrest conditions but is modified differently. Chromatin immunoprecipitations (ChIP) for phosphor(P)-ser15, P-ser37 or P-ser315 p53 from Panc-1 cells 6 h post Cr(VI), Gem, Doxo, or γ-IR treatment. PCR was performed for both the distal (-2.2 kb) p21 and proximal (-1.3 kb) p21 promoter regions. Error bars for each graph represents standard deviation obtained from three independent experiments.
Figure S3. Recruitment of p53 isoforms to the MDM2, PUMA and Bax promoter. ChIP assays were conducted for P-ser15 or P-ser37 from Panc-1 cells 6 h post Cr(VI), Gem, Doxo, or γ-IR treatment. PCR was performed for the MDM2, PUMA or Bax promoter regions. Error bars for each graph represent standard deviation obtained from three independent experiments for each promoter of interest.
Figure S4. ATM/ATR activation following DNA damage is independent of DNA-PK<sub>CS</sub>. (A) Left: Immunoblot for ATM and phosphor-ser1981 ATM in [p53<sup>+/+</sup>] HCT116 cells 12 h post DNA damage. + indicates 6 h pre-incubation with the DNA-PK inhibitor NU-7026 (10 µM) or the ATM/ATR inhibitor CGK-733 (20 µM). Total ATM was used as loading control. Right: Immunoblot for ATR and phosphor-ser428 ATM in [p53<sup>+/+</sup>] HCT116 cells 12 h post DNA damage. + indicates 6 h pre-incubation with the DNA-PK inhibitor NU-7026 (10 µM) or the ATM/ATR inhibitor CGK-733 (20 µM). (B) Immunoblot for total p53 using the A549 or Panc-1 cell extracts described in (A). (C) Top: Co-immunoprecipitation (Co-IP) from A549 or Panc-1 cells using either DNA-PK<sub>CS</sub> or p53 at the time points indicated. Bottom: Immunoblot for p53 or DNA-PK<sub>CS</sub> from the same cell lines 12 hours post damage.
Figure S5. DNA-PK<sub>CS</sub> forms a complex with p53 on the p21 promoters under pro-apoptotic conditions. (A) Top: ChIP assays for DNA-PK<sub>CS</sub> from A549 cells 6 h post Cr(VI), Gem, Doxo, or γ-IR treatment. PCR was performed for the distal (-2.2 kb) and the proximal (-1.3 kb) p21 promoter. Bottom: ChIP assay for P-thr2609 DNA-PK<sub>CS</sub> from A549 cells 6 h post Cr(VI) or Gem treatment. (B) Top: ChIP assay for DNA-PK<sub>CS</sub> from Panc-1 cells 6 h post Cr(VI), Gem, Doxo, or γ-IR treatment. PCR from performed for the distal (-2.2 kb) or the proximal (-1.3 kb) p21 promoter. Bottom: ChIP assay for P-thr2609 DNA-PK<sub>CS</sub> from Panc-1 cells 6 h post Cr(VI) or Gem treatment. Error bars on each indicated graph represent the standard deviation obtained from three independent experiments.
Figure S6. DNA-PK<sub>CS</sub> is recruited to the <i>p21</i> promoter under pro-apoptotic conditions. (A) Left: ChIP assay for p53 or DNA-PK<sub>CS</sub> from Panc-1 cells following Gem exposure for the time points indicated. + indicates 6 h pre-incubation with the ATM/ATR inhibitor CGK-733 (20 µM) or the DNA-PK inhibitor NU-7026 (10 µM). PCR was performed for the distal (-2.2 kb) <i>p21</i> promoter region. Right: Quantification of PCR bands shown on the left. (B) Left: ChIP assay for p53 or DNA-PK<sub>CS</sub> was conducted as in (A). PCR was performed for the proximal (-1.3 kb) <i>p21</i> promoter region. Right: Quantification of PCR bands shown on the left. (C) Left: ChIP assay for p53 or DNA-PK<sub>CS</sub> from Panc-1 cells following Cr(VI) exposure for the time points indicated. + indicates 6 h pre-incubation with the ATM/ATR inhibitor CGK-733 (20 µM) or the DNA-PK inhibitor NU-7026 (10 µM). PCR was performed for the distal (-2.2 kb) <i>p21</i> promoter region. Right: Quantification of PCR bands shown on the left. (D) Left: ChIP assay for p53 or DNA-PK<sub>CS</sub> was conducted as in (C). PCR was performed for the proximal (-1.3 kb) <i>p21</i> promoter region. Right: Quantification of PCR bands shown on the left. Error bars on each indicated graph represent standard deviation obtained from three independent experiments.
**Figure S7. DNA-PK<sub>cs</sub> inhibition restores p21 gene expression and increases cell survival.** (A-D) Fold change in Bax and PUMA mRNA levels in [p53<sup>+/+</sup>] HCT116, [p53<sup>-/-</sup>] HCT116, A549 or Panc-1 cells 12 h post Cr(VI), Gem, Doxo or γ-IR exposure. The DNA-PK inhibitor (NU-7026 [10 µM]) or the ATM/ATR inhibitor (CGK-733 [20 µM]) were pre-incubated for 6 h prior to DNA damage. All the mRNA expression levels were normalized to GAPDH mRNA and are represented as fold increase or decrease over untreated cells. Error bars represent the standard deviation obtained from three independent experiments.
Figure S8. Inhibition of DNA-PK<sub>cs</sub> restores p21 expression under pro-apoptotic conditions. Immunoblot for p21, PUMA or Bax in Panc-1 cells 12 h post DNA damage. + indicates 6 h pre-incubation with the DNA-PK inhibitor NU-7026 (10 µM) or the ATM/ATR inhibitor CGK-733 (20 µM). For each gel β-actin indicates loading control.
Figure S9. Myc/Miz-1 does not mediate p21 repression following chromium or gemcitabine exposure. (A) *p53*+/+ HCT116, A549 or Panc-1 cells were treated with Cr(VI), Gem or Doxo for 24 h. Cell extracts were prepared and subjected to immunoblot analysis for Myc and β-actin expression. (B) *p53*+/+ HCT116, A549 or Panc-1 cells were exposed to Cr(VI), Gem, or Doxo for 18 h. Trizol RNA extraction was carried out and cDNA was generated and the expression level for Myc was measured. (C) Myc co-immunoprecipitation was conducted using A549 or Panc-1 cells following Cr(VI), Gem or Doxo treatment for 24 h and probed for Miz-1 interaction. (D) ChIP was carried out with *p53*+/+ HCT116, A549 or Panc-1 cells following mock or 18 h post DNA damage with the indicated modalities using the α-Miz-1 antibody. PCR was performed for the distal (-2.2 kb) p21 promoter region. Quantification of the bands following each DNA damage modality was determined for triplicate studies and represented for this promoter.