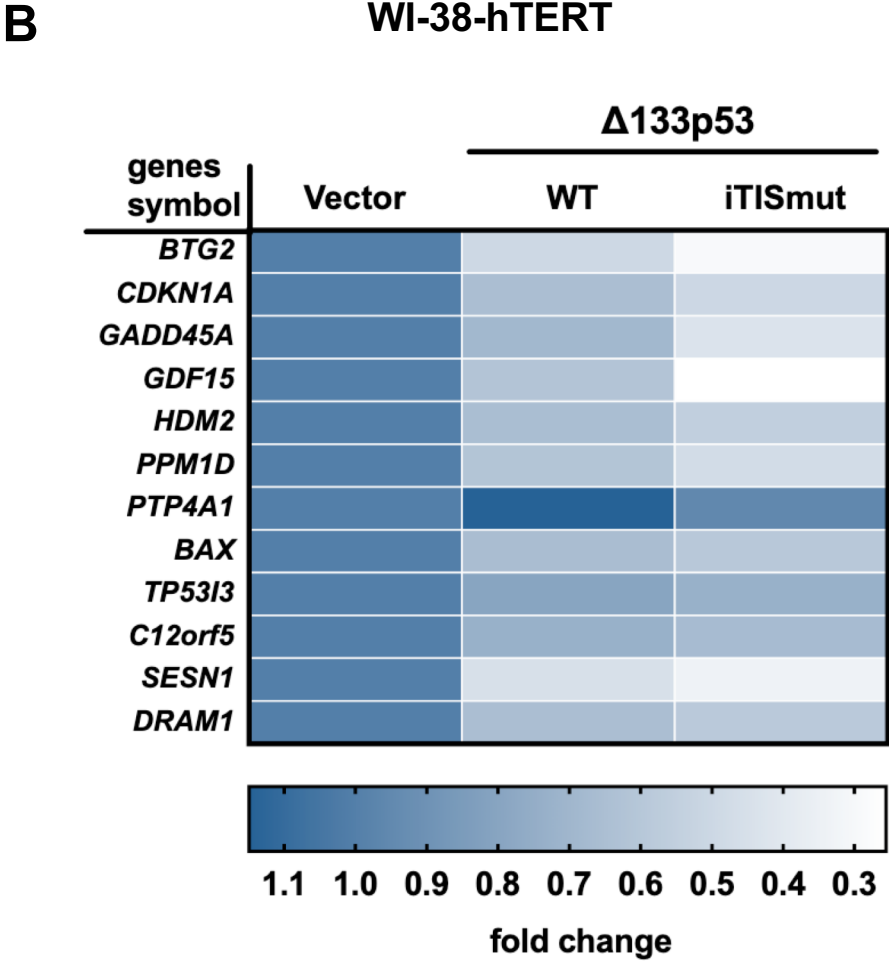
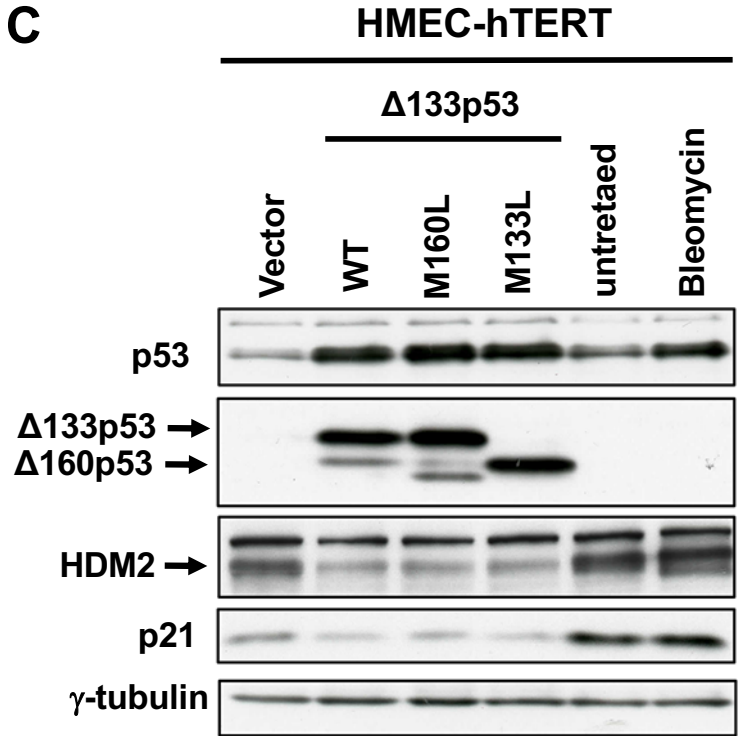
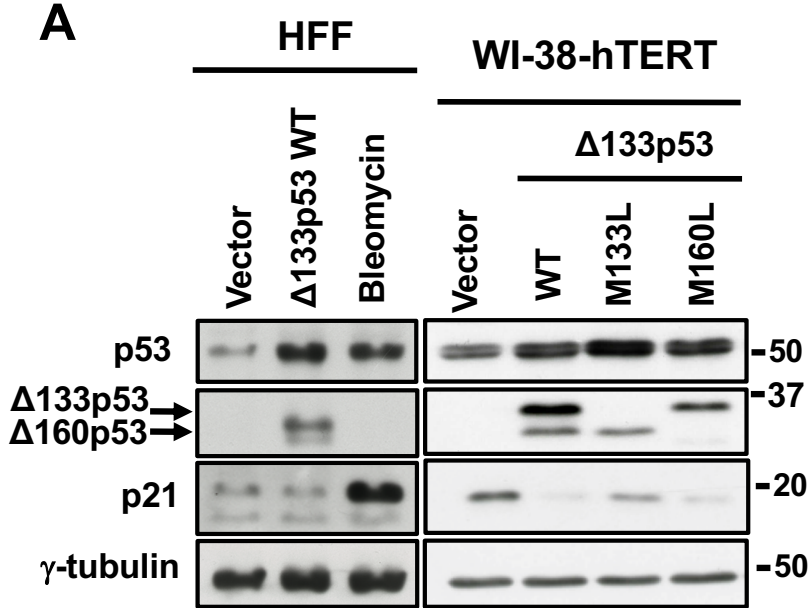


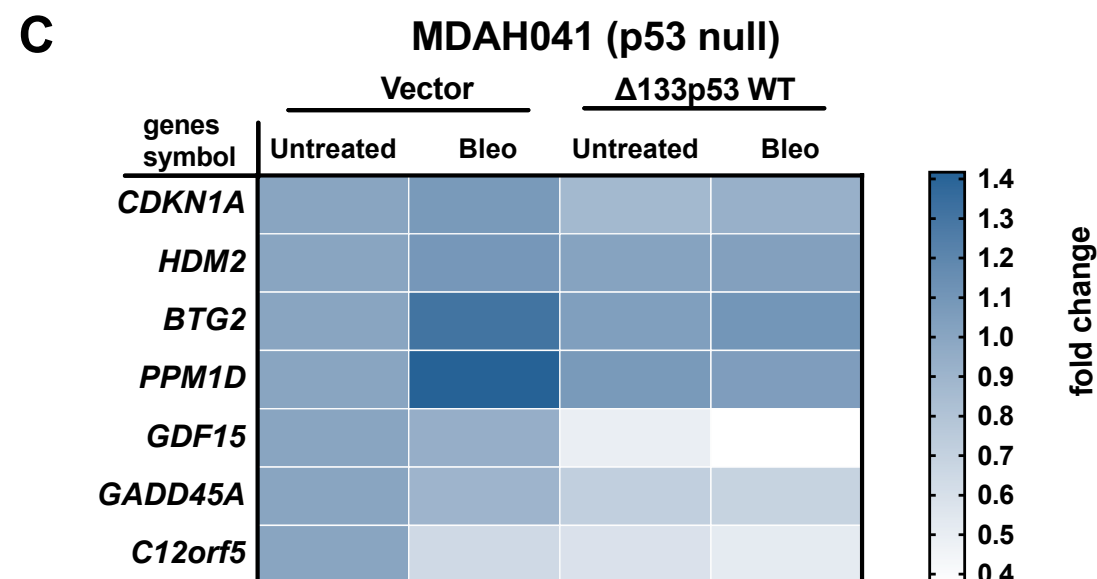
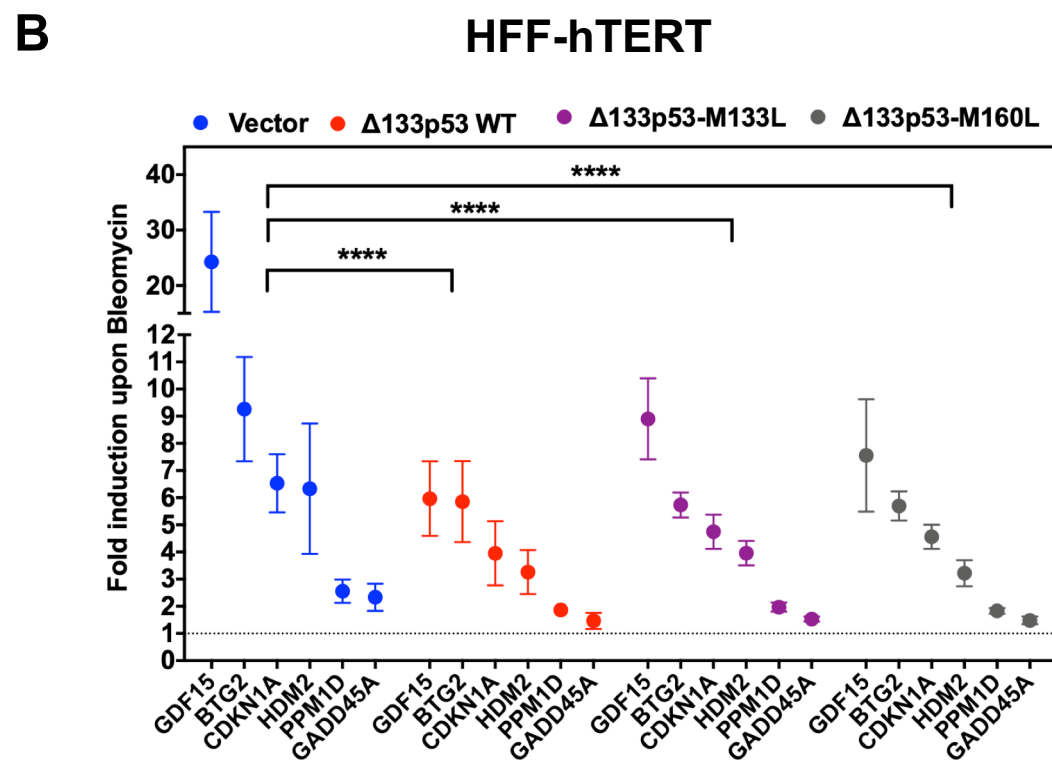
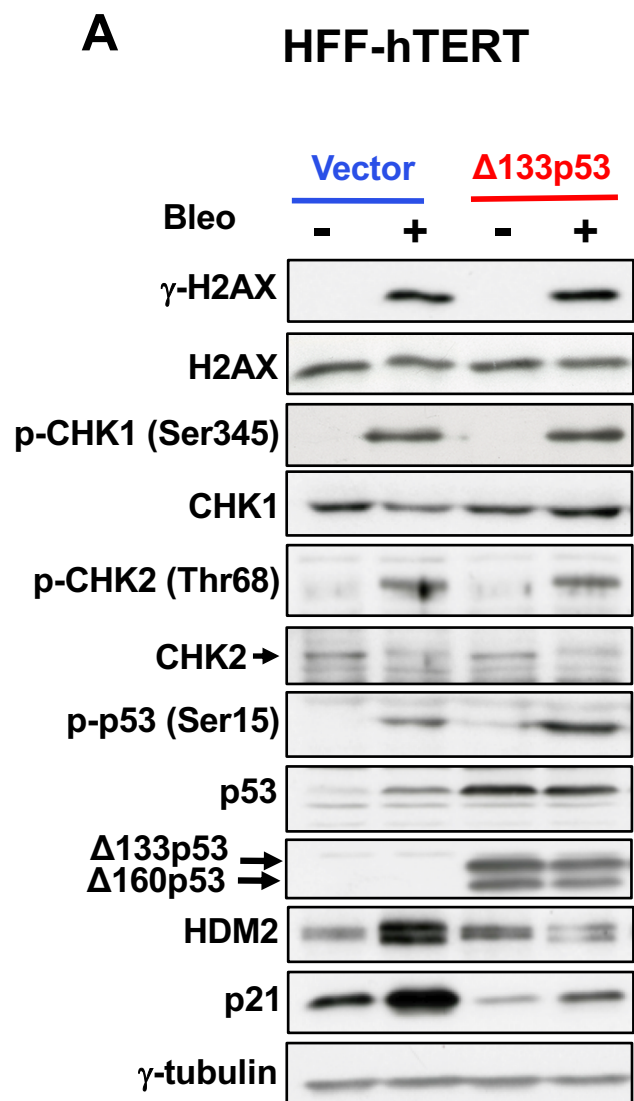


Supplementary Figure S1 (complements Figure 1). p53 is stabilized but its transcriptional activity is reduced in HFF and WI38-hTERT cells that express $\Delta 133p53\alpha$ and $\Delta 160p53\alpha$ isoforms.

(A) HFF and telomerase-immortalized WI-38 (WI38-hTERT) cells were infected with retroviral vectors expressing $\Delta 133p53\alpha$ -WT (WT), $\Delta 133p53\alpha$ -M133L (M133L), $\Delta 133p53\alpha$ -M160L (M160L) cDNA, or empty vector (vector). At day 8 post-infection, cells were harvested and p53 (DO1 antibody), $\Delta 133p53\alpha$ and $\Delta 160p53\alpha$ (DO11 antibody), and p21 levels were determined by immunoblotting. γ -tubulin, loading control. Bleomycin-treated cells were used as a positive control for p53 stability and activity. Images are representative of three independent experiments.

(B) The mRNA levels of a panel of p53 target genes were determined by RT-qPCR in WI-38-hTERT cells that overexpress $\Delta 133p53\alpha$ -WT (WT), $\Delta 133p53\alpha$ -iTISmut (iTISmut) or vector alone (vector) at day 8 post-infection. In the heatmap, colors indicate the fold-change compared with control (vector only) cells (n =3).

(C) Telomerase-immortalized HMEC cells (HMEC-hTERT) were infected with the $\Delta 133p53\alpha$ -WT (WT), $\Delta 133p53\alpha$ -M133L (M133L), $\Delta 133p53\alpha$ -M160L (M160L) constructs or empty vector. At day 8 post-infection, cells were harvested and endogenous p53 (DO1 antibody), $\Delta 133p53\alpha$ and $\Delta 160p53\alpha$ (DO11 antibody), p21 and HDM2 levels were detected by immunoblotting. γ -tubulin, loading control. Cells incubated with bleomycin were used as positive control for p53 stability and activity. Untreated, asynchronously growing, non-infected HMEC-hTERT cells were also used as controls.



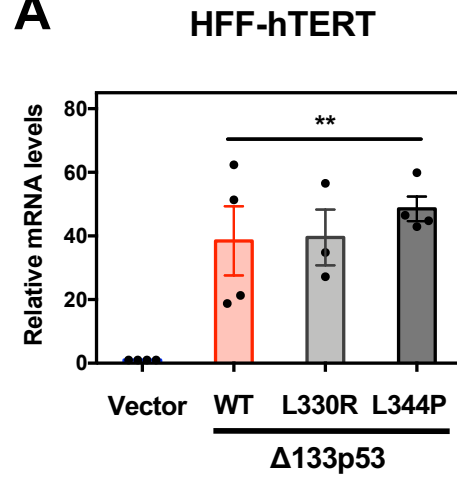
Supplementary Figure S2 (complements Figure 2). $\Delta 133p53\alpha$ and $\Delta 160p53\alpha$ controls cell proliferation by regulating p53 activity

(A) The p53-dependent DDR pathway upstream of p53 is functional in HFF-hTERT cells that express $\Delta 133p53\alpha$ -WT. HFF-hTERT cells that express $\Delta 133p53\alpha$ -WT or vector alone (control) were incubated or not with bleomycin for 6 hours and the levels of γ -H2AX, H2AX, CHK1 phosphorylated at Ser345 (p-Chk1 (Ser345)), CHK1, CHK2 phosphorylated at threonine 68 (p-Chk2 (Thr68)), CHK2, p53 phosphorylated at Ser15 (p-p53 (Ser15)), p53, $\Delta 133p53\alpha$, $\Delta 160p53\alpha$ and the p53 targets HDM2 and p21 were determined by immunoblotting. γ -tubulin, loading control. Images are representative of three independent experiments.

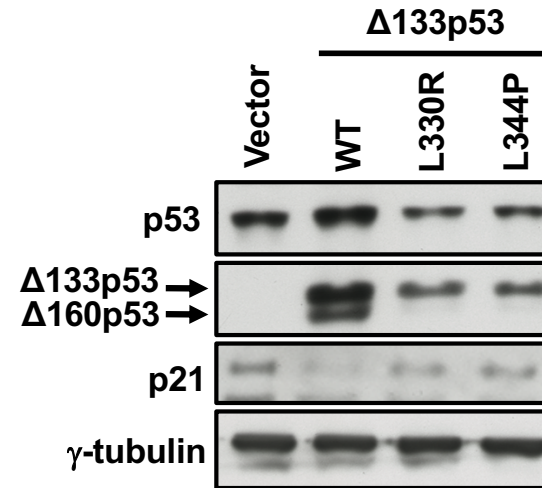
(B) p53 transcriptional activity is impaired in cells that express $\Delta 133p53\alpha$ -WT, $\Delta 133p53\alpha$ -M133L, $\Delta 133p53\alpha$ -M160L constructs or vector alone (vector). RT-qPCR analysis of genes activated by p53 and involved in cell cycle arrest in HFF-hTERT cells that express $\Delta 133p53\alpha$ -WT, $\Delta 133p53\alpha$ -M133L or $\Delta 133p53\alpha$ -M160L and in control cells (vector only) incubated or not with bleomycin for 6 hours. mRNA expression was normalized to *TBP* values and expressed as fold induction after 6 hours of incubation relative to untreated cells. Data are the mean $\pm$ SEM (n = 3); ****, $p < 0.0001$, two-way ANOVA with Dunnett's multiple comparison test.

(C) RT-qPCR analysis of a panel of p53 target genes in MDAH041 (p53-null) fibroblasts that overexpress $\Delta 133p53\alpha$ -WT or in control cells (vector only), untreated or incubated with bleomycin for 6 hours. mRNA expression was normalized to *TBP* values and in the heatmap, colors indicate the fold-change compared with control (vector only) cells (n = 4).

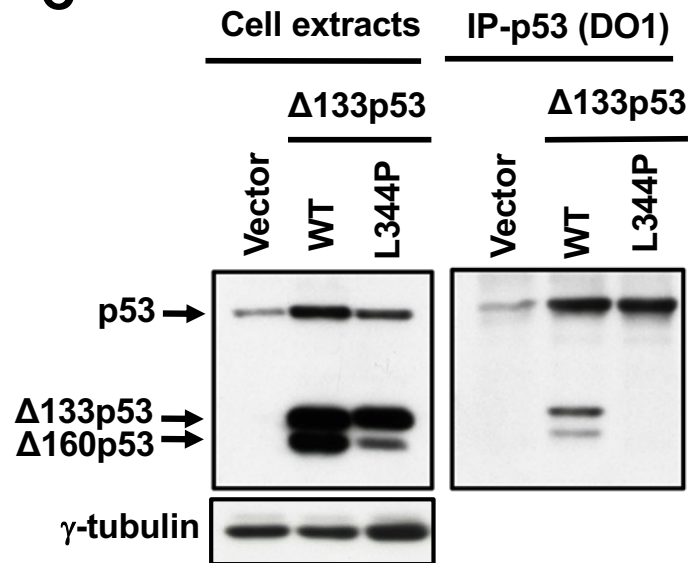
A



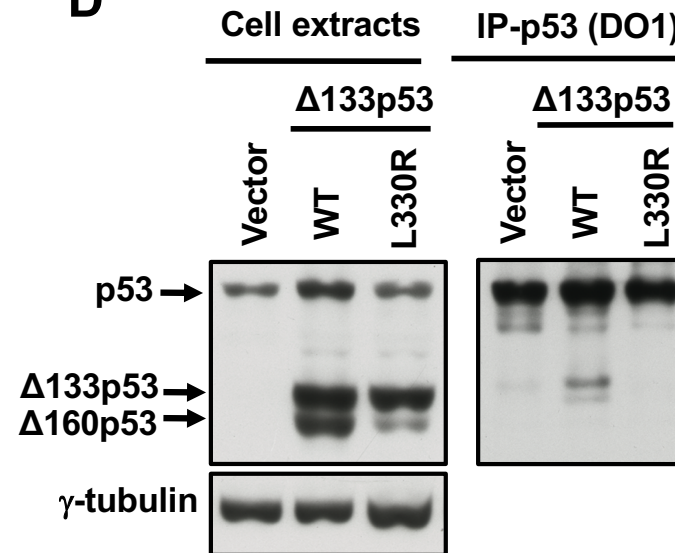
B



C



D



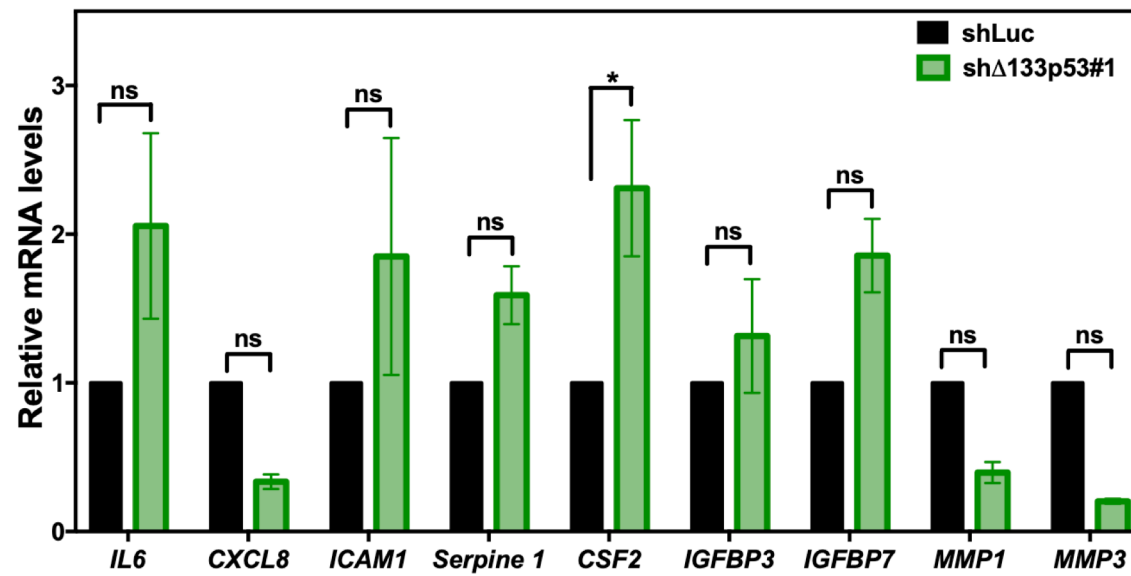
Supplementary Figure S3 (complements Figure 3). Validation of the $\Delta 133p53\alpha$ -L344P and $\Delta 133p53\alpha$ -L330R oligomerization mutants

(A) $\Delta 133TP53$ mRNA levels were determined by RT-qPCR in HFF-hTERT cells that express $\Delta 133p53\alpha$ -WT or its oligomerization mutants L344P or L330R. Data are the mean $\pm$ SEM ($n \geq 3$); ** $p < 0.01$, one-way ANOVA with Dunnett's multiple comparison test.

(B) $\Delta 133p53\alpha$ and $\Delta 160p53\alpha$ protein levels are lower in HFF-hTERT cells that express the $\Delta 133p53\alpha$ -L344P or $\Delta 133p53\alpha$ -L330R oligomeric mutant than in cells that express $\Delta 133p53\alpha$ -WT (WT). Western blot analysis was performed using the same cell samples described in (A) to measure $\Delta 133p53\alpha$ and $\Delta 160p53\alpha$, endogenous p53 and p21 protein levels. Images are representative of three independent experiments.

(C) The $\Delta 133p53\alpha$ -L344P oligomeric mutant cannot form a complex with endogenous p53. Total cell extracts from HFF-hTERT cells that express $\Delta 133p53\alpha$ -WT (WT), $\Delta 133p53\alpha$ -L344P or control cells (vector) were immunoprecipitated with the anti-p53 antibody (DO1). Note that the amounts of total extracts from $\Delta 133p53\alpha$ -L344P expressing cells and $\Delta 133p53\alpha$ -WT-expressing cells were corrected to have comparable levels of expressed isoforms. Total cell extracts (left panel) and immunoprecipitates (right panel) were immunoblotted with the DO1 and DO11 antibodies. γ -tubulin, loading control. Images are representative of three independent experiments.

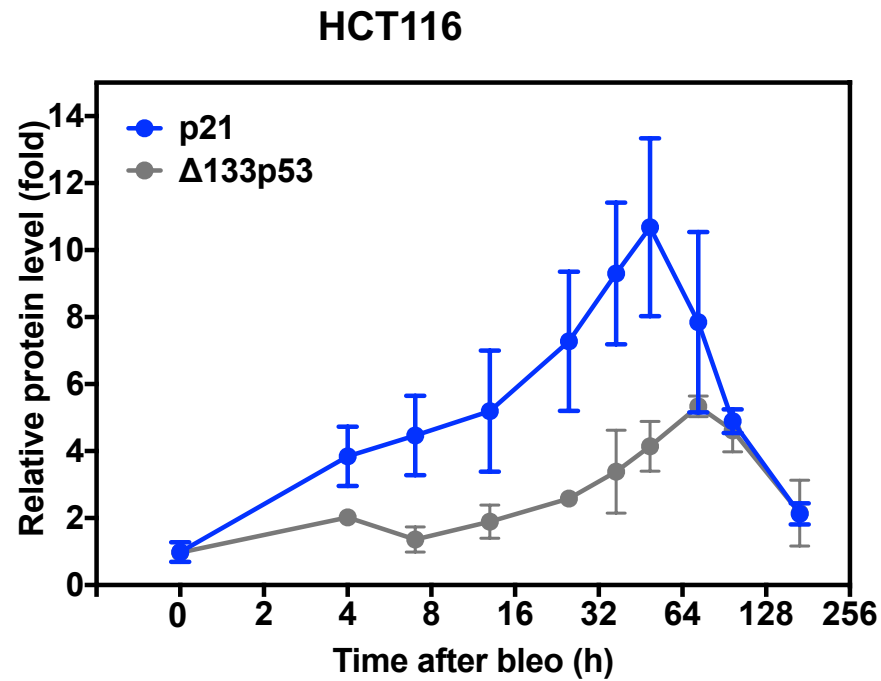
(D) Same analysis as in (C) of cells that express $\Delta 133p53\alpha$ -L330R. This oligomeric mutant also cannot form a complex with endogenous p53. Images are representative of three independent experiments.



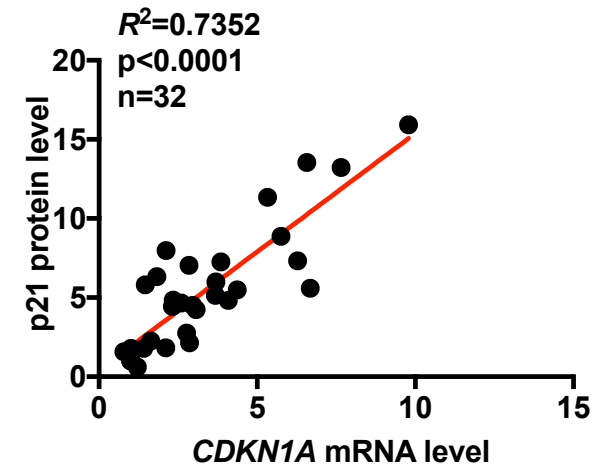
Supplementary Figure S4 (complements Figure 5). Depletion of $\Delta 133TP53$ mRNA does not result in a SASP response

HFF cells were infected with $\Delta 133p53\#1$ shRNA or sh*Luc* and the expression of the indicated SASP genes was determined by RT-qPCR at day 20 post-infection. Data are the mean $\pm$ SEM (n=3); *p <0.05, ns, not significant, two-way ANOVA with Sidak's multiple comparison test.

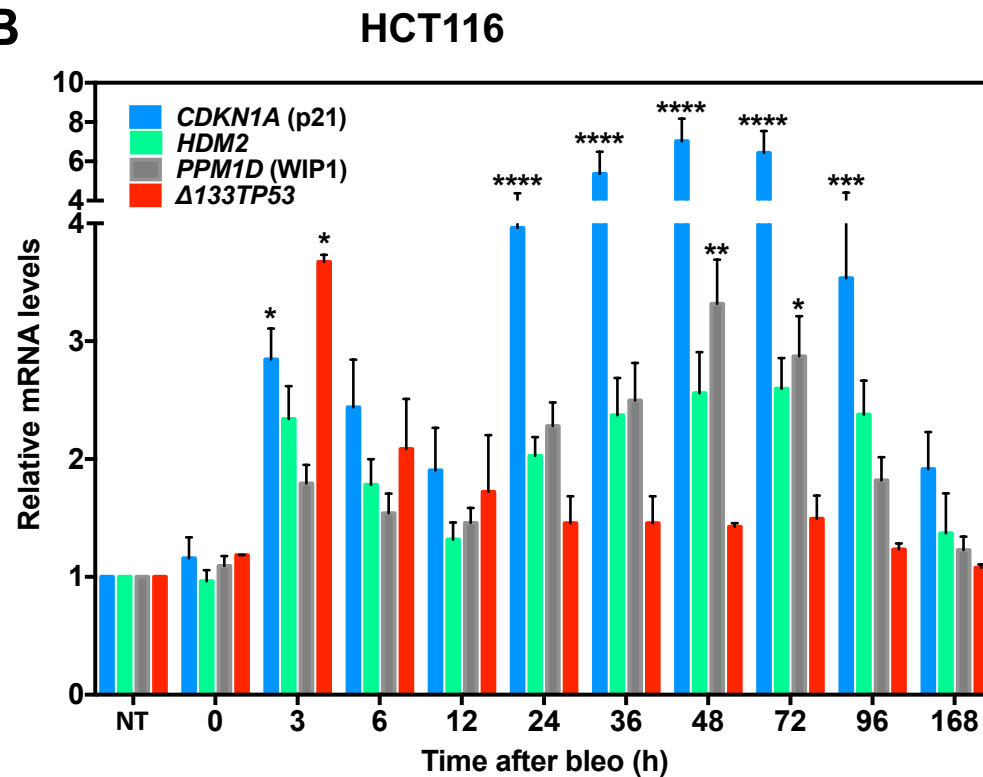
A



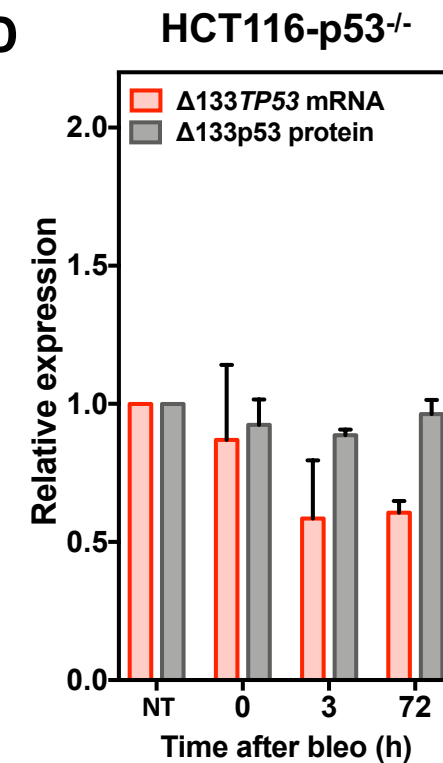
C



B



D



Supplementary Figure S5 (complements Figure 6).

(A) HCT116 cells were incubated or not with bleomycin for 1 hour, released and collected at different time points, and p21 and $\Delta 133p53\alpha$ expression levels were assessed by western blotting as in Fig. 6A. Immunoblots were quantified and p21 and endogenous $\Delta 133p53\alpha$ levels were normalized to γ -tubulin levels and were relative to the levels in untreated HCT116 cells. Data are the mean $\pm$ SEM (n = 3).

(B) HCT116 cells were incubated or not with bleomycin for 1 hour, released and collected at different time points, and the relative levels of *CDKN1A*, *HDM2*, *PPM1D* (encoding WIP1) and $\Delta 133TP53$ mRNAs were determined by RT-qPCR. Expression is relative to untreated HCT116 cells. Data are the mean $\pm$ SEM (n = 3); *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, two-way ANOVA with Dunnett's multiple comparison test (vs no treatment; NT).

(C) Correlation between p21 protein and *CDKN1A* mRNA levels in the experiment shown in (Fig. 6A and Supplementary Fig. S6A, B). The red line represents the linear regression.

(D) HCT116 p53^{-/-} cells were incubated or not with bleomycin for 1 hour, released and collected at 3 and 72 hours after bleomycin treatment. The relative levels of $\Delta 133TP53$ mRNA and protein were determined by RT-qPCR and immunoblotting, respectively. Data are the mean $\pm$ SEM (n = 2). NT; no treatment.