

SUPPLEMENTARY DATA

Anillin Recedes in p53-Dependent Senescence of Tumor Cells and Reappears in Cells Escaping from Senescence

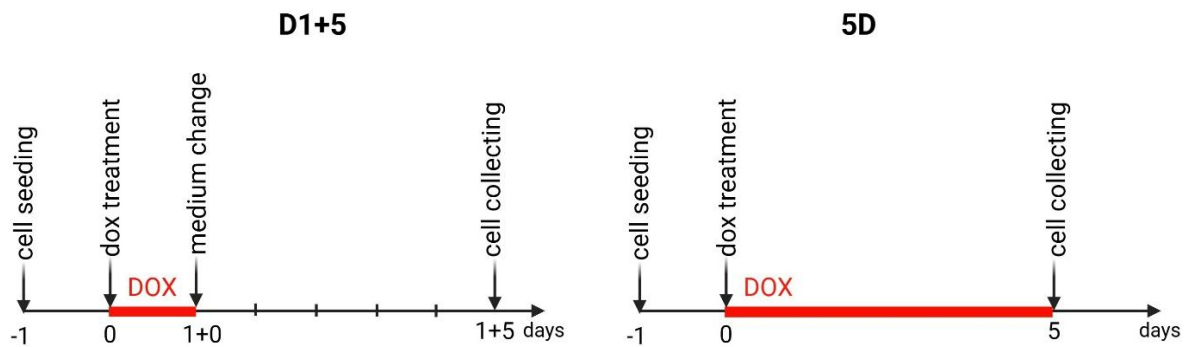
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SUPPLEMENTARY DATA

Supplementary Table

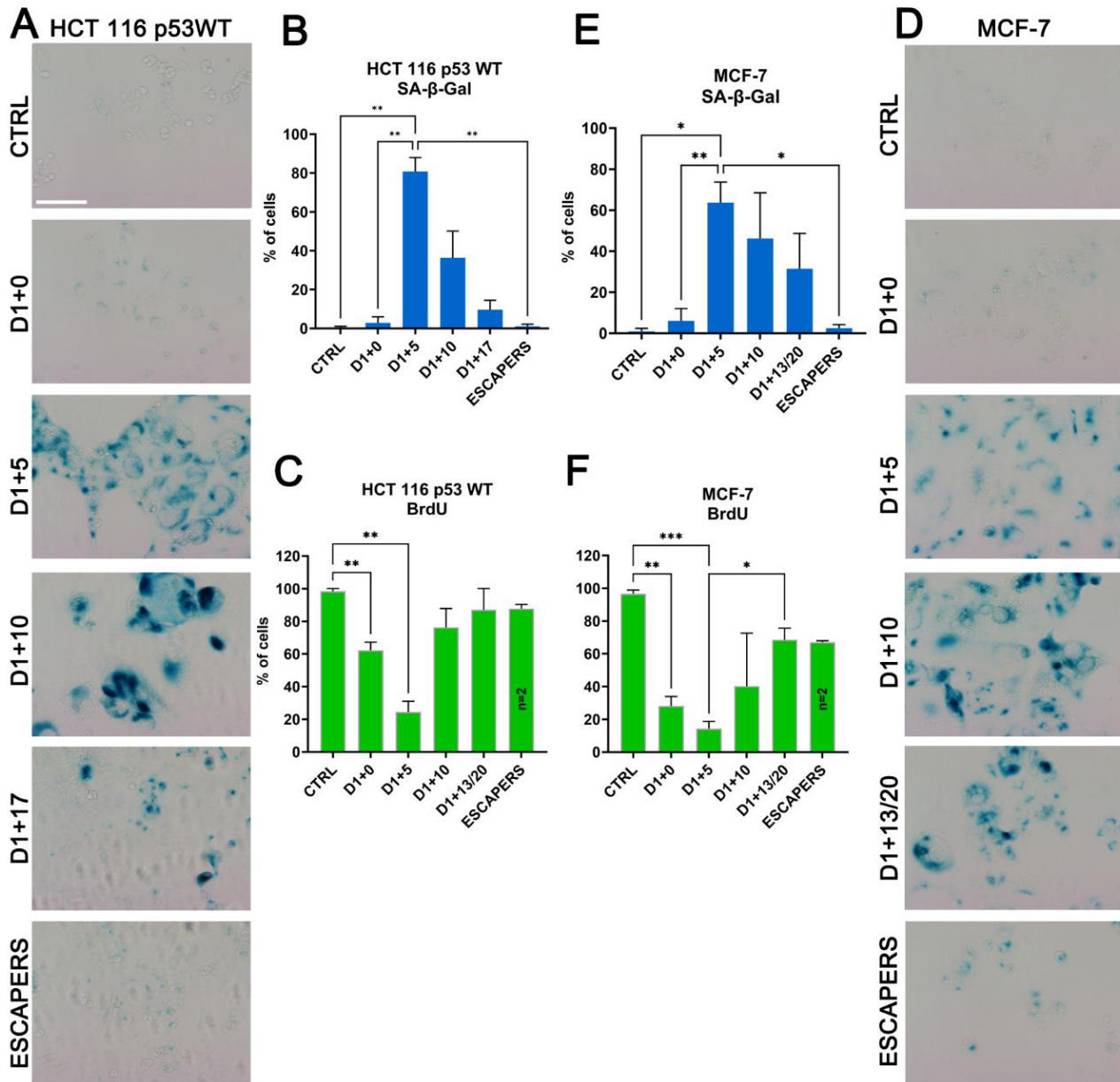
Supplementary Table 1. Comparison of two experimental approaches (D1+5 and 5D) inducing cell senescence. D1+5 – 1-day treatment with doxorubicin and then culture for 5 days in fresh medium. 5D – treatment with doxorubicin for 5 days (diagrams below the table show the experimental kinetics). Statistical analysis was performed using a one-tailed t-Student test. Statistical significance (relative to control): *** $p \leq 0.001$, **** $p \leq 0.0001$

cell types	analysis	D1+5 [% of positive cells]	5D [% of positive cells]
MCF-7	SA- β -Gal	63.75 \pm 9.98 $p=0.0004$ ***	83.98 \pm 7.84 $p<0.0001$ ****
	BrdU	14.30 \pm 4.37 $p<0.0001$ ****	8.30 \pm 0.10 $p<0.0001$ ****
HCT116 p53WT	SA- β -Gal	80.75 \pm 7.18 $p<0.0001$ ****	61.92 \pm 4.58 $p<0.0001$ ****
	BrdU	24.45 \pm 6.61 $p=0.001$ ***	14.86 \pm 3.70 $p<0.0001$ ****
HCT116 p53KO	SA- β -Gal	36.49 \pm 12.01 $p=0.065$ NS	47.23 \pm 7.23 $p=0.0004$ ***
	BrdU	50.10 \pm 6.01 $p=0.0003$ ***	43.41 \pm 4.63 $p<0.0001$ ****



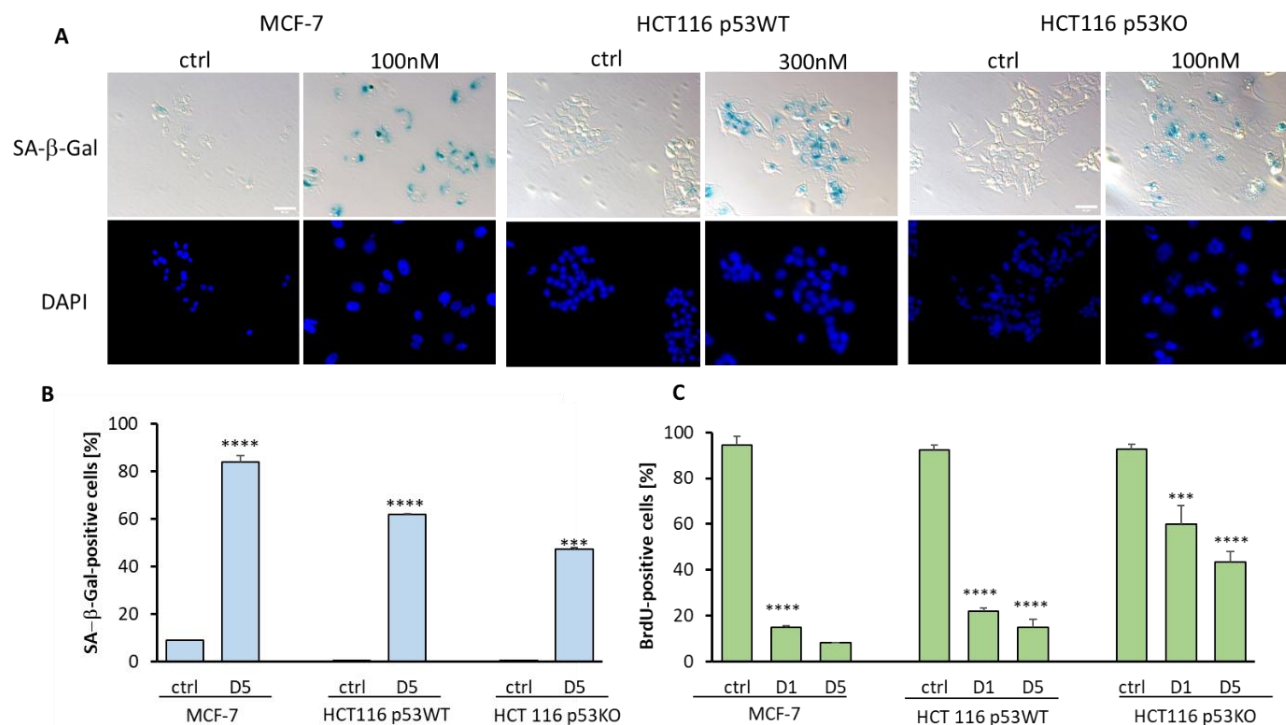
SUPPLEMENTARY DATA

Supplementary Figures



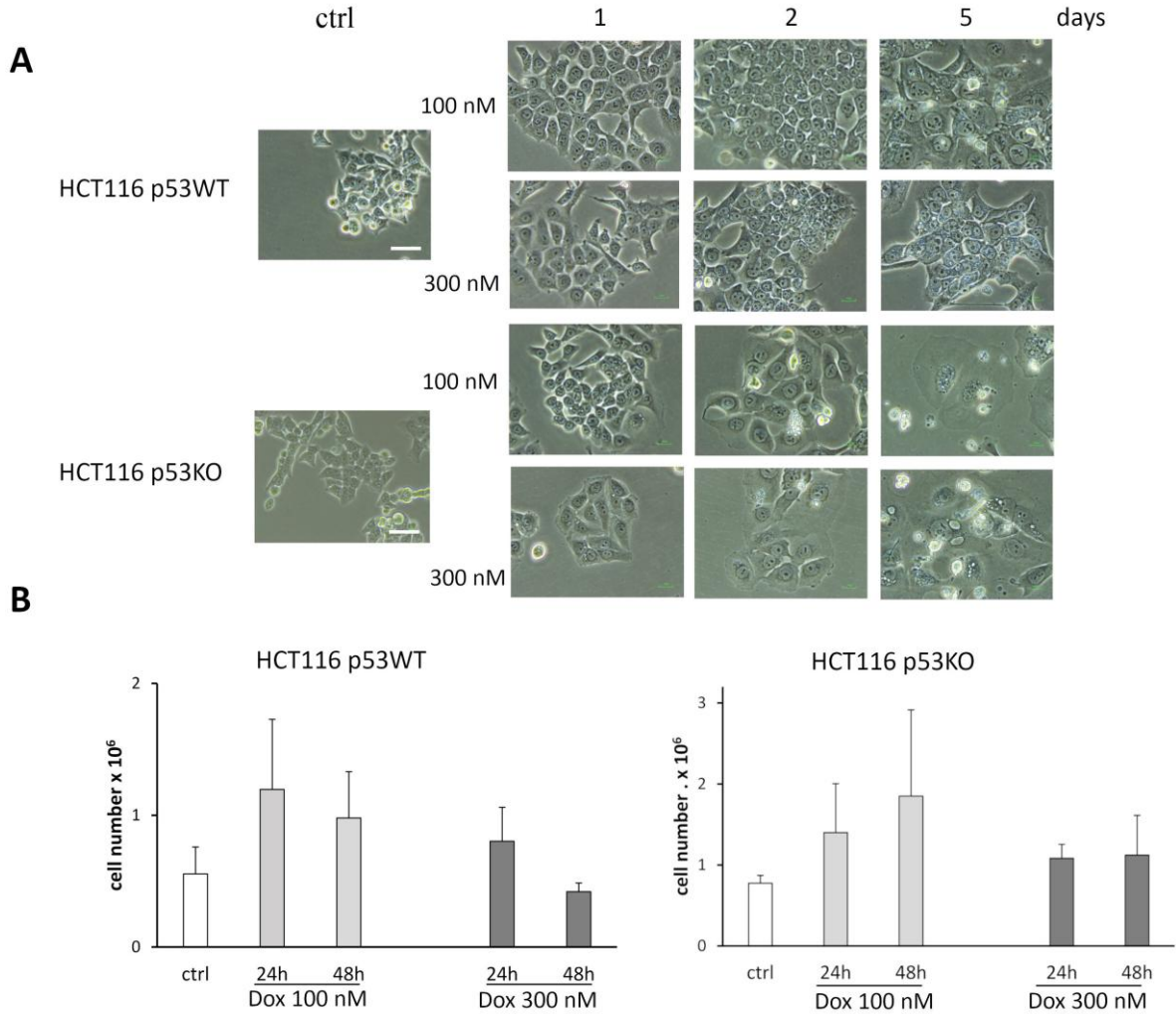
Supplementary Figure 1. Senescence efficiency assessment in HCT p53WT and MCF-7 cells treated for 1 day with doxorubicin (100 and 300 nM, respectively) and then cultured in fresh medium (see Fig. 1). The activity of SA-β-Gal and BrdU incorporation during senescence and the escape from senescence. (A) Representative phase contrast images (blue staining represents SA-β-Gal activity) in HCT116 p53WT cells. (B) Quantitative analysis of the number of SA-β-Gal-positive HCT116 p53WT cells. (C) Quantitative analysis of the number of BrdU-positive HCT116 p53WT cells. (D) Representative phase contrast images (blue staining represents SA-β-Gal activity) in MCF-7 cells. (E) Quantitative analysis of the number of SA-β-Gal -positive MCF-7 cells. (F) Quantitative analysis of the number of BrdU-positive MCF-7 cells. Data were calculated as the percentage of the total cell population and presented as means ± SD. n=4 except when indicated otherwise. Statistical analysis was performed using one-way ANOVA followed by post hoc analysis (Tukey's honest significant difference test; HSD test). Statistical significance of differences between subsequent days of treatment: * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001. Scale – 100 μm

SUPPLEMENTARY DATA

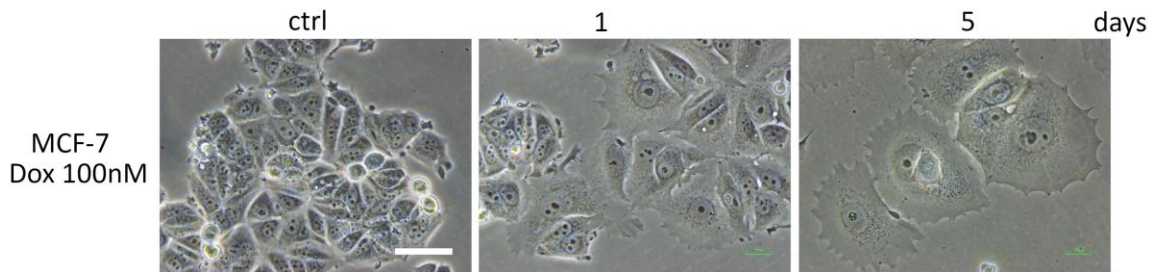


Supplementary Figure 2. Senescence efficiency assessment in MCF-7, HCT p53WT and HCT p53KO cells treated 5 days with doxorubicin (100, 300, 100 nM, respectively). Analysis of the activity of SA-β-Gal and BrdU incorporation during senescence induction. **(A)** Representative phase contrast (blue staining represents SA-β-Gal activity) and fluorescent microscope (nuclei were stained with DAPI) images of control and treated cells. **(B)** Quantitative analysis of the number of SA-β-Gal -positive cells. **(C)** Quantitative analysis of the number of BrdU-positive cells. Data were calculated as the percentage of the total cell population and presented as means \pm SD. n=3; statistical analysis was performed using a paired one-tailed t-Student test. Statistical significance relative to control: *** $p \leq 0.001$, **** $p \leq 0.0001$. Scale – 50 μ m.

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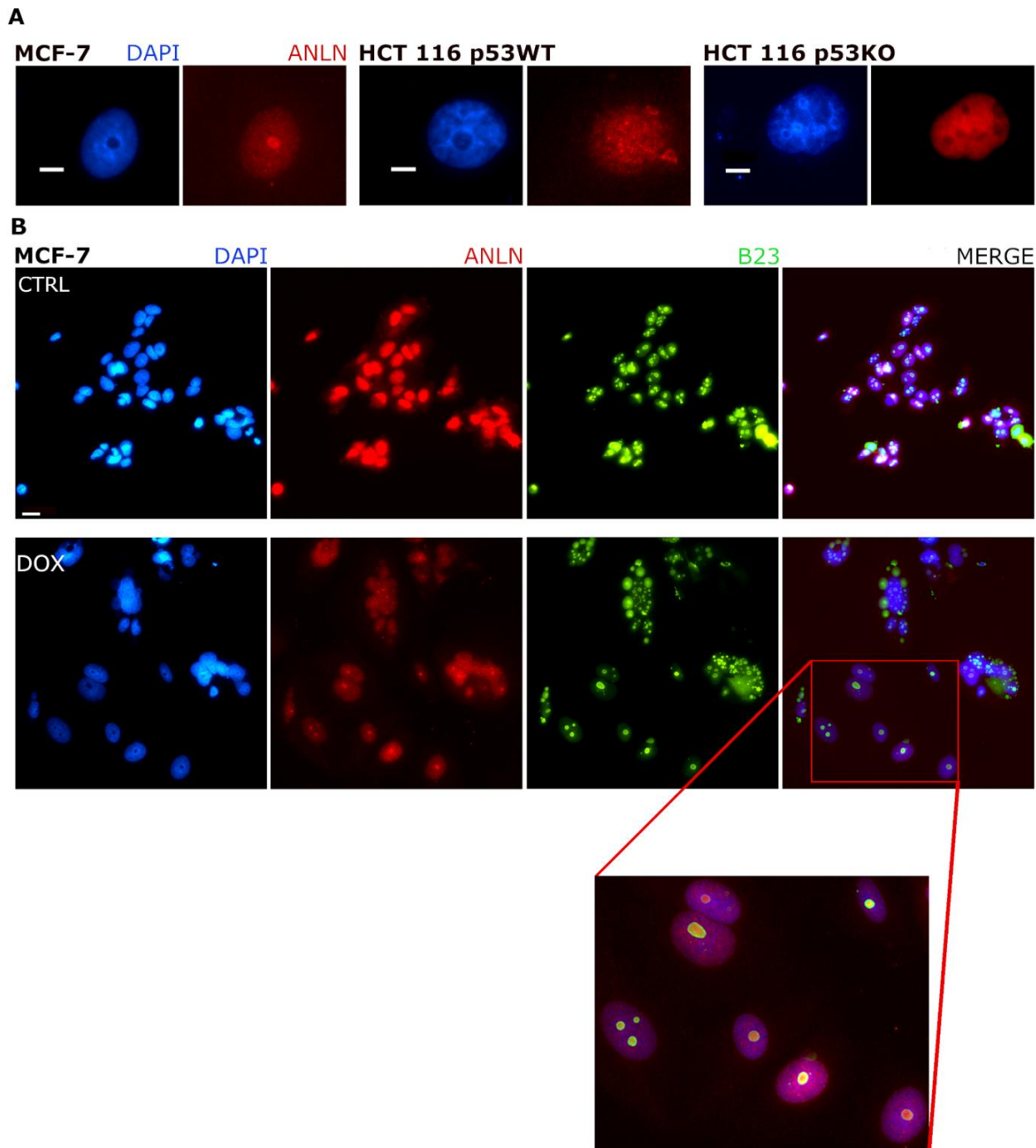


Supplementary Figure 3. Selection of senescence-inducing concentration of doxorubicin (100 vs. 300 nM, permanent treatment without changing the medium) in HCT116 p53WT and HCT116 p53KO cells. Analysis of short-time treatment -24 and 48 hours. **(B)** cell counting **(A)** morphological changes – representative phase contrast images. No statistical significance. n=3; statistical analysis was performed using a paired one-tailed t-Student test. Scale – 50 μ m. For HCT116 p53KO cells, the 100 nM concentration was efficient in stopping proliferation and inducing morphological changes; 300 nM concentration induced cell death rather than senescence, as evidenced by decreased number of cells **(B)** and the presence of floating cells in the culture **(A)**. In the case of HCT116 p53WT, 100 nM doxorubicin did not stop proliferation **(B)**; therefore, 300 nM concentration was used.



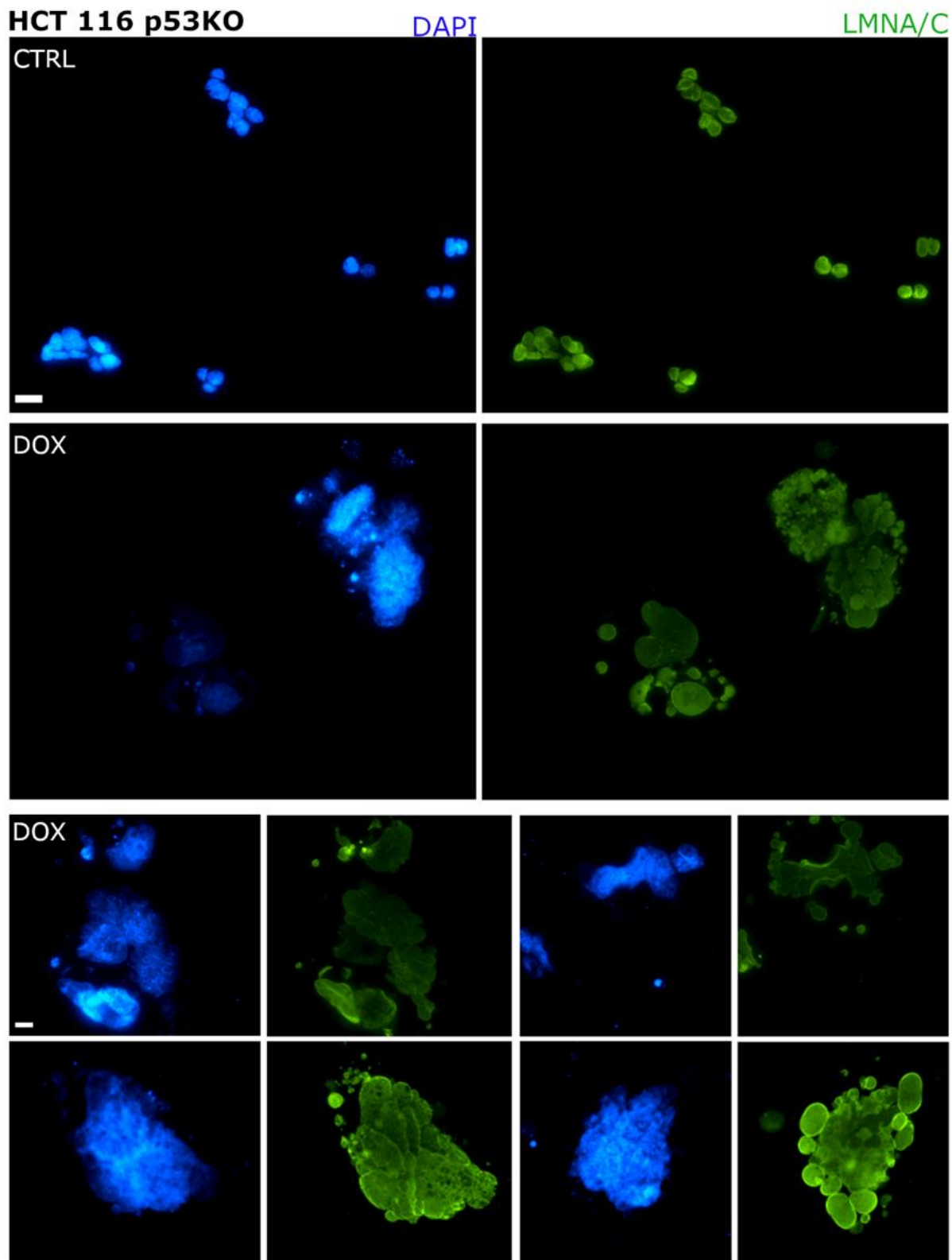
Supplementary Figure 4. Morphological changes in MCF-7 cells induced to senesce with doxorubicin treatment (100 nM, permanent treatment without changing the medium). Representative phase contrast images. Scale – 50 μ m.

SUPPLEMENTARY DATA



Supplementary Figure 5. Detection of anillin in the nucleoli of senescent cells (cells treated for 1 day with doxorubicin and then cultured in a fresh medium, D1+5). (A) representative images of MCF-7, HCT116 p53WT and HCT116 p53KO cells. Scale 10 μ m (B) MCF-7 cells. Scale 20 μ m. Blue – DAPI stained nucleus, red – anillin, green – B23 (nucleophosmin, a nucleolus marker)

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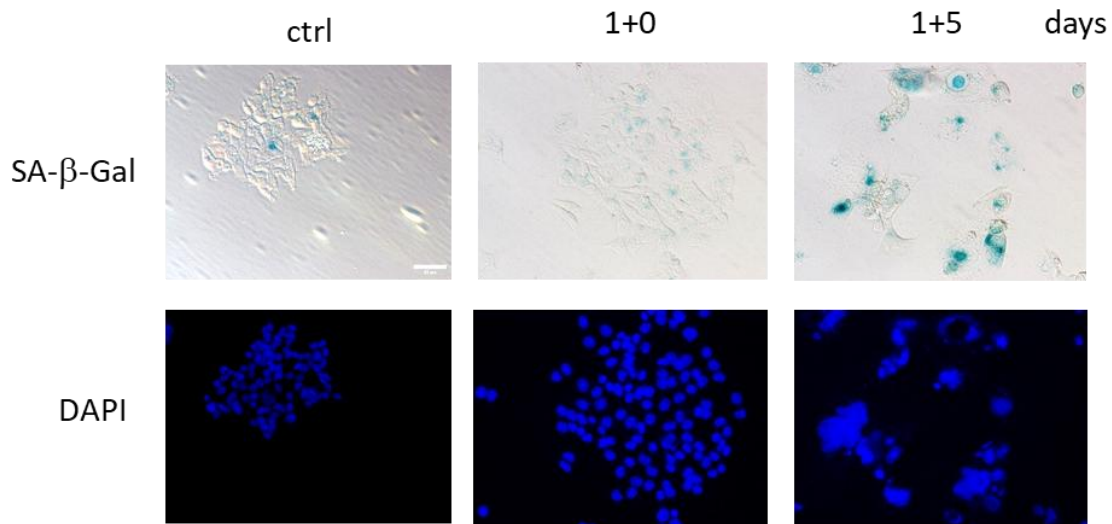


Supplementary Figure 6. Nucleus deformation in HCT116 p53KO cells treated with 100 nM doxorubicin for 1 day and analyzed after 5 days of culture in a fresh medium. Representative fluorescent microscope images of control and treated cells. Green – lamin A/C, blue – DAPI stained DNA. Scale – 20 μ m

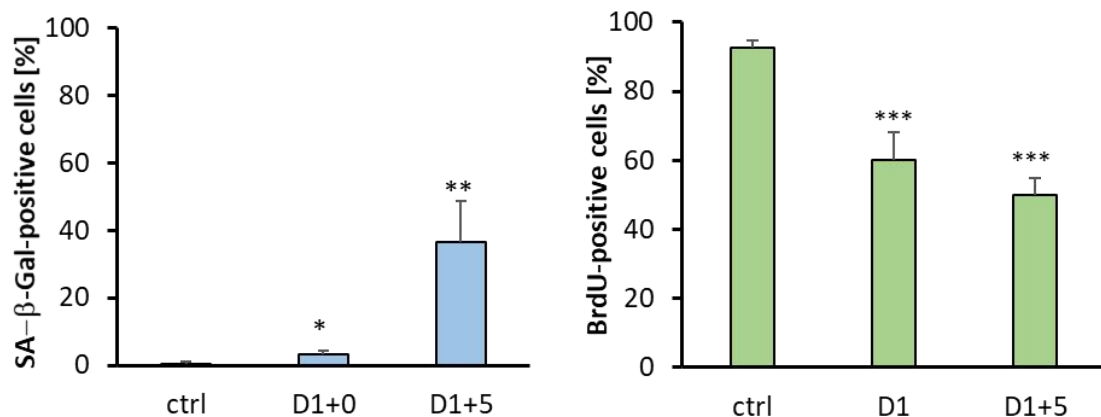
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HCT116 p53KO

A



B



Supplementary Figure 7. Senescence efficiency assessment in HCT116 p53KO cells, treated for 1 day with doxorubicin (100 nM) and then cultured in fresh medium, based on SA-β-Gal activity and BrdU incorporation. **(A)** Representative phase contrast (blue staining represents SA-β-Gal activity) and fluorescent microscope (nuclei were stained with DAPI) images of control and treated cells. **(B)** Quantitative analysis of the number of SA-β-Gal -positive cells. **(C)** Quantitative analysis of the number of BrdU-positive cells. Data were calculated as the percentage of the total cell population and presented as means \pm SD. n=3; statistical analysis was performed using a paired one-tailed t-Student test. Statistical significance relative to control: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Scale – 50 μ m.