Supplementary Figures S1-S10
Supplementary Tables S1-S2

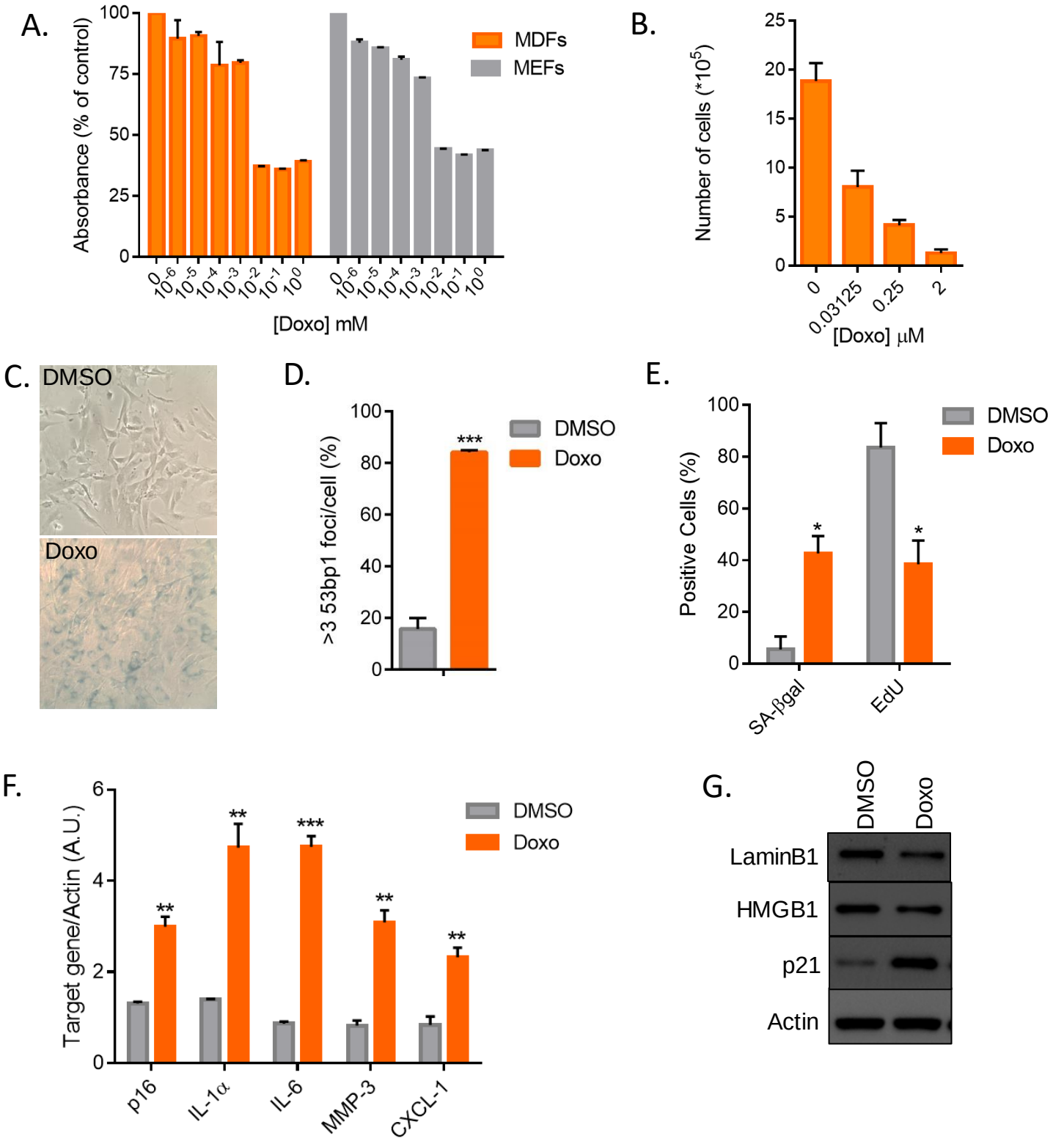


FIGURE S1. (A) Mouse dermal fibroblasts (MDFs) and Mouse embryonic fibroblasts (MEFs) were treated with the indicated concentrations of Doxo for 24 hours. 3 days later, cells were evaluated for viability using a MTS assay. The graph reports the absorbance of each sample as a percentage of control. N=3 independent experiments. (B) $5*10^5$ MDFs were treated with 250 nM Dox for 24 hours. 3 days later, cells were counted. N=3 independent experiments. (C) MDFs treated with 250 nM Doxo (right picture) or control (DMSO, left) were fixed and stained for SA-β-Gal. (D) Quantification of immunofluorescence of control- or Doxo-treated cells. Bars show the percentage of cells with more than 3 53BP1+ foci. (E-G) HCA2 cells were treated with DMSO or 250 nM of Doxo and analyzed 7 days later . (E) Cells were either fixed and stained for SA-β-Gal or incubated for 24 hours with EdU, fixed and stained. Shown is the percentage of positive cells relative to the total number of cells. N=3 independent experiments. (F) Quantitative real-time PCR (qRT-PCR) analysis of RNA. RNA was analyzed for mRNA levels of the indicated endogenous genes relative to actin mRNA (control for cDNA quantity). N=3 independent experiments. A.U.=arbitrary units. (G) LaminB1, HMGB1 and p21 protein levels were measured by immunoblotting. Actin served as a loading control. Data are shown as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

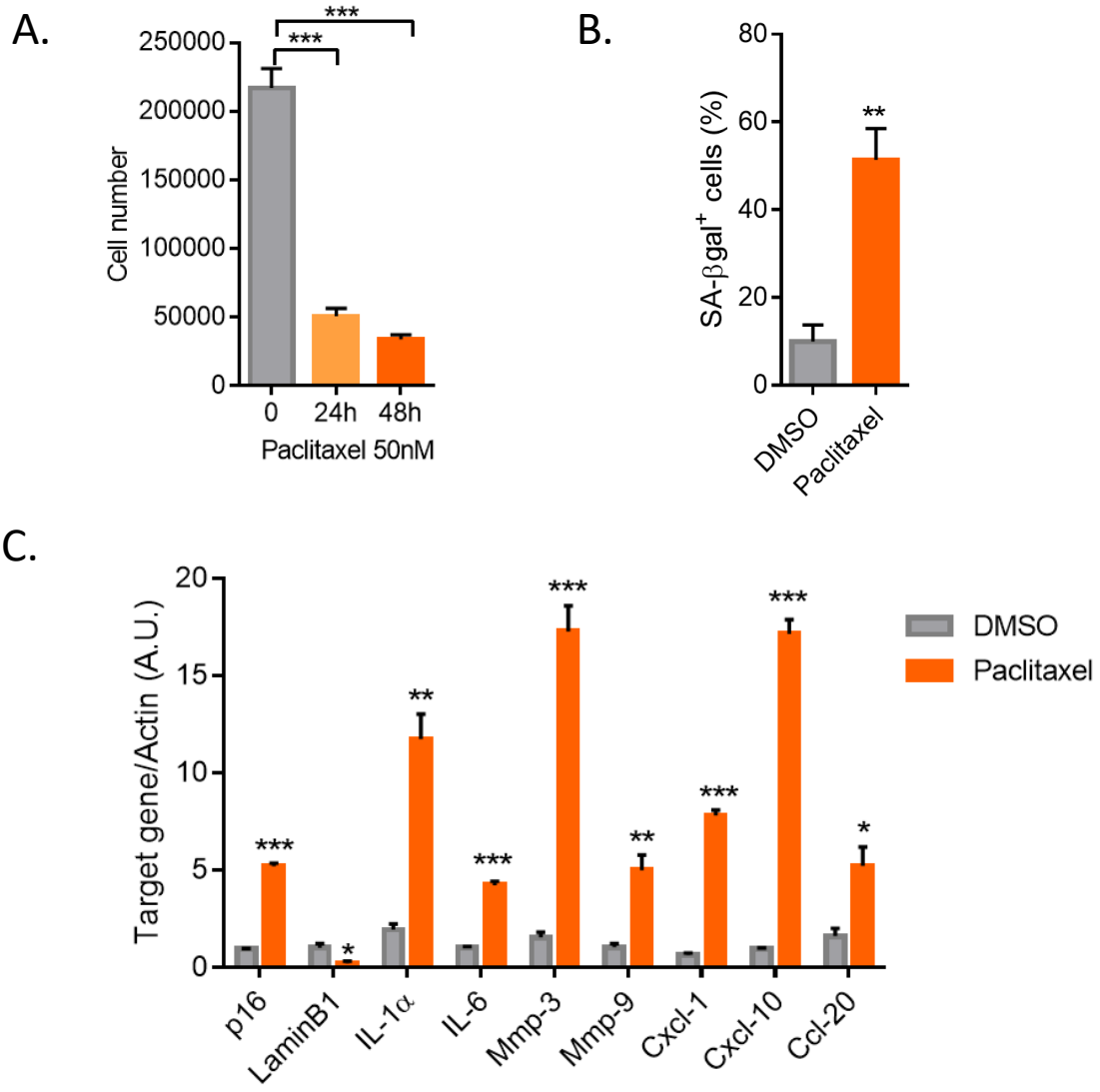


FIGURE S2. (A) Mouse dermal fibroblasts (MDFs) were treated with 50 nM Paclitaxel for 24 or 48 hours. 2 days later, cells were counted. N=3 independent experiments. (B) MDFs were treated with 50 nM Paclitaxel for 48 hours and stained for SA-β-Gal 7 days later. Shown is the percentage of positive cells relative to the total number of cells. N=3 independent experiments. (C) Quantitative real-time PCR (qRT-PCR) analysis of RNA isolated from control and Paclitaxel-treated (50 nM, 48 hours) MDFs. RNA was analyzed for mRNA levels of the indicated endogenous genes relative to actin mRNA (control for cDNA quantity). N=3 independent experiments. A.U.=arbitrary units. Data are shown as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

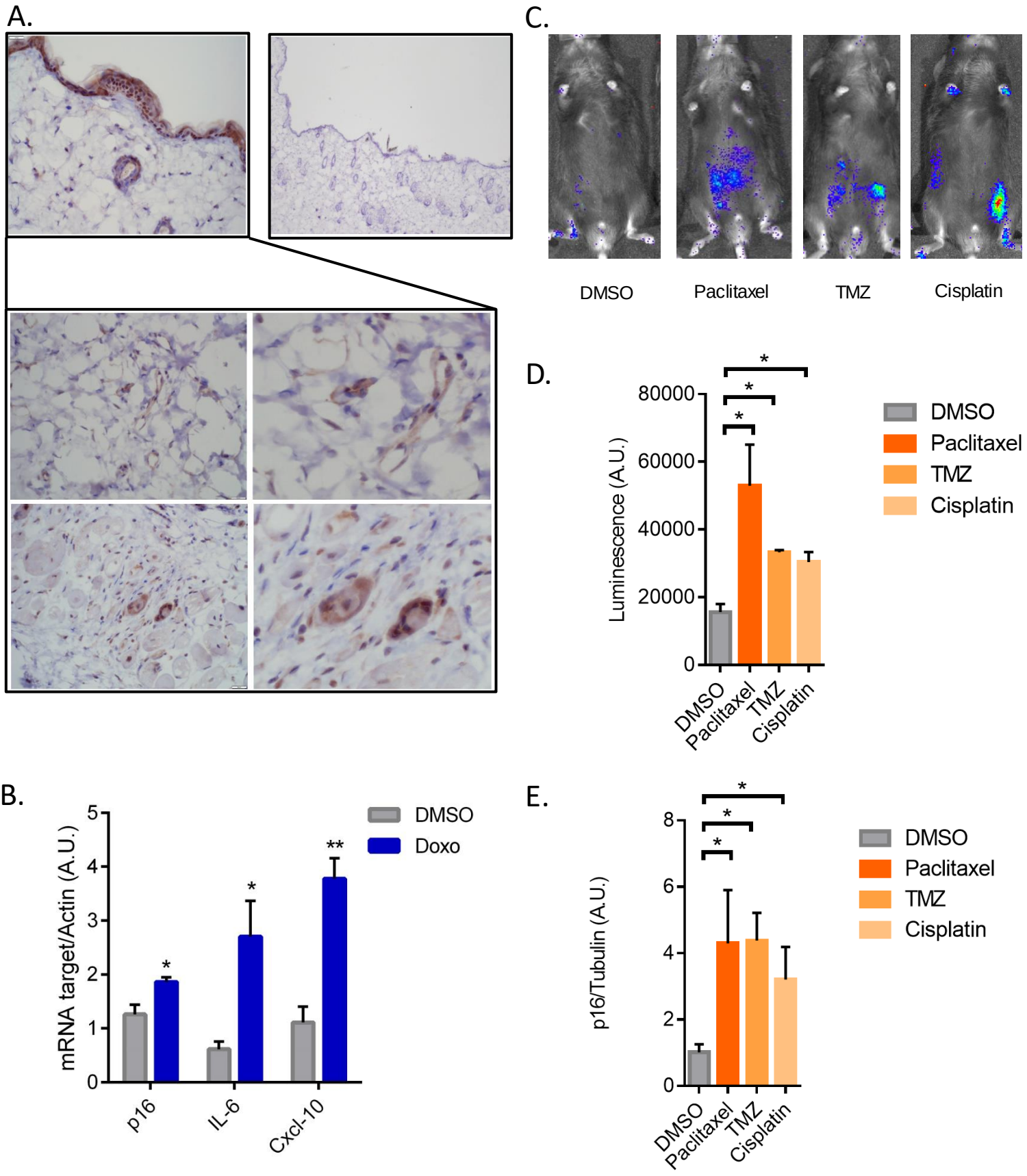


FIGURE S3. (A) Immunohistochemistry was performed on Doxo-treated skin using a p21 antibody (top left panel, 4X magnification) and non-treated skin as control (top right panel, 4X). In Doxo-treated animals, p21 nuclear staining is evident in keratinocytes (top panel), endothelial cells (middle panels, 10X and 20X) and fibroblasts/smooth muscle cells (bottom panels, 10X and 20X). (B) RNA was extracted from the liver of control- or Doxo- (10 mg/kg) treated mice 3 weeks after treatment, and quantified by qRT-PCR for mRNA encoding $p16^{Ink4a}$, IL-6 and Cxcl-10. mRNA encoding tubulin was used as a control. N=5. (C-D) p16-3MR mice, 10 days after treatment with Paclitaxel (3x10 mg/kg), Temolozomide (TMZ, 3x10 mg/kg) or Cisplatin (3x2.3 mg/kg) were injected with coelentarazine, and luminescence was quantified using a Xenogen Imaging system. (C) Representative image. (D) Quantification of total luminescence. A.U. = Arbitrary Units. N=4. (E) RNA was extracted from the skin and lung of control or Paclitaxel-treated (3x10 mg/kg) mice and quantified by qRT-PCR for mRNA levels of endogenous $p16^{Ink4a}$. Tubulin mRNA was used as a control. N=4. Data are shown as mean ± SEM. *p≤0.05; **p≤0.01

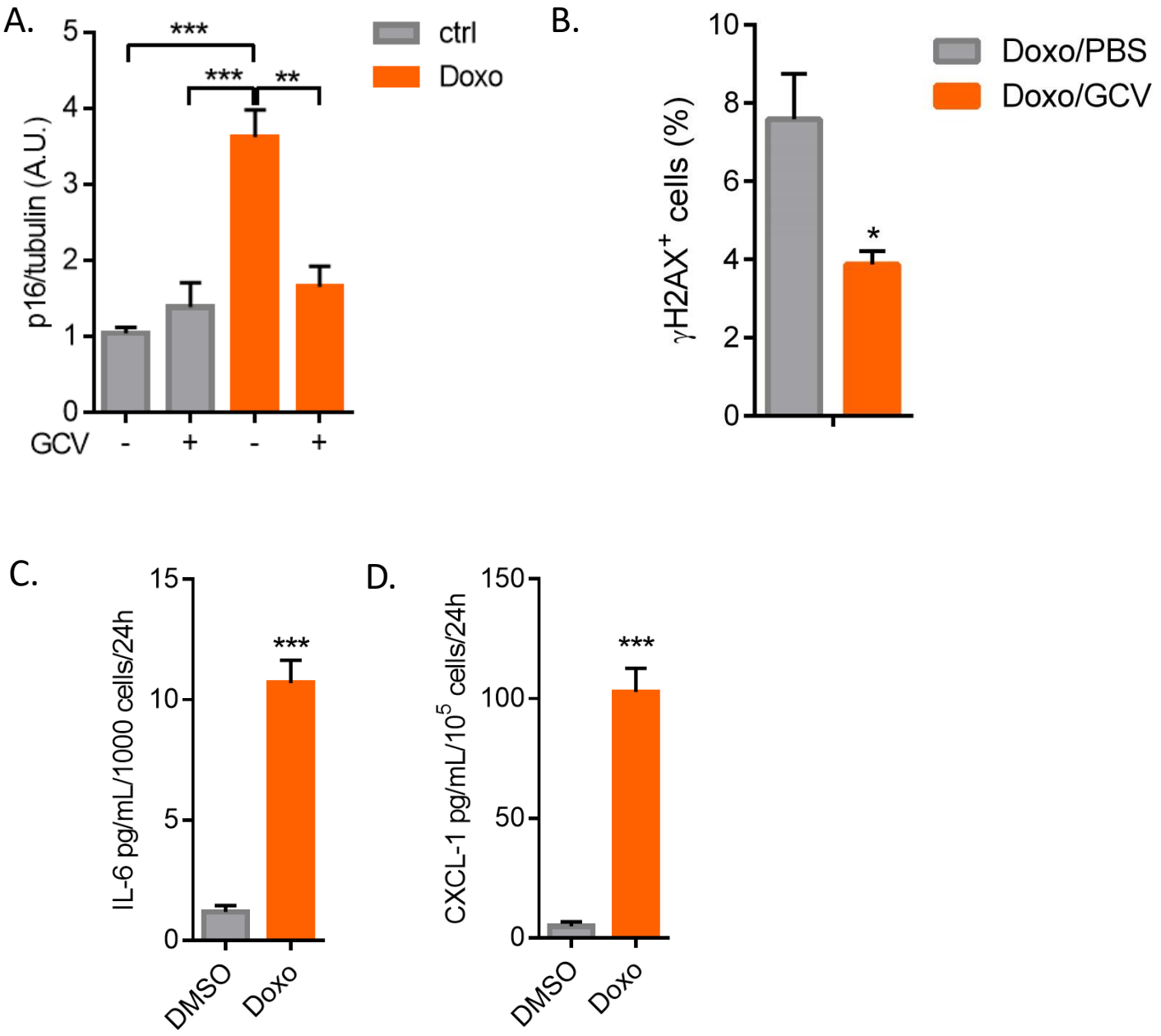


FIGURE S4. (A) RNA was extracted from the skin of control or Doxo-treated (10 mg/kg) p16-3MR mice after GCV (5x25 mg/kg) or vehicle (PBS) administration and quantified by qRT-PCR for mRNA levels of endogenous $p16^{Ink4a}$. Tubulin mRNA was used as a control. N=4. (B) Lungs were fixed in paraffin and stained for γH2AX. Bars show the percentage of positive cells relative to DAPI staining. N=4. (C-D) MEFs were treated with Doxo or PBS (ctrl) for 24 hours. 7 d after irradiation, conditioned media were collected and analyzed for IL-6 and CXCL-1 secreted proteins by ELISA. N=4. Data are shown as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

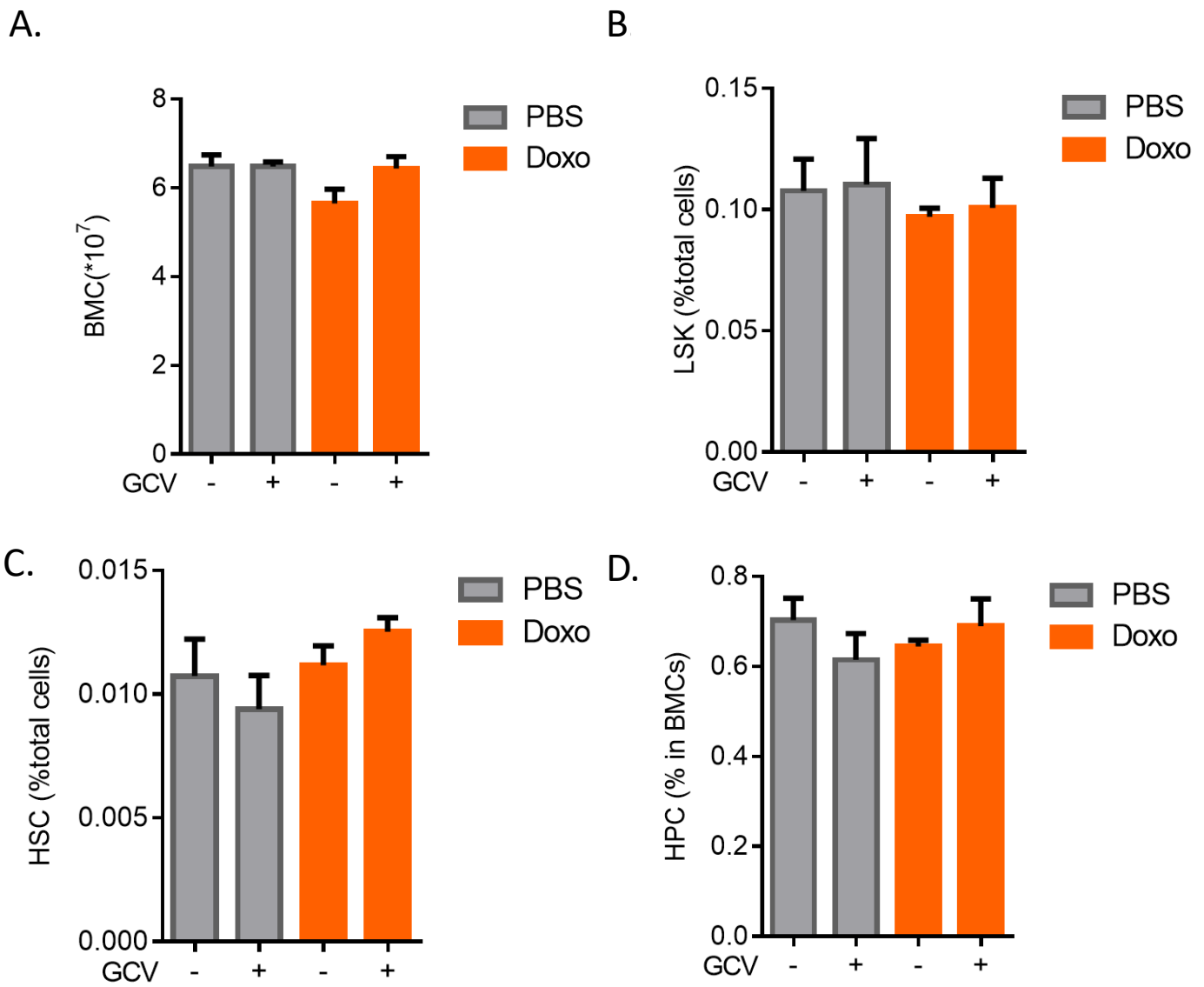


FIGURE S5. (A) Control or Doxo-treated p16-3MR mice (10 mg/kg) were treated with vehicle (PBS) or 25 mg/kg of GCV for 5 days (daily i.p. injections; GCV). Bone marrow cells (BMCs) were harvested from the mice 3 days after the last PBS or GCV treatment. (A) The number of BMCs in the two hind legs of the mice. (B-D) The frequency of lineage-Sca1+c-kit+ cells (LSK cells), hematopoietic stem cells (HSCs, CD150+CD48-LSK cells), and hematopoietic progenitor cells (lineage-Sca1-c-kit+ cells) in BMCs. N=3. Data are shown as mean ± SEM.

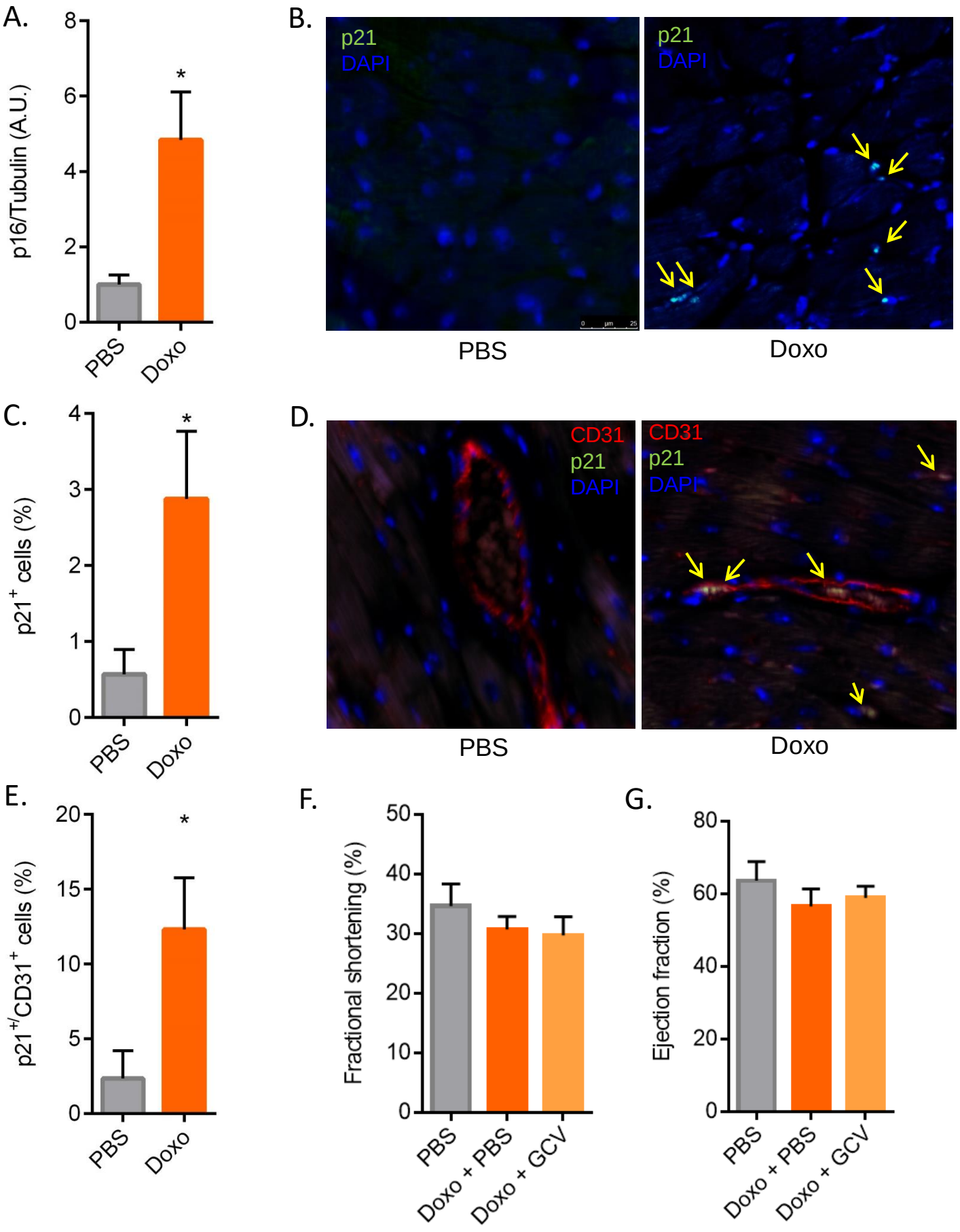


FIGURE S6. (A) RNA was extracted from the heart of control- or Doxo- (10 mg/kg) treated mice 4 weeks after treatment, and quantified by qRT-PCR for mRNA encoding $p16^{Ink4a}$. mRNA encoding tubulin was used as a control. (B) Heart sections from control- or Doxo- (10 mg/kg) treated mice 4 weeks after treatment were stained with an antibody against p21 (green), and counterstained with DAPI (blue). Cells with nuclear p21 staining are indicated by yellow arrows. (C) Quantification of p21+ cells (N=5). (D) Heart sections from control- or Doxo- (10 mg/kg) treated mice 4 weeks after treatment were stained with antibodies against p21 (green), CD31 (red) and counterstained with DAPI (blue). Cells with nuclear p21 staining are indicated by yellow arrows. (E) Quantification of p21+/CD31+ cells (N=5). (F-G) Two-dimensional transthoracic echocardiography was performed in mice 2 weeks after Doxo treatment. Graphs show fractional shortening (F) and ejection fraction (G). N=10. Data are shown as mean ± SEM. *$p<0.05$.

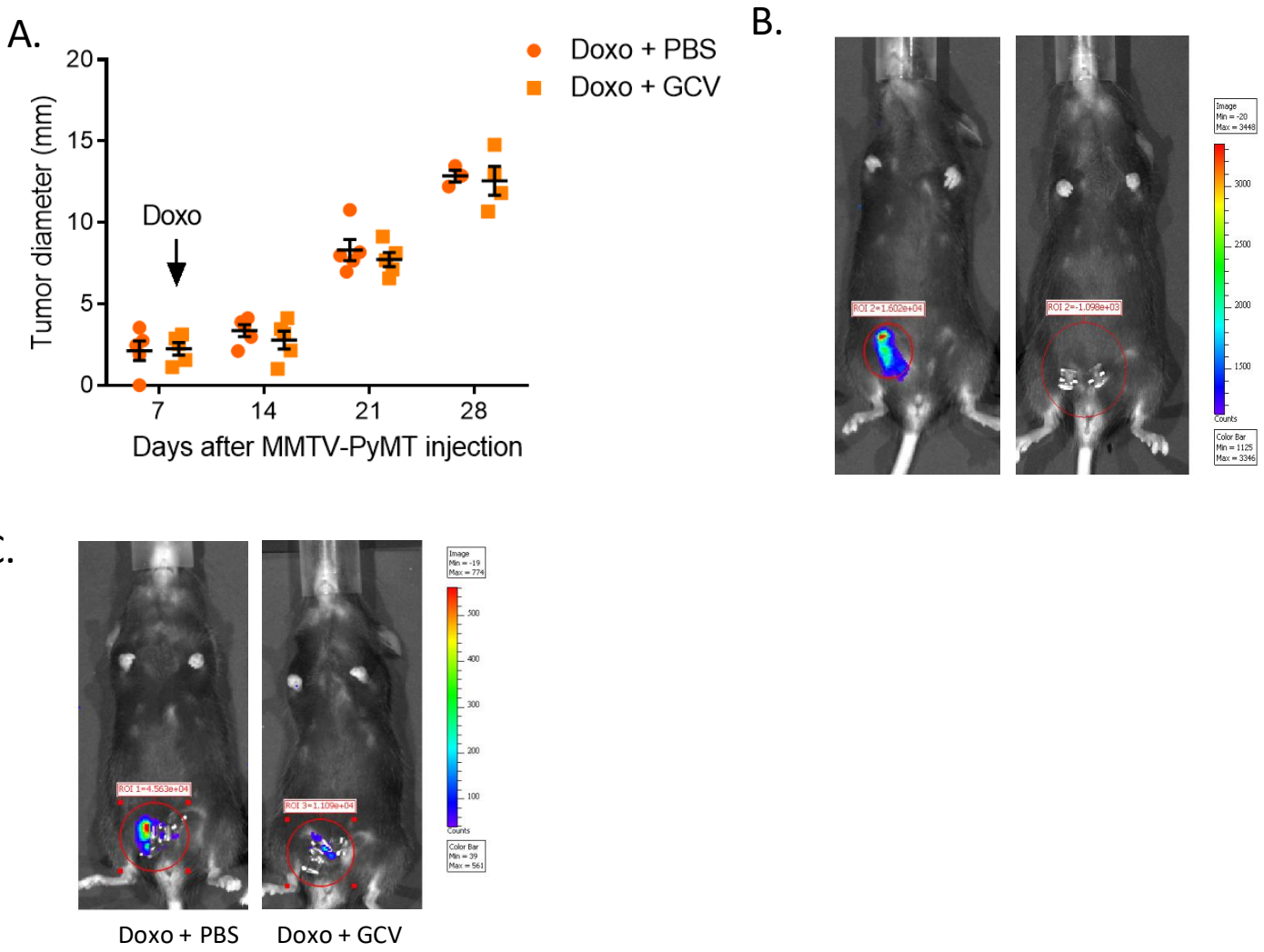
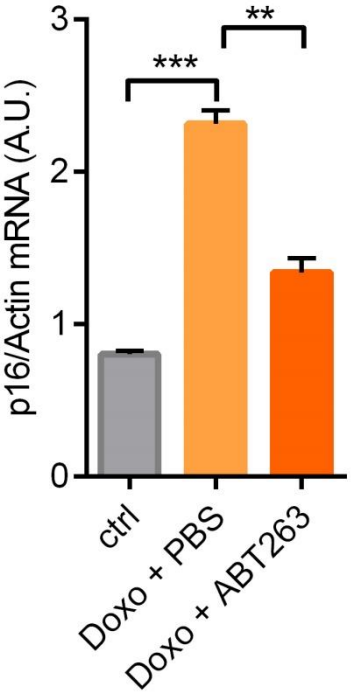


FIGURE S7. (A) fLUC-MMTV-PyMT cells (10^5) were injected in the mammary fat pad of p16-3MR mice and treated with PBS or Doxo (10 mg/kg) and with PBS or 25 mg/kg of GCV for 5 days (daily i.p. injections) 7 days after cancer cell injection. Tumor diameters were measured using a caliper at the indicated time points. (B-C) fLUC-MMTV-PyMT cells (10^5) were injected into the mammary fat pad of p16-3MR mice. 10 days later, primary tumors were surgically removed. Mice were administered D-Luciferin and luminescence measured using the Xenogen imaging system was evaluated prior (left panel) and 2 days after (right panel) the surgery. Luminescence signals identify fLUC-MMTV-PyMT in the mammary gland (B). Mice were then treated with Doxo (10 mg/kg) and with PBS or 25 mg/kg of GCV for 5 days (daily i.p. injections). Mice were administered D-Luciferin and luminescence measured using the Xenogen system 2 weeks after the indicated treatments (C). Data are shown as mean ± SEM.

A.



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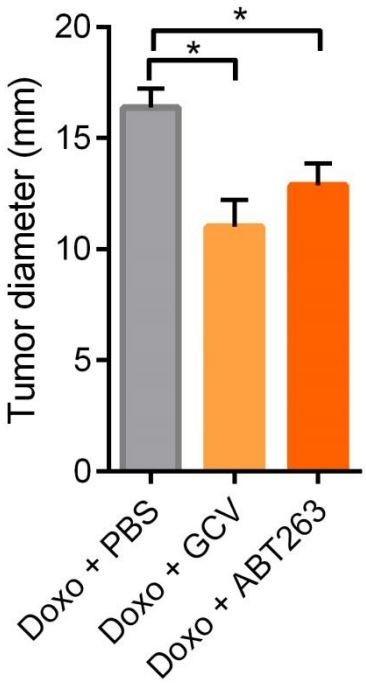

C.

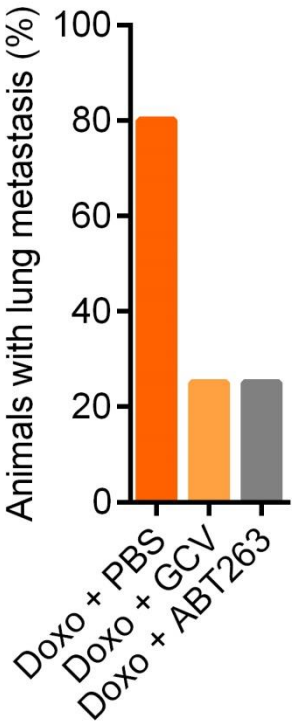

FIGURE S8. (A) Control or Doxo-treated p16-3MR mice (10 mg/kg) were treated with vehicle (PBS), 25 mg/kg of GCV for 5 days (daily i.p. injections; GCV), or 50 mg/kg ABT-263 for 7days (daily i.p. injections; ABT263). 2 weeks after Doxo treatment, RNA was extracted from the skin and quantified by qRT-PCR for mRNA levels of endogenous $p16^{Ink4a}$. Tubulin mRNA was used as a control (N=3). (B-C) fLUC-MMTV-PyMT cells (10^5) were injected into the mammary fat pad of p16-3MR mice. 10 days later, primary tumors were surgically removed. Mice were treated with PBS or Doxo (10 mg/kg) and with PBS, 25 mg/kg of GCV for 5 days (daily i.p. injections) or 50 mg/kg of ABT-263 for 7 days (daily i.p. injections). Tumor diameter (B) was measured using a caliper, and metastasis evaluated by luminescence of fLUC-MMTV-PyMT cells (C) 4 weeks after Doxo treatment. N=4-5. Data are shown as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

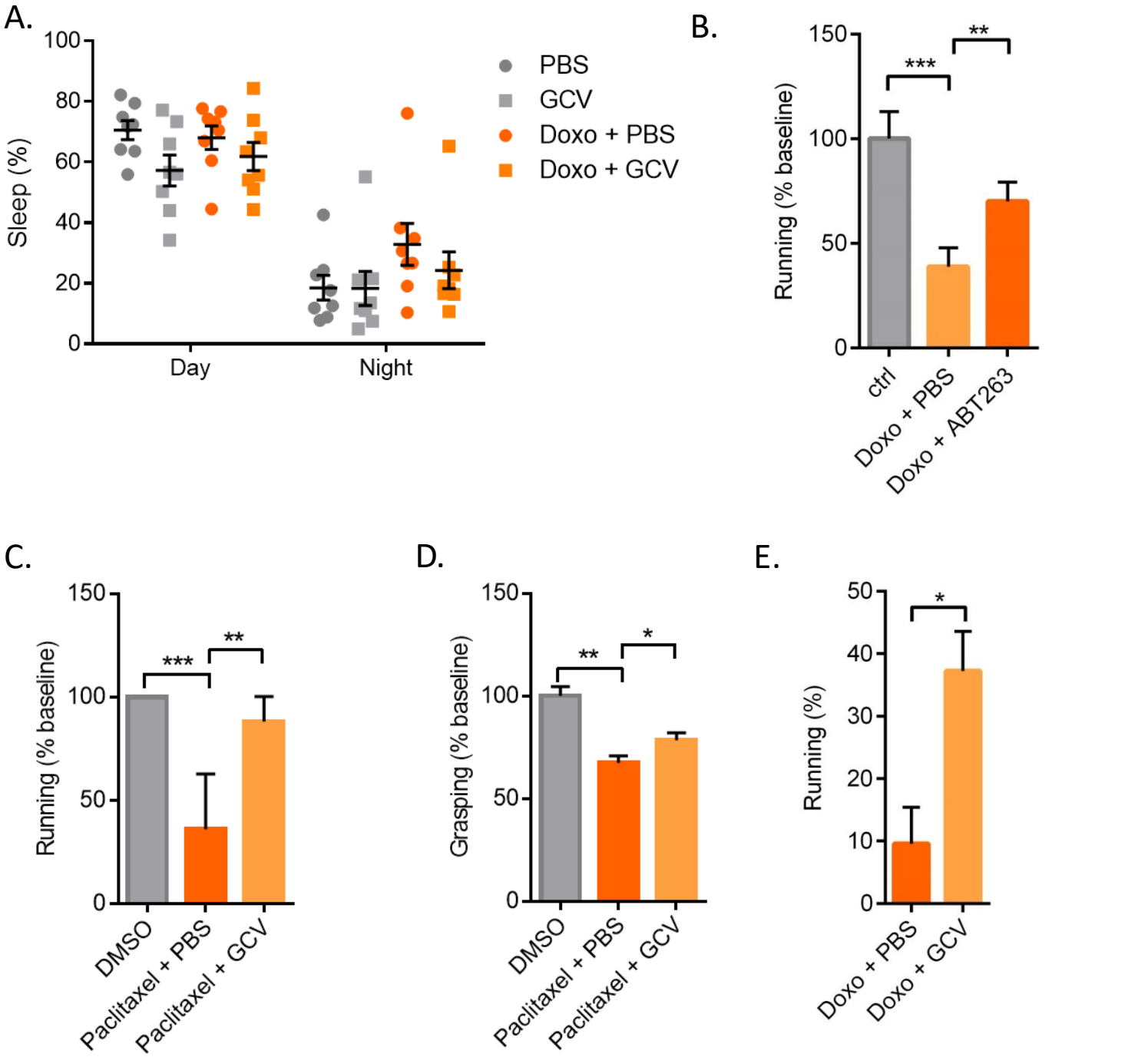

FIGURE S9. (A) Control or Doxo-treated p16-3MR mice (10 mg/kg) were treated with vehicle (PBS) or 25 mg/kg of GCV for 5 days (daily i.p. injections; GCV). Mice were single housed in metabolic cages, and sleep measured for 4 consecutive days. Data are the average of 4 cycles (day or night) and show the percentage of total time spent sleeping. N=8. (B) Control or Doxo-treated p16-3MR mice (10 mg/kg) were treated with vehicle (PBS) or 50 mg/kg ABT-263 for 7days (daily i.p. injections; ABT263). Mice were single housed, and the activity measured using running wheels. Bars show the percentage of time spent on the wheel compared to baseline. N=4.(C-D) Control or Paclitaxel-treated p16-3MR mice were treated with vehicle (PBS) or 25 mg/kg of GCV for 5 days (daily i.p. injections; GCV). (C) Mice were single housed in standard cages equipped with running wheels. The number of revolutions per each group was calculated before and 12 days after the Paclitaxel treatment . For each mouse, values are the average of 3 consecutive nights. The graph shows the ratio between the post- and pre-treatment running distance, expressed as a percentage. N=10. (D) Mice were assessed for time spent grasping a reversed cage grid. For each mouse, values are an average of 5 trials. The graph shows the ratio between the post- and pre-treatment grasping time, expressed as a percentage. N=10. (E) fLUC-MMTV-PyMT cells (10^5) were injected into the mammary fat pad of p16-3MR female mice and treated with PBS or Doxo (10 mg/kg), 7-10 days later, the animals were injected with PBS or 25 mg/kg GCV for 5 days (daily i.p. injections). 3 weeks after injecting fLUC-MMTV-PyMT cells, mice were single-housed in metabolic cages, and activities measured for 4 consecutive days. Shown are the average of 4 nocturnal cycles and the percentage of time spent running. N=5. Data are shown as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

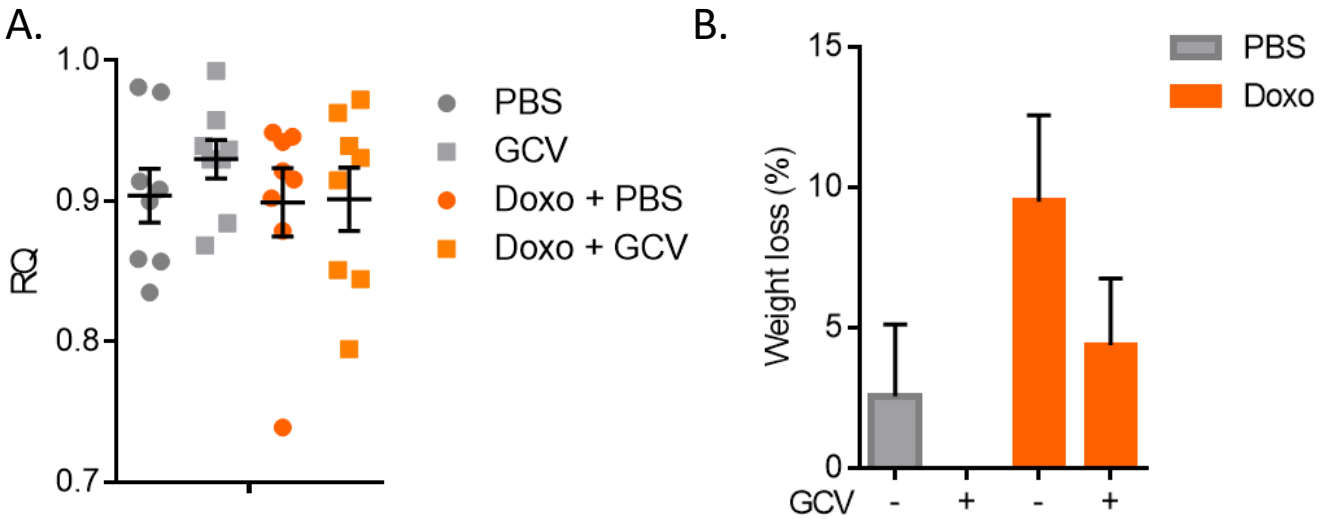

FIGURE S10. Control or Doxo-treated p16-3MR mice (10 mg/kg) were treated with vehicle (PBS) or 25 mg/kg of GCV for 5 days (daily i.p. injections; GCV). (A) Mice were single housed in metabolic cages, and RQ measured for 4 consecutive days. Data are the average of 4 nocturnal cycles. N=8. (B) Mice weights. The graph shows the weight loss 12 days after Doxo and GCV or vehicle treatments expressed in grams. N=8.

Variable	N (%)
Sample Size	89 (100)
Race	
White	68 (76)
A-A	17 (19)
Tumor Type	
ER/PR+	49 (55)
Triple negative	20 (23)
HER2 positive	20 (23)
Chemotherapy	
Neoadjuvant	35 (39)
Adjuvant	54 (61)
Chemo Type	
Anthracycline + Cyclophosphamide (±other)	4 (5)
Anthracycline + Cyclophosphamide+ Taxane (±other)	49 (55)
Taxane + Cyclophosphamide	26 (29)
Taxane (±other)	10 (11)
Her2 targeted	21 (24)
Growth Factors	
Pegfilgrastim	88 (92)
P16 mean(std)	7.53 (1.06)
3.29<= p16 <7.07	22 (25%)
7.07 <= p16 < 7.53	22 (25%)
7.53 <= p16 < 8.14	22 (25%)
8.14<= p16 <=9.64	23 (25%)

SUPPLEMENTARY TABLE S1. Clinical and demographic features of patients in LCCC 1027

Toxicity	Incidence of toxicity, N (%)	Mean p16 with tox	Mean p16 without tox	Wilcoxon p-value*	Relative Risk for highest vs lowest quartile of p16 values, adjusting for age	% with tox in highest vs lowest p16 quartile
Grade 3/4 Fatigue	23/89 (26%)	7.93	7.39	.02	9.63 P=0.03	44% v 5%
Grade 3/4 Neuropathy	9/89 (10%)	7.94	7.49	.11	N/A	9% v 0%
Grade 3/4 Any Non Heme	51/89 (57%)	7.58	7.47	.69	1.27 P=0.44	57% v 46%
Grade 3/4 Any Heme	34/89 (38%)	7.68	7.44	.77	1.09 P=0.85	35% v 27%

SUPPLEMENTARY TABLE S2. $p16^{INK4a}$ expression levels correlate with chemotherapy-induced fatigue in humans. *Report Wilcoxon since some small group sizes (ex. Neuropathy); ** p value from log-binomial model used to estimate relative risk; *** odds ratio reported here as an estimate of relative risk since this is a rare event