SUPPLEMENTARY MATERIAL

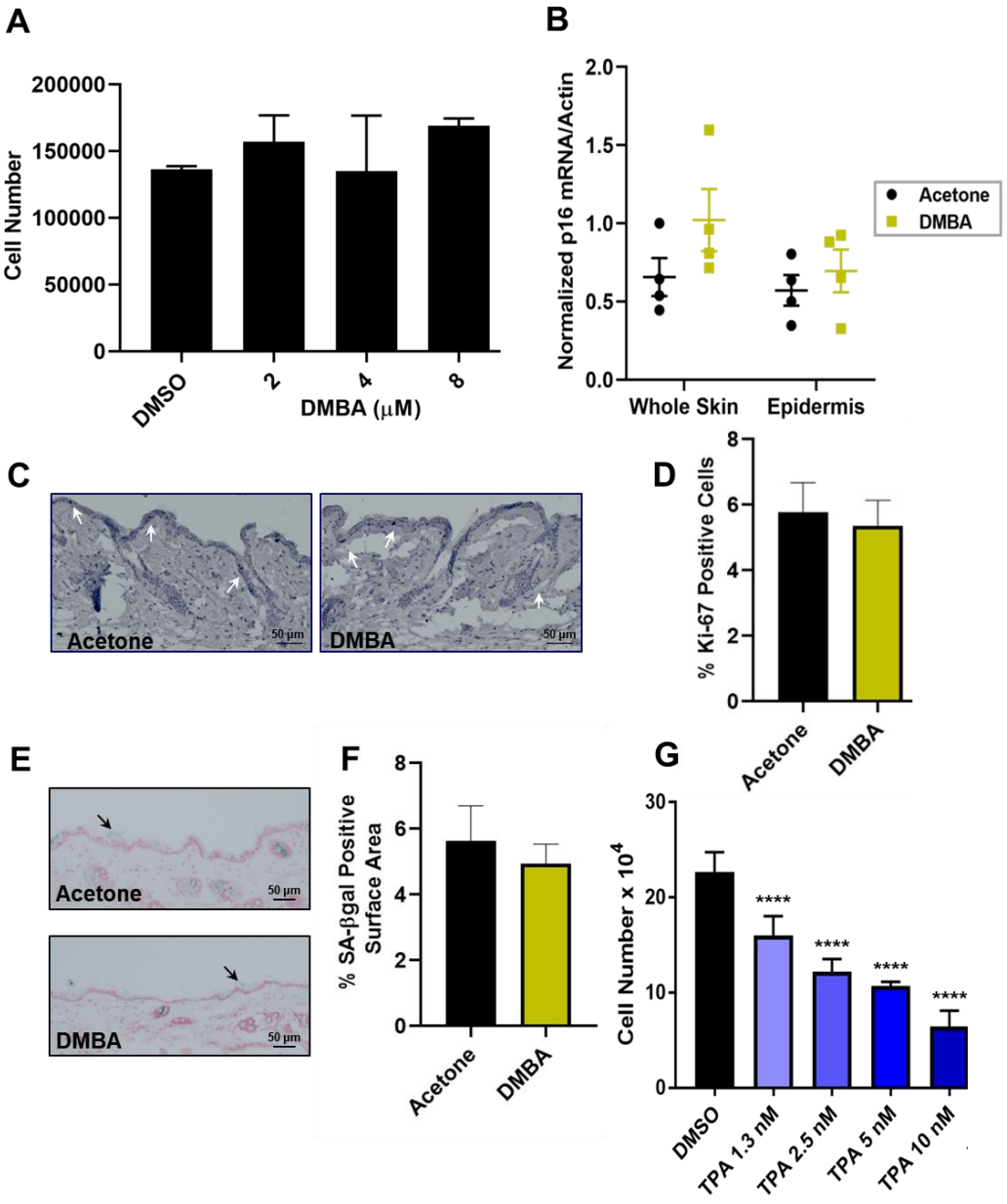

Figure S1. TPA, induces senescence in human keratinocytes and mouse skin

(A) Human keratinocytes were treated with either DMSO or increasing concentrations of DMBA (2-8 µM) for 24 h. Cell counts 10 days after DMBA treatment are shown. (B) The skin of p16-3MR female mice was treated with a single topical application of DMBA or acetone (control). One month later, whole skin or the epidermis was analyzed by qPCR for *p16* mRNA levels. N=4 per treatment group. (C) Ki-67 staining of skin treated with either acetone or DMBA, a representative image is shown. Positive cells are indicated by white arrows. (D) Percentage of Ki-67-positive cells in skin treated with either acetone or DMBA. (E) Acetone- or DMBA-treated skin was stained for SA-ß-gal (blue) and nuclei (red). Representative images are shown (50 µm), and arrows denote SA-ß-gal positive areas (hair follicles stain non-specifically for SA-ß-gal). (F) Percent of SA-ß-gal positive surface area of skin compared to non-stained skin. N=4 per treatment group. (G) Human keratinocytes were treated with DMSO or increasing concentrations (1.3-10 nM) of TPA for 48 h and counted on day 12. Shown are means ±SD, ****p<0.001 (Student t-test). N=3 per treatment group.

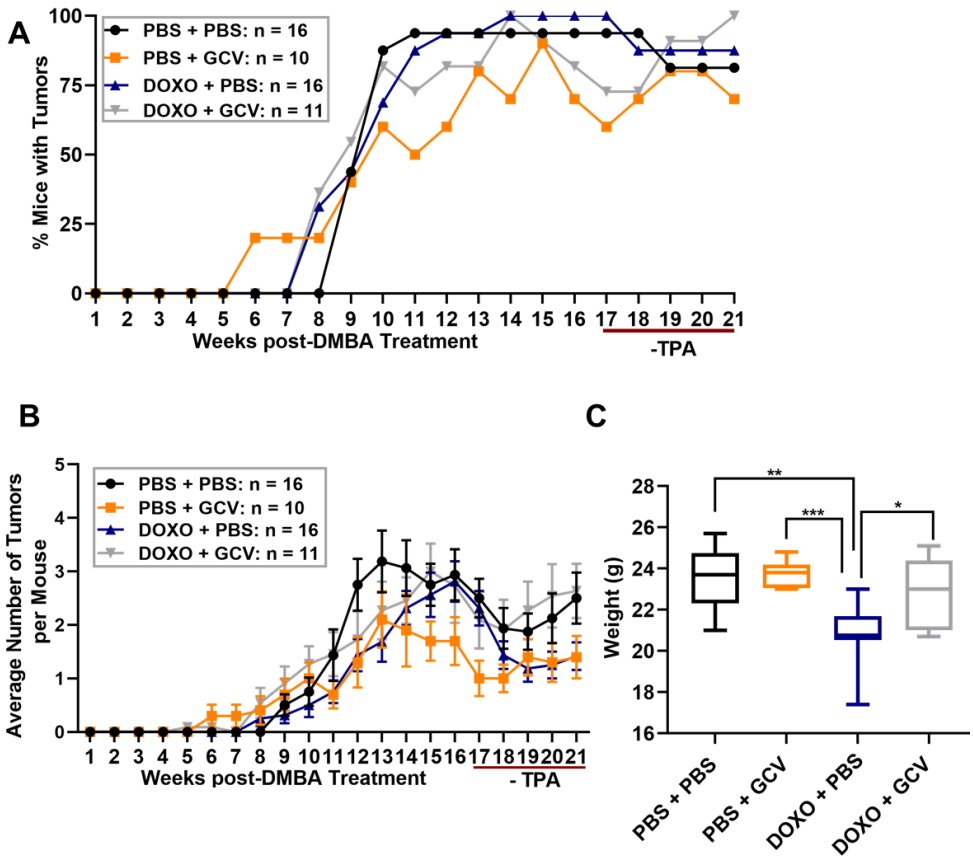

Figure S2. DOXO-induced senescence has no effect on tumor latency or number

(A-B) Senescence was induced in p16-3MR female mice with DOXO followed by elimination of p16-positive senescent cells with GCV (PBS served as control). Subsequently, the two-step skin carcinogenesis protocol was followed. Tumor incidence (A) and number (B) were recorded over 21 weeks for PBS + PBS, PBS +GCV, DOXO + PBS, DOXO + GCV groups. Percent tumor incidence and average number of tumors over time is shown. (C) The weight of (PBS + PBS: n=9, PBS + GCV: n=9, DOXO + PBS: n=9, DOXO + GCV: n=11) treated mice is shown 21 weeks after DMBA treatment. Shown are means ±SEM; *p<0.05, **p<0.01, ***p<0. 001 (one-way ANOVA, Sidak's multiple comparisons test was used post-analyses).

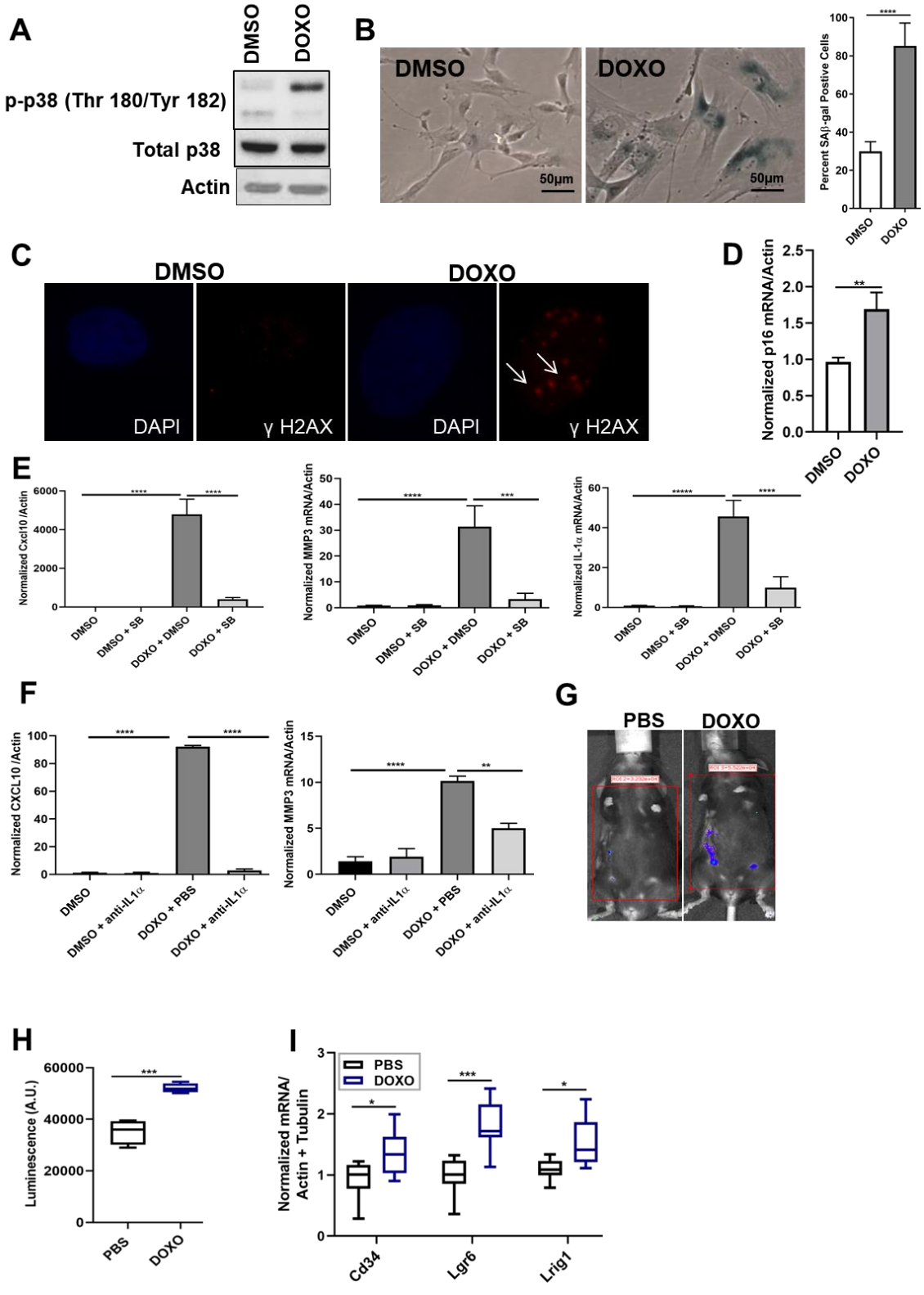
A
DMSO
DOXO
p-p38 (Thr 180/Tyr 182)
Total p38
Actin
B
DMSO
DOXO
50μm
50μm
Percent SAβ-gal Postive Cells
C
DMSO
DOXO
DAPI
γ H2AX
DAPI
γ H2AX
D
Normalized p16 mRNA/Actin
E
Normalized Cxcl10 /Actin
Normalized MMP3 mRNA/Actin
Normalized IL-1α mRNA/Actin
DMSO
DMSO + SB
DOXO + DMSO
DOXO + SB
F
Normalized CXCL10 /Actin
Normalized MMP3 mRNA/Actin
DMSO
DMSO + anti-IL1α
DOXO + PBS
DOXO + anti-IL1α
G
PBS
DOXO
H
Luminescence (A.U.)
PBS
DOXO
I
PBS
DOXO
Normalized mRNA/ Actin + Tubulin
Cd34
Lgr6
Lrig1

Figure S3. The SASP promotes skin carcinogenesis

(A) Human keratinocyte protein lysates treated with either DMSO (control) or DOXO were subjected to western blot analysis and analyzed for the indicated proteins. (B) HCA2 skin fibroblasts treated with either DMSO or DOXO were subjected to SA-ß-gal (blue) staining 10 days after treatment, and quantification is shown on the right panel. Shown are means ±SD, $p<0.0001$. (C) The same cells were immunostained with DAPI (nuclei) and gamma-H2AX (red), and arrows denote gamma-H2AX-positive foci. Representative images are shown. (D) Total RNA from the same cells was analyzed for p16 mRNA expression and normalized to actin control. Shown are means ±SD, $p<0.01$. (E-F) Total RNA isolated from HCA2 fibroblasts treated with either DMSO or DOXO in the absence or presence of SB or anti-IL-1α was analyzed for the indicated genes, normalized to actin and tubulin. Shown are means ±SD, $p<0.01$, $p<0.0001$. (G) Representative images of whole body luminescence of p16-3MR mice treated with PBS or DOXO 21 weeks after DMBA treatment. (H) Quantification of whole body luminescence of PBS- or DOXO-treated mice in arbitrary units (A.U.). (I) Total RNA was isolated from skin adjacent to tumors from the mice described above and analyzed for the indicated mRNAs, normalized to actin and tubulin. N=8 per treatment group.

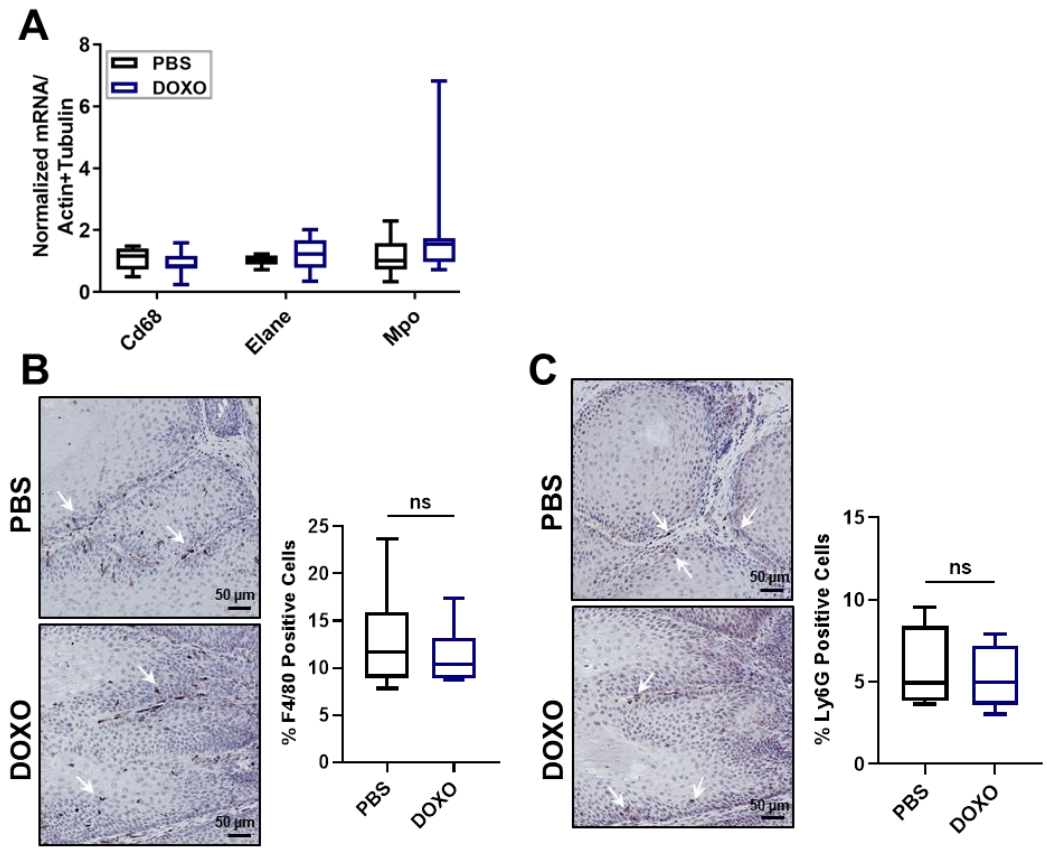

Figure S4. DOXO induces senescence and a SASP in the skin, independent of macrophages and neutrophils

(A) Total RNA was isolated from skin adjacent to tumors from mice treated with either PBS or DOXO, and analyzed for the indicated mRNAs, normalized to actin and tubulin. N=8 per treatment group. (B-C) Tissue sections from PBS or DOXO treated-mice were immunostained with either F4/80, a macrophage marker (B), or Ly6G, a neutrophil marker (C), and white arrows indicate positive staining. ns=not significant.

Table S1: Antibodies

Antibody	Source	Catalog #	Dilution
Actin Mouse mAb	Sigma-Aldrich	Cat# A2228	1:10000 (WB)
CD31 Rabbit mAb	Cell Signaling Technology	Cat# 77699	1:200 (IHC)
F4/80 Rabbit mAb	Cell Signaling Technology	Cat# 70076	1:200 (IHC)
Gamma-H2AX Rabbit mAb	Cell Signaling Technology	Cat#9718	1:1000 (IF)
Ki-67 Rabbit mAb	Cell Signaling Technology	Cat# 12202	1:200 (IHC)
Lamin B1 Goat pAb	Santa Cruz Biotechnology	Cat# sc-6216	1:2000 (WB)
Ly6G Rabbit mAb	Abcam	Cat#ab238132	1:200 (IHC)
p16 Rabbit mAb	Abcam	Cat# ab108349	1:500 (WB)
p38 Rabbit mAb	Cell Signaling Technology	Cat#9212	1:1000 (WB)
pp38 (Thr180/182) Rabbit mAb	Cell Signaling Technology	Cat#4631	1:200 (IHC), 1:1000 (WB)
pp44/42 Rabbit mAb	Cell Signaling Technology	Cat#4370	1:200 (IHC)
Tubulin Rabbit mAb	Cell Signaling Technology	Cat#11H10	1:5000 (WB)
Vimentin Rabbit mAb	Cell Signaling Technology	Cat# 5741	1:200 (IHC)
Anti-rabbit IgG HRP	Cell Signaling Technology	Cat# 7074	1:3000 (WB)
Anti-mouse IgG HRP	Cell Signaling Technology	Cat#7076P2	1:3000 (WB)
Anti-goat IgG HRP	Santa Cruz Biotechnology	Cat#sc2354	1:3000 (WB)

Table S2: “Human Oligonucleotides”

Human Primer	UPL Probe	Forward Sequence	Reverse Sequence
Actin	64	5’-ccaaccgcgagaagatga-3’	5’-tccatcacgatgccagtg-3’
CXCL10	34	5’-gaaagcagttagcaaggaaaggt-3’	5’gacatatactccatgtagggaagtga-3’
IL-1A	6	5’-ggttgagtttaagccaatcca-3’	5’-tgctgacctaggcttgatga-3’
IL-6	45	5’-gcccagctatgaactccttct-3’	5’gaaggcagcaggcaacac-3’
LMNB1	3	5’-gtgctgcgagcaggagac-3’	5’-ccattaagatcagattccttcttagc-3’
MMP9	6	5’-gaaccaatctcaccgacagg-3’	5’-gccacccgagtgtaaccata-3’
MMP3	36	5’-caaaacatatttctttgtagaggacaa-3’	5’-ttcagctatttgcttgggaaa-3’
p16	67	5’-gagcagcatggagccttc-3’	5’-cgtaactattcggtgcgttg-3’
p21	38	5’-caaacttaatggcatggacga-3’	5’-cgcctcgtacagggtgtc-3’

Table S3: “Mouse Oligonucleotides”

Mouse Primer	UPL Probe	Forward Sequence	Reverse Sequence
Actin	64	5’-ctaaggccaaccgtgaaaag-3’	5’-accagaggcatacagggaca-3’
Cd34	84	5’-gggtagctctctgcctgatg-3’	5’-tccgtggtagcagaagtcaa-3’
Cd68	6	5’-tgatcttgctaggaccgctta-3’	5’-taacggccttttgtgagga-3’
Cxcl10	3	5’-gctgccgtcattttctgc-3’	5’-tctcactggcccgtcatc-3’
Elastase	72	5’-tggaggtcatttctgtggtg-3’	5’-ctgcactgaccggaaatttag-3’
Il-1A	76	5’-tcctctagagctccatgctaca-3’	5’-agtgcaggaatgtacggagag-3’
Il-6	6	5’-gctaccaaactggatataatcagga-3’	5’-ccaggtagctatggtactccagaa-3’
Lgr6	1	5’-tgtgccaacagctgccta-3’	5’-aggctgggtaactcctcgat-3’
Lrig1	21	5’-acagctgccccacatacaac-3’	5’-gggatggtaggctgtgtca-3’
Mmp9	19	5’-acgacatagacggcatcca-3’	5’-gctgtggttcagttgtggtg-3’
Mmp3	76	5’-aagggtcttccggtcctg-3’	5’-atgcaatgggtaggatgagc-3’
Mmp3	95	5’-cgatggacagaggatgtcac-3’	5’-cagccttggctgagtggt-3’
Mpo	79	5’-gatggaatggggagaagctc-3’	5’-gcaggtagtcccggtatgtg-3’
p16	FAM	5’-aactctttcggtcgtacccc-3’	5’-tcctcgcagttcgaatctg-3’
p21	9	5’-ttgccagcagaataaaaggtg-3’	5’-tttgctcctgtgcggaac-3’

p21	21	5'-tccacagcgatatccagaca-3'	5'-ggacatcaccaggattggac-3'
Tubulin	88	5'-ctggaacccacggtcatc-3'	5'-gtggccacgagcatagttatt-3'

SUPPLEMENTARY METHODS

TPA-Induced Senescence in Mice: The dorsal skin of 3 mo old p16-3MR female mice was painted with TPA (100 µl (0.16 mM, n = 7) or acetone (n = 5) twice a week for 8 wks. Skin was isolated one month after the last treatment to exclude any inflammatory effects of TPA.

TPA-Induced Senescence and Two-Step Skin Carcinogenesis: Senescence was induced in the dorsal skin of p16-3MR mice as described above. One wk after the last TPA treatment, the mice were intraperitoneally (i.p.) injected with GCV (25 mg/kg) or PBS for two cycles of 5 consecutive days (10 d total) to clear p16-positive senescent cells. In total, 4 groups of mice were treated as follows: (1) Acetone + PBS: n = 9, (2) Acetone + GCV: n = 9, (3) TPA + PBS: n = 10, (4) TPA +GCV: n = 10. Three days after the last PBS or GCV injection, mice were subjected to the two-step skin carcinogenesis protocol: a single dose of DMBA (100 µl, 0.25 mg/ml) was applied to the same area pre-treated with TPA, followed a week later by promotion with TPA (100 µl, 0.16 mM) twice a week for 16 wks. To remove senescent cells, mice were treated with PBS or GCV every 3 wks until the end of the study. Tumor growth, incidence and number were monitored and recorded once a week for 24 wks.

DOXO-Induced Senescence and Two-Step Skin Carcinogenesis: To induce senescence, 3-mo old p16-3MR female mice were i.p. injected with DOXO (12 mg/kg) followed by a second injection (5 mg/kg) 3 d later. Three days after the last injection, mice were i.p. injected with PBS or GCV (25 mg/kg) for 5 consecutive d. Four days after the last PBS or GCV injection, the two-step skin carcinogenesis protocol was implemented. PBS or GCV treatments (2 cycles of 5 consecutive d) were repeated every 3 wks until the end of the study at 21 wks. To exclude

possible inflammatory effects of TPA on tumor growth, the experiment was terminated 5 wks after TPA treatment. Tumor growth, incidence and number were recorded once a wk for 21 wks. In total, 4 groups of mice were treated as follows: (1) PBS + PBS: n = 16, (2) PBS + GCV: n = 10, (3) DOXO + PBS: n = 16, (4) DOXO + GCV: n = 11.