Supplementary Materials and Methods

Animal studies

Body composition (fat and lean) of non-anesthetized mice was measured using the Bruker Minispec NMR analyzer (Bruker). For exercise testing, 22 wk old male mice were acclimated to a treadmill for 5 d by daily running at 10 m/min for 10 min. After 2 d of rest, maximum treadmill exercise capacity was determined as previously described (1). Blood lactate levels before and after sub-maximal exercise were measured using the Lactate Plus meter (Nova Biomedical).

Antibodies

Antibodies were obtained from the following sources: Lamin B1 rabbit pAb (#AB16048), Caspase3 mouse mAb (#9664) (Cell Signaling Technology); Cu/Zn-SOD rabbit pAb (#SOD-100) (Stressgen); CYP2E1 mouse mAb (#AB28146) (Abcam); p53 mouse mAb (DO1, #sc-126) (Santa Cruz Biotechnology).

p53 genomic PCR for loss of heterozygosity in liver tumors

Genomic DNA was isolated from tumor and adjacent normal liver tissue (~50 mg) using the DNeasy Blood & Tissue kit (#69506, Qiagen). The purified genomic DNA (~100 ng) was PCR amplified using the *p53* R334H genotyping primers.

Mouse embryonic fibroblast senescence assay

MEFs were prepared from *p53*$^{334R/R}$, *p53*$^{334H/H}$ and *p53*$^{-/-}$ mice. Senescence in non-transduced MEFs was quantified after 9 passages by staining for senescence-associated β-galactosidase activity (#9860, Cell Signaling Technology). To examine oncogene-induced senescence, MEFs were transduced with control or oncogenic *Hras*^{G12V} retrovirus for 1 d, selected with puromycin for 3 d, and then grown in normal medium for 6 d prior to staining for β-galactosidase (2). β-galactosidase stained MEFs were divided by total cell number to obtain the fraction of senescent cells in 3 non-overlapping fields by microscopy per sample.

Nuclear and cytosolic fractionation

Nuclear and cytoplasmic fractions were prepared from fresh liver tissues of control and DEN treated (100 mg/kg i.p., 24 h) mice using the NE-PER nuclear extraction kit (#78833, Thermo Fisher Scientific) per manufacturer's protocol.

Non-denaturing polyacrylamide gel electrophoresis and immunoblotting

Frozen liver tissue samples (~50 mg) were homogenized in 1 ml cold lysis buffer (20 mM Tris-HCl (pH 7.4), 137 mM NaCl, 1% NP-40, 2 mM EDTA, 10% glycerol, protease/phosphatase inhibitor cocktail (#04-693-116-001 and #04-906-837-001, Roche)) with a IKA Ultra-Turrax homogenizer (Millipore Sigma) for 15 s and centrifuged at 12,000x g at 4 ^{o}C for 10 min. The supernatant was depleted of mouse IgG with protein G-agarose (#sc-2002, Santa Cruz Biotechnology) and mixed with NativePAGE sample buffer (#BN2003, Invitrogen) and G-250 sample additive (0.5% final concentration) (#BN2004, Invitrogen) to a final protein concentration of 1 mg/ml. Samples (10 µg protein) were loaded into 4-16% NativePAGE Bis-Tris gel (#BN1002, Invitrogen) and resolved for ~3 h at 4 ^{o}C according to manufacturer's

protocol. Proteins were transferred to PVDF membrane at 4 ^{o}C for ~3 h using NuPAGE transfer buffer (#NP0006, Invitrogen). The membrane was destained using 100% methanol before immunoblotting with mouse monoclonal p53 antibody (#sc-126, Santa Cruz).

References

1. Park JY, Wang PY, Matsumoto T, Sung HJ, Ma W, Choi JW*, et al.* p53 improves aerobic exercise capacity and augments skeletal muscle mitochondrial DNA content. Circ Res **2009**;105:705-12, 11 p following 12
2. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell **1997**;88:593-602

Supplementary Table 1. Primer sequences used in this study

	Forward (5′ → 3′)	Reverse (5′ → 3′)
RT-PCR		
BAX	CCGGCGAATTGGAGATGAACT	CCAGCCCATGATGGTTCTGAT
CYP2E1	CTTAGGGAAAACCTCCGCAC	GGGACATTCCTGTGTTCCAG
MDM2	GCCATTGAACCTTGTGTGATTT	CATACTGGGCAGGGCTTATT
p21	CTGTGGGTCTCTGCCAGCTGC	GAGGCCTGTCTCACCACCAAG
PUMA	AGCAGCACTTAGAGTCGCC	CCTGGGTAAGGGGAGGAG
TIF	CTGAGGATGTGCTGTCTGGGAA	CCTTTGCCTCCACTTCGGTC
Genotyping		
p53 R334H	CCTGTTAAGCGTCTGTTCCC	CATTCAGCTCCCGGAACATCTC
ChIP-PCR		
p53RE of *p21*	GCAAGGCTGCATCAGTCCTCC	GGTCTCTGTCTCCATTCATGCTC

Supplementary Table 2. Genotype and gender of pups derived from heterozygous *p53*$^{334R/H}$ parents

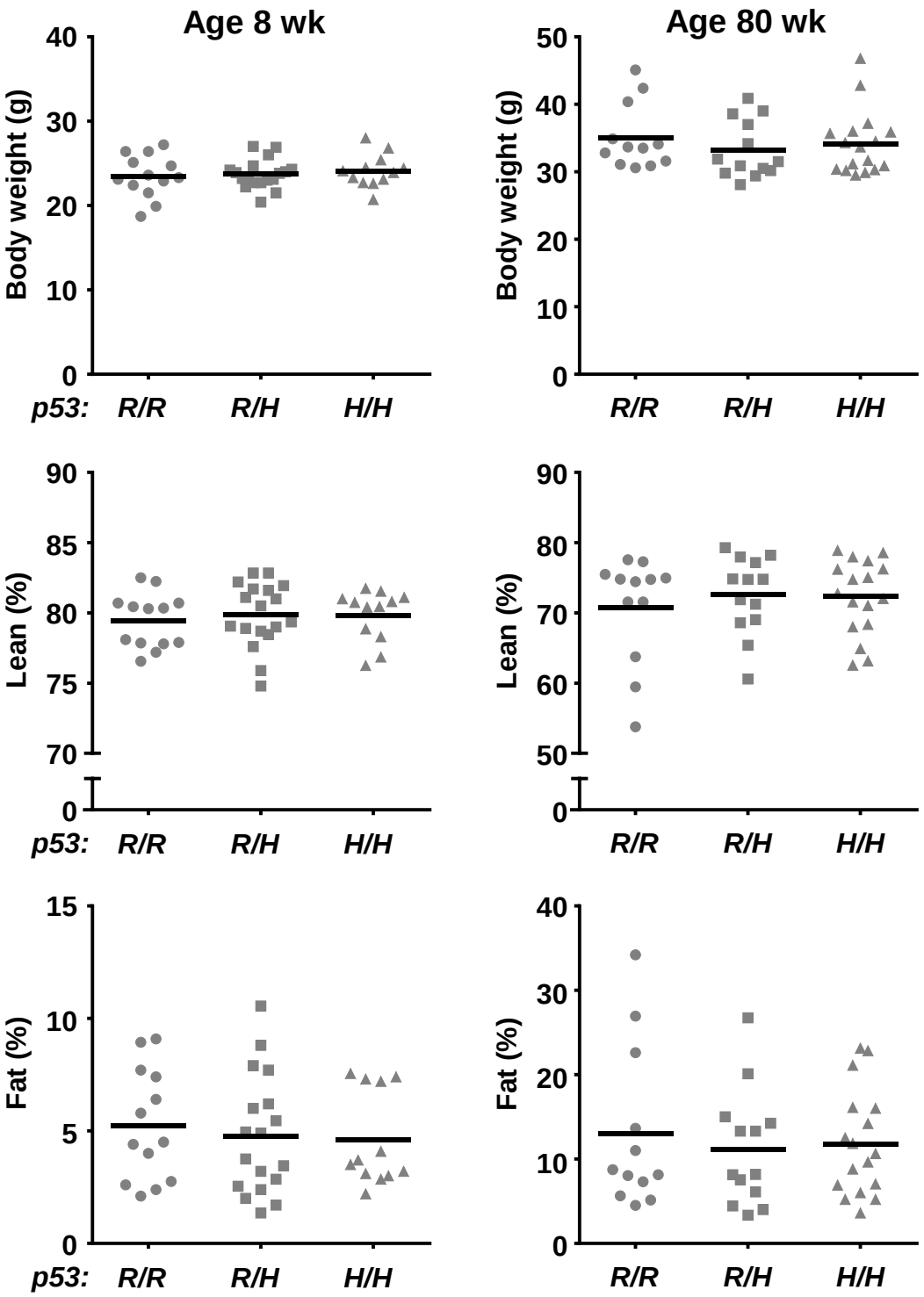
	Male	Female
p53$^{334R/R}$	57 (22%)	63 (26%)
p53$^{334R/H}$	130 (51%)	131 (55%)
p53$^{334H/H}$	70 (27%)	46 (19%)
***P*-value**	0.50	0.11

A total of 497 pups derived from heterozygous *p53*$^{334R/H}$ crossings were genotyped. Statistical significance was tested by Chi-square test.

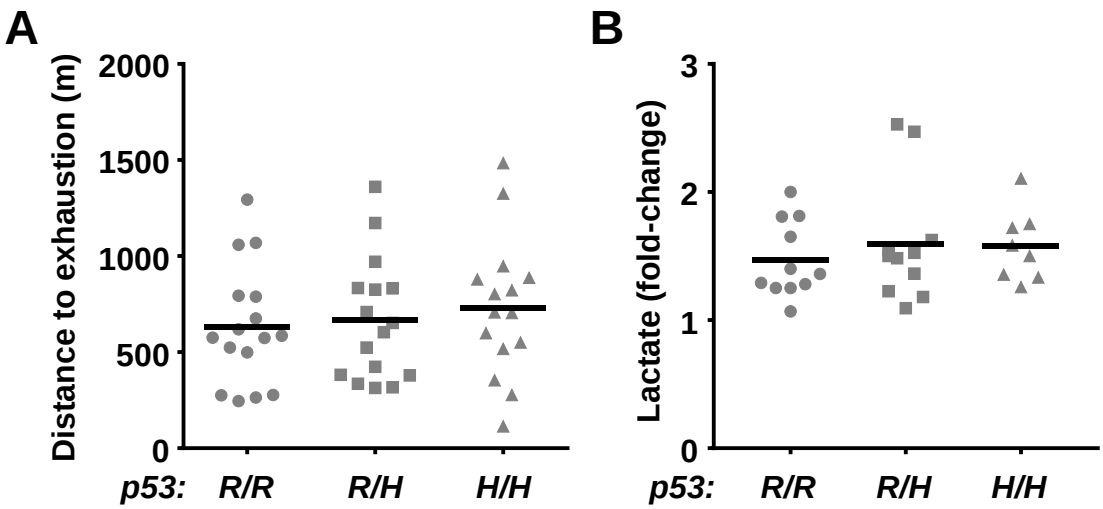
Supplementary Table 3. Incidence of adrenal abnormalities

	$p53^{334R/R}$	$p53^{334R/H}$	$p53^{334H/H}$
Total number of mice	24	39	24
Number with adrenal abnormalities (%)	16 (67%)	22 (56%)	14 (58%)
***P*-value**		0.44	0.77

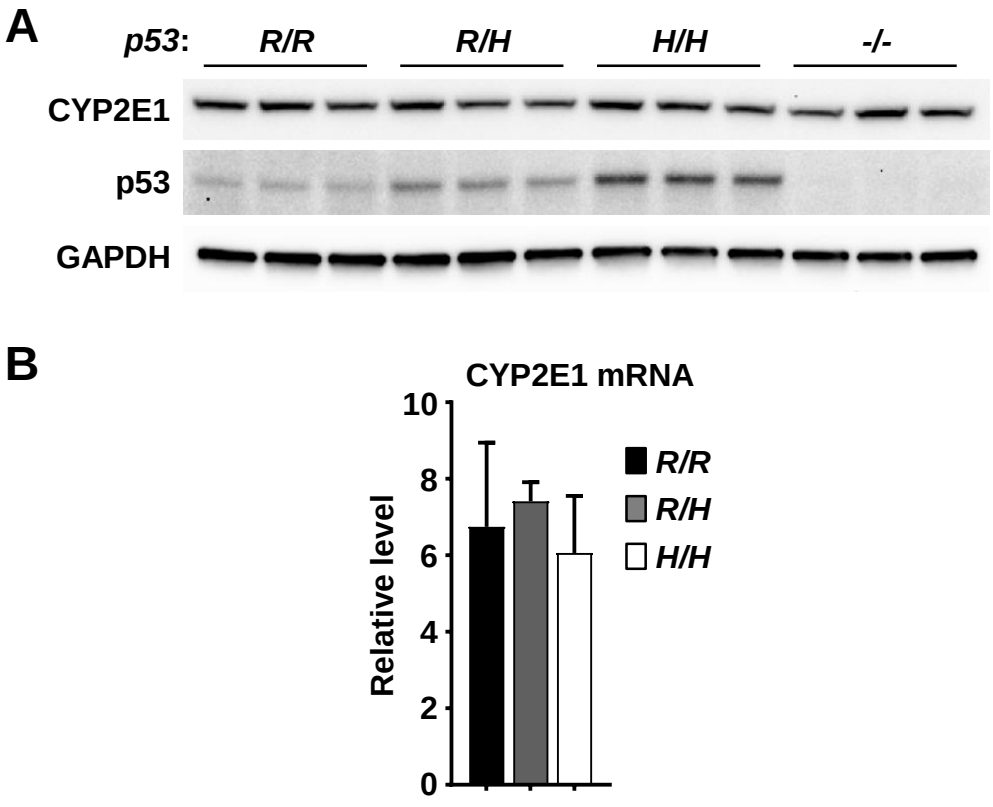
The incidence of adrenal abnormalities at the endpoint of the mouse survival study was defined by the presence of adrenal cortical hyperplasia and/or hypertrophy at necropsy. *P*-values are shown for the respective genotype compared with wild-type $p53^{334R/R}$ mice. Statistical significance was determined by Fisher's exact test.



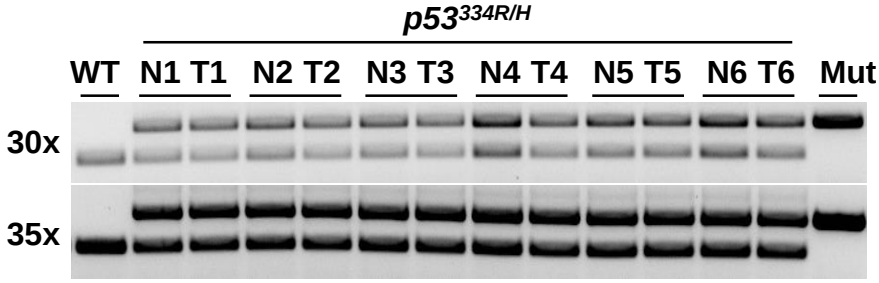
Supplementary Figure S1. Body weight and composition of male mice at age 8 and 80 wk. Body mass composition is shown as percent of total body weight. *p53* R334 genotype: wild-type (*R/R*); heterozygous mutant (*R/H*); and homozygous mutant (*H/H*) (*n* = 12-18). Horizontal bars indicate mean.



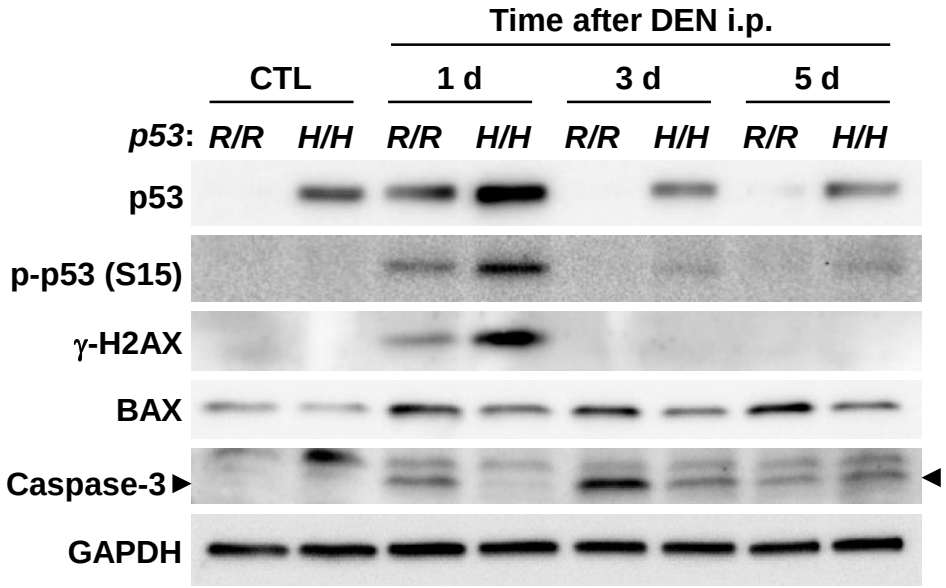
Supplementary Figure S2. Endurance exercise and aerobic metabolic capacity of male mice. A, maximum running distance was determined using a motorized treadmill (*n* = 15-16). **B**, fold-change in blood lactate level after submaximal exercise compared with basal lactate level are shown as an index of aerobic metabolic capacity (*n* = 8-11). *p53* R334 genotype: wild-type (*R/R*); heterozygous mutant (*R/H*); and homozygous mutant (*H/H*). Horizontal bars indicate mean.



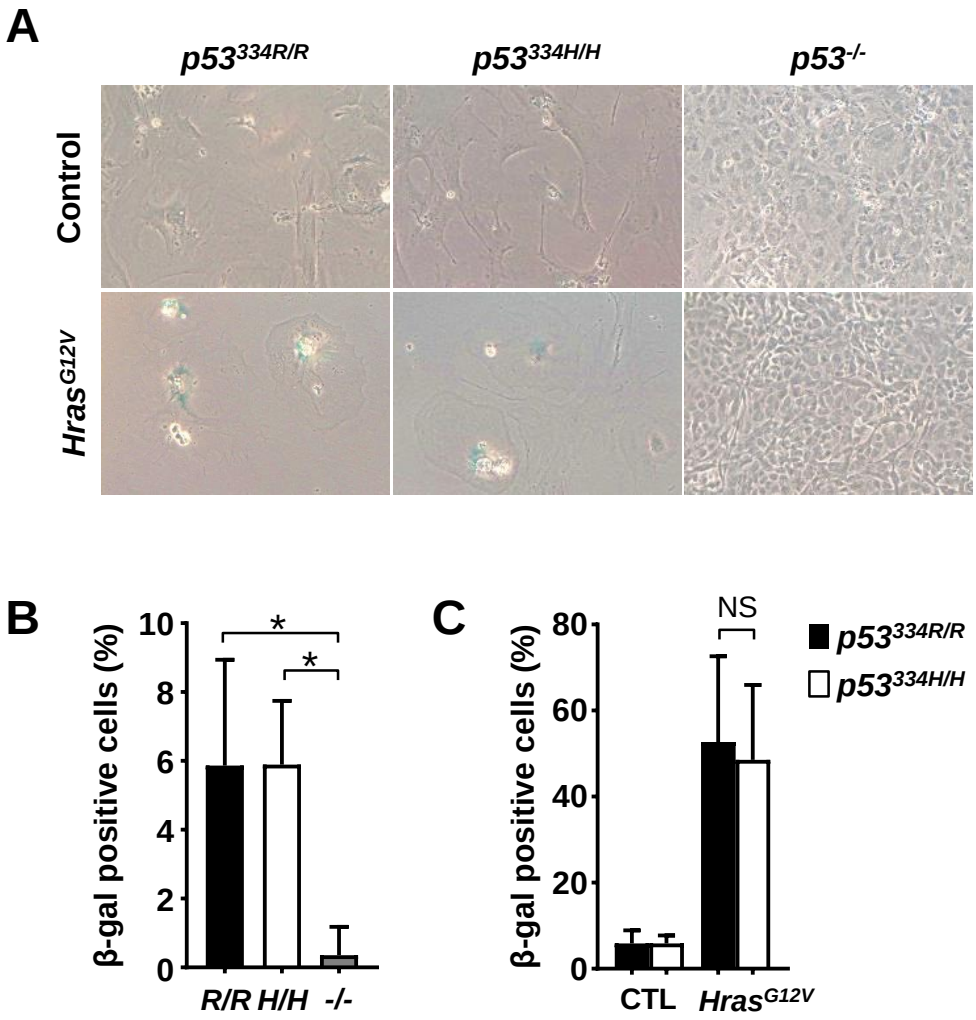
Supplementary Figure S3. Expression of CYP2E1 in mouse liver by p53 genotype. **A**, livers were collected from 6 wk old male mice and immunoblotted with the indicated antibodies. **B**, CYP2E1 mRNA was quantified by RT-PCR (*n* = 3). *p53* R334 genotype: wild-type (*R/R*); heterozygous mutant (*R/H*); and homozygous mutant (*H/H*). Values are mean ± SD.



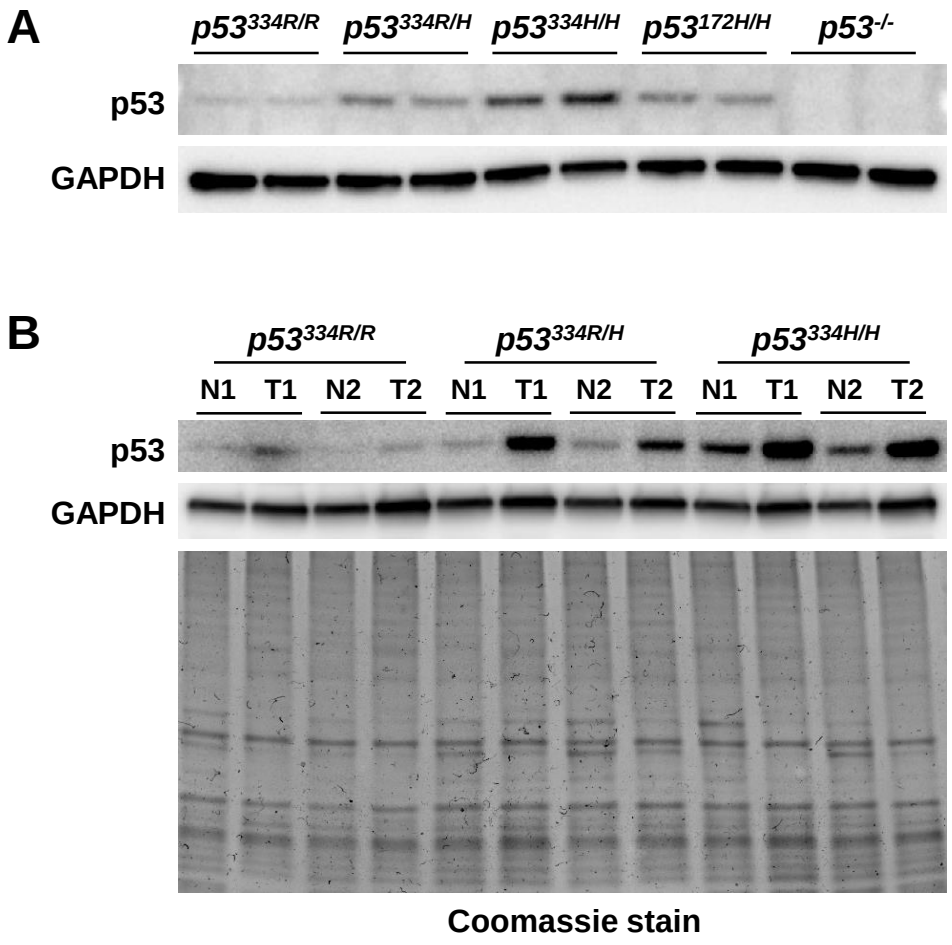
Supplementary Figure S4. Genomic PCR analysis for *p53* loss of heterozygosity in liver tumors of *p53*$^{334R/H}$ mice. DEN-induced tumor (T) and adjacent normal (N) liver tissues were collected from heterozygous *p53*$^{334R/H}$ mice. Genomic DNA was amplified by the indicated number of PCR cycles. *p53* PCR bands from *p53*$^{334R/R}$ (WT, 305 bp) and *p53*$^{334H/H}$ (Mut, 611 bp) liver samples are shown as markers.



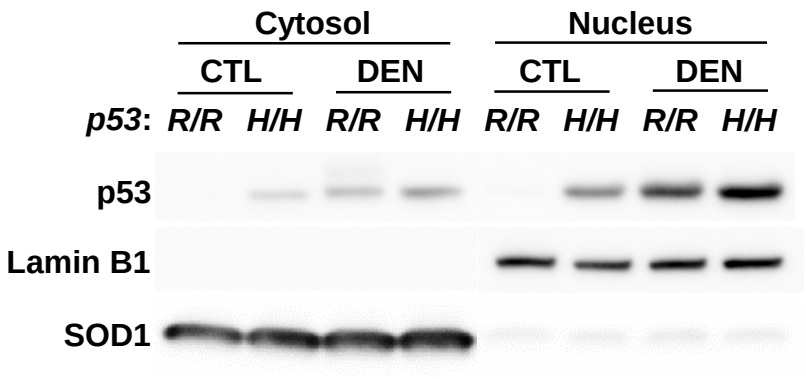
Supplementary Figure S5. Time course of DNA-damage response and apoptotic protein expression in liver after DEN treatment. Control (CTL) and DEN treated (100 mg/kg i.p.) mouse livers were harvested and immunoblotted at the indicated time points. *p53* R334 genotype: wild-type (*R/R*) and homozygous mutant (*H/H*).



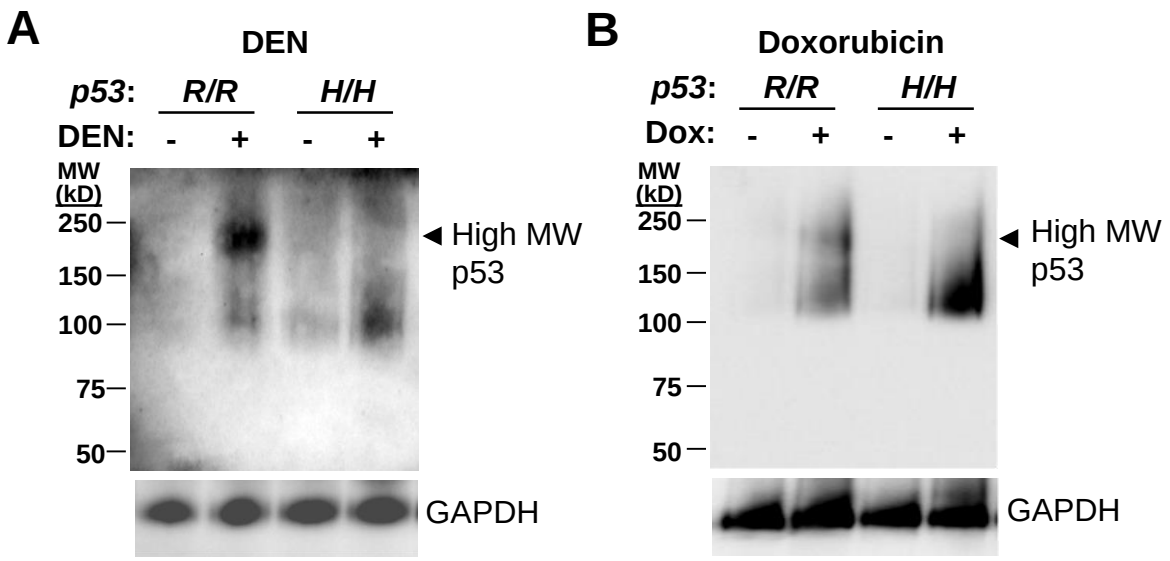
Supplementary Figure S6. Oncogenic *Hras*^{G12V}-induced senescence in mouse embryonic fibroblasts. **A**, representative images of *p53*$^{334R/R}$, *p53*$^{334H/H}$ and *p53*$^{-/-}$ MEFs that were transduced with control or oncogenic *Hras*^{G12V} retrovirus and stained for senescence-associated β-galactosidase activity (10x objective). **B**, quantification of senescence in non-transduced MEFs of the indicated p53 genotypes (*n* = 3). **C**, quantification of senescence in control or oncogenic *Hras*^{G12V} retrovirus-transduced MEFs of the indicated p53 genotypes (*n* = 3). Values are mean ± SD. NS, nonsignificant; **P* < 0.01.



Supplementary Figure S7. Basal levels of p53 protein in normal liver and DEN-induced liver tumor tissues. A, livers from 6-10 wk old male mice of the indicated p53 genotypes were immunoblotted with p53 and GAPDH antibodies. **B,** tumor (T) and adjacent normal (N) liver tissues were immunoblotted. Coomassie blue stained gel is shown as additional control for protein loading.



Supplementary Figure S8. Nuclear localization levels of wildtype and mutant p53 in liver before and after DEN-treatment. Male mice (6 wk old) were treated with DEN (100 mg/kg i.p.) 24 h prior to liver tissue harvesting, nuclear fractionation, and immunoblotting with the indicated antibodies. Lamin B1 and SOD1 serve as nuclear and cytosolic markers, respectively. *p53* R334 genotype: wild-type (*R/R*) and homozygous mutant (*H/H*).



Supplementary Figure S9. Assessment of p53 oligomerization in liver by non-denaturing polyacrylamide gel electrophoresis. A, control and DEN-treated (24 h after 100 mg/kg i.p.) mouse liver samples. **B,** control and doxorubicin-treated (Dox 6 h, 20 mg/kg i.p.) mouse liver samples. Liver protein samples were resolved by non-denaturing polyacrylamide gel electrophoresis, transferred to PVDF membrane, and immunoblotted to assess for formation of high molecular weight p53 complex. *p53* R334 genotype: wild-type (*R/R*) and homozygous mutant (*H/H*).