Supporting Information

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SI Materials and Methods

Generation of Mice. A 19-kb genomic DNA fragment containing exons 6–10 of the *TRP53* gene was cloned from a mouse 129Sv/Ev λ genomic library. The targeting vector was constructed using a 1.1-kb DNA fragment as the short arm, which was a PCR fragment from primers 53P20 and 53P19. Primer 53P20 is located 330 bp downstream of exon 10, with a sequence of 5′-CCACCACCATCATCACC-3′. Primer 53P19 is located 670 bp upstream of exon 10, with a sequence of 5′-CTCATGCTCTGAGGCTGTGCC-3′. The short arm was inserted into the 5′ end of the Neo gene cassette (flanked by LoxP sites) using a PspOMI site. The codon mutation (TCT → GCC) was introduced by PCR-directed mutagenesis. The PCR fragment was cloned into the 3′ end of the neo cassette using a natural BamHI site, and a 6.5-kb BamHI genomic fragment was cloned into the BamHI site to serve as the long arm.

The targeting vector was linearized using NotI and transfected into 129Sv/Ev (iTL1) embryonic stem (ES) cells. After G418 selection, surviving clones were screened by PCR to identify recombinants. A correctly targeted ES cell line was microinjected into C57BL6/J blastocysts to produce chimeras, which were mated to generate germline transmission of the mutation. The neo cassette was removed by mating the mice carrying the mutated codon with EIIaCre loxP deleter mice (FVB) (The Jackson Laboratory). The progeny were genotyped to verify Neo deletion and mated with C57BL/6 mice to identify non-mosaic Neo-deleted mice.

Genotyping. The TGT → GCC mutation was genotyped by PCR. A common PCR primer, 5′-CCCTTAAACCCCAATGTCCT-3′, was used with either primer mp53S312 (5′-GCCCACCTGCACAAGCGCC**TCT**-3′) to detect the WT allele or with primer mp53A312 (5′-GCCCACCTGCACAAGCGCC**GCC**-3′) for the mutant allele. Bases in bold text indicate either the wild type (TCT) or mutant (GCC) codons.

Tumor Induction and Analysis. Mice were exposed to 4 Gy IR from an X-ray source at 3 wk of age and observed for signs of tumor growth or general ill health. Postsacrifice, all tissues were fixed for histological analysis as described previously (1). Statistical significance was determined using the log-rank test.

Quantitative Real-Time PCR. The primer sequences used for quantitative real-time PCR (qRT-PCR) were mGAPDH (forward: AAC TTT GGC ATT GTG GAA GG; reverse: ACA CAT TGG GGG TAG GAA CA), m-βActin (forward: TGT TAC CAA CTG GGA CGA CA; reverse: GGG GTG TTG AAG GTC TCA AA), mp21 (forward: TCT TTG GCT TTG ACA CGG; reverse: CTC TTC AGT CGC TTT CAC A), and m-Cyclin G1 (forward: AGG TCT GCG GCT TGA AAC TA; reverse: TCA GTC CAA CAC ACC CAA GA).

Gene Expression Profiling. The Affymetrix GeneChip Whole Transcript Sense Target Labeling Assay was used to amplify and reverse-transcribe total RNA and to biotinylate sense-strand DNA targets. Arrays were hybridized with the labeled-target hybridization mixture by rotation at 60 rpm in an Affymetrix Gene Chip hybridization oven at 45 °C for 16 h, washed in an Affymetrix GeneChip Fluidics station FS 450, and scanned by an Affymetrix Gene Chip scanner 3000 7G system. Scanned image files (.CEL) were processed, normalized (RMA-Sketch Quantile), and $\log_2$-transformed by Expression Console Software (Affymetrix). Normalized intensity files were statistically analyzed to calculate *t*-statistics and *P* values. The *P* values were further adjusted by the false discovery rate (<5%) approach and enriched for data displaying over twofold change.

Cell Culture. Mouse embryonic fibroblasts (MEFs) were prepared from embryonic day 13.5 embryos generated by intercrossing $p53^{312A/+}$ mice and were cultured in DMEM + 10% FBS. The MEFs were transformed using the retroviral vector pBabe-puro E1A. Colony analysis was performed on Giemsa-stained dishes after 3 wk selection in puromycin. Growth analysis was performed by seeding the cells in 6-cm dishes at 3×10^5, counting the total number of cells after 3 d before reseeding the cells again at 3×10^5 cells/dish.

Assessment of Apoptosis. Thymocytes were removed from mice (at 7–12 wk) and, after exposure to apoptotic stimuli, incubated with Annexin V-FITC (Abcam) and analyzed by FACS.

Immunoblotting and Antibodies. Lysates were prepared in a solution of 8 M urea, 1 M thiourea, 0.5% CHAPS, 50 mM DTT, and 24 mM spermine. p53 was immunoprecipitated using anti-p53 mAb 242 in Nonidet P-40 lysis buffer. The p53 phospho-Ser312 polyclonal antibody was made by immunizing rabbits with the peptide CTSApSPPQKKKP, followed by purification against the nonphosphorylated peptide to remove nonspecific antibodies. Other antibodies used were CM5 (anti-p53), anti-p21 SX118 (mAb), anti-actin (C-2) (Santa Cruz Biotechnology), and anti-CD3 rabbit polyclonal antibody (Abcam).

RNA Extraction. Total RNA was extracted from mouse liver by homogenization in TRIzol (Invitrogen). Genomic DNA was removed with DNaseI and RNA enriched with RNeasy columns (Qiagen). Quantity and integrity of extracted RNA was assessed using a NanoDrop Spectrophotometer and an Agilent 2100 Bioanalyzer, respectively.

Quantitative Real-Time PCR. For cDNA synthesis, 2 μg of total RNA was reverse-transcribed with random primers using a Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase kit (Invitrogen). qRT-PCR was carried out with 1 μl of cDNA, using SYBR Green master mix (Applied Biosystems), and analyzed using the StepOne Plus Real Time PCR Detection System (Applied Biosystems). The relative abundance of specific mRNA levels was calculated by normalizing to both GAPDH and β-actin expression by the $2^{-\Delta\Delta Ct}$ method (2).

Gene Expression Profiling. Gene expression profiling was determined using Mouse Gene 1.0 ST (MoGene) arrays (Affymetrix).

1. Vives V, et al. (2006) ASPP2 is a haploinsufficient tumor suppressor that cooperates with p53 to suppress tumor growth. *Genes Dev* 20:1262–1267.

2. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402–408.

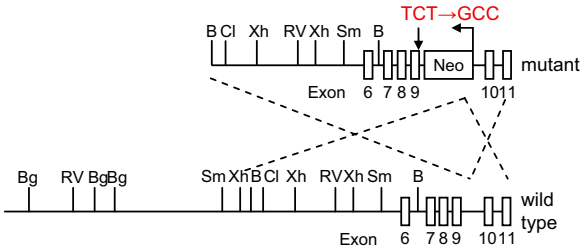

Fig. S1. Generation of p53$^{312A/A}$ mutant mice. Schematic showing the targeted mutation of the murine p53 gene and illustrating the targeting vector ("mutant") containing the TCT → GCC mutation in exon 9.

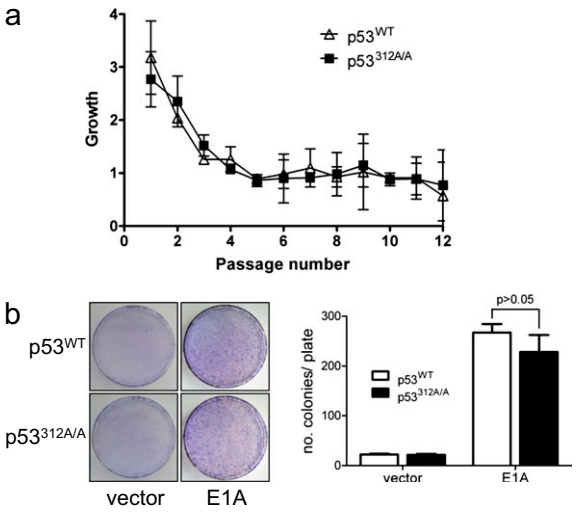

Fig. S2. Impact of p53$^{312A/A}$ mutation on cell growth and senescence. (*A*) Nontransformed MEFs were counted and reseeded at 3-d intervals, and the fold growth in the intervening period was calculated. (*B*) Colony assay of E1A immortalized MEFs. MEFs were immortalized with retroviral E1A alongside an empty vector control, and colonies were allowed to grow under antibiotic selection before being stained with Giemsa and counted. Data are representative of three independent experiments. The *p* value was calculated using the Student's *t* test.

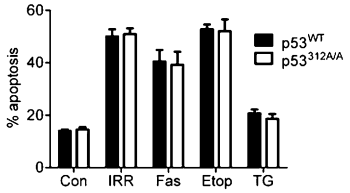

Fig. S3. Induction of apoptosis in p53$^{312A/A}$ thymocytes. Apoptosis in thymocytes from p53^{WT} and p53$^{312A/A}$ mice detected by Annexin V binding. Apoptosis was assessed 6 h after exposure to 5 Gy ionizing radiation (IRR), anti-Fas (clone JO-1; 0.5 μg/mL) (Fas), etoposide (10 μM) (Etop), or thapsigargin (50 nM) (TG). Con indicates untreated cells.

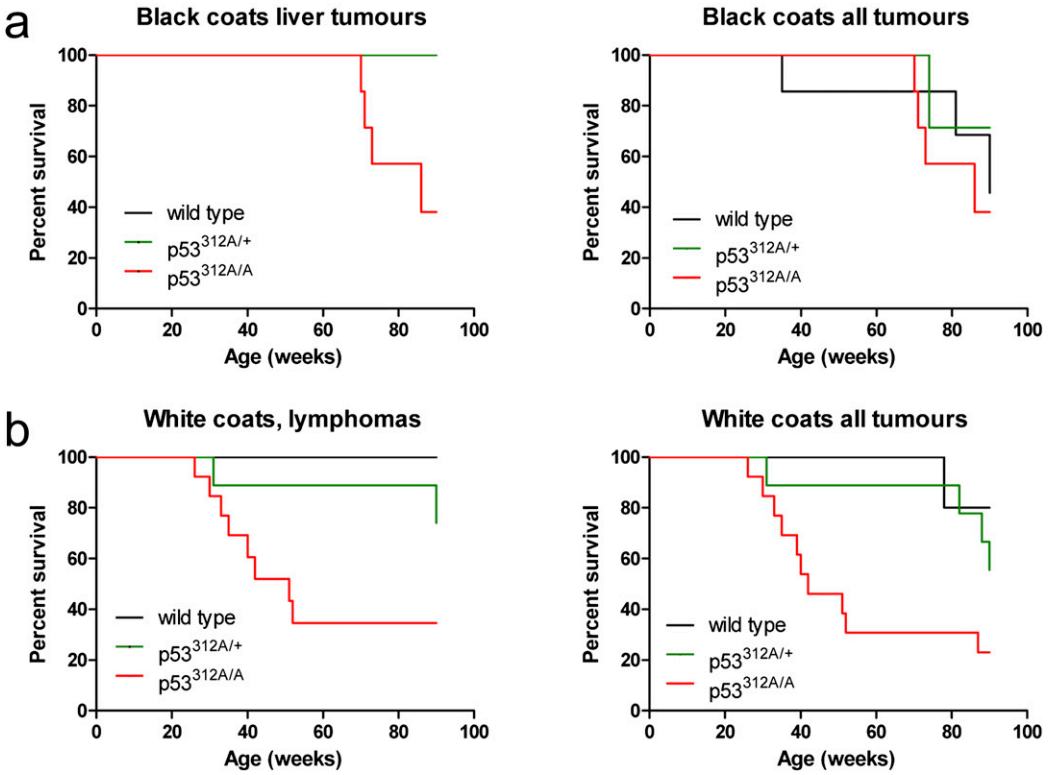

Fig. S4. Susceptibility of mice to tumors by coat color. (*A*) Black $p53^{312A/A}$ mice are significantly more liable to develop liver tumors ($P < 0.05$) (*Left*) than black wild type or $p53^{312A/+}$ mice, although there is no difference in their overall tumor susceptibility (*Right*). No black wild-type mice developed liver tumors. (*B*) White $p53^{312A/A}$ mice are significantly more susceptible to both lymphomas ($P < 0.05$) (*Left*) and tumors in general ($P < 0.05$) (*Right*) than either wild-type or $p53^{312A/+}$ mice with white coats.

Table S1. Coat-color distribution versus genotype in the cohort of mice used in the tumor study

	$p53^{WT}$	$p53^{312A/+}$	$p53^{312A/A}$	Total
Agouti	3	5	2	10
Black	7	7	7	21
White	6	9	13	28
Total	16	21	22	59

Table S2. Summary of the array analysis data of two representative p53 target genes

				$p53^{WT}$		$p53^{312A/A}$	
			$p53^{wt}$ vs. $p53^{312A/A}$	Treated vs. untreated		Treated vs. untreated	
Gene symbol	Ref Seq ID	Affymetrix ID	Fold change	Fold change	*P* value	Fold change	*P* value
CCNG1 (Cyclin G1)	NM_009831	10385271	NC	3.25	0.001023	2.15	0.010745831
CDKN1A (p21)	NM_007669	10443463	NC	9.90	0.004587	6.6	5.72293E-05

NC, no change.