Supplementary material and methods

Generation of LSL wild type p53 mice

The LSLp53 allele was obtained as a by-product of our efforts to generate a R270H point mutant p53 allele [30]. All p53 exons and intron-exon boundaries were sequenced to ensure the absence of mutations. Oligonucleotide sequences for genotyping are available upon request.

Generation of R26-Cre-ER^{T2} mice

A plasmid containing the Cre-ER^{T2} cDNA (pCre-ER^{T2}) was obtained from the laboratory of P. Chambon[20]. The Cre-ER^{T2} cDNA was amplified using the following oligonucleotides

5'-CATATGTTGCCAGACCGCGGACCATGTCCAATTTACTGACCGTACACC and

5'-GCTAGCTATAAATAATACGCGTTCAAGCTGTGGCAGGGAAACC. These oligonucleotides introduced an NdeI site, a SacII site and a Kozak sequence at the 5' end of the PCR product and an MluI site and an Nhe1 site at the 3' end. The PCR product was cloned into the pCR-Blunt II-TOPO vector (Invitrogen). Site-directed mutagenesis was performed to destroy an XbaI site at position 1329 bp of the Cre-ER^{T2} cDNA using the QuickChange kit (Stratagene) with the oligonucleotides

5'-CCCTCCATGATCAGGTCCACCTTCTTGAATGTGCCTGGC and

5'-GCCAGGCACATTCAAGAAGGTGGACCTGATCATGGAGGG (pMB48). A double-stranded oligonucleotide containing the adenovirus splice acceptor (SA) site flanked by NdeI and XbaI sites on the 5' end and a SacII site on the 3' end was separately

cloned into the pCR-Blunt II-TOPO vector (pMB54). Both pMB54 and pMB48 were digested with NdeI and SacII, and the Nde1/SacII fragment containing the SA was ligated to the NdeI/SacII-digested pMB48 vector, placing the SA 5' of the Cre-ER^{T2} cDNA (pMB61).

A floxed PGK-Puro cassette was amplified from pGEM$_T$-lox^{2}Puro using the oligonucleotides 5'-GCTAGCATAACTTCGTATAATGTATGC and 5'-TCTAGAATTATACGAAGTTATGCTAGAGTCGACCAGC.
These oligonucleotides introduced an NheI site at the 5' end of the PCR product and an XbaI site at the 3' end and deleted an XbaI site at position 1740 bp. The PCR product was cloned into the pCR-Blunt II-TOPO vector (pMB33). A double-stranded oligonucleotide with overhanging ends containing the PMC polyadenylation (PA) site flanked by BamHI and MluI sites on the 5' end and an NheI site on the 3' end was cloned directly into the pMB33 vector digested with BamHI and NheI, placing the PA 5' of the floxed PGK-Puro cassette (pMB43). Due to an error in the sequence of the 3' loxP site, PA-floxed PGK-Puro was reamplified with the oligonucleotides 5'-ACGCGTGGCAATAAAAAGACAGAATAAAACGCACGGGTGTTGGG and 5'-TCTAGAATAACTTCGTATAGCATACATTATACGAAGTTATGCTAGAGTCG, and reinserted into the pCR-Blunt II-TOPO vector (pMB75).

The final cloning step was a triple ligation with the XbaI/MluI SA-Cre-ER^{T2} fragment of pMB61, the MluI/XbaI PA-floxed PGK-Puro fragment of pMB75, and an XbaI-digested, CIP-treated, modified version pROSA26-1 vector from the laboratory of P. Soriano (pMB80). In the modified version of pROSA26-1, the SacII site has been

replaced by an AscI linearization site. The sequence of the entire insert from XbaI to XbaI site was confirmed by bidirectional sequencing.

The R26-Cre-ER^{T2} targeting vector was linearized at the unique AscI site, and electroporated into J1 ES cells. ES cells were selected in 2 μg/ml puromycin and appropriately targeted clones were identified with a 5' external probe and EcoRI-digested genomic DNA. Germline transmission was achieved in 2/2 clones harboring the floxed R26-CRE-ER^{T2} allele. The floxed PGK-Puro cassette was deleted in vivo by crossing to protamine-Cre transgenic mice, which express Cre recombinase in haploid sperm.

To determine the presence of the R26-Cre-ER^{T2} allele, genomic DNA was amplified using the following oligonucleotides to generate a wild-type band of 650 bp and a mutant band of 825 bp:

R26R-univF (5'-AAAGTCGCTCTGAGTTGTTAT),

R26R-wtR (5'-GGAGCGGGAGAAATGGATATG),

CreER-R1 (5'-CCTGATCCTGGCAATTTCG).

Tamoxifen Treatment.

All animal studies and procedures were approved by the MIT Institutional Animal Care and Use Committee and the Subcommittee on Research Animal Care at Massachusetts General Hospital. Mice were of a mixed 129Sv/Jae and C57/B6 background. After detecting a tumor by MRI, mice were treated with tamoxifen (SIGMA) by intraperitoneal injection. 100 μl of tamoxifen (10 mg/ml in corn oil) was injected every two to three days. Follow-up MRI was generally obtained 7 to 10 days after the first tamoxifen treatment.

β-Galactosidase histochemistry

For whole-mount X-gal staining, organs were dissected and fixed in 4% paraformaldehyde (PFA) for 1 h at 4ºC, rinsed, and then assayed for β-galactosidase activity as previously described (MacPherson et al., 2004). For X-gal staining of frozen sections, organs were dissected, fixed in 4% PFA for 24 h at 4ºC, cryopreserved in 0.5 M sucrose for 24 h at 4ºC, and frozen in OCT. Seven micron sections were cut and assayed for β-galactosidase activity as previously described (MacPherson et al., 2004).

Cell culture and tumor cell lines derivation

MEFs and tumor cell lines were cultivated in DME containing 10% fetal calf serum supplemented with Penicillin/Streptomycin and L-Glutamine. To derive cell lines, tumors were minced with a razor blade, filtered through a 70 μm filter, and then plated in complete medium. 4-OHT (SIGMA) was diluted in ethanol at 1mM concentration and added to the cells at a final concentration of 250 nM.

TUNEL and senescence-associated b-galactosidase assays

TUNEL assay was performed using the In situ cell death detection kit, AP (Roche cat. 11684809910) according to manufacturer's instructions.

Staining for senescence-associated beta-galactosidase activity was performed on either formalin fixed cells (*in vitro* experiments) or on cryosections using the Senescence beta-galactosidase Staining kit (Cell Signaling) according to manufacturer's instructions.

BrdU incorporation

Cells were infected with Adeno-eGFP or Adeno-Cre-eGFP. 24 hours later GFP-positive cells were sorted by FACS and plated at a density of 2x 10^5 cells/well into 6 wells plates. 2 days later cells were incubated with 10 μg/ml BrdU for 1 hour, fixed in 4% paraformaldehyde, permeabilized in 0.25% triton-X100, blocked in .025% Tween20, 1% Bovine Serum Albumin for 30 minutes and then incubated with anti-BrdU (mouse monoclonal, 1:50, BD Bioscience) for 2 hours at room temperature. After incubation, cells were washed in PBS and incubated with a rhodamine-conjugated anti-mouse antibody (1:500) for 1 hour. After washing in PBS, nuclei were counterstained in DAPI and cells were examined by fluorescence microscopy. Images were acquired using the Openlab software and analyzed using ImageJ software. Recombinant Adenoviral stocks were purchased from the Gene Transfer Vector Core facility of the University of Iowa College of Medicine.

Western Blot and Immunohistochemistry

Antibodies used for Western Blot were: p53 (a mouse monoclonal antibody generated and kindly provided by K. Helin, Biothec Research and Innovation Center, Copenhagen) used at a dilution of 1:500, vinculin (V9131, 1:10,000, Sigma), beta-actin (1: 2000, Santa Cruz), cleaved caspase 3 (9661, 1:1000 , Cell Signalling), p21 (F5: sc-6246, 1:2000, Santa Cruz), p15Ink4b (ab-3102, 1:50, Abcam), Dec1 (rabbit polyclonal, 1: 200, kindly provided by Dr. Adrian Harris, CRUK, Oxford), DcR2 (AAP-371, 1:2000, Stressgen, Canada). Antibodies used for immunohistochemistry were p19Arf (NB 200-106, 1:100,

Novus Biologicals), p53 (CM5, 1:500, Novocastra), p16Ink4A (sc-1207. 1:200. Santa Cruz. Used at the same dilution for WB), p15Ink4b (rabbit polyclonal, 1:200 kindly provided by Dr. Mariano Barbacid, CNIO, Madrid), phospho-Histone-H3 (Cell Signaling, 1:200), Ki-67 (VP-K452, 1:200, Vector Laboratories).

Magnetic Resonance Imaging (MRI) and Tumor Volume Analysis

All animals were sequentially imaged using a 4.7T Bruker Pharmascan (Bruker BioSpin, Billerica, MA) to screen for tumor growth and treatment response to tamoxifen. Multislice T2- and T1-weighted sequences were obtained in coronal and axial planes, using the following parameters: T1weighted: TR / TE = 900 / 14 ms, matrix size 192x192 (or 128 for axial images), 1mm slice thickness, field of view 4.6 x 4 (axial 4.6 x 3.1), 8 repetitions. T2-weighted: TR / TE = 2000 / 44 ms, matrix size 192x192 (or 128 for axial images), 1mm slice thickness, field of view 4.6 x 4 (axial 4.6 x 3.1), 6 repetitions. After intraperitoneal injection of Gadolinium-DTPA contrast, the T1 sequences were repeated, using the same parameters as described. Chelated Gadoliniuim was used to reduce the risk of toxic effects on the immune system. Tumor volume measurements were performed using T1- and T2-weighted coronal and axial image stacks. The tumors were manually segmented using Amira software (TGS, San Diego) to obtain the tumor volume in cm^3. For each time point, all measurements from the available sequences were used to calculate a mean volume +/- standard deviation in order to limit errors due to the manual segmentation process. As a measure of the amount of contrast taken up by the tumor, we placed a region of interest (ROI) in the tumor or in the adjacent paraspinal muscles on the same MRI slice. The relative signal to noise ratio

(SNR) was calculated as the ratio of the SNR of the tumor to the SNR of normal muscle before and after gadolinium-DTPA injection.

SUPPLEMENTARY FIGURES AND MOVIES LEGENDS

Supplementary Figure 1. Generation, targeting, and verification of the R26-CreER^{T2} allele. a, Targeting strategy for the generation of the R26-Cre-ER^{T2} allele. Position of the 5' probe, an EcoRI-PacI genomic fragment, is shown. A, AscI; E, EcoRI; P, PacI; X, XbaI; CreER^{T2}, adenovirus splice acceptor-Cre-ER^{T2}-PMC polyadenylation site; PURO, PGK-puromycin; DTA, PGK-DTA. **b,** Southern blot analysis of genomic DNA from a floxed R26-Cre-ER^{T2} ES cell clone treated with vehicle alone, C; 250 nM β-estradiol for 3 days, E; or 250 nM 4-hydroxytamoxifen for 3 days, T. Genomic DNA was digested with EcoRI yielding a 15-kb wild-type ROSA26 allele, wt; a 6.4-kb floxed R26-Cre-ER^{T2} allele, 2lox; and a 17-kb excised R26-Cre-ER^{T2} allele, 1lox. **c,** Limited background recombination in R26-Cre-ER^{T2} mice in the absence of tamoxifen. Organs from wild-type C57JBl/6 mice (first panel); male R26-Cre-ER^{T2} mice carrying a LSL-LacZ reporter [Gt(ROSA)26Sor^{tm1Sor} (Jackson Laboratories)] (middle panel); and female R26-Cre-ER^{T2} mice carrying a LSL-LacZ reporter (third panel) were harvested at 6 weeks of age. Whole mount X-gal staining was performed. (A) Brain, (B) heart, (C) lungs, (D) liver, (E) kidney, (F) pancreas, (G) intestine, (H) spleen, (I) uterus, (J) testis.

Supplementary Figure 2. Movies of the MRI scans with 3D reconstructions of the LSLp53; Cre-ER^{T2} mouse described in Fig. 2B are available at http://web.mit.edu/jacks-lab/protocols/Venturamovies.html**.** The entire sequence of the coronal, T2-weighted MRIs taken on Day 0, 12, 18, and 28 after tamoxifen administration are shown. At Day 0, a large lymphoma is observed below the

right kidney, which completely responds to tamoxifen treatment. Movies of the corresponding 3D reconstruction of the tumor on Day 0 and Day 12 are also shown.

Supplementary Figure 3. Two stable and one progressive LSLp53; Cre-ER^{T2} tumors after tamoxifen treatment. a, T1-weighted MRI with gadolinium-DTPA contrast of a mouse with a vertebral osteosarcoma before (left) and after (right) tamoxifen administration. No significant change in the volume of the tumor was evident (middle panel), but significantly less contrast was observed in this bony tumor following tamoxifen (lower panel) consistent with treatment effect. **b, c** T2-weighted MRI images of mice with thymic lymphomas before and after tamoxifen treatment show stable (**b**) or progressive (**c**) disease. **d, e** Immunohistochemistry for p53 of the tumors shown in **b** and **c** demonstrated limited (**d**) or no (**e**) p53 expression correlating with the degree of response to tamoxifen. **f,** PCR analysis of normal tissues or tumor from the mouse (AV 620) with the thymic lymphoma that progressed in **c.** Although normal tissues in this mouse retained the CRE-ER^{T2} allele, the thymic lymphoma lost CRE-ER^{T2}, thereby explaining why this tumor progressed during tamoxifen treatment. Tumor samples from two other LSLp53; CRE-ER^{T2} mice (AV 438 and AV 579) are included as positive controls.

Supplementary Figure 4. Movies of the MRI scans with 3D reconstructions of the LSLp53; Cre-ER^{T2} mouse described in Fig. 4C are available at http://web.mit.edu/jacks-lab/protocols/Venturamovies.html**.** The entire sequences of the coronal, T1-weighted MRI with gadolinium-DTPA taken on Day 0 and

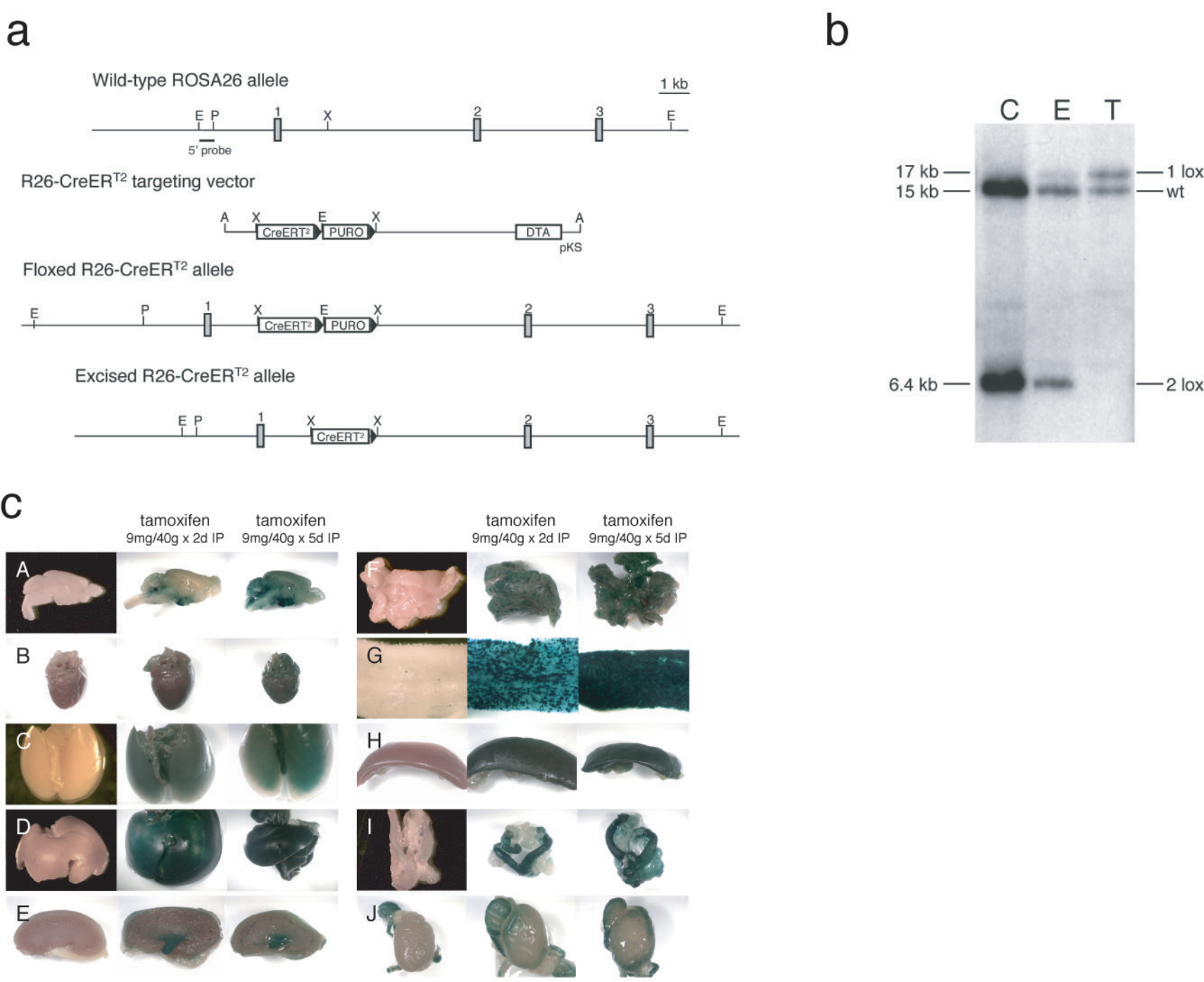
12 after tamoxifen administration are shown. At Day 0, a large sarcoma is observed in the right abdominal wall, which significantly regresses following tamoxifen treatment. Movies of the corresponding 3D reconstruction of the tumor on Day 0 and Day 12 are shown.

Supplementary Figure 5. Prolonged Tamoxifen treatment leads to sarcoma regression. a, A p53LSL/LSL;Cre-ER^{T2} soft tissue sarcoma (STS) (asterisk) before (right) and 12 days after tamoxifen treatment (left). **b.** H & E staining of the residual mass in **c** demonstrates necrosis.

Supplementary Figure 6. Senescence-associated beta-galactosidase staining. A Cre-ERT2-negative (left) and a Cre-ERT2-positive (right) sarcoma from p53LSL/LSL animals treated with Tamoxifen for 5 days were stained for SA-beta-gal and counterstained with nuclear fast red. Only the Cre-ER positive sarcoma shows clusters of senescent cells.

Supplementary Figure 7.

p19^{Arf} expression in tumors, but not in normal tissues of p53LSL/LSL mice. p19Arf immunohistochemistry was performed on a thymic lymphoma (**a**), a sarcoma (**b**) as well as normal liver (**c**) and intestine (**d**) obtained from p53 LSL/LSL mice.


a
Wild-type ROSA26 allele
1 kb
E P 1 X 2 3 E
5' probe
R26-CreER^{T2} targeting vector
A X E X A
CreERT2
PURO
DTA
pKS
Floxed R26-CreER^{T2} allele
E P 1 X E X 2 3 E
CreERT2
PURO
Excised R26-CreER^{T2} allele
E P 1 X X 2 3 E
CreERT2
b
C E T
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15 kb
6.4 kb
1 lox
wt
2 lox
c
tamoxifen
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tamoxifen
9mg/40g x 5d IP
tamoxifen
9mg/40g x 2d IP
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A
B
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Figure S1

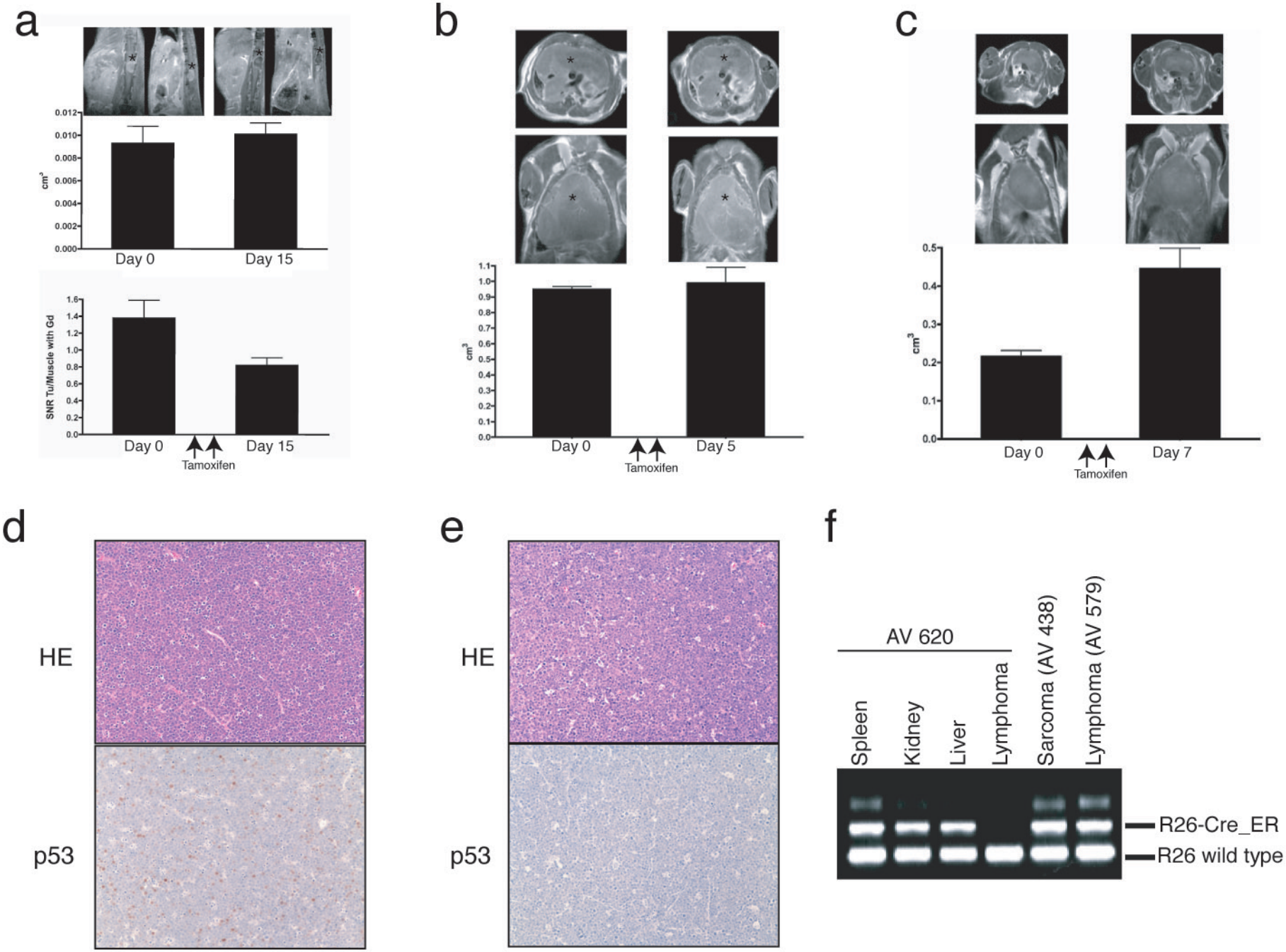
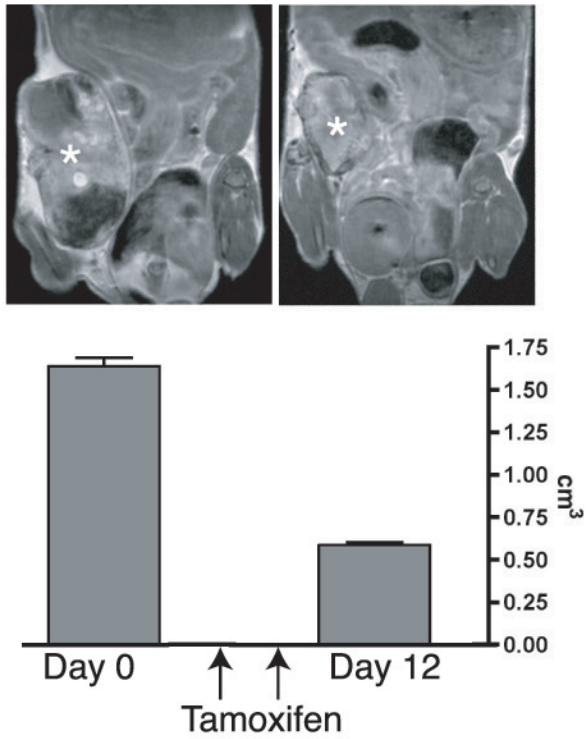

a
b
c
Day 0
Day 15
Day 5
Day 7
Tamoxifen
SNR Tu/Muscle with Gd
cm3
d
e
f
HE
p53
AV 620
Spleen
Kidney
Liver
Lymphoma
Sarcoma (AV 438)
Lymphoma (AV 579)
R26-Cre_ER
R26 wild type

Figure S3

a


1.75
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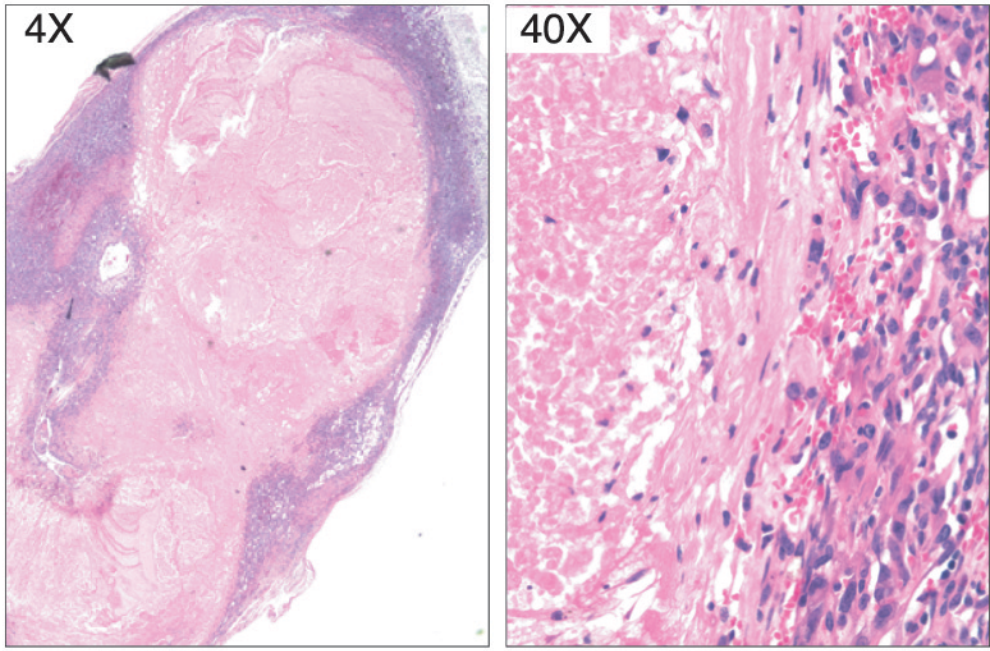
1.25
1.00
0.75
0.50
0.25
0.00
cm3
Day 0
Day 12
Tamoxifen

b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40X

Figure S5

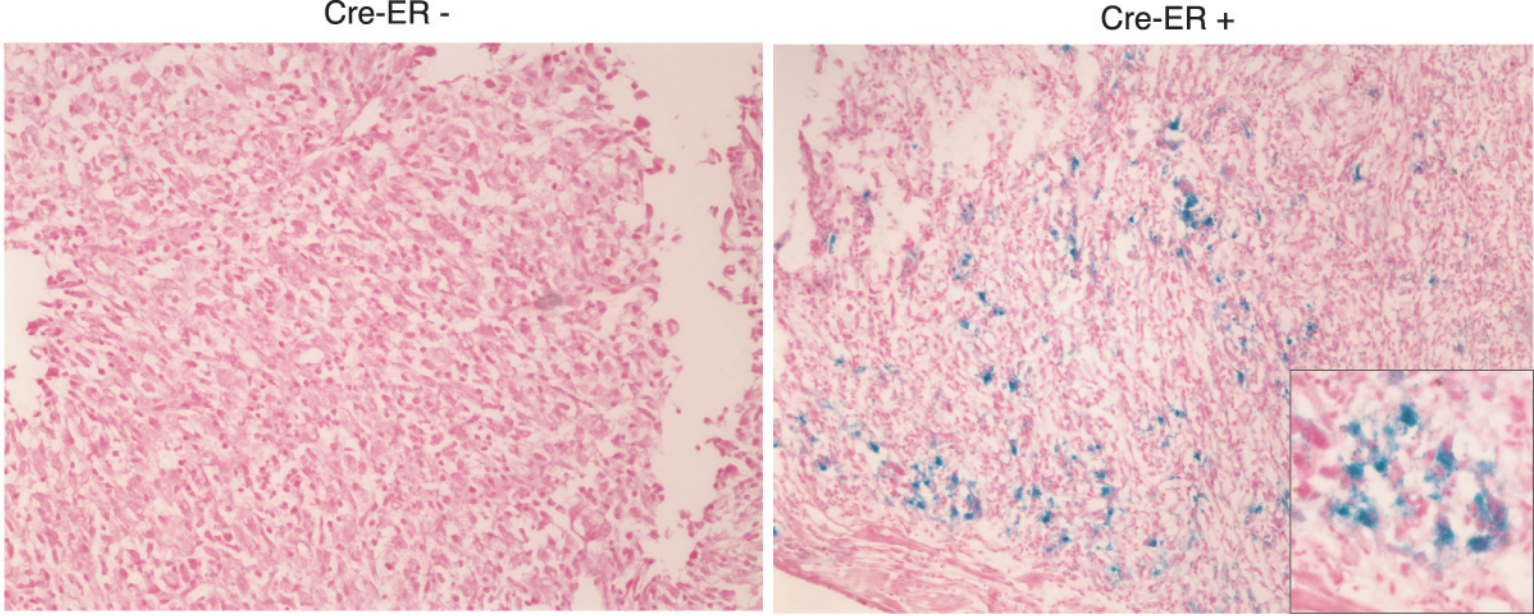
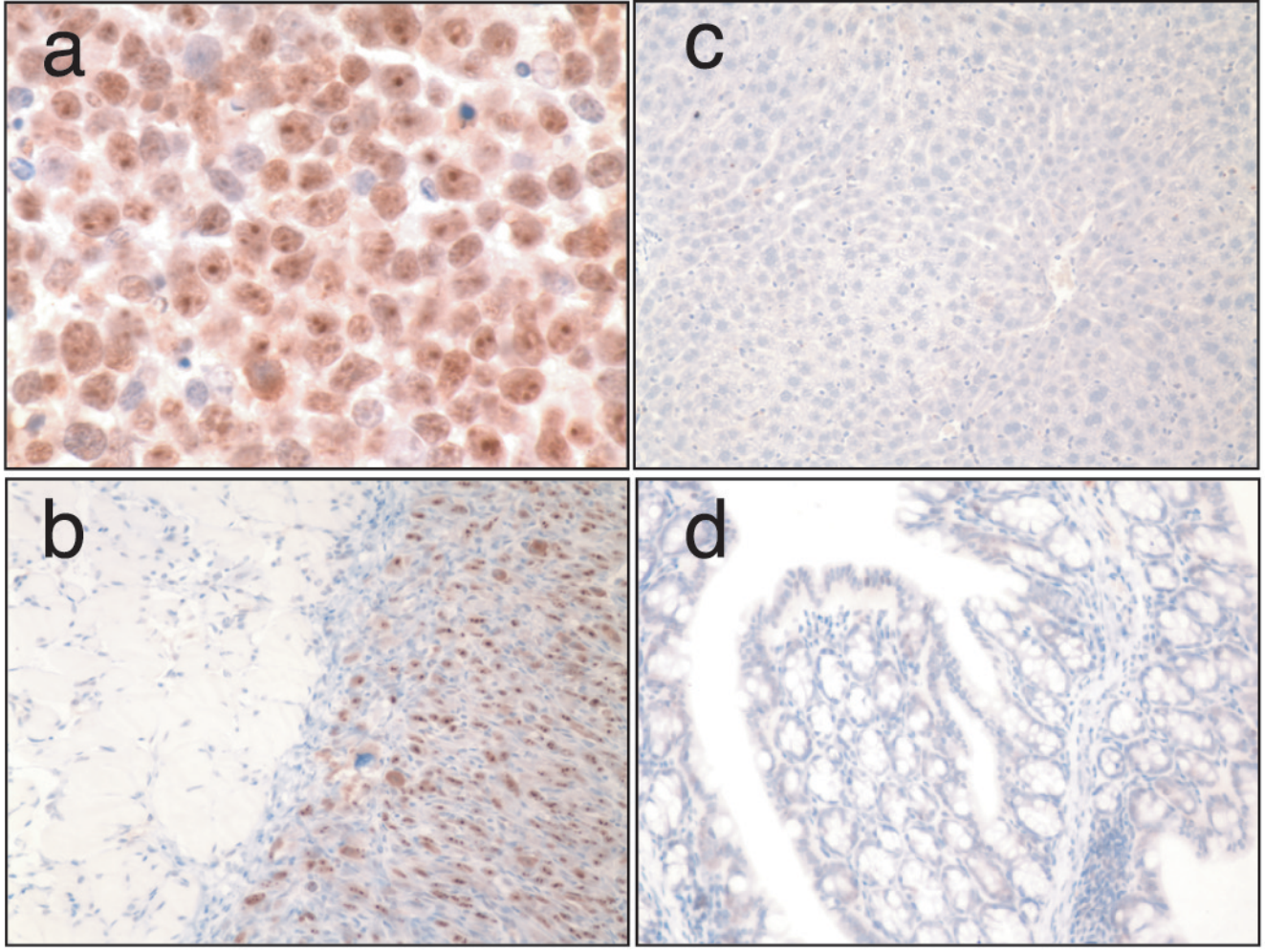


Figure S6



a = lymphoma
b = sarcoma + normal skeletal muscle
c = liver
d = colon

Figure S7