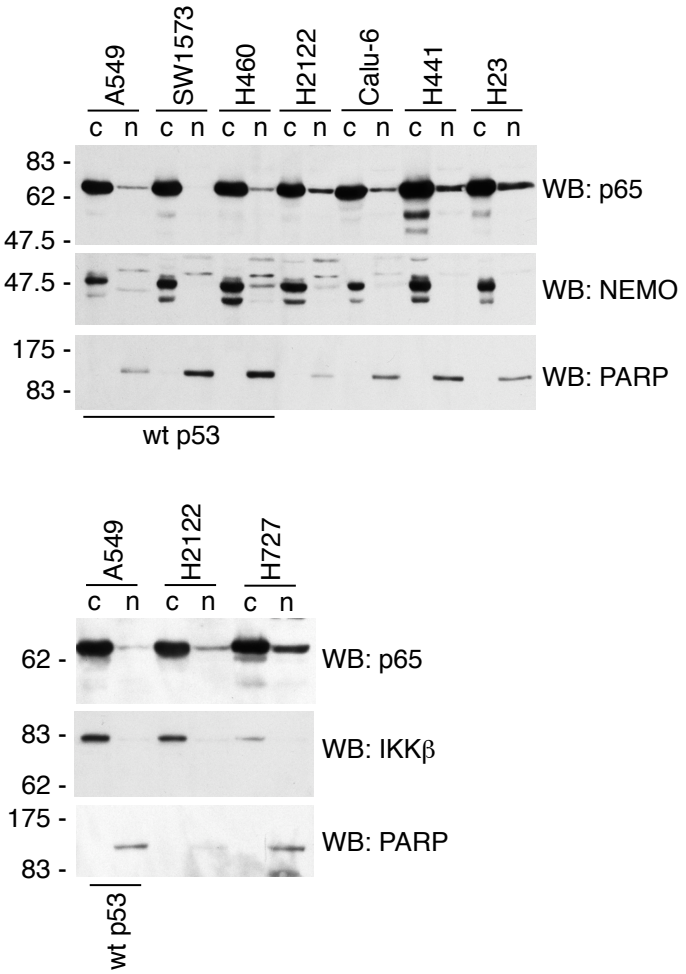
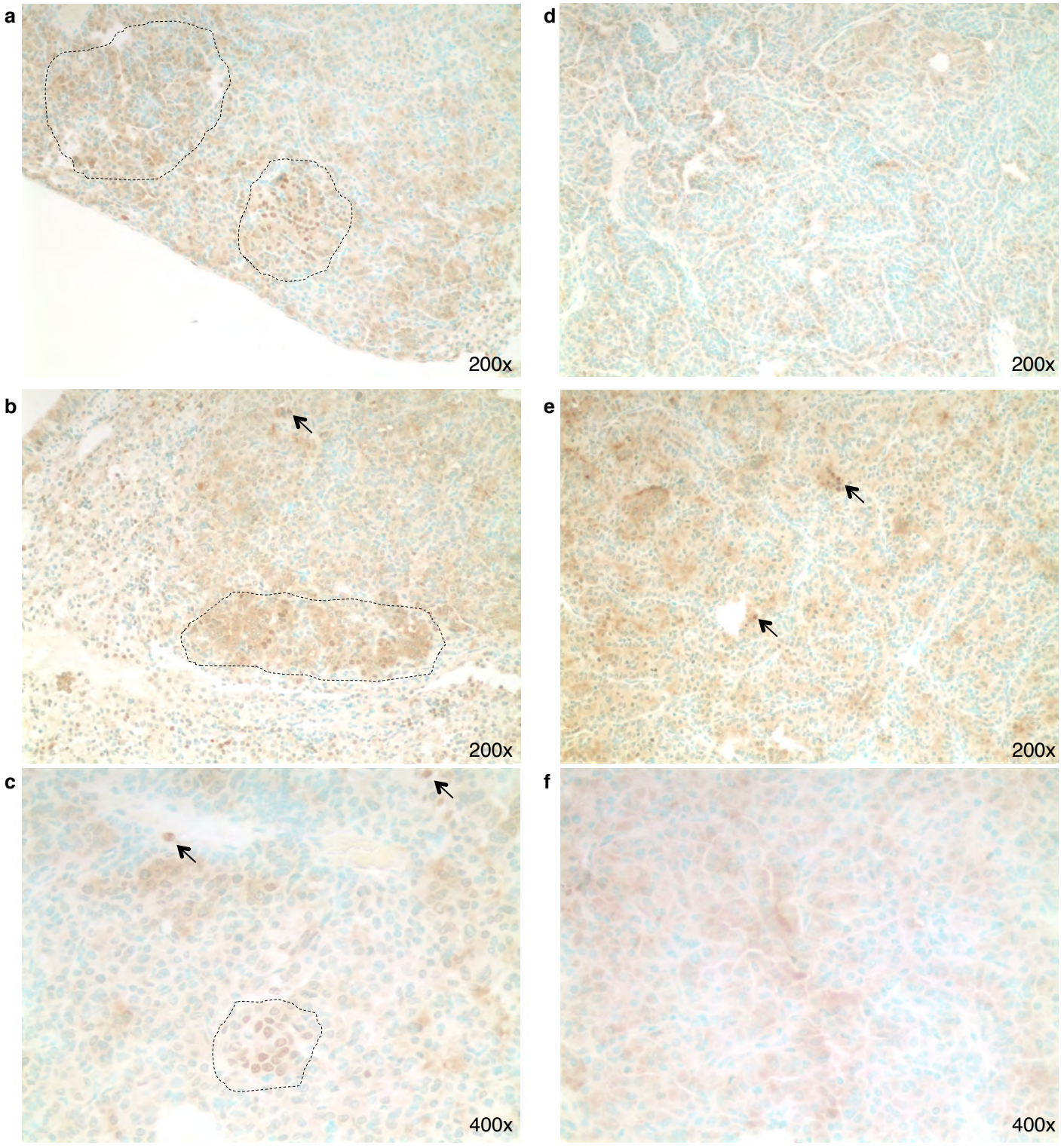
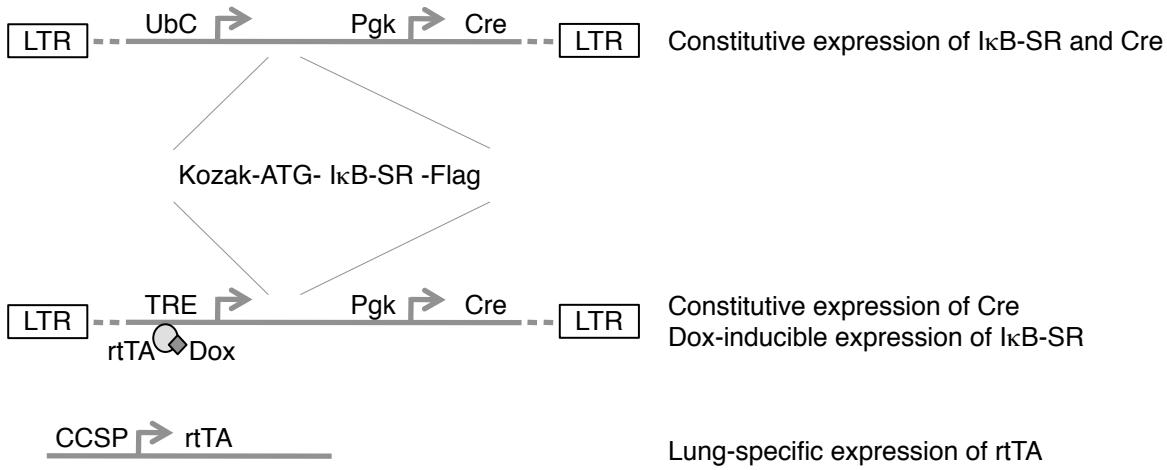


Supplementary Figure 1. Increased nuclear localization of p65 in p53 mutant cell lines. Human NSCLC cell lines expressing WT p53 (A549, SW1573, H460) or mutant p53 (H727 (lower panel), H2122, Calu-6, H441, H23) were lysed to obtain cytoplasmic (c) and nuclear (n) fractions. Lysates were analyzed for the presence of nuclear p65 by western blot. The purity of the fractions was determined by blotting for NEMO or IKKβ (cytoplasmic) and PARP (nuclear).

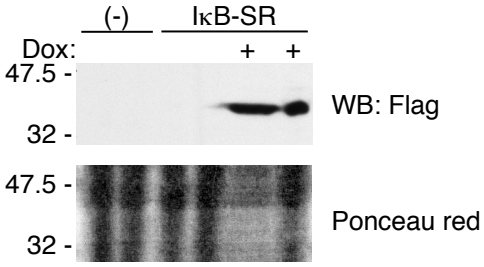


Supplementary Figure 2. Enhanced p65 nuclear staining in K-ras^{G12D}-expressing tumors lacking p53. (**a**-**c**) K-ras$^{LSL-G12D/WT}$; p53$^{Flox/Flox}$ or (**d**-**f**) K-ras$^{LSL-G12D/WT}$ mice were infected with 10^7 adenoviral particles expressing Cre (AdCre). Four months later (in the case of K-ras$^{LSL-G12D/WT}$; p53$^{Flox/Flox}$ mice) or five months later (in the case of K-ras$^{LSL-G12D/WT}$ mice) the mice were sacrificed, and immunohistochemistry for p65 was performed on lung sections. The tissues were counterstained with methyl green. Dashed circles show areas with nuclear p65; arrows show isolated nuclear p65 staining. The magnifications are indicated.

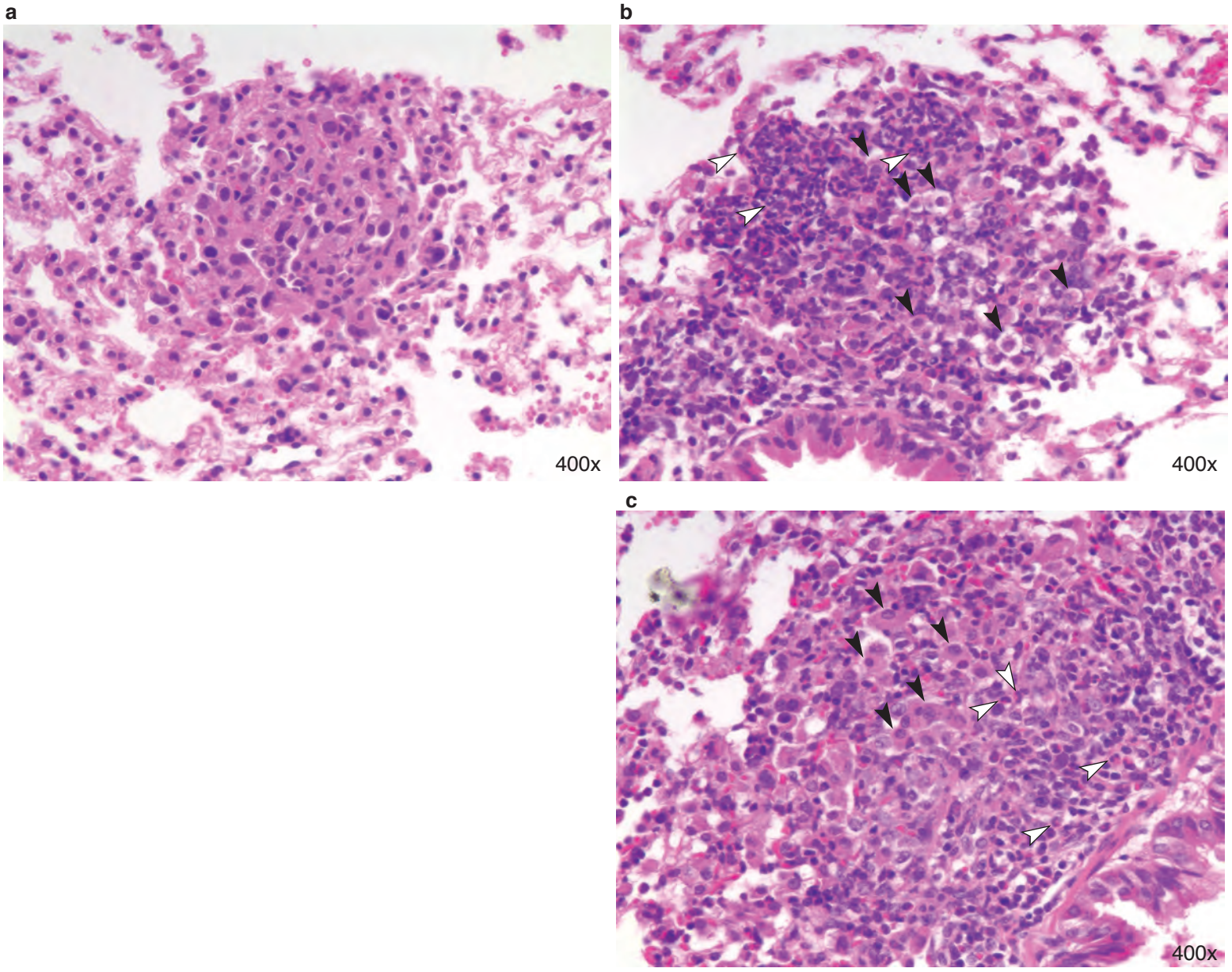
a



b



Supplementary Figure 3. (**a**) **Schematic representation of the lentiviral vectors used in this study.** IκB-SR was cloned downstream of a UbC promoter to allow for constitutive expression (top) or downstream of a TRE promoter to generate a doxycycline-mediated inducible construct (bottom). CCSP, Clara Cell Secretory Protein; Dox, doxycycline; LTR, long terminal repeat; Pgk, phosphoglycerate kinase; rtTA, reverse tetracycline transactivator; UbC, Ubiquitin C. (**b**) **TRE.IκB-SR; Pgk.Cre lentiviral vectors allow for inducible expression of IκB-SR.** 293T cells were infected with lentiviruses containing Cre alone (-) or in combination with a doxycycline-inducible version of Flag-IκB-SR (IκB-SR). 24 h post-infection, the cells were transfected with pSLIK vectors expressing rtTA under the control of the UbC promoter (purchased from the ATCC collection). 24 h later, cells were stimulated with doxycycline, where indicated, at a concentration of 2 μg/ml for 24 h, after which cells were lysed and the inducible expression of IκB-SR was assayed by western blot using anti-Flag antibodies.



Supplementary Figure 4. Recruitment of inflammatory cells in tumors acutely expressing IκB-SR. (**a**-**c**) 14 weeks post-infection with TRE.IκB-SR; Pgk.Cre lentiviruses, mice were put on a doxycycline diet for one week. (**a**) KP; CCSP-rtTA- and (**b**, **c**) KP; CCSP-rtTA+ mouse lung tumor sections were stained with hematoxylin and eosin. Arrowheads indicate neutrophils (white) or macrophages (black). The magnification is indicated.

Supplementary Methods

Measurement of lung tumor volumes. Mice were anesthetized using isoflurane, and were under anesthesia during the entire scanning procedure. Lungs were imaged at the Koch Institute Microscopy and Imaging Core Facility by μ-CT. Image acquisition was performed using eXplore Scan Control software, using a 45 μm voxel size program, and 3D reconstruction was performed using eXplore Reconstruction Utility software (both from GE Healthcare). High-resolution files of the lung scans were generated and individual tumor volumes were measured and calculated using MicroView software (GE Healthcare). For Fig. 4b, the lung surface opacity was set to 25%.

Histology and immunohistochemistry. Mouse lungs were inflated and then fixed in 3.6% formaldehyde in PBS for 24 hours, transferred to 70% EtOH, and embedded in paraffin. 4 μm tissue sections were either deparaffinized and stained with hematoxylin and eosin to count tumor numbers (Fig. 4a), or used for immunohistochemistry. In this case, sections were deparaffinized, boiled in 10 mM Na-citrate, washed in H_2O, treated with 1% H_2O_2 for 10 minutes, washed in H_2O and in PBS. Blocking was done for 1 hour in PBS with 5% goat serum, followed by incubation with anti-p65 antibodies (C-20, 1/200) for 1 hour, then by washing and staining with biotin-conjugated secondary antibodies (1 hour). After washing, avidin-biotinylated HRP complexes were added during 30 minutes (ABC kit, Vectastain), and the complexes were revealed with a DAB peroxidase substrate kit (#SK-4100, Vector Laboratories). The tissues were counterstained with methyl green and mounted.

Expression vectors. Super Repressor IκB (IκB-SR) was cloned from a mouse expressed sequenced tag with a 5' oligo that added an EcoRI site followed by a Kozak sequence prior to the START codon, and mutating codons for Ser32 and Ser36 into Alanine-encoding ones. The 3' oligo introduced a silent mutation removing an EcoRI site in the gene sequence, deleted the IκB STOP codon, added a FLAG tag sequence followed by a STOP and a NotI site. IκB-SR was amplified using Pfx polymerase (Invitrogen), and was cloned into EcoRI-NotI sites located next to a UbC promoter in a dual-promoter lentiviral vector that also expresses Cre from the Pgk promoter. To generate a doxycycline-responsive FLAG-IκB-SR version, IκB-SR was PCR amplified with oligos adding EcoRI sites on both ends, and cloned into an EcoRI-digested TRE; Pgk.Cre lentiviral vector (pCW22), downstream of the TRE promoter. shRNAs to NEMO, c-Rel and p65 were designed by the pSICOLIGOMAKER 1.5 program (http://web.mit.edu/jacks-lab/protocols/pSico.html; created by A. Ventura, Memorial Sloan-Kettering Cancer Center, New York). Oligos were annealed and ligated into pSicoRev retroviral vectors, as described [22]. The fidelity of the PCR amplifications and oligo syntheses were confirmed by sequencing.

Cell culture conditions. The human embryonic kidney (HEK) 293T and mouse fibroblasts 3TZ cells were grown in Dulbecco's modified Eagle's Medium (DMEM). LKR (LKR10, LKR13) and KP cell lines were grown in Minimum Essential Medium (MEM). Lung cells derived from *K-ras*$^{LA2/WT}$; *p53*$^{LSL/LSL}$; *Cre-ER*^{T2} (not published) tumors were grown in DMEM. A549 and SW1573 cell lines were grown in a 1:1 mixture of F12 and DMEM. H23, H2122, H441, H460, Calu-6, H1944, H2009 and H727 were grown in RPMI. All human cell lines were obtained from the ATCC. Early passage mouse embryonic fibroblasts (MEFs, all obtained from the indicated

genotypes in pure 129 backgrounds) were cultured with DMEM. All cell media were supplemented with 1% glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin and 10% heat inactivated FBS.

Statistics. P-values were all determined by Student's t tests.

Nuclear-cytoplasmic fractionations. Cells in 10 cm dishes were washed with cold PBS and harvested by scraping following addition of lysis buffer A (HEPES pH 7.6 20 mM, 20% glycerol, 10 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, 0.1% NP-40, and a proteinase inhibitor cocktail (Roche)). Lysis was completed on ice for 10 minutes. Supernatants containing the cytoplasmic fractions were collected after centrifugation (2000 rpm, 4^0C, 5 minutes). The pellets were washed three times in lysis buffer A and then lysed in buffer B (HEPES pH 7.6 20 mM, 20% glycerol, 500 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, 0.1% NP40, and a proteinase inhibitor cocktail (Roche)) for 30 minutes on ice. After centrifugation (13000 rpm, 4^0C, 15 minutes), the recovered supernatants containing the nuclear lysates were collected and frozen (-70^0C) until use. For Fig. 1a, the data are representative of two independent experiments each performed on two different MEF preparations. For Fig. 1b, the data are representative of results obtained from three independent cell lines.

Cell viability assay. Cells were split into 96-well plates (3000 cells per well). 24 hours later, cells were infected with the indicated retro- or lentiviruses, and 72 hours later cells were fixed in Methanol (10 minutes), dried 15 minutes, stained 10 minutes in Methylene blue dye (0.05% Methylene blue in 1x borate buffer (borate buffer 10x, pH8.4, consists of 100 mM H_3BO_3, 25 mM Na_2BO_7, 120 mM NaCl)), washed three times under gentle tap water, and dried (2 hours, 37^0C). 0.1 M HCl was added for

solubilization, after which cell viability was measured on a spectrophotometer (O.D. 650 nm). Non-infected cell values were set to 1 (100% viability).

RNA purification, reverse transcription and real-time PCR amplification. RNA was purified using Trizol (Invitrogen), according to the manufacturer's instructions. 1 μg RNA was reverse-transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). 10 ng cDNA was used for real-time PCR amplification, using commercially available Taqman probes for mouse *Gapdh*, *Tnf*, *Glut3*, *Ikba*, *Bcl2*, *Bclx*, *Xiap*, *Il6* and *Cxcl1* (Applied Biosystems). Data were normalized to the *Gapdh* levels, and analyzed using the comparative C_T method [30], except for Fig. 4d, where data are presented as fold changes ($2^{-\Delta CT}$ (rtTA+)/$2^{-\Delta CT}$ (rtTA-); mean rtTA- set to 1).

NF-κB p65 DNA-binding activity assay. 5 μg of nuclear extracts were used to determine p65 DNA-binding activity using an ELISA-based assay, according to the manufacturer's instructions (Active Motif TransAM, #40096). Briefly, κB oligonucleotide-coated plates (in a 96-well format) were incubated for 1 hour with the nuclear extracts. Specificity was achieved through incubation with anti-p65 primary antibodies for 1 hour. HRP-conjugated secondary antibodies were used for the detection of p65 bound to the κB sequences.

Reagents. Mouse TNF (used at 50 ng/ml) was from Apotech. Antibodies to cleaved caspase-3 (#9661), IKKβ (2C8) and PARP (46D11) were purchased from Cell Signaling. Antibodies to p65 (C-20), c-Rel (C) and NEMO (FL-419) were purchased from Santa Cruz. Mouse anti-p53 was a kind gift from K. Helin (Copenhagen,

Denmark). Rabbit anti-Flag (F7425) antibodies, 4-hydroxytamoxifen and doxycycline were purchased from Sigma.

Infection of MEFs. In Fig. 1a, primary MEFs (passage 2) of the indicated genotypes were infected with adenoviruses (100 MOI) expressing Cre recombinase (AdCre) or FlpO recombinase (AdFlpO) (University of Iowa, Gene Transfer Vector Core, http://www.uiowa.edu/~gene/). 6 days later, cells were harvested and subjected to the cytoplasmic-nuclear fractionation protocol.

	IkB	Bcl2	Bclx	Xiap	Tnf	Il6	Cxcl1
mean rtTA+	5.26	6.04	2.19	-1.24	12.98	11.79	9.96
s.d. rtTA+	1.10	4.45	2.36	0.76	7.40	6.98	8.25
mean rtTA-	1	1	1	1	1	1	1
s.d. rtTA-	0.21	0.26	0.22	0.09	0.18	0.21	0.31
P-value	0.001				0.04	0.05	

Supplementary Table 1. mRNA expression changes by real-time PCR in rtTA+ compared to rtTA- lung tumors at one week post-Dox feeding. The values correspond to the experiment presented in Fig. 4d. Data show means of relative mRNA expression in rtTA+ (n=4) and rtTA- (n=3) tumors. Mean rtTA- values were set to 1 for each gene analyzed. The P-value shows statistically-significant differences. s.d., standard deviation.