Supporting Information

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SI Materials and Methods

shRNA Library Screen. shRNA "barcode" screens were performed as described using the Netherlands Cancer Institute (NKI) mouse shRNA library containing shRNAs against 14,128 unique mRNA transcripts, selected from the ENSEMBL database, release 24 (1, 2). Two unique 19-mers for each target were selected and cloned into the retroviral vector pRETRO-SUPER-chloramphenicol (pRSC). This vector is derived from pRETRO-SUPER and carries an additional chloramphenicol resistance gene to prevent recombination of hairpin inserts. Additional information about the NKI mouse shRNA library can be found at http://screeninc.nki.nl. Two independent screens were performed. For each screen, 10^6 mouse embryonic fibroblasts (MEFs) expressing K-Ras (K-Raslox) were infected with the retroviral shRNA library, selected in 2 μg/mL puromycin, and plated at low density (5×10^4 cells per 15-cm plate) before treatment with 4-hydroxytamoxifen (4OHT) for 4 wk. Reference samples were harvested from untreated cells. The shRNA inserts were amplified from genomic DNA by PCR using common forward (CCCTTGAACCTCCTCGTTCGACC) and reverse (GACGTCATCAACCCGCTCC) primers, and used as templates for an in vitro linear amplification reaction (MEGAscript T7 Kit; Ambion) to generate RNA. RNAs were subsequently labeled with cyanine-3 (untreated cells) or cyanine-5 (cells treated with 4OHT) fluorescent groups using the Universal Linkage System (Kreatech Biotechnology). These RNA probes were purified, combined, and hybridized to oligonucleotide arrays containing all specific 19-mer sequences. shRNA barcode hits, defined as outliers in both screens, were recloned into pRSC and individually tested for their ability to allow proliferation of MEFs lacking Ras (Rasless). The shRNAs targeting p53 present in the library were found not to inhibit p53 sufficiently.

Plasmids and Viral Vectors. The WT human p53 (Hsp53) cDNA, as well as the Hsp53-6P-NT (N-terminal region), Hsp53-6P-NT/4P-CT (C-terminal region), Hsp53-6KR, and Hsp53-K120/K164/6KR mutants, were kindly provided by S. Llanos and M. Serrano [Centro Nacional de Investigaciones Oncológicas (CNIO)]. These cDNAs were cloned into pWZLblast. Additional mutant Hsp53 cDNAs, including Hsp53-K164R, Hsp53-K319-321R, and Hsp53-R248W, were generated by introducing the corresponding mutations into the WT Hsp53 cDNA using a QuikChange Site-Directed Mutagenesis Kit (Agilent). shRNAs were cloned into pRETRO-SUPER unless otherwise specified. The shRNA sequences and vectors are listed in Table S2. Retroviruses were generated as previously described (3). Lentiviruses were generated using the ViraPower Lentiviral Expression System (Invitrogen). Lentiviral vectors expressing p53 shRNAs have been published (4). Adenoviruses expressing GFP or Cre recombinase have also been described (3). Adenoviruses expressing murine p53 (Mmp53)-GFP (Adeno-Mmp53-GFP) were generated by PCR amplification of a mouse p53 cDNA lacking the STOP codon (kindly provided by S. Velasco, CNIO), followed by cloning into pEGFP-N3 (Clontech). The resulting Mmp53-GFP cDNA was subsequently cloned into pShuttle-CMV.

ChIP Assays. ChIP assays were performed as described previously (5). For precipitation of endogenous mouse p53, we used polyclonal anti-p53 FL-393 antibodies (sc-6243; Santa Cruz Biotechnology). Immunoprecipitated chromatin was analyzed by quantitative real-time PCR and quantified using the comparative threshold cycle (ΔΔCt) method (6), with normalization to input DNA. Primer sequences included *Cdkn1a* promoter 5′ p53 response element: 5′-TGGCCTTCAGGAACATGTCTT-3′, 5′-CACCACCCTGCACTGAAGC-3′; *Cdkn1a* promoter 3′ p53 response element: 5′-TCTAGGTCAGCTAAATCCGAGGA-3′, 5′-GAGTTCTGACATCTGCTCTCCGA-3′; proximal *Cdkn1a* promoter: 5′-GGTTCTAGCTGTCTGGCG-3′, 5′-CTCACACCTCTCGGCTGCT-3′; and *Gapdh* promoter: 5′-AACGACCCCTTCATTGACCTC-3′, 5′-CTTGACTGTGCCGTTGAATTTG-3′.

BrdU Incorporation Assay. To determine the percentage of cells in S phase, cells were maintained in the presence of 50 μM BrdU (Roche) for 3 h. Labeled cells were detected by immunofluorescence staining with anti-BrdU antibodies from BD Pharmingen (556028, 1:1,000) and counterstained with Hoechst 33342 (Molecular Probes).

Protein Analysis. For Western blot analysis, cells were lysed in 150 mM NaCl, 50 mM Tris·HCl (pH 7.5), 0.5% Nonidet P-40 supplemented with 5 mM NaF, 100 μM PMSF, and 2 mM sodium orthovanadate plus protein inhibitor mixture Complete Mini (Roche); separated by SDS/PAGE; and transferred to nitrocellulose membranes. Antibodies were purchased from Cell Signaling Technologies [p53 1C12 (2524, 1:250), p-p53S15 (9284, 1:1,000), Ac-p53K379 (2570, 1:1,000), p-Erk1/2 (9101, 1:250), p-Mek1/2 (9154, 1:250), p-Elk-1 (9181, 1:250), Elk-1 (9182, 1:500)], Santa Cruz Biotechnology [p53 FL-393 (sc-6243, 1:200), p53 DO-1 (sc-126, 1:200), p21 Cdk-interacting protein 1 (Cip1) (sc-397, 1:200), p27Kip1 (sc-528, 1:200), Erk1 (sc-93, 1:500), Mek1 (sc-219, 1:500), Mek2 (sc-525, 1:500), B-Raf (sc-5284, 1:250)], Sigma–Aldrich [GAPDH (G8795, 1:10,000)], Abcam [Mdm2 (ab16895, 1:500), A-Raf (ab19880, 1:250)], Invitrogen [GFP (A11122, 1:500)], Calbiochem [Pan-Ras (OP40, 1:250)], and BD Biosciences [c-Raf (610151, 1:250)]. Anti-p53 1C12 antibodies were used to detect murine p53 and FL-393, or p53 DO-1 antibodies were used to detect Hsp53. Immunofluorescence stainings were performed as described (3) using anti-p21Cip1 antibodies (M7202, 1:400; Dako).

Protein Purification. For purification of Mmp53-GFP fusion proteins, K-Raslox and Rasless cells were infected with adenoviruses expressing Mmp53-GFP. Cells were harvested 48 h later and lysed in radioimmunoprecipitation assay buffer [50 mM Tris·HCl (pH 8), 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% Nonidet P-40] supplemented with Complete Mini Protease Inhibitor Mixtures (Roche) as well as Phosphatase Inhibitor Mixtures 2 and 3 (Sigma–Aldrich). For immunoprecipitation of Mmp53-GFP, 2 mg of total lysate from both cell types was precleared for 1 h with blocked agarose beads (ChromoTek) and incubated for 2 h with 2 μL of GFP-Trap agarose beads (ChromoTek) at 4 °C on a rotating wheel. Beads were subsequently washed according to the manufacturer's guidelines and subjected to LC-tandem MS (MS/MS) analysis.

LC-MS/MS Analysis. Trypsin-digested samples were subjected to LC-MS/MS using an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled to an Eksigent NanoLC (AB Sciex). The nanospray source was fitted with an emitter tip of 10 μm, and voltage of 1.4 kV was applied. Peptide samples, reconstituted in 0.1% formic acid, were loaded on a trap column (300 μm × 10 mm) and run on a 120-min gradient. Data were acquired using an Xcalibur 2.2 (Thermo Fisher Scientific). In the scan range of m/z 250–1,750, the six most abundant ions were selected for

fragmentation. MS data were acquired with an Orbitrap analyzer at an Fourier transformation (FT) resolution of 60,000 at 400 *m/z*. The lock mass was enabled for accurate mass measurements. Polydimethylcyclosiloxane (445.1200026 *m/z*) ions were used for internal calibration. MS data analysis was carried out using Proteome Discoverer (version 1.3; Thermo Fisher Scientific) data using Mascot algorithms. The data were searched against the UniProt *Mus musculus* database. The parameters used for data analysis include trypsin as a protease, which allowed one missed cleavage. Carbamidomethyl cysteine was specified as a fixed modification. Phosphorylation at Ser, Thr, and Tyr; deamidation of Asn and Gln; oxidation of Met; and protein N-terminal and lysine acetylation were specified as variable modifications. The precursor mass tolerance was set to 20 ppm, and the fragment mass tolerance was set to 0.1 Da. The false discovery rate (FDR) was calculated using a decoy database at less than 1% FDR.

1. Bernards R, Brummelkamp TR, Beijersbergen RL (2006) shRNA libraries and their use in cancer genetics. *Nat Methods* 3(9):701–706.
2. Huang S, et al. (2009) ZNF423 is critically required for retinoic acid-induced differentiation and is a marker of neuroblastoma outcome. *Cancer Cell* 15(4):328–340.
3. Drosten M, et al. (2010) Genetic analysis of Ras signalling pathways in cell proliferation, migration and survival. *EMBO J* 29(6):1091–1104.
4. Dirac AMG, Bernards R (2003) Reversal of senescence in mouse fibroblasts through lentiviral suppression of p53. *J Biol Chem* 278(14):11731–11734.
5. Cuadrado A, et al. (2007) A new p38 MAP kinase-regulated transcriptional coactivator that stimulates p53-dependent apoptosis. *EMBO J* 26(8):2115–2126.
6. Yuan JS, Reed A, Chen F, Stewart CN, Jr (2006) Statistical analysis of real-time PCR data. *BMC Bioinformatics* 7:85.

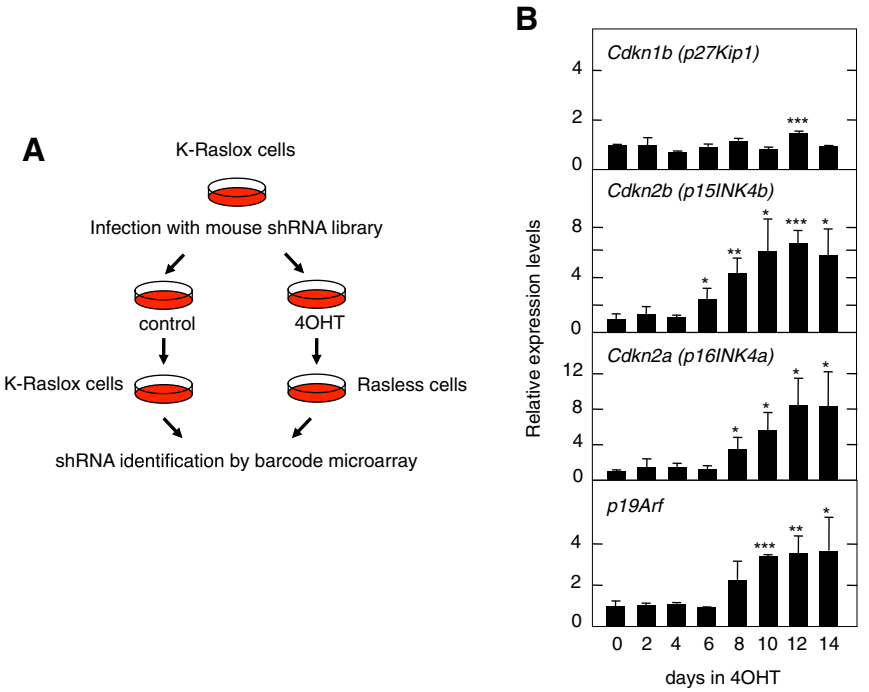

Fig. S1. Schematic outline of the shRNA barcode screen and analysis of cell cycle regulators in K-Raslox and Rasless cells. (*A*) Schematic outline of the shRNA barcode screen. K-Raslox cells were infected with the NKI mouse shRNA library (1), selected with puromycin, and either left untreated (control) or treated with 4OHT for 4 wk to ablate the K-*Ras*^{lox} alleles and generate Rasless cells. shRNA inserts from K-Raslox and Rasless cultures were recovered, labeled, and hybridized to DNA oligonucleotide barcode arrays. (*B*) Quantitative real-time PCR assay showing relative expression levels of the indicated mRNAs in K-Raslox MEFs treated for the indicated times with 4OHT. β-Actin mRNA expression levels were used for normalization. Data are represented as mean ± SD. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (unpaired Student *t* test).

1. Huang S, et al. (2009) ZNF423 is critically required for retinoic acid-induced differentiation and is a marker of neuroblastoma outcome. *Cancer Cell* 15(4):328–340.

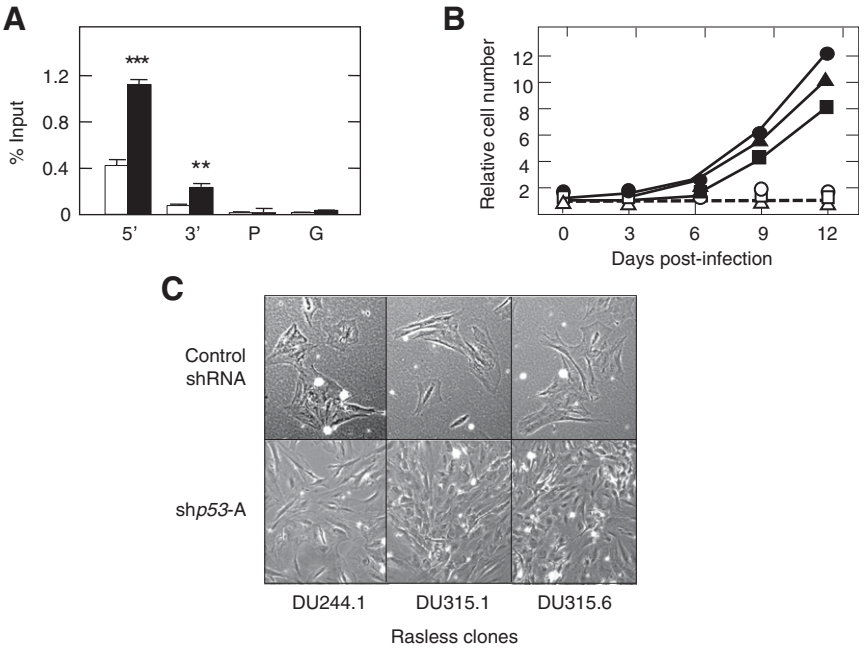

Fig. S2. Rasless cells proliferate after lentiviral infection with an shRNA against p53. (*A*) ChIP assay using p53 antibodies in K-Raslox cells (white bars) or Rasless cells (solid bars). Binding of the 5′ p53-response element to the *Cdkn1a* promoter (5′); the 3′ p53-response element (3′); the proximal *Cdkn1a* promoter (P); and the *Gapdh* promoter (G), which lacks p53 binding sites, was analyzed by quantitative real-time PCR and normalized to the amount of input DNA. Data are represented as mean ± SD. $^{**}P < 0.01$; $^{***}P < 0.001$ (unpaired Student *t* test). (*B*) Growth curve of Rasless cells obtained from three independent cultures of K-Raslox cells (DU244.1, DU315.1, and DU315.6) infected with lentiviruses expressing a control shRNA (open symbols) or an shRNA against p53 (sh*p53*-A; solid symbols). DU244.1 cells (○ and ●), DU315.1 cells (□ and ■), and DU315.6 (△ and ▲) are shown. (*C*) Representative images of the three cultures of Rasless cells indicated in *B*.

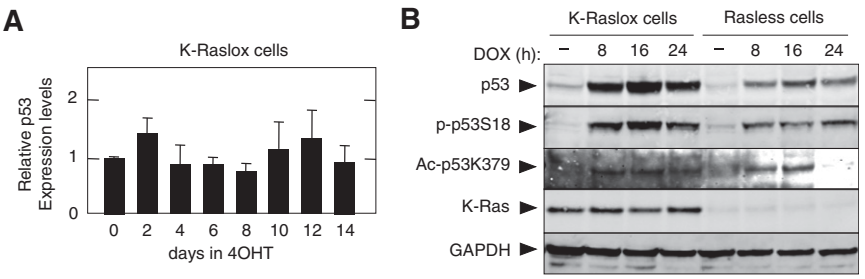

Fig. S3. Characterization of p53 in K-Raslox and Rasless cells. (*A*) Quantitative real-time PCR analysis showing relative expression levels of *p53* mRNA in K-Raslox MEFs treated for the indicated time with 4OHT. β-Actin mRNA expression levels were used for normalization. Data are represented as mean ± SD. (*B*) Western blot analysis of p53, p-p53S18, Ac-p53K379, and K-Ras expression in K-Raslox or Rasless MEFs treated for the indicated time with 5 μg/mL doxorubicin (DOX). GAPDH expression served as a loading control.

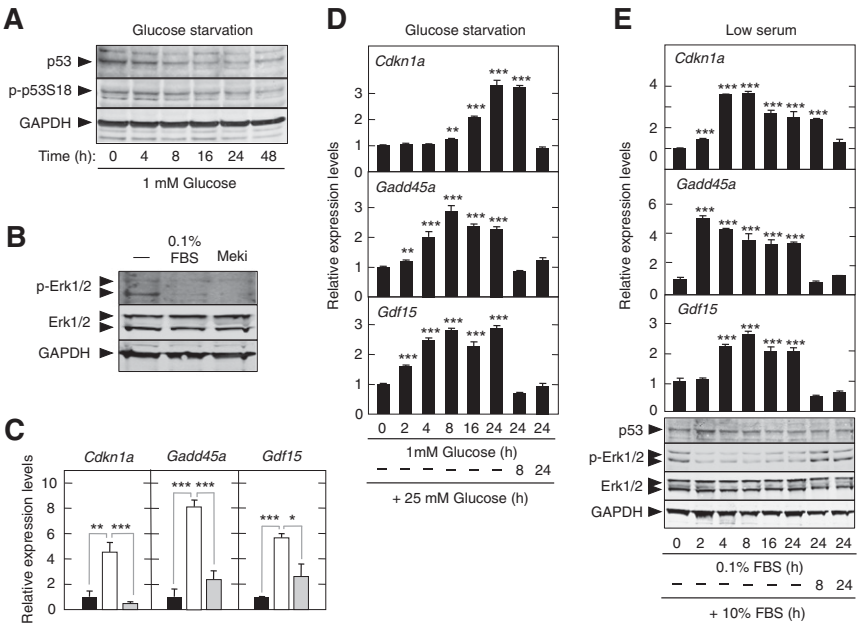

Fig. S4. Reversibility of p53 activation under growth factor or nutrient deprivation. (*A*) Western blot analysis of p53 and p-p53S18 expression in K-Raslox MEFs incubated for the indicated times in the presence of 1 mM glucose. GAPDH expression served as a loading control. (*B*) Western blot analysis of p-Erk1/2 and Erk1/2 expression in untreated K-Raslox cells, in K-Raslox cells grown in 0.1% FBS for 24 h (0.1% FBS), or in K-Raslox cells treated with 0.4 μM Mek inhibitor PD0325901 for 24 h (Meki). GAPDH expression served as a loading control. (*C*) Quantitative real-time (qRT) PCR analysis showing relative expression levels of *Cdkn1a* (p21Cip1), *Gadd45a*, and *Gdf15* mRNA in untreated K-Raslox MEFs (solid bars), Rasless cells (open bars), and Rasless cells expressing a p53 shRNA (sh*p53*-A; gray bars). Data are represented as mean ± SD. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (unpaired Student *t* test). (*D*) qRT-PCR analysis showing relative expression levels of *Cdkn1a* (p21Cip1), *Gadd45a*, and *Gdf15* mRNA in K-Raslox MEFs incubated in the presence of 1 mM glucose for the indicated times and after re-stimulation with 25 mM glucose for the additional indicated times. β-Actin expression levels were used for normalization. Data are represented as mean ± SD. $**P < 0.01$; $***P < 0.001$ (unpaired Student *t* test). (*E*, *Upper*) qRT-PCR analysis showing relative expression levels of *Cdkn1a* (p21Cip1), *Gadd45a*, and *Gdf15* mRNAs in K-Raslox MEFs incubated in the presence of 0.1% FBS for the indicated times and after restimulation with 10% FBS for the additional indicated times. β-Actin expression levels were used for normalization. Data are represented as mean ± SD. $***P < 0.001$ (unpaired Student *t* test). (*E*, *Lower*) Western blot analysis of p53, p-Erk1/2, and Erk1/2 expression levels in K-Raslox MEFs treated as indicated above. GAPDH expression served as a loading control.

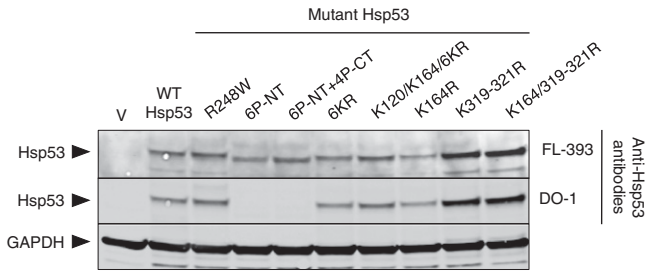

Fig. S5. p53 phosphorylation and acetylation mutants used in this study. Western blot analysis of p53 expression in Rasless cells expressing an shRNA against p53 (sh*p53*-A) and infected with an empty retrovirus [vector (V)], a retrovirus expressing the WT Hsp53 protein, or retroviruses expressing the indicated mutant Hsp53 cDNAs using antibodies that specifically recognize Hsp53 is shown. The anti-Hsp53 FL-393 antibody recognized all Hsp53 proteins. The anti-Hsp53 DO-1 antibody recognizes an epitope in the transactivation domain that is mutated in the Hsp53-6P-NT and Hsp53-6PNT+4P-CT (C-terminal region) cDNAs. GAPDH expression served as a loading control.

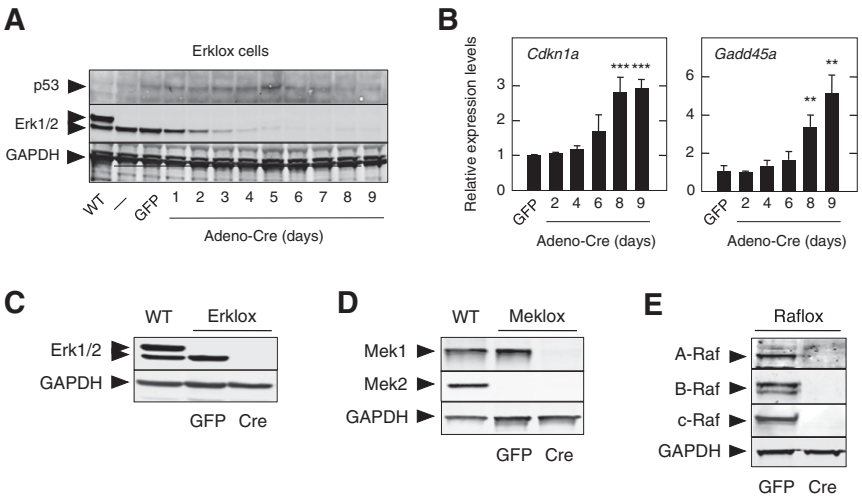

Fig. S6. p53 activation in Erkless (*Erk1*$^{-/-}$;*Erk2*$^{-/-}$) cells and generation of Erkless, Mekless (*Mek1*$^{-/-}$;*Mek2*$^{-/-}$), and Rafless (*A-Raf*$^{-/-}$;*B-Raf*$^{-/-}$;*c-Raf*$^{-/-}$) cells. (*A*) Western blot analysis of Erk1/2 and p53 expression levels in Erklox (*Erk1*$^{-/-}$;*Erk2*$^{lox/lox}$) MEFs left untreated (–), 48 h after infection with Adeno-GFP (GFP), or after the indicated times following infection with Adeno-Cre particles. WT MEFs were used as a positive control. GAPDH expression served as a loading control. (*B*) qRT-PCR showing relative expression levels of p21Cip1 (*Cdkn1a*) and *Gadd45a* mRNA in Erklox MEFs either 48 h after infection with Adeno-GFP (GFP) or after the indicated times following infection with Adeno-Cre particles. Data are represented as mean ± SD. $^{**}P < 0.01$; $^{***}P < 0.001$ (unpaired Student *t* test). (*C*) Western blot analysis of Erk1/2 expression in Erklox MEFs 7 d after infection with Adeno-GFP (GFP) or Adeno-Cre (Cre). WT MEFs were used as a positive control. GAPDH expression served as a loading control. (*D*) Western blot analysis of Mek1/2 expression in Meklox (*Mek1*$^{lox/lox}$;*Mek2*$^{-/-}$) MEFs 7 d after infection with Adeno-GFP (GFP) or Adeno-Cre (Cre). WT MEFs were used as a positive control. GAPDH expression served as a loading control. (*E*) Western blot analysis of A-Raf, B-Raf, and c-Raf expression in Raflox (*A-Raf*$^{lox/lox}$;*B-Raf*$^{lox/lox}$;*c-Raf*$^{lox/lox}$) MEFs 7 d after infection with Adeno-GFP (GFP) or Adeno-Cre (Cre). GAPDH expression served as a loading control.

Table S1. Quantitative Real-Time PCR Primers

Name	Sequence
14-3-3σ-F	5′-GGAGAGAGCCAGTCTGATC-3′
14-3-3σ-R	5′-TCTCCTTCTTGCTGATGTCC-3′
β-*Actin*-F	5′-GACGGCCAGGTCATCACTATTG-3′
β-*Actin*-R	5′-AGGAAGGCTGGAAAAGAGCC-3′
Adhb4-F	5′-AGGATCCTCCAGTGTCTCCA-3′
Adhb4-R	5′-AGCCATGTACCATCACCAGA-3′
Aif-F	5′-AGCAACTGCACAAGACAACC-3′
Aif-R	5′-TCTGTCTCACTCTCCGAACG-3′
Aldh4-F	5′-CCCATCATGAAGGAGGAGAT-3′
Aldh4-R	5′-AGGCCATAGCTGGTAGTGCT-3′
Apaf1-F	5′-AGCTTGTGCACACCTACGAC-3′
Apaf1-R	5′-ATCCCAGAGCTTGAGGAAGA-3′
Bax-F	5′-TAGCAAACTGGTGCTCAAGG-3′
Bax-R	5′-TCTTGGATCCAGACAAGCAG-3′
Bid-F	5′-AGACATCTCGCCCAAATAGG-3′
Bid-R	5′-TTTGTCTTCCTCCGACAGG-3′
Bnip3l-F	5′-CCACCCAAAGAGTTCCATTT-3′
Bnip3l-R	5′-CCTTTCTTCATGGCTCCACT-3′
Btg2-F	5′-GGCTCCATCTGTGTCCTGTA-3′
Btg2-R	5′-CAGCATCATCTGGTTCTTGC-3′
Ccng1-F	5′-AAGTGCTCCAAACCTAACGG-3′
Ccng1-R	5′-GAATCGTTGGGAGGTGAGTT-3′
Cdkn1a ($p21^{Cip1}$)-F	5′-TTCCGCACAGGAGCAAAGT-3′
Cdkn1a ($p21^{Cip1}$)-R	5′-CGGCGCAACTGCTCACT-3′
Cdkn1b ($p27^{Kip1}$)-F	5′-AAGGGCCAACAGAACAGAAG-3′
Cdkn1b ($p27^{Kip1}$)-R	5′-GGATGTCCATTCAATGGAGTC-3′
Cdkn2a ($p16^{INK4a}$)-F	5′-CGTACCCCGATTCAGGTGAT-3′
Cdkn2a ($p16^{INK4a}$)-R	5′-TTGAGCAGAAGAGCTGCTACGT-3′
Cdkn2b ($p15^{INK4b}$)-F	5′-AGATCCCAACGCCCTGAAC-3′
Cdkn2b ($p15^{INK4b}$)-R	5′-CCCATCATCATGACCTGGATT-3′
Clca2-F	5′-CAACTGAAATCAAGAGGGACTATG-3′
Clca2-R	5′-CTGGGAATTGAACTCAGGAC-3′
Cpt1c-F	5′-AGCTTCATCCAGTTGGTCCT-3′
Cpt1c-R	5′-TCTCTGGTGCAAGACCTCAC-3′
Dec1-F	5′-GGCAAGAAATGTTCTGCTCA-3′
Dec1-R	5′-ATTTCAGGTCCCGAGTGTTC-3′
Dhrs3-F	5′-AAGCTTGATGGACAGTGACG-3′
Dhrs3-R	5′-CCTTGGTGGTCCAGAATTG-3′
Dram-F	5′-CTTCCTCCTGGTGACTTGGT-3′
Dram-R	5′-CTGGAGGAGTTGTTCCTGTG-3′
Dusp6-F	5′-CGGATCACTGGAGCCAAAAC-3′
Dusp6-R	5′-GCCTCGGGCTTCATCTATGA-3′
Egfr-F	5′-CAGCGCCTTCAATCCTCAAG-3′
Egfr-R	5′-GCGATGTCAGAAAAGGACTCTGT-3′
Etv4-F	5′-GGACCTCAGTCACTTCCAAGAGA-3′
Etv4-R	5′-AAATCAGGAACAAACTGCTCATCA-3′
Etv5-F	5′-GTTTGTCCCAGATTTTCAGTCTGA-3′
Etv5-R	5′-GCAGCTCCCGTTTGATCTTG-3′
Fas/CD95-F	5′-AACATCAAGGAGGGCAAGAT-3′
Fas/CD95-R	5′-ATGCATCACTCTTCCCATGA-3′
Fosl1-F	5′-AAACCGAAGAAAGGAGCTGACA-3′
Fosl1-R	5′-CCCGATTTCTCATCCTCCAA-3′
Gadd45a-F	5′-CCGAAAGGATGGACACGGTG-3′
Gadd45a-R	5′-TTATCGGGGTCTACGTTGAGC-3′
Gdf15-F	5′-CGAGAGGACTCGAACTCAGA-3′
Gdf15-R	5′-CCCAATCTCACCTCTGGACT-3′
Gamt-F	5′-AGCTGGCTTCCTGAAAGAAA-3′
Gamt-R	5′-GATCATCTGAGGGAAGGCAT-3′
Gls2-F	5′-AATGCCACATTCCAGTCAGA-3′
Gls2-R	5′-TAACCTCCACAGAGCACAGC-3′
Gpx1-F	5′-CAGGAGAATGGCAAGAATGA-3′
Gpx1-R	5′-GAAGGTAAAGAGCGGGTGAG-3′
Hzf-F	5′-AGGAAGAGGAGGAGAAAGCC-3′

Table S1. Cont.

Name	Sequence
Hzf-R	5′-GGCCTCCAGAATTGTGTTGT-3′
Lats2-F	5′-GAACCACATGAGACAGGACAG-3′
Lats2-R	5′-CACAGCGACAGTTGGAAAC-3′
Lpin1-F	5′-GGGAGTGTCCCTGAATAGGA-3′
Lpin1-R	5′-CGTACGAGCTGATGTTGGTC-3′
Mdm2-F	5′-TGTCTGTGTCTACCGAGGGTG-3′
Mdm2-R	5′-TCCAACGGACTTTAACAACTTCA-3′
Ndrg1-F	5′-GTGCTCATGAATGTGAACCC-3′
Ndrg1-R	5′-GTATCTCCTCCTTGCCGAAG-3′
Noxa-F	5′-GCAGAGCTACCACCTGAGTTC-3′
Noxa-R	5′-CTTTTGCGACTTCCCAGGCA-3′
Myc-F	5′-TGACCTAACTCGAGGAGGAGCTGGAATC-3′
Myc-R	5′-AAGTTTGAGGCAGTTAAAATTATGGCTGAAGC-3′
p19^{Arf}-F	5′-GCCGCACCGGAATCCT-3′
p19^{Arf}-R	5′-TTGAGCAGAAGAGCTGCTACGT-3′
Pai1-F	5′-TTCAGCCCTTGCTTGCCTC-3′
Pai1-R	5′-ACACTTTTACTCCGAAGTCGGT-3′
Park2-F	5′-AGGAAGCATACCATGAAGGG-3′
Park2-R	5′-CCTGTAGGCCTGAGAAGTGG-3′
Perp-F	5′-TGGCTGCCATATTCCAGAT-3′
Perp-R	5′-GAAGCCATAGGCCCAGTTAT-3′
Phlda1-F	5′-ACCTCCAACTCTGCCTGAAGAG-3′
Phlda1-R	5′-CACTTTCCGAAGTCCTCAAAACC-3′
Phlda3-F	5′-TCAAGAATCAACAGGCCATC-3′
Phlda3-R	5′-TAGGACACAAGGGTCCCAGT-3′
Pidd-F	5′-TTGGACAGCTTCCTTGTGAC-3′
Pidd-R	5′-GGGCAACCAGAGTCGATAGT-3′
Pml-F	5′-GAGCAGGAGGCTTCTCAGAC-3′
Pml-R	5′-TGTCCTCGTTCTCCTCTGTG-3′
Prkab1-F	5′-CATCTCTTGTGACCCAGCAC-3′
Prkab1-R	5′-TGTAGAGGAGGGTGGTGACA-3′
Ptprv-F	5′-CCCACACGTGCTACCTTATG-3′
Ptprv-R	5′-ATAGCCTGGGATGAAGTTGG-3′
Puma-F	5′-AGCAGCACTTAGAGTCGCC-3′
Puma-R	5′-CCTGGGTAAGGGGAGGAGT-3′
Redd1-F	5′-CTGGACAGCAGCAACAGTG-3′
Redd1-R	5′-ATCAGGTTGGCACACAGGT-3′
Rprm-F	5′-TTCCTGGTCAACAGTAGCGA-3′
Rprm-R	5′-ACACGCATGATGTACAGGCT-3′
Sco2-F	5′-AGGGCTGAGAAGGAACAGTG-3′
Sco2-R	5′-GCTGGCCTTTGTGGTCTAGT-3′
Sesn1-F	5′-CTGAAAGCAGAGGAGCACAG-3′
Sesn1-R	5′-ATTCCACAGCCAAATGTGAA-3′
Sesn2-F	5′-GCTGATCCAGAGGCTCTACC-3′
Sesn2-R	5′-GCATGGCGATGGTGTTATAG-3′
Sidt2-F	5′-CTGGAACATAGCCACCATTG-3′
Sidt2-R	5′-CATTCACCACCGTCTGGTAG-3′
Sod2-F	5′-ATTAACGCGCAGATCATGCA-3′
Sod2-R	5′-TGTCCCCCACCTTGAACTT-3′
Spry2-F	5′-CAGAGTTTGGAAAGAAGGAAAAAGTT-3′
Spry2-R	5′-CAGGTCTTGGCAGTGTGTTCA-3′
Tigar-F	5′-GAAAGCTCTTTGGCAGAGGT-3′
Tigar-R	5′-GCCATGGCTCACAACTAAGA-3′
Tp53-F	5′-AAGATCCGCGGGCGTAA-3′
Tp53-R	5′-CATCCTTTAACTCTAAGGCCTCATTC-3′
Tsc2-F	5′-TTCAGCAACTCCAGAACCTG-3′
Tsc2-R	5′-GTCTGCATAGCTCTCCACCA-3′
Vdr-F	5′-TCACTGGACATGATGGAACC-3′
Vdr-R	5′-CTGACAAGATCAGCCAGGTG-3′
Ypel3-F	5′-TGCTGACAGGTCTTCATGCT-3′
Ypel3-R	5′-CAAAGGCTTGCTCATATTTCC-3′
Zmat3-F	5′-GACTCGGAAAGAAGGGAGTG-3′
Zmat3-R	5′-AGTAAGGGCCTGAATTGCTC-3′

F, forward; R, reverse.

Table S2. shRNA sequences and vectors

shRNA	Vector	Sequence
sh*p21*-A	pRETRO-SUPER	5′-GAGCAAAGTGTGCCGTTGT-3′
sh*p21*-B	pRETRO-SUPER	5′-GCCCTCACTCTGTGTGTCT-3′
sh*p53*-A	pRETRO-SUPER	5′-GTACATGTGTAATAGCTCC-3′
sh*p53*-B	pRETRO-SUPER	5′-GACGTGCCCTGTGCAGTTG-3′
sh*p27*-A	pLKO.1	MISSION TRC0000071063 (Sigma)
sh*p27*-B	pLKO.1	MISSION TRC0000071064 (Sigma)
sh*p15*-A	pRETRO-SUPER	5′-CGCAGATCCCAACGCCCTGAA-3′
sh*p15*-B	pRETRO-SUPER	5′-CTGCCGGTAGACTTAGCTGAA-3′
sh*p16*-A	pRETRO-SUPER	5′-ATCAAGACATCGTGCGATA-3′
sh*p16*-B	LMP	5′-GCCCAGGGCCGTGTGCATGACT-3′
sh*Arf*-A	pRETRO-SUPER	5′-GTTCGTGCGATCCCGGAGA-3′
sh*Arf*-B	LMP	5′-CGCTCTGGCTTTCGTGAACATT-3′
Control	pLKO.1	Nontarget shRNA control SHC002 (Sigma)

Arf, alternate reading frame of the *Cdkn2a* locus; LMP, MSCV/LTRmir30-PIG.