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Supplementary Information for

Loss of p53 function at late stages of tumorigenesis confers ARF-dependent vulnerability to p53 reactivation therapy

Boris Klimovich[a,1], Samet Mutlu[a,1], Jean Schneikert[a], Sabrina Elmshäuser[a], Maria Klimovich[a], Andrea Nist[b], Marco Mernberger[a], Oleg Timofeev[a,2], Thorsten Stiewe[a,b,2,3]

Corresponding author: Thorsten Stiewe
Email: stiewe@uni-marburg.de

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Supplementary Information Text

Materials and Methods

Plasmids. p14ARF CDS (NM_058195.3) was amplified by PCR (primers TTAGGATCCA CCATGGTGCGCAGGTTCTTGGTG, TATGATGAATTCTCAGCCAGGTCCACGGGCAG), subcloned into pCR™8/GW/TOPO® TA vector (Thermo Fischer Scientific) and shuttled into pInducer20 vector (1) using gateway cloning.

Cell culture. To establish lymphoma cell lines, primary lymphoma cells where plated on feeder layers of irradiated 3T3 fibroblasts (30 Gy) in B cell medium (40% IMDM, 40% DMEM, 20% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.05 mM 2-Mercaptoethanol) supplemented with 1 ng/ml IL-7 (Immunotools) and passaged until cell viability reached 90%. In all *in vitro* experiments, p53ER^{TAM} was activated with 100 nM 4-hydroxytamoxifen (4-OHT, Sigma-Aldrich). To establish tamoxifen-resistant late-OFF cell lines, late-OFF lymphoma cells were cultured in the presence of 3 nM 4-OHT. 4 independent TAM-resistant cell pools were established after 2 weeks of culture. DNA damage was induced by treatment with 3 µg/ml mafosfamide (Santa-Cruz Biotechnologies). HCT116 and RKO cells (ATCC) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin and 1 mg/l amphotericin B. Mdm2 inhibitors were used as follows: Nutlin-3a (10 µM, Sigma-Aldrich), AMG 232 (0.75 µM, MedChem Express), MI-773 (1 µM, Selleckchem), RO5963 (10 µM, Merck-Millipore).

Retroviral and lentiviral infection. For retrovirus production, Platinum-E cells (Cell Biolabs) were transfected with retroviral plasmids using a calcium phosphate protocol (2). Supernatants were collected 2-3 days after transfection, 0.45 µM filtered and supplemented with 8 µg/ml polybrene for infection. Infection of lymphoma cells was performed in 12-well suspension plates (Greiner) precoated with retronectin (40 µg/ml,Takara) using spinoculation (1 h, 600 g, 37ºC). Cells were infected twice daily with 6 h interval for 2 days (total 4 infections). Lentiviruses were packaged in HEK293-T cells. Lentiviral packaging plasmids pMD2.G and psPAX2 were a gift from Didier Trono (Addgene plasmids # 12259, 12260). Lentiviral supernatants were collected 24-48 h after transfection and concentrated as described (2). Target cells were infected with 20 µl of concentrated lentivirus in 6-well plates.

RNA interference. Late-OFF lymphoma cells were transduced with TtRMPVIR retrovirus (Addgene plasmid # 27995 (3)) allowing Tet-inducible expression of shRNA (target sequence p19Arf: CGCUCUGGCUUUCGTGAACAU, control: UCUCGCUUGGGCGAGAGUAAG) coupled to dsRed. Cells were treated with 1 µg/ml doxycycline (Sigma-Aldrich) for 24 h to induce shRNA expression before activating p53ER^{TAM} with 3 nM 4-OHT for up to 3 days. Viable dsRed-positive (shRNA-expressing) cells were quantified by flow cytometry.

CRISPR/Cas9 gene editing. HCT116 and RKO cells were co-transfected with pX330 (Addgene plasmid #42230 (4)) encoding *TP53*-exon11-specific sgRNA (UGUCAGUGGGGAACAAGAAG) and a donor DNA for homology-directed repair containing 1000 bp *TP53* gene sequence (GRCh38/hg38 chr17:7.669.112-7.670.111) with the ER^{TAM} domain inserted at position chr17:7.669.611 immediately before the endogenous stop codon. The donor was custom-synthesized (Thermo Fischer Scientific), excised from backbone plasmid with BamHI and PvuI (NEB), gel-purified and used for co-transfection. Transfected cells were expanded as single-cell clones and analyzed by PCR and Sanger sequencing for correct recombination.

RTqPCR and RNAseq. RNA from lymphoma tissues and cultured cells was isolated using RNeasy Mini kit (Qiagen). For RTqPCR, cDNA was synthesized using SuperScript VILO cDNA Synthesis kit (Thermo Fisher Scientific) according to manufacturer's instructions. RTqPCR was performed using ABsolute QPCR Mix, SYBR Green (Thermo Fisher Scientific) and LightCycler

480 (Roche). Expression data were normalized against β-actin. For RNAseq, RNA quality was assessed using the Experion RNA StdSens Analysis Kit (BioRad). RNAseq libraries were prepared from total RNA using the TruSeq Stranded mRNA LT kit (Illumina) according to the manufacturer's instructions. Quality of sequencing libraries was controlled on a Bioanalyzer 2100 using the Agilent High Sensitivity DNA Kit (Agilent). Pooled sequencing libraries were quantified with digital PCR (QuantStudio 3D, Thermo Fisher) and sequenced on the HiSeq 1500 platform (Illumina) in Rapid-Run mode with 50 bases single reads. Obtained sequencing data were aligned to the Ensembl *Mus musculus* reference genome, revision 92 (mm10) using STAR and read counts were obtained for exonic regions. Genes with less than 10 reads in all samples were pruned from the count table. Subsequently, differential expression was assessed using DEseq2 (5) and false discovery rate was controlled via Benjamini-Hochberg correction. Sequencing data were deposited at EBI ArrayExpress (accession number E-MTAB-8015).

Primer sequences for RT-qPCR:

Gene	sense	antisense
Actb	CATTGCTGACAGGATGCAGAAGG	TGCTGGAAGGTGGACAGTGAGG
Cdkn2a/p19ARF	TGGTCACTGTGAGGATTCAGC	GTTGCCCATCATCATCACCTGG
Sesn2	ATTACCTGCTGCTGCATACG	GGTCTGCAGGAACTCAGTCA
Ddit4	GCCGGAGGAAGACTCCTCATA	CATCAGGTTGGCACACAGGT
Phlda3	TTCGCCCGATCAAAGCCGT	AGGGGGCAGCGGAAGTCGAT
Pmaip1/Noxa	GAGTGCACCGGACATAACTG	CTCGTCCTTCAAGTCTGCTG
Bbc3/Puma	GTACGAGCGGCGGAGACAAG	GCACCTAGTTGGGCTCCATTTCTG
Mdm2	CTAGCTTCTCCCTGAATGCC	TTGCACACGTGAAACATGAC
Gls2	GAACAAGATGGCTGGGAACGA	CGGAGCCGATGGCGTAATTCCG
Bax	TAGCAAACTGGTGCTCAAGG	TCTTGGATCCAGACAAGCAG
Sfn/14-3-3s	CCTGCTTTCCGTAGCTTACA	TCCCGGTACTCTTTCACCTC
Aldha4	CGCCTGGCTGGAGAGTGTGGCG	GGCCGCCGTACTCGAATG
Dram1	GGGCATCGTAGCCAACTTCC	CGGTGAAAGCCAGAAGCGCACCG
Mx2	TCAATCCGACTCCACTTCAA	TGCCATGCTTTGTCTTCTTC
Ccng1	AAGTGCTCCAAACCTAACGG	GAATCGTTGGGAGGTGAGTT
Cdkn1a	CAAGAGGCCCAGTACTTCCT	ACACCAGAGTGCAAGACAGC

Analysis for genomic *Cdkn2a* deletion. Genomic DNA was isolated from primary lymphoma tissues or in vitro cultured cell lines with peqGOLD Tissue DNA Kit (PeqLab) and used as template for qPCR with primers mapping to different regions of the *Cdkn2a* gene locus or a reference region 50 kb upstream. ΔCt values were used as a measure for *Cdkn2a* deletion.

Primer sequences:

pair #	sense	antisense
1	CTTTCGCTCCGGTTAACTTTC	CCTCGCCGATCTTCCTATTT
2	AGTGCTGGGACTAAAGGGATTTG	CACCATTAAGGAGGACGCCTAG
3	CAGCTGCTCTTCTTCCTCTCC	AGAATACAGCAGGATCAGGTACG
4	GCTGACAGTAGATGGGAGAAAC	GGAGCCACCCATTAAACTAACT
5	GGGACATCAAGACATCGTGC	GTTCCCAGCGGTACACAAAG
6	CAATCCCAAGAGCAGAGCTAAATC	TTAAAGCCACATGCTAGACACG
Cdkn2a-50kb	GGTGCTAGTGAGGGCTTGGC	ACATTTCCCTACCGCCGCTG

***Cdkn2a* methylation analysis.** Genomic DNA isolated from lymphoma tissues was subjected to bisulfite conversion with EpiTect Bisulfite Kit (Qiagen) according to the manufacturer's protocol. To control conversion efficacy, 1 µg of genomic DNA was methylated in vitro in the presence of 4 IU of CpG Methyltransferase M.SssI (NEB) and 1.6 mM S-adenosylmethionine for 1 h at 37°C, purified by phenol-chloroform extraction and used for bisulfite conversion. Primers for amplification of converted DNA were designed using MethPrimer software (6). Converted DNA was amplified with primers GTTTTTGGTTATTGTGAGGATTTAG and AACCTTTCCTACCTAATCCAAAATT and cloned into the pCR2.1 vector using TOPO-TA Cloning Kit (Thermo Fisher). Single plasmid clones were analyzed by Sanger sequencing (LGC).

Chromatin immunoprecipitation. For chromatin preparation, 50-200 mg of fresh-frozen lymphoma tissues (lymph nodes) were transferred in tissueTUBE TT1 (Covaris), submerged in liquid nitrogen for 10 s and then pulverized with a hammer. Pulverized tissue was resuspended in 10 ml of PBS supplemented with protease inhibitors (cOmplete ULTRA Tablets Mini, Roche) and crosslinked by addition of 0.5 ml of 18.5% paraformaldehyde (10 min, RT). After incubation for 5 min, 1 ml of 1.25 M glycine was added and after 5 min incubation samples were centrifuged. The supernatant was discarded and the pellets were frozen. Pellets were then lysed in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris pH 8.1) supplemented with protease inhibitor (1.6 ml buffer/70 mg tissue). Samples were then sonicated using Bioruptor® (Diagenode) (5 cycles of 30 sec "ON" and 30 sec "OFF"). After centrifugation, 100 µl sheared chromatin was diluted 1:10 with dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.1, 167 mM NaCl) and pre-cleared for 1 h with Protein G Sepharose™ 4 Fast Flow (GE Healthcare) at 4°C. Afterwards, 1% input was sampled. Proteins were precipitated with 2.5 µg antibody for H3K27me3 (Merck-Millipore, 07-449), H2AK119ub (Cell Signaling, D27C4) and normal rabbit IgG (Cell Signaling, 2729) overnight at 4°C. Next day, 50 µl of beads pre-blocked in 0.5% BSA were added and samples were incubated 4 h at 4°C. Beads were pelleted and washed once with low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl), once with high salt wash buffer (0.1% SDS, 1%Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 500 mM NaCl), once with LiCl wash buffer (0.25 M LiCl, 1% IGEPAL-CA630, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH 8.1), and twice with TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA was de-crosslinked from proteins by adding 100 µl of 10% Chelex suspension to ChIP and input samples, 10 min incubation at 99°C followed by proteinase K digestion for 30 min at 55°C and inactivation of proteinase K at 99°C for 10 min. Precipitated DNA was recovered by two rounds of elution with 2x100 µl sterile water. DNA was further diluted 1:2 with water. 3 µl DNA were used as a template for qPCR. The abundance of each modification was calculated as % input.

ChIP primer sequences:

pair #	sense	antisense
1	GGGTCTCGAGGTGCCTCAAC	TCCCGCTGCTGTACTCCCTCAG
2	CTTTCGCTCCGGTTAACTTTC	CCTCGCCGATCTTCCTATTT
3	GCTGACAGTAGATGGGAGAAAC	GGAGCCACCCATTAAACTAACT
4	GGGACATCAAGACATCGTGC	GTTCCCAGCGGTACACAAAG
5	CAATCCCAAGAGCAGAGCTAAATC	TTAAAGCCACATGCTAGACACG

Flow cytometry. Cell viability was determined by propidium iodide (1 µg/ml) exclusion. Apoptosis was detected by Annexin-V-APC (MabTag) and FITC-VAD-FMK (CaspGLOW™

Fluorescein Active Caspase Staining Kit, Biovision) according to manufacturer's instructions. All flow cytometry experiments were performed using an Accuri C6 (BD Biosciences).

Live cell imaging. Real-time monitoring of tumor cell proliferation was performed using an IncuCyte S3 Live-Cell Analysis System (Sartorius). Cells were seeded on 96-well plates overnight and treated with 1 μM 4-OHT and/or 10 μM nutlin-3a the next day. Doxycycline treatment of HCT116_p53ER^{TAM} pInd-p14ARF was started 24 h before addition of 1 μM 4-OHT. 4 phase contrast images per well were recorded every 2 hours at 10x magnification, with 3 replicate wells per treatment condition. Confluence analysis was performed with IncuCyte S3 2018A software in Phase Object Confluence mode, using a segmentation score of 0.7 and excluding objects smaller than 500 μm^2. Proliferation curves were normalized to the confluence of (not TAM-treated) reference cells at the end of the time course.

Western blot and immunohistochemistry. Cells were lysed in NP-40 Lysis Buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 2% NP-40, pH 8.0) supplemented with protease inhibitor (complete ULTRA tablets EASYpack, Roche) and phosphatase inhibitor (PhosSTOP, Roche). Tissue samples were homogenized in RIPA-buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, pH 7.4) supplemented with protease inhibitor using TissueLyser LT (Qiagen), sonicated with Bioruptor® (Diagenode) and clarified with centrifugation (13 000 g, 10 min). Protein yield was determined by Bradford assay (Bio-Rad). Total protein (5-100 μg) was separated on NuPAGE SDS Gels (Life Technologies) and tank-blotted to nitrocellulose membranes. Following blocking in TBST (5 mM Tris, 15 mM NaCl, 0.1% Tween 20, pH 7.5) with 5% nonfat dry milk, membranes were incubated with primary antibodies diluted in TBST/5% nonfat dry milk and incubated overnight at 4°C. Antibodies: mouse p53 (CM5, Leica Biosystems), human p53 (DO1, gift from B. Vojtesek), p19Arf (5-C3-1, Santa Cruz), p14 ARF (4C6/4, Cell Signaling), cleaved caspase-3 Asp175 (5A1E, Cell Signaling), β-actin (AC-15, Abcam). Proteins were detected with secondary antibody (anti-mouse IgG-HRP, anti-rabbit IgG-HRP, GE Healthcare, 1:5000) or anti-rat IgG-HRP (Rockland, 1:5000) using WesternBright ECL Substrat Sirius kit (Biozym). Anti-actin antibodies were detected using goat anti-mouse-Alexa-488 conjugate (Thermo Fisher Scientific, A-11029). Tissues were fixed with 4% buffered formalin overnight, embedded in paraffin, sectioned and immunostained for p53 (CM5, #p53-CM5P-L, Leica Biosystems), p19Arf (5-C3-1, Santa Cruz) and cleaved caspase-3 Asp175 (5A1E, Cell Signaling) using standard procedures.

Animal studies. Eμ-Myc (B6.Cg-Tg(IghMyc)22Bri/JThst) mice were crossed with p53ER$^{TAM/+}$ knock-in (B6;129Sv-*Trp53*^{tm1Gev}/Gev) animals (7) to generate Eμ-Myc;p53ER$^{TAM/+}$ males that were further crossed with p53ER$^{TAM/+}$ females to obtain Eμ-Myc;p53ER$^{TAM/TAM}$ embryos. To maintain an active p53 status during development of the hematopoietic lineage, pregnant mice were injected intraperitoneally (i.p.) with 50 μg/kg tamoxifen (TAM, Sigma-Aldrich) dissolved in corn oil (Sigma-Aldrich) starting from day E7.5. At E13.5, embryos where genotyped by PCR and fetal livers cells (FLCs) were isolated. Following lysis of red blood cells with ACK buffer (Thermo Fischer Scientific), FLCs were cultured in B cell medium (40% IMDM, 40% DMEM, 20% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.05 mM 2-Mercaptoethanol) supplemented with 0.2 ng/ml IL-3, 2 ng/ml IL-6, 20 ng/ml SCF (all cytokines from Immunotools) and 4% WEHI-3B supernatant. For establishment of p53-ON lymphomas all media were continuously supplemented with 100 nM 4-hydroxytamoxifen (4-OHT, Sigma-Aldrich). FLCs were transduced with luciferase-expressing retrovirus (pMSCV-Firefly-T2A-Gaussia) to enable longitudinal monitoring of disease progression. FLCs were plated in 6-well plates precoated with retronectin (40 μg/ml, Takara) and transduced with fresh virus supernatant (1 ml/well supplemented with 8 μg/ml polybrene) using spinoculation (25 min, 600xg, 37ºC). Cells were infected twice daily with 6 h interval for 2 days (in total 4 infections). Infected FLCs were transplanted into B6-albino mice (B6N-Tyr^{c-Brd}/BrdCrCrl, Charles River and C57BL/6Brd CrlHsd-Tyr^{c}, Envigo) lethally irradiated

with 7 Gy X-rays 6 hours before transplantation. Recipients were kept on neomycin-supplemented water (1.6 g/L, pH 3) starting 2 days before transplantation until 3 weeks after transplantation. For generation of p53-ON lymphomas, mice were constantly fed with tamoxifen-supplemented chow (LASvendi CreActive TAM400). For bioluminescence imaging (BLI) of lymphomas, mice were i.p. injected with 200 µl of D-luciferin (15 mg/ml in PBS, PJK). Animals were anesthetized with 2% isoflurane and imaged 5 min after injection with IVIS 50 system (Xenogen) for 30 seconds. Primary FLC recipients were euthanized by cervical dislocation when reaching the humane endpoint specified by the experimental protocol. Lymphoma cells were isolated from lymph nodes and spleens and used for establishment of cell lines or further propagation by serial transplantation. For reactivation experiments, cohorts of B6-albino mice were intravenously transplanted with lymphoma cells, fed with normal chow and monitored by BLI. Animals were treated for 7 days with daily i.p. injections of tamoxifen (100 µl of 10 mg/ml solution in corn oil) or vehicle. Treatment started 4 days after transplantation (Fig. 2G) or once bioluminescence was clearly detectable in spleen and cervical lymph nodes (Fig. 2E,F). All animal experiments were performed according to the German Animal Welfare Act and approved by the Regional Board Giessen.

Statistical analysis. For animal experiments, an *a priori* power analysis was performed to calculate the group size needed to measure an estimated effect size (Cohen's *d*) of 1.0 with sufficient statistical power (α=0.05, 1-β=0.80). GraphPad Prism 8 Software was used to generate all plots and perform statistical analysis. False discovery rates were calculated with the two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (8). *P* or FDRq values <0.05 were considered significant.

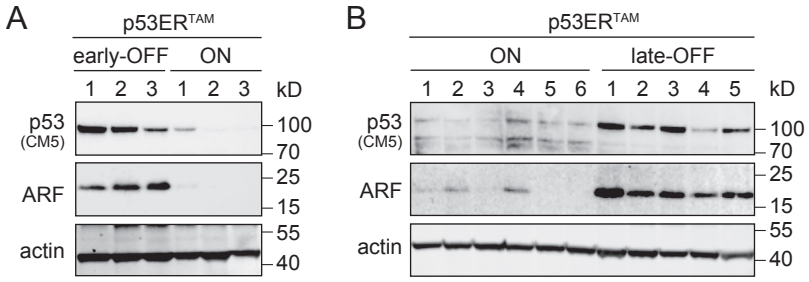

Fig. S1. Western blot for p53ER^{TAM} and ARF expression in Eμ-Myc lymphomas. (A) Shown are independent early-OFF (n=3) and p53-ON (n=3) Eμ-Myc lymphomas. (B) Shown are independent p53-ON (n=6) and late-OFF (n=5) lymphomas. p53ER^{TAM} was stained with anti-p53 (CM5, Leica) antibody.

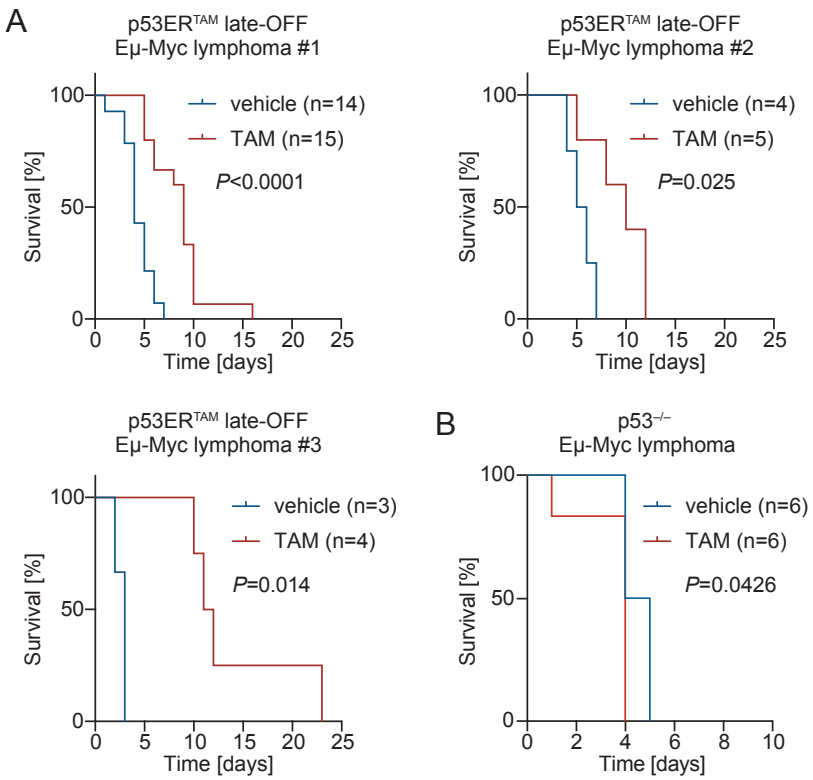

Fig. S2. p53 reactivation response in multiple independent late-OFF and a p53$^{-/-}$ Eμ-Myc lymphoma.

(A) Three independent primary p53-ON Eμ-Myc lymphomas from moribund TAM-fed mice were transplanted into recipient mice which were fed with normal chow to inactivate p53ER^{TAM}. The resulting p53 late-OFF lymphomas were further transplanted into cohorts of recipient mice fed with normal chow. Once bioluminescence was clearly detectable in spleen and cervical lymph nodes, the animals were treated for 7 days with daily i.p. injections of tamoxifen (100 µl of 10 mg/ml solution in corn oil) or vehicle.

(B) Animals with p53$^{-/-}$ Eμ-Myc lymphoma were treated for 7 days with daily i.p. injections of tamoxifen (100 µl of 10 mg/ml solution in corn oil) or vehicle.

Shown is time after start of treatment and *P*-values of Log-Rank test.

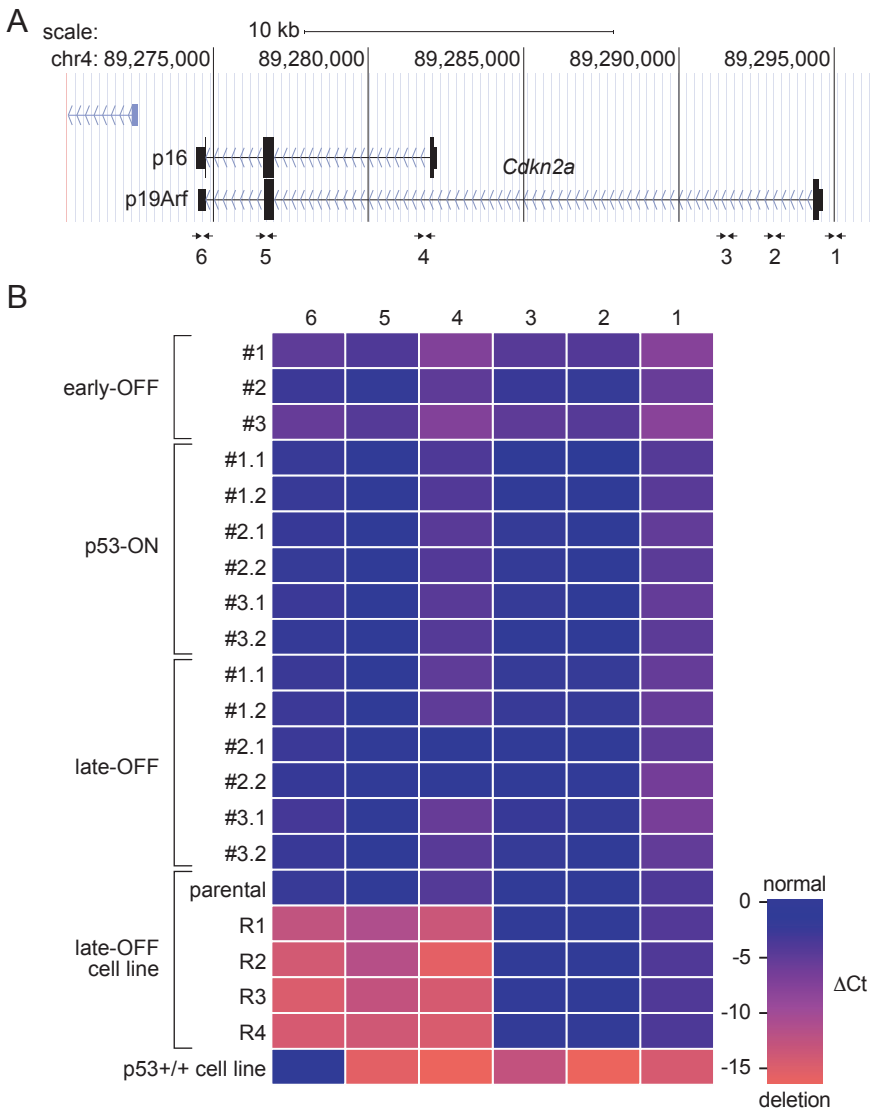

Fig. S3. Deletion of *Cdkn2a* gene locus in TAM-resistant Eμ-Myc lymphomas. Genomic DNA from lymphoma tissue of early-OFF (n=3), p53-ON (n=6) and late-OFF (n=6) Eμ-Myc mice was analyzed by quantitative PCR using primer pairs for the *Cdkn2a* locus and a reference locus 50kb upstream. (A) Map of the gene gene locus depicting location of primer pairs used for qPCR. (B) Heatmap depicts ΔCt values (Ct of reference minus Ct of *Cdkn2a*) of individual lymphoma samples. In addition, the graph shows results for a (*parental*) late-OFF lymphoma cell line before treatment with 3 nM 4-OHT (TAM) and 4 independent TAM-resistant cell pools (R1-R4) established after 2 weeks of culture. A $p53^{+/+}$ Eμ-Myc lymphoma cell line is shown as a positive control for a *Cdkn2a/p19Arf* deletion.

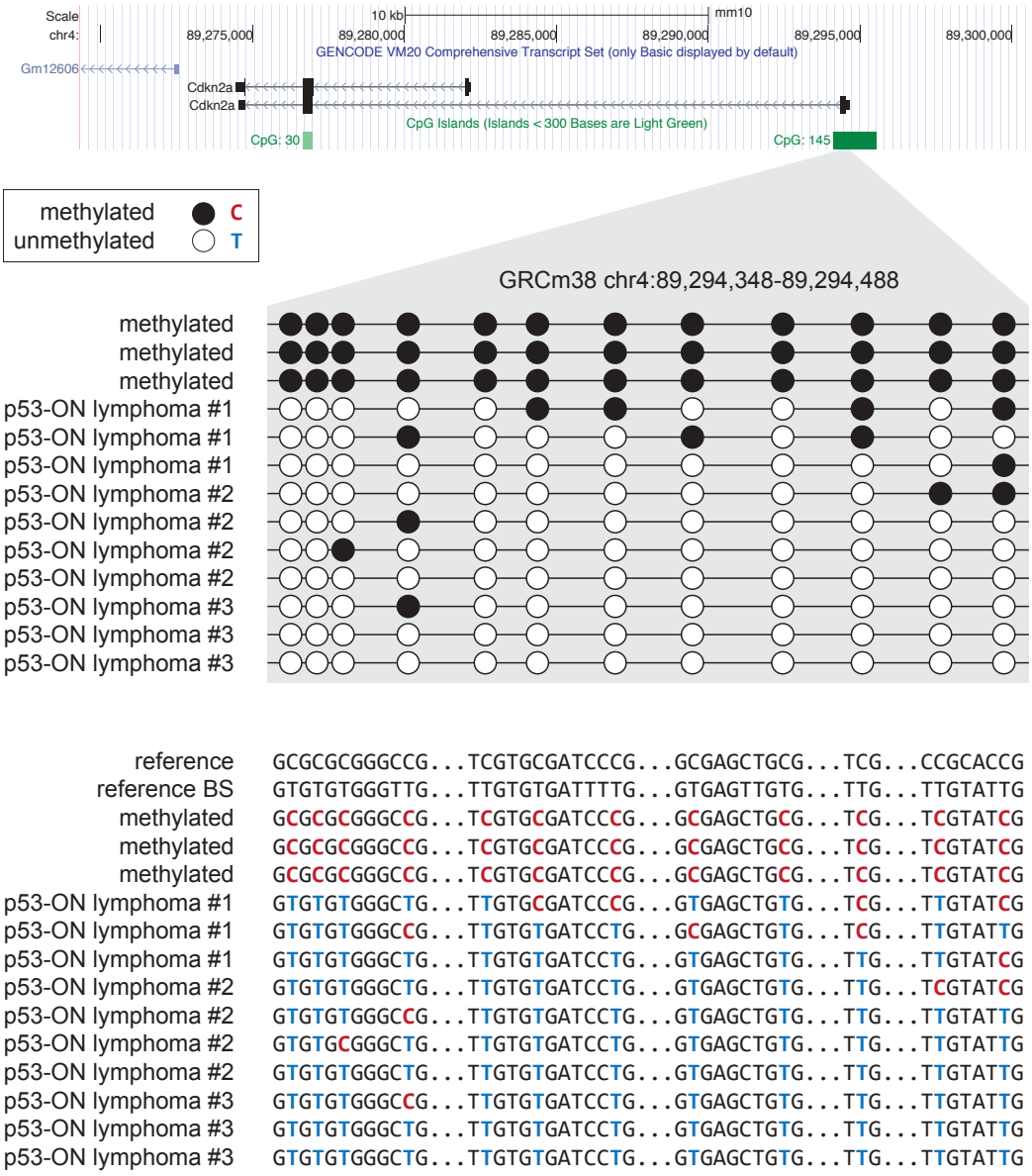

Fig. S4. Lack of *Cdkn2a* methylation in p53-ON lymphomas. Genomic DNA from p53-ON Eμ-Myc lymphomas was analyzed by bisulfite (BS) sequencing for methylation at the CpG island spanning *Cdkn2a* exon 1β. Shown is a map of the *Cdkn2a* gene locus (*top*), a symbolic representation of CpG methylation in the amplified region (*middle*) and sequences of multiple clones from 3 independent p53-ON lymphomas (*bottom*). Reference and bisulfite converted reference sequences are shown for comparison. Enzymatically methylated genomic DNA was used as a positive control for successful bisulfite conversion.

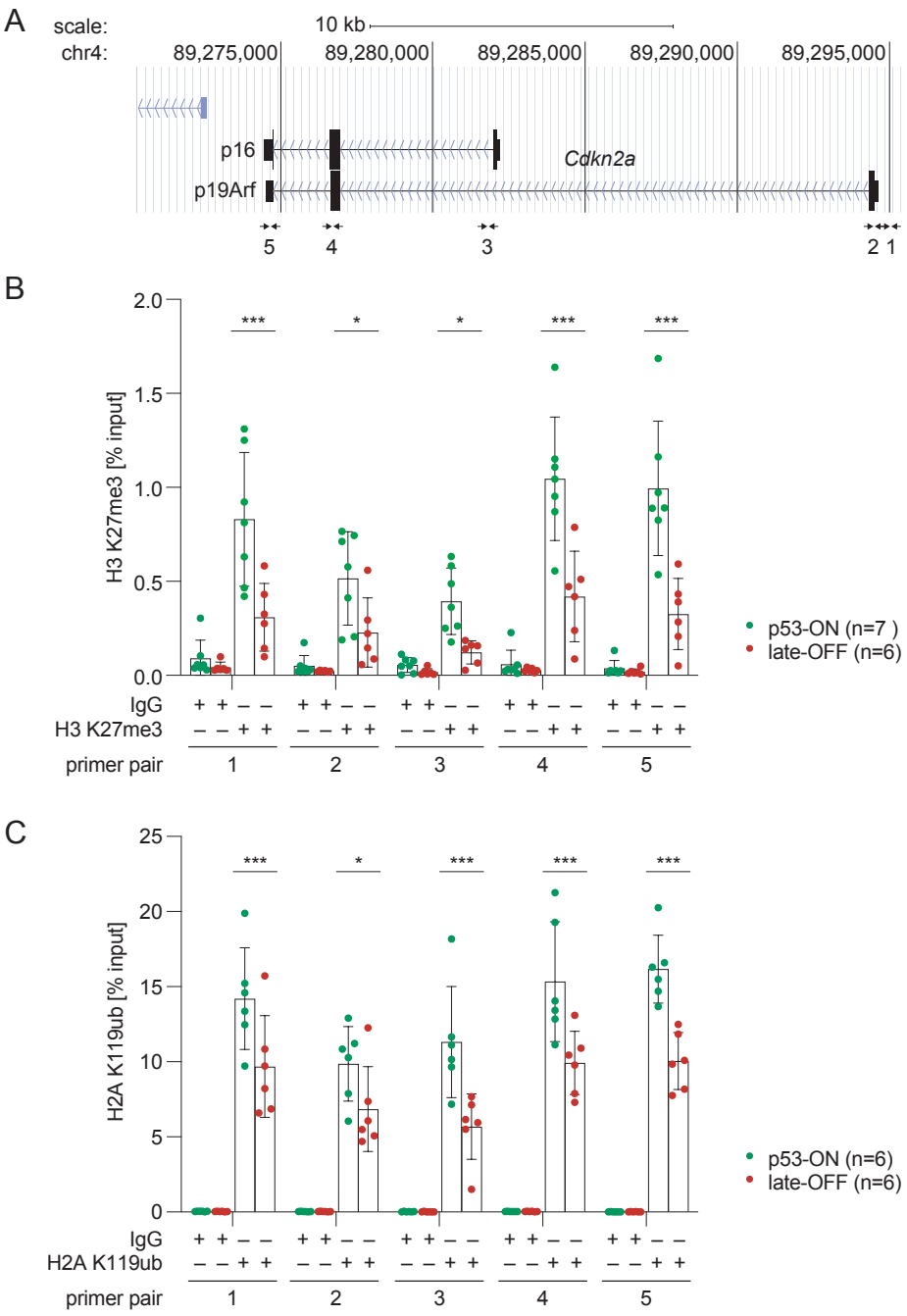

Fig. S5. Polycomb group protein-mediated repression of *Cdkn2a* locus is reduced upon late p53 inactivation. p53-ON and late-OFF Eμ-Myc lymphomas were subjected to chromatin immunoprecipitation analysis for Polycomb group protein-mediated histone modifications. (A) Map of the *Cdkn2a* gene locus depicting location of primer pairs used for ChIP-qPCR. (B,C) ChIP results for (B) H3K27me3 and (C) H2AK119ub. IgG is shown as negative control. All results are shown as % input chromatin. Bars graphs depict the mean±SD and data for all individual lymphomas. Statistical significance was assessed with multiple two-sided t-test in combination with False Discovery Rates: *, FDR q<0.05, ***, FDR q<0.001.

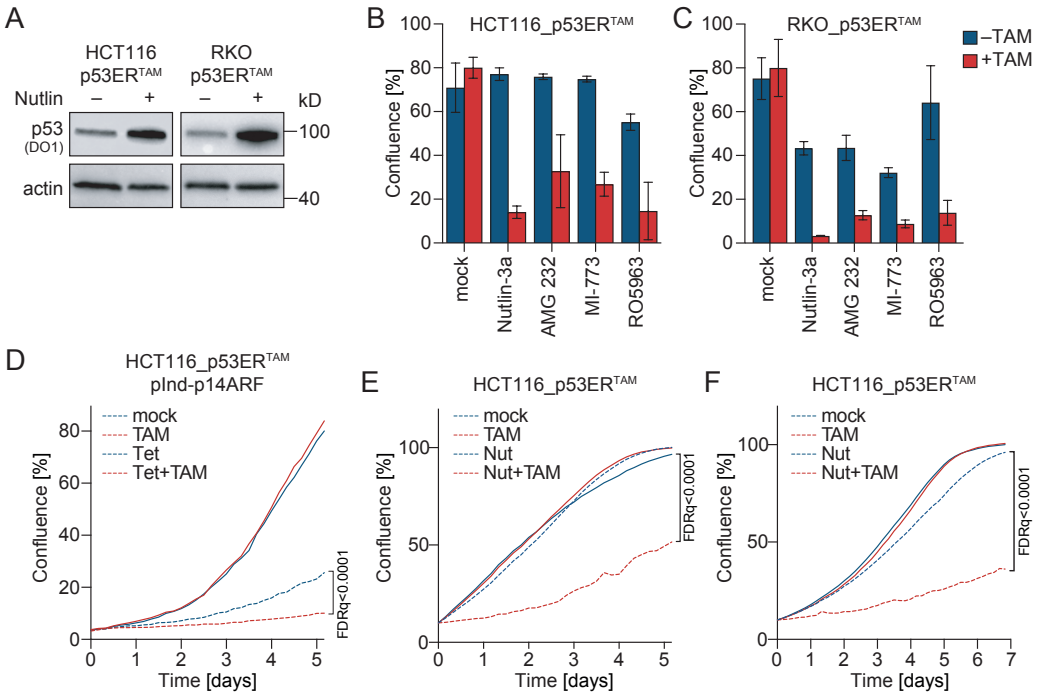

Fig. S6. Mdm2 inhibition sensitizes human ARF-deficient p53ER^{TAM} tumor cells to p53 reactivation.

(A) Western Blot demonstrating stabilization of p53ER^{TAM} following 48 h-treatment of HCT116/RKO_p53ER^{TAM} cells with 10 µM Nutlin-3a.

(B, C) Indicated human p53ER^{TAM} cell lines were cultured in the presence of indicated Mdm2 inhibitors ± 4-OHT (TAM). Shown is the confluence of cell cultures (mean± SD, n=3) determined by automated live cell imaging.

(D) HCT116_p53ER^{TAM} cells with Tet-inducible expression of p14ARF were cultured in the presence of doxycycline (Tet) and/or 4-OHT (TAM), respectively. Doxycycline treatment was started 24 h before addition of TAM. (E, F) Indicated p53ER^{TAM} cell lines were cultured in the presence of Nutlin-3a (Nut) and/or 4-OHT (TAM), respectively. Shown is the confluence of cell cultures (mean± SD, n=3) determined by automated live cell imaging. Statistical significance was assessed with multiple two-sided t-tests in combination with False Discovery Rates. Reported are FDRq-values for the confluence obtained at the end of the observation period.

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