Supplementary Information for

SIRT7-dependent deacetylation of NPM promotes p53 stabilization following UV-induced genotoxic stress

Alessandro Ianni, Poonam Kumari, Shahriar Tarighi, Nicolas G. Simonet, Daniela Popescu, Stefan Guenther, Soraya Hölper, Andreas Schmidt, Christian Smolka, Shijing Yue, Marcus Krüger, Claudia Fiorillo, Alejandro Vaquero, Eva Bober and Thomas Braun

Corresponding author names: Alessandro Ianni and Thomas Braun
Email: Alessandro.Ianni@mpi-bn.mpg.de; Thomas.Braun@mpi-bn.mpg.de

This PDF file includes:

Supplementary Information Text

SI Material and Methods

UV irradiation of the skin. For UV irradiation experiments, age-matched wild type and SIRT7-/- littermates were anesthetized with 120 mg/Kg ketamine and 16 mg/Kg xylazin. The dorsal skin of the mice was shaved using an electric clipper and the mice were either left untreated or exposed to 365 nm wave length irradiation with a radiant exposure of 5000 J/m^2 using a UV-crosslinker (Biolink-365; Vilber). Mice were sacrificed 12h post irradiation. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (1) and were approved by the local authorities (Regierungspraesidium Darmstadt).

Cell culture and treatments. Primary MEFs were cultivated in DMEM medium containing 1g/L glucose supplemented with 10% fetal calf serum (FCS, Sigma-Aldrich), 100U/ml penicillin, 0.1 mg/ml streptomycin and 2mM glutamine (Sigma-Aldrich) at 37 °C in a humidified atmosphere with 5% CO_2. MEFs were used in all experiments at passage 2. U2OS and 293T HEK cells were grown in DMEM medium containing 4.5 g/L glucose supplemented with 10% FCS, 100U/ml penicillin, 0.1mg/ml streptomycin and 2mM glutamine (Sigma-Aldrich) at 37 °C in a humidified atmosphere with 5% CO_2. For UVC irradiation, cells were grown in Petri dishes and the medium was removed from the plates prior to UV exposition. Cells were exposed to 254 nm irradiation with the radiant exposure indicated in each experiment using a CX-2000 UV-crosslinker (UVP Inc., USA). Cells were supplemented with fresh medium after irradiation, returned into incubators and harvested at indicated time points after exposition. MG132 proteasome inhibitor (Calbiochem), Trichostatin A (Sigma-Aldrich), Cycloheximide (Calbiochem), Actinomycin D (ActD; Sigma-Aldrich), ATR inhibitor (ATRi; Millipore #118510) and ATM inhibitor (ATMi; Millipore #118502) were used at the concentration and times indicated in the figure legends. 293T HEK cells were transfected using the calcium phosphate method as previously described (2). U2OS cells were transfected with lipofectamine 3000® (Invitrogen) following the manufacturer's instructions. Cell proliferation was assessed by EdU incorporation assay using the Click-it Plus EdU imaging kit (Invitrogen) following the manufacturer´s instructions.

Plasmids and cloning. The SIRT7-YFP constructs have been described previously (3). The SIRT7-YFP phosphomimetic mutant (S137D) was generated with the QuickChange site-directed mutagenesis kit (Stratagene) and verified by DNA sequencing. Mouse NPM cDNA N-terminally fused with 2x Flag-tag or EGFP was cloned into the pcDNA™5/TO Mammalian Expression Vector (Thermofisher). NPM point mutants were generated with the QuickChange site-directed mutagenesis kit (Stratagene) and verified by DNA sequencing. pCMV-myc3-HDM2 was a gift of Dr. Yue Xiong and was obtained from Addgene (plasmid # 20935). pcDNA3-ATR WT was a gift from Aziz Sancar (Addgene plasmid # 31611).

***In vitro* deacetylation assay and mass spectrometry analysis.** For in vitro deacetylation assay, SIRT7-Flag WT and HY mutant proteins were purified after transfection in 293T HEK cells. 48h after transfection, cells were either left untreated or exposed to 40 J/m^2 UV and harvested 5h post-irradiation. Flag-tagged NPM was overexpressed in 293T HEK cells. 48 hours after transfection cells were treated with 5mM Nicotinamide (NAM; Sigma-Aldrich) for 24 hours and for additional 3h with 5mM NAM and 5μM Trichostatin A (TSA) to achieve hyperacetylation.

Flag-NPM and SIRT7-Flag proteins were immunoprecipitated with Flag resin. After precipitation, beads were washed 3 times in BC100 buffer (10mM Tris-HCl pH 7.8, 0.5mM EDTA, 0.1mM PMSF, 0.1mM DTT, 10% Glycerol, 100mM KCl) supplemented with 0.5% NP-40 and with complete

proteasome inhibitor cocktail (Roche) and 5 times in BC500 buffer (10mM Tris-HCl pH 7.8, 0.5mM EDTA, 0.1mM DTT, 10% glycerol, 500mM KCl) with 0.5% NP-40 and supplemented with proteasome inhibitors as above. Proteins were eluted in deacetylation buffer (10mM Tris-HCl pH 8.0, 150mM NaCl and 10% glycerol) using 0.4 µg/µL of Flag peptide (Sigma-Aldrich) for 30 min at 4°C. Eluted Flag-SIRT7 proteins were incubated with hyper-acetylated Flag-NPM in presence of 5mM NAD+ in deacetylation buffer for 90 min at 37°C. The reaction was stopped by addition of Laemmli buffer. Samples were then separated by SDS-PAGE and the levels of acetylation of NPM were assessed by western blotting using anti-acetylated lysine antibody.

For mass spectrometry analysis of NPM acetylated lysines, 293T HEK cells were transfected either with empty vector or SIRT7-Flag WT and SIRT7-Flag HY. 48h post transfection cells were exposed to 40 J/m^2 UVC irradiation and incubated for 5h in presence of 5µM Trichostatin A (TSA) before harvest. Endogenous NPM was immunoprecipitated with a specific antibody and immunoprecipitates were resolved by SDS-PAGE electrophoresis. The levels of acetylated NPM were assessed after in gel-digestion following described procedures (3). For mass spectrometry analysis of SIRT7 phosphorylation sites, 293T HEK cells were transfected with human SIRT7-Flag construct. 48h post-transfection, cells were pre-treated for 1h with 4µM ATR inhibitor (ATRi), exposed to UVC irradiation (40 J/m^2) and grown for additional 5h in presence of vehicle or ATRi before harvest. Exogenous SIRT7 was immunoprecipitated with anti-Flag antibody and immunoprecipitates were resolved by SDS-PAGE electrophoresis. The levels of phosphorylated Sirt7 were assessed after in gel digestion with trypsin following published procedures (4). Peak areas of the identified phosphopeptides were extracted from raw data using Skyline (5) and normalized to protein concentrations.

Luciferase assay. p53 transcriptional activity was measured using a dual luciferase assay. Cells were transfected in a 96 well plate with 20ng/well of a luciferase reporter containing 13 copies of the p53-binding consensus sequence (Addgene plasmid #16442) and 1ng/well of *Renilla*-based control vector (pRL-TK, Promega) for internal normalization. Cells were treated 24h post-transfection as indicated in each experiment and subjected to luciferase assay as described (6).

SI Figures and Legends

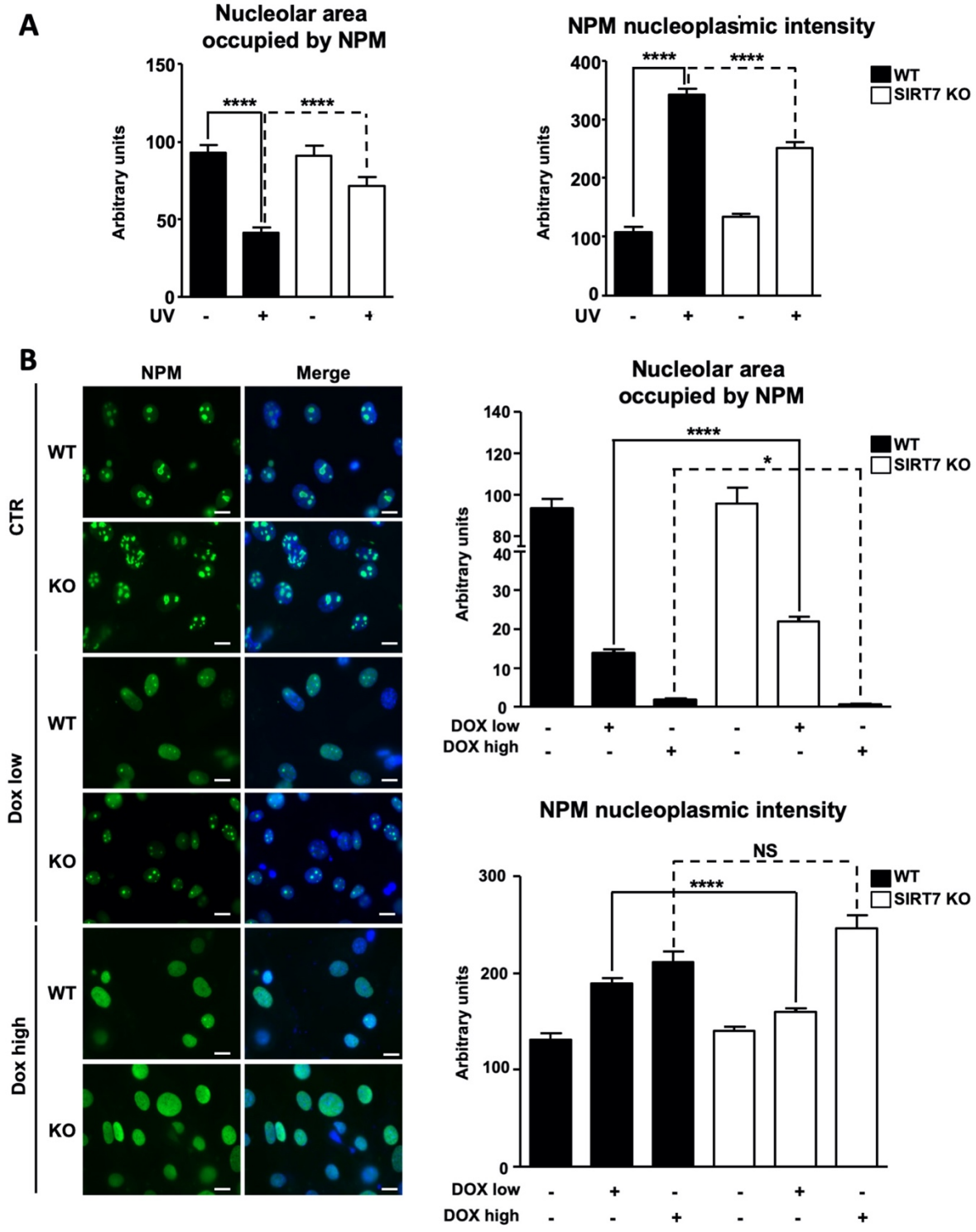

Fig. S1. SIRT7 mediates exclusion of NPM from nucleoli following genotoxic stress. (A) Quantification of the nucleolar area occupied by NPM (left panel) and the nucleoplasm intensity of the NPM staining (right panel) in primary WT and SIRT7 KO MEFs 12h after exposure to 80 J/m^2 UVC irradiation. The nucleolar area and the nucleoplasmic intensity were measured using ImageJ

Fiji software. 100 cells for each group were measured, ***p<0.001. **(B)** Immunofluorescence (IF) staining of NPM in SIRT7 KO primary MEFs after exposure to 0.2µg/mL (Dox low) or 2µg/mL (Dox high) of doxorubicin for 5h. Nuclei were counterstained with DAPI (n=3; scale bar=20µm). Note that NPM is retained in the nucleolus in SIRT7 knockout cells following treatment with low concentration of doxorubicin while no difference in NPM localization could be observed between WT and SIRT7 KO cells at high doxorubicin concentration (scale bar=20µm). The histograms on the right present quantification of the nucleolar area (upper panel) and nucleoplasm intensity (lower panel) of NPM staining measured as in (A). *p<0.05; ***p<0.001; NS: Not significant.

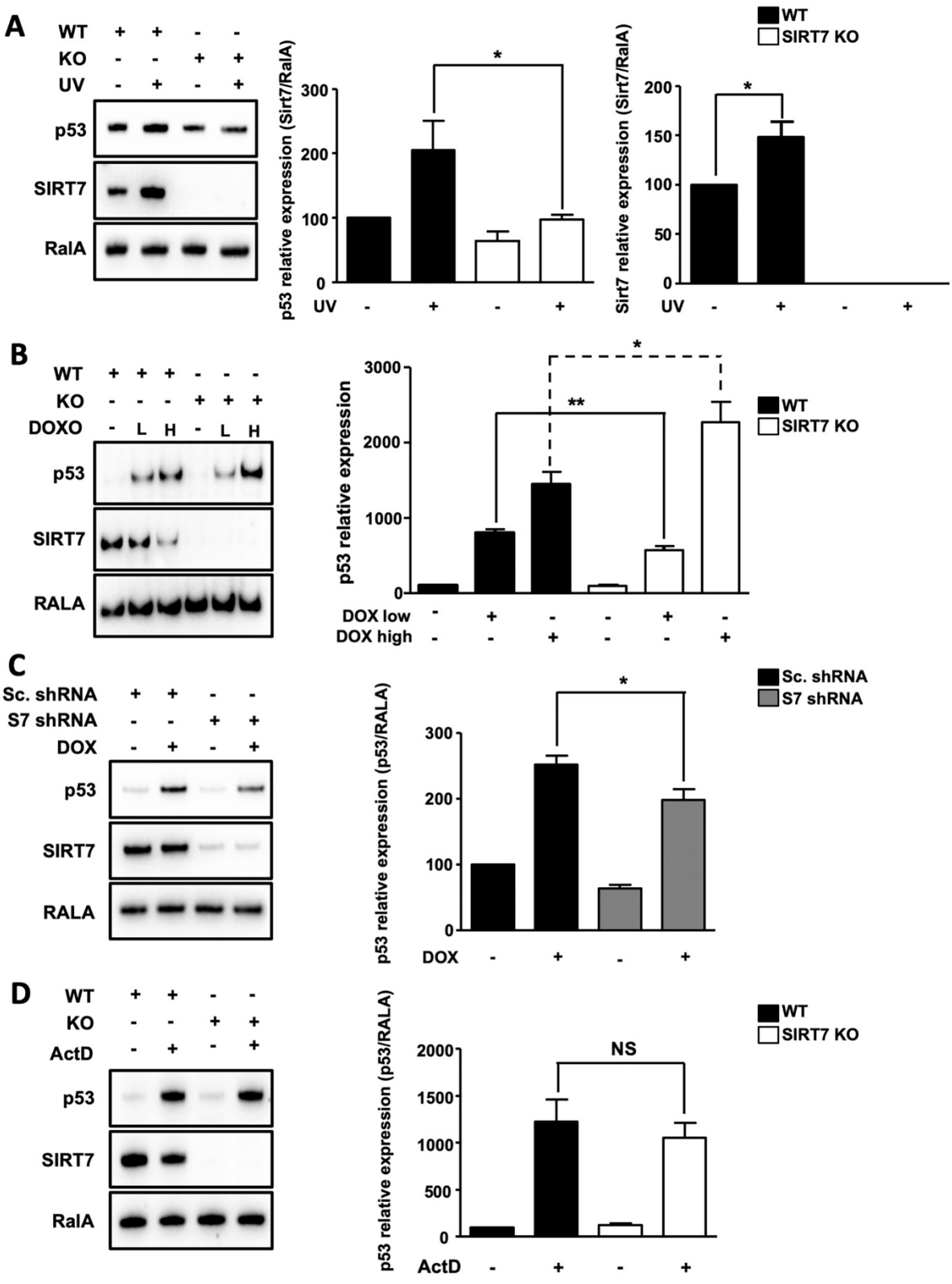

Fig. S2. SIRT7 promotes stabilization of p53 under genotoxic stress. (A) Western blot analysis of SIRT7 and p53 levels in WT and SIRT7 KO MEFs 12h after 40000 J/m^2 UVA irradiation. RALA was used as a loading control. Quantification of average p53 and SIRT7 levels relative to RALA ±

SD (n=3; *p<0.05, Student´s t test). **(B)** Western blot analysis of p53 levels in WT and SIRT7 KO MEFs after 8h treatment with 0.2µg/mL (L) or 2µg/mL (H) doxorubicin. RALA was used as loading control. Quantification of average p53 levels relative to RALA ± SD (n=8 for low and n=4 for high concentration; *p<0.05; **p<0.01, Student´s t test). **(C)** Western blot analysis of p53 levels in control and SIRT7 KD U2OS cells after 8h treatment with 0.2µg/mL doxorubicin. RALA was used as loading control. Quantification of average p53 levels relative to RALA ± SD (n=4; *p<0.05, **P<0.01, Student´s t test). **(D)** Western blot analysis of p53 levels in WT and SIRT7 KO MEFs after exposure to 8nM actinomycin D (ActD) for 6h. Quantification of the average p53 levels relative to RALA ± SD is shown in the histogram on the right (n=4; NS: Not significant).

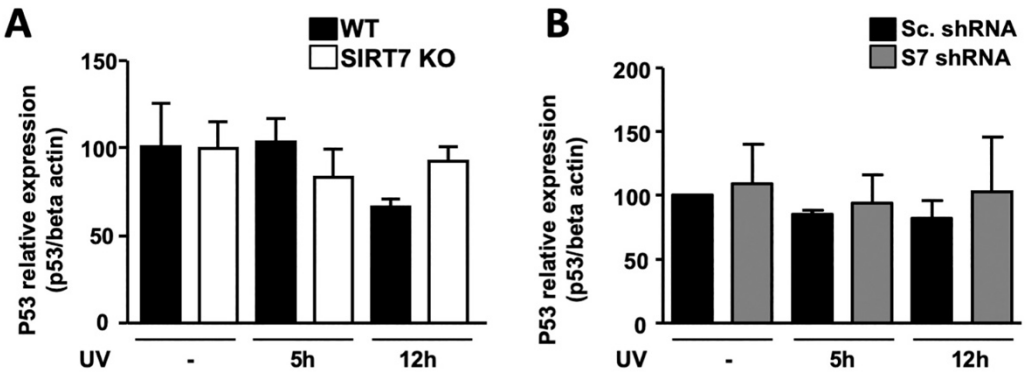

Fig. S3. SIRT7 does not control *p53* mRNA expression in response to UV irradiation. (A) RT-qPCR analysis of *p53* mRNA expression in WT and SIRT7 KO MEFs after exposure to UV irradiation. **(B)** RT-qPCR analysis of *p53* mRNA expression in control (scrambled) and SIRT7 KD U2OS cells after UV irradiation. *ß-actin* was used as a loading control. Quantification of average *p53* mRNA levels relative to *ß-actin* ± SD (n=3 in A and B).

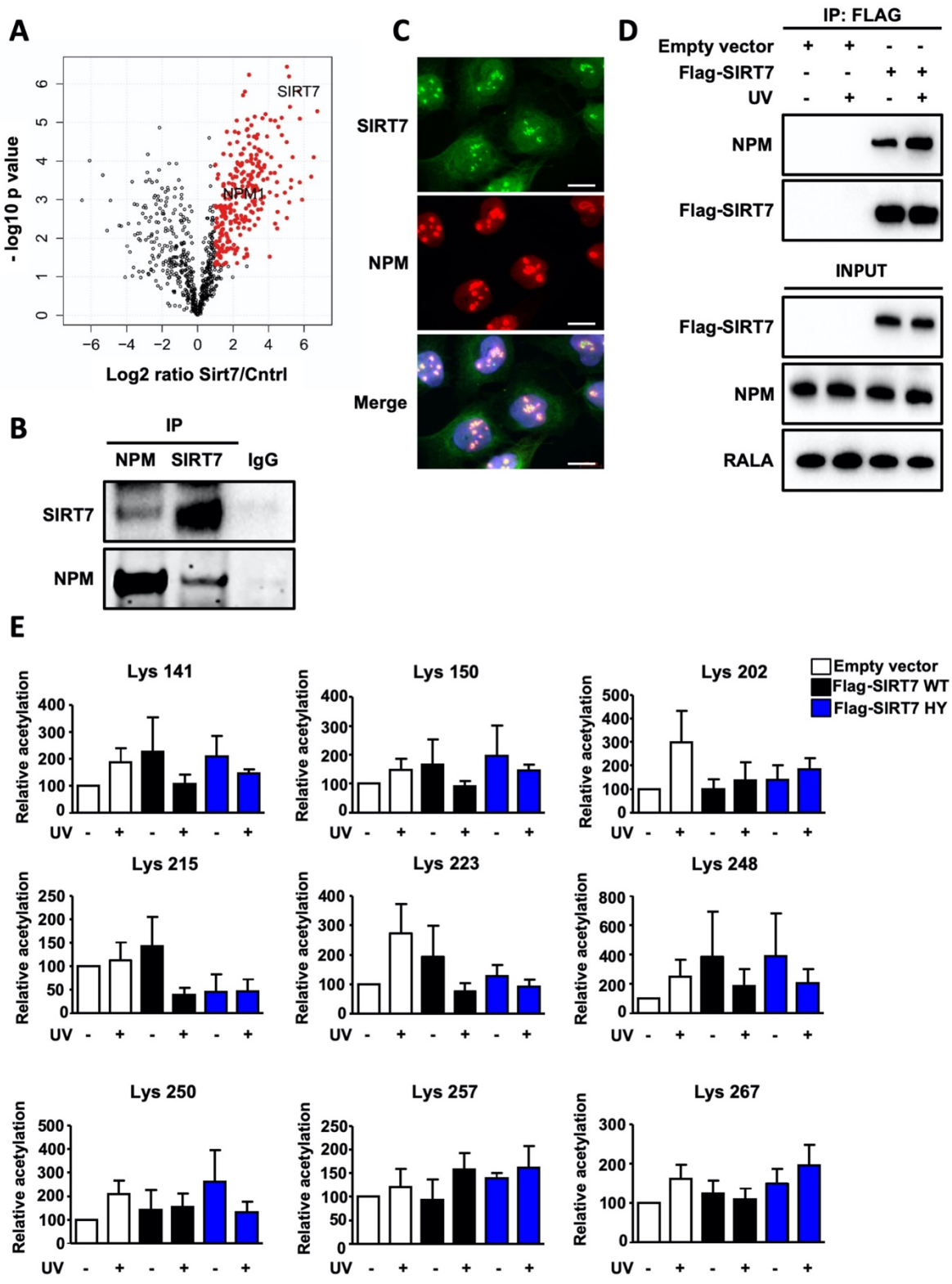

Fig. S4. SIRT7 forms a molecular complex with NPM. (A) Volcano plot depicting mass spectrometry measurements of proteins co-precipitating with transfected Flag-tagged SIRT7 in 293T HEK cells (n=3). The positions of SIRT7 and NPM are indicated (n=3). **(B)** Coupled immunoprecipitation (IP) (anti-SIRT7 or anti-NPM antibodies) and western blot analysis (anti-

SIRT7 or anti-NPM antibodies) of U2OS cells demonstrating interaction of SIRT7 and NPM (n=3). **(C)** IF staining for endogenous SIRT7 (green) and NPM (red) in U2OS cells showing a co-localization of the two proteins in the nucleoli. Cellular nuclei were counterstained with DAPI. Scale bar=10µm. **(D)** Coupled IP (ANTI-FLAG® M2 Affinity beads) and western blot analysis (antibody against the endogenous NPM) of 293T HEK cells transfected with SIRT7-Flag 5h after UVC irradiation (n=3). **(E)** Acetylated lysines in NPM protein identified by mass spectrometry. No significant differences in acetylation of indicated lysines were observed after overexpression of WT and SIRT7 catalytic inactive mutant SIRT7 HY and subsequent UVC exposure.

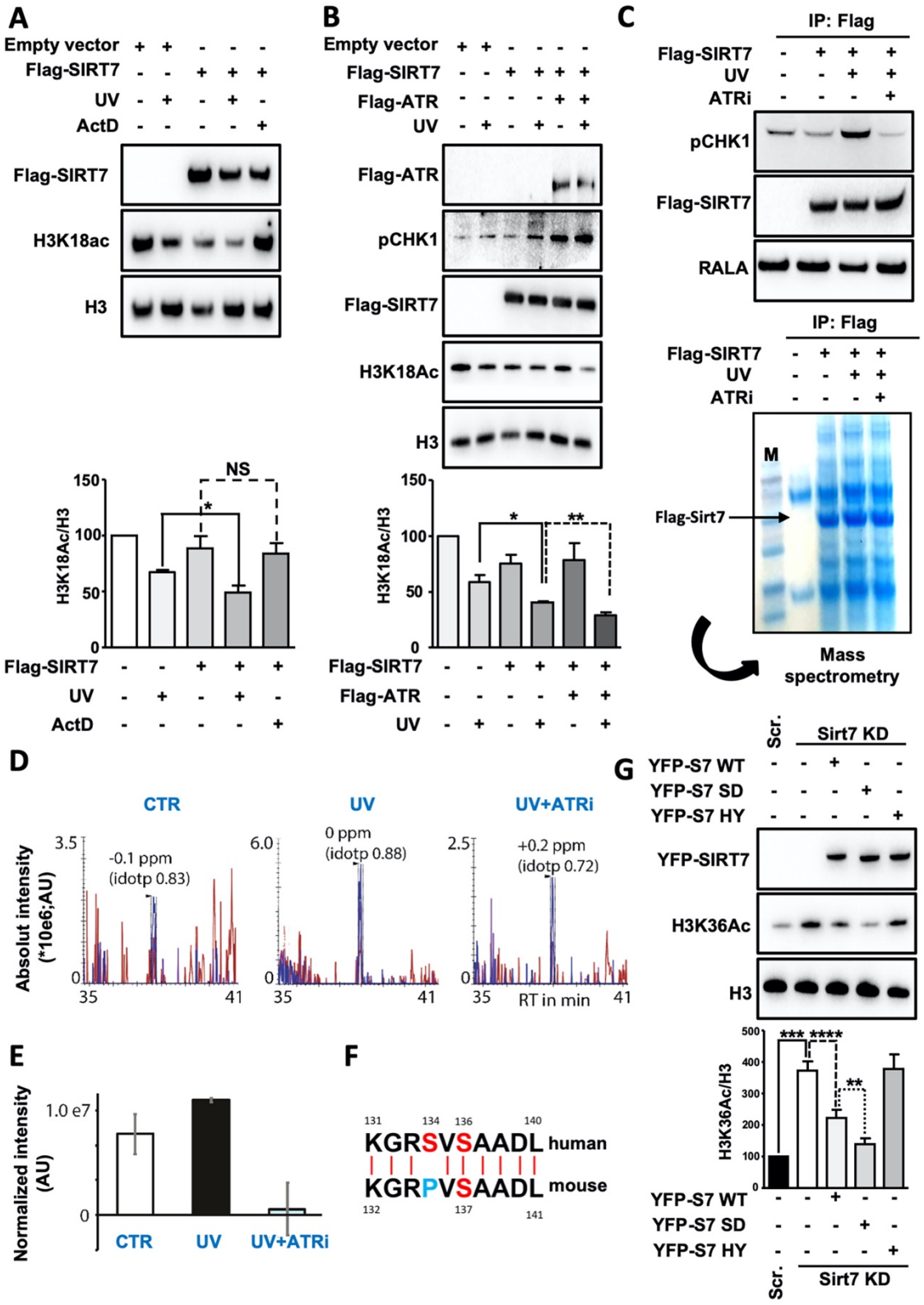

Fig. S5. ATR-mediated phosphorylation enhances SIRT7 catalytic activity following UV irradiation. (A) Western blot analysis of H3K18Ac in 293T HEK cells transfected with an empty vector or with Flag-tagged wildtype SIRT7 (Flag-SIRT7 WT) 5h after exposure to UVC irradiation (40J/m^2) or after incubation for 5h with 8nM ActD as indicated. The histogram below represents a

quantification of the average H3K18Ac levels relative to total H3 ± SD (n=5; *p<0.05, NS: Not significant). **(B)** Western blot analysis of H3K18Ac in 293T HEK cells transfected with wildtype Flag-SIRT7 alone or in combination with Flag-tagged ATR and collected 5h after exposure to UVC irradiation (40 J/m^2). **(C)** Scheme depicting the experimental approach used for mass spectrometry-based analysis of ATR-dependent phosphorylation of SIRT7. 293T HEK cells were transfected either with an empty vector or Flag-tagged human SIRT7. 48 hours post-transfection, cells were pre-treated for 1 hour with 4μM ATR inhibitor (ATRi), exposed to UVC irradiation (40 J/m^2) and grown for additional 5 hours in presence of vehicle or ATRi. Western blot analysis of ATR target pCHK1 demonstrated efficient inhibition of ATR in presence of the inhibitor (upper panel). Cellular lysates were subjected to immunoprecipitation using an anti-Flag antibody. Immunoprecipitates were resolved by SDS-PAGE electrophoresis and subjected to in-gel digestion and mass spectrometry analysis (lower panel). (**D**). Retention time (RT)/intensity plots for the phosphopeptide GR**S**(134)V**S**(136**)**AADLSEAEPTLTHMSITR (peptide mass 2588.1343 Da; ΔM 0.13429711; MaxQuant score 62.831) under control conditions and following UVC irradiation, in presence or absence of ATR inhibitor (ATRi). Retention time, mass accuracy and idot-product information are shown. (**E**) Histogram displaying the relative phosphorylation levels at Ser134 and Ser136 of human SIRT7 in control and UV irradiated cells (40J/m^2; 5 hours) in presence or absence of ATR inhibitor. Peak areas from (D) were extracted in Skyline and normalized to respective protein concentrations. (**F**) Alignment of the amino acid sequence of human and mouse SIRT7. ATR-dependent phosphorylation sites of SIRT7 are indicated in red. Note that only Ser136 of human SIRT7 protein is conserved in mice (corresponding to Ser137), while Ser134 is substituted by a proline at position 135 (blue). (**G**) Western blot analysis of acetylated H3K36 (H3K36Ac) in scramble (Scr) and Sirt7 KD U2OS cells 48 hours after transfection with an empty vector, with YFP-tagged wildtype SIRT7 (YFP-S7 WT), the catalytic inactive mutant SIRT7 HY (YFP-S7HY) and the phosphomimetic mutant S137D. Quantification of H3K36Ac normalized to histone 3 (H3) ± SD is shown in the histogram below (n=6; **p<0.01; ***p<0.001, ****p<0.0001).

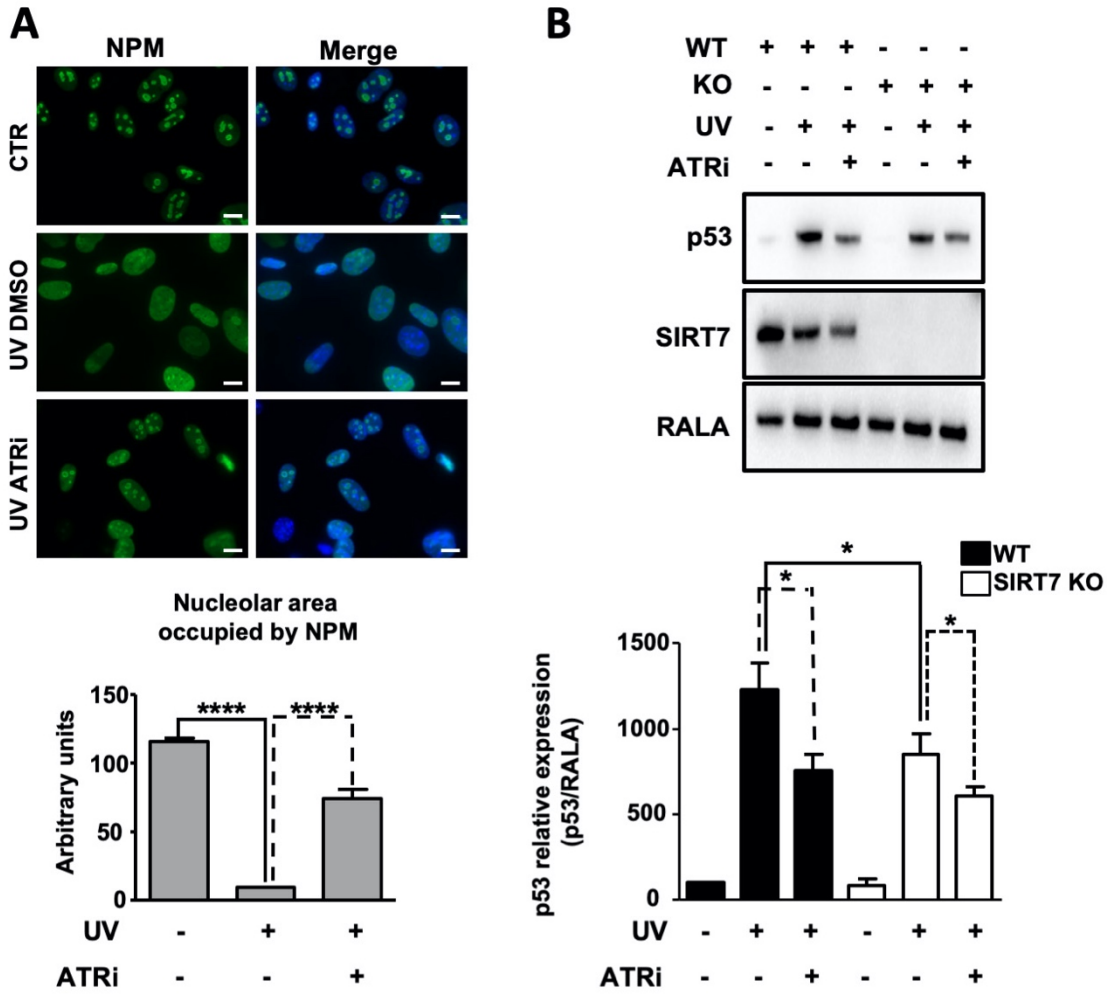

Fig. S6. ATR-mediated phosphorylation of Sirt7 is required for NPM exclusion from nucleoli and p53 stabilization (A) IF staining for NPM in wildtype primary MEFs pre-incubated for 1h with 4μM ATR inhibitor (ATRi), exposed to UVC irradiation (80 J/m^2), and grown for additional 14h in presence of vehicle or ATRi. Note the impaired translocation of NPM in ATRi treated cells. Nuclei were counterstained with DAPI. Quantification of the nucleolar area occupied by NPM is shown in the histogram below (n=3; ****p<0.0001; scale bar=10μm). **(B)** Western blot analysis of p53 levels in WT and SIRT7 KO MEFs pre-treated for 1h with ATR inhibitor or vehicle DMSO, exposed to UVC irradiation (80 J/m^2), and grown for additional 12h in the presence of vehicle or ATRi. RALA was used as loading control. Quantification of normalized p53 expression ± SD is shown in the graph (n=4; *p<0.05; Student´s t test).

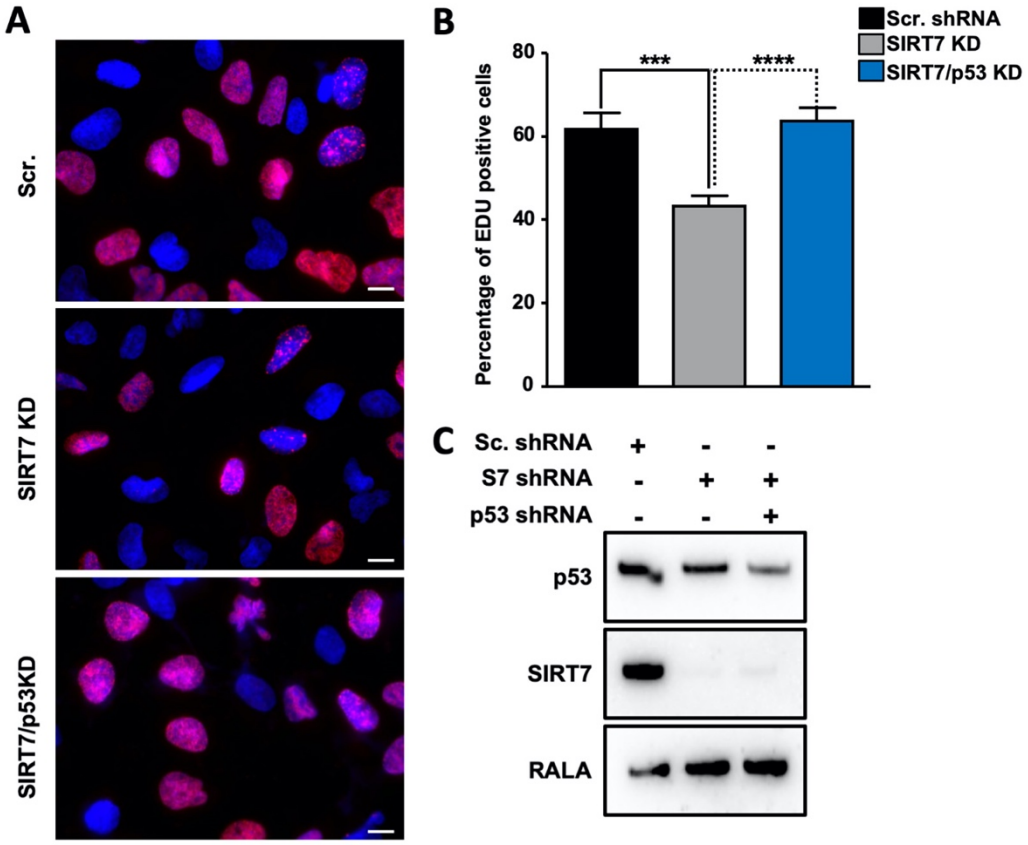

Fig. S7. SIRT7 deficiency impairs cancer cell proliferation in a p53-dependent manner. (A) EdU staining of SIRT7 KD and SIRT7/p53 double KD cells. Cell nuclei were counterstained with DAPI. **(B)** The percentages of BrdU positive cells in (A) are shown in the histograms. A minimum of 100 cells for each experiment was analysed (n=3; ***P<0.001, ****p<0.0001). **(C)** Western blot analysis of efficient shRNA inhibition of p53 and SIRT7 expression. RALA was used as a loading control.

SI Tables

Table S1. List of shRNA sequences used in this study

shRNA	Sequence (5´-3´)
Scrambled shRNA	CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG
SIRT7 shRNA#1	CCGGGTCCAGCCTGAAGGTTCTAAACTCGAGTTTAGAACCTTCAGGCTGGAC TTTTTG
SIRT7 shRNA#2	CCGGTCCACGGGAACATGTACATTGCTCGAGCAATGTACATGTTCCCGTGGA TTTTTG
NPM shRNA	CCGGCCTAGTTCTGTAGAAGACATTCTCGAGAATGTCTTCTACAGAACTA GGTTTTTG
p53 shRNA #1	CCGGGTCCAGATGAAGCTCCCAGAACTCGAGTTCTGGGAGCTTCATCTGGACTTTTT
p53 shRNA #2	CCGGCACCATCCACTACAACTACATCTCGAGATGTAGTTGTAGTGGATGG TGTTTTT

Table S2. List of antibodies used for Western blot, immunoprecipitation and co-immuno-precipitation

Name of antibody	Manufacturer (catalog number)
SIRT7	Cell Signaling Tech.(# 5360)
NPM/B23	Santa Cruz Biotech. (sc-6013-R and sc-56622)
Acetylated-lysine	Cell Signaling Tech. (#9441),
Monoclonal ANTI-FLAG® M2	Sigma-Aldrich (F1804),
RALA	BD Transduction laboratories (610221)
MDM2/HDM2	Santa Cruz Biotech. (sc-965)
p53 Do-1	Santa Cruz Biotech. (sc-126)
p53 FL-393	Santa Cruz Biotech. (sc-6243)
Myc-Tag	Cell Signaling Tech. (#2278),
Actin	Sigma-Aldrich (A2103)
anti-ubiquitin P4D1	Cell Signaling Tech (#3936)
H3K18Ac	Abcam (ab1191)
histone 3 (H3)	Cell Signaling Tech. (#9715)
Anti-Tag(CGY)FP	Evrogen (ab121)
phospho-CHK1 (Ser317)	Cell Signaling Tech. (#2344)
phosphor-CHK2 (Thr387)	Cell Signaling Tech. (#2668)
Anti-phosphoserine	Millipore (05-1000X)
H3K36Ac	Cell Signaling Tech. (#27683)

Table S3. List of primers used in this study

Name	**Sequence**
human p53	Forward: 5′-TAACAGTTCCTGCATGGGCGGC-3′ Reverse: 5′-AGGACAGGCACAAACACGCACC-3′
mouse p53	Forward: 5′-CTCCGTCATGTGCTGTGACT-3′ Reverse: 5′-GCAACTATGGCTTCCACCTG-3′
human β-actin	Forward: 5′- ATCGTCCACCGCAAATGCTTCTA-3′ Reverse: 5′-AGCCATGCCAATCTCATCTTGTT-3′
mouse β-actin	Forward: 5′- CAACGAGCGGTTCCGATG-3′ Reverse: GCCACAGGATTCCATACCCA.
human *P21Waf1*	Forward: 5′- TCTTGTACCCTTGTGCCTC-3′ Reverse: 5′- AACCTCTCATTCAACCGCC-3′
human *EI24*	Forward: 5′- TGCCAGAGGAATCAAAGACTCC-3′ Reverse: 5′- TCTCTTGCTTCCGCTCTATACT-3′
human *DDB2*	Forward: 5′-AGCATCACTGGGCTGAAGTT-3′ Reverse: 5′-GTGACCACCATTCGGCTACT-3′

Table S4. List of antibodies used for immunofluorescence analysis

Name of antibody	Manufacturer (catalog number)
SIRT7	Cell Signaling Tech.(# 5360)
NPM/B23	Santa Cruz Biotech.(sc-56622)
MDM2/HDM2	Sigma-Aldrich (M4308)
Anti-Tag(CGY)FP	Evrogen (ab121)
Keratin 5	(Biolegend; 905501)

SI References

1. J. M. Worlein, K. Baker, M. Bloomsmith, K. Coleman, T. L. Koban, The Eighth Edition of the Guide for the Care and Use of Laboratory Animals (2011); Implications for Behavioral Management. *Am J Primatol* **73**, 98-98 (2011).
2. J. Sambrook, D. W. Russell, Calcium-phosphate-mediated Transfection of Eukaryotic Cells with Plasmid DNAs. *CSH Protoc* **2006** (2006).
3. J. Fang *et al.*, Sirt7 promotes adipogenesis in the mouse by inhibiting autocatalytic activation of Sirt1. *Proc Natl Acad Sci U S A* **114**, E8352-E8361 (2017).
4. J. L. Wiederstein *et al.*, Skeletal Muscle-Specific Methyltransferase METTL21C Trimethylates p97 and Regulates Autophagy-Associated Protein Breakdown. *Cell Rep* **23**, 1342-1356 (2018).
5. B. MacLean *et al.*, Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* **26**, 966-968 (2010).
6. I. Salwig *et al.*, Bronchioalveolar stem cells are a main source for regeneration of distal lung epithelia in vivo. *EMBO J* **38** (2019).