SI Appendix

Supplemental Material and Methods

Plasmids and cloning

Plasmids pTag-CFP-C and pTag-YFP-N were obtained from BioCAT (Evrogen, Heidelberg). Sirt1- and Sirt7-CFP were generated by C-terminal insertion of mouse Sirt1 and Sirt7 cDNAs; Sirt7-YFP was constructed by N-terminal insertion of the mouse Sirt7 cDNA. Plasmid expressing an untagged wt Sirt1 (Sir2αcDNA-pUSEamp) was obtained from Upstate Biotechnology. pBabe-Flag-HA-mPPARγ and Myc-tagged Set7/9 were obtained from Addgene. Sirt7-myc was made by insertion of the 5´-6x myc-tag fused with mouse Sirt7 into the StuI and XhoI sites of the pCS2+ vector. pQsupR-Scramble, pQsupR-mSirt1shRNA and pCMVTag2a-hSirt7-Flag plasmids were provided by I. Grummt, Heidelberg (1). pCMV-sport6-mSirt1-Flag and pCMV-sport6-mSirt1H355Y-Flag were gifts from O. Leo, Bruxelles. For overexpression of human Sirt7, the pCemmCTAP-hSirt7Flag vector was constructed using human Sirt7 cDNA combined with a Flag-Tag derived from pCMVTag2a-hSirt7-Flag. Point mutants of pTag-mSirt7-YFP and mSirt1-CFP were generated using the QuickChange site-directed mutagenesis kit (Stratagene) and verified by DNA sequencing.

Cell culture

Primary preadipocytes were isolated from subcutaneous adipose tissue of 7 weeks old Sirt7(+/-), Sirt7(-/-), and Sirt7(-/-)//Sirt1(+/-) mice by collagenase digestion. Cells were plated in chamber slides at a density of 2×10^4 per cm^2 for 4 days before induction of adipogenesis. At day 0, cells were treated with medium supplemented with 0.5mM isobutylmethylxanthine (IBMX), 1μM (dexamethasone) DEX, 5μg/ml insulin, 0.2nM T3, and 0.5μM rosiglitazone. Two days later, cells were changed to medium containing insulin, T3, and rosiglitazone. The medium was replaced at 2 days intervals for 8 days. MEFs or 3T3-L1 cells were induced to differentiate into adipocytes two days after reaching confluence (day 0) with MDI medium

supplemented with 0.5mM IBMX, 1μM DEX, 10μg/ml insulin, and 0.5μM rosiglitazone. Two days later, the medium was replaced by medium containing 5μg/ml insulin and 0.5μM rosiglitazone and changed every 2 days for 8 days. Fat accumulation was visualized by staining of lipids with Oil Red O. To inhibit sirtuins and HDACs 5mM nicotinamide (NAM, Sigma-Aldrich) or 5μM Trichostatin A (TSA) were used. HATs were inhibited using the following reagents: 1mM Cyclopentylidene-(4-(4′-chlorophenyl)thiazol-2-yl)hydrazine (CPTH2, Millipore), 100μM Sodium-4-(3,5-bis(4-hydroxy-3-methoxystyryl)-1H-pyrazol-1-yl)benzoate (CTK7A, Millipore), 40μM 5-(1,2-thiazol-5-yldisulfanyl)-1,2-thiazole (NU 9056, Tocris). Sirtuin 1 activator II {(3-(Benzenesulfonyl)-1-(4-fluorophenyl)pyrrolo[4,5-b]quinoxalin-2-amine, Millipore)} was used at the concentration of 40 μM. Cell proliferation was monitored by EdU incorporation using 10μM EdU and the Click-it Plus EdU imaging kit (Invitrogen). Cells were stained with 0.1% crystal violet (Sigma-Aldrich) for 20 min after fixation in 4% formaldehyde in PBS for 15 min. Absorbance of the samples was measured at 590 nm after extraction of the stain by addition of 1 ml 10% acetic acid for 20 min and 1:4 dilution in water.

Sirt 1 deacetylase assay and mass spectrometric analysis

Recombinant Sirt1 was mixed with a buffer containing 50mM HEPES pH 7.0, 150mM NaCl, 2mM $MgCl_2$, 0.05% TWEEN-20; 3% Glycerol; 1mM DTT; and 0.1% BSA to a final concentration of 500nM. 0.5% DMSO was added and the mixture incubated for 30min at RT. 50nM peptides and 500μM NAD^+ were added to start the reaction and incubated for 4h at 37ºC. Reactions were diluted with 8M urea and digested with LysC and/or Trypsin after alkylation as described (2). Peptides were desalted by stop and go extraction tips (3). Mass spectrometric experiments were performed on a nano-flow HPLC system (Agilent) connected to an LTQ-Orbitrap Velos instrument (Thermo Fisher Scientific) equipped with a nanoelectrospray source (Proxeon) following published procedures (4).

Raw data were processed using MaxQuant 1.5.3.8 (5) and the implemented Andromeda (6) search engine. MS/MS spectra were correlated against the human reference proteome Uniprot database including a list of common contaminants. We used 7 ppm and 4.5 ppm MS/MS tolerances for first and main search, respectively. The FDR at the peptide-spectrum-match and the protein level to 1%. Match-between runs, Re-quantify and LFQ quantification algorithms were enabled and used by default settings. LFQ intensities were log2 transformed. To overcome the problem of missing values in proteomics data we replaced missing quantitative data from a slightly down-shifted (1 standard-deviation from the whole population) Gaussian distribution (width: 0.3). Since we quantified a significantly higher number of proteins in the SIRT7 immunoprecipitated data, replacing data from a stronger down-shifted Gaussian distribution might introduce a bias towards false positive interaction partners. Due to this, the replacement does not mimic the detection limit of the mass spectrometer. Then a two-sided t-test was applied to identify significant differently pulled down proteins between the control and SIRT7-FLAG. To correct for multiple testing, we used a permutation based FDR calculation. We used a fudge-factor s0 of 1 and a FDR cutoff of 0.1% (# of permutations: 500).

Chromatin Immunoprecipitation

For each ChIP experiment 1 x 10^8 cells were cross-linked with 1% formaldehyde for 10 min at room temperature with gentle shaking. The reaction was quenched with 125mM glycine for 5 min. Cells were washed 3 times with ice-cold PBS buffer before isolation of nuclei and fragmentation of DNA to a 0.2-0.5 kb range using the Diagenode Bioruptor. 10% of the chromatin sample was saved as input and 50-100 μg chromatin samples were incubated with the respective primary antibody overnight at 4°C. Immuno-complexes were precipitated with protein A/G Sepharose beads (Diagenode) for 3 h at 4°C. Beads were washed sequentially with low salt washing buffer, high salt washing buffer, LiCl washing buffer and TE buffer. Bead-bound DNA complexes and the input sample were boiled and treated with proteinase K.

After proteinase K inactivation, ChIP samples and corresponding input DNA were analyzed by quantitative PCR with the iCycle Real Time PCR machine (BioRad) using the standard curve method. Primer sequences and antibodies are listed in (Table S2) and (Table S3), respectively.

Table S1. List of identified Sirt1 and Sirt7 peptides after co-expression of both proteins and Sirt1 immunoprecipitation.

IP	Protein	Peptides control	Unique control	LFQ Int. control	Peptides Sirt1_IP	Unique Sirt1_IP	LFQ Int. sirt1_IP	ratio
Sirt1	Sirt1	6	6	1,4E+05	24	24	2,3E+09	15827,3
	Sirt7	1	1	0	2	2	3,3E+06	no ratio
Sirt1-YFP	Sirt1	0	0	0	10	10	2,52E+08	no ratio
	Sirt7	2	2	6,14E+05	2	2	2,71E+06	4,4
Sirt7	Sirt1	1	1	0	10	10	2,7E+09	no ratio
	Sirt7	0	0	0	5	5	3,6E+07	no ratio

Table S2. List of primers used in the study.

Name	Sequence	Application
mouse PPARγ2 (promoter)	Forward: 5'- CTGTACAGTTCACGCCCCTC-3' Reverse: 5'- TCACACTGGTGTTTTGTCTATG-3'	ChIP
TK promoter	Forward: 5'- GGCGAATTCGAACACGCAGATGC-3' Reverse: 5'- CTTCCAGCGGATAGAATGGCGCCG-3'	ChIP
mouse β-actin	Forward: 5'-CAACGAGCGGTTCCGATG-3' Reverse: 5'-GCCACAGGATTCCATACCCA-3'	QRT-PCR
mouse Sirt1	Forward: 5'-AAAAGATAATAGTTCTGACTGGAGCTG-3' Reverse: 5'-GGCGAGCATAGATACCGTCT-3'	QRT-PCR
mouse Sirt7	Forward: 5'- CCCCGGACCGCCATCTCAG -3' Reverse: 5'- ATCTCCAGGCCCAGTTCATTCAT -3'	QRT-PCR
mouse PPARγ2	Forward: 5'-TGCGGAAGCCCTTTGGTGAC-3' Reverse: 5'-CTTGGCGAACAGCTGAGAGGAC-3'	QRT-PCR
mouse C/EBPα	Forward: 5'-GGATTCCTGCTTCCTCTCG-3' Reverse: 5'-CGGGATCTCAGCTTCCTGTA-3'	QRT-PCR
mouse aP2	Forward: 5'-GAAAACGAGATGGTGACAAGC-3' Reverse: 5'-GCCCTTTCATAAACTCTTGTGG-3'	QRT-PCR
mouse adiponectin	Forward: 5'-GGAGAGAAAGGAGATGCAGGT-3' Reverse: 5'-CTTTCCTGCCAGGGGTTC-3'	QRT-PCR
mouse FASN	Forward: 5'-GTGCCCTGAGCTGGACTACT-3' Reverse: 5'-AAGCCGTAGTTGCTCTGTCC-3'	QRT-PCR
mouse adiposin	Forward: 5'-GTGGCTGGTTGGGGTGTGGTCA-3' Reverse: 5'-AAGTGTCCCTGCGGTTGCTCTC-3'	QRT-PCR
luciferase	Forward: 5'-GCACTGATCATGAACTCCTCTGGATCTAC-3' Reverse: 5'-GAGAATAGGGTTGGCACCAGCAGCGCAC-3'	QRT-PCR

Table S3. List of antibodies used the study.

Primary antibodies			
Name	**Type**	**Company or other resource**	**Application**
Sir 2 (Sirt1)	rabbit, polyclonal	Upstate (Cat.07-131)	WB (1:1000), IP, ChIP
Sirt1	rabbit, polyclonal	Cell Signaling Techn. (Cat.2028)	WB (1:1000), IP, IF
Sirt7 (D3K5A)	rabbit, monoclonal	Cell Signaling Techn. (Cat.5360	WB (1:1000)
RalA	mouse, monoclonal	BD (Cat.610221)	WB (1:5000)
GAPDH	rabbit, polyclonal	Cell Signaling Techn. (Cat.2118)	WB (1:2000)
β-actin	mouse, monoclonal	Sigma (Cat.A-5441)	WB (1:5000)
β-tubulin	mouse, monoclonal	Sigma (Cat.T-4026)	WB (1:5000)
aP2	Chicken, IgY	Millipore (Cat.ab3515)	WB (1:1000)
FLAG M2	mouse, monoclonal	Sigma (Cat.F-1804)	WB (1:5000), IP
Myc-tag	rabbit, monoclonal	Cell Signaling Techn. (Cat.2278)	WB (1:1000), IP
Set7/9	rabbit, polyclonal	Cell Signaling Techn. (Cat.2813)	WB (1:1000), IP
p53	rabbit, polyclonal	Cell Signaling Techn. (Cat.9282)	WB (1:1000)
p53 (acetyl K373 + K382)	mouse, monoclonal	Abcam (Cat.ab4276)	WB (1:1000)
Acetyl-p53 (Lys382)	rabbit, polyclonal	Cell Signaling Techn. (Cat. 2525)	WB (1:1000)
Tag G(CY)FP	rabbit, polyclonal	Evrogen (Cat.ab122)	WB (1:5000), IP
PPARγ	rabbit, polyclonal	Santa Cruz (Cat.sc-7196)	IF
PPARγ (D9)	rabbit, polyclonal	Cell Signaling Techn. (Cat. 2430)	WB (1:1000)
HA	mouse, monoclonal	Covance (Cat.NMS-101R)	WB (1:1000)
Acetyl-lysine	rabbit, polyclonal	Immunochem (Cat.ICP0380)	WB (1:250)
Histone acH3K9	rabbit, polyclonal	Abcam (Cat.ab10812)	ChIP
Histone H3K9me3	rabbit, polyclonal	Abcam (Cat.ab8898)	ChIP
Histone acH4K16	mouse, monoclonal	Diagenode (Cat.C15200219)	ChIP
Histone acH1K26	rabbit, affinity isolated	Sigma (Cat.H7789)	ChIP

Histone H3	rabbit, polyclonal	Abcam (Cat.ab1791)	ChIP
Secondary antibodies and IgGs			
anti-rabbit IgG	goat,HRP-conjugated	Pierce (Cat.1858415)	WB (1:5000)
anti-mouse IgG	goat,HRP-conjugated	Pierce (Cat.1858413)	WB (1:5000)
anti-Chicken IgY	goat,HRP-conjugated	Abcam(Cat.ab6877-1)	WB (1:1000)
anti-goat IgG	dankey,HRP-conjugated	Rockland (Cat.600667625)	WB (1:1000)
anti-rabbit IgG	goat,Alex594-conjugated	Invitrogen (Cat.A11012)	IF (1:1000)
anti-rabbit IgG	goat,Alex488-conjugated	Invitrogen (Cat.A11070)	IF (1:1000)
Rabbit non-immune IgG		Diagenode (Cat.kch-504-100)	ChIP

Supplementary Figures and datasets.

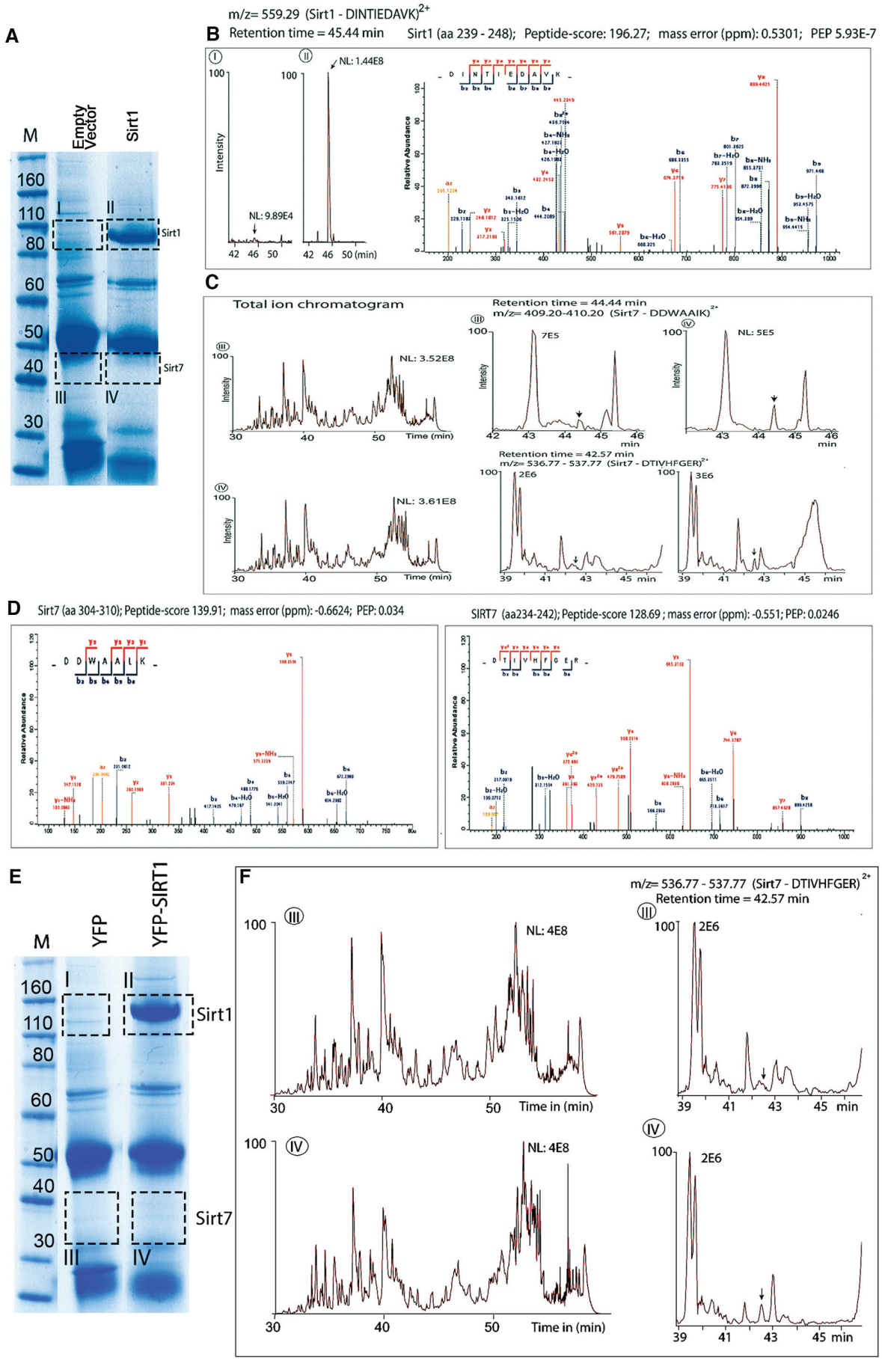

Fig. S1. Identification of Sirt1 binding partners and mass spectrometry analysis of Sirt7 after Sirt1 and YFP-Sirt1 immunoprecipitation. (A) Commassie-stained SDS-PAGE after immunoprecipitation of YFP in control YFP-plasmid (empty vector) transfected cells and of Sirt1 after transfection of a plasmid expressing an untagged wild type Sirt1 using anti-YFP and anti-Sirt1 antibodies, respectively. Bands corresponding to the molecular weight of Sirt1 (indicated as I-II) and Sirt7 (III-IV) were excised and subjected to in-gel digestion with trypsin. Peptides were extracted and measured with an LTQ-Orbitrap Velos instrument. **(B)** Ion chromatogram of a Sirt1 peptide from control (I) and Sirt1 (II) immunoprecipitations. Right panel in (B) indicates a selected MS/MS spectrum for Sirt1. Black arrows mark retention of selected Sirt1 peptide mass in control (band I) and Sirt1 IPs (band II). **(C)** Total ion chromatograms of gel bands (III, control and IV, Sirt7) indicate similar peak intensities. The right panel shows selected ion chromatograms for two Sirt7 peptides. Black arrows mark retention of selected Sirt7 peptides in control (band III) and Sirt1 IPs (band IV). **(D)** MS/MS spectra of Sirt7. Label free quantification data were analysed with the MaxQuant software Version 1.2.2.5. **(E)** Commassie stained SDS-PAGE after immunoprecipitation of YFP control and YFP-Sirt1. Dotted lines indicate analysed gel bands. **(F)** Total ion chromatogram of a control (III) and SIRT7 (IV) bands. Right panel shows Sirt7 peptide mass.

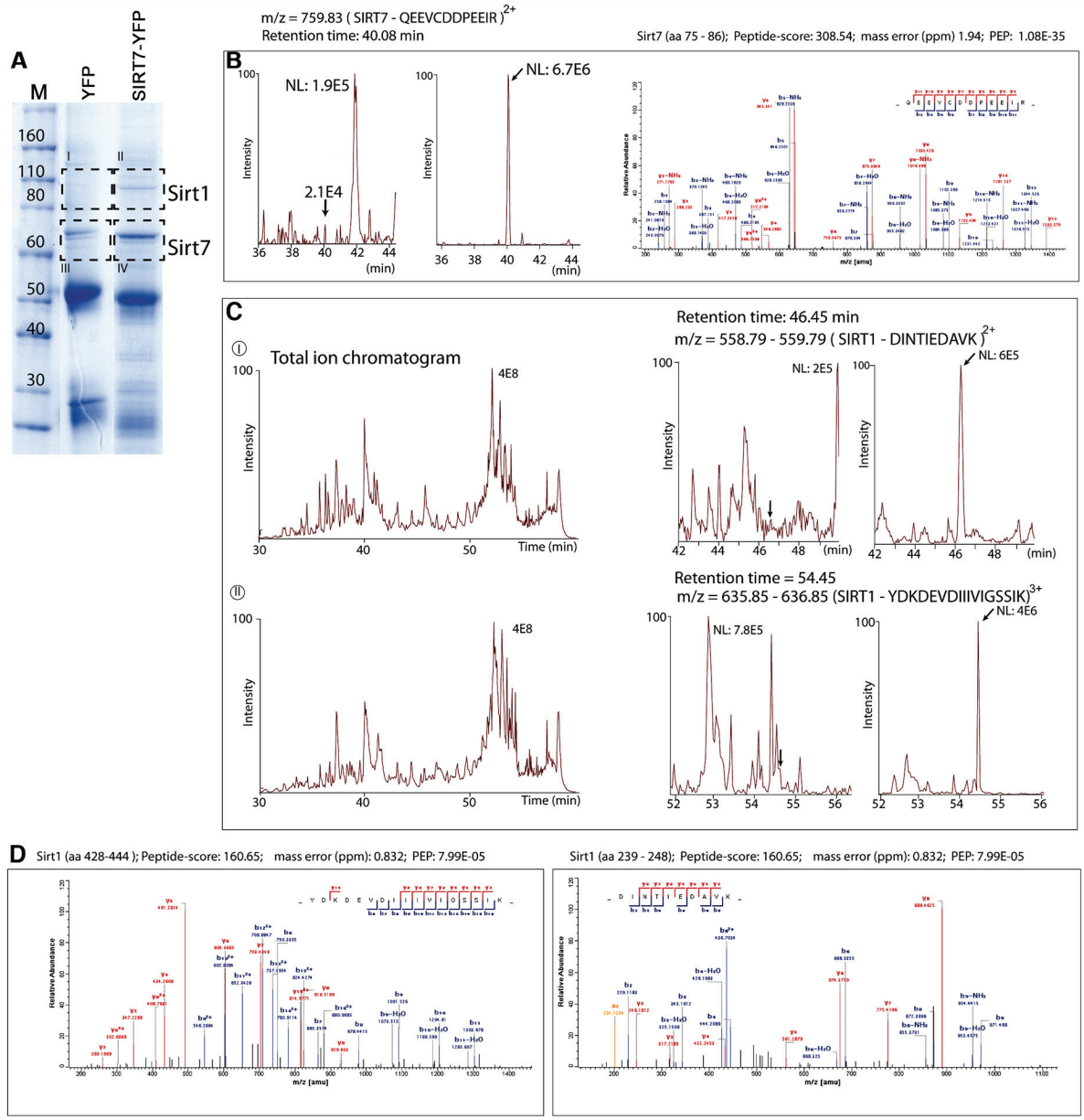

Fig. S2. Mass spectrometry analysis of Sirt1 after Sirt7-YFP immunoprecipitation. (A) Commassie stained SDS-PAGE. **(B)** Ion chromatogram of a Sirt7 peptide and MS/MS spectrum (right panel). **(C)** Total ion chromatogram of control (I) and Sirt1 positive bands (II). The right panels show two Sirt1 peptides, located in the indicated band (II). **(D)** MS/MS spectra from two Sirt1 peptides. Black arrows mark retention of selected Sirt1 peptides in control and Sirt7 IPs.

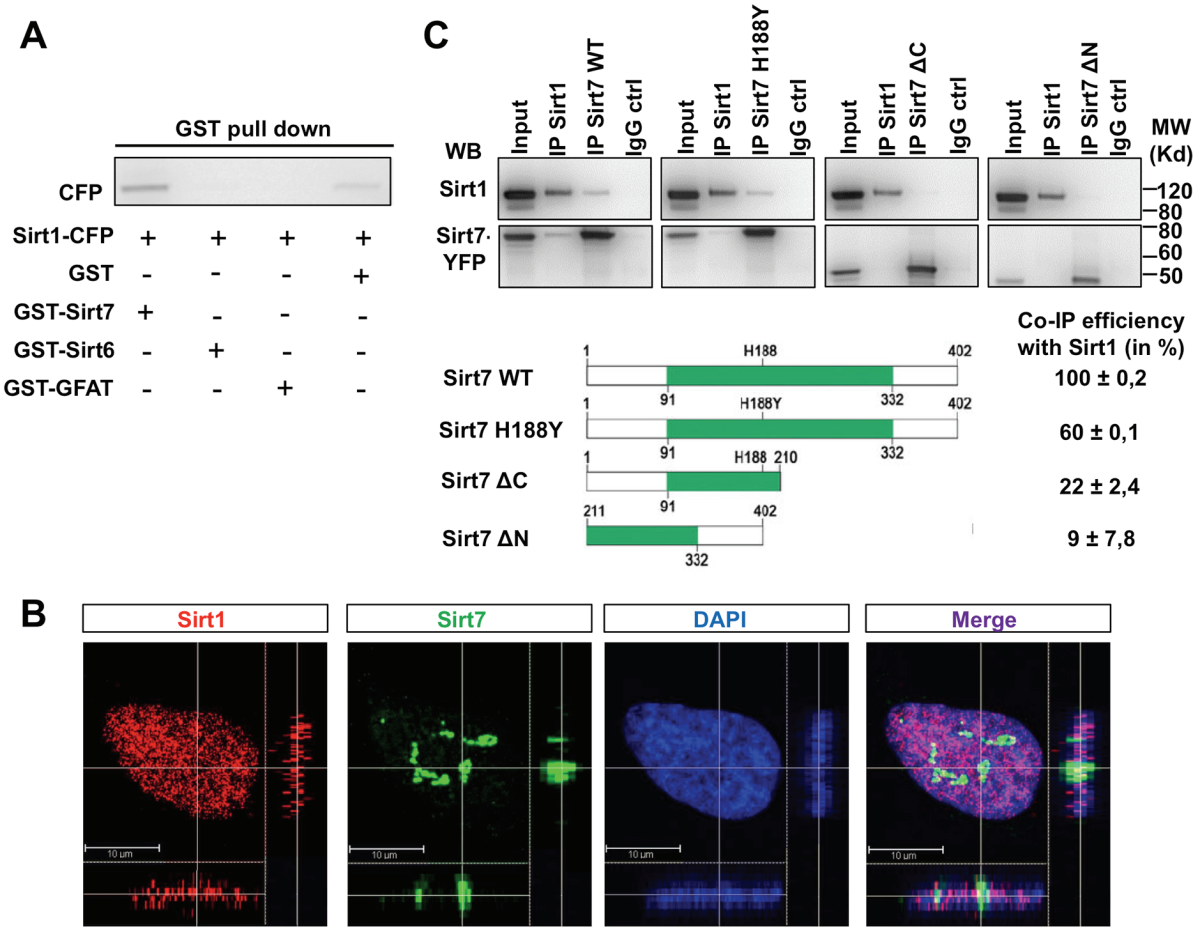

Fig. S3. Sirt7 interacts with Sirt1 and inhibits its auto-deacetylase activity. (A) GST pull-down experiment using bacterially produced Sirt7, Sirt6, GFAT, and Sirt1-CFP. Sirt1 interacts with Sirt7 but not with Sirt6 or GFAT (n=3). **(B)** Co-localization of endogenous Sirt1 and Sirt7 proteins in the nucleus of U2OS cells. Confocal microscopy images of cells stained with anti-Sirt1 (red) and anti-Sirt7 (green) antibodies are shown. **(C)** Coupled immuno-precipitation/western blot analysis of Sirt1-Sirt7 interactions in HEK293 cells transfected with untagged Sirt1 and different YFP-Sirt7 constructs depicted below. Interactions of Sirt1 with full-length Sirt7, Sirt7 H188Y and Sirt7 deletion mutants (Sirt7deltaC contains amino acids 1-210 and Sirt7deltaN amino acids 211-402) are shown. Anti-Sirt1 and anti-YFP antibodies were used for co-immunoprecipitation and for western blot analysis as indicated (n=3). Densitometric measurements reveal reduced interaction of the Sirt7 H188Y mutant with Sirt1 compared to WT Sirt7 while deletion of the C- and the N-

terminus of Sirt7 virtually abolished the interaction between both molecules. Quantification represents the ratio of Sirt7 normalized to Sirt1 (+/- SD); n=3.

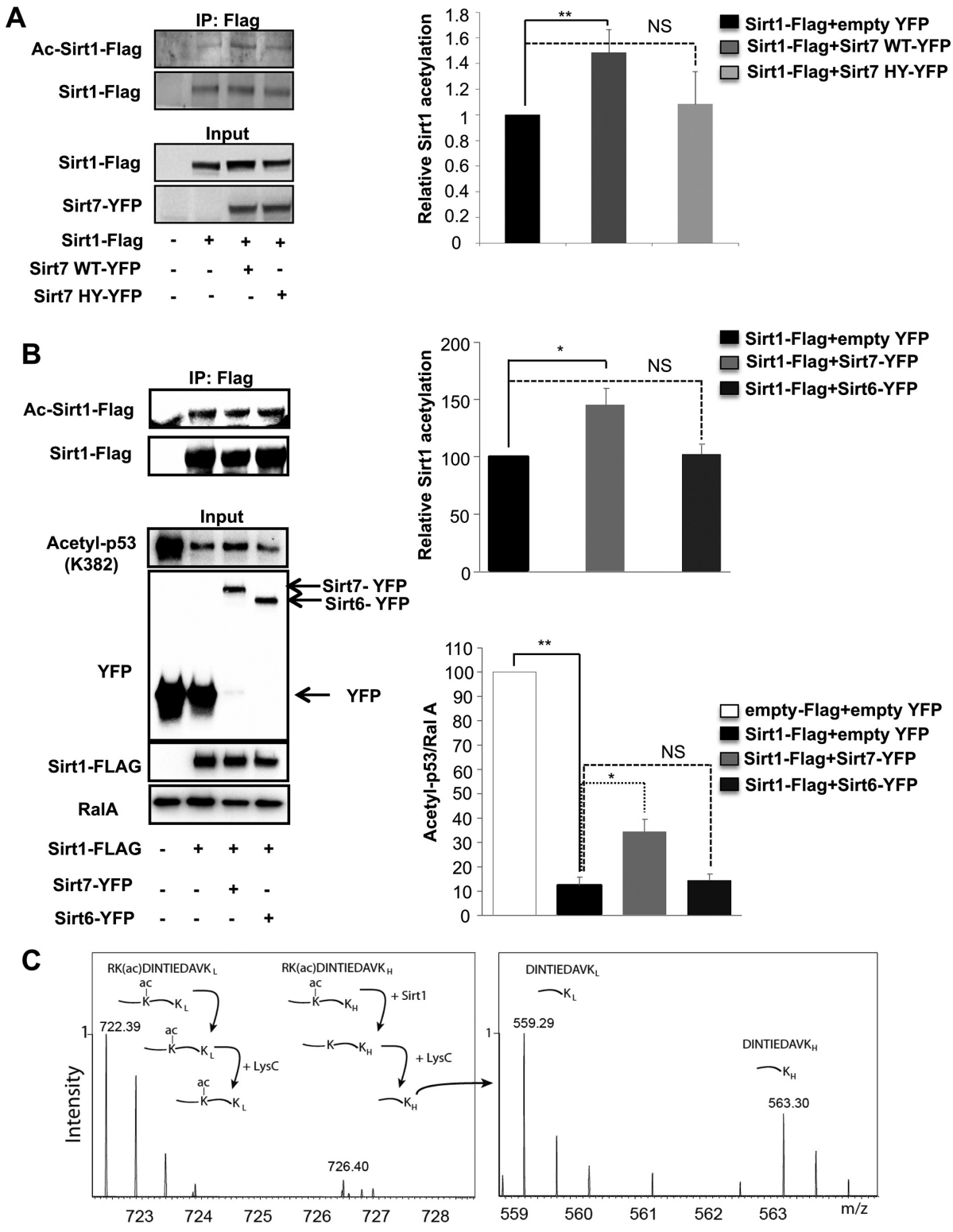

Fig. S4. Sirt1 auto-deacetylation. (A) Increased acetylation (WB: anti-AcK) of Flag-tagged Sirt1 after co-expression with wild type Sirt7 and, to a lesser extent, with H188Y-Sirt7 mutant (n=3); ** p< 0.001. **(B)** Sirt6 does not affect activity of Sirt1. WB analysis of Sirt1 and p53 acetylation levels (quantifications shown on the right) after overexpression of Sirt7 or Sirt6.

(n=3); *p<0.05; ** p< 0.001. **(C)** Quantitative mass spectrometry analysis of deacetylation of Sirt1-derived peptides containing acetylated K230 residue by recombinant Sirt1.

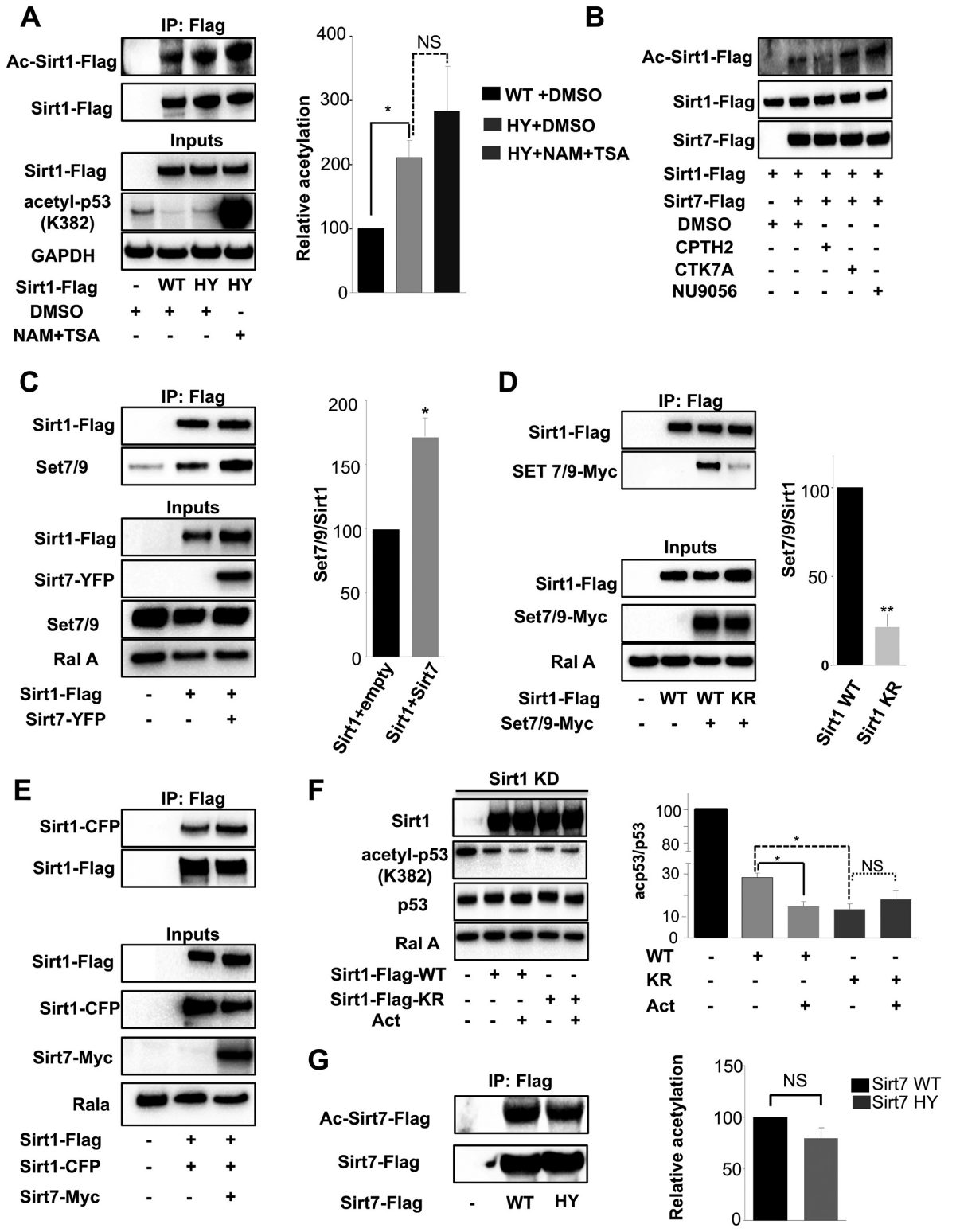

Fig. S5. Sirt7 inhibits Sirt1 activity. (A) The Sirt1 HY mutant does not show (auto)catalytic activity. Levels of Sirt1 acetylation were visualized by immunoprecipitation (Flag) and anti-acetyl-lysine antibody. p53 acetylation (inputs) was proved by specific antibody against p53 acetylated at K382. Quantifications are shown on the right (n=3); *p<0.05; NS - not

significant. **(B)** Acetylation levels of Sirt1-Flag after treatment with HAT inhibitors, with and without addition of Sirt7 as indicated. A representative WB is shown, (n=3). **(C)** Sirt7 expression promotes interaction of Set7/9 with Sirt1. Quantification of Sirt1/Set7/9 binding is shown on the right. (n=3); *p<0.05. **(D)** The Sirt1 hyperactive mutant K230R (Sirt1 KR) shows weaker binding to Set7/9 compared to wildtype Sirt1. (n=3); **p< 0.001. **(E)** Sirt7 stimulates oligomerization of Sirt1 (note the stronger Sirt1-CFP band co-immunoprecipitated with the anti-Flag antibody in presence of Sirt7). (n=3); a representative WB is shown. **(F)** Wild type Sirt1 or Sirt1 hyperactive mutant (Sirt1 KR) were transfected into Sirt1 knockdown (Sirt1 KD) cells. Cells were treated with Sirt1 activator II (Act) as indicated. The intensity of p53 acetylation was quantified (right panel). (n=3); *p<0.05; NS - not significant. **(G)** Sirt7 is an acetylated protein but lacks autodeacetylation activity. Acetylation of Sirt7 wildtype (WT) and its catalytical inactive mutant (HY) was quantified by immunoprecipitation and WB analysis (right). (n=3); NS - not significant**.**

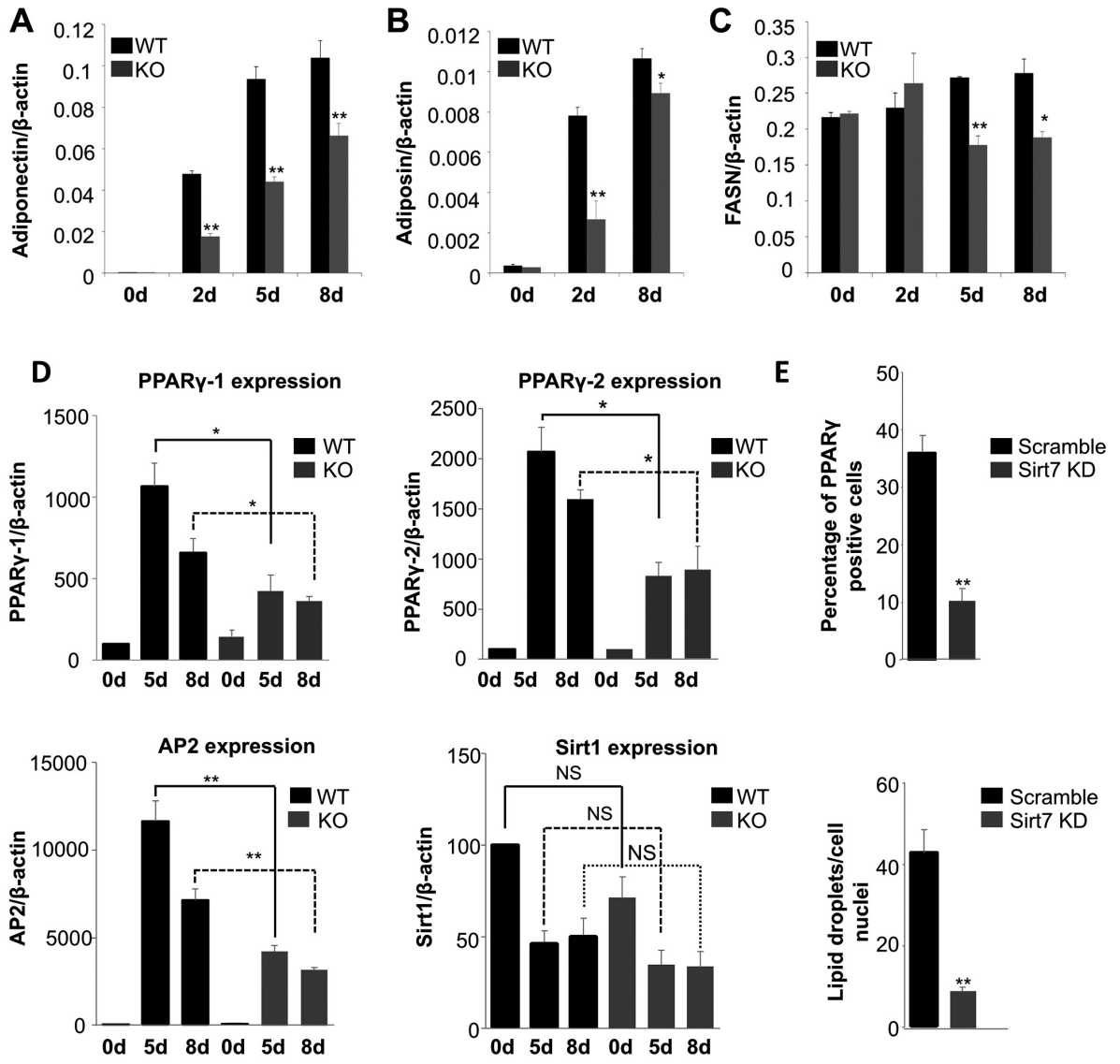

Fig. S6. Expression of adipogenic markers is decreased in Sirt7 knockout MEFs. A quantitative RT-PCR analysis of adiponectin **(A)**, adiposin **(B)** and FASN **(C)** expression in cultured wild type and Sirt7 knockout MEFs is shown (mean values +/- SD); (n=3); *p<0.05; **p<0.01. Data were analyzed using Student´s *t* test (two-tailed paired t-test). **(D)** Quantification of PPARγ, AP2 and Sirt1 expression levels presented in the main Fig. 5E. **(E)** Quantification of PPARγ positive cells and the number of lipid droplets per cell. The data correspond to the experiment shown in the main Fig. 5G. n=3; *p<0.05; ** p< 0.001; NS – not significant.

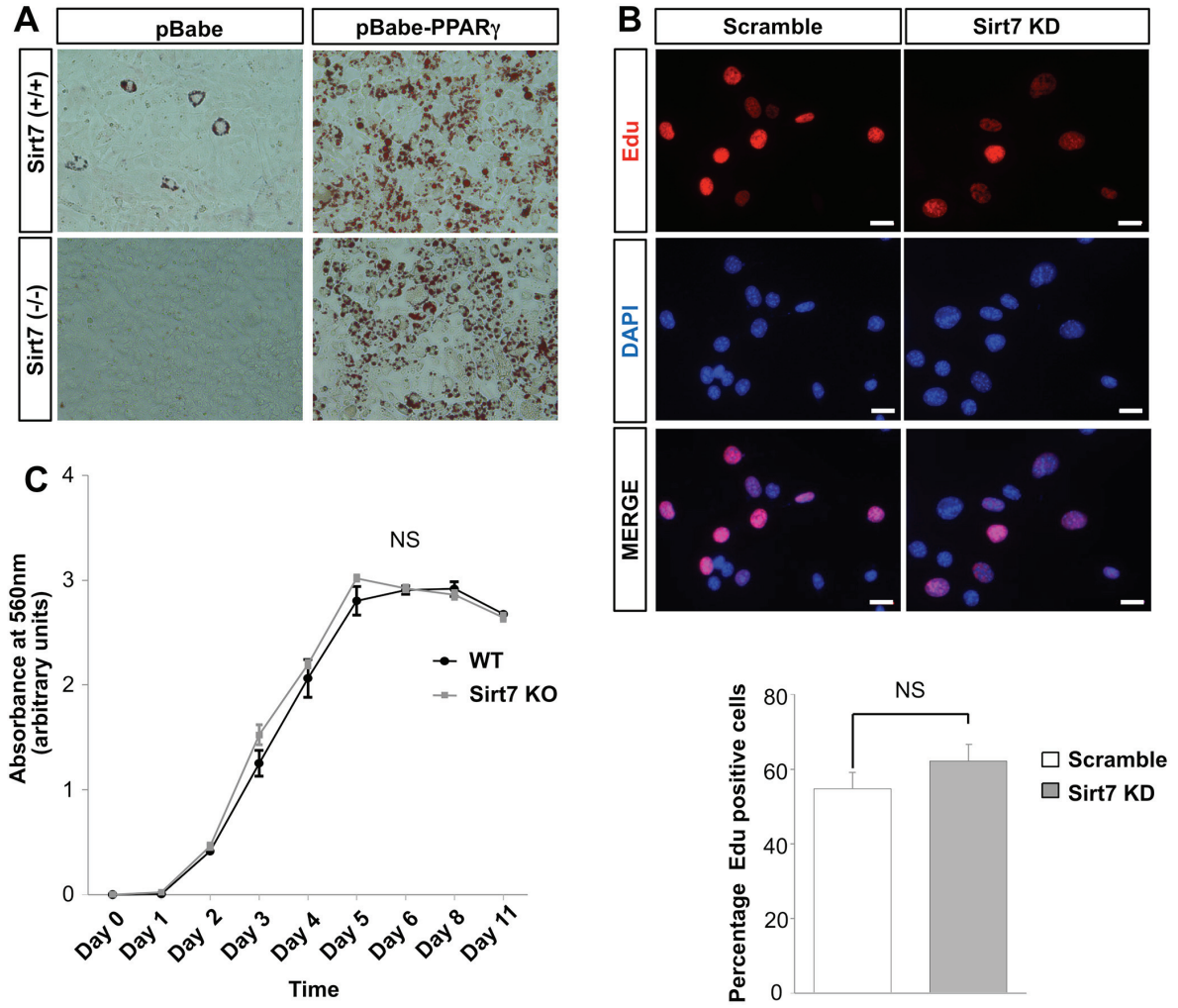

Fig. S7. Sirt7-/- preadipocytes do not reveal overt cellular defects. (A) Overexpression of PPARγ rescues adipocyte differentiation. Oil-red-O staining indicates differentiation of adipocytes after overexpression of PPARγ in Sirt7 knockout MEFs. (n=3). **(B)** Scramble or Sirt7 knockdown (Sirt7 KD) 3T3 L1 preadipocytes were stained for EdU incorporation (red). Nuclei were counterstained with DAPI (blue). A representative picture from 3 independent experiments is shown. Quantification of EdU positive cells is shown below. **(C)** Growth curves of wildtype and Sirt7 KO MEFs after crystal violet staining at indicated days in culture.

Dataset 1. List of proteins interacting with Sirt7. Provided as an additional Excel table.

References

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