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Supplemental Information

SIRT7 Facilitates CENP-A Nucleosome Assembly and Suppresses Intestinal Tumorigenesis

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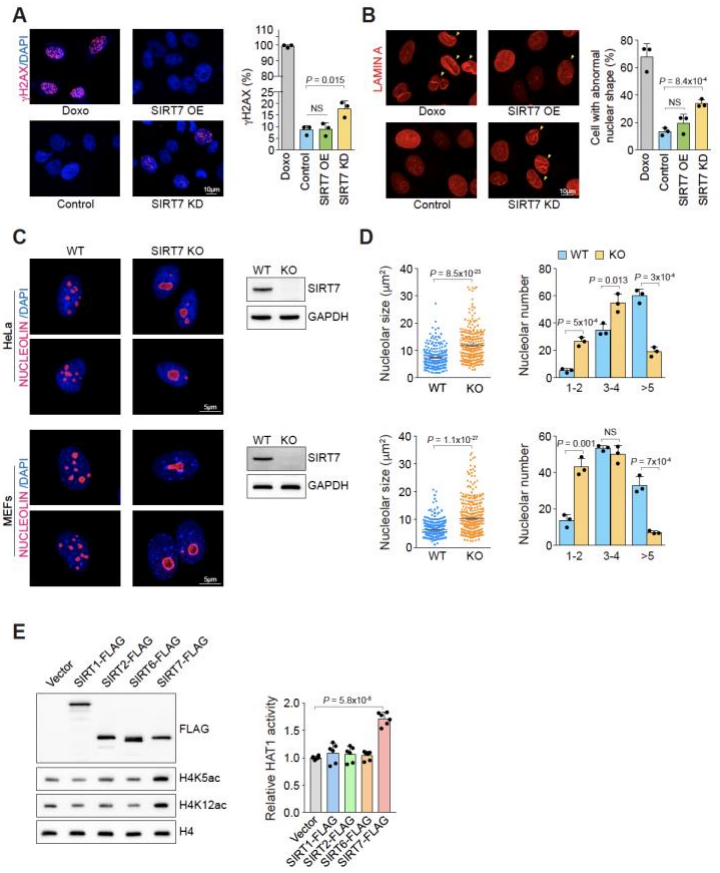

Figure S1. SIRT7 deletion triggers DNA damage response and nucleolar expansion. Related to Figure 1 and 2

(A) Immunofluorescence analysis of γH2AX in SIRT7 overexpressing or knockout Rpe-1 cells. 24 hour doxorubicin (Doxo, 1μM) were used as a positive control. Cell with γH2AX foci>5 was set as γH2AX+ cell (n>100 per experiment). (B) Lamin A stained images for irregular nuclear shape in *Sirt7* knockdown and doxorubicin-treated Rpe-1 cells. (n>100 per experiment). (C) Immunofluorescence of Nucleolin (magenta) in HeLa and MEF cells, and immunoblot demonstrations of SIRT7 expression. (D) Nucleolar size was analyzed by Nucleolin immunofluorescence, and the nucleolus number per cell was quantified (n>50 per experiment). (E) Immunoblot analysis of histone H4K5Ac and H4K12Ac levels from HeLa cells expressing SIRT1, 2, 6 and 7 in SIRT7 KO background (left). Comparison of HAT1 activity in SIRT7 KO HeLa cells with expressing SIRT1, 2, 6 and 7. SIRT7 KO activity was set as 1. Unpaired Student's t-test was used Nucleolar size results are presented as mean ± s.e.m; other results are presented as mean ± s.d.

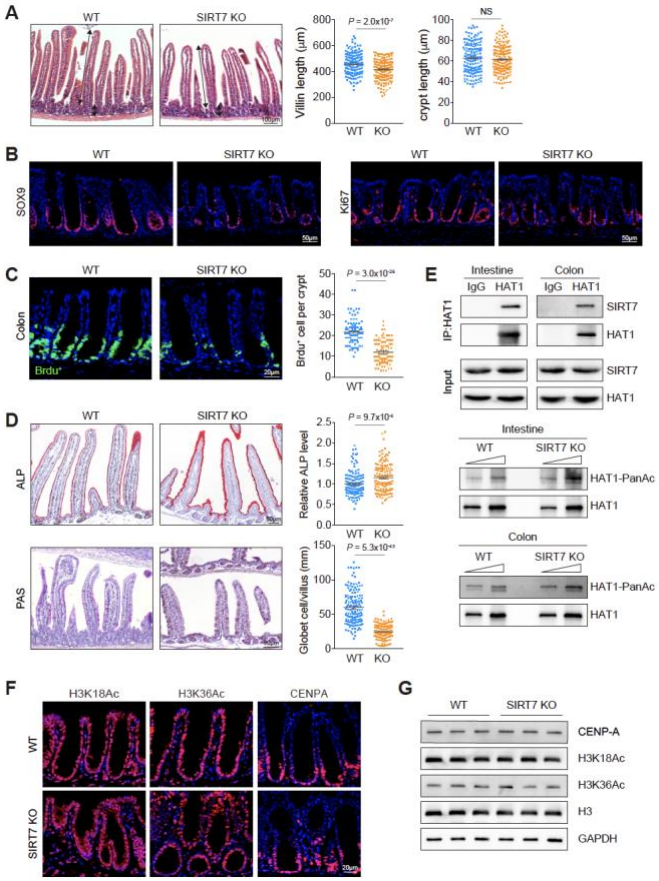

Figure S2. SIRT7 deficiency lead to shortened villus and altered intestinal cell specificity. Related to Figure 3

(A) Images of H&E staining and the quantification of villus and crypt length of wild-type versus SIRT7 knockout intestine samples. Immunohistostaining of (B) SOX9 and Ki67 (C) BrdU in colon samples from wild-type and SIRT7 KO mice. (D) Immunohistostaining of enterocyte marker ALP, and goblet cell marker PAS in intestine samples from wild-type and SIRT7 KO mice. (E) Immunoprecipitation of HAT1-associated proteins from wild-type intestinal extracts. Immunoblot demonstration of HAT1 associated with SIRT7 (upper). Elevated acetylation level of HAT1 in SIRT7 KO revealed by immunoblot analysis (lower). (F) Immunoblot analysis of the indicated proteins from intestinal crypt lysates (n=3). (G) Immunostaining analyses of CENP-A, H3K18Ac and H3K36Ac in colon from wild-type and SIRT7 KO mice (n=3). Unpaired Student's t-test was used. Results are presented as mean ± s.e.m.

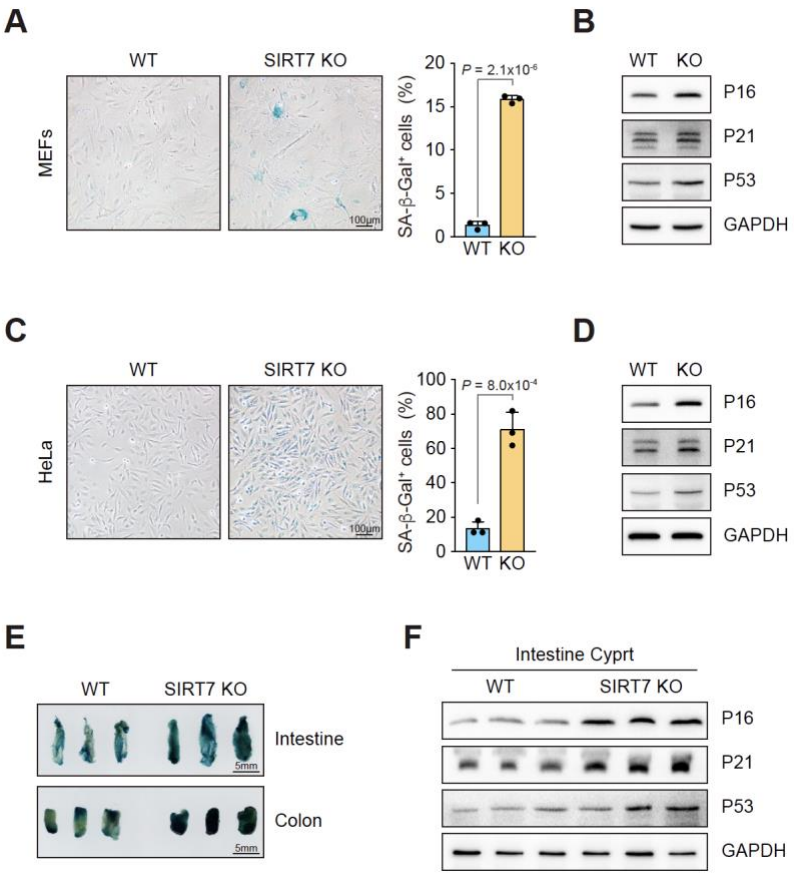

Figure S3. The loss of SIRT7 triggers senescence in vitro and in vivo. Related to Figure 3

(A) Senescence-associated β-galactosidase (SA-β-Gal) stain was performed in wild-type and SIRT7 KO MEF cells, and the SA-β-Gal positive cells were scored. (B) Immunoblots indicated elevated p16, p21 and p53 in SIRT7 KO MEF cells. (C) SA-β-Gal stain in wild-type and SIRT7 KO HeLa cells and the positive cell counting. (D) Immunoblots indicated elevated p16, p21 and p53 in SIRT7 KO HeLa cells. (E) Images of SA-β-Gal stained small intestine and colon samples from wild-type and SIRT7 KO mice (n=3 per group). (F) Immunoblots of p16, p21 and p53 from small intestine crypts. Unpaired Student's t-test was used. Results are presented as mean ± s.e.m.

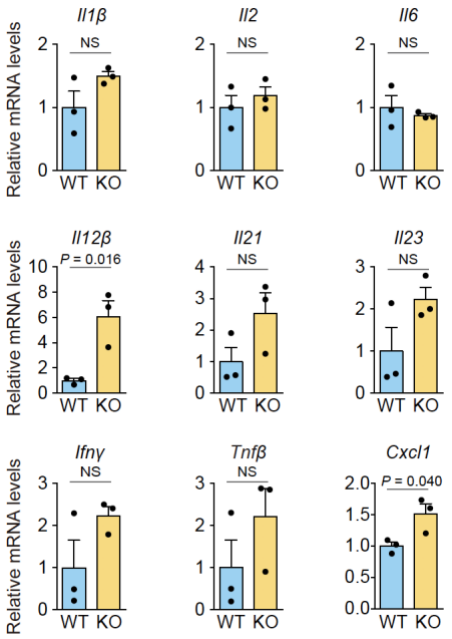

Figure S4. SIRT7 loss elicits subtle intestinal inflammation. Related to Figure 4

Quantitative RT-PCR analysis of cytokine genes from untreated wild-type and SIRT7 KO colon homogenates (n=3). Unpaired Student's t-test was used (NS, not significant). Results are presented as mean ± s.e.m.

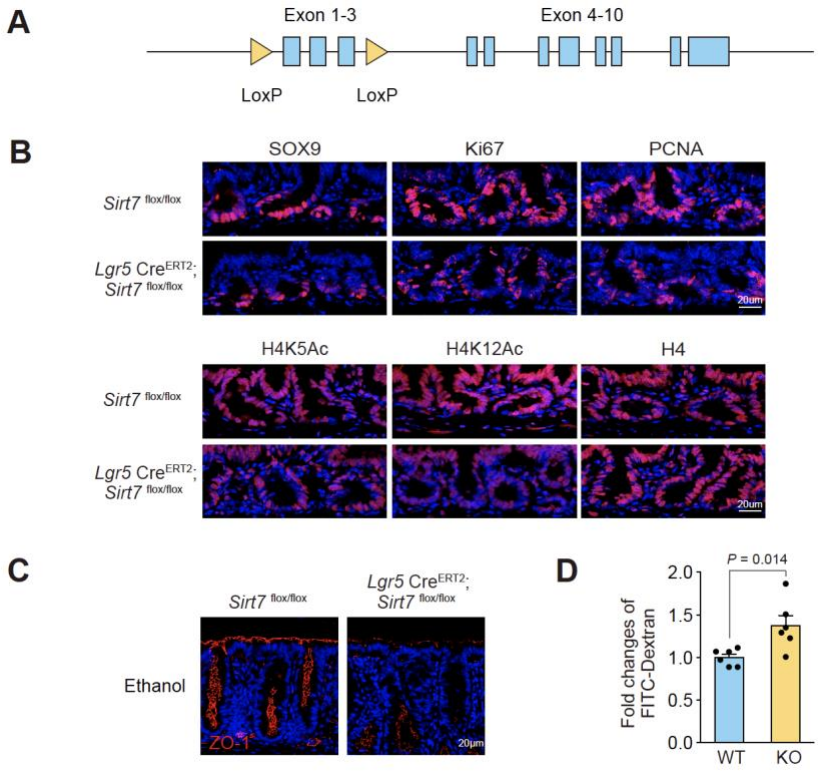

Figure S5. Reduced stem cell marker and histone H4K5/12Ac in the *Sirt7*-conditional KO intestine. Related to Figure 6

(A) Design of the conditional *Sirt7* knockout strategy, with Exon 1-3 flanked by loxP sites. (B) Immunohistostaining of crypt stem cell marker SOX9, TA cell markers Ki67 and PCNA, and histone H4, H4K5Ac and H4K12Ac in intestine samples from wild-type and Lgr5 Cre-mediated *Sirt7*-conditional ISC KO. (C) ZO-1 staining in colon sections from wild-type and SIRT7-ISC KO mice that were treated with ethanol for 5 days (n=3 per group). (D) Intestinal permeability measured by the concentration of FITC-dextran in the blood serum (n=6 per group). Unpaired Student's t-test was used, and data are shown as mean ± s.e.m.

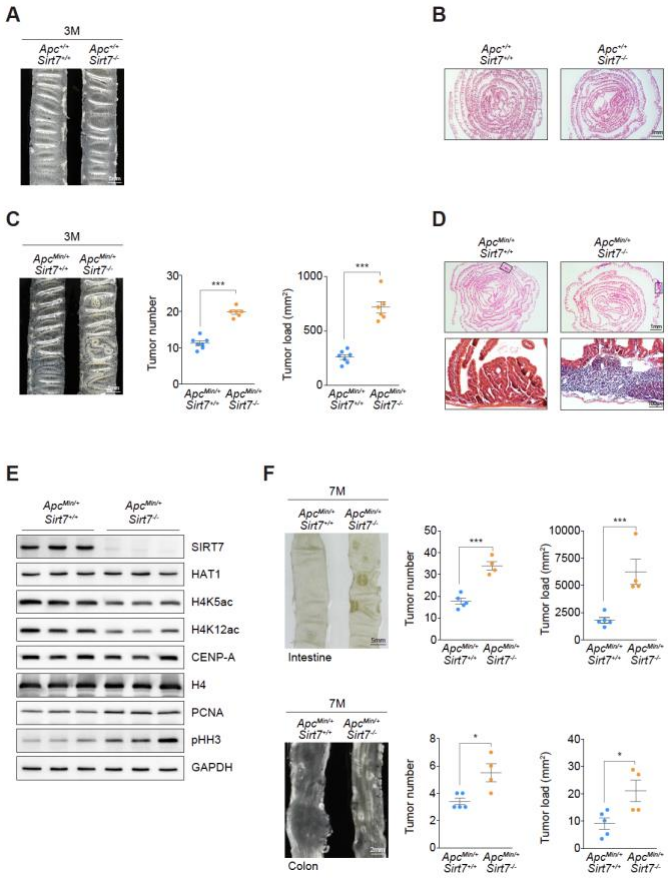

Figure S6. SIRT7-ablation accelerates tumor incident in *Apc*$^{Min/+}$ mice. Related to Figure 7

(A) Macroscopic images of the small intestine samples from 3 month-old wild-type and SIRT7 KO mice (n≥4 per group), no apparent tumor was identified in the KO samples. (B) H&E images of the representative small intestine samples. (C) Macroscopic image, tumor number, and tumor load from the 3 month-old *Apc*$^{Min/+}$ and *Apc*$^{Min/+}$*; Sirt7*$^{-/-}$ small intestine samples. (n≥6 per group). (D) H&E images of the representative small intestines. (E) Immunoblot analysis of the indicated proteins from *Apc*$^{Min/+}$ and *Apc*$^{Min/+}$*; Sirt7*$^{-/-}$ intestinal lysates (n=3). (F) Macroscopic image, tumor number, and tumor load from the 7 month-old *Apc*$^{Min/+}$ and *Apc*$^{Min/+}$*; Sirt7*$^{-/-}$ small intestine (upper) and colon (lower). (n≥4 per group). Unpaired Student's t-test was used, and data are shown as mean ± s.e.m.

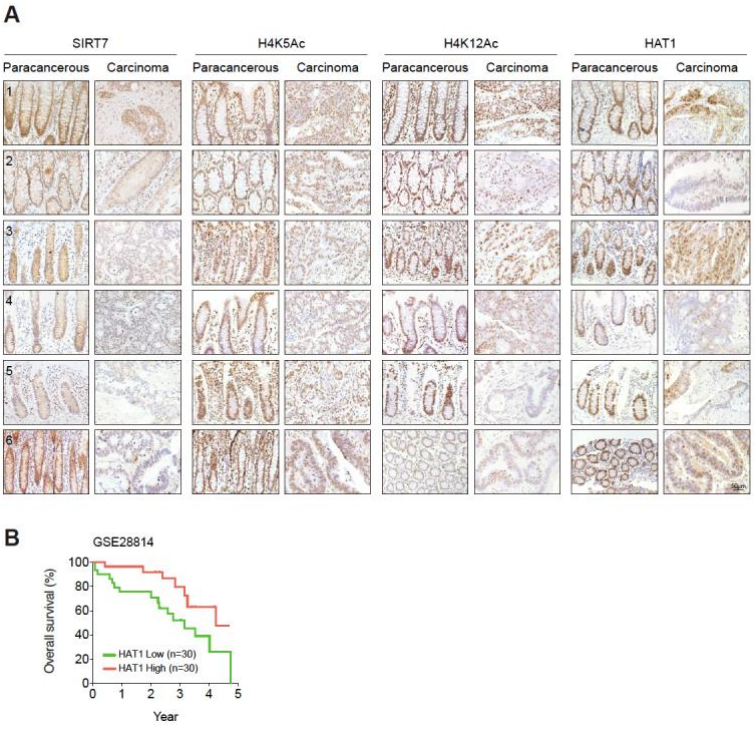

Figure S7. Lower SIRT7 expression in the human colorectal cancer biopsy specimens. Related to Figure 7

(A) Immunohistostaining analyses of SIRT7, histone H4K5Ac, H4K12Ac, and HAT1 levels in human CRC biopsy specimens (n=6 per group). SIRT7, histone H4K5Ac, H4K12Ac are declined in the carcinoma samples. Scale bars: 50 μm. (B) Survival analysis (log-rank test) of colorectal cancer patients with high or low levels of HAT1.

TRANSPARENT METHODS:

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
BrdU	Abcam	# ab6326, RRID:AB_2313786
human CENP-A	Abcam	# ab13939; RRID:AB_300766
mouse CENP-A	Cell Signaling Technology	# 2048, RRID:AB_1147629
CENP-E	Abcam	# ab133583
HAT1	Abcam	# ab194296
Histone H3	Cell Signaling Technology	# 4499, RRID:AB_10544537
Histone H3K18ac	Cell Signaling Technology	# 13998, RRID:AB_2783723
Histone H3K36ac	Abcam	# ab177179
Histone H4	Abcam	# ab10158, RRID:AB_296888
Histone H4K5ac	Cell Signaling Technology	# 8647, RRID:AB_11217428
Histone H4K12ac	Cell Signaling Technology	# 13944, RRID:AB_2798350
Ki67	Cell Signaling Technology	#12202, RRID:AB_2620142
Lamin A+C	Abcam	# ab133256, RRID:AB_2813767
NPM1	Cell Signaling Technology	# 3542, RRID:AB_2155178
Nucleolin	Cell Signaling Technology	# 14574, RRID:AB_2798519
Pan anti-acetyllysine	PTM BIO	# PTM-101
PCNA	Abcam	#ab29, RRID:AB_303394
pHH3	Cell Signaling Technology	#9701, RRID:AB_331535
RBBP4/7	Cell Signaling Technology	# 9067; RRID: AB_11178523
SIRT7	Cell Signaling Technology	# 5360, RRID:AB_2716764
SOX9	Abcam	# ab185966, RRID:AB_2728660
Villin	Santa Cruz	# sc-7672, RRID:AB_2215973
Chemicals, Peptides, and Recombinant Proteins		
BrdU	Sigma	B9285
anti-FLAG M2 magnetic beads	Sigma	M8823
FLAG peptide	Sigma	F4799
DSS	MP Biomedicals	216011010
FITC-conjugated dextran	Sigma	FD4
Tamoxifin	Sigma	T5648
TSA Plus Fluorescein System	PerkinElmer	NEL741001KT
TSA Plus Cyanine 3 System	PerkinElmer	NEL744001KT
Critical Commercial Assays		
DAB Peroxidase Substrate Kit	Vector Laboratories	SK-4100

HAT Activity Assay Kit	Enzo Life Sciences	ALX-850-326-KI01
H&E Stain Kit	Abcam	ab245880
ImmPACT Vector Red Alkaline Phosphatase Substrate Kit	Vector Laboratories	SK-5105
Omniscript RT Kit	Qiagen	205113
Periodic Acid Schiff (PAS) Stain Kit	Abcam	ab150680
RNeasy Mini Kit	Qiagen	74106
Senescence β-Galactosidase Staining Kit	Cell Signaling Technology	9860S
SYBR Green PCR Kit	Qiagen	204057
Vectastain Elite ABC Peroxidase Kit	Vector Laboratories	PK-6101
Deposited Data		
RNA-seq data available at GEO	This paper	GEO: GSE138289
Experimental Models: Cell Lines		
SIRT7 KO Mouse Embryonic Fibroblasts	This paper	N/A
Human: HeLa	ATCC	#CCL-2
Human: U2OS	ATCC	#HTB-96
Human: HEK293T	ATCC	#CRL-11268
Human: Caco2	ATCC	#HTB-37
Human: hTERT RPE-1	ATCC	#4000
Experimental Models: Organisms/Strains		
Male C57BL/6 strain	SHANGHAI MODEL ORGANISMS CENTER	N/A
Sirt7 knockout strain	Jackson Laboratory	JAX-012771
Sirt7-floxed strain	This paper	N/A
$APC_{Min/+}$ strain	Jackson Laboratory	JAX-002020
Lgr5-EGFP-IRES-creERT2 strain	Jackson Laboratory	JAX-008875
Oligonucleotides		
SIRT7 shRNA	gcacctttctgtgagaacggaa	N/A
SIRT7 sgRNA-1	cgttaccaggtccgcgctct	N/A
SIRT7 sgRNA-1	gcttcaggccctcgcgccgc	N/A
SIRT7 sgRNA-1	ggccctgcagctccgttacc	N/A
SIRT7-Fwd-NotI	gctgcggccgcatggcagccgggggtctgag	N/A
SIRT7-Rev-MluI	catacgcgtcgtcactttcttccttttg	N/A
SIRT7 H187Y-Fwd	ctccgagctctacgggaacatg	N/A
SIRT7 H187Y-Rev	catgttcccgtagagctcggag	N/A
HAT1-Fwd-NotI	gctgcggccgcatggcgggatttggtg	N/A
HAT1-Rev-MluI	tcgacgcgtctcttgagcaagtcg	N/A
HAT1 K15R-Fwd	tagaatataggagtgcagtgga	N/A
HAT1 K15R-Rev	actgcactcctatattctaccaa	N/A

HAT1 K15Q-Fwd	gtagaatatcagagtgcagtgg	N/A
HAT1 K15Q-Rev	ctgcactctgatattctaccaa	N/A
HAT1 K280R-Fwd	aagatccatccagaagctatgtgaa	N/A
HAT1 K280R-Rev	ttcacatagcttctggatggatctt	N/A
HAT1 K280Q-Fwd	aagatccatcccaaagctatgtgaa	N/A
HAT1 K280Q-Rev	ttcacatagctttgggatggatctt	N/A
Il1β-Fwd	agcttcaggcaggcagtatc	N/A
Il1β-Rev	cgtcacacaccagcaggtta	N/A
Il2-Fwd	ctgcggcatgttctggatttg	N/A
Il2-Rev	tggcactcaaatgtgttgtcag	N/A
Il6-Fwd	cacaagtccggagaggagac	N/A
Il6-Rev	ctgcaagtgcatcatcgttg	N/A
Il12β-Fwd	agaccctgcccattgaactg	N/A
Il12β-Rev	caggagtcagggtactccca	N/A
Il21-Fwd	gcacatagctaaatgcccttcc	N/A
Il21-Rev	ggtacccggacacaacatgg	N/A
Il23-Fwd	agaccttggcggatcctttg	N/A
Il23-Rev	ccagcagctctctcggaatc	N/A
Ifnγ-Fwd	agacaatcaggaaatcagca	N/A
Ifnγ-Rev	tggacctgtgggttgttgac	N/A
Tnfβ-Fwd	ctctctggtgtccgcttctc	N/A
Tnfβ-Rev	gagcagtgagttctgcttgc	N/A
Cxcl1-Fwd	ctgggattcacctcaagaacatc	N/A
Cxcl1-Rev	cagggtcaaggcaagcctc	N/A
Sirt7-Fwd	gctgctagcaaagcagacac	N/A
Sirt7-Rev	gttggtgggagcggttgtag	N/A
Lgr5-Fwd	acgtagctgatgtggttgg	N/A
Lgr5-Rev	gcctcaaagtgcttatgctg	N/A
Ascl2-Fwd	gcctactcgtcggaggaa	N/A
Ascl2-Rev	ccaactggaaaagtcaagca	N/A
Sox9-Fwd	ctcgcttcagatcaactttgc	N/A
Sox9-Rev	actccccacattcctcct	N/A
Ki67-Fwd	ccagcactccaaagaaaccc	N/A
Ki67-Rev	attttgtagggtcgggcagg	N/A
Cdk4-Fwd	ccaatgttgtacggctgatgg	N/A
Cdk4-Rev	tgaagaaaatccaggccgct	N/A
Muc2-Fwd	acctccaggttcaacaccag	N/A
Muc2-Rev	gttggccctgttgtggtct	N/A
Clca3-Fwd	gggagcatgctgaacgatga	N/A
Clca3-Rev	ccctcctgcagatactgtgg	N/A
Chga-Fwd	cgatccagaaagatgatggtc	N/A
Chga-Rev	ccttcagacggcagagctt	N/A

Reg4-Fwd	ggcgtgcggctactcttac	N/A
Reg4-Rev	gaagtacccatagcagtggga	N/A
Trpm5-Fwd	cctgtagaatggtgccctcg	N/A
Trpm5-Rev	gtggatgagccgaagtgtga	N/A
Dclk1-Fwd	agctcagttaatggaacccctg	N/A
Dclk1-Rev	tgccttctcccaagctcatc	N/A
Alpi-Fwd	acctccatctttggtctggc	N/A
Alpi-Rev	cctgctgcttgtagttggga	N/A
Apoa1-Fwd	gccaacagctgaacctgaatc	N/A
Apoa1-Rev	tcccagaagtcccgagtcaa	N/A
Rpl19-Fwd	aagcctgtgactgtccattc	N/A
Rpl19-Rev	cttcttggattcccggtatc	N/A
Recombinant DNA		
pCAGGS-mCherry	Addgene	# 41583
pCAGGS-Sirt7 WT-FLAG	This paper	N/A
pCAGGS-Sirt7	This paper	N/A
pCAGGS-shSirt7	This paper	N/A
pCAGGS-Sirt7 H187Y	This paper	N/A
pCAGGS-Hat1	This paper	N/A
pCAGGS-Hat1 K15R	This paper	N/A
pCAGGS-Hat1 K15Q	This paper	N/A
pCAGGS-Hat1 K280R	This paper	N/A
pCAGGS-Hat1 K280Q	This paper	N/A
pCAGGS-Hat1 K15R,K280R	This paper	N/A
pCAGGS-Hat1 K15Q,K280Q	This paper	N/A
pLVX-Tight-Puro	Clontech	# 632162
pLVX-Sirt7	This paper	N/A
pLVX-shSirt7	This paper	N/A
pLVX-HP1□-mcherry	This paper	N/A
Software and Algorithms		
Graphpad Prism 7 software	https://www.graphpad.com/	N/A
ImageJ	https://imagej.nih.gov/ij/	N/A
Leica LAS AF 2.4.1	Leica	N/A
Zen 2011 SP2	Zeiss	N/A
NIS-Elements F3.2	Nikon	N/A

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plasmid Construction

Constructs for inducible mouse Sirt7 or Sirt7 shRNA were cloned into pLVX-Tight-Puro

vector (#632162, Addgene) via NotI and MluI sites. The rtTA expressing construct pMA2640 (#25434); the helper plasmids for retroviral production, pUMVC (#8449) and pCMV-VSV-G (#8454); and the helper plasmids for lentiviral production, pMD2.G (#12259) and psPAX2 (#12260) were purchased from Addgene. SIRT7 and SIRT7-Flag plasmids were constructed by cloning the Sirt7 cDNA into a pCAGGS backbone (#41583, Addgene) via NotI and MluI sites. HP1β were constructed in a pLVX-mcherry vector (Invitrogen) via NotI and BamHI sites. SIRT7 H187Y mutant, HAT1 and HAT1 point mutations (K15R, K15Q, K280R, K280Q, K15/K280R, K15/K280Q) were generated using PCR based site-directed mutagenesis method with primers designed as listed, then subcloning into the pCAGGS vector.

Cell culture

HEK293T, HeLa and RPE1 cell lines were obtained from ATCC. HEK293T, HeLa and RPE1 cells were cultured in DMEM/F12 or DMEM (Invitrogen) with 10 % FBS and 100 U/ml penicillin/streptomycin, and maintained at 37°C with 5 % CO2 in a humidified environment. All cell lines were examined with a PCR-based method (MP0035, Sigma) to ensure mycoplasma-free condition.

Animal model

Whole body Sirt7 knockout (JAX-012771), $APC^{Min/+}$ (JAX-002020) and Lgr5-EGFP-IRES-creERT2 (JAX-008875) mouse strains were obtained from the Jackson Laboratory. The Sirt7-floxed mice were generated by Viewsolid Biotech (Beijing, China) via introducing a loxP-flanked exon 1-3 cassette into ES cells. All mice were maintained in a pathogen-free facility, and all experiments were conducted according to the approved protocols under the regulation of laboratory animal care and use guidelines issued by the Institutional Biomedical Research Ethics Committee of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

METHOD DETAILS

Generation of SIRT7 knockout HeLa cell line by CRISPR-Cas9

Small guide RNAs (sgRNAs 1-3) that were used to target different human SIRT7 gene regions were designed with software (www.tools.genome-engineering.org). Oligos corresponding to the sgRNAs were cloned into the lentiCRISPR v2 vector (#52961, Addgene), containing the hSPCas9 gene and a puromycin selection marker gene. HeLa cells that were transfected with the sgRNAs were isolated for single clones under 2 days of puromycin selection, then expanded for the analysis of SIRT7 ablation via immunoblotting.

Retroviral and lentiviral transduction

For retrovirus production, HEK293T cells were transfected with the rtTA-encoding pMA2640 plasmid, together with the packaging plasmids pUMVC and pCMV-VSV-G using a Fugene HD transfection reagent (Promega). Retroviral particles were collected 36-48 hours after transfection, and passed through a 0.45 μM filter to remove cell debris. After transduction, blasticidin (5 μg/ml) was used to select for stable rtTA-expressing HeLa and RPE1 cells. Lentiviruses were generated by co-transfecting the pLVX-Tight-Puro shRNA vectors together with the packaging plasmids psPAX2 and pMD2.G into HEK293T cells using the Fugene HD transfection reagent. Lentiviruses were collected 36 hours after transfection. After passing through 0.45 μM filters, the cleared supernatants were used to transduce the rtTA-expressing cells for doxycycline inducible purposes. Puromycin (1 μg/ml) was applied for the selection of stable cell lines.

Metaphase spread and chromosome counting

Exponentially growing MEF or HeLa cells were treated with colchicine (50μg/ml, Sigma) for 6-8 hours. Cells were collected and swelled with 10 ml of pre-warmed hypotonic solution (75 mM KCl) for 25 min at 37°C. Following swelling in hypotonic solution, cells were washed in fix solution (3:1 methanol: acetic acid) for 3 times and resuspended in 100-200μl of fix solution. Slide for chromosome-counting was generated by adding 8μl of fixed metaphase cells onto a clean glass slide, and spread by gently tilting the slide. The dried slide was rapidly held cells-side down over a boiling water bath for 3-5 seconds, then transferred to a heating block set at 90°C for 2-5 min for mild cell disruption. Slides were allowed to dry at room temperature overnight, before the staining with Geimsa (Sigma) or DAPI (Vector

Lab) for visualizing chromosomes.

Immunoprecipitation

HEK293T cells were transfected with pCAGGS-Sirt7-FLAG plasmid for the expression of SIRT7-FLAG for 48 hours. $1x10_7$ cells were then harvested and lysed with 400 μl chilled lysis buffer (50 mM Tris-HCl at pH 7.5, 150 mM NaCl, 5 mM MgCl2, 0.5% Nonidet P-40 and 1% Triton X-100) supplemented with protease inhibitors (Roche). Approximately 3 mg cell lysates were then incubated with 20 μl anti-FLAG M2 magnetic beads (Sigma) for 2 hours at 4°C. The beads were washed with TBS buffer (50 mM Tris at pH 7.5, 150 mM NaCl) for three times, before the elution of protein complex from the beads by 100 μl of 3X FLAG peptide (150 ng/μl, Sigma). The eluted SIRT7-FLAG protein complexes were subjected to the analyses of mass spectrometry (LC-MS/MS) and immunoblot.

Immunoblot

Samples from intestinal tissues or culture cells were lysed in chilled lysis buffer (50 mM Tris at pH 7.5, 150 mM NaCl, 5 mM MgCl2, 0.5% Nonidet P-40 and 1% Triton X-100) supplemented with protease inhibitors (Roche). The lysates were measured for protein concentration by Bradford assay, then adjusted for equal protein content before immunoblot analysis with antibodies described above.

Immunofluorescence staining

Cells were fixed with ice-cold 4% paraformaldehyde for 10 min, permeabilized with 0.5% Triton X-100 for 5 min, then blocked with 5% BSA at room temperature for 1hour. Cells were incubated with primary antibodies at 4°C for overnight, followed by PBS rinse for two times. The secondary antibody (Donkey anti-mouse antibody conjugated to Alexa488 or Alexa555, Thermo Fisher Scientific, 1:500) was applied and incubated for 2 hours at room temperature. Cell nuclei were stained with DAPI in antifade mounting medium (Vector Lab). All fluorescence images were acquired using a Zeiss inverted confocal microscope (LSM 710). Single plane images were exported and further analyzed using Image J. All representative images were obtained from at least three successful repeats.

Purification of the CENP-A -H4 complex

To isolate the CENP-A -H4 complex containing pre-nucleosome fraction, HeLa and HeLa SIRT7 KO cells ($2x10_7$) were cultured with transiently expressing HJURP-FLAG31, then the cell extracts were prepared as indicated in the immunoprecipitation section. Cell lysates were incubated with anti-FLAG M2 magnetic beads (Sigma) at 4°C for 2 hours, then washed the beads with TBS buffer for four times, and eluted the CENP-A-H4 complex with 3X FLAG peptide for immunoblot analysis.

In vitro deacetylation assays

$1x10_6$ WT and SIRT7 KO HeLa cells were seeded and cultured in 10 cm dishes for 24 hours before the transfection of plasmids pCAGGS (4 μg) encoding either SIRT7-FLAG, SIRT7 H187Y-FLAG or HAT1-FLAG by using a Fugene HD transfection reagent (Promega). After 48 hours, SIRT7-FLAG, SIRT7 H187Y-FLAG and HAT1-FLAG-expressng cells were harvested, then the proteins were purified with anti-FLAG M2 magnetic beads (Sigma). Purified HAT1 from SIRT7 KO cells was applied as the substrate for deacetylation assay. 1 μg HAT1 was incubated with 300 ng SIRT7 or SIRT7 H187Y in deacetylation buffer (2 mM NAD+, 25 mM Tris–HCl pH 8.0, 150 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, and 25 μM $ZnSO_4$) in a total volume of 30 μL. After 3 h-incubation at 37°C, 20 mM nicotinamide was added to the reactions to quench further deacetylation. HAT1 acetylation level was detected by immunoblotting using a pan anti-acetyllysine antibody (PTM BIO).

HAT activity assay

The HAT activity was performed using HAT activity kit (ALX-850-326, Enzo Life Sciences). Culture cells or intestinal crypts were homogenized in hypotonic buffer (20 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2) on ice for 15 min. After centrifugation at 1000g for 10 min, nuclear pellet was re-suspended in lysis Buffer for nucleus disruption for 30 min on ice. After centrifugation at 14,000g for 30 min, 25 μg of nuclear extracts were incubated with the reaction substrates for 30 min at 37°C, then measured for absorbance at 440 nm in a plate reader (Synergy Mx, BioTek), according to the manufacturers' protocol.

Mouse treatment and experiment

For dextran sodium sulfate (DSS)-induced colitis experiment, mice at 8-10 weeks of age were fed with 3% (w/v) DSS (MP Biomedicals) solution prepared in double distilled water for 5 days, followed by 5 days of regular drinking water recovery before. For permeability experiments, FITC-conjugated dextran (4,000 MW, Sigma) was gavaged at 0.2 g kg-1. After 4 hours, the FITC-dextran levels of serum were measured at excitation wavelength of 485 nm and emission wavelength of 528 nm as described previously(Liu et al., 2017). For acute ethanol exposure, mice (8-10 weeks) were injected intraperitoneally with ethanol (2.9 g kg-1, Sigma) twice within 4 hours as described previously (Garaycoechea et al., 2018). For chronic ethanol exposure, mice (8-10 weeks) were injected the same dose daily for 5 days. For tamoxifen induction, mice (>8 weeks) were injected intraperitoneally with 200 µl tamoxifen (T5648, Sigma) in sunflower oil at 10 mg ml-1 (Barker et al., 2007). For BrdU injection, mice (>8 weeks) were injected intraperitoneally four times at 6-hour intervals with 200 µl BrdU (B9285, Sigma) in PBS at 5 mg ml-1 (Barker et al., 2007).

Haematoxylin and eosin staining and immunohistochemistry

Mice intestine and colon tissues were flushed and fixed in 4% paraformaldehyde for overnight. Samples were subsequently dehydrated in ethanol, embedded in paraffin, sectioned at 7 µm thickness, and then stained with haematoxylin and eosin with a kit (Sigma). For immunohistochemistry staining, sample slides were deparaffinized in xylene and ethanol, and rehydrated in water. Slides were first quenched with 3% hydrogen peroxide to block endogenous peroxidase activity. Epitope retrieval was performed by heating slides in sub-boiling condition in sodium citrate buffer (10mM, pH 6.0) for 15 min, followed by a cooling down step for at least 30 minutes at room temperature. Slides were then rinsed with PBS before blocking, primary antibody and secondary antibody incubation steps, further visualized with Tyramide Signal Amplification (TSA) system (PerkinElmer) according to the manufacturer's instructions. ALP activity assay was performed using the Alkaline Phosphatase Staining Kit (Vector), and Periodic Acid Schiff (PAS) staining was performed using PAS staining kits (Muto Pure Chemicals, Japan), respectively, according to the

manufacturers' protocols.

Quantitative RT-PCR analysis

Total RNA was extracted using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Approximately 3 μg RNA was applied for cDNA synthesis using Omniscript RT Kit (Qiagen). Real-time PCR reactions were prepared with the use of SYBR Green PCR Kit (Qiagen), then analyzed in an ABI ViiATM 7. The QuantStudioTM Real-Time PCR Software was used to determine gene expression levels and normalized to a ribosomal reference gene, Rpl19. Primer sequences are listed in Key Resource Table.

Tight junction barrier assay in Caco-2 cell

$1x10_5$ Caco-2 cells were seeded and cultured in 6-well chamber slides. After 24h, 1μg pCAGGS vectors encoding either Sirt7, shSirt7, HAT1, HAT1 K15R or HAT1 K15Q were transfected into Caco-2 cells using a Fugene HD transfection reagent (Promega). After 48 hours, the transfected cells were treated with or without 7.5% alcohol in RPMI-1640 medium for 1 hour, then replaced with fresh RPMI-1640 medium for tight junction recovery as described (Ma et al., 1999). After 2 hours, the cells were labeled with anti-ZO-1 antibody followed by immunofluorescence staining procedures as described above, to visualize the structural change of tight junction.

RNA-Sequencing and GSEA analysis

Intestinal total RNA was obtained from 5-month-old $Apc^{Min/+}$ and $Apc^{Min/+}$; $Sirt7^{-/-}$ mice. The extracted total RNA samples were first examined with Agilent 2100 Bioanalyzer to ensure RNA integrity number (RIN) > 8.0, before subjected to cDNA library construction and further RNA sequencing analysis. Libraries were prepared using Illumina TruSeq RNA Library Prep Kit v2, then sequenced via Illumina Hiseq platform at 150 bp paired-end reads (CAS-MPG Partner Institute for Computational Biology Omics Core, Shanghai, China.). Reads were then mapped with the Tophat algorithm (TopHat v2.1.1) to target sequences. Gene expression levels were quantified by the software package HTSeq (v0.6.1p1). The list of differentially expressed genes were generated by DESeq2 with $P<0.05$. The gene datasets

were further analyzed by Gene Set Enrichment Analysis (GSEA), and the significant genes were ranked by "Signal2Noise" and permutation type was "gene set" and other sets using the GSEA default ($P < 0.05$ and $q < 0.25$). Data from this experiment has been deposited in the GEO database (GSE138289) and NODE database (OEP000258) (https://www.biosino.org/node/project/detail/OEP000258).

Human colorectal specimen analysis

The human colorectal specimens with fully annotated with clinical and pathological information were obtained from Shanghai outdo Biotech Co., Ltd (Shanghai, China). A detailed description of the patient was given in the Supplementary Table 2. Immunohistochemical stainings of SIRT7, HAT1, H4K5Ac and H4K12Ac were performed as describe above. Staining was visualized with Vectastain Elite ABC Kit (Vector) or DAB Peroxidase Substrate Kit (Vector).

QUANTIFICATION AND STATISTICAL ANALYSIS

Individual in vitro experiments were performed at least three times unless otherwise indicated. Animal experiments were independently repeated at least two times with similar results. Numbers are indicated in the figure legends. Results are presented as mean ± s.e.m. Statistical analyses were performed using Prism version 7.0 (GraphPad) with an unpaired two-tailed Student's t test. Statistical significances are labeled as ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Supplemental References

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