SIRT1 is downregulated by autophagy in senescence and ageing

Caiyue Xu1,2,18, Lu Wang1,2,18, Parinaz Fozouni3,4, Gry Evjen5, Vemika Chandra6,7, Jing Jiang6,7,17, Congcong Lu1,8, Michael Nicastri9, Corey Bretz10, Jeffrey D. Winkler9, Ravi Amaravadi11, Benjamin A. Garcia1,8, Peter D. Adams10, Melanie Ott3,4, Wei Tong6,7, Terje Johansen5, Zhixun Dou1,2,12,13,14 and Shelley L. Berger1,2,15,16

SIRT1 (Sir2) is an NAD+-dependent deacetylase that plays critical roles in a broad range of biological events, including metabolism, the immune response and ageing1–5. Although there is strong interest in stimulating SIRT1 catalytic activity, the homeostasis of SIRT1 at the protein level is poorly understood. Here we report that macroautophagy (hereafter referred to as autophagy), a catabolic membrane trafficking pathway that degrades cellular components through autophagosomes and lysosomes, mediates the downregulation of mammalian SIRT1 protein during senescence and in vivo ageing. In senescence, nuclear SIRT1 is recognized as an autophagy substrate and is subjected to cytoplasmic autophagosome-lysosome degradation, via the autophagy protein LC3. Importantly, the autophagy-lysosome pathway contributes to the loss of SIRT1 during ageing of several tissues related to the immune and haematopoietic system in mice, including the spleen, thymus, and haematopoietic stem and progenitor cells, as well as in CD8+CD28− T cells from aged human donors. Our study reveals a mechanism in the regulation of the protein homeostasis of SIRT1 and suggests a potential strategy to stabilize SIRT1 to promote productive ageing.

Sirtuins are an evolutionarily conserved family of NAD+-dependent deacetylases and ADP-ribose transferases that play important roles in a broad range of biological activities6–10. Among the mammalian sirtuins, SIRT1 is well-characterized as a regulator of several cellular and organisational processes, including metabolism and ageing11,12. SIRT1 functions through deacetylation of its substrates, which include histone substrates, such as acetylated histones H4K16 and H3K56, and non-histone targets, such as p53 (ref. 13). We have previously reported that the levels of the SIRT1 orthologue in yeast Sir2 declines following replicative ageing, which is a cause of yeast ageing6. However, the regulatory mechanisms of SIRT1 protein homeostasis during mammalian ageing remain unclear. Here we investigated SIRT1 homeostasis in mammalian senescence and ageing.

Cellular senescence is a stable form of cell-cycle arrest induced by telomere shortening or cellular stress14. Aged tissues are typically characterized by the accumulation of senescent cells15. Clearance of senescent cells delays age-related pathologies16, suggesting a critical association between cellular senescence and organismal ageing. Overexpression of SIRT1 in primary human lung fibroblasts (IMR90 cell line; Extended Data Fig. 1a) resulted in delayed senescence, as shown by reduced senescence-associated β-galactosidase staining (Extended Data Fig. 1b,c), consistent with previous observations13. We observed that the levels of SIRT1 protein gradually decreased in IMR90 cells following replicative senescence (Fig. 1a), in stress-induced senescence triggered by the expression of the activated oncogene HRasV12 (oncogene-induced senescence, OIS; Fig. 1b) and by the DNA damaging agent etoposide (Fig. 1c) as well as in senescent primary B lymphoid foreskin fibroblasts (Extended Data Fig. 1d). In contrast, the levels of SIRT1 protein remained unchanged in quiescence induced by contact inhibition (Extended Data Fig. 1e), suggesting that SIRT1 protein is specifically lost in cellular senescence.

To address the mechanism of SIRT1 loss in senescence, we first examined whether SIRT1 is downregulated at the messenger RNA level by RNA sequencing17 and quantitative PCR with reverse transcription (RT–qPCR). The levels of SIRT1 mRNA did not decrease in OIS or DNA damage-induced senescence (Fig. 1d and Extended Data Fig. 1f–h). The SIRT1 mRNA levels in the cells in replicative senescence were reduced but to a lesser extent than the reduction in protein levels (Fig. 1e and Extended Data Fig. 1i). These results suggest that mechanisms other than the regulation of mRNA synthesis or stability are primarily involved in modulating SIRT1 protein homeostasis during cellular senescence.

1Penn Epigenetics Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. 2Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. 3Gladstone Institute of Virology, Gladstone Institutes, San Francisco, CA, USA. 4Department of Medicine, University of California, San Francisco, San Francisco, CA, USA. 5Molecular Cancer Research Group, Department of Medical Biology, University of Tromsø—The Arctic University of Norway, Tromsø, Norway. 6Division of Hematology, Children’s Hospital of Philadelphia, Philadelphia, PA, USA. 7Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA. 8Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. 9Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA. 10Sanford Burnham Prebys Medical Discovery Institute, San Diego, CA, USA. 11Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. 12Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, USA. 13Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA. 14Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA. 15Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. 16Department of Biology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, USA. 17Present address: Institute of Translational Medicine, School of Medicine, Yangzhou University, Yangzhou, China. 18These authors contributed equally: Caiyue Xu, Lu Wang. ✉e-mail: zdou@mgh.harvard.edu; bergers@pennmedicine.upenn.edu
We then investigated the possible mechanisms of SIRT1 protein downregulation. We first tested whether proteasome-mediated degradation is involved. Treatment with the proteasome inhibitor MG132 failed to restore SIRT1 protein in senescent cells (Fig. 2a). We then tested whether lysosome-mediated degradation contributes to the downregulation of SIRT1 protein. Treatment with the autophagy inhibitor 3-MA failed to restore SIRT1 protein in senescent cells (Fig. 2b).

**Fig. 1 | SIRT1 protein is reduced during cellular senescence.** a, Western blot showing SIRT1 expression in primary IMR90 fibroblasts with the indicated population doublings. PD, population doubling; n = 2 independent experiments. b, Western blot showing SIRT expression in IMR90 cells stably expressing ER:HRasV12. The number of days of 4-hydroxytamoxifen (4OHT) induction are indicated; n = 2 independent experiments. The asterisk indicates the SIRT1 band. c, Western blot showing SIRT1 expression in DNA-damage-induced senescent cells. The cells were treated with 100 μM etoposide for 48 h and harvested at the indicated days following treatment; n = 2 independent experiments. d, Gene expression levels of SIRT1 and CDKN2A mRNA in cells in OIS (d) and replicative senescence (RS); e, conditions compared with proliferating cells (Pro). FPKM, fragments per kilobase million. The mRNA levels were normalized to ACTB. Data are the mean ± s.d.; n = 3 biologically independent samples; unpaired two-tailed Student’s t-test. Statistical information and unprocessed blots are provided.

**Fig. 2 | SIRT1 is subjected to autophagosome–lysosome degradation during cellular senescence.** a, Western blots showing SIRT1 expression in proliferating and DNA-damage-induced senescent IMR90 cells (day 6 after etoposide treatment) treated with MG132 (a) and Lys05 (b). a, MG132 was added at 0.125 (+), 0.25 (++) and 0.5 μM (++++) for 48 h. The proteasomal substrate MCL1 serves as a control showing the effects of MG132. b, Lys05 was added at 2 (+), 3 (++) and 5 μM (++++) for 48 h. c, Western blots (bottom) showing changes in SIRT1 expression in IMR90 cells expressing inducible short-hairpin RNA (shRNA) to Atg7 (shAtg7) and a non-targeting control (shNTC) in response to OIS induced by 4-hydroxytamoxifen (4OHT) treatment for the indicated number of days (c) and after the establishment of senescence induced by etoposide (d). Haerin expression was induced by Dox treatment for 5 d. The experimental scheme is shown (top). d, Doxycycline (Dox) was added on day 4 after treatment with etoposide. a–d, Each experiment was repeated twice. e, f, Confocal microscopy analysis of cytoplasmic SIRT1 in proliferating and senescent IMR90 cells at day 9 after etoposide treatment (e), and quantification of the percentage of cells with cytoplasmic SIRT1 puncta (f). The arrows indicate co-localization of cytoplasmic SIRT1 puncta and LC3. g, h, Confocal microscopy analysis of IMR90 cells stably expressing mCherry–GFP–SIRT1 under proliferation and at day 14 after etoposide treatment (g), and quantification of the percentage of cells with cytoplasmic mCherry signals (h). The cells were co-stained with antibodies to LC3 and LAMP1. I, Magnified views of the labelled regions in the white boxes are shown (bottom). e, f, h. Data are the mean ± s.d.; more than 500 cells were analysed. Each data point (n) represents cells in ten random fields; n = 7, 5, and 8 from left to right (f), and n = 5 (h). i, The relative intensities of the mCherry and GFP signals of a typical senescent cell as in g were quantified using the LAS X Core software (right). The fluorescence intensities of GFP and mCherry signals were measured at the location of the white arrow, in the direction from arrow tail to tip. The origin of the relative intensity-distance chart indicates the arrow tail and the 15 μm mark indicates the arrow tip. j, k, Each experiment was repeated twice. j, Confocal microscopy analysis of senescent cells (etoposide) stably expressing mCherry–GFP–SIRT1 with and without Lys05 treatment. The cells were treated with 5 μM Lys05 for 48 h on day 6 after etoposide treatment; n = 2 independent experiments. j, k, The arrows indicate cytoplasmic SIRT1 puncta with strong mCherry signals and fading GFP signals. Pro, proliferating cells; Sen, senescent cells; Eto, etoposide. Source Data are provided.
Lys05 (a dimeric form of chloroquine) rescued the loss of SIRT1 protein in senescent cells (Fig. 2b), indicating that SIRT1 is degraded through lysosomes during senescence.

Macroautophagy (hereafter referred to as autophagy) is a central lysosomal-mediated process that is activated following cellular senescence. We recently showed that autophagy plays a role in degrading nuclear components such as lamin B1 (ref. 15). We therefore hypothesized that autophagy contributes to SIRT1 downregulation following senescence.

To test this hypothesis, we first genetically knocked down an upstream autophagy protein, Atg7, to block autophagy activity and then induced senescence to examine the SIRT1 protein levels. Compared with the non-targeting control, Atg7 knockdown impaired SIRT1 downregulation following senescence (Fig. 2c). Furthermore, we examined whether Atg7 knockdown in already-established senescent cells can restore the levels of SIRT1 protein. After the induction of senescence by etoposide, Atg7 was inactivated using short-hairpin RNA. Atg7 knockdown did not reverse senescence-associated cell-cycle arrest, as indicated by cyclin A loss, but rescued the SIRT1 protein levels (Fig. 2d). Atg7 inactivation also rescued the deacetylation activity of SIRT1, which correlated with the increased levels of SIRT1 protein (Extended Data Fig. 1j). Together, these findings suggest that autophagy is required for SIRT1 degradation during cellular senescence.
We performed immunofluorescence staining of endogenous SIRT1 to test whether SIRT1 is an autophagy substrate in senescence. Although SIRT1 was primarily localized in the nucleus of proliferating cells, cytoplasmic SIRT1 puncta were observed in senescent cells (Fig. 2e,f). Importantly, the cytoplasmic SIRT1 puncta co-localized with LC3, an autophagy protein required for recognition and delivery of substrates to autophagosomes (Fig. 2e). We then used an mCherry–green fluorescent protein (GFP)–SIRT1 tandem-tag construct\(^1\) to investigate the autophagic trafficking of SIRT1. Due to the sensitivity of GFP to low pH, mCherry-only signals of the tandem-tagged protein represent localization within acidic autolysosomes and lysosomes. Confocal imaging of mCherry–GFP–SIRT1 expressed in proliferating IMR90 cells showed predominantly nuclear localization, with a merged yellow signal (Fig. 2g, upper panels). In contrast, senescent cells showed cytoplasmic red-only SIRT1 puncta (Fig. 2g–i) that co-localized with LC3 or LAMP1 (Fig. 2g). Neutralization of the lysosome pH with Lys05 prevented GFP quenching from the mCherry–GFP–SIRT1 protein in senescent cells and led to the retention of its merged yellow signals in the cytoplasm (Fig. 2j), which further indicates that cytoplasmic SIRT1 is targeted by lysosomal degradation. Together, these results indicate that nuclear SIRT1 is subjected to cytoplasmic autophagy–lysosome degradation during cellular senescence.

As binding to autophagy proteins is essential for degradation of autophagy substrates\(^2\), we investigated potential interactions between SIRT1 and several autophagy proteins by immunoprecipitation (IP). The results showed that SIRT1 associates with LC3 but not with other proteins in the autophagy cascade—including Atg5, beclin 1 and ULK1 (Fig. 3a). Furthermore, glutathione-S-transferase (GST) pull-down using bacteria-expressed and purified GST–SIRT1 and LC3 proteins showed that SIRT1 directly binds to LC3 in vitro (Fig. 3b).

We performed a bimolecular fluorescence complementation assay (BiFC)\(^3\) to visualize the intracellular location of the SIRT1–LC3 interaction. SIRT1 and LC3 were fused to the amino (N)- or carboxy (C)-terminal domains of Venus (VN and VC, respectively) to generate VN–SIRT1 and VC–LC3 fusion proteins. Although the two fusion proteins do not fluoresce when co-transfected with VN or VC, co-expression of VN–SIRT1 and VC–LC3 exhibited fluorescence of Venus in the nucleus, showing that SIRT1 and LC3 interact in the nucleus (Fig. 3c,d).

Given that our findings indicated that SIRT1 and LC3 interact at the basal state, we next investigated their interaction following cellular senescence by performing co-IP in the two states. Although the levels of SIRT1 protein in the input of senescent cells were reduced compared with proliferating cells, LC3 immunoprecipitated SIRT1 at similar levels in senescent cells, suggesting that the LC3–SIRT1 interaction was enhanced during senescence (Fig. 3e and Extended Data Fig. 2a). We confirmed comparable and complete IP of LC3 from the lysates of both proliferating and senescent cells (Fig. 3e). In contrast, the interaction of LC3 with another autophagy substrate, p62, was not enhanced in senescence compared with the proliferating control (Fig. 3e). Moreover, contact inhibition did not enhance the LC3–SIRT1 interaction (Extended Data Fig. 2b,c). The LC3–SIRT1 interaction, but not the LC3–p62 interaction, was also increased in the nuclear fraction of senescent cells compared with proliferating control (Fig. 3f and Extended Data Fig. 2d–f). These results suggest that the SIRT1–LC3 association is specifically enhanced in cellular senescence, which potentially promotes SIRT1 degradation in senescent cells.

Next, we examined the potential mechanisms for the enhanced SIRT1–LC3 interaction during senescence. At least in some cases, LC3 interactions with autophagy substrates are known to be modulated by substrate phosphorylation\(^2\). We therefore performed an endogenous LC3 IP in proliferating cells treated with lambda protein phosphatase to reduce the overall protein phosphorylation. We observed that LC3–SIRT1 binding was drastically increased following the phosphatase treatment, when the overall phosphorylation levels were lowered (Extended Data Fig. 2g), indicating that dephosphorylation enhances SIRT1–LC3 binding. We subsequently evaluated the phosphorylation status of endogenous SIRT1 in senescent cells using mass spectrometry. The quantities of peptides from proliferating and senescence samples that were injected for mass spectrometry were comparable (Extended Data Fig. 2h). Five of six SIRT1 peptides showed decreased phosphorylation levels in senescent samples (Extended Data Fig. 2i). The results suggest that the overall SIRT1 phosphorylation levels are reduced in senescence, consistent with our observation that the LC3–SIRT1 interaction is enhanced following phosphatase treatment (Extended Data Fig. 2g).

SIRT1 was previously shown to deacetylate LC3, which leads to the exportation of LC3 from the nucleus to execute its roles in the cytoplasmic autophagy cascade during starvation\(^1\). Paradoxically, in the context of cellular senescence, we propose that LC3 may instead facilitate the degradation of SIRT1. To dissect the differential responses of LC3 and SIRT1 in starvation versus senescence, we disrupted SIRT1 and evaluated the consequences in starvation and senescence conditions. Gene inactivation of SIRT1, mediated by clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR associated protein 9 (Cas9), resulted in the loss of SIRT1 protein and a corresponding elevation of histone H4K16 acetylation (Extended Data Fig. 3a). Following starvation, LC3-II expression was induced in control cells (the induction was further revealed by Lys05 addition), whereas SIRT1 depletion suppressed the induction of LC3-II expression (Extended Data Fig. 3b), which is consistent with previous literature\(^2\). In contrast, depletion of SIRT1 during senescence did not suppress LC3-II induction (Extended Data Fig. 3c), suggesting that a different pathway from the SIRT1-mediated deacetylation of LC3 may be employed to induce LC3-II during senescence. Furthermore, immunofluorescence staining of endogenous LC3 in starved cells showed that LC3 was predominantly transported to the cytoplasm (Extended Data Fig. 3d,e). However, although increased cytoplasmic localization...
was observed, LC3 was not completely translocated out of the nuclei of senescent cells (Extended Data Fig. 3d,e). Last, in contrast to SIRT1 protein reduction following cellular senescence, cells cultured in various nutrient-deprived conditions did not lose SIRT1 (Extended Data Fig. 3f). Together, these data demonstrate that the nuclear SIRT1–LC3 interaction has distinct dynamics and functions in senescence compared with starvation. Hence, we went on to investigate the potential role of LC3 in reducing SIRT1 protein levels during cellular senescence in this study.

We subsequently identified the amino acid residues responsible for the LC3–SIRT1 interaction. We first disrupted two essential residues on LC3, which are involved in substrate binding (F52A substitution) or in the lipidation process that facilitates the association of LC3 with autophagic membranes (G120A substitution). Although the G120A substitution did not affect SIRT1 binding, suggesting that LC3 lipidation is not required for SIRT1 interaction, the F52A substitution showed impaired interaction with SIRT1 (Fig. 3g).
We next investigated the region in SIRT1 that interacts with LC3. Many autophagy substrates bind to LC3 through an LC3-interacting region (LIR) motif. The LIR motifs typically have a core consensus sequence of (W/F/Y)XX(I/L/V), and acidic-charged residues (E/D) at the N terminus of the LIR motif further facilitate the binding. The aromatic residue (W/F/Y) binds to a hydrophobic pocket in LC3, while the hydrophobic residue (I/L/V) binds to another hydrophobic pocket containing the crucial F52 residue. Therefore, reduced binding between SIRT1 and LC3 F52A implies that SIRT1 may bind to LC3 through an LIR or LIR-like motif. We identified LC3-binding regions on SIRT1 employing a peptide array approach that has been used to characterize the LC3 interaction. Using the array, we identified eight regions in SIRT1 that potentially associate with LC3 (Fig. 4a; peptide locations shown in Fig. 4c; peptide sequences shown in Extended Data Fig. 4a). To narrow down the essential regions, we performed peptide competition IP by adding synthetic peptides of approximately 30 amino acids that correspond to the individual regions (Extended Data Fig. 4a) into the SIRT1–LC3 IP assays, to examine their ability to compete with the full-length SIRT1–LC3 interaction. Using this assay, we identified three target LIR/LIR-like motifs, where the addition of synthetic peptides reduced full-length SIRT1–LC3 binding (Fig. 4b). To further confirm the role of these motifs in LC3 binding, we substituted the individual key residues of the three LIR/LIR-like motifs within full-length SIRT1 (Extended Data Fig. 4a) and performed IP between LC3 and full-length SIRT1 substitutions. The IPs showed that the W221A and V224A double substitution (hereafter referred to as WV mutant), which disrupts the aromatic and hydrophobic positions of an LIR motif at amino acid positions 221–224 of SIRT1, had an impaired interaction with LC3, whereas substitutions of the other two motifs—F474A, D475A and V476A—did not affect LC3 binding (Fig. 4c). The crucial LIR motif (221–224) is located at the N-terminal region of SIRT1, with the acidic E214, D216 and D217 residues present at the N terminus of this motif (Fig. 4c); substitution of these residues also impaired LC3 binding (Fig. 4d). Moreover, introduction of the WV substitution on a synthetic peptide spanning amino acid positions 205–233 demonstrated an impaired ability to compete with the full-length SIRT1–LC3 interaction (Fig. 4f). Overall, these results show that the region 221–224 on SIRT1 is essential for the SIRT1–LC3 interaction.

We next questioned whether this LIR motif is required for the degradation of SIRT1 in senescent cells. The 221–224 LIR motif resides adjacent to the catalytic domain of SIRT1 and a broader region covering this motif (183–229) has been reported to bind to SIRT1-activating compounds. We therefore assessed the deacetylation activity of the SIRT1 WV mutant and found that the mutant maintained approximately 36% deacetylation activity compared with wild-type SIRT1 (Extended Data Fig. 4b). Treatment with a SIRT1-activating compound, resveratrol, did not disrupt the SIRT1–LC3 interaction or significantly rescue the levels of SIRT1 protein during senescence (Extended Data Fig. 4c–e). Given that the catalytic activity of SIRT1 suppresses senescence, we introduced a previously characterized catalytic dead mutation, I347A, to eliminate potential differences in the induction of senescence when stably expressing the various SIRT1 mutants in IMR90 cells. The SIRT1 I347A mutant showed no deacetylation activity compared with wild-type protein (Extended Data Fig. 4f), while still maintaining its interaction with LC3 (Extended Data Fig. 4g).

To assess the degradation of the I347A and the WV + I347A (W221A, V224A and I347A substitutions) mutants, we stably expressed haemagglutinin (HA)-tagged I347A and WV + I347A SIRT1 mutants in IMR90 cells and examined their levels following senescence. Although p16 induction was at similar levels in cells expressing either mutants, indicating similar induction of senescence, the WV + I347A mutant showed impaired downregulation compared with the I347A mutant (Fig. 4g). Confocal imaging of mCherry–GFP-tagged I347A and WV + I347A SIRT1 mutants in IMR90 cells showed that, at the same time points following senescence induction, cells with the WV + I347A mutant had stronger fluorescence intensities of nuclear SIRT1 and there were fewer cells exhibiting cytoplasmic SIRT1 puncta compared with cells with the I347A mutant (Fig. 4h,i). Together, these data show that the SIRT1–LC3 interaction is required for nuclear SIRT1 degradation in senescent cells.

In addition to senescence of fibroblasts, we quantified the levels of SIRT1 protein in mice following natural ageing. We dissected several organs and tissues from young (2–4 months old) and naturally aged mice (19–26 months old) and performed IP of SIRT1 protein and mRNA levels. The spleens, testes and thymus of the older mice showed reduced levels of SIRT1 protein compared with the younger mice (Fig. 5a,b and Extended Data Fig. 5a). The decline in SIRT1 protein was not observed in other tissues—including the heart, liver, kidneys, pancreas, uterus, lungs and muscle (Extended Data Fig. 5c). In contrast, the SIRT1 protein levels were not reduced in the spleens and testes of mice subjected to 24 h of fasting (Extended Data Fig. 5d,e), which is consistent with previous reports, indicating that SIRT1 protein downregulation is specific to ageing of some tissues. The levels of SIRT1 mRNA in the spleen, testes and thymus of the older mice were not significantly different from the younger mice (Fig. 5a,b and Extended Data Fig. 5b).

Next, we injected aged mice intraperitoneally with Lys05 or PBS and harvested corresponding tissues for analysis to examine the effects of Lys05 on the levels of SIRT1. The spleens, testes and thymus of the older mice showed reduced levels of SIRT1 protein compared with the younger mice (Fig. 5a,b and Extended Data Fig. 5a). The decline in SIRT1 protein was not observed in other tissues—including the heart, liver, kidneys, pancreas, uterus, lungs and muscle (Extended Data Fig. 5c). In contrast, the SIRT1 protein levels were not reduced in the spleens and testes of mice subjected to 24 h of fasting (Extended Data Fig. 5d,e), which is consistent with previous reports, indicating that SIRT1 protein downregulation is specific to ageing of some tissues. The levels of SIRT1 mRNA in the spleen, testes and thymus of the older mice were not significantly different from the younger mice (Fig. 5a,b and Extended Data Fig. 5b).
role of autophagy–lysosome in SIRT1 downregulation. Autophagy inhibition was confirmed by the accumulation of p62 in the spleens and testes of the treated mice (but not the thymus, possibly due to issues in drug uptake in the aged thymus; Fig. 5c,d). Spleens and testes from Lys05-injected mice showed elevated SIRT1 protein levels compared with samples from the PBS-injected group; the trends of SIRT1 protein were consistent with changes in p62 levels (Fig. 5c,d). The SIRT1 mRNA levels remained unaltered following Lys05 treatment (Fig. 5c,d). Notably, Lys05 treatment did not affect SIRT1 protein levels in young mice (Extended Data Fig. 5f,g), suggesting that the lysosomal degradation of SIRT1 is specific to aged tissues. Together, these results indicate that the SIRT1 protein

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is downregulated through lysosomal-mediated degradation during ageing in several mouse tissues.

Furthermore, we examined SIRT1 in haematopoietic stem and progenitor cells (HSPCs). It has been reported that SIRT1 deletion in young HSPCs results in ageing-like phenotypes. To determine whether SIRT1 levels change during HSPC ageing, we isolated Lin−Sca-1−c-KIT+ (LSK) cells from young and aged mice to represent HSPC populations (Extended Data Fig. 5h) and examined the levels of SIRT1 protein and mRNA. The LSK cells from the older mice displayed significantly decreased levels of SIRT1 protein compared with cells from the younger mice (Fig. 5e), whereas the mRNA levels in both groups did not differ significantly (Fig. 5e). Importantly, short-term Lys05 treatment of aged LSK cells led to the elevation of SIRT1 protein levels compared with control cells from the same pool of aged mice (Fig. 5e). Treatment of young LSK cells with Lys05 did not change the SIRT1 protein levels (Extended Data Fig. 5i). These results indicate that SIRT1 downregulation during HSPC ageing is mediated by a lysosome-mediated pathway.

The above studies implicate a loss of SIRT1 in the ageing of the immune and haematopoietic system. To explore this idea further, we investigated T cells from older human donors. The accumulation of terminally differentiated CD8−CD28− memory T cells is one of the hallmarks of immune ageing. These cells are highly cytotoxic but have a reduced proliferative response to antigen-specific activation. SIRT1 protein levels are reported to be downregulated in CD8−CD28− T cells, whereas the mRNA levels remain unaltered. Therefore, we questioned whether the autophagy–lysosome pathway contributes to a loss of SIRT1 in CD8−CD28− T cells from older human donors. Consistent with previous studies, the levels of SIRT1 protein were markedly reduced in CD8−CD28− T cells compared with CD8+CD28+ T cells (Fig. 5f). Importantly, transient treatment of these cells with low doses of Lys05 increased the SIRT1 protein levels (Fig. 5f). Because MG132 treatment failed to increase SIRT1 in these cells, our results indicate that SIRT1 is degraded at least in part through the autophagy–lysosome pathway during T-cell ageing in humans.

In conclusion, we report that autophagy contributes to the downregulation of SIRT1 protein during cellular senescence, during ageing of several mouse tissues and cells, and in human CD8−CD28− T cells. Although the loss of SIRT1 protein has also been previously observed in several cellular models of senescence and ageing, our work demonstrates that SIRT1 is a nuclear substrate of autophagy, which involves SIRT1 recognition by LC3 and transport to cytoplasmic autophagosomes for degradation following cellular senescence. In addition to lamin B1, our discovery of a second major nuclear substrate of LC3-mediated selective autophagy indicates that the nuclear autophagy pathway may have a general role in cell physiology.

Although we showed that SIRT1 dephosphorylation in senescence could be the trigger of its degradation (Extended Data Fig. 2h,i), we note that the SIRT1 region 199–236, which includes the WQIV motif responsible for LC3 interaction, was not detected in mass spectrometry samples due to the high charge sequence at the region. Moreover, the specific dephosphorylation site on SIRT1 remains unidentified. Future studies are needed to characterize the post-translational modification(s) and signalling pathway that trigger SIRT1 degradation through autophagy during senescence.

As a quality-control pathway, autophagy is generally considered to delay the functional decline associated with ageing; the elevation of basal autophagy activity promotes an increase in the health span and longevity of mice. Our study suggests that selective degradation of certain autophagy substrates, such as SIRT1, in certain tissues may paradoxically be detrimental during ageing. One plausible explanation for the discrepancy could be the differences between bulk and selective autophagy. While stimulation of the overall autophagy flux is beneficial for cell and tissue fitness, selective degradation of nuclear constituents such as lamin B1 and SIRT1 leads to the arrest of cell cycles and induction of inflammation. Further studies are needed to dissect the mechanisms underlying cytoplasmic versus nuclear autophagy and their roles in senescence and ageing.

The downregulation of SIRT1 in senescent cells may be associated with biological functions of cellular senescence. SIRT1 was reported to negatively regulate the expression of several senescence-associated secretory phenotype factors, including IL-6, IL-8 and IL-1β. Given the critical functions of the senescence-associated secretory phenotype in the immunosurveillance of pre-malignant cells, we speculate that the downregulation of SIRT1 is a programmed event of senescence to alarm the immune system to restrain tumorigenesis. This idea is consistent with the distinct dynamics of the SIRT1–LC3 interaction in starvation versus senescence (Extended Data Fig. 3a–f); that is, as the pro-inflammatory response is not induced during starvation, SIRT1 is not degraded. Although the acute induction of senescence restrains tumorigenesis, the accumulation of senescent cells during ageing promotes chronic inflammation and age-associated diseases. Hence, although the loss of SIRT1 in senescence serves as a potential tumour-suppressive mechanism, SIRT1 loss in aged tissues may promote ageing and age-related pathologies, consistent with the 'antagonistic pleiotropy hypothesis'.

Immunosenescence is an important aspect of ageing. The decline of the immune system, such as dysfunction of HSCs and T cells, contributes to age-related diseases. SIRT1 is a critical regulator of haematopoietic-lineage generation from HSPCs, and the skewed myeloid and lymphoid differentiation associated with ageing is linked to a defective immune response. Furthermore, the SIRT1–FOXO1 axis has been shown to contribute to the immune dysfunction in human CD8−CD28− T cells, the accumulation of which is a common event in ageing and age-related diseases. In addition, the reduction of SIRT1 protein in critical immune organs, including the spleen and thymus (Fig. 5a and Extended Data Fig. 5a), further suggests a deterioration of the adaptive immune system during ageing. Thus, restoration of the levels of SIRT1 protein may provide a critical means to reprogramme aged immune system function.

**Fig. 5 | SIRT1 undergoes lysosomal degradation during ageing in mice and humans.** a,b. The spleens (a) and testes (b) of young (2–4 months) and aged (19–26 months) C57BL/6 mice were analysed by western blotting (top) and RT-qPCR (bottom). Data are the mean ± s.e.m.; unpaired two-tailed Student’s t-test; n = 4 animals. c,d. The spleens (c) and testes (d) of aged (19–24 months) mice subjected to daily intraperitoneal injections of 10 mg kg−1 Lys05 in PBS or PBS control in a 100-μl volume for 2 weeks were analysed by western bloting (top) and RT-qPCR (bottom) for SIRT1 expression. Data are the mean ± s.e.m.; two-tailed Mann–Whitney test. Spleen protein, n = 8 (control) and 7 (Lys05 treatment) animals; spleen RNA, n = 8 (control) and 6 (Lys05 treatment) animals; testes protein and mRNA, n = 6 animals for both groups. e. HSPC populations were isolated from young (2–4 months) and aged (20–26 months) C57BL/6 mice, cultured with or without 2 μM Lys05 for 24 h and analysed by western blotting (left and middle) and RT-qPCR (right). Data are the mean ± s.e.m.; n = 6 (protein) and 4 (mRNA) independent experiments; protein, one-way ANOVA coupled with Tukey’s test; mRNA, unpaired two-tailed Student’s t-test. f. Freshly sorted human CD8−CD28− (control) and CD8−CD28+ T cells were treated with Lys05 at doses of 0 and 5 μM for 14 h, and then harvested and analysed by western blotting. Donor ages, 53, 54 and 66 years. Data are the mean ± s.d.; unpaired two-tailed Student’s t-test; n = 3 human donors. a–f, Western blot quantifications, the SIRT1 bands were normalized to the GAPDH bands; for the testes, both bands of SIRT1 were considered. a–e, RT-qPCR quantification, data were normalized to 18S rRNA. Statistical information and unprocessed blots are provided.
cells, shedding light on a potential therapeutic intervention to delay the process of immune ageing/senescence. Stabilization of SIRT1 protein levels—for example, through the interruption of the SIRT1–LC3 interaction—could be a new direction for the design of anti-ageing compounds.

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Methods

Cell lines, culture and treatments. IMR90, B) and HEK293T cells were described previously\(^{13}\). IMR90 and BJ cells with a population doubling of less than 40 were used, except for the replicative senescence experiments. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen). IMR90 and BJ cells were cultured under physiological oxygen (3%). Stable cell lines were made by retrovirus or lentivirus infection, as previously described\(^{14,15}\).

For replicative senescence, the cells were subjected to continuous passaging to induce senescence. For OIS, cells expressing ER:HRasV12 were treated with 4-hydroxytamoxifen to induce HRasV12 expression. For DNA damage–triggered senescence, the cells were treated with 100 μg/ml etoside for 48 h.

For contact inhibition, the cells were continuously cultured at 100% confluency to induce growth arrest and quiescence. The medium was replenished with fresh medium every 2 d to maintain the cellular metabolic activities.

For amino acid starvation, the cells were cultured in Hank’s buffer (with calcium and glucose) supplemented with 10% dialysed FBS and 1% HEPES (Invitrogen). For amino acid and serum starvation, the cells were cultured in Hank’s buffer containing 1% HEPES. For the 2-deoxy-D-glucose (2-DG) treatment, the cells were incubated in complete medium supplemented with 10 mM 2-DG. For the Torin 1 treatment, the cells were incubated in complete medium supplemented with 250 nM Torin 1.

Reagents and antibodies. The following reagents were used: 4-hydroxytamoxifen (Sigma-Aldrich), etoside (Sigma-Aldrich), 2-DG (Sigma-Aldrich), Torin 1 (Selleckchem), Resveratrol (Sigma-Aldrich), MG132 (Calbiochem) and Lys05 (a gift from R. Amaravadi and J. D. Winkler, and also purchased from MedKoo Biosciences, Inc).

The antibodies used in this study are described in Supplementary Table 1.

Plasmids. GFP, GFP–LC3 wild-type and mutants, FLAG–LC3, GFP–Atg5, GFP–beclin 1, GFP–ULK1, Tet-pLKO–shAtg8 and the constructs used for the BiFC assay were described previously\(^{16}\). The SIRT1 sequence was cloned into pLPC–HA, LPC–FLAG, PETDuett–GST and previously described phabe–mCherry–GFP vectors\(^{17}\). All SIRT1 mutants were cloned from the SIRT1 sequence and verified by DNA sequencing.

The following CRISPR guide sequences were cloned into the lentiCRISPR v2 vector: PRM1, CACCGGACAAAGAAGTCGCAGACGA and AAACCTGCTCAGCGACTTCTTGTCC; SIRT1, CACCGCTCCCCGGCGGGGGACGACG and AAACTCGTCGTCCCCCGCCGGGGAGC. The constructs were incorporated into IMR90 cells through lentivirus infection for single-guide-RNA knockdown.

Western blotting. Western blotting was performed as previously described\(^{18}\) with modifications. Briefly, the cells were lysed in buffer containing 20 mM Tris pH 7.5, 137 mM NaCl, 1 mM MgCl\(_2\), 1% NP-40 and 1% NTA supplemented with 1:100 Halt protease and phosphatase inhibitor cocktail (Thermo Scientific) and benzamidine (Novagen) at 12.5 μM. For lysis, the cells were rotated at 4 °C for 30 min and boiled with 1% SDS. The supernatants were then subjected to centrifugation and transferred to a 0.2 μm nitrocellulose membrane. The membrane was incubated overnight with primary antibodies in TBS supplemented with 0.1% Tween 20 at 4 °C, probed with horseradish peroxidase-conjugated secondary antibodies and then developed with SuperSignal west pico PLUS chemiluminescent substrate (Thermo Fisher) and imaged using an Amersham Imager 600.

RNA sequencing and RT–qPCR. The results of the RNA sequencing were based on data from Rai et al. (Gene Expression Omnibus accession nos GSE52848 and GSE53350)\(^\text{15}\). For stabilizing cells, IMR90 cells were used at a population doubling of 32. For replicative senescence, IMR90 cells at a population doubling of 88 were used. For oncogene-induced senescence cells, IMR90 cells expressing the ER:HRasV12 construct were induced by 4-hydroxytamoxifen. For RNA-sequencing data analysis, quantification of fragments per kilobase million were used and normalization was performed using the ACTB.

For RT–qPCR, RNA was extracted using a Qiagen RNeasy mini kit (cat. no. 74104). Reverse transcription was performed using an Applied Biosystems (a gift from R. Amaravadi and J. D. Winkler, and also purchased from MedKoo Biosciences, Inc).

The antibodies used in this study are described in Supplementary Table 1.

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Peptide array and peptide competition. The peptide array experiment was performed following a previously described procedure\(^{19}\). Twenty-amino-acid peptides, with a three-amino-acid shift, covering the full length of SIRT1 were synthesized. The peptides were synthesized and immobilized on a cellular membrane using a MultiPep automated peptide synthesizer (INTAVIS Bioanalytical Instruments AG). GST–LC3B was used as an overlay probe for the binding, and the interaction signals were detected using specific GST antibody, developed by chemiluminescence and imaged by camera.

For peptide competition IP, peptides of approximately 30 amino acids were synthesized by GenScript and dissolved in DMSO. The peptides (500 μM) or equal volumes of DMSO were added to the IP system at the overnight–incubation step. The other steps of the IP were performed as per the regular procedure.

Mouse experiments. Mice in a C57BL/6 background were used in this study. Aged mice were obtained from National Institute on Aging-aged rodent colonies. The mice were housed under a 12-h light and 12-h dark cycle, with lights on at 7:00 and lights off at 19:00. Water and standard chow were provided ad libitum following the regulations and guidelines of the University of Pennsylvania. Tumor-free mice, both male and female, were used in the study.

For the Lys05 injection experiment, the mice were injected intraperitoneally daily with 10 mg/kg\(^{-1}\) Lys05 in PBS or PBS alone in a 100-μl volume for 2 weeks, after which they were euthanized and their tissues were harvested. All procedures
were approved and performed following the regulations and guidelines of the University of Pennsylvania.

For LSK cell isolation and culture, the mice were euthanized and dissected to excise the tibia, femur and hip bones. Bone marrow cells were harvested and subjected to lineage depletion using a Lineage cell depletion kit (Miltenyi Biotec, 130-090-858) following the manufacturer's instructions. Briefly, the harvested cells were incubated with a biotinylated monoclonal antibody cocktail against lineage-specific cells (CD5, CD11b, B220, Gr1 and Ter119). The cells were then washed and incubated with anti-biotin magnetic beads, followed by magnetic separation on an autoMACS pro separator (Miltenyi Biotec, 130-092-545). The eluted lineage-depleted cells were stained with surface markers to define the c-Kit and Sca-1 populations. The LSK cells were sorted using a BD FACS Aria flow cytometer with DiVa software (Becton Dickinson). Forward and side scatter parameters were used to exclude the doublets. Purity was maintained at >95–97%. The sorted LSK cells were cultured in StemSpan SFEM II (STEMCELL Technologies, 09600) supplemented with 10% FBS (Sigma-Aldrich, 12103C), penicillin–streptomycin, 1-glutamine (Gibco, 15140-122), 2-mercaptoethanol, 100 ng ml−1 SCF (PeproTech, 250-03), 20 ng ml−1 IL-7 (PeproTech, 315-14), 20 ng ml−1 IL-3 (PeproTech, 213-13) and 20 ng ml−1 IL-6 (PeproTech, 216-16), at a cell concentration of 1 × 10^6 cells ml−1. For Lys05 treatment, the cells were collected at 24 h post incubation, resuspended in medium containing 2 μM Lys05 at a cell concentration of 1 × 10^6 cells ml−1, and incubated for 24 h. The cells were then harvested for analysis. The gating strategy is illustrated in Supplementary Fig. 1a.

Senescence-associated β-galactosidase assay. The assays were performed using a Cellular senescence assay kit (Millipore, KA0002), and imaged and quantified under a regular light microscope.

SIRT1 deacetylation activity assay. Cells were lysed in buffer containing 20 mM Tris pH 7.5, 157 mM NaCl, 1 mM MgCl2, 1% NP-40 and 10% glycerol (Thermo Scientific) at 4°C for 1 h. The supernatant was incubated overnight with antibody-conjugated agarose beads (Thermo Fisher) at 4°C. The next day, the beads were washed four times for 10 min at 4°C. 5 μl buffer was added and the beads were subjected to SIRT1 activity detection using a SIRT1 activity assay kit (Abcam, ab156065). The deacetylation activity of the samples was determined from the average slopes of the fluorescence intensity curves, which were calculated through lineage regression analyses.

Mass spectrometry. Elution samples of IPs were separated by 4–12% Bis-Tris NuPAGE and stained using G-250. The SIRT1 bands (confirmed by western blotting) were analysed using a standard protocol of in-gel digestion, C18 stage tip and label-free quantitative liquid chromatography with tandem mass spectrometry. Tryptic peptides were run on a Thermo Fisher Orbitrap Fusion system equipped with a Dionex UHPLC. The samples were separated on a home-packed capillary column (75 µm × 20 cm) containing C18-AQ resin (3 µm) at a flow rate of 400 nl min−1. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in 80% acetonitrile. A gradient of 70 min was preceded by a 2-min loading period (2% buffer B). The mobile phase was 300–1800 was analysed in the Orbitrap at 60,000 FWHM (200 z) resolution and 4.0 × 10^5 AGC target value with a maximum injection time of 100 ms. Determined charge states between two and six were required and a dynamic exclusion window of 30 s was used with isotopes excluded. Tandem mass spectrometry was performed in the ion trap in the Rapid mode with the TopSpeed mode (3 s) using data-dependent acquisition. The HCD collision energy was set to 30%, the AGC target to 1.0 × 10^6 and maximum injection time to 150 ms. Raw files were analysed using MaxQuant 1.6.0.16 against SIRT2 and a common contaminant database. The search included fixed modification of carbamidomethyl cysteine and variable modifications of methionine oxidation, N-terminal acetylation and N-terminal phospho-tyrosine phosphorylation. All of the other values used default settings. The msms.txt generated by MaxQuant was sent to Skyline software for label-free quantification. All peptides from SIRT1 were manually checked before extracting the peak area to compute the relative abundance of unmodified and phosphorylated status.

Human T-cell-population sorting and culture. Blood samples were collected at the Vitalant in San Francisco. All donors were 50-ys old or older, provided written informed consent and were de-identified. Total human CD8+ T cells were enriched using a RosetteSep human CD8+ T cell enrichment cocktail (STEMCELL Technologies, 15063). The following antibodies were then used to stain the CD8+ and CD8+CD28+ T cells: fixable viability dye eFlour506 (Invitrogen, 6-0866-14), CD3–PECy5 (BD Biosciences, 553534), CD8–V500 (BD Biosciences, 560347) and CD28–PE (Invitrogen, 12-0289-42). Sorting was performed on an ARIA II system (BD Biosciences). The gating strategy is illustrated in Supplementary Fig. 1b.

The T cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 2 mM l-glutamine and penicillin–streptomycin. Lys05 was used at 5 μM for 14 h.

Statistics and reproducibility. Unpaired two-tailed Student's t-tests and Mann–Whitney tests were used for comparisons between two groups. One-way ANOVAs coupled with Tukey’s multiple comparison test or two-way ANOVAs coupled with Sidak’s multiple comparisons test were used for comparisons of more than two groups. All bar graphs show the mean values with error bars (s.d. or s.e.m., as indicated in the figure legends). Confidence intervals of 95% were used and significance was considered when the P value was less than 0.05. The number of times an experiment was repeated is indicated in the figure legends.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
RNA sequencing data were referenced to the Gene Expression Omnibus under accessions GSE52848 and GSE53356. The mass spectrometry data have been deposited in ProteomeXchange with the primary accession code PXD020081. The authors declare that the data that support the findings of this study are available within the manuscript. No restriction on data availability applies. All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

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Author contributions
C.X., Z.D. and S.L.B. conceived the project. C.X. performed most of the experiments. Z.D. and L.W. performed cell-culture experiments. L.W., P.F. and M.O. performed human T-cell experiments. V.C., J.J. and W.T. performed HSPC isolation. M.N., J.D.W. and R.A. contributed autophagy reagents. T.J. and G.E. performed peptide arrays. C.I. and B.A.G. performed mass spectrometry analysis. C.B. and P.D.A. contributed to the mouse experiments. C.X., L.W., P.D.A., Z.D. and S.L.B. contributed to the experimental design.

C.X., L.W. and S.L.B. wrote the manuscript. All authors discussed the results and reviewed the manuscript.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to Z.D. or S.L.B.

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Extended Data Fig. 1 | Characterization of SIRT1 role and mRNA levels in cellular senescence. **a**, Western blot showing SIRT1 expression in IMR90 cells stably expressing LPC vector or LPC-HA-SIRT1 construct; n = 3 independent experiments. **b**, β-gal-stained cells at day 9 post etoposide treatment were imaged by microscopy; n = 2 independent experiments. OE: overexpression. **c**, Percentages of β-gal staining-positive cells were quantified at indicated days after etoposide treatment. Data are mean ± s.d.; more than 500 cells and 5 fields were counted; two-way ANOVA with Sidak’s multiple comparisons test (p values). **d**, Western blot showing SIRT1 expression in primary BJ fibroblasts treated with 100 μM etoposide for 48 h in time course as indicated; n = 2 independent experiments. **e**, Western blot showing SIRT1 expression in IMR90 cells cultured at 100% confluency for indicated days; n = 2 independent experiments. **f–i**, RT-qPCR time course analysis of proliferating and senescent IMR90 cells induced by overexpressing ER-HrasV12 treated with 4OHT (**f**), by etoposide for 48 h (**g**), and by etoposide for 48 h (**h**), and in IMR90 replicative senescent cells (**i**). Data were normalized to GAPDH; the bar indicates the average of three technical replicates. PD, population doubling. **j**, SIRT1 deacetylation activity assay of endogenous SIRT1 protein immunoprecipitated from extracts of proliferating or senescent IMR90 cells expressing inducible hairpins of shNTC and shAtg7. For senescent cells, cells were induced by Dox for 5 days, and then were subjected to etoposide treatment for 48 h; Cells at Day 8 after etoposide treatment were harvested for analysis. For proliferating cells, cells were induced by Dox for 5 days and then were harvested for analysis. Proteins loaded were analysed by western blotting. This experiment has been repeated for two times. Statistical information and unprocessed blots are provided as source data.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Characterization of the SIRT1–LC3 interaction. a, IP of extracts from proliferating and senescent IMR90 cells. Quantification: SIRT1 IP bands were normalized to LC3 IP and SIRT1 input bands. Mean ± s.d.; n = 6 independent experiments; paired two-tailed Students’ t-test. b, IP of extracts from proliferating and contact-inhibited IMR90 cells (at 100% confluency for 8 days). This experiment has been repeated once. Excessive beads and antibodies were used in the IP to capture nearly 100% of LC3 protein in the lysates. Flow: flow-through. c, IP of extracts from proliferating and contact-inhibited cells; n = 3 independent experiments. d, IP of nuclear extracts from proliferating and senescent cells. Excessive beads and antibodies were used to capture nearly 100% of LC3 protein in the lysates. This experiment has been repeated once. Flow: flow-through. e, IP of nuclear extracts from proliferating and senescent cells; n = 2 independent experiments. In a,d,e, senescent cells were harvested at day 8 after etoposide treatment. f, Western blotting of nuclear (Nuclear) and cytoplasmic (Cyto) extracts from proliferating and senescent cells; n = 2 independent experiments. g, Endogenous LC3 IP of IMR90 cell extracts with or without protein phosphatase Lambda treatment. Quantification: SIRT1 IP bands were normalized to LC3 IP and SIRT1 input bands. Mean ± s.d.; paired one-tailed Students’ t-test; n = 5 independent experiments. h-i, Mass spectrometry analysis of SIRT1 immunoprecipitated from proliferating and senescent IMR90 cells. h, Boxplot showing the peptide intensity distribution of SIRT1. N = 54 peptides; p value = 0.25; unpaired two-tailed Student’s t-test. The median of the data was indicated as the line in the box, and edges stand for the 25th/75th percentile. i, Phosphorylated peptides identified by mass spec and their phosphorylation levels in proliferating and senescence states. AA: amino acid. Source data are provided.
Extended Data Fig. 3 | Characterization of the SIRT1 deacetylation role in starvation and senescence. a–c, IMR90 cells that undergo CRISPR/Cas9-mediated gene inactivation of non-targeting control (PRM1, as PRM1 is involved in spermatogenesis and is not expressed in IMR90 cells) or SIRT1 were analysed under starvation and senescence conditions. This experiment has been repeated for two times. a, Cells were analysed by western blotting. b, Cells were subjected to 250 μM T orin 1 and 5 μM Lys05 treatment for 24 h, and analysed by western blotting. Relative LC3-II intensities to GAPDH were quantified. c, Cells at day 6 after etoposide-treated senescence were subjected to 2 μM or 5 μM Lys05 treatments for 24 h, and analysed by western blotting. Relative LC3-II intensities to GAPDH were quantified. d–e, IMR90 cells under proliferating, starvation (T orin 1 250 μM for 24 h) and senescence (induced by etoposide treatment, harvested at day 7) conditions were stained with LC3 antibody and analysed. d, Cells were imaged by confocal microscopy. Scale bar: 10 μm. e, Percentages of cells with nuclear LC3 signals were quantified. Starv: starvation. Mean ± s.d.; more than 500 cells were counted; each data point (n) represents cells in 10 random fields, n = 5 for all conditions; one-way ANOVA coupled with Turkey’s multiple comparisons test. f, IMR90 were treated as indicated ways for 24 h and analysed by western blotting; n = 2 independent experiments. CT: control. A.A.: amino acids. 2-DG: treatment of 10 mM 2-DG. T orin 1: treatment of 250 μM T orin 1. Statistical information and unprocessed blots are provided as source data.
Extended Data Fig. 4 | Characterization of SIRT1 mutants and peptides. a, Information of potential SIRT1–LC3 interaction regions identified in the peptide array as in Fig. 4a, and the corresponding synthetic peptides and mutants. Key amino acid residues are labelled in red. Potential region: LC3-binding regions on SIRT1 identified in the peptide array as in Fig. 4a. Peptide region: synthetic peptides tested in the peptide competition IP as in Fig. 4b. Peptide competition: results of the peptide competition IP as in Fig. 4b. Substitution generated: SIRT1 mutants tested in the IP as in Fig. 4c.

b, SIRT1 deacetylation activity assay of SIRT1 WT or WV mutant immunoprecipitated from extracts of HEK293T expressing corresponding HA-tagged constructs. Proteins loaded were analysed by western blotting. This experiment has been repeated for two times.

c, IP of HEK293T cells expressing HA-SIRT1 and Flag-LC3 constructs; n = 2 independent experiments. Cells were pre-treated with 20 μM resveratrol for 6 h.

d, IMR90 cells at day 6 after etoposide-initiated senescence were subjected to treatment with 20 μM resveratrol for 48 h; n = 2 independent experiments. Cells were then harvested for western blotting.

e, HEK293T cells expressing HA-SIRT1 and Flag-LC3 were treated with 20 μM resveratrol for 6 h, and were then harvested for SIRT1 activity assay. This experiment has been repeated for two times.

f, SIRT1 deacetylation activity assay of SIRT1 WT or I347A mutant immunoprecipitated from extracts of HEK293T expressing corresponding HA-tagged constructs. Proteins loaded were analysed by western blotting; n = 2 independent experiments. g, IP of HEK293T cell lysates expressing Flag-tagged LC3 and HA-tagged SIRT1 WT or I347A, WV+I347A, or WV mutants. This experiment has been repeated for two times. Statistical information and unprocessed blots are provided as source data.
Extended Data Fig. 5 | Analysis of SIRT1 in mouse tissues and HSPCs. a,b, Thymus from young (2-4 months) and aged (19-26 months) C57BL/6 mice were lysed and analysed by western blotting (a) and RT-qPCR (b); n = 3 biologically independent animals in each group. RT-qPCR data were normalized to 18S; mean ± s.e.m.; unpaired two-tailed Students’ t-test. c, Indicated organs and tissues of young (3 months) and aged (19 months) mice were dissected and analysed by western blotting; n = 2 independent experiments. SE: short exposure; LE: long exposure. d,e, Young (3 months) mice were fed or fasted for 24 h. Spleens (d) and Testes (e) were harvested for western blotting; n = 2 biologically independent animals in each group. f, Young (2-4 months) mice were subjected to daily i.p. injection of 10 mg/kg Lys05 in PBS or PBS control in 100 μL volume for two weeks. Spleens (f) and Testes (g) were analysed by western blotting. Western blot quantification: SIRT1 bands were normalized to GAPDH bands. For spleens, data are mean ± s.e.m.; control group n = 3 animals, Lys05 group n = 5 animals; two-tailed Mann-Whitney test. For testes, data are mean ± s.e.m.; n = 4 animals; two-tailed Mann-Whitney test. h, Representative flow cytometry plots of cell sorting of lineage-depleted bone marrow cells from young and aged mice to isolate Lin-Sca-1+c-Kit+ cells (HSPC populations). Boxes indicate cell populations isolated. i, HSPC populations were isolated from young (2-4 months) mice, cultured with or without 2 μM Lys05 for 24 hours and analysed by western blotting. This experiment has been repeated once. Statistical information and unprocessed blots are provided as source data.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

The following softwares were used in the study: FACSDIVA (8.0); MaxQuant (1.6.0.16); Skyline (19.1.1.283); IAS X Core (3.3.0.16799).

Data analysis

The following softwares were used: Prism (7.0c, 8.4.2) ; Flowjo (v10, v10.6.1).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNA sequencing data were referenced to GEO under accession number GSE52848 and GSE53356. Mass spect data have been deposited in ProteomeXchange with the primary accession code PXD0070081. Source data are provided in the Source Data files. The authors declare that the data that support the findings of this study are available within the manuscript. No restriction on data availability applies. All other data supporting the findings of this study are available from the corresponding author on reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences  - Behavioural & social sciences  - Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**
Sample sizes used were determined based on previous experience, literatures [DOI: 10.1038/nature15548 and DOI: 10.1038/nature24050] and current standards of cell and mouse experiments. No statistical test was used to pre-determine the sample sizes.

**Data exclusions**
For mouse experiments, data from mice bearing tumors were excluded. Since critical cell signaling pathways in natural aging are often misregulated in cancerous cells and organs, these samples cannot provide reliable results in our observation of normal mouse aging. Therefore, we excluded these mice in our experiments.

**Replication**
All data was successfully replicated in at least two independent experiments [unless indicated otherwise]. Specific numbers of replication done for each experiments are included in the figure legends.

**Randomization**
In mouse experiments, no pre-established selection criteria for mice were used other than age. Mice in the same age group were randomly divided into experimental groups. In cell experiments, no pre-established selection criteria were used other than proliferating / senescent conditions. Cells in the same condition were randomly divided into experimental groups.

**Blinding**
No blinding was used by the investigators, since the phenotypes between proliferating and senescent cells or between young and aged mouse samples are physiologically distinct, so it is impossible to perform blinding. The data were examined by at least two investigators.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology         |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

**Antibodies**

Detailed information is available in Supplementary Table 1.

- Antibody: Source - Clone - Use - Concentration
- SirT1 Cell Signaling Technology 2496 C14H4 WB 1:1000
- SirT1 Cell Signaling Technology 8469 F3 WB/JF 1:1000; 1:100
- SirT1 Millipore 07-131 n/a WB 1:1000
- LC3 Cell Signaling Technology 3868 D11 LP; IF 1:100 for comparable IP, 5ug for complete IP; IF 1:150
- LC3 Cell Signaling Technology 2775 n/a WB 1:1000
- Lamin B1 Abcam ab16048 n/a WB 1:1000
- Cyclin A Abcam ab38 E23.1 WB 1:1000
- Cyclin A Santa Cruz Biotechnology sc-751 n/a WB 1:1000
- p16 Abcam ab16123 DC50.1 WB 1:1000
- Atg7 Cell Signaling Technology B558 D12B3 WB 1:1000
- p62 Abcam ab56416 n/a WB 1:10000
- GAPDH Cell Signaling Technology S174 016H11 WB 1:1000
- β-tubulin Sigma-Alrich T4026 TUB 2.1 WB 1:1000
- β-tubulin Abcam ab6161 YOYO34 WB 1:1000
- β-actin Cell Signaling Technology 4970 13E5 WB 1:1000
- Ras Millipore 05-516 RAS10 WB 1:500
- MCL1 Cell Signaling Technology 4572 n/a WB 1:1000
Eukaryotic cell lines

Policy information about cell lines

- Cell line source(s): IMR90 cell line was obtained from Coriell Institute for Medical Research, Camden, NJ. BJ and HEK293T cell lines were obtained from ATCC.
- Authentication: Cell lines were not further authenticated.
- Mycoplasma contamination: All cell lines were tested negative for mycoplasma contamination.
- Commonly misidentified lines: No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

- Laboratory animals: C57BL/6 mice were used in this study. Both sex were included in the study. Tumor-free mice with ages of 2-4 months or 19-26 months were used.
- Wild animals: No wild animals were used in this study.
- Field-collected samples: No field-collected samples were used in this study.
- Ethics oversight: University Laboratory Animal Resources of the University of Pennsylvania

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

- For Lin-Sca-1+ c-Kit+ (LSK) cell isolation and culture, mice were euthanized and dissected to excise the tibia, femur and Hip bones. Bone marrow cells were harvested and subjected to lineage depletion using Lineage Cell Depletion Kit (Miltenyi Biotec) following the manufacturer's instructions. Briefly, harvested cells were incubated with biotinylated monoclonal antibodies cocktail against lineage specific cells (CD5, CD11b, B220, Gr1 and Ter119). Cells were then washed and incubated with anti-biotin Magnetic beads followed by magnetic separation on autoMACS Pro. Eluted Lineage depleted cells were stained with surface markers to define c-Kit and Sca-1 population.

- Total human CD8+ T cells were enriched using the RosetteSep Human CD8+ T Cell Enrichment Cocktail (15063; Stemcell Technologies). Then, the following antibodies were used to stain CD8+CD28+ and CD8+CD28- T cells: fixable viability dye
eFluor506 (65-0866-14, Invitrogen), CD3-PECy5 (555334, BD Biosciences), CD8-V450 (560347, BD Biosciences), and CD28-PE (12-0289-42, Invitrogen).

**Instrument**
BD FACS Aria/II flow cytometer

**Software**
FlowJo and DIVA software (Becton Dickinson)

**Cell population abundance**
For LSK cell isolation, purity was maintained at > 95-97%.
For human T cells, the post-sort fractions (CD3+CD8+CD28+ & CD3+CD8+CD28-) were >99% pure. This was determined by running a sample from the post-sort fractions on the flow cytometer and confirming the relevant staining.

**Gating strategy**
Lineage depleted (Lin-) bone marrow cells stained with c-Kit and Sca-1 were first selected in FSC-A/SSC-A dot plot, and then in FSC-H/FSC-W and SSC-H/SSC-W dot plots for singlet selection and FSC-A/DAPI dot plot for live cell selection. The selected cells were then gated with Sca-1-PE/c-Kit-PerCP, and the double positive cells were collected for further analysis.

For human T cells, we first gated on appropriately sized cells in SSC-A/SSC-A. We then gated on single cells (vs doublets) on FSC-H/FSC-A. We subsequently gated on live cells using FSC-A/Viability-eFluor506; then gated on double positive CD3+CD8+ cells (CD3-PECy5/c-CD8-V450), and then sorted both CD28-PE +/- cells [FSC-A on y axis] into separate tubes. We used fluorescent minus one controls to set gates and determine the boundaries between positive/negative staining. Our gating strategy has been previously described and published in Jeng et al. [2018] doi: 10.1084/jem.20161066.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.