SIRT3 deSUMOylation sustains hematopoietic stem cell activities under stressful conditions via the DHX58-IRF7 axis

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Abstract

Hematopoietic stem cells (HSC) sustain physiological hematopoiesis by generating all hematopoietic cells. However, the connection between HSC stemness/activities and organismal lifespan, especially under stress, is still not completely understood. We previously revealed a unique mutation at the SUMOylation site of SIRT3 (lysine 223 to arginine, K223R) that could enhance its deacetylase activity. Here, using *Sirt3*-K223R transgenic mice, we demonstrate that SIRT3 deSUMOylation promotes HSC self-renewal, inhibits myeloid differentiation, and delays HSC senescence under multiple stress conditions. Notably, these effects are associated with extended lifespan in mice, though the underlying link between preserved HSC function and extending lifespan needs further investigation. Mechanistically, SIRT3 regulates mitochondrial metabolic activity and promotes the deacetylation of H3K9 and H3K27. This epigenetic modification attenuates the RIG-I signaling pathway by down-regulating DHX58 and IRF7, sustaining HSC activities. Our study uncovers a unique SIRT3-DHX58-IRF7 axis that sustains HSC activities to delay organismal aging under stress, providing insights into potential anti-aging strategies that could target hematopoietic homeostasis.

Introduction

Throughout the aging process, the functionality of various organs and the stemness/activity of adult stem cells (e.g., hematopoietic stem cells, HSC) undergo significant deterioration.[1] Aging, as well as other stress conditions (i.e., chemotherapy, irradiation), usually affects the cell fate decisions of different types of adult stem cells.[2] HSC, characterized

by their unique ability to self-renew and differentiate into all hematopoietic lineages, play a pivotal role in the aging of both the entire hematopoietic system and other important organs.[3,4] Aging of HSC has been linked to multiple hematologic dysfunctions, including lymphoid deficiency, diminished immune competence, increased DNA damage, and leukemia.[5-7] HSC aging is a key driver of systemic aging in the hematopoietic system and broader organismal homeostasis,[4,8] highlighting the importance of understanding HSC aging for health and longevity. Elucidating the molecular mechanisms underlying HSC aging is crucial for combating aging-related disorders and advancing therapeutic strategies.

Sirtuins, a family of NAD^+-dependent protein deacetylases, play a crucial role in regulating various aging-related cellular processes, particularly under stress conditions.[9,10] For instance, SIRT2 enhances the self-renewal ability of HSC during aging by inhibiting NLRP3 inflammasome activation,[11] SIRT6 maintains HSC homeostasis by inhibiting the Wnt signaling pathway,[12] and SIRT7 reinforces the regenerative capacity of HSC during aging by regulating the mitochondrial unfolded protein response (UPRmt).[13] Among sirtuins, SIRT3 is of particular interest due to its mitochondrial localization and role in metabolic regulation. Notably, inhibition of SIRT3 is closely associated with the progression of age-related diseases, including cancer, cardiovascular diseases, and neurodegenerative diseases.[14,15] Consistently, *Sirt3*-deficient mice exhibit a shortened lifespan,[16] whereas elevated SIRT3 expression under conditions of calorie restriction can mitigate oxidative damage and prevent age-related diseases.[17] These findings underscore the protective role of SIRT3 in organismal aging. However, the specific mechanisms by which SIRT3 regulates HSC aging and fate determination remain elusive.

SIRT3 is a known target of small ubiquitin-like modifier (SUMOylation), a post-translational modification that negatively regulates its deacetylase activity. The murine *Sirt3*-K223R mutant prevents SIRT3 SUMOylation and renders it constitutively active.[17] However, the role of SIRT3 SUMOylation in determining HSC fate and aging has not been established. Specifically, how constitutive SIRT3 activation affects HSC fate determination under stress conditions, and whether enhanced HSC activity through SIRT3 activation directly contributes to extended lifespan, remain unknown.

In this study, we investigated the role of SIRT3 in HSC activities and its direct impact on mouse lifespan using *Sirt3*-K223R mutant mice (*Sirt3*-KR) with constitutively active SIRT3, especially under stress conditions such as aging, serial transplantation, and chemotherapy.

Methods

Aging model and competitive transplantation assays

To investigate the role of SIRT3 in extending recipient lifespan, $2x10^6$ bone marrow (BM) cells from *Sirt3*-WT or *Sirt3*-KR donor mice were transplanted into lethally irradiated CD45.2 recipient mice. Body weight was monitored every four weeks from 12 weeks post transplantation, and post-transplant survival of the recipient mice was meticulously monitored and documented. At 12 months post transplantation, the frequency of $Lin^-Sca\text{-}1^+c\text{-}Kit^+CD34^-CD135^-$ long-term HSC (LT-HSC) in BM, and myeloid ($Mac\text{-}1^+Gr\text{-}1^+$), B ($B220^+$) and T ($CD3e^+$) lineage chimerism in peripheral blood (PB) were quantified by flow cytometry. To assess the functional activity of HSC from primary recipients, $2x10^5$ $CD45.2^+$ donor BM cells (*Sirt3*-WT or *Sirt3*-KR) were transplanted with $2x10^5$ $CD45.1^+$ competitor cells into lethally irradiated CD45.1 mice via retroorbital injection. PB chimerism ($CD45.2^+$) was analyzed at 3-16 weeks post transplantation. At 16 weeks, donor-derived myeloid ($Mac\text{-}1^+Gr\text{-}1^+$), B ($B220^+$) and T ($CD3e^+$) lineage subsets were further examined using anti-CD45.1, CD45.2, Mac-1, Gr-1, B220, and CD3e antibodies. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Jiao Tong University School of Medicine and performed in accordance with the Guidelines for Animal Care.

Metabolic analysis

Oxygen consumption rate (OCR) was determined in *Sirt3*-WT and *Sirt3*-KR hematopoietic cells using the XF Cell Mito Stress Test Kit (Seahorse #103015-100) on a Seahorse XF96 analyzer, following the manufacturer's instructions. In brief, for OCR measurement, $3x10^5$ *Sirt3*-WT or *Sirt3*-KR hematopoietic cells were seeded into 96-well plates pre-coated with BD Cell-Tak (BD Biosciences). The cells were maintained in XF basic medium supplemented with 10 mM glucose, 1 mM sodium pyruvate, and 2 mM glutamine (pH 7.4 at 37°C). The injection port A on the sensor cartridge was loaded with 1.5 μM oligomycin, 2 μM of FCCP was loaded into port B, and 0.5 μM rotenone/antimycin A was loaded into port C. Adenosine triphosphate (ATP) level was analyzed using the ATP Bioluminescence Assay Kit HS II (Roche) according to the manufacturer's protocol, and data were normalized to cell count.

Functional, biochemical, and molecular assays

Competitive repopulation assays, flow cytometric analyses (including cell cycle, apoptosis, mitochondrial membrane potential, and reactive oxygen species [ROS] detection), senescence-associated β-galactosidase staining, immunofluorescence staining, RNA sequencing and quantitative RT-PCR, luciferase reporter assays, chromatin immunoprecipitation (ChIP) assays, western blotting, and cleavage under targets and tagmentation (CUT&Tag) analyses were performed as detailed in the *Online Supplementary Appendix*. Primer sequences for genotyping, quantitative RT-PCR (qRT-PCR), dual-luciferase reporter assay, and CUT&Tag are listed in *Online Supplementary Table S1*.

Statistical analysis

Statistical analysis was performed using GraphPad software

(Prism 10.0). Data are represented as mean ± standard deviation unless indicated elsewhere. All the experiments were performed independently at least 3 times. Data were analyzed with two-tailed Student *t* test, one-way ANOVA with Tukey multiple comparison test, or two-way ANOVA with Sidak multiple comparison test, as appropriate. $P<0.05$ was considered statistically significant.

Results

SIRT3 constitutive activation inhibits hematopoietic stem cell aging under stress conditions and markedly extends the overall lifespan of mice

We utilized a murine model with a constitutive activation of SIRT3 introduced by CRISPR-Cas9 targeting the SUMOylation site at lysine 223 (*Sirt3*-K223R or *Sirt3*-KR[17]) to investigate the role of SIRT3 in HSC (*Online Supplementary Figure S1A*). We explored the impact of the SIRT3 SUMOylation site mutation on HSC aging by assessing the multilineage differentiation potential, HSC frequency, the proportion of cells positive for β-galactosidase (SA–β-Gal) staining, and the expression levels of the senescence-associated genes *p16* (INK4a) and *p21* (CIP1). 24-month old wild-type (WT) mice showed significantly higher HSC frequency than young (6-month old) WT mice, whereas HSC frequency in *Sirt3*-KR mice remained stable with age (*Online Supplementary Figure S1B*). Aged *Sirt3*-KR mice displayed attenuated myeloid skewing, with no significant differences observed in young mice (*Online Supplementary Figure S1C*). Notably, SA-β-Gal positivity in Lin⁻ progenitor cells and HSC was robustly reduced in both young and aged *Sirt3*-KR mice relative to WT controls (*Online Supplementary Figure S1D, E*), accompanied by decreased expression of *p16* and *p21* in HSC (Figure 1A). These findings demonstrate that constitutive activation of SIRT3 attenuates HSC senescence during aging.

Aged HSC are known to exhibit impaired repopulation

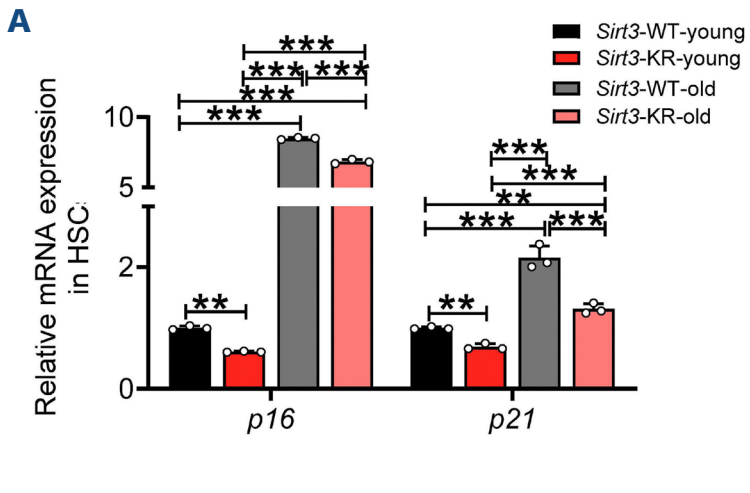


Figure 1. SIRT3 activation inhibits hematopoietic stem cell aging under stress conditions and markedly extends the overall lifespan of mice. (A) The relative mRNA levels of *p16* and *p21* were measured in the hematopoietic stem cells (HSC) of young or old *Sirt3*-wild-type (WT) and *Sirt3*-K223R mutant (*Sirt3*-KR) mice by quantitative RT-PCR (N=3). (B) Frequencies of immunophenotypic Lin⁻Sca-1⁺c-Kit⁺CD34⁻CD135⁻ long-term (LT)-HSC from recipient mice receiving *Sirt3*-WT and *Sirt3*-KR BM donor cells at 12 months after transplantation. (C) The multilineage contribution of donor cells in peripheral blood (PB) quantified at 12 months after transplantation. (D) Lifespan of the recipient mice receiving *Sirt3*-WT or *Sirt3*-KR BM donor cells (N=15). (E) The repopulation ability of donor cells from *Sirt3*-WT and *Sirt3*-KR mice was determined by flow cytometric analysis at the indicated time points (N=5). (F) The multilineage contribution of donor cells in the PB was quantified at 16 weeks after transplantation (N=5). *$P<0.05$, **$P<0.01$, ***$P<0.001$.

capacity and a myeloid differentiation bias.[18,19] However, to what extent aged HSC contribute to a long lifespan remains largely unknown. To address this, we conducted BM transplantation (BMT) with total BM cells from *Sirt3*-KR or *Sirt3*-WT donor mice into lethally irradiated syngeneic recipient mice to evaluate HSC frequency, the lineage distributions, repopulation abilities, and overall lifespan (*Online Supplementary Figure S1F*). At 12 months post transplantation, recipients reconstituted with *Sirt3*-KR BM exhibited reduced HSC and myeloid cell frequencies, accompanied by elevated B-cell frequencies compared to WT controls (Figure 1B, C). Strikingly, the median lifespan of recipients engrafted with *Sirt3*-KR BM cells was markedly extended (432 days *vs*. 382 days) (Figure 1D). Analysis of body weight revealed that weight gain gradually slowed by 42 weeks post transplantation, indicating the onset of senescence (*Online Supplementary Figure S1G*). Terminal-stage animals showed significant weight loss (*Online Supplementary Figure S1H*) accompanied by decreased HSC frequency (*Online Supplementary Figure S1 I*) and a strong myeloid differentiation bias (*Online Supplementary Figure S1J*). These observations collectively suggest that improved HSC function may delay age-related hematopoietic decline and contribute to extended lifespan.

We further performed secondary competitive BMT assays using cells from the primary recipients at 12 months post transplantation and showed that *Sirt3*-KR HSC exhibited enhanced repopulating capabilities (Figure 1E) and a reduced tendency toward myeloid lineage differentiation (Figure 1F) as compared with *Sirt3*-WT HSC. Competitive transplantation demonstrated that aged *Sirt3*-KR HSC exhibited markedly higher donor-derived repopulation capacity than aged WT HSC (*Online Supplementary Figure S1K*). Collectively, these findings indicate that deSUMOylation of SIRT3 sustains HSC function, delays HSC aging, and contributes to an extended lifespan in recipient mice.

The deSUMOylation of SIRT3 preserves hematopoietic stem cell self-renewal under other stress conditions

To further examine whether SIRT3 deSUMOylation also preserves HSC self-renewal under additional stress, we performed serial transplantation assays (*Online Supplementary Figure S2A*). Although the engraftment levels were similar between *Sirt3*-KR and *Sirt3*-WT donor HSC after 1st transplant (*Online Supplementary Figure S2B*), donor cells derived from *Sirt3*-KR mice presented better repopulation at 8, 12, and 16 weeks after 2nd transplant (Figure 2A). The BM reconstitution ability of *Sirt3*-KR cells dramatically exceeded that of *Sirt3*-WT cells after 3rd and 4th transplant (Figure 2B, C). Meanwhile, no significant differences in lineage differentiation were observed between *Sirt3*-WT and *Sirt3*-KR donor cells after 1st transplant (*Online Supplementary Figure S2C*). Interestingly, lymphoid reconstitution from *Sirt3*-KR donor cells gradually increased after 2nd, 3rd, and 4th transplants (Figure 2D, *Online Supplementary Figure S2D, E*), consistent with the delayed aging phenotype of HSC. We also explored the influence of SIRT3 on HSC cell cycle kinetics and apoptosis under stress conditions and demonstrated that SIRT3 could suppress HSC apoptosis as evaluated by the flow cytometric analysis at 16 weeks post transplantation (*Online Supplementary Figure S2F*). An increased frequency of HSC in G0 phase was observed in *Sirt3*-KR mice compared with *Sirt3*-WT mice (*Online Supplementary Figure S2G*). After 2nd transplant, donor HSC from *Sirt3*-KR mice displayed similar inhibition of apoptosis and enhancement of quiescence (*Online Supplementary Figure S2H, I*). These findings indicate that the mutation in the SIRT3 SUMOylation site inhibits HSC apoptosis and enhances quiescence under stress conditions, highlighting the importance of SIRT3 in different HSC fates.

We further tested the impact of SIRT3 on HSC fates using other stress conditions, including *in vitro* culture and chemotherapy. Primary LT-HSC were cultured *ex vivo* and then transplanted into recipient mice. After eight days of culture, the number and frequency of immunophenotypic Lin$^-$Sca-1$^+$c-Kit$^+$ (LSK) cells were significantly increased in *Sirt3*-KR-derived HSC (*Online Supplementary Figure S2J, K*). More importantly, recipient mice showed an improved reconstitution ability and increased lymphoid differentiation with *Sirt3*-KR donor cells (Figure 2E, *Online Supplementary Figure S2L, M*). We continued to treat *Sirt3*-KR mice with two chemotherapeutic agents, including 5-fluorouracil (5-FU) or cyclophosphamide (CTX), to evaluate the HSC activities by competitive transplantation assays. Compared with that observed in *Sirt3*-WT HSC, the frequency of HSC in *Sirt3*-KR mice was significantly increased after 5-FU treatment (*Online Supplementary Figure S2N*). Moreover, PB chimerism from *Sirt3*-WT donors was notably decreased compared with that from *Sirt3*-KR donors at 16 weeks post transplantation (*Online Supplementary Figure S2 O*). After CTX treatment, the frequency of HSC in *Sirt3*-KR mice was significantly higher than that in *Sirt3*-WT mice (*Online Supplementary Figure S2P*). More importantly, HSC from *Sirt3*-KR mice exhibited enhanced repopulation abilities and lymphoid differentiation potential after CTX treatment (Figure 2F, *Online Supplementary Figure S2Q*). Collectively, these findings show that HSC from *Sirt3*-KR mice exhibit enhanced self-renewal and delayed aging under different stress conditions.

SIRT3 sustains hematopoietic stem cell activities and delays aging by regulating inflammation-associated pathways

To elucidate the molecular mechanisms by which SIRT3 deSUMOylation regulates HSC function and aging, we performed RNA-sequencing using HSC from *Sirt3*-WT and *Sirt3*-KR mice. A total of 593 differentially expressed genes (DEG) were identified, including 305 up-regulated and 288 down-regulated genes in *Sirt3*-KR HSC, which were highly enriched in immune- and inflammation-related pathways

(*Online Supplementary Figure S3A, B*). Several immune-related regulators, including *Mx1, Mx2, Irf7, Oas1g, Oasl2, Dhx58, Oas2, Ifit1* and *Ifi44* were significantly reduced in *Sirt3*-KR HSC (*Online Supplementary Figure S3C*). Given that SIRT3 is also reported to modulate gene expression by modifying the chromatin status through the deacetylation of H3K9 and H3K27,[20] we further performed CUT&Tag analysis. The results revealed markedly lower enrichment of H3K9ac and H3K27ac in *Sirt3*-KR HSC and hematopoietic stem/progenitor cells (HSPC) compared with *Sirt3*-WT cells (Figure 3A, *Online Supplementary Figure S3D*). Western blot analyses also demonstrated decreased acetylation of H3K9 and H3K27 in *Sirt3*-KR cells (*Online Supplementary Figure S3E*). Conjoint analysis of RNA-seq and CUT&Tag data identified 90 commonly down-regulated genes in *Sirt3*-KR HSC, which were strongly enriched in the RIG-I-like receptor signaling pathway, including *Dhx58, Gbp5, Irf7, Mx1, Trim30a* and *Ifi44* (Figure 3B, *Online Supplementary Figure S3F*). RT-qPCR further verified the reduced expression of these genes in *Sirt3*-KR HSC (Figure 3C). Given that *Dhx58* encodes a RIG-I-like receptor that modulates interferon and pro-inflammatory cytokine expression via *Irf7*,[21,22] we hypothesized that SIRT3 deSUMOylation may suppress the RIG-I-like receptor signaling pathway.

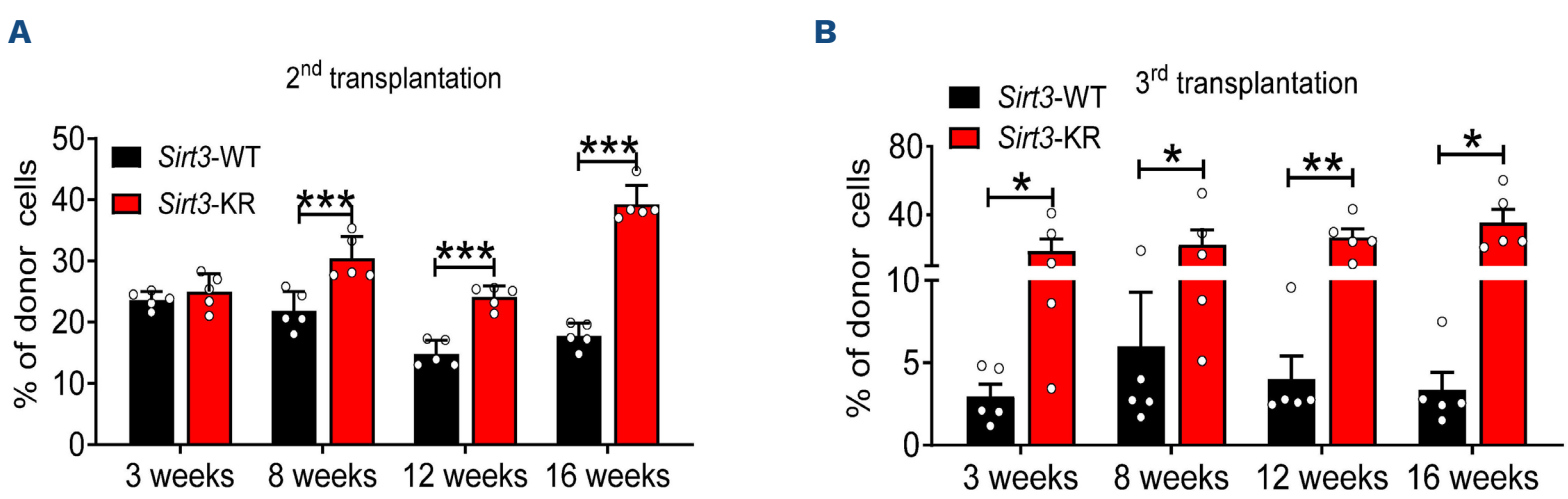


Figure 2. The deSUMOylation of SIRT3 preserves hematopoietic stem cell self-renewal under stress conditions. (A-C) The repopulation of *Sirt3*-wild-type (WT) and *Sirt3*-K223R mutant (*Sirt3*-KR) donor cells was measured 3-16 weeks after the 2nd (A), 3rd (B), and 4th (C) transplantations (N=5). (D) The multilineage differentiation of donor cells into myeloid cells (Mac-1+ and Gr-1+), B cells (B220+), and T cells (CD3e+) in peripheral blood (PB) quantified by flow cytometry at 16 weeks after the 4th transplant (N=5). (E) The repopulation ability of cultured hematopoietic stem cells (HSC) from *Sirt3*-WT and *Sirt3*-KR mice at the indicated time points after transplantation (N=5). (F) The repopulation ability of donor cells from CTX-treated *Sirt3*-WT and *Sirt3*-KR mice was determined by flow cytometric analyses at the indicated time points (N=5). *P<0.05, **P<0.01, ***P<0.001.

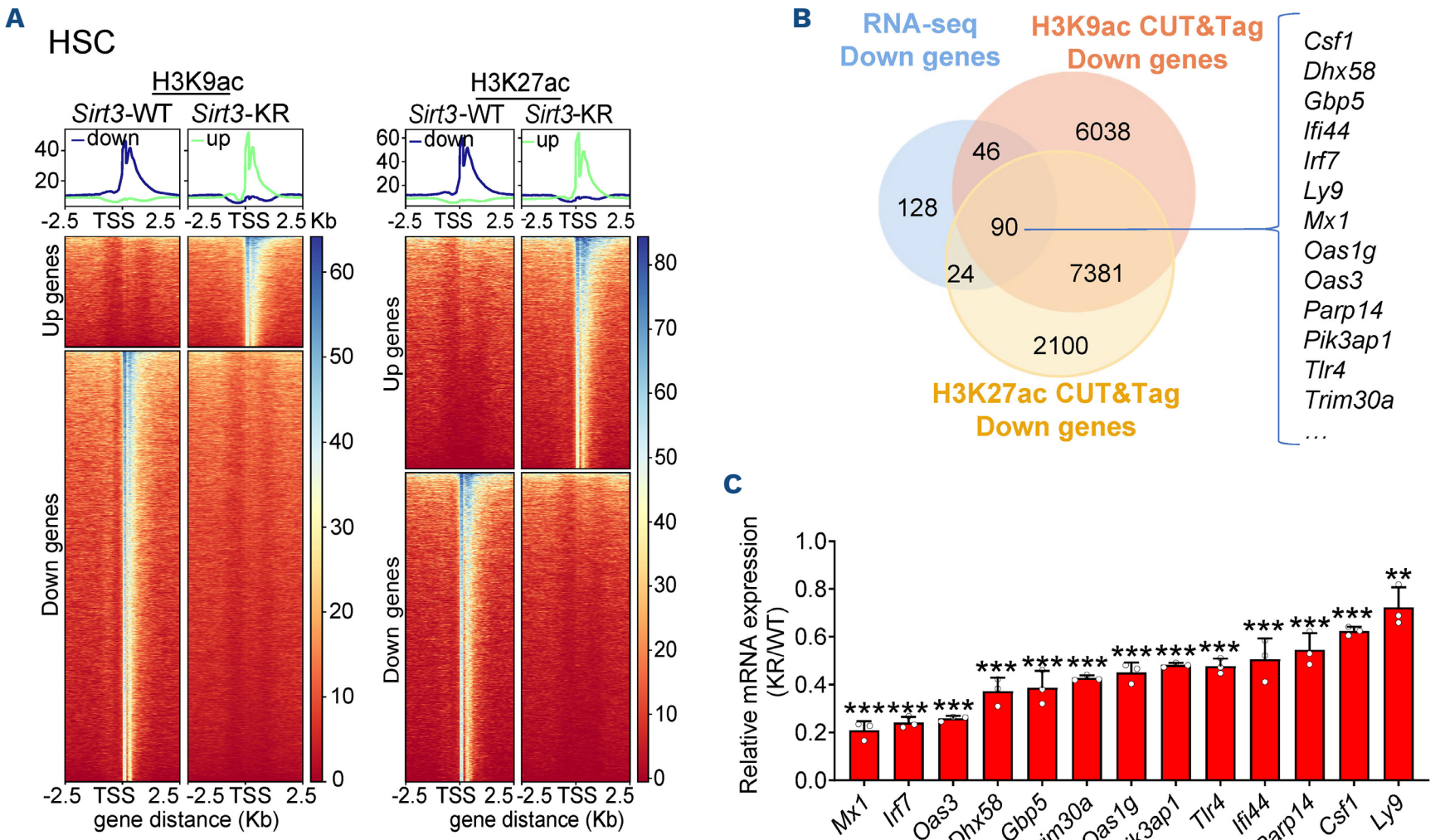


Figure 3. SIRT3 sustains hematopoietic stem cell activities and delays aging by regulating inflammation-associated pathways. (A) Heatmap showing cleavage under targets and tagmentation (CUT&Tag) sequencing (-seq) coverage at sites with altered H3K-9ac and H3K27ac modifications in *Sirt3*-K223R mutant (*Sirt3*-KR) hematopoietic stem cells (HSC) compared with the *Sirt3*-wild-

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type (WT) group. (B) Venn diagram of matching differentially expressed genes among RNA-seq, H3K9ac CUT&Tag-seq and H3K27ac CUT&Tag-seq data. (C) Relative mRNA expression of potential candidates (*Dhx58*, *Oas3*, *Irf7* and *Mx1*) in long-term (LT)-HSC purified from *Sirt3*-WT and *Sirt3*-KR mice, as measured by quantitative RT-PCR (N=3). (D) Analysis of H3K9ac and H3K27ac CUT&Tag-seq at peaks in the genomic regions of DHX58 and IRF7. (E) ChIP-qPCR analysis of H3K9ac or H3K27ac levels at the promoter of *Dhx58* in HSC isolated from *Sirt3*-WT and *Sirt3*-KR mice. (F) Chromatin immunoprecipitation (ChIP)-qPCR analysis of H3K9ac or H3K27ac levels at the promoter of *Irf7* in HSC isolated from *Sirt3*-WT and *Sirt3*-KR mice. ***P*<0.01, ****P*<0.001.

CUT&Tag data revealed an enrichment of the *Dhx58* and *Irf7* genes in *Sirt3*-WT HSC and HSPC, with the higher transcript abundance and increased levels of the active histone marks H3K9ac and H3K27ac (Figure 3D, *Online Supplementary Figure S4A*). ChIP-qPCR revealed a greater enrichment of acetylated H3K27 and H3K9 at the *Dhx58* or *Irf7* promoter in *Sirt3*-WT LT-HSC than in *Sirt3*-KR HSC (Figure 3E, F, *Online Supplementary Figure S4B, C*). These findings suggest that decreased H3K9ac and H3K27ac levels are involved in the transcriptional repression of the *Dhx58* and *Irf7* genes upon SIRT3 deSUMOylation.

It has been reported that SIRT3 governs a mitochondrial protective program in stem cells and prevents stem cell aging.[23] Moreover, mitochondrial dysfunction can trigger cGAS-STING activation.[24] We speculated that the SUMOylation site mutation of SIRT3 may prevent the activation of the cGAS-STING pathway. Indeed, we found that *cGas* expression level was down-regulated in *Sirt3*-KR mice, with no significant changes in *Sting*, *Tbk1* and *Irf3* expression according to RNA-Seq (*Online Supplementary Figure S4D*). However, immunofluorescence staining and western blotting further confirmed that mutation of the SIRT3 SUMOylation site reduced STING and phosphorylated TBK1 (p-TBK1) levels (*Online Supplementary Figure S4E, F*). Cytosolic mtDNA levels (*mtND1*, *mtCOX2*) and mitochondrial ROS (MitoSOX) were significantly lower in *Sirt3*-KR HSPC compared with WT controls (*Online Supplementary Figure S4G-I*). These results indicate that SIRT3 deSUMOylation plays a role in inhibiting the activation of the cGAS-STING signaling pathway.

SIRT3 promotes hematopoietic stem cell self-renewal and delays hematopoietic stem cell aging through downregulation of DHX58

We then evaluated how DHX58 regulates HSC self-renewal and aging and found that the overexpression of *Dhx58* in *Sirt3*-KR HSC significantly impaired the BM reconstitution capacity of *Sirt3*-KR cells (Figure 4A, *Online Supplementary Figure S5A*) and reversed the tendency to myeloid differentiation (Figure 4B). Meanwhile, we found that *Dhx58* overexpression in *Sirt3*-KR HSC increased the proportion of SA-β-Gal positive cells (Figure 4C, *Online Supplementary Figure S5B*) and up-regulated the expression of the senescence-associated marker genes *p16* and *p21* (Figure 4D). These results indicate that SIRT3 suppresses DHX58 expression to preserve HSC activities and delays HSC aging. Senescent cells secrete a plethora of growth factors, cytokines, and proteases, contributing to a senescence-associated secretory phenotype (SASP). Notably, IL-1β, and IL-6 are known to trigger cellular senescence.[25,26] We speculated that the secretion of IL-1β and IL-6 could lead to HSC senescence in a paracrine manner. Indeed, the addition of IL-1β and IL-6 to the culture medium promoted 32D cells (a myeloid progenitor cell line) to start the process of senescence in a dose-dependent manner (*Online Supplementary Figure S5C, D*). Consistently, *Dhx58*-over-expressing 32D cells (32D-*Dhx58*-OE) presented increased expression of *Il-1β* and *Il-6* (*Online Supplementary Figure S5E, F*), elevated levels of the DNA damage response (DDR) factor γ-H2A.X (*Online Supplementary Figure S5G, H*), and a higher proportion of senescent cells positive for β-galactosidase staining (*Online Supplementary Figure S5I, J*). *Sirt3*-KR HSC showed significantly reduced *Il-1β* and *Il-6* expression compared with *Sirt3*-WT HSC. Notably, this reduction was rescued by *Dhx58* overexpression (Figure 4E). Collectively, these findings demonstrate that SIRT3 inhibits HSC senescence and SASP by suppressing DHX58.

IRF7 serves as a DHX58 downstream target to regulate hematopoietic stem cell stemness and aging

DHX58 acts as a co-factor to co-ordinate the RIG-1 and MDA5 function in RNA recognition and activates downstream IRF7 signaling to promote the secretion of inflammatory factors, such as IL-6 and INF-γ.[27] We noticed that *Irf7* expression was significantly reduced in *Sirt3*-KR HSC (Figure 3C), suggesting IRF7 may act as a downstream effector of SIRT3. Indeed, the overexpression of *Irf7* markedly reduced the BM reconstitution capacity of *Sirt3*-KR HSC but exerted no significant effect on *Sirt3*-WT HSC (Figure 5A, *Online Supplementary Figure S6A*). The overexpression of *Irf7* also led to a decrease in B-lymphoid differentiation and an increase in myeloid differentiation (Figure 5B). Furthermore, *Irf7* overexpression accelerated senescence of *Sirt3*-KR HSC, as shown by elevated SA-β-galactosidase activity and increased expression of *p16*, *p21*, *Il-1β,* and *Il-6* (Figure 5C, D, *Online Supplementary Figure S6B, C*). These results show that, similarly to *Dhx58*, *Irf7* overexpression triggers cellular senescence, suggesting that it serves as a critical downstream effector of SIRT3.

We then investigated how SIRT3 regulates HSC activities, including enhanced self-renewal and reduced tendency to myeloid cells. Indeed, RNA-sequencing data showed that genes related to HSC self-renewal (*Hoxb4, Bmi1, Mllt3 and Gata2*) were significantly up-regulated in *Sirt3*-KR HSC, whereas myeloid differentiation-related genes (*Pu.1* and *Cebpa*) were down-regulated. Additionally, lymphoid dif-

ferentiation-related genes (*Ikaros, E2a, Sox4* and *Bcl11a*) were up-regulated in *Sirt3*-KR HSC (*Online Supplementary Figure S6D*). Quantitative RT-PCR assays further confirmed that *Hoxb4*, *Mllt3* and *Gata2* were significantly up-regulated, whereas *Pu.1*, *Gata1* and *Cebpa* were significantly down-regulated. Additionally, *Pax5*, *Ebf1*, *E2a*, *Sox4* and *Bcl11a* were notably increased in *Sirt3*-KR HSC (*Online Supplementary Figure S6E*).

We next examined how IRF7 mediates the effects of SIRT3 on HSC self-renewal and differentiation. We found that *Irf7* overexpression inhibited the expression of key self-renewal- and B-cell differentiation-related genes, including *Hoxb4*, *Mllt3*, *Gata2*, *Pax5*, *Ebf1*, *E2a* and *Sox4*, while promoting the expression of the myeloid differentiation-related genes *Pu.1* and *Gata1* (*Online Supplementary Figure S6F, G*). Dual-luciferase reporter assay showed that IRF7 could repress the transcription of *Gata2* and *Mllt3* while transactivating *Pu.1* and *Gata1* expression (Figure 5E, *Online Supplementary Figure S6H, I*). ChIP-PCR analysis further confirmed the direct binding of IRF7 to the promoters of these genes (*Online Supplementary Figure S6J-M*). Overall, these results suggest that SIRT3 can inhibit the DHX58-

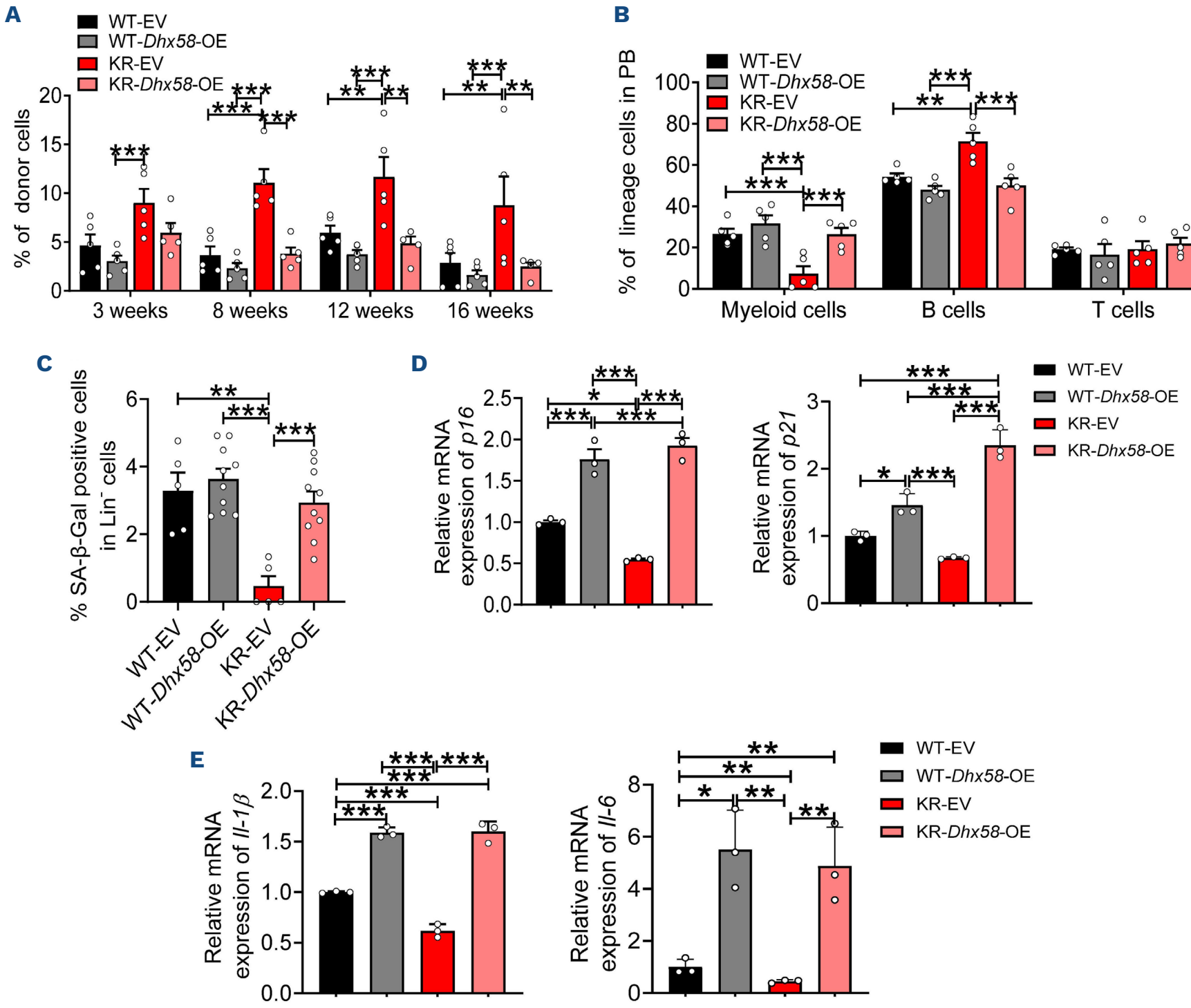


Figure 4. SIRT3 promotes hematopoietic stem cell self-renewal and delays hematopoietic stem cell aging through the downregulation of DHX58. (A) Quantification of hematopoietic stem cell (HSC) repopulation capacity from *Sirt3*-wild-type (WT) and *Sirt3*-K223R mutant (*Sirt3*-KR) mice with/without *Dhx58* overexpression (RFP^+ cells) at the indicated time points post transplantation (N=5). (B) The multilineage contribution of donor cells in peripheral blood (PB) was quantified at 16 weeks post transplantation (N=5). (C) Frequencies of SA-β-Gal-expressing *Sirt3*-WT, *Sirt3*-KR, *Dhx58*-over-expressing *Sirt3*-WT and *Sirt3*-KR Lin^- cells were measured. (D) The relative mRNA expression of aging-related genes *p16* and *p21* in *Sirt3*-WT, *Sirt3*-KR, *Dhx58*-over-expressing *Sirt3*-WT or *Sirt3*-KR HSC was measured by quantitative RT-PCR (N=3). (E) The relative mRNA levels of the senescence-associated secretory phenotype (SASP) factors *Il-1*β and *Il-6* were measured in *Sirt3*-WT, *Sirt3*-KR, *Dhx58*-over-expressing *Sirt3*-WT and *Sirt3*-KR HSC via quantitative RT-PCR (N=3). **P*<0.05, ***P*<0.01, ****P*<0.001.

IRF7 signaling by regulating histone modifications, thereby increasing self-renewal and inhibiting senescence in HSC.

SIRT3 enhances mitochondrial metabolism and α-KG generation in hematopoietic stem cells

SIRT3 has been reported to play important roles in mitochondrial metabolism to further initiate downstream signaling pathway.[17,28] To understand how SIRT3 regulates DHX58-IRF7 signaling, we then analyzed the respiratory status of *Sirt3*-KR and showed that *Sirt3*-KR HSC exhibited significantly higher OCR, ATP levels, and mitochondrial membrane potential than *Sirt3*-WT counterparts (Figure 6A-C). Consistently, we also observed a notable increased mitochondrial count (*Online Supplementary Figure S7A, B*) and a larger mitochondrial area and greater quantity in *Sirt3*-KR HSC by electron microscopy (Figure 6D).

We had previously established a metabolic SoNar sensor that could sensitively and specifically detect the ratio of NADH and NAD^+ both *in vitro* and *in vivo*. The higher/lower SoNar ratio ($NADH/NAD^+$) reflected the higher/lower activities of glycolysis and tricarboxylic acid cycle (TCA), respectively,[29,30] while the iNap sensor is capable of accurately detecting the levels of reduced NADPH.[31] We further generated *Sirt3*-KR/SoNar and *Sirt3*-KR/iNap double transgenic mice and demonstrated that *Sirt3*-KR HSC had much higher levels of TCA (lower SoNar ratio; *Online Supplementary Figure S7C, D*), and higher NADPH levels (higher iNap ratio; *Online Supplementary Figure S7E, F*) than those of WT controls. The increased NADPH levels likely contribute to the markedly reduced mitochondrial ROS levels observed in *Sirt3*-KR HSC (Figure 6E), strengthening their antioxidant capacity. Notably, α-KG levels were increased by 1.5-2-fold in *Sirt3*-KR HSC compared to WT controls, a phenomenon consistently observed in both young (2-month-old) and aged (20-month-old) mice (Figure 6F). This is consistent with reports that α-KG declines with age and that its homeostasis supports longevity.[32,33]

Consistently, RT-qPCR confirmed that genes involved in antioxidant defense (e.g., *Sod1, Sod2, Prdx1, Gpx1*) and oxidative phosphorylation (e.g., *Mdh2, Idh2, Aco2*) were up-regulated in *Sirt3*-KR HSC, while pro-oxidant genes (e.g., *Glrx2, Nox2*) were down-regulated. Notably, among genes involved in oxidative phosphorylation, *Idh2* showed the highest expression level (*Online Supplementary Figure S7G*). IDH2 catalyzes the conversion of isocitrate to α-KG with concomitant NADPH production,

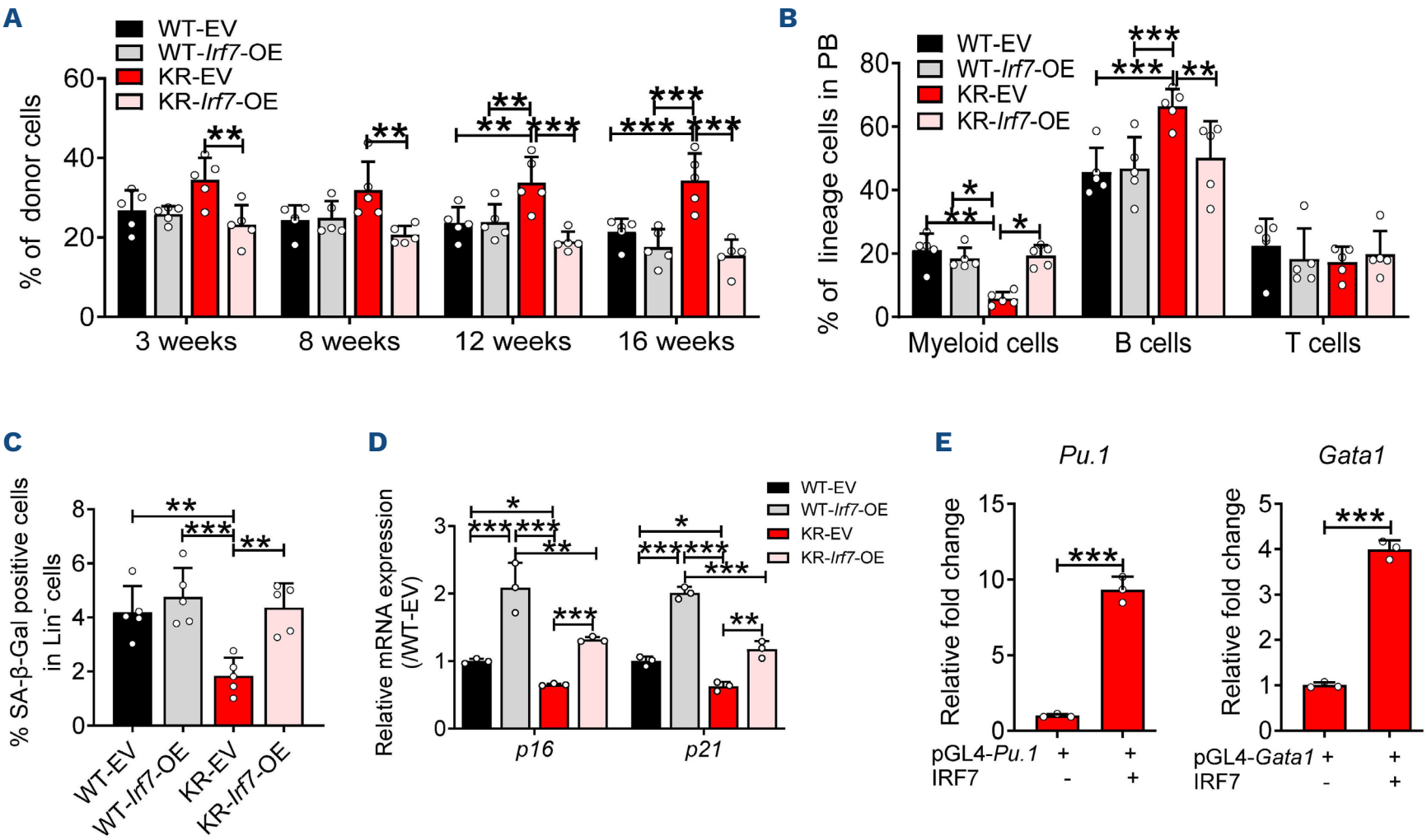


Figure 5. IRF7 serves as a DHX58 downstream target to regulate hematopoietic stem cell stemness and aging. (A) Quantification of hematopoietic stem cell (HSC) repopulation capacity from *Sirt3*-wild-type (WT) and *Sirt3*-K223R mutant (*Sirt3*-KR) mice with/without *Irf7* overexpression (RFP^+ cells) at the indicated time points post transplantation (N=5). (B) The multilineage contribution of donor cells in peripheral blood (PB) was quantified at 16 weeks after transplantation (N=5). (C) Frequencies of SA-β-Gal-expressing *Sirt3*-WT, *Sirt3*-KR, *Irf7*-over-expressing *Sirt3*-WT and *Sirt3*-KR Lin^- cells were measured. (D) The relative mRNA expression levels of aging-related genes (*p16* and *p21*) in *Sirt3*-WT, *Sirt3*-KR, *Irf7*-over-expressing *Sirt3*-WT or *Sirt3*-KR HSC were measured by quantitative RT-PCR (N=3). (E) A luciferase reporter assay was performed to evaluate the transcriptional activity of IRF7 at the promoters of *Pu.1* and *Gata1* (N=3). **P*<0.05, ***P*<0.01, ****P*<0.001.

indicating that IDH2 may play an important role in regulating histone deacetylation in *Sirt3*-KR HSC. Collectively, these findings demonstrate that SIRT3 deSUMOylation enhances mitochondrial metabolism, boosts antioxidant capacity, and promotes α-KG generation, which in turn drives epigenetic repression of the DHX58-IRF7 axis to sustain HSC function and delay aging (Figure 7). These observations establish a mechanistic link between adult stem cell homeostasis and organismal lifespan under stress and provide a theoretical framework for developing interventions to delay aging.

Discussion

The senescence of HSC is considered a key factor leading to the dysregulation and aging of the entire hematopoietic

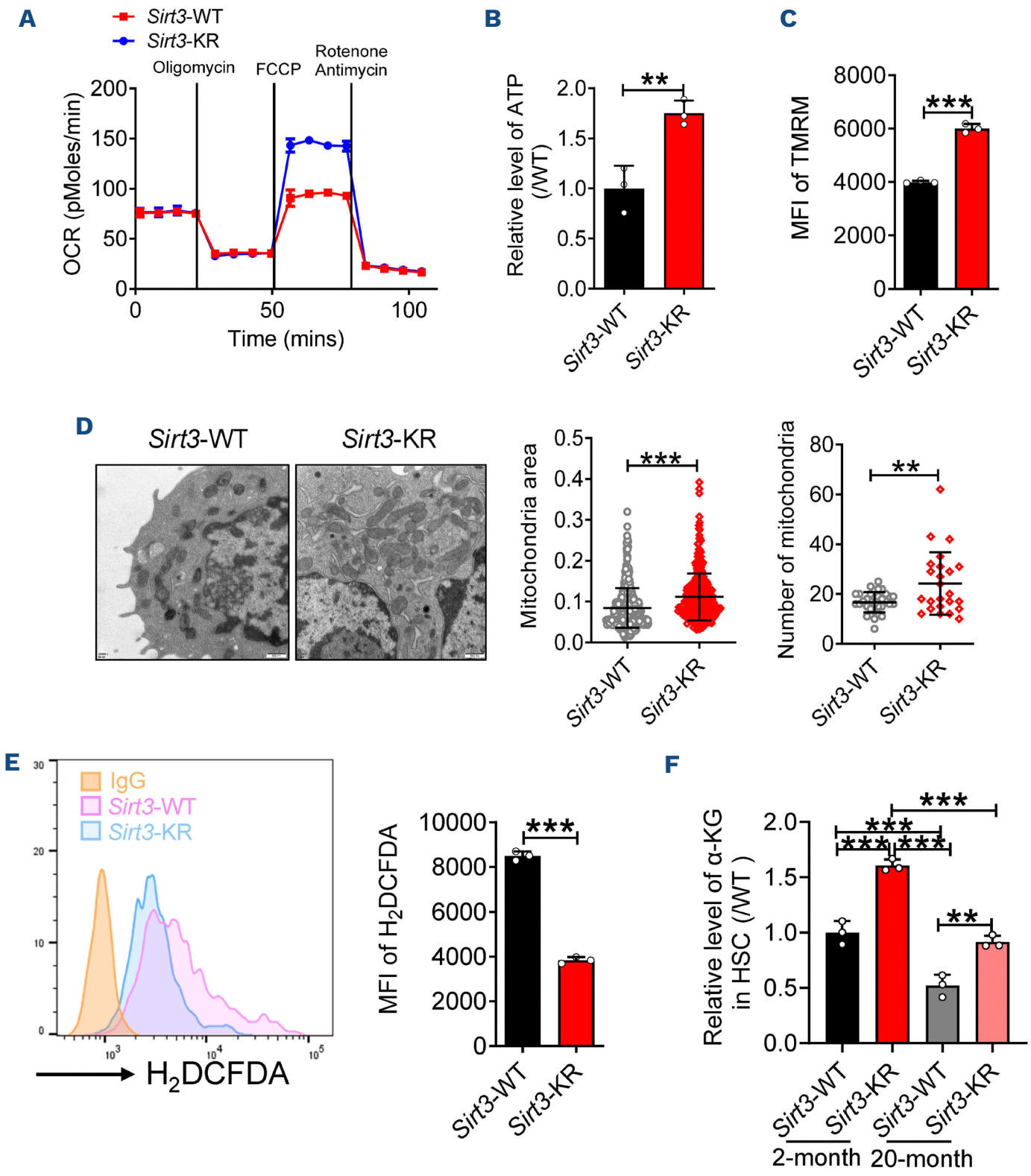


Figure 6. SIRT3 serves as a key regulator of mitochondrial metabolism in hematopoietic stem cells. (A) Oxygen consumption rate (OCR) was measured in *Sirt3*-wild-type (WT) (red) and *Sirt3*-K223R mutant (*Sirt3*-KR) (blue) hematopoietic stem/progenitor cells (HSPC) using a Seahorse XF96 analyzer under basal conditions and in response to sequential addition of 1.5 μM oligomycin, 2 μM FCCP, and 0.5 μM rotenone/antimycin A. (B) Adenosine triphosphate (ATP) levels in *Sirt3*-WT and *Sirt3*-KR hematopoietic stem cells (HSC) were measured with an ATP assay kit (N=3). (C) Mean fluorescence intensity (MFI) of the tetramethylrhodamine methyl ester (TMRM) in *Sirt3*-WT and *Sirt3*-KR HSC (N=3). (D) Mitochondrial morphology as well as quantitative analyses of mitochondrial area and number in HSC from *Sirt3*-WT and *Sirt3*-KR mice were examined by transmission electron microscopy. Scale bar, 500 nm. (E) Measurement of 2',7'-dichlorofluorescein diacetate (H_2DCFDA) levels in *Sirt3*-WT and *Sirt3*-KR HSC by flow cytometry (N=3). (F) Relative α-KG levels in HSC isolated from *Sirt3*-WT and Sirt3-KR mice at 2 and 20 months of age (N=3). ***P*<0.01, ****P*<0.001.

system, as well as other organismal disorders. Sirtuins regulate a variety of cellular responses, including DNA damage repair, telomere maintenance, and metabolism.[34] SIRT2, SIRT6, and SIRT7 exert significant effects on the regulation of HSC homeostasis and aging.[11-13] Using a *Sirt3*-K223R mouse model, we demonstrated that SIRT3 deSUMOylation enhances HSC self-renewal capacity and maintains lineage-balanced differentiation through the DHX58-IRF7 signaling, ultimately prolonging lifespan in this transgenic model. Given that IRF7 inhibitors are in clinical trials for autoimmune diseases,[35] our findings suggest repurposing these compounds to ameliorate myeloid skewing in elderly patients. This effect may translate to reduced frailty and risk of hematologic malignancy in elderly humans, highlighting the potential of targeting SIRT3 for improving HSC function in age-related BM disorders.

Previous studies have linked SIRT3 to cellular senescence and age-related diseases,[14,36,37] and SIRT3 deletion was reported to shorten mouse lifespan.[16] However, whether specific adult stem cell populations such as HSC contribute to organismal longevity remains poorly defined. Here, we found that HSC from 24-month-old *Sirt3*-KR mice presented a reduced proportion of senescent cells and down-regulated expression of the senescence-associated genes *p16* and *p21*. These findings suggest that SIRT3 deSUMOylation inhibits HSC aging, and this protection correlates with extending lifespan in a *Sirt3*-KR model. Whether HSC aging directly affects organismal lifespan remains to be confirmed.

Mitochondria play a central role in HSC maintenance, stress response, and aging through metabolic and epigenetic regulation.[38] Metabolites, such as acetyl-CoA, α-KG, and ROS are crucial for enzymes that modify the chromatin structure to affect gene expression.[39,40] SIRT3 is essential for maintaining mitochondrial homeostasis by regulating metabolic activity through multiple substrate proteins,[41] and this function has attracted intense interest in senescence and aging research.[15,42] Notably, we observed that *Sirt3*-KR HSC displayed elevated mitochondrial activity (Figure 6) and enhanced quiescence (*Online Supplementary Figure S2G*), which appears to contradict the general model that quiescent HSC rely on glycolysis. Future studies should examine whether this reflects a unique metabolic state in stress-resistant HSC (e.g., high mitochondrial quality but low respiratory rate).

The RIG-I signaling pathway has emerged as a key regulator of aging, linking DNA damage to inflammatory senescence.[43] Several inflammatory factors, such as IL-1, IL-6, IL-11, CCL4 and IL-8, are known to promote the progression of senescence.[25,26,44,45] Interestingly, our study demonstrated that SIRT3 deSUMOylation leads to reduced secretion of IL-1β and IL-6. However, the cellular source of these cytokines (e.g., HSC or other hematopoietic cells) and their direct impact on lifespan require further study. Consistent with previous studies showing that inhibition of IL-1 signaling or administration of anti-IL-11 antibodies can attenuate HSC senescence and promote longevity,[45,46] our

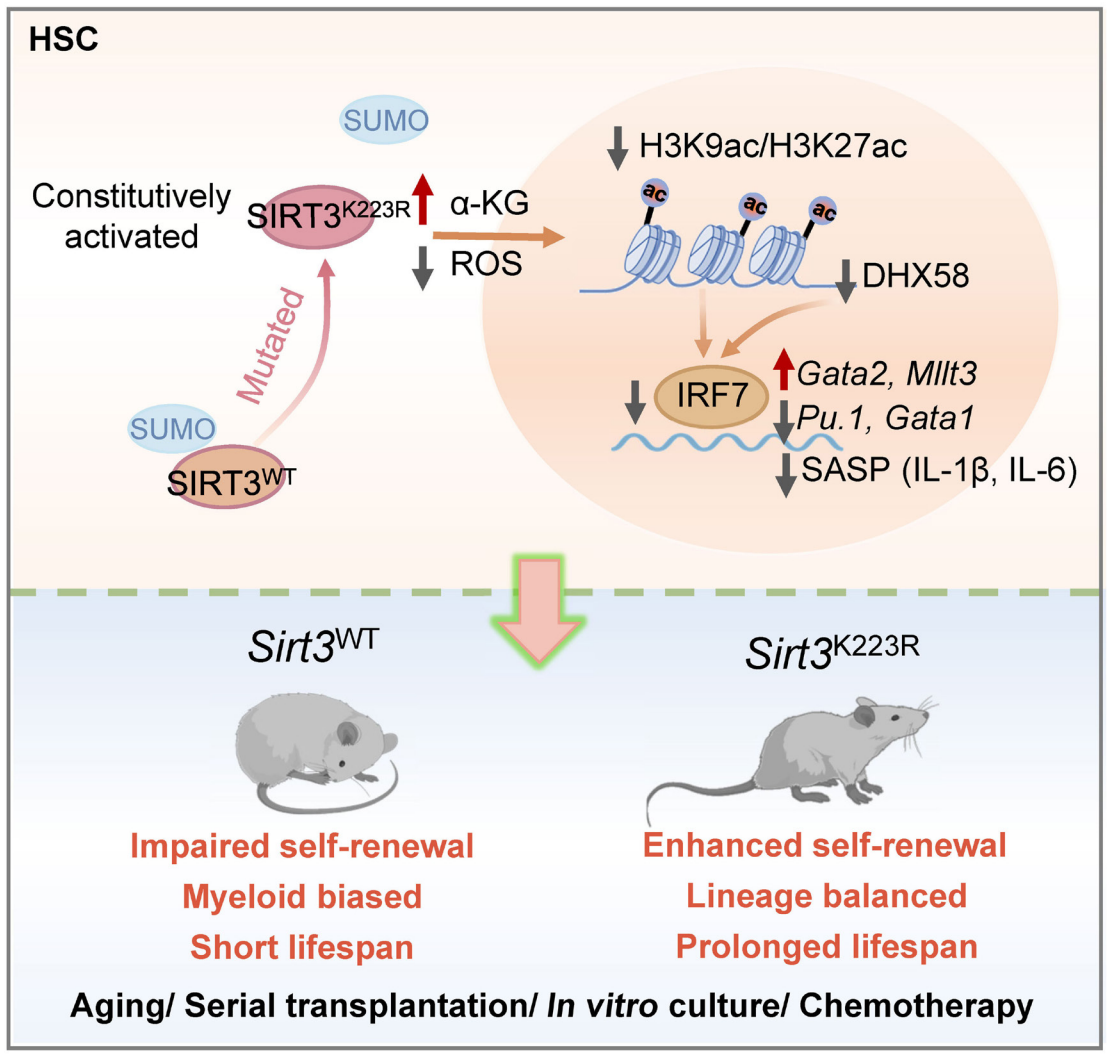


Figure 7. Working model. A working model for the roles of SIRT3 in hematopoietic stem cell (HSC) activities, aging and mouse lifespan. Under stress conditions such as serial transplantation, chemotherapy or aging, the deSUMOylation of SIRT3 enhances HSC self-renewal and lymphoid differentiation potential, while suppressing HSC aging and extending lifespan. Mechanistically, deSUMOylated SIRT3, achieved by constitutive activation of the SIRT3 mutant (K223R), inhibits senescence-associated inflammatory responses through a novel mechanism involving increased α-KG levels, which in turn suppresses the acetylation of H3K9 and H3K27. This leads to inhibition of key genes in the RIG-I signaling pathway, including *Dhx58* and *Irf7*. IRF7 induces the expression of myeloid differentiation-related genes such as *Pu.1* and *Gata1*, while repressing HSC self-renewal-related genes including *Gata2* and *Mllt3*. The SIRT3-DHX58-IRF7 signaling axis down-regulates the expression of the senescence-associated secretory phenotype (SASP) factors IL-1β and IL-6, which are critical for maintenance of HSC stemness and longevity.

findings highlight the potential of targeting inflammatory pathways as a strategy to delay aging. Additionally, SIRT3 mitigates senescence via cGAS-STING suppression,[47] and we observed down-regulated cGAS, STING, and TBK1 in *Sirt3*-KR HSC. However, the extent to which cGAS-STING signaling impacts lifespan remains to be verified.

We had previously demonstrated that SIRT3 deSUMOylation is specifically regulated by the AMPK-SENP1 pathway,[48] which provides a basis for further screening and for exploring the role of SIRT3 SUMOylation agonists or antagonists in determining HSC fate. Our future focus will be on exploring the potential of small-molecule compounds and gene-therapeutic strategies that precisely target this signaling axis. Importantly, SUMOylation sites are conserved in human SIRT3, and mutation of these sites activates SIRT3 deacetylase activity.[17] Investigating the role of SIRT3 SUMOylation in human HSC aging will be crucial for translating these findings into interventions to extend lifespan.

In conclusion, SIRT3 deSUMOylation serves as a key regulator of HSC stress resistance, acting via the DHX58-IRF7 axis and mitochondrial metabolism. These findings advance our understanding of HSC functional maintenance under stress and provide a mechanistic basis for developing strategies to preserve hematopoietic homeostasis during aging.

Disclosures

No conflicts of interest to disclose.

Contributions

XH, JYu, YXu, EG, YZ, JC and JZ designed and performed the experiments, analyzed the data, and wrote the paper. TW, YL, JW, SD, YXi, XM, LX, CC, ZY and JYe performed the experiments, provided reagents, and helped with manuscript preparation.

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Data-sharing statement

Raw RNA-seq and CUT&Tag-seq data have been deposited in the NCBI Sequence Read Archive (SRA) database (PRJNA1259606, PRJNA1260697).

References

1. Campisi J, Kapahi P, Lithgow GJ, Melow S, Newman JC, Verdin E. From discoveries in ageing research to therapeutics for healthy ageing. Nature. 2019;571(7764):183-192.
2. Kuijk E, Kranenburg O, Cuppen E, Van Hoek A. Common anti-cancer therapies induce somatic mutations in stem cells of healthy tissue. Nature Commun. 2022;13(1):5915.
3. Rodriguez-Fraticelli AE, Wolock SL, Weinreb CS, et al. Clonal analysis of lineage fate in native haematopoiesis. Nature. 2018;553(7687):212-216.
4. Zhang B, Lee DE, Trapp A, et al. Multi-omic rejuvenation and life span extension on exposure to youthful circulation. Nat Aging. 2023;3(8):948-964.
5. Ross JB, Myers LM, Noh JJ, et al. Depleting myeloid-biased haematopoietic stem cells rejuvenates aged immunity. Nature. 2024;628(8006):162-170.
6. Mejia-Ramirez E, Florian MC. Understanding intrinsic hematopoietic stem cell aging. Haematologica. 2020;105(1):22-37.
7. Liang C, Liu Z, Song M, et al. Stabilization of heterochromatin by CLOCK promotes stem cell rejuvenation and cartilage regeneration. Cell Res. 2021;31(2):187-205.
8. Kollman C, Howe CW, Anasetti C, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. Blood. 2001;98(7):2043-2051.
9. Fang Y, An N, Zhu L, et al. Autophagy-Sirt3 axis decelerates hematopoietic aging. Aging Cell. 2020;19(10):e13232.
10. Finkel T, Deng CX, Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. Nature. 2009;460(7255):587-591.
11. Luo H, Mu WC, Karki R, et al. Mitochondrial stress-initiated aberrant activation of the NLRP3 inflammasome regulates the functional deterioration of hematopoietic stem cell aging. Cell Rep. 2019;26(4):945-954.e4.
12. Wang H, Diao D, Shi Z, et al. SIRT6 controls hematopoietic stem cell homeostasis through epigenetic regulation of Wnt signaling. Cell Stem Cell. 2016;18(4):495-507.
13. Mohrin M, Shin J, Liu Y, et al. Stem cell aging. A mitochondrial UPR-mediated metabolic checkpoint regulates hematopoietic stem cell aging. Science. 2015;347(6228):1374-1377.
14. McDonnell E, Peterson BS, Bomze HM, et al. SIRT3 regulates progression and development of diseases of aging. Trends Endocrinol Metab. 2015;26(9):486-492.
15. Brown K, Xie S, Qiu X, et al. SIRT3 reverses aging-associated degeneration. Cell Rep. 2013;3(2):319-327.
16. Benigni A, Cassis P, Conti S, et al. Sirt3 deficiency shortens life span and impairs cardiac mitochondrial function rescued by Opa1 gene transfer. Antioxid Redox Signal. 2019;31(17):1255-1271.
17. Wang T, Cao Y, Zheng Q, et al. SENP1-Sirt3 signaling controls mitochondrial protein acetylation and metabolism. Mol Cell. 2019;75(4):823-834.e5.
18. de Haan G, Lazare SS. Aging of hematopoietic stem cells. Blood. 2018;131(5):479-487.
19. DeGregori J. Aging, inflammation, and HSC. Blood.

2020;136(2):153-154.
20. Palomer X, Román-Azcona MS, Pizarro-Delgado J, et al. SIRT3-mediated inhibition of FOS through histone H3 deacetylation prevents cardiac fibrosis and inflammation. Signal Transduct Target Ther. 2020;5(1):14.
21. Lefkopoulos S, Polyzou A, Derecka M, et al. Repetitive elements trigger RIG-I-like receptor signaling that regulates the emergence of hematopoietic stem and progenitor cells. Immunity. 2020;53(5):934-951.e9.
22. Xiao Z, Chen H, Xu N, Chen Y, Wang S, Xu X. MATR3 promotes liver cancer progression by suppressing DHX58-mediated type I interferon response. Cancer Lett. 2024;604:217231.
23. Jiang DQ, Wang Y, Li MX, et al. SIRT3 in neural stem cells attenuates microglia activation-induced oxidative stress injury through mitochondrial pathway. Front Cell Neurosci. 2017;11:7.
24. Domizio JD, Gulen MF, Saidoune F, et al. The cGAS-STING pathway drives type I IFN immunopathology in COVID-19. Nature. 2022;603(7899):145-151.
25. Caiado F, Manz MG. IL-1 in aging and pathologies of hematopoietic stem cells. Blood. 2024;144(4):368-377.
26. Kuilman T, Michaloglou C, Vredeveld LC, et al. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell. 2008;133(6):1019-1031.
27. Rehwinkel J, Gack MU. RIG-I-like receptors: their regulation and roles in RNA sensing. Nat Rev Immunol. 2020;20(9):537-551.
28. Peng F, Liao M, Jin W, et al. 2-APQC, a small-molecule activator of Sirtuin-3 (SIRT3), alleviates myocardial hypertrophy and fibrosis by regulating mitochondrial homeostasis. Signal Transduct Target Ther. 2024;9(1):133.
29. Gu H, Chen C, Hao X, et al. MDH1-mediated malate-aspartate NADH shuttle maintains the activity levels of fetal liver hematopoietic stem cells. Blood. 2020;136(5):553-571.
30. Hao X, Gu H, Chen C, et al. Metabolic imaging reveals a unique preference of symmetric cell division and homing of leukemia-initiating cells in an endosteal niche. Cell Metab. 2019;29(4):950-965.e6.
31. Chen C, Lai X, Zhang Y, et al. NADPH metabolism determines the leukemogenic capacity and drug resistance of AML cells. Cell Rep. 2022;39(1):110607.
32. Chin RM, Fu X, Pai MY, et al. The metabolite alpha-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. Nature. 2014;510(7505):397-401.
33. Lee I, Piao S, Kim S, et al. IDH2 deficiency promotes endothelial senescence by eliciting miR-34b/c-mediated suppression of mitophagy and increased ROS production. Antioxidants (Basel). 2023;12(3):585.
34. Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol. 2012;13(4):225-238.
35. Werth VP, Furie RA, Romero-Diaz J, et al. Trial of anti-BDCA2 antibody litifilimab for cutaneous lupus erythematosus. N Engl J Med. 2022;387(4):321-331.
36. Yang L, Wang B, Guo F, et al. FFAR4 improves the senescence of tubular epithelial cells by AMPK/SirT3 signaling in acute kidney injury. Signal Transduct Target Ther. 2022;7(1):384.
37. Zhang J, Xiang H, Liu J, Chen Y, He R-R, Liu B. Mitochondrial sirtuin 3: new emerging biological function and therapeutic target. Theranostics. 2020;10(18):8315-8342.
38. Chandel NS, Jasper H, Ho TT, Passegué E. Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. Nat Cell Biol. 2016;18(8):823-832.
39. Fu ZW, Li JH, Feng YR, Yuan X, Lu Y-T. The metabolite methylglyoxal-mediated gene expression is associated with histone methylglyoxalation. Nucleic Acids Res. 2021;49(4):1886-1899.
40. Kaelin WG Jr, McKnight SL. Influence of metabolism on epigenetics and disease. Cell. 2013;153(1):56-69.
41. Yang W, Nagasawa K, Münch C, et al. Mitochondrial sirtuin network reveals dynamic SIRT3-dependent deacetylation in response to membrane depolarization. Cell. 2016;167(4):985-1000.e21.
42. Wiley CD, Velarde MC, Lecot P, et al. Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. Cell Metab. 2016;23(2):303-314.
43. Liu F, Wu S, Ren H, Gu J. Klotho suppresses RIG-I-mediated senescence-associated inflammation. Nat Cell Biol. 2011;13(3):254-262.
44. Chang TT, Lin LY, Chen C, Chen J-W. CCL4 contributes to aging related angiogenic insufficiency through activating oxidative stress and endothelial inflammation. Angiogenesis. 2024;27(3):475-499.
45. Widjaja AA, Lim WW, Viswanathan S, et al. Inhibition of IL-11 signalling extends mammalian healthspan and lifespan. Nature. 2024;632(8023):157-165.
46. Mitchell CA, Verovskaya EV, Calero-Nieto FJ, et al. Stromal niche inflammation mediated by IL-1 signalling is a targetable driver of haematopoietic ageing. Nat Cell Biol. 2023;25(1):30-41.
47. Zhou Q, Yi G, Chang M, et al. Activation of sirtuin3 by honokiol ameliorates alveolar epithelial cell senescence in experimental silicosis via the cGAS-STING pathway. Redox Biol. 2024;74:103224.
48. He J, Shangguan X, Zhou W, et al. Glucose limitation activates AMPK coupled SENP1-Sirt3 signalling in mitochondria for T cell memory development. Nat Commun. 2021;12(1):4371.