

Aging-related decrease of histone methyltransferase SUV39H1 in adipose-derived stem cells enhanced SASP

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ABSTRACT

Aging-related diseases are closely associated with the state of inflammation, which is known as “inflammaging.” Senescent cells are metabolically active, as exemplified by the secretion of inflammatory cytokines, chemokines, and growth factors, which is termed the senescence-associated secretory phenotype (SASP). Epigenetic regulation, especially the structural regulation of chromatin, is closely linked to the regulation of SASP. In our previous study, the suppressor of variegation 3–9 homolog 1 (*SUV39H1*) was elucidated to interact with Lhx8 and determine the cell fate of mesenchyme stem cells. However, the function of *SUV39H1* during aging and the underlying mechanism of its epigenetic regulation remains controversial. Therefore, the C57BL/6 J CAG-Cre; *SUV39H1*^{fl/fl} knockout mice and irradiation-induced cellular senescence model were built in this study to deepen the understanding of epigenetic regulation by *SUV39H1* and its relation to SASP. In vivo and in vitro studies demonstrated that *SUV39H1* decreased with aging and served as an inhibitor of SASP, especially IL-6, MCP-1, and Vcam-1, by altering H3K9me3 enrichment in their promoter region. These results provide new insights into the epigenetic regulation of SASP.

1. Introduction

Aging is a complex multifactorial biological process with a gradual decline of normal physiological functions, thus causing the aging-related diseases (B. Huang et al., 2019), including Alzheimer's, Parkinson's, tumors, and immune diseases (Kusumoto et al., 2021). Epigenetic changes are considered one of the “hallmarks of aging” in different tissues and species (López-Otín et al., 2013, 2023), as a result of epigenetic programming, DNA methylation patterns (Hannum et al., 2013; Horvath, 2013), histone modification landscapes (Kawahara et al., 2009; Sen et al., 2015; Zhang et al., 2015), chromatin architecture organization (Chandra and Kirschner, 2016; Criscione et al., 2016), and non-coding RNAs influence senescence. The suppressor of variegation 3–9 homologue 1 (*SUV39H1*) is a specific methyltransferases of histone 3 lysine-9 (H3K9), which mediates H3K9 trimethylation to H3K9me3

(Qin et al., 2020). It has been reported that the trimethylation of lysine 9 and 27 in histone H3 (H3K9me3 and H3K27me3) usually suppresses gene expression via repressive chromatin structure (Abdelmohsen et al., 2013; Greer and Shi, 2012). Some studies have shown that *SUV39H1* insufficiency negatively impacted aging progression, whereas others have demonstrated the opposite. For instance, it has been discovered that hematopoietic stem cells (HSCs) isolated from the aging people over 70 showed an approximately 50% reduction in *SUV39H1* compared with those under 35 years (Djegloul et al., 2016). Additionally, a similar decrease in the *SUV39H1* and H3K9me3 expression is observed in HSCs harvested from the old mice compared to the young mice (Djegloul et al., 2016). However, it has also been found that depleting the methyltransferase *SUV39H1* improves the DNA repair and extends the lifespan in a progeria mouse model (Liu et al., 2013). Therefore, *SUV39H1* is believed to play a crucial role in the epigenetic regulation of aging, but

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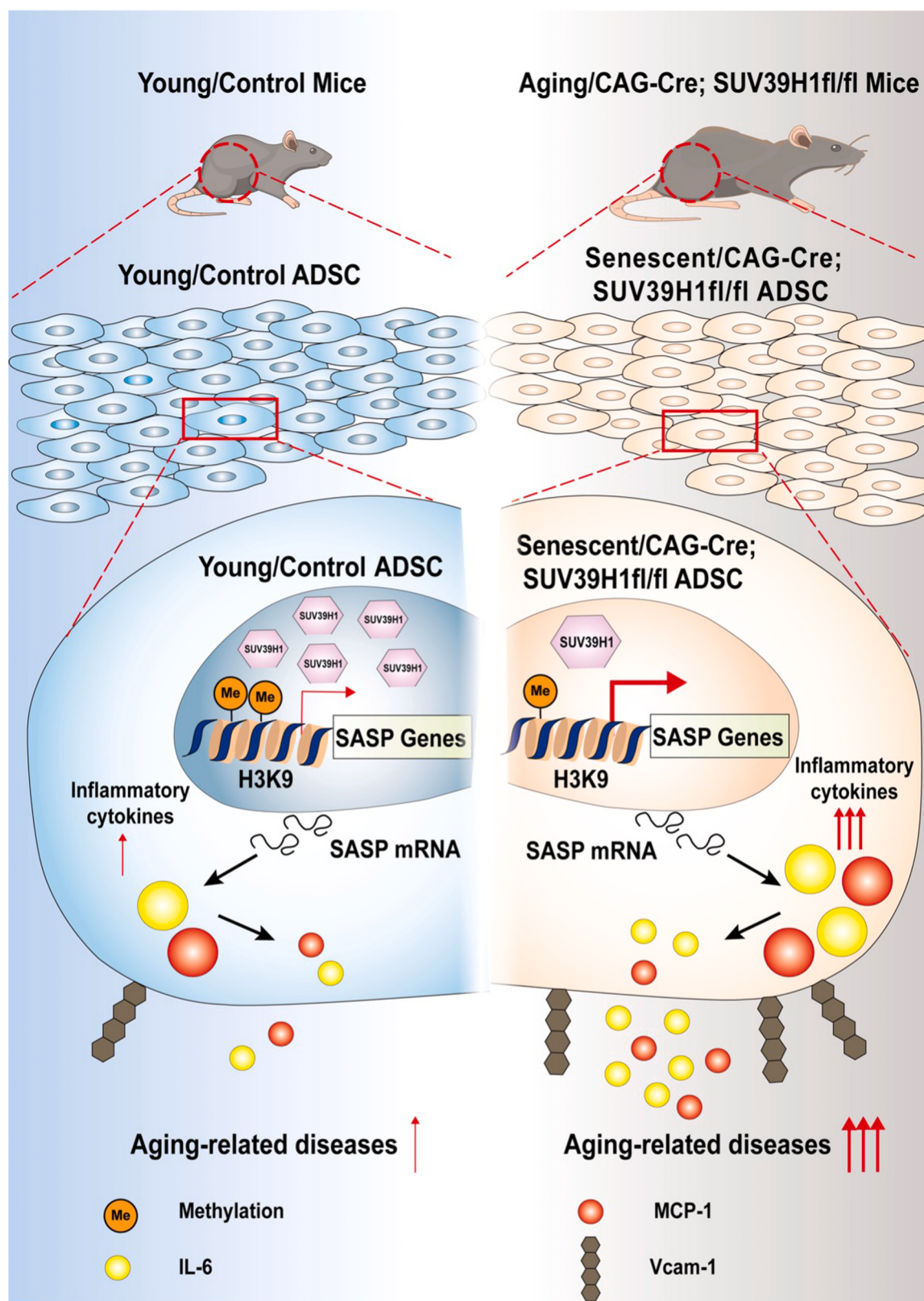


Fig. 1. Schematic illustration of the current study. *SUV39H1* decreased in ADSCs from the aging mice or the *CAG-Cre; SUV39H1^{fl/fl}* mice, compared to those from the young mice or the control mice. *SUV39H1* served as an inhibitor of SASP, especially IL-6, MCP-1, and Vcam-1, by altering the H3K9me3 enrichment in their promoter region. SASP was significantly enhanced in ADSCs from aging mice or *CAG-Cre; SUV39H1^{fl/fl}* mice, which might give rise to more aging-related diseases. Our study indicated that *SUV39H1* plays a pivotal role in the epigenetic regulation of ADSCs during aging.

its mechanism is still elusive. Therefore, more studies are needed on the function of *SUV39H1* to deepen our understanding of how aging occurs and how rejuvenation can be achieved.

Although cellular senescence plays beneficial roles in embryogenesis, wound healing, host immunity, and tumor suppression, it also brings the consequences such as the inflammatory cytokines accumulation (He and Sharpless, 2017; Jat, 2021) related to numerous diseases. Senescence-associated secretory phenotype (SASP) is the secretory feature during the cellular senescence progress characterized by the secreting of various inflammatory cytokines (e.g., IL-1 α , IL-1 β , IL-6 and IL-8), growth factors (e.g., HGF, TGF- β and GM-CSF), chemokines (e.g., CXCL-1/3 and CXCL-10) and matrix remodeling enzymes (e.g., metalloprotein) (Coppé et al., 2010). Deciphering the mechanism of SASP and reducing its impact on SASP may be an approach to helping healthy aging and tumor suppression (W. Huang et al., 2022). Epigenetic regulation, especially the structural regulation of chromatin, is considered as a key integration mechanism underlying the SASP. Recently, in TAD-related chromatin rearrangements, H3K27ac-enriched SAFs (senescence-activated enhancers) modify the adjacent SASP gene kinetics by remodeling epigenetic enhancer repertoires (Guan et al., 2020). Moreover, the methyltransferase polycomb repressive complex 2 (PRC2) exhibited significant enrichment of SASP, which was associated with the transcriptional repression via the tri-methylation of histone H3 on lysine 27 residue (H3K27me3) (Chu et al., 2022). DOT1L modulates SASP through the epigenetic regulation of IL1A in the oncogene-induced senescence (OIS) (Leon et al., 2021). Consequently, epigenetic regulation of SASP expression in senescent cells could effectively regulate the aging microenvironment. Previously, we have found that *SUV39H1* interacts with homeobox *Lhx8*, a stemness and cell fate regulator, balancing the differentiation and proliferation of dental mesenchyme via H3K9 methylation (Zhou et al., 2015), and have presented a scheme for the comprehensive control of mesenchymal stem cells (MSCs) biology via histone modifications (Ren et al., 2020).

We constructed the *SUV39H1* knockout mice in this study. We used an irradiation-induced cellular senescence model, focusing on the epigenetic regulation of cellular senescence and SASP of adipose-derived stem cells (ADSCs) via *SUV39H1*, aiming to provide insights into *SUV39H1* function in aging. Ultimately, we found that *SUV39H1* decreased in ADSCs from the aging mice or *CAG-Cre; SUV39H1^{fl/fl}* mice, compared to those from the young mice or the control mice. *SUV39H1* served as an inhibitor of SASP, especially IL-6, MCP-1 and Vcam-1, by altering of the H3K9me3 enrichment in their promoter region. SASP was significantly enhanced in ADSCs from the aging mice or the *CAG-Cre; SUV39H1^{fl/fl}* mice, which might give rise to more aging-related diseases. Our study indicated that *SUV39H1* plays a pivotal role in the epigenetic regulation of ADSCs during aging (Fig. 1) and (Figure. S1).

2. Materials and methods

2.1. Mice

The aging mice came from the Laboratory Animal Center of Sun Yat-Sen University. The *SUV39H1-flox* transgenic mice were first built with loxP sites flanking exon 1–2 of the *SUV39H1* gene via microinjection of Cas9/sgRNA and targeting vectors into mouse fertilized eggs. Mice were genotyped using the following primers: P1 5'loxP-F: TGTCTATGCCAGGAATTCGCCTAA, P1 5'loxP-R: AGCCTACACACCAGGAAGCATCAAC, P1 3'loxP-F: AGGTCTGGGCATAAGG-TTGCAGAAC, P1 3'loxP-R: GGGGTCA TTCTATCCCCAAAGGGGT using a thermomixer with the following cycling conditions: Initial denaturation 94 °C for 5 min, followed by 15 cycles of 98 °C for 30 s, 67 °C for 30 s (–0.7 °C/cycle), 68 °C 1 kb/min, followed by 25 cycles of 98 °C for 30 s, 57 °C for 30 s, 68 °C 1 kb/min, followed by a final hold of 68 °C for 10 min. Reaction were separated on 2% agarose gels yielding the following band sizes: P1 5'loxP+ : 527 bp, P1 5'loxP-: 441 bp, P1 3'loxP+ : 264 bp, and P1 3'loxP-: 179 bp. *CAG-Cre* transgenic mice were purchased from GemPharmatech (Nanjing, China) and expressed the

Cre enzyme in the entire body driven by a *CAG* promoter. *CAG-Cre* was genotyped using protocols as described by GemPharmatech using the following primers: P1 F: CCTGCTGTCCATTCCTTATTCCATA, P1 R: ATATCCCCTTGTTC-CTTCTGC using a thermomixer with the following cycling conditions: Initial denaturation 95 °C for 5 min, followed by 20 cycles of 98 °C for 30 s, 65 °C for 30 s (–0.5 °C/cycle), 72 °C for 45 s, followed by 20 cycles of 98 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, followed by a final hold of 72 °C for 5 min. Reactions were separated on 2% agarose gels yielding the following band sizes: P1 + : 337 bp, P1 - : / bp. To generate *CAG-Cre; SUV39H1^{fl/fl}* mice and littermates, we mated *SUV39H1-flox* and *CAG-Cre* mice to obtain fl heterozygous mice. Fl heterozygous mice were further mated to obtain homozygous mice, and 5'loxP-, 5'loxP-F/3'loxP-R+, *Cre*- mice were considered the experimental group and their littermates with a mix of *CAG-Cre; WT* and no *Cre SUV39H1^{fl/fl}* mice as the control group. Base on the PCR results to generate homozygous *CAG-Cre; SUV39H1^{fl/fl}* mice for further research. All mice were fed in specific pathogen-free conditions and maintained at a temperature- and humidity-controlled facility alternated day and night every 12 h. All mice used in this study had a C57BL/6 J genetic background. Three to five-week-old transgenic mice were used for experiments. All animal studies followed the guidelines defined by the Institutional Animal Care and Use Committees of Sun Yat-Sen University.

2.2. Chromatin immunoprecipitation

Chromatin immunoprecipitation was performed as per the instructions. We collected proliferating ADSCs and cross-linked protein DNA with formaldehyde before being subjected to ChIP. First, cells were lysed and their nuclei isolated. Then, the cells were then sheared with chromatin from 200 to 1000 bp by an ultrasonicator. Ten percent of the sonicated samples were used as inputs, and the rest were used for the next steps. The Magna ChIP A/G beads were prebonded with antibodies for 2 h at 4 °C and then incubated with samples overnight at 4 °C on a rotating platform. The equivalent amount of IgG (Millipore, USA) and anti-H3K9me3 (CST, USA) were used for immunoprecipitation. Next, the immunocomplexes were further removed from the supernatant on the magnetic separator. Finally, 45 μ L ChIP Elution Buffer (Millipore, USA) and 1 μ L proteinase K were added, and the immunocomplexes were put in a thermomixer at 65 °C for 2 h and then at 95 °C for 15 min for reversal of cross-linking. The ChIP-PCR primers are listed in Table supplement 1.

2.3. Hematoxylin & eosin (H & E) and immunohistochemistry (IHC) staining

After being fixed with 4% paraformaldehyde for 24 h, the samples were dehydrated by graded alcohol series and were embedded in paraffin to cut into 4- μ m sections for staining. The sections were stained with hematoxylin & eosin (H & E) according to the manufacturer's protocol (Servicebio, China). After dewaxing, rehydrating and antigen retrieval, the slices were incubated overnight with anti-*SUV39H1* for immunohistochemistry (1:200, Affinity, China) at 4 °C. After incubation with the appropriate secondary antibodies for 1 h at room temperature, diaminobenzidine (DAB) (Servicebio, China) was used for staining, followed by counterstaining with hematoxylin (Servicebio, China). The Aperio AT2 slide scanner (Leica Biosystems, Germany) was used for imaging the tissue slices.

2.4. Isolation, identification, and cultivation of ADSCs

Isolation, identification and cultivation of ADSCs were conducted using available reported protocols (Sugii et al., 2010, 2011). Specifically, the adipose tissues of the groin were isolated from the C57BL/6 mice (3–5 weeks of age) after cervical dislocation. After cutting the inguinal fat tissues with micro-scissors, the chips were suspended in 3 ml of DMEM-F12 containing 10% v/v FBS in the presence of 1 mg ml⁻¹ w/v

of collagenase I. The chips were digested for 1–2 h at 37 °C. After digesting, a pasteurized tube was used to blow the shredded tissue adequately. Adipose-derived cells were cultured with the complete DMEM-F12 medium (containing 10% FBS, 1% v/v GlutaMAX™ and 1% v/v penicillin/streptomycin) at 37 °C in a 5% CO₂ humidified cell culture incubator. The adherent cells were collected and passaged, with the cells from passages 2–3 (P2–3) used for the subsequent experiments. The medium was changed every 2–3 days during culturing. A phase-contrast microscope (Zeiss, Germany) was used to image ADSCs after reaching 90% confluency. The multiple differentiation ability of ADSCs was confirmed by the alizarin red, oil red and alcian blue staining after 21 days of culturing in different induction mediums. The surface markers of the ADSCs were analyzed by flow cytometry (Beckman CytoFLEX, USA) based on CD29, CD34, CD44, CD45 and CD90.2 (BioLegend, USA).

2.5. SA-β-gal staining

The SA-β-gal staining was performed using a kit according to the manufacturer's protocol (Biosharp, China). Specifically, after ADSCs reached 70–80% confluency, the cells were fixed with stationary liquid of the kit for 15 min at room temperature and then stained for 24 h using staining solution including 940 μL dye A, 10 μL dye B and 50 μL X-gal solution working fluid per unit volume at 37 °C. After staining, the cells were observed under a phase-contrast microscope (Zeiss, Germany) to identify positive cells and images.

2.6. Cell viability, proliferation and migration assessment

The CCK-8 cell proliferation assay was performed according to the manufacturer's instructions. Specifically, ADSCs were cultured in 96-well plates at a density of 1×10^3 cells/well. The medium was removed after one, three, five and seven days of incubation. Next, a fresh culture medium containing 10% v/v Cell Counting Kit-8 (CCK-8, Yeasen, China) was added to the 96-well plates, followed by incubation for 1 h at 37 °C in a 5% CO₂ humidified cell culture incubator in the absence of light. Subsequently, 100 μL supernatant medium was transferred to another 96 well plates. Absorbance at a wavelength of 450 nm was measured using a microplate spectrophotometer (Bio-Tek, UK).

The transwell assay was performed in the following steps. First, cell migration assays were performed in transwell chambers (8 μm pores, Corning, USA). In detail, 200 μL serum-free medium containing 1×10^5 cells was added to the upper chambers, and 800 μL medium containing 10% FBS was placed in the lower chambers. After 24 h of incubation, the invading cells were fixed with 4% paraformaldehyde for 30 min, stained with crystal violet for 1 h, and observed under a phase-contrast microscope (Zeiss, Germany).

2.7. Quantitative real-time PCR

The genes encoding suppressor of variegation 3–9 homolog 1 (*SUV39H1*), cyclin-dependent kinase inhibitor 1 A (*P21*), cyclin-dependent kinase inhibitor 2 A (*P16*), vascular cell adhesion molecule 1 (*Vcam-1*), intercellular adhesion molecule 1 (*Icam-1*), C-C motif chemokine ligand 2 (*MCP-1*), nitric oxide synthase 2 (*Nos2*), interleukin 6 (*IL-6*), interleukin 8 (*IL-8*) of ADSCs were confirmed by the quantitative real-time PCR analysis and β-actin was used as an internal control for PCR. The primer sequences are shown in Table supplement 2. Total RNA was isolated from ADSCs using an RNA-quick purification kit (ESscience Biotech, China) following the manufacturer's instructions. A total of 2 μg RNA was synthesized from cDNA using HiScript III-RT SuperMix (Vazyme, China). Quantitative real-time PCR was performed by using the AceQ qPCR SYBR Green Master Mix (Vazyme, China) under the following thermocycling conditions: 95 °C for 5 min, 40 cycles at 95 °C for 20 s, 60 °C for 30 s and 72 °C for 30 s. The relative expression of the genes was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.8. Western Blot

The total protein of ADSCs was extracted using RIPA lysis buffer supplemented with 1% protease inhibitors. The protein concentration was then determined using the BCA protein assay. Next, the proteins were resolved by SDS-PAGE (ACE, China) and transferred to a 0.22 μm polyvinylidene fluoride (PVDF) membrane (Millipore, USA) by using a wet transfer blotting system (Bio-Rad, USA). Next, the membranes were placed in a blocking solution (TBST containing 5% non-fat dry milk) for 1 h. Subsequently, the membranes were incubated at 4 °C overnight with appropriate primary antibodies: monoclonal rabbit anti-H3K9me3 (1:1000, CST, USA), polyclonal rabbit anti-SUV39H1 (1:1000, Affinity, China), polyclonal rabbit anti-Vcam-1 (1:1000, Zenbio, China), polyclonal rabbit anti-P16 (1:1000, Zenbio, China), polyclonal rabbit anti-P21 (1:1000, Zenbio, China) and polyclonal rabbit anti-β-Actin (1:1000, Servicebio, China). Subsequently, the membranes were washed and incubated with the relevant secondary antibodies (1:10000, CST, USA) for 1 h at room temperature. Finally, the band signals were detected using an ECL Western blotting substrate kit (Millipore, USA).

2.9. ELISA

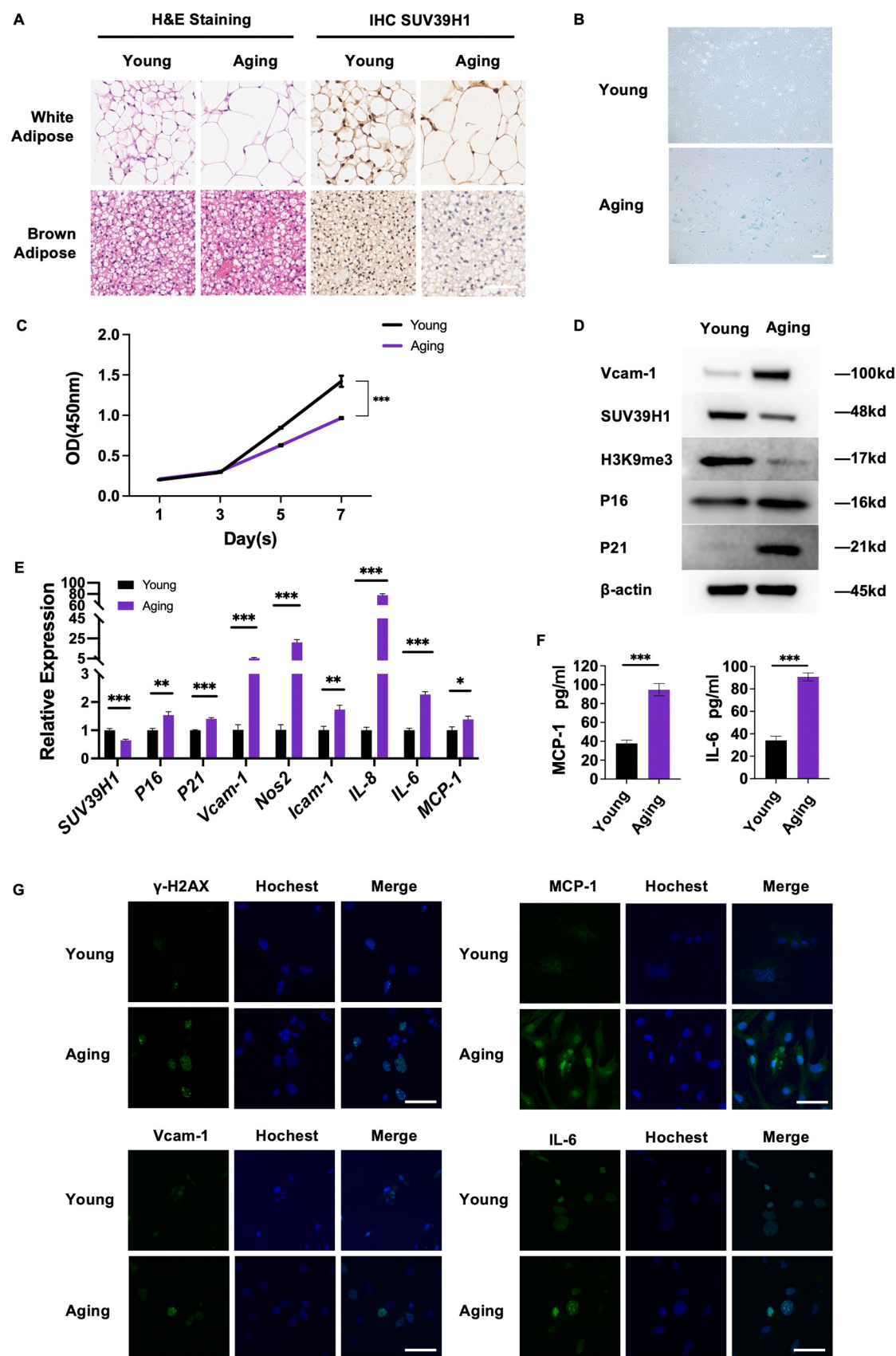
IL-6 and MCP-1 levels were measured using the mouse IL-6 ELISA kit and the mouse MCP-1 ELISA kit. (MEI MIAN, China), according to the manufacturer's instructions. Specifically, we added standard products and prepared the test cell supernatant to each well of the ELISA plate. Then, we put the plate in a humidified box at 37 °C for 1 h. Discard the solution in the ELISA plate and wash the plate 5 times. Then, we added 100 μL HRP-labeled secondary Ab to each well and incubated it to avoid the light in a humidified box under 37 °C for 15 min. At length, added 50 μL stop buffer was added to each well, and absorbance at a wavelength of 450 nm was measured using a microplate spectrophotometer (Bio-Tek, UK).

2.10. Immunofluorescence

The ADSCs were cultivated on cell crawling and then washed with PBS solution before being fixed with 4% paraformaldehyde for 15 min. The cells were then incubated with 0.3% PBST for 10 min and blocked in a 0.3% PBST solution containing 5% goat serum. Subsequently, cells were incubated with primary antibodies anti-IL-6 (1:200, Zenbio, China), anti-Vcam-1 (1:200, Zenbio, China), anti-MCP-1 (1:200, Zenbio, China) and anti-γ-H2AX (1:200, CST, USA) overnight at 4 °C. Finally, the secondary antibodies conjugated to FITC and Cy3 were incubated at room temperature for 1 h, and the nucleus was stained with Hoechst33422 (Beyotime, China). Cells were observed using a laser-scanning confocal microscope (Olympus, Japan).

2.11. Small interfering RNA and adenovirus transfection

The *SUV39H1* overexpression adenovirus and the control adenovirus were commercially synthesized and identified by Hanbio Biotechnology (Shanghai, China). The virus titer was 400 MOI. For the siRNA knock-down of *SUV39H1* in ADSCs, the siRNA of *SUV39H1* and the negative control were designed and synthesized by Generay Biotechnology (Shanghai, China). The sequences of the siNC used in our experiments were sense, 5'-UUCUCCGAACGUGUCACGUTT-3', and antisense, 5'-ACGUGACACGUUCGGAGAATT-3'. The sequences of the *SUV39H1* siRNA used in our experiments were sense, 5'-GCCUUUGUACUC-AGGAAAGAA-3', and antisense, 5'-UUCUUUCUGAGUACAAAGGC-3'. Opti-MEM medium (Gibco, USA) and Rfect reagent (BAIDAI, China) were used to transfect siRNA.



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Fig. 2. *SUV39H1* decreased with aging and upregulation of SASP. (A) Representative H&E and immunohistochemical staining images of *SUV39H1* in the brown and white fat tissues of young mice and aging mice. Scale bar = 50 μ m. (B) SA- β -gal staining in ADSCs from young mice and aging mice. Scale bar = 50 μ m. (C) CCK-8 assay of ADSCs from young mice and aging mice after one, three, five, and seven days of seeding. (D) Immunoblotted image for *Vcam-1*, *SUV39H1*, *H3K9me3*, *P16*, *P21* and β -actin of ADSCs from young and aging mice. (E) qPCR analysis of senescence associated SASP, and *SUV39H1* genes expression of ADSCs among groups. (F) MCP-1 and IL-6 concentration analysis by ELISA. (G) Representative immunofluorescent images for γ -H2AX, MCP-1, *Vcam-1*, and IL-6 among groups. γ -H2AX/MCP-1/*Vcam-1*/IL-6 are marked by green fluorescence, and the cell nuclei were dyed blue by Hoechst. Scale bar = 50 μ m. Statistical analyses in (C, E) and (F) were performed using Student's two-tailed t-test. (n = 3 from biological replicates, statistical analyses were performed using Student's two-tailed t-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the control group).

2.12. Statistical analysis

Data were expressed as mean $\pm$ standard deviation (SD) of at least three independent experiments. The comparisons were performed by using the GraphPad Prism software. Statistical analyses were performed using Student's two-tailed t-test. The differences between groups or treatments were reported as ns/Ns (non-significant) or significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control).

3. Results

3.1. *SUV39H1* decreased in multiple organs with aging, especially in brown fat tissue

In aging mice, *SUV39H1* expression decreased in tissues from multiple organs, including liver, kidney, and heart tissue, accompanied by aging characterizations of cell atrophy and fatty degeneration, thickening of the basement membrane, and dilation of the renal lumen (Figure. S2A). Compared to young mice, the maximum reduction in *SUV39H1* was observed in the adipose tissue of aging mice by immunohistochemical staining, especially in brown fat (Fig. 2A). The volume of fat cells, as well as the number of fat drops, increased with aging, both in the white and brown fats (Fig. 2A).

ADSCs were further isolated from mice to demonstrate the relationship between *SUV39H1* and cellular senescence. The SA- β -gal activity was detected to be stronger in the aging group than in the young group (Fig. 2B), and the statistical analysis of the number of SA- β -gal positive cells was also presented (Figure. S2B). By CCK-8 assay, the proliferation rate of ADSCs from the aging mice was found to be significantly lower than those from the young mice (Fig. 2C). The cell cycle-related factors *P16* and *P21* also accumulated more in the ADSCs from aging mice than those from young mice at both protein and mRNA levels (Figs. 2D and 2E). However, *SUV39H1* decreased at both the protein and mRNA levels in ADSCs with aging. Accordingly, decreased *H3K9* trimethylation to *H3K9me3* was also detected (Figs. 2D and 2E). SASP-related inflammatory cytokines, including *Vcam-1*, *MCP-1*, *IL-6*, *Nos2*, *Icam-1* and *IL-8* were found to increase in the ADSCs from aging mice at the transcriptional level, among which *Vcam-1*, *MCP-1* and *IL-6* also increased at the post-transcriptional level detected by both Western blot assay and immunofluorescence staining (Fig. 2D–2G). The DNA damage marker, γ -H2AX, was also detected using immunofluorescence staining, showing higher γ -H2AX fluorescence intensity in the nucleus of ADSCs from aging mice than those from the young mice (Fig. 2G), and the relevant statistical analysis results were shown in Figure S2D.

These results indicated that *SUV39H1* expression decreased in association with adipose senescence, especially in the brown fat tissue, both *in vivo* and *in vitro*. Moreover, the decline of *H3K9me3* coincided with the change in *SUV39H1* and the SASP-related inflammatory cytokines in the ADSCs from aging mice, implying a possible regulatory relationship in-between.

3.2. *SUV39H1* decreased with cellular senescence and upregulation of SASP-related inflammatory cytokines

An irradiation-induced cellular senescence model was constructed using ADSCs from young C57/BL6 mice to investigate the role of *SUV39H1* during cellular senescence. Additionally, the detection of SA-

β -gal activity was used to verify senescence (Fig. 3A), and the statistical analysis of the number of SA- β -gal positive cells was also presented (Figure. S3B).

The proliferation rate of ADSCs from the senescent group was significantly lower than those from the non-senescent group by the CCK-8 assay (Fig. 3B). *P16* and *P21* also accumulated more in ADSCs from the senescent group than those from the non-senescent group at both protein and mRNA levels (Figs. 3C and 3D). *SUV39H1* decreased at both protein and mRNA levels during ADSCs cellular senescence and *H3K9* trimethylation to *H3K9me3* (Figs. 3C and 3D). SASP-related inflammatory cytokines, including *Vcam-1*, *MCP-1*, *IL-6*, *Nos2*, *Icam-1* and *IL-8* were found to increase in the ADSCs from the senescent group at the transcriptional level, among which *Vcam-1*, *MCP-1* and *IL-6* also increased at the post-transcriptional level detected by both Western blot assay and immunofluorescence staining (Fig. 3C–3F). Immunofluorescence staining showed higher γ -H2AX fluorescence intensity in the nucleus of ADSCs from the senescent group than those from the non-senescent group (Fig. 3E), and the results of the statistical analysis were presented in Figure S3C–3D.

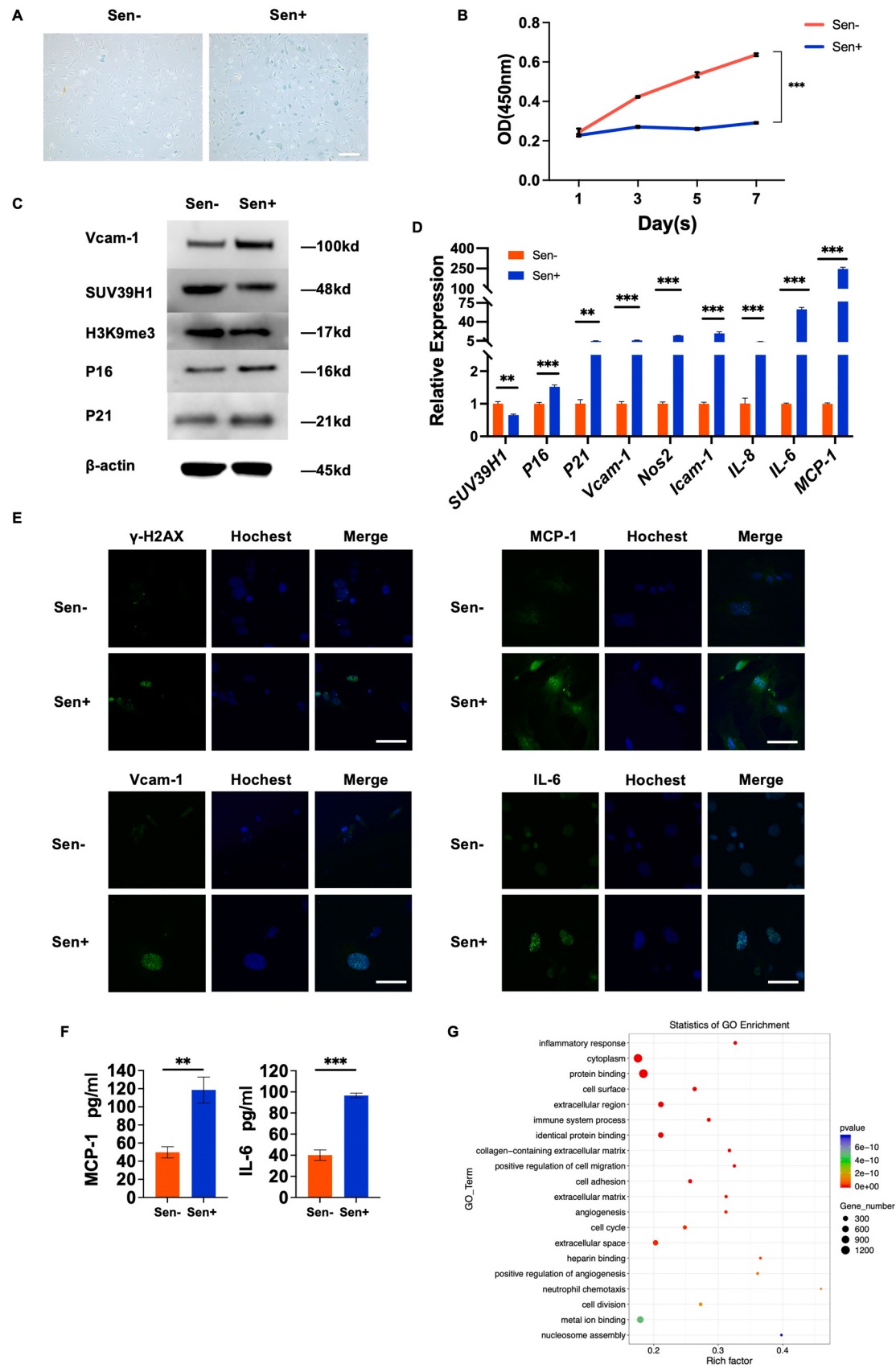
In the transwell assay (Figure. S3A), cell migration ability significantly decreased with senescence, especially in the Sen+ group compared to the Sen- group, likely due to altered cell morphology, impaired adhesion properties, and dysregulated senescence-associated secretory factors. Accordingly, the Gene ontology (GO) enrichment analysis results showed significant diversity of inflammatory responses in the senescent group compared with the non-senescent group, as well as protein binding, positive regulation of cell migration and cell cycle (Fig. 3G).

These results indicated that, compared with the non-senescent group, *SUV39H1* expression decreased in the senescent cells with lower *H3K9me3* and higher SASP-related inflammatory cytokines, similar to the changes of ADSCs from aging mice than young ones.

3.3. Loss of *SUV39H1* in CAG-Cre; *SUV39H1*^{fl/fl} transgenic mice and *SUV39H1* knockdown elevated SASP-related inflammatory cytokines

CAG-Cre; *SUV39H1*^{fl/fl} transgenic mice were constructed by the breeding protocol and their littermates with a mix of CAG-Cre; WT and no Cre *SUV39H1*^{fl/fl} mice were used as control (Fig. 4A). CAG-Cre; *SUV39H1*^{fl/fl} transgenic mice were selected, and ADSCs were extracted and cultured for follow-up experiments. Based on the results of the CCK-8 assay, the proliferation rate of ADSCs from CAG-Cre; *SUV39H1*^{fl/fl} transgenic mice was lower than the control (Fig. 4B), in the transwell assay (Figure. S4A), cell migration ability significantly decreased with *SUV39H1* insufficient, similar to the changes of ADSCs from senescent group than non-senescent ones. In ADSCs from the CAG-Cre; *SUV39H1*^{fl/fl} group, the expression of *SUV39H1* decreased with reduced *H3K9me3* and enhanced *Vcam-1* (Fig. 4C). *MCP-1* and *IL-6* concentrations from the culture media of ADSCs from CAG-Cre; *SUV39H1*^{fl/fl} mice were found to be elevated by ELISA (Fig. 4D), and the results of the statistical analysis were presented in Figure S4B. qPCR analysis confirmed *SUV39H1* knockout efficiency and showed that inflammatory cytokines related to SASP were increased, including *Vcam-1*, *Nos2*, *Icam-1*, *IL-8*, *IL-6*, and *MCP-1* (Fig. 4E).

Next, we knocked down *SUV39H1* in ADSCs from wild-type mice by siRNA *in vitro*, which showed similar results to CAG-Cre; *SUV39H1*^{fl/fl} transgenic mice. At the protein level, *SUV39H1* was knocked down with



(caption on next page)

Fig. 3. *SUV39H1* decreased in senescent ADSCs associated with increased SASP-related inflammatory cytokines. (A) SA- β -gal staining in ADSCs from non-senescent group and senescent group. Scale bar = 50 μ m. (B) CCK-8 assay of ADSCs from non-senescent group and senescent group after one, three, five and seven days of seeding. (C) Immunoblotted image for Vcam-1, *SUV39H1*, H3K9me3, P16, P21 and β -actin of ADSCs from the senescent group and non-senescent groups. (D) qPCR analysis of senescence-associated, SASP and *SUV39H1* genes expression of ADSCs among groups. (E) MCP-1 and IL-6 concentration analysis by ELISA. (F) Representative immunofluorescent images for γ -H2AX, MCP-1, Vcam-1, and IL-6 among groups. γ -H2AX/MCP-1/Vcam-1/IL-6 are marked by green fluorescence, and the cell nuclei were dyed blue by Hoechst. Scale bar = 50 μ m. (G) GO enrichment analysis of the non-senescent group and the senescent group. Statistical analyses in (B, D) and (F) were performed using Student's two-tailed t-test. (n = 3 from biological replicates, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the control group).

decreased H3K9me3, but Vcam-1 increased (Fig. 4F) and MCP-1 and IL-6 (Fig. 4G), and the results of the statistical analysis were presented in Figure S4C. At the mRNA level, SASP-related inflammatory cytokines *Vcam-1*, *MCP-1*, *IL-6*, *Nos2*, *Icam-1* and *IL-8* were upregulated by *SUV39H1* knockdown (Fig. 4H).

These results were consistent with the findings of aging and senescence-related changes in ADSCs, which indicated that the upregulation of SASP-related inflammatory cytokines was probably due to insufficient *SUV39H1*.

3.4. *SUV39H1* regulates SASP by altering H3K9me3 enrichment in promoters

The overexpression of *SUV39H1* was induced in senescent ADSCs through the use of adenovirus. This manipulation resulted in a significant increase in the expression levels of both *SUV39H1* and global H3K9me3 (Fig. 5A). Subsequently, a downregulation of MCP-1 and IL-6 was observed consequent to the heightened expression of *SUV39H1* (Fig. 5B). Moreover, a decrease in the expression levels of SASP-related genes, including *Vcam-1*, *Nos2*, *Icam-1*, *IL-8*, *IL-6*, and *MCP-1*, was detected upon the overexpression of *SUV39H1* (Fig. 5C).

In-depth epigenetic analysis utilizing ChIP-qPCR further unveiled that the promoters of *Vcam-1*, *IL-6*, and *MCP-1* exhibited higher levels of H3K9me3 enrichment in non-senescent ADSCs when compared to their senescent counterparts (Fig. 5D). This observation suggests that *SUV39H1* overexpression may play a pivotal role in modulating the epigenetic landscape of SASP genes, particularly by regulating the levels of H3K9me3 at specific gene promoters.

Taken together, although after the overexpression of *SUV39H1*, the expression levels of most SASP-related genes and proteins could not fully return to the non-senescent state, these findings provide compelling evidence for the involvement of *SUV39H1* in the regulation of cellular senescence and SASP-associated genes, shedding light on its potential as a promising target for therapeutic interventions aimed at ameliorating senescence-related inflammatory responses in ADSCs.

4. Discussion

In 2013, the hallmarks of aging were first published (López-Otín et al., 2013), which sparked significant interest in aging research. A decade later, an updated version of the hallmarks was published (López-Otín et al., 2023), adding three new marks, including loss of proteostasis, chronic inflammation, and dysbiosis. Among these hallmarks, inflammation in aging, termed "inflammaging," has gained attention and consensus. Senescent cells secrete various factors collectively known as senescence-associated secretions (SASP) (Coppé et al., 2008), which can influence the senescence program, including cell cycle arrest (Acosta et al., 2008; Kuilman et al., 2008). Non-coding RNAs, particularly microRNAs, also play a role in SASP regulation (Gorgoulis et al., 2019; Panda et al., 2017).

Many scientists worldwide search for ways to reverse cellular senescence and aging (Kennedy et al., 1997; Ocampo et al., 2016; Sen et al., 2016). Dafni et al. (Chondronasiou et al., 2022) reported that a single period of transient *OKSM* expression was sufficient to reverse DNA methylation changes occurring upon aging in the pancreas, liver, spleen, and blood and furthermore reverse senescence. Lu et al. (Lu

et al., 2020) used the three genes *Oct4*, *Sox2* and *Klf4* transmitted to the mouse retina (*OSK* therapy) through an adeno-related virus (AAV), inducing ganglion cell reprogramming, restoring young epigenetic information, and reversing vision loss caused by glaucoma and aging.

Rather than genomic instability, epigenetic alterations, as a potential cause of aging, caused intensive attention much later until the 1990 s (Kennedy et al., n.d.). We all know that each cell basically has the same DNA for organisms, but the phenotypes of different cells are significantly different. This is the switch between epigenetic molecules and genetic activity, which helps determine the types and functions of different cells. Furthermore, epigenetic factors can determine which genes of specific cells are active or inactive at a specific time (Marchini et al., 2016). Various epigenetic changes contribute to aging, including changes in DNA methylation patterns, abnormal posttranslational modifications of histones, and aberrant chromatin remodeling. Many enzymatic systems generate and maintain epigenetic patterns, including DNA methyltransferases, histone acetylases, deacetylases, methylases, and demethylases, and protein complexes implicated in chromatin remodeling or ncRNA synthesis and maturation (López-Otín et al., 2023). Age-related epigenetic changes (Pal and Tyler, 2016; Sen et al., 2015), including changes in DNA methylation patterns, H3K4me3, H3K9me3, and H3K27me3, are also associated with multiple disease-associated expressions observed in biological cells.

In this study, we investigated the role of *SUV39H1* in regulating SASP in adipose mesenchymal stem cells (ADSCs) during cellular senescence and aging. Our findings revealed that *SUV39H1* downregulation was associated with the induction of SASP-related genes, while its overexpression ameliorated SASP through H3K9me3 enrichment on gene promoters. These results suggest that *SUV39H1* plays a critical role in modulating the inflammatory state during cellular senescence.

The identification of *SUV39H1* as a regulator of SASP adds to the growing understanding of the molecular mechanisms underlying cellular aging. By elucidating how *SUV39H1* influences SASP expression, we have provided insights into potential therapeutic targets for mitigating age-related inflammation and its associated pathologies.

Moreover, our study highlights the importance of considering epigenetic changes in the context of aging. While genomic instability has long been associated with aging, we found that epigenetic alterations, such as changes in histone methylation patterns, also significantly contribute to the senescent phenotype. This emphasizes the need to explore the epigenome's role in aging and provides a new perspective for aging-related research.

Although we have demonstrated the impact of *SUV39H1* on SASP regulation through ChIP-qPCR analysis, it is essential to acknowledge the limitations of this approach. ChIP-seq analysis, which provides a more comprehensive and genome-wide view of *SUV39H1* binding sites and H3K9me3 enrichment patterns, would yield a deeper understanding of the regulatory network involved. Future studies employing ChIP-seq could offer more insights into the broader epigenetic landscape governing cellular senescence and aging.

In conclusion, our study contributes to the current knowledge of aging and inflammation by identifying *SUV39H1* as a regulator in regulating SASP. The findings pave the way for further investigations into epigenetic mechanisms involved in aging and provide potential avenues for developing interventions to combat aging-related

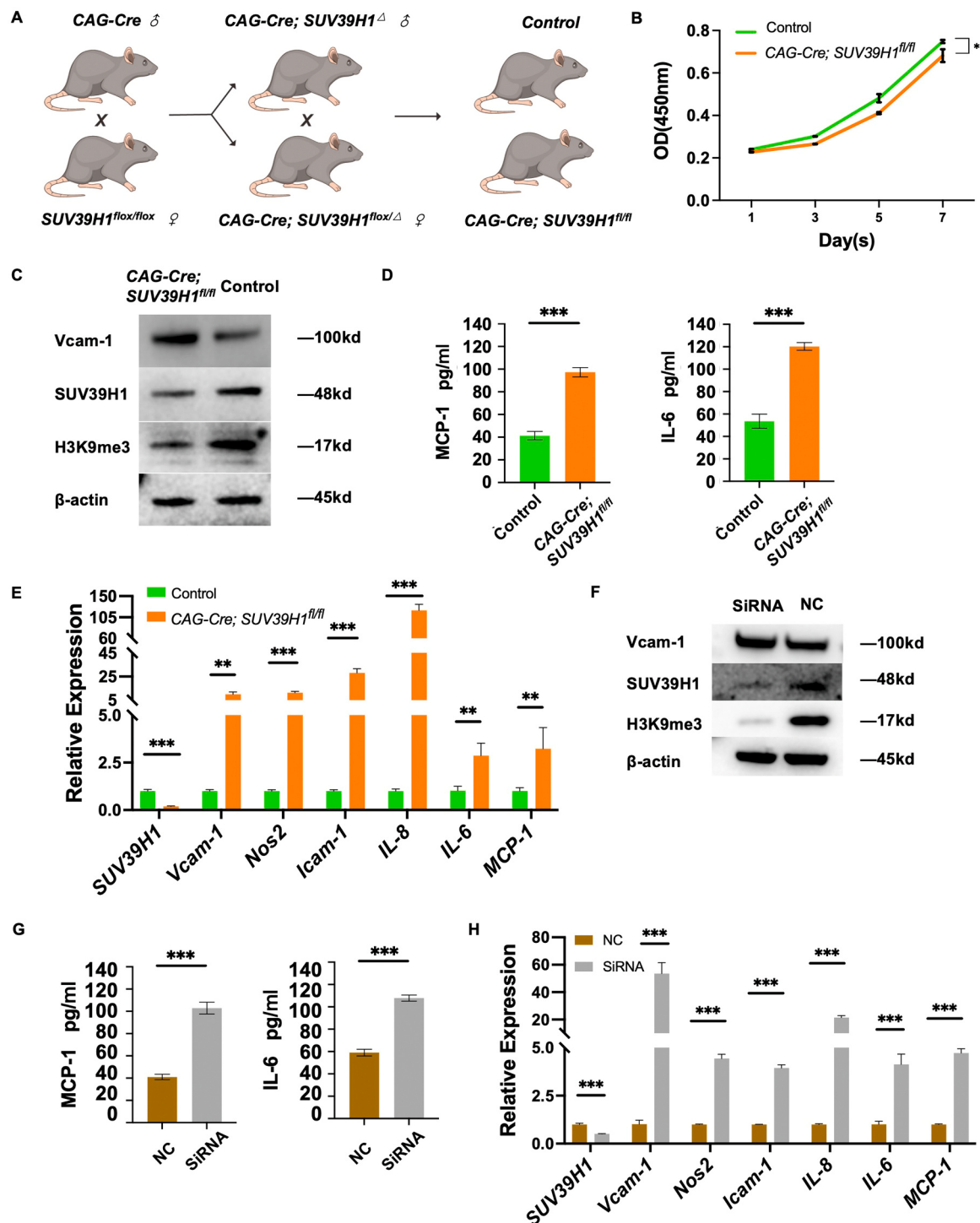


Fig. 4. *SUV39H1* deletion and inhibition in ADSCs led to overactivated SASP-related inflammatory cytokines. (A) Schematic illustration of *CAG-Cre; SUV39H1^{fl/fl}* mice construction. (B) CCK-8 assay of ADSCs from the *CAG-Cre; SUV39H1^{fl/fl}* and control groups after one, three, five and seven days of seeding. (C) Immunoblotted image for Vcam-1, SUV39H1, H3K9me3, and β -actin of ADSCs from the *CAG-Cre; SUV39H1^{fl/fl}* group and control group. (D) MCP-1 and IL-6 concentration analysis by ELISA. (E) qPCR analysis of senescence associated SASP and *SUV39H1* genes expression of ADSCs among groups. (F) Immunoblotted image for Vcam-1, SUV39H1, H3K9me3, and β -actin of ADSCs from the siRNA group and NC groups. (G) MCP-1 and IL-6 concentration analysis by ELISA. (H) qPCR analysis of senescence associated SASP and *SUV39H1* genes expression of ADSCs among groups. Statistical analyses in (D, E) and (G, H) were performed using Student's two-tailed t-test, $n = 3$ from biological replicates. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the control group).

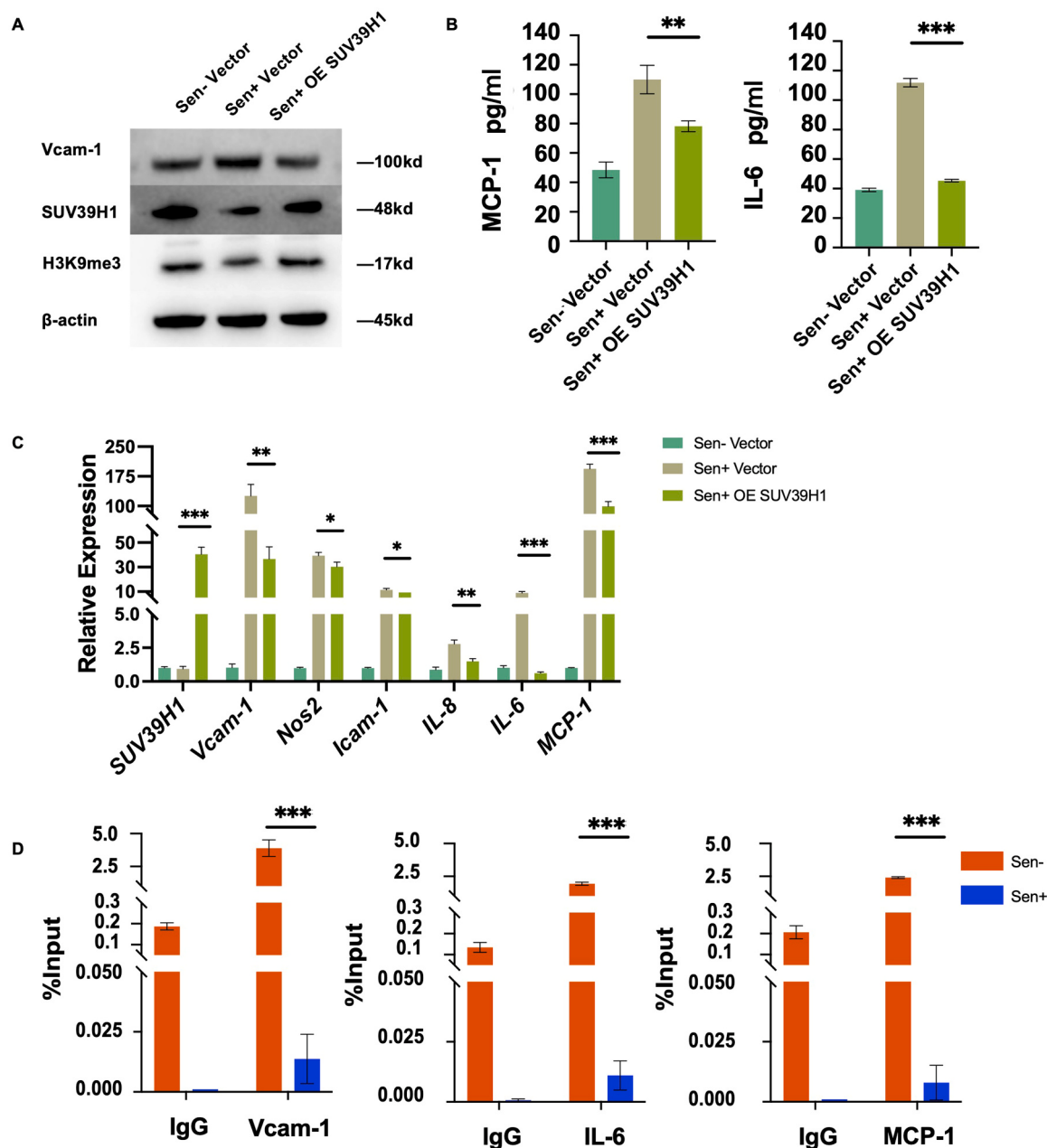


Fig. 5. SASP alleviated *SUV39H1* overexpression by altering H3K9me3 enrichment on promoters of *Vcam-1*, *IL-6*, and *MCP-1*. (A) Western blotting analysis of *Vcam-1*, *SUV39H1*, H3K9me3, and β -actin of ADSCs from the non-senescent and senescent vector groups and the senescent *SUV39H1* overexpression group. (B) MCP-1 and IL-6 concentration analysis by ELISA. (C) The mRNA levels of SASP and *SUV39H1* in ADSCs by qPCR analysis. (D) The enrichment of H3K9me3 in the *Vcam-1*, *IL-6* and *MCP-1* promoters was analyzed by ChIP. Statistical analyses in (B, C) and (D) were performed using Student's two-tailed t-test, $n = 3$ from biological replicates. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the control group.).

inflammatory conditions. However, additional research is warranted to explore the full scope of SUV39H1's regulatory effects and its potential as a therapeutic target for aging-related pathologies.

CRedit authorship contribution statement

Ruoyu Li: Conceptualization, Investigation, Visualization, Methodology, Writing – original draft. **Yungshan Teng:** Conceptualization, Data curation; **Yuqing Guo:** Investigation, Writing – original draft; **Jianhan Ren:** Formal analysis, Validation, Writing – review & editing. **Runze Li:** Investigation, Methodology; **Haotian Luo:** Data curation, Formal analysis, Validation; **Danying Chen:** Formal analysis, Validation; **Zhicai Feng:** Funding acquisition. **Zheng Fu:** Data curation, Methodology. **Xuenong Zou:** Funding acquisition, Validation; **Weicai Wang:** Formal analysis, Funding acquisition. **Chen Zhou:** Resources, Funding acquisition, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability

No data was used for the research described in the article.

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Ethics

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Sun Yat-sen University (Reference: SYSU-IACUC-2023-000584).

References

- Abdelmohsen, K., Panda, A., Kang, M.-J., Xu, J., Selimyan, R., Yoon, J.-H., Martindale, J. L., De, S., Wood, W.H., Becker, K.G., Gorospe, M., 2013. Senescence-associated lncRNAs: senescence-associated long noncoding RNAs. *Aging Cell* 12 (5), 890–900. <https://doi.org/10.1111/ace.12115>.
- Acosta, J.C., O'Loughlin, A., Banito, A., Guijarro, M.V., Augert, A., Raguz, S., Fumagalli, M., Da Costa, M., Brown, C., Popov, N., Takatsu, Y., Melamed, J., d'Adda di Fagnola, F., Bernard, D., Hernandez, E., Gil, J., 2008. Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell* 133 (6), 1006–1018. <https://doi.org/10.1016/j.cell.2008.03.038>.
- Chandra, T., Kirschner, K., 2016. Chromosome organisation during ageing and senescence. *Curr. Opin. Cell Biol.* 40, 161–167. <https://doi.org/10.1016/j.cob.2016.03.020>.
- Chondronasiou, D., Gill, D., Mosteiro, L., Urdinguio, R.G., Berenguer-Llugo, A., Aguilera, M., Durand, S., Aprahamian, F., Nirmalathasan, N., Abad, M., Martin-Herranz, D.E., Stephan-Otto Attolini, C., Prats, N., Kroemer, G., Fraga, M.F., Reik, W., Serrano, M., 2022. Multi-omic rejuvenation of naturally aged tissues by a single cycle of transient reprogramming. *Aging Cell* 21 (3), e13578. <https://doi.org/10.1111/ace.13578>.
- Chu, L., Qu, Y., An, Y., Hou, L., Li, J., Li, W., Fan, G., Song, B.-L., Li, E., Zhang, L., Qi, W., 2022. Induction of senescence-associated secretory phenotype underlies the therapeutic efficacy of PR2 inhibition in cancer. *Cell Death Dis.* 13 (2), 155. <https://doi.org/10.1038/s41419-022-04601-6>.
- Coppé, J.-P., Patil, C.K., Rodier, F., Sun, Y., Muñoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.-Y., Campisi, J., 2008. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* 6 (12), e301. <https://doi.org/10.1371/journal.pbio.0060301>.
- Coppé, J.-P., Desprez, P.-Y., Krtolica, A., Campisi, J., 2010. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu. Rev. Pathol.: Mech. Dis.* 5 (1), 99–118. <https://doi.org/10.1146/annurev-pathol-121808-102144>.
- Crisicione, S.W., De Cecco, M., Siranosian, B., Zhang, Y., Kreiling, J.A., Sedivy, J.M., Neretti, N., 2016. Reorganization of chromosome architecture in replicative cellular senescence. *Sci. Adv.* 2 (2), e1500882. <https://doi.org/10.1126/sciadv.1500882>.
- Djehghoul, D., Kuranda, K., Kuzniak, I., Barbieri, D., Naguibneva, I., Choisy, C., Bories, J.-C., Dosquet, C., Pla, M., Vanneaux, V., Socié, G., Porteu, F., Garrick, D., Goodhardt, M., 2016. Age-associated decrease of the histone methyltransferase SUV39H1 in HSC perturbs heterochromatin and B lymphoid differentiation. *Stem Cell Rep.* 6 (6), 970–984. <https://doi.org/10.1016/j.stemcr.2016.05.007>.
- Gorgoulis, V., Adams, P.D., Alimonti, A., Bennett, D.C., Bischof, O., Bishop, C., Campisi, J., Collado, M., Evangelou, K., Ferbeyre, G., Gil, J., Hara, E., Krizhanovsky, V., Jurk, D., Maier, A.B., Narita, M., Niedernhofer, L., Passos, J.F., Robbins, P.D., Demaria, M., 2019. Cellular senescence: defining a path forward. *Cell* 179 (4), 813–827. <https://doi.org/10.1016/j.cell.2019.10.005>.
- Greer, E.L., Shi, Y., 2012. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat. Rev. Genet.* 13 (5), 343–357. <https://doi.org/10.1038/nrg3173>.
- Guan, Y., Zhang, C., Lyu, G., Huang, X., Zhang, X., Zhuang, T., Jia, L., Zhang, L., Zhang, C., Li, C., Tao, W., 2020. Senescence-activated enhancer landscape orchestrates the senescence-associated secretory phenotype in murine fibroblasts. *Nucleic Acids Res.* 48 (19), 10909–10923. <https://doi.org/10.1093/nar/gkaa858>.
- Hannun, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., Klotzle, B., Bibikova, M., Fan, J.-B., Gao, Y., Deconde, R., Chen, M., Rajapakse, I., Friend, S., Ideker, T., Zhang, K., 2013. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol. Cell* 49 (2), 359–367. <https://doi.org/10.1016/j.molcel.2012.10.016>.
- He, S., Sharpless, N.E., 2017. Senescence in health and disease. *Cell* 169 (6), 1000–1011. <https://doi.org/10.1016/j.cell.2017.05.015>.
- Horvath, S., 2013. DNA methylation age of human tissues and cell types. *Genome Biol.* 14 (10), R115. <https://doi.org/10.1186/gb-2013-14-10-r115>.
- Huang, B., Wang, B., Yuk-Wai Lee, W., Pong U, K., Leung, K.T., Li, X., Liu, Z., Chen, R., Lin, J., Cheng, Tsang, L.L., Liu, B., Ruan, Y., Chun, Chan, H.C., Li, G., Jiang, X., 2019. KDM3A and KDM4C regulate mesenchymal stromal cell senescence and bone aging via condensin-mediated heterochromatin reorganization. *iScience* 21, 375–390. <https://doi.org/10.1016/j.isci.2019.10.041>.
- Huang, W., Hickson, L.J., Eirin, A., Kirkland, J.L., Lerman, L.O., 2022. Cellular senescence: the good, the bad and the unknown. *Nat. Rev. Nephrol.* <https://doi.org/10.1038/s41581-022-00601-z>.
- Jat, P., 2021. Mechanisms of cellular senescence: cell cycle arrest and senescence associated secretory phenotype. *Front. Cell Dev. Biol.* 9, 24.
- Kawahara, T., Michishita, E., Adler, A.S., Damian, M., Berber, E., Lin, M., McCord, R. A., Ongaiqui, K.C.L., Boxer, L.D., Chang, H.Y., Chua, K.F., 2009. SIRT6 links histone H3 lysine 9 deacetylation to NF- κ B-dependent gene expression and organismal life span. *Cell* 136 (1), 62–74. <https://doi.org/10.1016/j.cell.2008.10.052>.
- Kennedy, B.K., Gotta, M., Sinclair, D.A., Mills, K., McNabb, D.S., Murthy, M., Pak, S.M., Laroche, T., Gasser, S.M., Guarente, L., 1997. Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S. cerevisiae*. *Cell* 89 (3), 381–391. [https://doi.org/10.1016/S0092-8674\(00\)80219-6](https://doi.org/10.1016/S0092-8674(00)80219-6).
- Kuilman, T., Michaloglou, C., Vredeveld, L.C.W., Douma, S., van Doorn, R., Desmet, C.J., Aarden, L.A., Mooi, W.J., Peeper, D.S., 2008. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* 133 (6), 1019–1031. <https://doi.org/10.1016/j.cell.2008.03.039>.
- Kusumoto, D., Seki, T., Sawada, H., Kunitomi, A., Katsuki, T., Kimura, M., Ito, S., Komuro, J., Hashimoto, H., Fukuda, K., Yuasa, S., 2021. Anti-senescence drug screening by deep learning-based morphology senescence scoring. *Nat. Commun.* 12 (1), 257. <https://doi.org/10.1038/s41467-020-20213-0>.
- Leon, K.E., Buj, R., Lesko, E., Dahl, E.S., Chen, C.-W., Tangudu, N.K., Imamura-Kawawara, Y., Kossenkova, A.V., Hobbs, R.P., Aird, K.M., 2021. DOT1L modulates the senescence-associated secretory phenotype through epigenetic regulation of IL1A. *J. Cell Biol.* 220 (8), e202008101. <https://doi.org/10.1083/jcb.202008101>.
- Liu, B., Wang, Z., Zhang, L., Ghosh, S., Zheng, H., Zhou, Z., 2013. Depleting the methyltransferase Suv39h1 improves DNA repair and extends lifespan in a progeria mouse model. *Nat. Commun.* 4 (1), 1868. <https://doi.org/10.1038/ncomms2885>.
- López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. *Cell* 153 (6), 1194–1217. <https://doi.org/10.1016/j.cell.2013.05.039>.
- López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2023. Hallmarks of aging: an expanding universe. *S0092867422013770 Cell*. <https://doi.org/10.1016/j.cell.2022.11.001>.
- Lu, Y., Brommer, B., Tian, X., Krishnan, A., Meer, M., Wang, C., Vera, D.L., Zeng, Q., Yu, D., Bonkowski, M.S., Yang, J.-H., Zhou, S., Hoffmann, E.M., Karg, M.M., Schultz, M.B., Kane, A.E., Davidsohn, N., Korobkina, E., Chwalek, K., Sinclair, D.A., 2020. Reprogramming to recover youthful epigenetic information and restore vision. *Nature* 588 (7836), 124–129. <https://doi.org/10.1038/s41586-020-2975-4>.
- Marchini, A., Ogata, T., Rappold, G.A., 2016. A track record on SHOX: from basic research to complex models and therapy. *Endocr. Rev.* 37 (4), 417–448. <https://doi.org/10.1210/er.2016-1036>.
- Ocampo, A., Reddy, P., Martinez-Redondo, P., Platero-Luengo, A., Hatanaka, F., Hishida, T., Li, M., Lam, D., Kurita, M., Beyret, E., Araoka, T., Vazquez-Ferrer, E., Donoso, D., Roman, J.L., Xu, J., Rodriguez Esteban, C., Nuñez, G., Nuñez Delicado, E., Campistol, J.M., Izpisua Belmonte, J.C., 2016. In Vivo Amelioration of age-associated hallmarks by partial reprogramming. *e12 Cell* 167 (7), 1719–1733. <https://doi.org/10.1016/j.cell.2016.11.052>.

- Pal, S., Tyler, J.K., 2016. Epigenetics and aging. *Sci. Adv.* 2 (7), e1600584 <https://doi.org/10.1126/sciadv.1600584>.
- Panda, A.C., Abdelmohsen, K., Gorospe, M., 2017. SASP regulation by noncoding RNA. *Mech. Ageing Dev.* 168, 37–43. <https://doi.org/10.1016/j.mad.2017.05.004>.
- Qin, B., Yu, J., Nowsheen, S., Zhao, F., Wang, L., Lou, Z., 2020. STK38 promotes ATM activation by acting as a reader of histone H4 ufmylation. *Sci. Adv.*, 6(23), eaax8214. <https://doi.org/10.1126/sciadv.aax8214>.
- Ren, J., Huang, D., Li, R., Wang, W., Zhou, C., 2020. Control of mesenchymal stem cell biology by histone modifications. *Cell Biosci.* 10 (1), 11. <https://doi.org/10.1186/s13578-020-0378-8>.
- Sen, P., Dang, W., Donahue, G., Dai, J., Dorsey, J., Cao, X., Liu, W., Cao, K., Perry, R., Lee, J.Y., Wasko, B.M., Carr, D.T., He, C., Robison, B., Wagner, J., Gregory, B.D., Kaerberlein, M., Kennedy, B.K., Boeke, J.D., Berger, S.L., 2015. H3K36 methylation promotes longevity by enhancing transcriptional fidelity. *Genes Dev.* 29 (13), 1362–1376. <https://doi.org/10.1101/gad.263707.115>.
- Sen, P., Shah, P.P., Nativio, R., Berger, S.L., 2016. Epigenetic mechanisms of longevity and aging. *Cell* 166 (4), 822–839. <https://doi.org/10.1016/j.cell.2016.07.050>.
- Sugii, S., Kida, Y., Kawamura, T., Suzuki, J., Vassena, R., Yin, Y.-Q., Lutz, M.K., Berggren, W.T., Izpisua Belmonte, J.C., Evans, R.M., 2010. Human and mouse adipose-derived cells support feeder-independent induction of pluripotent stem cells. *Proc. Natl. Acad. Sci.* 107 (8), 3558–3563. <https://doi.org/10.1073/pnas.0910172106>.
- Sugii, S., Kida, Y., Berggren, W.T., Evans, R.M., 2011. Feeder-dependent and feeder-independent iPS cell derivation from human and mouse adipose stem cells. *Nat. Protoc.* 6 (3), 346–358. <https://doi.org/10.1038/nprot.2010.199>.
- Zhang, W., Li, J., Suzuki, K., Qu, J., Wang, P., Zhou, J., Liu, X., Ren, R., Xu, X., Ocampo, A., Yuan, T., Yang, J., Li, Y., Shi, L., Guan, D., Pan, H., Duan, S., Ding, Z., Li, M., Belmonte, J.C.I., 2015. A Werner syndrome stem cell model unveils heterochromatin alterations as a driver of human aging. *Science* 348 (6239), 1160–1163. <https://doi.org/10.1126/science.aaa1356>.
- Zhou, C., Yang, G., Chen, M., Wang, C., He, L., Xiang, L., Chen, D., Ling, J., Mao, J.J., 2015. Lhx8 mediated Wnt and TGF β pathways in tooth development and regeneration. *Biomaterials* 63, 35–46. <https://doi.org/10.1016/j.biomaterials.2015.06.004>.