

RESEARCH PAPER

Dynamic tRNA modification landscapes reveal METTL1 in fibroblasts as a central regulator of aging

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RNA modifications have emerged as crucial regulators of cellular processes linked to aging. However, the dynamic changes in global RNA modification patterns during aging and their functional implications remain underexplored. Here, we comprehensively deciphered the RNA modification landscapes of total RNA, poly(A)-enriched mRNA, and tRNA-enriched fragments across six tissues in both young and aged mice, as well as in multiple cellular senescence models. Aged tissues exhibit overall decline of RNA modification abundance in tRNA, rather than in total RNA or mRNA, when compared to young tissues. Correspondingly, the expression of tRNA methyltransferases, METTL1 and TRMT1, also declines with aging and senescence. Modulation of these methyltransferases affects cellular senescence phenotypes; specifically, Mettl1 depletion results in accelerated aging in mice. Mechanistically, fibroblasts are identified as the primary cell type responsible for the aging traits induced by Mettl1 deficiency in mice. Reduced m⁷G modification in fibroblasts leads to a decrease in the abundance of m⁷G-modified tRNAs, which in turn impairs translation efficiency and protein synthesis, and contributes to an accumulation of tRNA-derived small RNAs (tsRNAs). Remarkably, these alterations affected the translation of genes involved in senescence and aging pathways. Our study provides a comprehensive landscape of tRNA modifications during aging and demonstrates that decreased activity of METTL1 in fibroblasts drives aging. Thus, modulation of tRNA modifications may be a promising strategy for improving healthy aging and alleviating age-associated disorders.

RNA modifications | aging | METTL1 | tRNA | fibroblasts | senescence | m⁷G

INTRODUCTION

Aging is a complex and multifactorial biological process characterized by progressive metabolic dysregulation, tissue dysfunction, and increased susceptibility to age-related diseases across multiple organ systems (Ajoalabady et al., 2024; López-Otín et al., 2023; Pan et al., 2025; Xu et al., 2025). Unraveling its underlying mechanisms is crucial for developing strategies to extend healthspan and delay age-associated decline (López-Otín et al., 2023). Among the key regulatory mechanisms, epigenetic modifications, such as DNA methylation, histone modifications, chromatin remodeling, and RNA modifications, dynamically respond to environmental stimuli (e.g., stress, cellular damage, and metabolic alterations). These modifications serve as a bridge between environmental stimuli and genetic information, under-

scoring the plasticity of the genome during aging, and providing crucial insights into the mechanisms of aging as well as potential therapeutic strategies (Akbarian et al., 2013; Wang et al., 2022a). While DNA methylation has received substantial attention and forms the basis of widely used aging clocks (Duan et al., 2022; Hannum et al., 2013; Horvath, 2013; Horvath and Raj, 2018; Pierce, 2022), RNA modifications have recently emerged as an important but underexplored layer of epigenetic regulation (Birkedal et al., 2015; Kellner et al., 2010; Linder et al., 2015; Malbec et al., 2019).

To date, over 170 RNA modifications have been identified across various RNA species, including messenger RNA (mRNA), structural non-coding RNA (such as transfer RNA (tRNA), ribosomal RNA (rRNA), and small nucleolar RNA (snoRNA)), as well as regulatory non-coding RNA (ncRNA), primarily long

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non-coding RNA (lncRNA) and small RNA (Boccalletto et al., 2022). These RNA modifications play a critical role in modulating key aspects of RNA metabolism, including stability, splicing, translation, and degradation (Boccalletto et al., 2022), and are essential for the regulation of gene expression and the maintenance of cellular homeostasis (Roundtree et al., 2017). Among these modifications, *N*⁶-methyladenosine (m⁶A) is the most prevalent internal modification in mRNA, catalyzed by the METTL3-METTL14 complex, and its function is tightly regulated by “reader” and “eraser” proteins. The role of m⁶A in aging has been extensively studied, with changes in its levels influencing protein synthesis, telomere stability, and the senescence-associated secretory phenotype (SASP), all of which impact aging processes (Chen et al., 2022; Huang et al., 2023; Li et al., 2022; Liu et al., 2021; Wang et al., 2022b; Wu et al., 2023; Wu et al., 2020). However, the roles of other RNA modifications, particularly those in tRNAs, remain less well defined.

tRNA modifications, among the most abundant RNA modifications (Zhang et al., 2022a), are particularly significant in the anticodon loop, where they are critical for translation efficiency and accuracy. Modifications within the tRNA body also influence its structure and stability (Wang et al., 2023b). Despite their potential relevance, the dynamics of tRNA modifications during aging and their regulatory mechanisms remain largely unexplored. Moreover, the contribution of tRNA-modifying enzymes to aging processes is not fully understood.

Recently, Fu et al. (2024) demonstrated that perturbation of METTL1-mediated tRNA m⁷G methylation induced cellular senescence and accelerated organismal aging. In that study, METTL1 and its cofactor WDR4 were downregulated in several senescence models and aged mouse tissues, and loss of METTL1/WDR4 reduced tRNA m⁷G, promoted rapid tRNA degradation, and activated integrated and ribotoxic stress responses, ultimately shortening lifespan in an inducible whole-body Mettl1 knockout model. These findings established METTL1-dependent tRNA m⁷G as a causal regulator of aging. However, Fu et al. (2024) primarily focused on tRNA m⁷G in a limited set of tissues and did not address how different classes of RNA are remodeled across organs, nor which specific cell types translate METTL1 deficiency into systemic aging phenotypes. The multi-tissue modification landscapes of diverse RNA species during aging, and the cellular specificity of the METTL1-m⁷G aging axis, therefore, remain incompletely defined.

In this study, we substantially extend these observations by constructing a comprehensive analysis of RNA modification profiles. Across this dataset, we profiled total RNA, poly(A)-enriched mRNA, and tRNA-enriched fragments in six tissues from both young and aged mice, as well as in multiple cellular senescence models. We identified a conserved signature of age-associated tRNA (rather than total RNA or mRNA) hypomodification, encompassing not only m⁷G but also other regulatory marks such as m²₂G, m⁵C, and m¹A, several of which exhibit sex-specific dynamics during early adulthood. Correspondingly, the expression of tRNA methyltransferases, METTL1 and TRMT1, was also reduced with aging and cellular senescence. Altering these methyltransferases significantly impacts cellular senescence phenotypes. Notably, fibroblast-specific deletion of METTL1 was sufficient to induce systemic premature aging, indicating that fibroblasts represent a critical cell type mediating the organismal effects of RNA modification loss. Mechanistically, METTL1 deficiency led to enhanced production of tRNA-derived

small RNAs (tsRNAs) and disrupted decoding of specific codons, thereby linking altered tRNA modification states to translational reprogramming in aging cells.

Together, these findings highlight tRNA modifications as central modulators of aging biology. By combining multi-tissue epitranscriptomic profiling with functional interrogation of key enzymes and cell types, this study bridges descriptive and mechanistic understanding of the RNA epitranscriptome in aging and establishes a foundation for future therapeutic exploration.

RESULTS

RNA modification landscapes among mouse tissues during aging

To unveil the RNA modification landscapes among mouse tissues during aging, we employed our previously established high-performance liquid chromatography-mass spectrometry (LC-MS/MS) methodology to precisely quantify RNA modification abundances across various RNA classes, including total RNA (with rRNA comprising over 80%), mRNA (derived from poly(A)-enriched RNA fractions), and tRNA (with ~80-nt enriched fragments). The quantification of RNA modification abundances was conducted across six tissues (brain, heart, kidney, liver, lung, and skin) (Figure 1A). Using our RNA modification detection platform, 26 nucleobase standards enabled the quantification of 22, 22, and 14 RNA modifications in tRNA-enriched fragments, total RNA, and mRNA, respectively (Figure 1B; Figures S1A and S2A). Consistent with previous studies (Guo et al., 2023), we observed that tRNA displays the most enrichment of RNA modifications compared to mRNA and total RNA. Notably, tRNA, rather than mRNA or total RNA, showed an overall reduction in abundances of various types of RNA modifications across young and aged tissues from both sexes (Figure 1B; Figures S1A and S2A), except for the 2'-O-methylation (2'-O-Me) modifications, including Im, Am, Cm, and Gm (but not Um), which show increased levels in aged tissues (Figure 1B). Moreover, the uniform manifold approximation and projection (UMAP) analysis revealed that RNA modification signatures of tRNA, rather than total RNA and mRNA, are capable of accurately distinguishing young and aged groups across various mouse tissues (Figure 1C; Figures S1B and S2B), suggesting a pivotal role of tRNA modification signatures during aging. Interestingly, the expression correlation between different types of RNA modifications in tRNA and total are enhanced across mouse tissues during aging, such as m⁷G vs. m²G, m⁵C vs. m³C on tRNA, and m⁷G vs. Cm, m³U vs. ac⁴C in total RNA, while mRNA showed fewer associations (Figure 1D–G; Figures S1C–F and S2C–F). To further identify the prevalent patterns of downregulated RNA modifications in aged tissues, we observed a consistent decrease of twelve specific RNA modifications on tRNA across 6 aged tissues (Figure 1H and I; Figures S1G and S2G). Interestingly, these modifications exhibit sexual dimorphisms in young mice, which may have implications for our understanding of sex-specific aging. In 2-month-old mice, RNA modifications show clear sexual differences, with female mice having higher Um levels in the brain and higher i⁶A and m⁵Um levels in the skin compared to males. Conversely, in the liver, male mice have higher Um levels than females. These differences were no longer detectable in 23-month-old mice, suggesting that

Figure 1 tRNA modification landscapes in young and aged mouse tissues. A, Total RNA, tRNA (enriched by PAGE), and mRNA (enriched by poly(A) selection) were extracted from six mouse tissues (brain, heart, kidney, liver, lung, and skin) of both young and aged male and female mice. RNA modifications were quantified using LC-MS/MS. B, Heatmaps showing the relative expression levels of each tRNA modification across six mouse tissues in young and aged mice stratified by sex. Young: 2-month-old mice; aged: 23-month-old mice. $n=3$ mice per sex per age group (3 young males, 3 young females, 3 aged males, and 3 aged females). C, Principal component analysis (PCA) of tRNA modifications in six tissues (brain, heart, kidney, liver, lung, and skin) from young and aged mice. Each dot represents one tissue sample. Young and aged: $n=6$ mice per group (3 male and 3 female mice). D and E, Linear correlation analysis of tRNA modifications in six tissues (brain, heart, kidney, liver, lung, and skin) from young and aged mice (6 mice per group, including 3 male and 3 female mice). F and G, Analysis of the linear correlation between m^2G and m^7G modifications in tRNA for young (left) and aged (right) mice (6 mice per group across 6 tissues, including 3 male and 3 female mice), as well as between m^3C and m^5C modifications in the same age groups. H, Upset plot showing the overlap of downregulated tRNA modifications in aged mice compared to young mice across different tissues (6 mice per group across 6 tissues, including 3 male and 3 female mice). Numbers indicated the count of marks shared by the indicated tissue combinations. I, Box plots analyzing the downregulation of specific tRNA methylation marks (m^7G , m^2G , m^2_2G , m^1G , m^5C , m^3C , Ψ , m^5U , m^5Um , m^1I , m^1A , and i^6A) in aged compared to young mice across various tissues (6 mice per group across 6 tissues, including 3 male and 3 female mice). Data were presented as mean $\pm$ SEM. Correlation analysis was performed using Pearson correlation coefficients (D–G). *P* values were determined by a two-tailed unpaired Student's *t*-test (I).

sex-specific variations in RNA modifications are most prominent during early stages of the aging process rather than persisting into advanced age (Figure 1B).

Taken together, our data suggest that aging induces dynamic changes in RNA modifications specifically in tRNA across various tissues, highlighting their potential role in the aging process.

Uniform tRNA modification signature during cellular senescence

To determine whether dynamic alterations in tRNA modifications during cellular senescence align with the patterns observed across various tissues during aging, we conducted a comparative analysis of RNA modification profiles in multiple models of cellular senescence, including replicative senescence (RS), oncogene-induced senescence (OIS), and stress-induced premature senescence (SIPS), utilizing LC-MS/MS (Figure 2A). Our results showed that the tRNA modification patterns were strikingly similar to those observed in various tissues. Specifically, during senescence, the levels of 2'-O-Me modifications—such as Am, Gm, and Cm—were progressively elevated, while other modifications exhibited a reverse trend, decreasing with aging (Figure 2B). Notably, a marked abundance reduction of 7 types of tRNA modifications was consistently observed in OIS and SIPS human dermal fibroblasts (HDFs) and IMR90 cells (Figure 2C), while 6 types of them are consistent with aged tissues, including m^7G , m^2_2G , m^2G , m^1G , m^1A , and m^5C (Figure 2D).

The tRNA modifiers METTL1 and TRMT1 are actively involved in cellular senescence

RNA modifications are enzymatically catalyzed by distinct families of enzymes, each responsible for the addition of specific chemical modifications to RNA molecules. Building upon our preliminary findings, we next investigated the alterations in the expression levels of RNA modification enzymes during the aging process. Immunoblotting analysis demonstrated a significant downregulation of the m^7G methyltransferase, METTL1, and the m^2_2G methyltransferase, TRMT1, in aged tissues, including skin, heart, liver, and lung (Figure 2E). Consistently, we observed a marked decrease in the protein levels of METTL1 and TRMT1 during RS, OIS, SIPS, and oxidative stress-induced senescence (OSIS) (Figure 2F–I; Figure S3A–D). To further elucidate the role of METTL1, WDR4 (serving as a scaffold for METTL1 and the tRNA T-arm), and TRMT1 in the aging process, we silenced these enzymes via lentiviral shRNA-mediated gene knockdown experiments in HDFs (Li et al., 2023). In these cells, RNA dot blot analysis showed that *METTL1* or *WDR4* knockdown reduced

tRNA m^7G , while TRMT1 knockdown reduced tRNA m^2_2G (Figure S3E–G). Our results showed that silencing any of the three enzymes, METTL1, WDR4, or TRMT1, induced cellular senescence, evidenced by increased P16 protein levels, enhanced senescence-associated β -galactosidase (SA- β -gal) activities, and reduction in cell proliferation (Figure 2J–L; Figure S3H–K), all classical markers of senescence (Gorgoulis et al., 2019). In contrast, simultaneous overexpression of METTL1 and WDR4 (but not their individual overexpression) rejuvenated these cells, increased tRNA m^7G and reduced P16, SA- β -gal expression, and promoted cell proliferation (Figure 2M; Figure S3L–N). TRMT1 overexpression in aged HDFs likewise restored tRNA m^2_2G and alleviated senescence markers (Figure 2N; Figure S3O–Q).

Finally, we tested whether these effects depend on methyltransferase activity. In aged HDFs, overexpression of wild-type METTL1 or TRMT1 increased their cognate tRNA m^7G or m^2_2G marks and attenuated senescence, whereas catalytic-site mutants failed to restore m^7G/m^2_2G and showed little effect on SA- β -gal or P16/P21 (Figure S4). Collectively, these findings indicate that METTL1/WDR4- and TRMT1-mediated tRNA modifications are downregulated during aging, and that their anti-senescence effects in fibroblasts are largely dependent on their enzymatic activity.

Given this functional link between METTL1 levels, tRNA m^7G level, and fibroblast senescence, we next considered upstream regulators that might be associated with the age-related reduction in METTL1. METTL1 expression is sensitive to the chromatin state of its promoter, including CpG methylation and recruitment of p300-containing activator complexes (Zhang et al., 2023; Zhao et al., 2024). We first interrogated promoter DNA methylation using publicly available Illumina methylation datasets from human whole blood spanning a wide age range and from fibroblasts undergoing RS (Hannum et al., 2013; Sakaki et al., 2017). In both contexts, CpGs across the *METTL1* promoter were largely hypomethylated and exhibited minimal variation with chronological age or population doublings (Figure S5A–D). We next assessed chromatin coactivator occupancy. In published p300 ChIP-seq profiles comparing proliferating and senescent fibroblasts (Sen et al., 2019), promoter-proximal p300 signal at the *METTL1* locus was reduced in the senescent state (Figure S5E).

METTL1 deficiency induces premature aging in mice

The results presented above prompted us to investigate the causal connections between METTL1 and aging *in vivo*. To experimentally mimic the decline in METTL1 expression and m^7G modification during physiological aging, we knocked out *Mettl1*

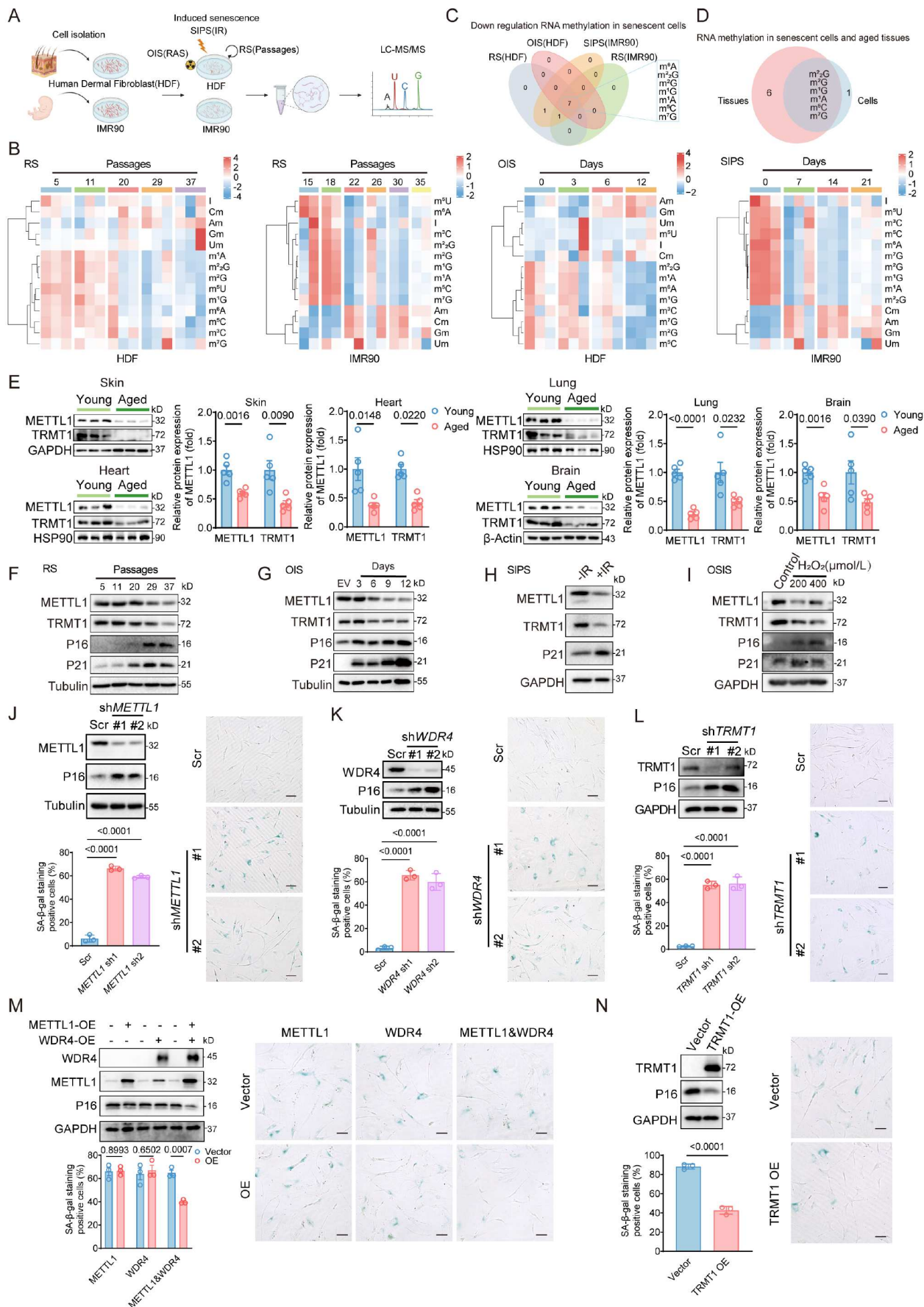


Figure 2 Comprehensive analysis of RNA modifications and protein expression in HDF and IMR90 under various senescence conditions. A, Schematic representation of the experimental workflow for analyzing RNA modifications in HDFs and IMR90 cells under replicative and induced senescence conditions. B, Heatmaps of tRNA modifications in RS HDF, OIS HDF, RS IMR90, and SIPS IMR90 reflecting changes in replicative aging and RAS/IR-induced senescence ($n=2-3$ independent replicates). C, Intersection analysis of RNA methylation marks downregulated in four cell types: RS(HDF), OIS(HDF), RS(IMR90), and SIPS(IMR90). D, Circular Venn diagram showing the overlap of downregulated RNA methylation marks in both senescent cells and aged tissues. E, Representative images of Western blot analysis for METTL1 and TRMT1 in skin, heart, brain, and lung from young and aged mice. For each group, $n=5$ individual mice were analyzed. Quantification of relative METTL1 protein levels was summarized on the right. F–I, Representative images of Western blot analysis for METTL1, TRMT1, and senescence-associated proteins in RS (F), OIS (G), SIPS (H), and OSIS (I) HDF. The quantitative analysis results were shown in Figure S3A–D ($n=3$). J–N, Representative images of Western blot analysis and SA- β -gal staining in HDFs to assess cellular senescence in response to knockdown or overexpression of METTL1 (J and M), WDR4 (K and M), and TRMT1 (L and N). Scale bar, 50 μ m. Left bottom, quantification of SA- β -gal positive cells ($n=3$). The quantitative analysis results of the Western blot were shown in Figure S3E–G, M, and P ($n=3$). All results are representative of at least three independent experiments. Data were presented as mean $\pm$ SEM. *P* values were determined by two-tailed unpaired Student's *t*-test (E, J, K, L, and N) and one-way ANOVA with Bonferroni's post hoc test (M).

in young adult mice. We employed a conditional knockout strategy using *CAG-CreER*, *Mettl1*^{fllox/fllox} (*Mettl1*^{fl/fl}) mice, hereafter referred to as *Mettl1* iKO mice. Tamoxifen was administered to induce METTL1 deletion at 8 weeks of age, with analyses conducted 11 weeks post-treatment (Figure 3A). Immunoblotting analysis revealed a substantial decrease in METTL1 protein levels across various tissues, including skin, heart, liver, lung, kidney, and brain, in *Mettl1* iKO mice compared to control adult mice (Figure S6A and B). In addition, m⁷G modification was significantly downregulated in tRNA-enriched fractions and total RNA from various tissues of *Mettl1* iKO mice, relative to controls, although no such alteration was observed in mRNA levels (Figure 3B). Compared to control mice, *Mettl1* iKO mice exhibited a markedly reduced lifespan compared to control adult mice, with all subjects perishing within 200 d (Figure 3C). *Mettl1* deficiency also resulted in pronounced premature aging phenotypes, including reduced body size, lower body weight, sparse fur, and cataracts, compared to control mice (Figure 3D; Figure S6C–G). Additionally, *Mettl1* iKO mice exhibited various age-related phenotypes typically observed in aged mice, such as motor impairments (evidenced by a marked decrease in the latency to fall during the rotarod test and diminished grip strength), skin atrophy (characterized by subcutaneous fat depletion and reduced hair follicle density), as well as fibrosis in the lungs and kidneys, and hepatic and renal steatosis (Figure 3E–Q). Moreover, *Mettl1* iKO mice displayed significantly elevated levels of P53 and reduced LaminB1 in multiple tissues (Figure S7), both of which are well-established biomarkers of aging.

Collectively, these results, along with the observed decline in METTL1 expression and global tRNA m⁷G modification during aging, suggest that METTL1 plays a crucial role in preventing premature aging.

Loss of METTL1 in fibroblasts drives premature systemic aging

We next investigated the cell types primarily responsible for the age-associated decline in *Mettl1* and m⁷G modification. To achieve this, we analyzed the *Tabula Muris Senis* dataset (Almanzar et al., 2020), which provides single-cell transcriptomic information from various organs, enabling us to track the dynamics of *Mettl1* expression across the murine lifetime. Our results demonstrated that *Mettl1* levels decrease with age, though this reduction is not universally observed across all cell types. Specifically, the decline is most pronounced in fibroblasts (Figure 4A), while *Mettl1* levels in other cell types, such as endothelial cells, lymphocytes, and smooth muscle cells, remained relatively stable with age (Figure S8A). The above results prompted further investigation into the causal connections

between METTL1 in fibroblasts and the aging process. To address this, we generated a fibroblast-specific *Mettl1* knockout mouse model (*Mettl1* Fb iKO mice) by combining *Mettl1*^{fl/fl} with the fibroblast-specific driver *Collagen1a2* (*Col1a2*)-*CreER* (Figure 4B) (Sladitschek-Martens et al., 2022). To assess the efficiency and cell-type specificity of *Mettl1* deletion in Fb iKO mice, we performed METTL1 co-staining with vimentin (VIM) in lung and kidney and with CD45 in spleen. METTL1 intensity was strongly reduced in VIM-positive (VIM⁺) cells in the lung and kidney of Fb iKO mice compared with controls (Figure S8B and C), but was unchanged in VIM-negative (VIM[−]) cells and CD45-positive (CD45⁺) hematopoietic cells (Figure S8D). Notably, *Mettl1* Fb iKO mice also exhibited a significantly shortened lifespan, with all individuals succumbing by 450 d (Figure 4C and D). Additionally, similar to *Mettl1* global knockout mice, *Mettl1* Fb iKO mice displayed multiple age-related pathologies, including skin atrophy (manifested by diminished subcutaneous fat and reduced hair follicle density), pulmonary and renal fibrosis, and hepatic and renal steatosis (Figure 4E–O). Immunoblotting and immunostaining analysis revealed elevated P16 levels and a loss of LaminB1 expression in various tissues (Figure S8E–L). To determine whether this senescence occurred exclusively in *Mettl1*-deficient fibroblasts, we performed VIM and LaminB1 co-staining in the lung and kidney. We then quantified LaminB1 fluorescence in VIM⁺ and VIM[−] cells and found reduced LaminB1 expression in both populations (Figure S8M and N). These observations suggested that loss of *Mettl1* in fibroblasts not only drove fibroblast senescence but may also trigger senescence in neighboring non-fibroblast populations via paracrine signaling. This propagation of senescence across cell types was associated with tissue-level dysfunction and may represent an important route by which fibroblast *Mettl1* decline promotes organismal aging. The premature aging phenotypes observed in *Mettl1* Fb iKO mice closely resemble those noted in *Mettl1* iKO mice, underscoring the critical role of fibroblast-specific m⁷G modification in the aging process of an entire organism.

METTL1 deficiency drives senescence via a decrease in the abundance of m⁷G tRNAs during cellular translation

As the final part of this study, we focused on elucidating the mechanism by which *Mettl1* deficiency in fibroblasts drives aging. To this end, we isolated mouse embryonic fibroblasts (MEFs) from *Mettl1* iKO mice to investigate their effects *in vitro*. Induction of *Mettl1* knockout with 4-OH tamoxifen resulted in notable senescence-associated phenotypes, including upregulation of P16 protein, an increase in SA- β -gal positive cells, and a decline in cell proliferation (Figure 5A–E; Figure S9A and B). As expected, Knockout of *Mettl1* led to a marked reduction in the

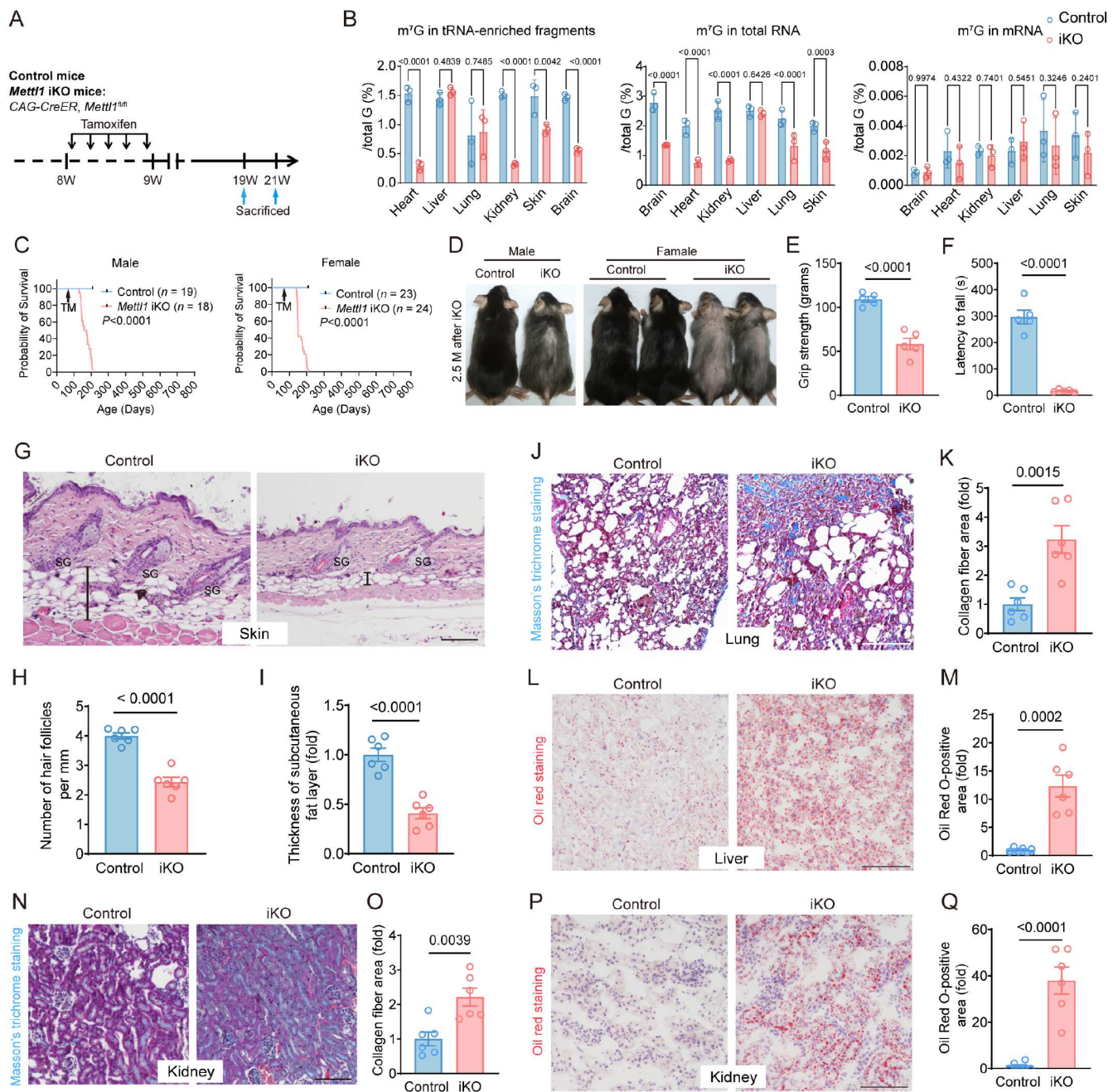


Figure 3 *Mettl1* deficiency decreases lifespan and aggravates age-related phenotypes in mice. **A**, Strategy for *Mettl1* knockout in adult mice. *Mettl1* iKO mice were 8-week-old CAG-CreER, *Mettl1*^{fl/fl} mice treated with tamoxifen to induce *Mettl1* deletion and analyzed 11 weeks later. **B**, Comparison of m⁷G modification between control and iKO groups in tRNA-enriched fragments, total RNA, and mRNA from different tissues. Control, *Mettl1*^{fl/fl} mice; iKO, CAG-CreER, *Mettl1*^{fl/fl} mice (n=3 per group). **C**, Kaplan-Meier curves of control and iKO mice. Control, 19 male and 23 female mice; *Mettl1* iKO: 18 male and 24 female mice. **D**, Representative images of the systemic phenotypes of control and iKO mice. Mice were photographed around 2.5 months after tamoxifen induction. **E**, Comparison of grip strength between control and iKO mice (n=5). **F**, Comparison of the rotarod test between control and iKO mice (n=5). **G–I**, Representative H&E staining images of dorsal skin sections from control and *Mettl1* iKO mice (**G**). Line segment: subcutaneous fat layer; scale bar, 200 μm. Quantification of hair follicle per mm (**H**) and subcutaneous fat layer (**I**) in skin sections from control and iKO mice (n=6). **J** and **K**, Representative Masson's trichrome staining images (**J**) and quantification of collagen fiber area (**K**) of lung sections from control and iKO mice (n=6). Scale bar, 100 μm. **L** and **M**, Representative oil red staining images (**L**) and quantification of positive area (**M**) of liver sections from control and iKO mice (n=6). Scale bar, 100 μm. **N** and **O**, Representative Masson's trichrome staining images (**N**) and quantification of collagen fiber area (**O**) of kidney sections from control and iKO mice (n=6). Scale bar, 100 μm. **P** and **Q**, Representative oil red staining images (**P**) and quantification of positive area (**Q**) of kidney sections from control and iKO mice (n=6). Scale bar, 100 μm. Data were presented as mean±SEM. P values were determined by two-tailed unpaired Student's *t*-test (**B**, **E**, **F**, **H**, **I**, **K**, **M**, **O**, and **Q**) and log-rank test (**C** and **D**).

m⁷G modification levels on tRNAs (Figure S9C). Additionally, nascent protein labeling revealed a global decrease in protein synthesis following *Mettl1* deletion in MEFs induced by 4-OH tamoxifen (Figure 5F and G).

To further explore the impact of *Mettl1* knockout on protein synthesis, we performed ribosome profiling. Ribosome-protected fragment (RPF) reads were predominantly located in the coding sequence (CDS) regions and exhibited strong trinucleotide

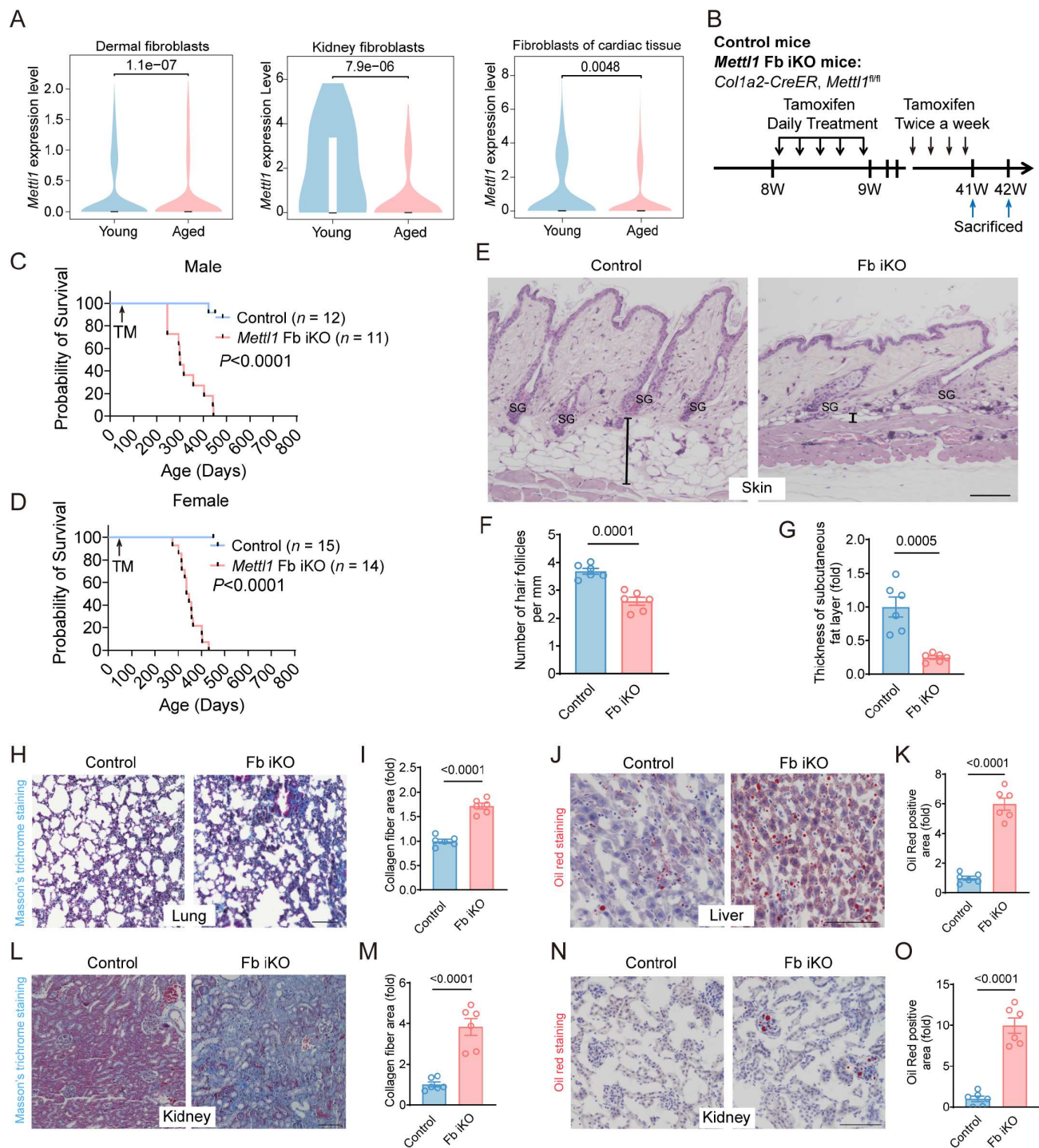


Figure 4 *Mettl1* deficiency in fibroblasts decreases lifespan and aggravates age-related phenotypes in mice. **A**, Violin plots showing the distribution of *Mettl1* expression in the indicated cell types from young and aged mice. Dermal fibroblast RNA-seq data were obtained from GEO (accession GSE165668) (young, $n=2,655$ cells; aged, $n=1,172$ cells). Data were retrieved from *Tabula Muris Senis* for kidney fibroblasts (young, $n=28$ cells; aged, $n=84$ cells) and fibroblasts of cardiac tissue (young, $n=476$ cells; aged, $n=1,933$ cells). **B**, Strategy for *Mettl1* Fb iKO mice. 8-week-old control and *Mettl1* Fb iKO mice were treated with tamoxifen to induce *METTTL1* deletion analyzed 33 weeks later. Control, *Mettl1^{fl/fl}* mice; *Mettl1* Fb iKO, *Col1a2-CreER, Mettl1^{fl/fl}* mice. **C** and **D**, Kaplan-Meier curves of control and *Mettl1* Fb iKO mice. Control, 12 male and 15 female mice; *Mettl1* Fb iKO, 11 male and 14 female mice. P values were determined by the log-rank test. **E–G**, Representative H&E staining images of dorsal skin sections from control and *Mettl1* Fb iKO mice ($n=6$). Line segment: subcutaneous fat layer; scale bar, 200 μm . Bottom, quantification of hair follicle per mm (**F**) and subcutaneous fat layer (**G**) in skin sections from control and *Mettl1* Fb iKO mice ($n=6$). **H** and **I**, Representative Masson's trichrome staining images (**H**) and quantification of collagen fiber area (**I**) of lung sections from control and *Mettl1* Fb iKO mice ($n=6$). Scale bar, 100 μm . **J** and **K**, Representative oil red staining images (**J**) and quantification of positive area (**K**) of liver sections from control and *Mettl1* Fb iKO mice ($n=6$). Scale bar, 100 μm . **L** and **M**, Representative Masson's trichrome staining images (**L**) and quantification of collagen fiber area (**M**) of kidney sections from control and *Mettl1* Fb iKO mice ($n=6$). Scale bar, 100 μm . **N** and **O**, Representative oil red staining images (**N**) and quantification of positive area (**O**) of kidney sections from control and *Mettl1* Fb iKO mice ($n=6$). Scale bar, 100 μm . Data were presented as mean $\pm$ SEM. P values were determined by two-tailed unpaired Student's *t*-test (**A**, **F**, **G**, **I**, **K**, **M**, and **O**) and log-rank test (**C** and **D**).

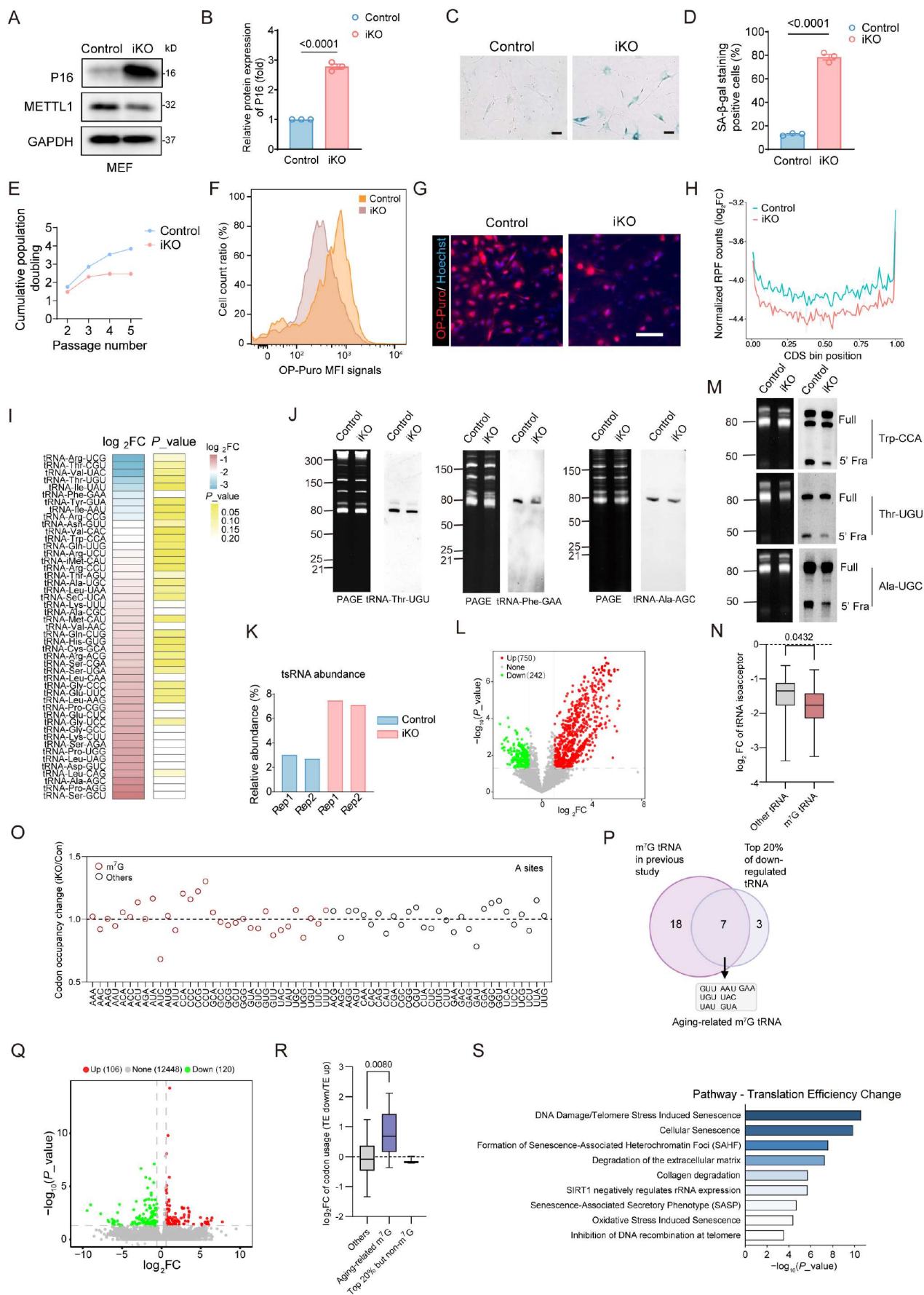


Figure 5 METTL1 regulates aging by affecting the decoding process of m⁷G tRNA. A, Representative images of Western blot analysis for the expression of METTL1 and the aging marker P16 in MEFs 8 d after 4-OH tamoxifen-induced *Mettl1* knockout (*n*=3). Control, MEFs treated with vehicle control (ethyl alcohol); iKO, MEFs treated with 4-OH tamoxifen to induce *Mettl1* knockout. B, Quantification of P16 protein level in (A) (*n*=3). C and D, Representative β -Gal staining images (C) and quantification of positive area (D) in MEFs following 4-OH tamoxifen-induced *Mettl1* knockout (*n*=3). E, Representative changes in cumulative population doubling capacity with increasing passage number after 4-OH tamoxifen-induced *Mettl1* knockout (*n*=3). F and G, Flow cytometry (F) and fluorescent staining to assess OP-Puro-labeled nascent protein (G) following 4-OH tamoxifen-induced *Mettl1* knockout. OP-Puro, red; Hoechst, blue (*n*=3). H, Meta-gene analysis showing changes in RPFs counts in the CDS region following 4-OH tamoxifen-induced *Mettl1* knockout (*n*=2 independent replicates). I, tRNA-seq showing changes in tRNA abundance following 4-OH tamoxifen-induced *Mettl1* knockout (*n*=2 independent replicates). J, Representative images of Northern blot analysis showing changes in the abundance of specific tRNAs following *Mettl1* knockout (*n*=3 independent replicates). K, PANDORA-seq detects changes in the proportion of tsRNAs among all small RNAs ranging from 15 to 50 nt in length following *Mettl1* knockout (*n*=2 independent replicates). L, The differential volcano plot shows changes in the abundance of specific tsRNAs following *Mettl1* knockout (*n*=2 independent replicates). M, Representative images of Northern blot analysis of specific tRNAs after m⁷G-TRAC to infer changes in m⁷G levels (*n*=3 independent replicates). N, log₂ fold change in the abundance of m⁷G tRNAs compared to other tRNAs following 4-OH tamoxifen-induced *Mettl1* knockout (*n*=2 independent replicates). O, Changes in occupancy of codons decoded by m⁷G tRNAs at the ribosomal A-site following 4-OH tamoxifen-induced *Mettl1* knockout (*n*=2 independent replicates). P, Venn diagram identifying a set of aging-related m⁷G tRNAs (*n*=2 independent replicates). Q, Differential volcano plot showing changes in gene translation efficiency following 4-OH tamoxifen-induced *Mettl1* knockout (*n*=2 independent replicates). R, Weighted codon usage changes for genes with decreased translation efficiency compared to those with increased translation efficiency following 4-OH tamoxifen-induced *Mettl1* knockout (*n*=2 independent replicates). S, Pathway terms enriched in genes with altered translation efficiency following 4-OH tamoxifen-induced *Mettl1* knockout (*n*=2 independent replicates). All results are representative of at least three independent experiments. Data were presented as mean $\pm$ SEM. *P* values were determined by a two-tailed unpaired Student's *t*-test.

periodicity between start and stop codons, confirming the reliability of the data (Figure S9D and E). Metagene analysis demonstrated a reduction in RPFs within the CDS, further supporting the global decline in protein synthesis following *Mettl1* knockout (Figure 5H).

We next investigated whether *Mettl1* deficiency affected the tRNA pool and thereby influenced translation. tRNA sequencing revealed a significant reduction in the overall tRNA pool, with isoacceptor tRNAs exhibiting downregulation (Figure 5I). Northern blot analysis also confirmed a reduction in tRNA abundance following *Mettl1* knockout (Figure 5J). Meanwhile, by employing PANDORA-seq (Shi et al., 2021), we observed a global increase in tsRNAs after *Mettl1* knockout (Figure 5K and L), which may result from the fragmentation of tRNAs due to their decreased stability in the absence of *Mettl1*, thus corroborating the observed overall reduction in tRNA abundance. We then examined the effects of *Mettl1* deficiency on m⁷G-containing tRNAs. Previous study identified a group of m⁷G tRNAs using m⁷G TRAC-seq (Figure S9F) (Huang et al., 2022; Kaneko et al., 2024; Lin et al., 2018; Orellana et al., 2021). m⁷G-TRAC combined with Northern blot analysis revealed a significant reduction in m⁷G modification on specific tRNAs following *Mettl1* knockout (Figure 5M). Importantly, the depletion of m⁷G tRNA isoacceptors was more pronounced than that of other tRNAs (Figure 5N).

The reduction in specific tRNA isoacceptor abundance prolonged ribosomal A-site occupancy at the corresponding codons, which in turn decreased translation efficiency (Giguère et al., 2024; Richter and Collier, 2015). To further explore this phenomenon, we analyzed codon occupancy at the ribosomal A-site following *Mettl1* knockout, and found a slight increase in the occupancy of codons decoded by m⁷G-modified tRNAs at the A-site, while no significant changes were observed at the A+1 site (Figure 5O; Figure S9G). Cross-referencing the m⁷G-modified tRNAs with the 20% most downregulated tRNAs identified in our tRNA-seq analysis, we identified seven m⁷G-modified tRNAs associated with aging (Figure 5P). By integrating ribosome profiling data with transcriptomic analysis, we assessed the changes in translation efficiency across individual genes (Figure 5Q). Weighted codon usage analysis of genes with altered translation efficiency revealed a preferential use of codons decoded by the aging-related m⁷G tRNAs for genes with reduced translation efficiency (Figure 5R; Figure S9H). These results provide evidence that *Mettl1* deficiency significantly decreases m⁷G tRNA levels, thereby impairing codon decoding and

translation efficiency. Pathway analysis of genes with altered translation efficiency highlighted senescence-related programmes, including SAHF formation and SASP-like signaling, as well as pathways involving core histones H3/H4 and SIRT1 (Figure 5S). Because several enriched terms were related to core histones H3 and H4, we examined global H3, H4, and SIRT1 protein levels in control and *Mettl1* iKO MEFs. We analysed these proteins at an early stage after *Mettl1* deletion, before overt senescence markers are typically detectable in this system. At this stage, METTL1 protein was already strongly reduced, and total H3, H4, and SIRT1 levels were lower in *Mettl1* iKO MEFs than in control cells (Figure S9I and J).

Together, these findings indicated that loss of METTL1-dependent m⁷G tRNA modification not only alters global translation profiles. In addition, it directly impacted the production of chromatin components (H3/H4) and the stress regulator SIRT1. This combination might predispose fibroblasts to enter the senescent state.

DISCUSSION

RNA modifications have emerged as an additional layer in gene expression, but how they change during aging is not well defined (Barbieri and Kouzarides, 2020; Delaunay et al., 2024; Jonkhout et al., 2017; Wang et al., 2023a; Wang et al., 2022c). Here, we generated an integrative atlas of RNA modifications across tissues, genders, and ages. We observed a conserved, non-random decline in several tRNA modifications during aging, most prominently m⁷G, m¹A, m⁵C, and m²G. These patterns suggested that age-associated RNA modification changes were structured rather than purely stochastic. Given the DNA methylation clocks in capturing biological age (Hannum et al., 2013; Horvath, 2013; Zheng et al., 2024), RNA modification profiles may represent another candidate readout of aging dynamics, although this will require larger human datasets and quantitative standardization.

Recently, Fu et al. (2024) demonstrated that loss of METTL1 reduced m⁷G tRNA modification, destabilized tRNAs, and promoted aging phenotypes. Here, our study extends the previous work at least in two main directions. First, we provide a cross-tissue atlas of tRNA modifications that place METTL1-dependent m⁷G within a coordinated programme of age-related tRNA remodeling. Second, we identify fibroblasts as a key cell type in which the loss of METTL1 was sufficient to accelerate organismal aging *in vivo*.

Aging did not affect RNA modifications uniformly. In this study, we focused on METTL1 and TRMT1, the writers of m⁷G and m²₂G, because both were downregulated with age and their perturbation promoted senescence. In fact, other age-sensitive tRNA-modifying (e.g., m¹A and m⁵C) enzymes have been linked to aging-relevant pathways. TRMT6/TRMT61A (m¹A writer) has been implicated in hematopoietic stem cell aging and in the regulation of the unfolded protein response (He et al., 2024; Su et al., 2022). For m⁵C, prior work connected cytosine methylation to type I interferon signaling (Zhang et al., 2022b) and implicated m⁵C writers such as NSUN2 and TRDMT1 in cell-cycle control (including p21 regulation) and DNA damage repair, respectively (Gao et al., 2019; Li et al., 2017; Yang et al., 2023). Together, these studies suggested that multiple tRNA-modifying enzymes beyond METTL1/TRMT1 may influence proteostasis, immune signaling, proliferation control, and genome maintenance during aging. Future work should define how these enzymes and their associated tRNA marks change in physiological aging, particularly in human systems.

Importantly, our data identified fibroblasts as the specific cell type of METTL1 decline during aging. Moreover, fibroblast-specific *Mettl1* loss was sufficient to induce systemic aging phenotypes. This is consistent with emerging paradigms that stromal cell states actively shape the aging microenvironment (Salminen, 2023a; Salminen, 2023b). Our pathway enrichment analyses in *Mettl1*-deficient fibroblasts point to coordinated changes in ECM/collagen homeostasis together with an amplified SASP, suggesting potential tissue-level effects of fibroblast METTL1 loss. Accumulating evidence indicates that senescent fibroblasts adopt a persistently activated, pro-fibrotic state that drives abnormal ECM deposition and disordered remodeling (Howes et al., 2024; Schafer et al., 2017; Takaya et al., 2022). Such matrix alterations can alter tissue stiffness and viscoelasticity, thereby influencing mechanotransduction, cytoskeletal tension, and nuclear integrity, which in turn can exacerbate mitochondrial stress, DNA damage, and further amplify SASP programs in fibroblasts and neighbouring cells (Cho et al., 2019; Du et al., 2025; Sladitschek-Martens et al., 2022; Swift et al., 2013; Zeng et al., 2025). Conversely, SASP components, including pro-inflammatory cytokines, chemokines, growth factors, and matrix-remodeling enzymes, can sustain chronic low-grade inflammation and reinforce fibroblast activation, thereby driving additional pathological ECM remodeling (Basisty et al., 2020; Chen et al., 2024b; Lee et al., 2021; Wiley et al., 2019; Zeng et al., 2025). Together, these findings support a feed-forward loop in which ECM remodeling and SASP signaling mutually reinforce one another, potentially accelerating fibrosis and functional decline in aging tissues. Based on these considerations, we propose that fibroblast-specific METTL1 downregulation may promote aging at the tissue level by altering fibroblast states and their ECM/secretory outputs. Future work using fibroblast-targeted *in vivo* models will be important to determine the extent to which these changes are sufficient to drive tissue aging phenotypes and whether they can be therapeutically mitigated.

Mechanistically, our data link loss of METTL1-dependent m⁷G to fibroblast senescence through three connected axes: translation/proteostasis, tsRNAs, and chromatin state. First, *Mettl1*-deficient fibroblasts showed reduced translation efficiency and depletion of mature tRNAs, consistent with impaired m⁷G-dependent decoding capacity. Global translational output

declines with age in multiple models and tissues. Ribosome-profiling studies have documented age-associated deregulation of protein synthesis and translation machinery, consistent with a progressive constraint on translational capacity. This decline is frequently linked to mTOR-dependent control of translation initiation and to reduced ribosome biogenesis programs (Anisimova et al., 2020; Di Fraia et al., 2025; Papadopoli et al., 2019; Zid et al., 2009). In this study, METTL1-dependent m⁷G reduction provided another direct mechanism for translational decline. Second, we observed robust accumulation of tsRNAs in *Mettl1*-deficient fibroblasts. In line with this, age-associated increases in tRNA-derived fragments have also been reported in worms and flies (Karaikos et al., 2015; Kato et al., 2011; Shin et al., 2021), and specific tsRNAs have been linked to functional decline in mammals. Specifically, an aging-induced mitochondrial Glu-5' tsRNA-CTC that impairs mitochondrial translation and promotes cognitive deficits, and aging-related changes of sperm tsRNA may contribute to the intergenerational transmission associated with behavioral and cardiac phenotypes (Guo et al., 2021; Li et al., 2024). Mechanistically, a prior study showed that reduced m⁷G modifications due to METTL1 downregulation increased tRNA fragility and cleavage under stress, thereby favoring tsRNA production (Zhao et al., 2024). These findings support a model in which METTL1-dependent m⁷G acts as an upstream safeguard of tRNA integrity, and its decline in aging fibroblasts shifts the balance from mature tRNAs toward tsRNAs, with potential consequences for mitochondrial translation and other aging-relevant pathways. Third, METTL1-dependent m⁷G-linked translational control to chromatin homeostasis. In *Mettl1*-deficient fibroblasts, transcripts with reduced translation efficiency were enriched for core histones H3/H4 and SIRT1, with early decreases in their protein levels after *Mettl1* loss. Loss of canonical histones and chromatin decompaction are recurrent features of aging, and maintaining histone supply can extend lifespan in model organisms (Debès et al., 2023; Feser et al., 2010; Lin et al., 2020; Oberdoerffer, 2010). SIRT1 likewise supports genomic stability and stress resistance in multiple systems (Giblin et al., 2014; Oberdoerffer et al., 2008; Satoh et al., 2017). Together, our findings suggested that METTL1-dependent tRNA m⁷G-coupled RNA stability, translation robustness to chromatin maintenance, and disruption of this axis promote a senescence-prone state in fibroblasts.

A key question is what drives the decline of METTL1 expression during aging. While public resources pointed away from a dominant contribution of classical promoter CpG hypermethylation and instead were more consistent with alterations in promoter-proximal chromatin activation (Sen et al., 2019; Zhang et al., 2023; Zhao et al., 2024). The upstream regulatory architecture of METTL1 in the aging context is likely multifactorial. Independent studies also indicate that METTL1 transcription can be regulated by promoter-bound factors. For example, YY1 has been reported to upregulate METTL1 expression (Wang et al., 2025; Yu et al., 2024). Hypoxia can repress METTL1 through HIF-1α binding to promoter HRE elements (Mi et al., 2024). Metabolic state may also influence METTL1 through chromatin-coupled pathways, as lactate-associated chromatin marks have been linked to METTL1 regulation in fibroblast-like cells (Xu et al., 2026). However, defining how METTL1 is controlled in aging tissues and senescent fibroblasts remains an important direction for future work.

This study has several limitations. First, although our RNA

modification atlas spans multiple tissues, sexes, and ages, it is generated from bulk samples and is therefore largely descriptive; it cannot distinguish single cell-intrinsic remodeling of RNA modifications from apparent changes driven by shifts in cellular composition with age. Second, our functional and mechanistic analyses focus primarily on METTL1 (and to a lesser extent TRMT1) in fibroblasts. Other age-sensitive tRNA marks and their enzymes, as well as additional stromal and parenchymal cell types, are likely to contribute to aging but were not systematically investigated here. Finally, while our data link reduced m⁷G with tsRNA accumulation, translational suppression, and decreased H3/H4 and SIRT1 protein levels, parts of this network remain correlative; the directionality and necessity/sufficiency of individual nodes have not been fully resolved. For example, we did not independently manipulate tsRNAs, histone dosage, or SIRT1 to determine whether each component is required to drive the downstream chromatin and stress-response phenotypes. These limitations motivate several future directions: (i) single-cell-resolved or spatially resolved mapping of RNA modifications and RNA-modifying enzymes to disentangle cell type-intrinsic changes from compositional effects; (ii) systematic dissection of additional age-sensitive tRNA modification pathways (including TRMT1/m²G) using conditional *in vivo* models; (iii) functional experiments to test causality between tRNA hypomodification, tsRNA biogenesis, translational control, and chromatin maintenance.

In summary, our study provides a multi-tissue, multi-model atlas of RNA modification changes during aging and establishes the METTL1-tRNA-translation axis in fibroblasts as a critical regulatory mechanism. By extending previous work with systems-level analyses, we show that tRNA modifications are not inert biochemical decorations but dynamic regulatory elements that can influence cell fate and organismal aging. Because these marks are enzymatically installed and reversible, they may represent tractable targets within the RNA landscape. We propose that tRNA modification patterns could serve not only as aging biomarkers, complementary to DNA methylation clocks, but also as functional levers to reshape the aging transcriptome. As interest in the epitranscriptome continues to grow, our results highlight the need to integrate tRNA modifications into the broader conceptual framework of aging, which may open new therapeutic avenues to maintain translational fidelity and cellular resilience in aging tissues.

CONCLUSIONS

This study provides a comprehensive profile of RNA modification changes across multiple mouse tissues during aging. We established a detailed atlas showing how RNA modifications, especially in tRNAs, change in a tissue- and cell-specific manner. Our results reveal a consistent decrease in many tRNA modifications with age, highlighting the important role of tRNA modifications in aging. Mechanistically, we found that the reduction of the tRNA m⁷G methyltransferase METTL1 in fibroblasts is associated with lower tRNA stability, impaired protein translation, and increased cellular senescence. These changes are associated with premature aging in mice, although additional work will be required to determine whether they are causally responsible. Our work advances understanding of aging by focusing on RNA modifications and identifies METTL1-mediated tRNA m⁷G modification as a key regulator of aging

and cell function. These findings may help develop new therapies targeting RNA modification to slow down aging and improve health span.

MATERIALS AND METHODS

Mice

C57BL/6J wild-type mice were purchased from Slac Laboratory Animal Co. (Shanghai, China). All mice were raised in specific pathogen-free conditions with ambient temperatures maintained between 20 and 25°C and humidity levels regulated at 50%±20%.

To produce inducible *Mettl1* knockout mice, the CAG-CreER mice (029567, Jackson Laboratory, USA) were hybridized with *Mettl1*^{fl/fl} mice (S-CKO-03718, Cyagen, USA). To generate fibroblast-specific METTL1 knockout mice, Col1a2-CreER mice (007900, Jackson Laboratory) were crossed with *Mettl1*^{fl/fl} mice. To induce gene deletion of *Mettl1*, the mice were given tamoxifen (125 mg/kg, T5648, Sigma, Germany) by intraperitoneal injection for 5 d at the same time every day, beginning at 8 weeks. The weighed tamoxifen powder was dissolved in 10% by volume anhydrous ethanol, then added with 90% by volume corn oil and thoroughly mixed.

tRNA m⁷G TRAC-treatment for Northern blot

The TRAC-treatment protocol was adapted from previously reported methodologies. METTL1 knockdown and control cells were harvested using Trizol-LS to isolate tRNA fragments. The RNA samples were treated with 0.2 mol/L NaBH₄ on ice for 30 min in the dark, with 2 mmol/L free m⁷GTP serving as the methylation carrier. NaBH₄-treated RNA was then incubated with an aniline-acetate solution (H₂O: glacial acetic acid: aniline, 7:3:1) at room temperature for 2 h to induce specific cleavage at the m⁷G site.

Following treatment, RNA was precipitated by adding 0.1 volume of 3 mol/L sodium acetate (pH 5.2) and 2.5 volumes of cold ethanol, and incubated at -80°C for 1 h. After precipitation, TRAC-treated RNA was denatured at 95°C for 2 min and separated by 10% urea-PAGE. The gels were stained with SYBR GOLD, imaged, and immediately transferred to Roche Nylon Membranes (11417240001, Roche, Switzerland), followed by UV-crosslinking twice at 0.12 J energy.

The membranes were pre-hybridized with Roche DIG Hybridization Buffer (11796895001, Roche) for 60 min at 42°C with shaking. DIG-labeled probes for tRNA (5'-DIG-CGC TCT ACC ACT GAG CTA CAC CCC C-3' for tRNA^{Ala}; 5'-DIG-CGC GCT ACC GTT GCG CCA CGA GGT C-3' for tRNA^{Trp}; 5'-DIG-GCT CTA ACC CCT GAG CTA TGG AGC C-3' for tRNA^{Thr}; 5'-DIG-CTC TCC CAA CTG AGC TAT TTC GGC-3' for tRNA^{Phe}) were added to the hybridization solution and incubated overnight at 42°C.

After discarding the hybridization solution, membranes were washed twice with low-stringency buffer (2×SSC with 0.1% (wt/vol) SDS) at 42°C for 15 min each, followed by two washes with high-stringency buffer (0.1×SSC with 0.1% (wt/vol) SDS) for 10 min each. The membranes were then washed with washing buffer (1×SSC) for 10 min.

Next, the membranes were blocked in Roche Blocking Buffer (11096176001, Roche) at room temperature for 3 h. DIG antibody (anti-Digoxigenin-AP Fab fragments, 11093274910,

Roche) was diluted 1:10,000 in blocking buffer and incubated with the membranes for 1 h at room temperature. The membranes were washed four times with DIG Wash Buffer (1×Maleic Acid Buffer, 0.3% Tween-20) at room temperature for 15 min each, followed by incubation in developing buffer for 10 min at room temperature.

Finally, the membranes were incubated with CSPD reagent (11755633001, Roche) at 37°C for 15 min in the dark and then imaged using a Bio-Rad imaging system (USA).

Detection and quantitative analysis of RNA modifications by LC-MS/MS

LC-MS/MS was conducted as previously described (Zhang et al., 2018). Briefly, the RNAs concentration was measured, and 100 ng RNAs was incubated in a 30 µL reaction mixture containing 3 µL of 10×RNA hydrolysis buffer (M0660S, NEB, USA), 0.2 IU of benzonase (D7121, Beyotime, Shanghai, China), 0.2 IU of alkaline phosphatase (M0525V, NEB), and 0.2 IU of phosphodiesterase I (M0660S, NEB) at 37°C for 3 h. Mass spectrometry analysis was carried out on a SCIEX Triple Quad 6500, coupled to a Waters ACQUITY-UPLC I-class (USA), with an electrospray ionization source. The expression levels of RNA modifications were quantified using a standard curve and the relative quantification methods for RNA modifications described in previous work (Guo et al., 2022). The modified nucleotide standards used in this study are provided in the Additional File.

tRNA-seq and RNA-seq

Total RNA was extracted using TRIzol (15596018, Invitrogen, USA) following the manufacturer's instructions. Deacylation was performed in 0.1 mol/L Tris-HCl (pH 9.0) for 30 min, followed by separation of tRNA bands on a 10% urea-PAGE gel. tRNA bands at ~80 nt were excised, eluted overnight in RNA Gel Extraction Buffer (300 mmol/L sodium acetate, pH 4.5, 0.25% SDS, 1 mmol/L EDTA), and recovered via ethanol precipitation. AlkB enzyme treatment was performed to remove specific methylation modifications on tRNAs (Behrens et al., 2021). The tRNA was purified using TRIzol LS (10296010, Invitrogen) and dissolved in 7 µL nuclease-free water. Concentration of enriched tRNAs was measured using NanoDrop One (Thermo Scientific, USA). Libraries were prepared using the VAHTS Small RNA Library Prep Kit for Illumina V2 (NR811, Vazyme, Nanjing, China) with minor modifications. Briefly, 3' adapter ligation was performed at 25°C for 3 h, followed by RT primer annealing at 75°C for 5 min, 37°C for 15 min, and 25°C for 15 min. 5' adapter ligation was conducted at 25°C for 1 h, followed by reverse transcription at 50°C for 1 h and PCR amplification. Libraries were sequenced on the Illumina Novaseq X Plus platform. RNA-seq libraries were prepared and sequenced by a commercial service provider.

Small RNA-seq

Small RNA extraction and cDNA library construction were performed according to our recently published protocol (Shi et al., 2021). Briefly, RNA loading dye (B0363S, NEB) was added to the RNA sample, mixed thoroughly, and incubated at 95°C for 5 min. The RNA mixture was then subjected to electrophoresis on a 10% urea polyacrylamide gel. SYBR Gold solution (S11494, Thermo Scientific) was used to stain and visualize the small RNA.

The small RNA fraction (15–50 nucleotides) was excised and eluted overnight at 4°C with sodium acetate (0.3 mol/L, AM9740, Invitrogen). After 10 min centrifugation (12,000×g, 4°C), the aqueous phase was collected. Subsequently, linear acrylamide, 3 mol/L sodium acetate, and 100% ethanol were added to the supernatant, followed by incubation at –80°C for 1 h. The RNA was precipitated and resuspended in nuclease-free water.

The RNA was added to a 50 µL reaction buffer containing α-ketoglutarate (1 mmol/L, K1128, Sigma), AlkB (4 µg/mL), sodium ascorbate (2 mmol/L), HEPES (50 mmol/L, 15630080, Thermo Scientific), bovine serum albumin (50 mg/L, A9647, Sigma), ferrous ammonium sulfate (75 µmol/L), and RNase inhibitor (2,000 U/mL). The mixture was incubated at 37°C for 20 min, after which 500 µL of TRIzol reagent was added to extract the RNA. The isolated RNA was transferred to another 50 µL reaction buffer containing 10× PNK buffer (5 µL, B0201S, NEB), ATP (1 mmol/L, P0756S, NEB), and T4 Polynucleotide Kinase (10 U, M0201L, NEB) and incubated at 37°C for 20 min. RNA was extracted again using 500 µL of TRIzol reagent.

Small RNA library construction and sequencing were performed by Illumina using the Vazyme Small RNA Sample Preparation Kit (NR811-01). Raw sequencing reads were processed using the SPORTS software (v1.2). Reads were aligned to the mouse reference genome, miRNA datasets, rRNA and YRNA datasets, genomic tRNA datasets, mitochondrial tRNA datasets, piRNA datasets, and ncRNA datasets to count the read numbers of small non-coding RNA (sncRNA) transcripts using Bowtie (v1.3.1) (<http://bowtie-bio.sourceforge.net/index.shtml>).

Ribosome profiling

Ribosome profiling libraries were prepared using the QEZ-seq 1.0 Kit (RB1012S, EzraBio, USA) following the manufacturer's instructions. In brief, cells were lysed in lysis buffer containing cycloheximide, and RNA was digested using RNase I. The resulting RPFs were separated on a 15% urea-PAGE gel, and fragments between 26–32 nt were extracted. Following polyadenylation, adapter ligation, reverse transcription, and library enrichment, sequencing was performed on the Illumina Novaseq X Plus platform.

High-throughput sequencing data analysis

For RNA-seq, data were trimmed using fastp v0.23.4, aligned with STAR v2.7.11, and assembled with StringTie v2.2.3 to generate raw count matrices for translation efficiency analysis.

For tRNA-seq, data were processed with cutadapt v4.4 using the parameters:

```
-m 18 -M 90 --trim-n --trimmed-only -a AGATCGGAAGAG-CACACGTCTGAACTCCAGTCAC.
```

Analysis was conducted with the mim-tRNAseq pipeline (Shi et al., 2021) using parameters:

```
--species Mmus --cluster-id 0.97 --threads 1 --min-cov 0.0005 --max-mismatches 0.1 --max-multi 1 --remap --remap-mismatches 0.075. Data were normalized using RPM.
```

For ribosome profiling, data were trimmed with cutadapt v4.4 using the parameters:

```
-m 15 -a GGG; e=1...AAAAAAAAAAAA -q 5 --trim-n, and analyzed with RiboToolkit (https://rnainformatics.org.cn/RiboToolkit/index.php), setting RPF length to 26–32 nt. Weighted
```

codon usage was calculated using:

$$W_i = \frac{\sum_{j=1}^n (p_{ij} \cdot \exp_j \cdot \text{len}_j)}{\sum_{i=1}^m \sum_{j=1}^n (p_{ij} \cdot \exp_j \cdot \text{len}_j)}.$$

Where m is the number of codons, n is the number of genes, i is the i -th codon, j is the j -th gene, p is codon frequency, $\exp$ is mRNA expression, and len is mRNA length. $\log_2$ fold change was used to represent differences in weighted codon usage between gene sets.

Protein synthesis assay

To assess the impact of *Mettl1* knockout on tRNA function, protein synthesis was measured in IMR90 cells using the O-propargyl-puromycin (OPP) assay 15 d after tamoxifen induction. Briefly, OPP (1416561-90-4, MCE, USA) was diluted 1:1,000 in 1 mL of DMEM supplemented with 10% FBS and 1% antibiotics, and a final working concentration of 20 $\mu\text{mol/L}$ was prepared. For incorporation into cells, 2 $\times$ OPP working solution (20 $\mu\text{mol/L}$) was pre-warmed to 37°C, and an equal volume was added to the 6-well plate, resulting in a final OPP concentration of 1 $\times$. The cells were incubated at 37°C for 15 min to allow OPP incorporation.

After OPP labeling, the culture medium was removed, and the cells were fixed with 1 mL of 4% paraformaldehyde (PFA) at room temperature for 15 min. The fixative was removed, and the cells were washed three times with 1 mL of wash buffer for 3–5 min per wash. The wash buffer was removed, and the cells were permeabilized with 1 mL of permeabilization buffer (0.3% Triton X-100 in phosphate-buffered saline (PBS)) at room temperature for 10–15 min. After permeabilization, cells were washed again with 1 mL of wash buffer for 1–2 washes, each for 3–5 min.

To detect OPP incorporation, a 500 μL OPP Click chemistry reaction mixture was prepared. The reaction mixture was incubated at room temperature, protected from light, for 30 min. After incubation, the reaction mixture was removed, and the cells were washed three times with wash buffer for 3–5 min per wash.

Finally, protein synthesis levels were analyzed using flow cytometry on a CytoFLEX LX flow cytometer (Beckman Coulter, USA).

DNA methylation analysis

Genome-wide DNA methylation data were obtained from two publicly available Illumina HumanMethylation450 BeadChip datasets. Whole-blood DNA methylation profiles were downloaded from GSE40279 ($n=656$). Probe-level β -values were obtained from the processed file provided by the authors, and sample metadata (including age and sex) were extracted from the corresponding series matrix file using the GEOquery package in R (version 4.5.2). For each individual, *METTL1* promoter methylation was calculated as the mean β -value across 18 CpG probes mapping to the genomic window chr12:58,164,000–58,168,000 (hg19). For age-stratified comparisons, individuals were grouped into “Young” (<45 years) and “Old” (≥ 60 years).

To examine *METTL1* promoter methylation in a fibroblast senescence model, we re-analyzed GSE81797, which profiles human fibroblasts at different population-doubling levels. Normalized β -values were extracted from the series matrix file. Based

on sample annotations, PDL36 fibroblasts were classified as “Young” and PDL85 fibroblasts as “Senescent”. CpG probe annotation was obtained from the Illumina Human Methylation 450 Kanno. ilmn12. hg19 package. *METTL1* promoter probes were defined as CpGs annotated to *METTL1* and assigned to promoter-associated categories (TSS1500, TSS200, 5'UTR, or 1stExon), yielding 66 probes in total. For each condition, mean promoter β -values were calculated across all 66 CpGs.

ChIP-seq data analysis

Public p300 ChIP-seq signal profiles for proliferating and senescent fibroblasts were downloaded from GSE106146. The *METTL1* promoter was defined as the genomic interval chr12:58,164,977–58,167,215 (hg19). For each condition, the mean normalized p300 signal within this promoter window was computed as a width-weighted average of bigWig scores.

Behaviour testing

Age-matched male and female control and *Mettl1* iKO mice were included in the study. All the mice underwent three consecutive days of acclimation training, once a day at the same time, and were tested on the fourth day. To improve the reliability and consistency of the results, all evaluations were performed by the same investigator following standardized protocols.

Rotating rod testing was performed using a programmable rotating rod apparatus (Shanghai Yuyan Instruments Co., Ltd., China) with variable speed settings. Mice were placed on a rotarod that gradually accelerated from 0 to 40 r/min over 1 min. Each mouse completed three trials, with a 15 min rest interval between each. The average latency to fall for each mouse was recorded across the trials.

Grip strength for all limbs was assessed using a grip strength meter (Shanghai Yuyan Instruments Co., Ltd.). Mice were allowed to grasp a grid with both their forelimbs and hindlimbs and were then gently pulled backward until they released their grip. The maximum force exerted (in grams) at the moment of release was recorded. The average grip strength from six consecutive trials, with a 10–15 s rest interval between each trial, was used as a measure of overall grip strength.

Histological examination

For histological analysis, mouse tissues were fixed in 4% PFA for 48 h and embedded in paraffin. Paraffin sections (4 μm thick) were prepared using a rotary microtome, deparaffinized, and stained with H&E according to standard protocols (Chen et al., 2024a). Hair follicle number and fat layer thickness were measured by the ImageJ software (version 2.0).

Masson's trichrome staining

Masson's trichrome staining was performed using the Masson staining kit (C0189M, Beyotime), following the manufacturer's instructions. The percent of positive area was measured by the ImageJ software.

SA- β -gal staining

Cells were seeded in six-well plates and stained with the β -

galactosidase staining kit (C0602, Beyotime) following the manufacturer's guidelines to evaluate SA- β -gal activity. ImageJ software was used to measure the percentage of positive cells per area in each group.

Immunofluorescence staining

Immunofluorescence was performed on 8- μ m frozen tissue sections from mice following standard protocols (Chen et al., 2024a). Sections were fixed with 4% PFA for 15 min, washed with PBS, and blocked with 5% normal donkey serum, 1% BSA, and 0.3% Triton X-100 for 60 min. Anti-Lamin B1 antibody (1:2,000, Abcam, UK) was incubated overnight at 4°C, followed by 60 min of incubation with Alexa Fluor 594-conjugated secondary antibodies at room temperature. After washing, the sections were counterstained with DAPI. Imaging was carried out using a Zeiss Axioplan 2 microscope (Germany), and fluorescence intensity was quantified using ImageJ software.

Multiplex immunohistochemistry

Multiplex immunohistochemistry of mouse tissue sections (4 μ m) was performed using the Opal™ 7-Color Manual IHC Kit (Australian) according to the manufacturer's instructions. Briefly, paraffin-embedded sections were deparaffinized and rehydrated through graded alcohols. Before epitope retrieval, slides were fixed in 10% neutral buffered formalin for 30 min at room temperature. Heat-induced epitope retrieval was then performed in AR buffer supplied with the kit. Slides were cooled to room temperature and washed twice with TBST (TBS containing 0.05% Triton X-100). Slides were incubated with blocking buffer for 10 min and then with primary antibodies diluted in blocking buffer overnight at 4°C. After washing in TBST, slides were incubated with Polymer HRP secondary antibody for 10 min at room temperature, followed by incubation with the appropriate Opal fluorophore to amplify the signal. The previous antibody complex was stripped by incubation in AR buffer according to the manufacturer's protocol. Slides were then reblocked and probed with the next primary antibody, followed by the same HRP/Opal detection steps. After completion of all staining cycles, slides were counterstained with DAPI for 5 min and coverslipped with antifade mounting medium. Images were acquired using a fluorescence or multispectral imaging system under identical settings for all groups.

To assess Lamin B1 expression in fibroblasts and non-fibroblasts, VIM⁺ and VIM⁻ cells were segmented based on VIM and DAPI signals. The mean nuclear Lamin B1 fluorescence intensity in VIM⁺ and VIM⁻ cells was quantified using Image J software from background-subtracted images.

The primary antibodies used in this study were as follows: anti-VIM antibody (1:300, 5741S, Cell Signaling Technology, USA), anti-Lamin B1 antibody (1:2,000, ab16048, Abcam), anti-CD45 antibody (1:300, AWA10976, Abiowell, Changsha, China), and anti-METTL1 antibody (1:100, 14994-1-AP, Proteintech, USA).

Single-cell transcriptomic data from *Tabula Muris Senis*

The single-cell RNA-seq data of *Microtus fortis* (*Tabula Muris Senis atlas*) were obtained from the Gene Expression Omnibus (GEO, GSE149590, 2020). The single-cell data of dermal fibroblasts

were obtained from a separate dataset (GSE165668) (Sladitschek-Martens et al., 2022).

Briefly, raw data from the 10 $\times$ Genomics H5AD object file downloaded from GSM4505404, containing single-cell raw counts and cell annotations, were processed and normalized using the Seurat R package (v3.1.558). The resulting data consisted of normalized gene expression values after preprocessing.

The single-cell dataset included the following information:

Kidney: fibroblasts, endothelial cells, and smooth muscle cells from young (1- and 3-month-old) and aged (18-, 21-, 24-, and 30-month-old) mice.

Heart and aorta: fibroblasts, capillary endothelial cells, and lymphocytes from young (1- and 3-month-old) and aged (18-, 21-, 24-, and 30-month-old) mice.

Cell cultures

IMR90 and HEK293T cells were acquired from the American Type Culture Collection (ATCC). HDFs were isolated from discarded foreskin tissue obtained from circumcised patients, following previously established protocol (Xie et al., 2022).

To isolate MEFs, E14.5 embryos were harvested, and the internal organs and heads were carefully excised, with the heads reserved for genotyping. The remaining body tissues were finely minced and digested in a 0.05% trypsin/EDTA solution at 37°C for 15 min. Digestion was terminated by adding complete medium prepared with DMEM (Gibco, USA), followed by centrifugation at 1,000 r/min for 5 min. The resulting cell pellet from one embryo was then plated into a 100-mm petri dish.

For *in vitro* *Mettl1* depletion experiments, MEFs were isolated from *Mettl1* iKO embryonic mice and treated with 0.5 μ mol/L 4-OH Tamoxifen (H7904, Sigma) for 24 h. The control group received an equivalent volume of anhydrous ethanol.

For RS, HDFs, and IMR90 cells were serially passaged at a 1:4 dilution ratio, with each passage corresponding to approximately two population doublings, until cell proliferation ceased. Initially, the average passage interval was three days.

As cellular aging progressed, proliferation slowed, and the interval between passages gradually increased. In the penultimate passage, the interval extended to 2–3 weeks.

For OIS, HDFs were infected with an HRAS-expressing lentivirus or a control vector. Following 12 d of puromycin selection, senescent and control cells were harvested.

For SIPS, cells were exposed to a 20 Gy dose of radiation. Senescent and control cells were collected 20 d post-irradiation.

For OSIS, cells at 40%–50% confluence were treated with 200 μ mol/L or 400 μ mol/L H₂O₂ for 2 h. After treatment, residual H₂O₂ was removed by washing twice with PBS, and fresh complete medium was added. Cells were then cultured for an additional 48 h before harvesting.

MEFs, HDFs, and HEK293T cells were cultured in DMEM, while IMR90 cells were cultured in EMEM. The complete culture medium contained 10% fetal bovine serum (Gibco). All cells were cultured at 37°C in a 5% CO₂ incubator.

Plasmids

The primers for shRNA targeting human *METTL1*, human *WDR4*, and human *TRMT1* were synthesized by Sangon Biotech (Shanghai, China) and later cloned into the lentiviral vectors

pLKO.1-Puro or pLKO.1-Neo. Using the wild-type METTL1 expression plasmid as a template, a catalytically inactive METTL1 mutant was generated by site-directed mutagenesis, in which amino acids 160–163 were substituted from LFPD to AFPA (Dai et al., 2021). Similarly, using the wild-type TRMT1 expression plasmid as a template, a catalytically inactive TRMT1 mutant was generated by site-directed mutagenesis by substituting the conserved catalytic residue Asp233 with alanine (D233A) (Zhang et al., 2025). The overexpression plasmids were purchased from Sangon Biotech. Details of the primers used for constructing the shRNA can be found in Table S1. All plasmids were verified via sequencing.

Dot blotting

Total RNA was isolated from cells or tissues using TRIzol reagent according to the manufacturer's instructions, quantified, and diluted to the indicated concentrations in nuclease-free water. RNA samples were denatured at 95°C for 5 min and immediately chilled on ice, and then spotted onto a positively charged nylon membrane (Hybond-N⁺). After air-drying, the membrane was UV-crosslinked (120 mJ/cm², twice) to immobilize the RNA. The membrane was then blocked in 5% non-fat milk in TBST (20 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.1% Tween-20, pH 7.5) for 1 h at room temperature, followed by incubation with primary antibodies diluted in blocking buffer overnight at 4°C. After three washes in TBST, the membrane was incubated with an HRP-conjugated secondary antibody for 1 h at room temperature. Signals were detected using enhanced chemiluminescence (ECL) and imaged with the Bio-Rad ChemiDoc XRS system. Methylene blue staining of total RNA on the membrane was used as a loading control.

The primary antibodies used in this study were as follows: anti-m⁷G antibody (1:1,000, RN017M, MBL, Japan), Anti-m²G antibody (1:1,000, ab211488, Abcam).

Western blot

Western blot was performed as previously described (Deng et al., 2021). The supernatant of cell and tissue lysates was centrifuged, mixed with sample buffer, and heated at 100°C for 5 min. Proteins were separated by SDS-PAGE and transferred to a PVDF membrane. To reduce non-specific binding, the membranes were blocked with 5% skim milk in 1×TBST and incubated at room temperature for 1 h. Following blocking, the membranes were incubated overnight at 4°C with the specific primary antibody. The membranes were then incubated with an HRP-conjugated secondary antibody at room temperature for 1 h. Protein bands were visualized using the Bio-Rad ChemiDoc XRS system. The primary antibodies used in this study were as follows: anti-METTL1 antibody (1:1,000, ab271063, Abcam), anti-WDR4 antibody (1:1,000, ab169526, Abcam), anti-CDKN2A/p16 INK4a antibody (1:1,000, ab108349, Abcam), anti-CDKN2A/p16 INK4a antibody (1:1,000, ab108349, Abcam), anti-TRMT1 antibody (1:1,000, 14970-1-AP, Protein-tech), anti-HSP90 antibody (1:1,000, ab203085, Abcam), anti-P21 antibody (1:1,000, 2947S, Cell Signaling Technology), anti-GAPDH (1:10,000, 60004-1, Proteintech), anti-β Actin (1:2,000, sc-47778, Santa Cruz Biotechnology, USA), anti-P53 antibody (1:500, sc-71820, Santa Cruz Biotechnology), anti-α Tubulin (1:2,000, sc-5286, Santa Cruz Biotechnology), anti-

Lamin B1 antibody (1:1,000, ab16048, Abcam), anti-H3 antibody (1:1,000, AWA11353, Abiowell), anti-H4 antibody (1:1,000, AWA13313, Abiowell), and anti-SIRT1 antibody (1:1,000, AWA10421, Abiowell).

Statistical analysis

GraphPad Prism (version 9) was used for statistical analysis, and results were shown as mean±SEM. We first assessed normality and variance similarity. A two-tailed unpaired Student's *t*-test was used for two-group comparisons. For comparisons involving more than two groups, one-way ANOVA followed by Bonferroni's multiple comparisons test. Survival analysis was performed using the Log-rank test. The sample size was not predetermined statistically, and mice were randomly assigned to groups. Unless otherwise stated, “*n*” denotes the number of biologically independent samples (individual mice or samples from an independent *in vitro* experiment). Technical replicates were used only to ensure assay robustness and are not plotted as separate data points.

Data availability

All data needed to evaluate the conclusions in this study are provided in the manuscript and/or the Supplementary Materials. Total RNA-seq, tRNA-seq, and Ribo-seq data were uploaded to the Genome Sequence Archive under accession number CRA024462. The single-cell RNA-seq data of *Microtus fortis* (*Tabula Muris Senis atlas*) were obtained from the GEO under accession number GSE149590. The single-cell data of dermal fibroblasts were obtained from a separate dataset under accession number GSE165668. Whole-blood DNA methylation profiles were downloaded from the GEO under accession number GSE40279, and human fibroblasts at different population-doubling methylation profiles were downloaded from the GEO under accession number GSE81797. Public p300 ChIP-seq signal profiles for proliferating and senescent fibroblasts were downloaded from the GEO under accession number GSE106146. Additional details supporting the findings of this study can be obtained from the corresponding author upon reasonable request.

Compliance and ethics

The authors declare that they have no conflict of interest. All animal experiments and human sample collections were conducted following institutional guidelines and approved by the Ethics Committee of the ethical committee of the Xiangya Hospital of Central South University (Approval IRB No. 2022020197 and No. 202103574). Informed consent was obtained from all human participants.

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Supporting information

The supporting information is available online at <https://doi.org/10.1007/s11427-025-3287-6>. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

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