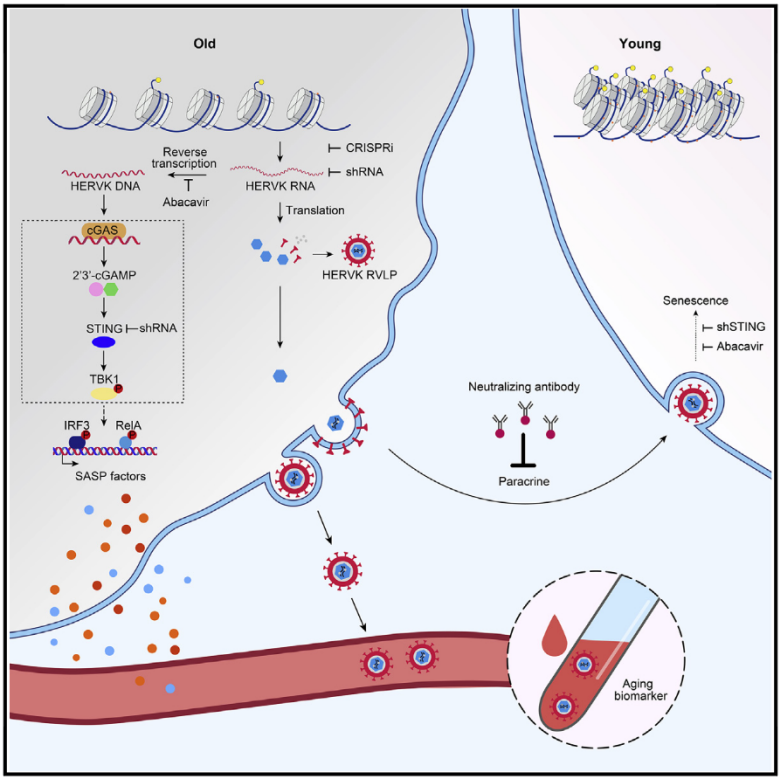
Cell

Resurrection of endogenous retroviruses during aging reinforces senescence

Graphical abstract



Highlights

- Derepression of the endogenous retrovirus contributes to programmed aging
- Upregulation of HERVK triggers the innate immune response and cellular senescence
- Extracellular HERVK retrovirus-like particles induce senescence in young cells
- Endogenous retrovirus serves as a potential target to alleviate aging

Authors

Xiaoqian Liu, Zunpeng Liu, Zeming Wu, ..., Weiqi Zhang, Jing Qu, Guang-Hui Liu

Correspondence

zhangwq@big.ac.cn (W.Z.), qujing@ioz.ac.cn (J.Q.), ghliu@ioz.ac.cn (G.-H.L.)

In brief

Liu and colleagues uncover the ways in which derepression of human endogenous retrovirus triggers cellular senescence and tissue aging; the findings provide fresh insights into therapeutic strategies for alleviating aging.

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Resurrection of endogenous retroviruses during aging reinforces senescence

Xiaoqian Liu,[1,5,6,7,21] Zunpeng Liu,[1,5,7,21] Zeming Wu,[2,5,7,21] Jie Ren,[3,5,7,21] Yanling Fan,[3,7] Liang Sun,[9] Gang Cao,[8] Yuyu Niu,[11,12,13] Baohu Zhang,[1,7] Qianzhao Ji,[2,7] Xiaoyu Jiang,[2,7] Cui Wang,[3,7] Qiaoran Wang,[3,7] Zhejun Ji,[1,5,7] Lanzhu Li,[2,7] Concepcion Rodriguez Esteban,[18] Kaowen Yan,[2,5,7] Wei Li,[4] Yusheng Cai,[2,5,7] Si Wang,[4,5,7,15,16] Aihua Zheng,[7,19] Yong E. Zhang,[7,14] Shengjun Tan,[14] Yingao Cai,[7,14] Moshi Song,[2,5,6,7] Falong Lu,[7,10] Fuchou Tang,[17] Weizhi Ji,[11,12,20] Qi Zhou,[1,5,6,7,20] Juan Carlos Izpisua Belmonte,[18,20] Weiqi Zhang,[3,5,7,*] Jing Qu,[1,5,6,7,*] and Guang-Hui Liu[2,4,5,6,7,22,*]

[1]State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[2]State Key Laboratory of Membrane Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[3]CAS Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences and China National Center for Bioinformation, Beijing 100101, China
[4]Advanced Innovation Center for Human Brain Protection, National Clinical Research Center for Geriatric Disorders, Xuanwu Hospital Capital Medical University, Beijing 100053, China
[5]Institute for Stem cell and Regeneration, Chinese Academy of Sciences, Beijing 100101, China
[6]Beijing Institute for Stem Cell and Regenerative Medicine, Beijing 100101, China
[7]University of Chinese Academy of Sciences, Beijing 100049, China
[8]State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China
[9]NHC Beijing Institute of Geriatrics, NHC Key Laboratory of Geriatrics, Institute of Geriatric Medicine of Chinese Academy of Medical Sciences, National Center of Gerontology/Beijing Hospital, Beijing 100730, China
[10]State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, The Innovative Academy of Seed Design, Chinese Academy of Sciences, Beijing 100101, China
[11]State Key Laboratory of Primate Biomedical Research, Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, Yunnan 650500, China
[12]Yunnan Key Laboratory of Primate Biomedical Research, Kunming, Yunnan 650500, China
[13]Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, Yunnan 650500, China
[14]Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[15]Aging Translational Medicine Center, International Center for Aging and Cancer, Xuanwu Hospital, Capital Medical University, Beijing Municipal Geriatric Medical Research Center, Beijing 100053, China
[16]The Fifth People's Hospital of Chongqing, Chongqing 400062, China
[17]Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China
[18]Altos Labs, Inc., San Diego, CA 94022, USA
[19]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[20]Senior author
[21]These authors contributed equally
[22]Lead contact
*Correspondence: zhangwq@big.ac.cn (W.Z.), qujing@ioz.ac.cn (J.Q.), ghliu@ioz.ac.cn (G.-H.L.)
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SUMMARY

Whether and how certain transposable elements with viral origins, such as endogenous retroviruses (ERVs) dormant in our genomes, can become awakened and contribute to the aging process is largely unknown. In human senescent cells, we found that HERVK (HML-2), the most recently integrated human ERVs, are unlocked to transcribe viral genes and produce retrovirus-like particles (RVLPs). These HERVK RVLPs constitute a transmissible message to elicit senescence phenotypes in young cells, which can be blocked by neutralizing antibodies. The activation of ERVs was also observed in organs of aged primates and mice as well as in human tissues and serum from the elderly. Their repression alleviates cellular senescence and tissue degeneration and, to some extent, organismal aging. These findings indicate that the resurrection of ERVs is a hallmark and driving force of cellular senescence and tissue aging.

INTRODUCTION

Aging is associated with a physiological decline and manifestations of chronic diseases, yet many of its underlying molecular changes and mechanisms remain poorly understood. With significant efforts over the past decades, several causal determinants of aging-related molecular changes have been identified, such as epigenetic alterations and stimulation of senescence-associated secretory phenotype (SASP) factors.[1–7] Although the majority of these studies describe aging determinants originating primarily from protein-coding genes, the non-coding part of the genome has started to garner attention as well. For example, silent long-interspersed element-1 (LINE1) retrotransposons, belonging to non-long terminal repeat (non-LTR) retrotransposons, can be activated during senescence, triggering the innate immune response that is responsible for part of the senescence-associated phenotypes.[8–15]

A different class of retroelements, endogenous retroviruses (ERVs), belonging to LTR retrotransposons are a relic of ancient retroviral infection, fixed in the genome during evolution, comprising about 8% of the human genome.[16–19] As a result of evolutionary pressure, most human ERVs (HERVs) accumulate mutations and deletions that prevent their replication and transposition function.[20,21] However, some evolutionarily young subfamilies of HERV proviruses, such as the recently integrated HERVK human mouse mammary tumor virus like-2 (HML-2) subgroup, maintain open reading frames encoding proteins required for viral particle formation, including Gag, Pol, Env, and Pro.[18,22] Except at specific stages of embryogenesis when DNA is hypomethylated and under certain pathological conditions such as cancer,[23–27] HERVs are transcriptionally silenced by host surveillance mechanisms such as epigenetic regulation in post-embryonic developmental stages.[28,29] Notably, whether ERVs can escape host surveillance during aging and, if so, what effects they may exert on cellular and organismal aging are still poorly investigated.

In this study, using cross-species models and multiple techniques, we revealed an uncharacterized role of endogenous retrovirus resurrection as a biomarker and driver for aging. Specifically, we identified endogenous retrovirus expression associated with cellular and tissue aging and that the accumulation of HERVK retrovirus-like particles (RVLPs) mediates the aging-promoting effects in recipient cells. More importantly, we can inhibit endogenous retrovirus-mediated pro-senescence effects to alleviate cellular senescence and tissue degeneration *in vivo*, suggesting possibilities for developing therapeutic strategies to treat aging-related disorders.

RESULTS

Upregulated HERVK expression in senescent hMPCs is associated with epigenetic derepression

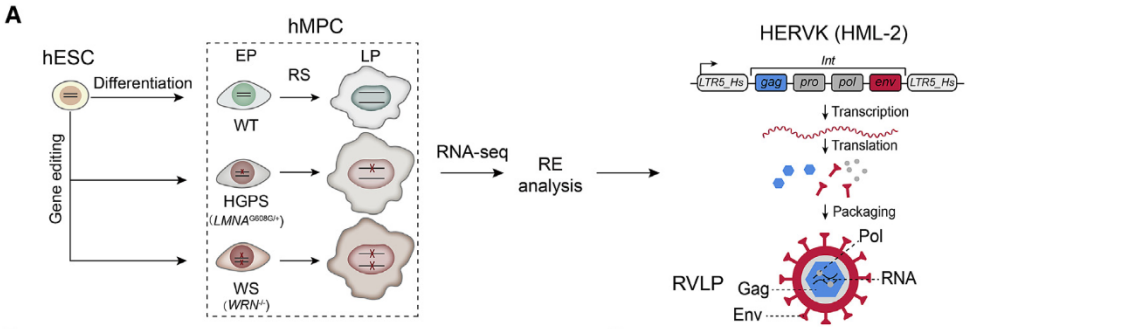
Cellular senescence is considered a major contributing factor to aging and a hallmark of human progeroid diseases, i.e., Hutchinson-Gilford progeria syndrome (HGPS) and Werner syndrome (WS).[2,30–36] We previously demonstrated that HGPS human mesenchymal progenitor cells (hMPCs) (*LMNA*$^{G608G/+}$ hMPCs) or WS hMPCs (*WRN*$^{-/-}$ hMPCs) recapitulated premature aging phenotypes (Figure 1A), characterized by increased senescence-associated β-galactosidase (SA-β-gal)-positive cells (Figure S1A). Reduced cellular proliferation rates were also observed in HGPS and WS hMPCs, as well as in replicatively senescent (RS) wild-type (WT) hMPCs, as evidenced by fewer Ki67-positive cells and decreased clonal expansion ability (Figures S1B–S1F).[31–35,37]

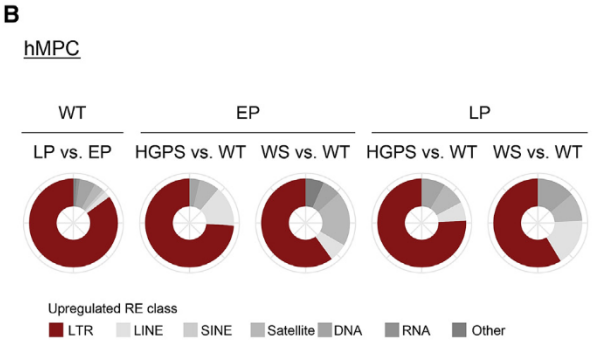
Here, we leveraged these premature aging models to test whether the activation of the ERVs is associated with human cellular senescence. We found an increased expression of several transposable elements in senescent hMPCs, such as LTRs (Figures 1B and S1G; Table S1). Specifically, we found that retroelement HERVK internal coding sequences (HERVK-int) were upregulated in both replicatively and prematurely senescent hMPCs (Figures 1C and S1H; Table S1). Using primers targeting different regions of HERVK transcripts, including *env*, *pol*, and *gag*, we confirmed by quantitative reverse transcriptase PCR (qRT-PCR) that HERVK retroelements were highly expressed during cellular senescence, with a similar increase of the senescence marker p21^{Cip1} (*CDKN1A*), whereas lamina-associated protein LAP2 (*TMPO*) decreased during senescence as reported previously (Figure 1D).[38] Likewise, an RNA fluorescence *in situ* hybridization (RNA-FISH) analysis also showed increased HERVK RNA signals in HGPS and WS hMPCs (Figure S1I). When we performed single-molecule RNA-FISH (smRNA-FISH) with different fluorescent probes targeting *LTR5_Hs*, *env*, and *pol*, we detected co-staining signals in close proximity, implying that mRNA molecules harboring *LTR5_Hs* (the transcriptional regulatory region of HERVK), *env*, and *pol* are present in senescent hMPCs (Figure 1E).

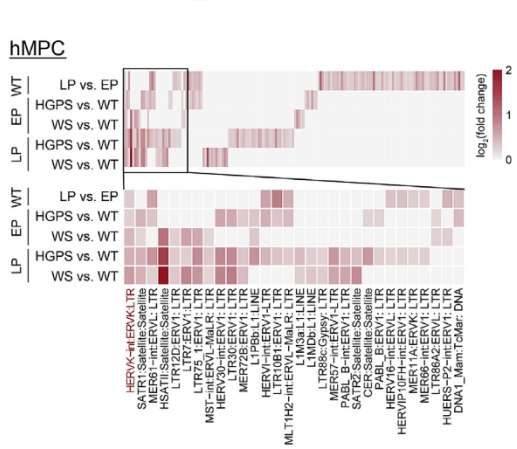
Consistent with the increased expression of HERVK transcription in senescent hMPCs, we observed reduced CpG DNA methylation levels in HERVK-int regions and those HERVK proviral loci (Figures 1F, S1J, and S1K),[39,40] alongside a decrease in the repressive histone mark (H3K9me3) and an increase in the transcriptionally active histone mark (H3K36me3) at HERVK-*LTR5_Hs* (Figures 1G and S1L). These data indicate that the epigenetic derepression of HERVK, likely contributing to HERVK transcription, is associated with cellular senescence.

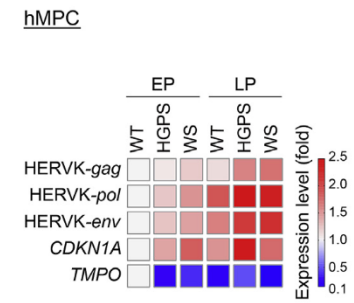
Accumulation of viral proteins and RVLPs of HERVK in various types of senescent human cells

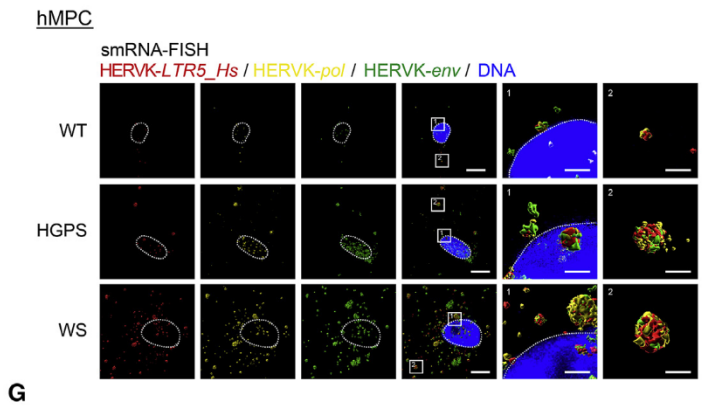
Next, we asked whether elevated endogenous retrovirus expression would elicit the production of HERVK protein components and even the formation of RVLPs. Western blotting and immunofluorescence staining analyses showed increased HERVK-Env protein levels in both prematurely senescent and RS hMPCs (Figures 2A, 2B, and S2A). Moreover, by co-staining HERVK-Env with SPiDER-βGal or p21^{Cip1}, we found that the elevation of HERVK-Env was more pronounced in the senescent cell population (Figures S2B and S2C). In line with the increased levels of HERVK mRNA and protein, we detected an accumulation of RVLPs in the cytoplasm of both prematurely senescent and RS hMPCs by transmission electron microscopy (TEM) analysis; by contrast, RVLPs were very rare in early-passage WT hMPCs that were phenotypically young (Figures 2C and S2D). Using immuno-TEM analysis with an anti-HERVK-Env antibody,[41] we found that these RVLPs, with a diameter ranging from 80 to 120 nm, were labeled with HERVK-Env antibody in senescent cells (Figures 2D,

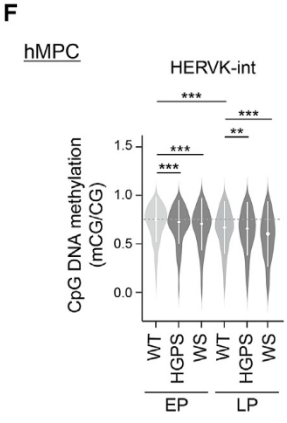


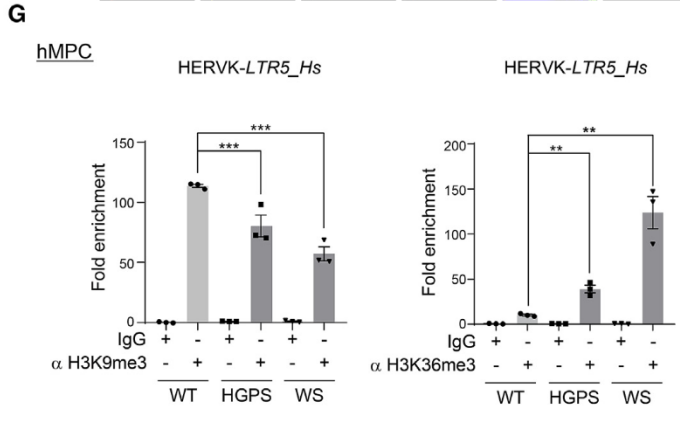












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S2E, and S2F). These findings demonstrate the increased production of both viral proteins and RVLPs of HERVK in senescent hMPCs.

In order to verify the expression of HERVK in other senescent cell types, we isolated primary human fibroblasts and hMPCs. Similar to senescent hMPC models, we observed increased HERVK-Env protein levels in the RS primary fibroblasts (Figures 2E, 2F and S2G–S2I). In addition, we found decreased occupancy of H3K9me3 and increased occupancy of H3K36me3 at HERVK-*LTR5_Hs* in human fibroblasts during replicative senescence (Figure 2G). We also observed an increased accumulation of RVLPs in these senescent primary fibroblasts (Figure 2H). Furthermore, we found prominent HERVK expression evidenced by a more than 5-fold increase in transcript levels in primary hMPCs derived from old individuals compared with their younger counterparts (Figures 2I and S2J; Table S2). Collectively, through multimodal experiments, we verified aberrant HERVK expression, as well as viral protein and RVLP accumulation in senescent cells.

Increased expression of HERVK drives cellular senescence

To determine how the activation of endogenous HERVK affects cellular senescence, we used a CRISPR-dCas9-mediated transcriptional activation (CRISPRa) system containing activation protein complexes (synergistic activation mediators [SAMs]) with sgRNAs targeting the HERVK-*LTR5_Hs* promoter regions in WT hMPCs (Figure S3A).[42] We confirmed the activation of HERVK endogenous retrotransposon elements by qRT-PCR and western blotting (Figures 3A and S3B). We found that targeted HERVK activation induced hMPC senescence, as evidenced by the induction of classic senescent features (Figures 3B, 3C, S3B, and S3C). To further assess whether suppression of endogenous HERVK inhibited cellular senescence, we used the CRISPR-dCas9-KRAB transcriptional inactivation (CRISPRi) system[43] (Figures 3D, S3D, and S3E) to repress HERVK in prematurely senescent hMPCs and found that targeted HERVK repression alleviated hMPC senescence (Figures 3E, 3F, S3E, and S3F). In addition, the knockdown of HERVK using HERVK-interfering shRNA[44,45] also antagonized premature senescence (Figures 3G, 3H, and S3G–S3I).

Given the observed aging-associated decrease of DNA methylation at the HERVK loci in hMPCs (Figures 1F and S1K), we conducted an independent experiment in which we treated early-passage WT hMPCs with the DNA methyltransferase inhibitor (DNMTi) 5-azacytidine (5-AZA) to mimic aging-related global hypomethylation (Figure 3I).[46,47] A whole-genome bisulfite sequencing (WGBS) analysis confirmed reduced CpG DNA methylation levels at HERVK elements after treatment (Figures 3J and S3J). Consistent with targeted HERVK activation by the CRISPRa system, 5-AZA treatment also led to upregulated HERVK RNA levels concomitant with premature cellular senescence phenotypes (Figures 3K–3M and S3K). By contrast, these senescence phenotypes were abrogated by HERVK knockdown (Figures S3L–S3N), suggesting that 5-AZA triggers cellular senescence at least partially via the DNA demethylation-induced transcriptional activation of HERVK. Moreover, we also activated endogenous HERVK in primary hMPCs derived from a young individual via CRISPRa or 5-AZA treatment (Figures S3O and S3P) and found that transcriptional activation of HERVK accelerated cellular senescence (Figures 3N–3Q). Taken together, through the disruption of the host epigenetic mechanism and targeted manipulation of HERVK transcriptional activity, we revealed that enhanced levels of endogenous HERVK are a driver of hMPC senescence.

HERVK expression triggers the innate immune response

HERVK-encoded Pol protein possesses reverse transcription activity that can reverse-transcribe HERVK RNA into DNA,[16,17] thereby generating additional HERVK DNA outside of the genome. Consistent with increased HERVK RNA (Figures 1D and S1I), we also observed increased HERVK DNA in the cytoplasm of senescent hMPCs by single-molecule DNA-FISH (Figures 4A and S4A). Therefore, we suspected that such excessive cytoplasmic DNA might be recognized by the DNA sensor cGMP-AMP synthase (cGAS) and trigger activation of the innate immune system (Figure 4B).[48–50] Indeed, by immunoprecipitation analysis, we verified the marked enrichment of cGAS on cytoplasmic HERVK DNA in senescent hMPCs, which was not the case in early-passage young hMPCs (Figure 4C). Supporting cytosolic HERVK DNA triggering the activation of the cGAS-Stimulator of interferon genes (STING) pathway, we detected increases in 2′3′-cGAMP content (Figure 4D) and the phosphorylation of TANK-binding kinase 1 (TBK1), RelA, and IFN regulatory factor 3 (IRF3) (Figure 4E). We also observed the upregulation of inflammatory cytokines, including *IL1B* (IL1β) and *IL6*, both of which are classified as SASP factors[51,52] (Figures 4F, 4G, and S4B; Table S3) in prematurely senescent hMPCs. The activation of the cGAS-STING-mediated innate immune response was also revealed in RS hMPCs (Figures S4C–S4E; Table S3) and RS fibroblasts (Figures S4F–S4H).

Consistently, the inhibition of HERVK via shRNA decreased the phosphorylation levels of TBK1 and RelA (Figure 4H). In addition, blocking one downstream effector of HERVK activation via the knockdown of STING reduced both the inflammatory response and SASP expression (Figures 4I and S4I), thus

Figure 1. Epigenetic derepression of HERVK is observed in senescent hMPCs

(A) Schematic diagram of human stem cell aging models and the HERVK proviral genome structure.

(B and C) Ring plots showing the percentages of upregulated repetitive elements in each class (B) and heatmap showing the relative expression levels for upregulated RepeatMasker-annotated repetitive elements (C) in RS and prematurely senescent hMPCs at early passage (EP) and late passage (LP).

(D) Heatmap showing the levels of HERVK transcripts and senescence marker genes in WT, HGPS, and WS hMPCs at EP and LP as detected by qRT-PCR.

(E) Representative z stack three-dimensional (3D) reconstruction images of smRNA-FISH in WT, HGPS, and WS hMPCs with probes targeting HERVK-*LTR5_Hs*, *-pol*, and *-env* with different fluorophores.

(F) Violin plot showing the CpG DNA methylation levels for HERVK-int in RS and prematurely senescent hMPCs.

(G) ChIP-qPCR analysis of H3K9me3 and H3K36me3 enrichment in HERVK-*LTR5_Hs* regions in WT, HGPS, and WS hMPCs.

Scale bars, 10 μm and 100 nm (zoomed-in image) in (E).

See also Figure S1 and Table S1.

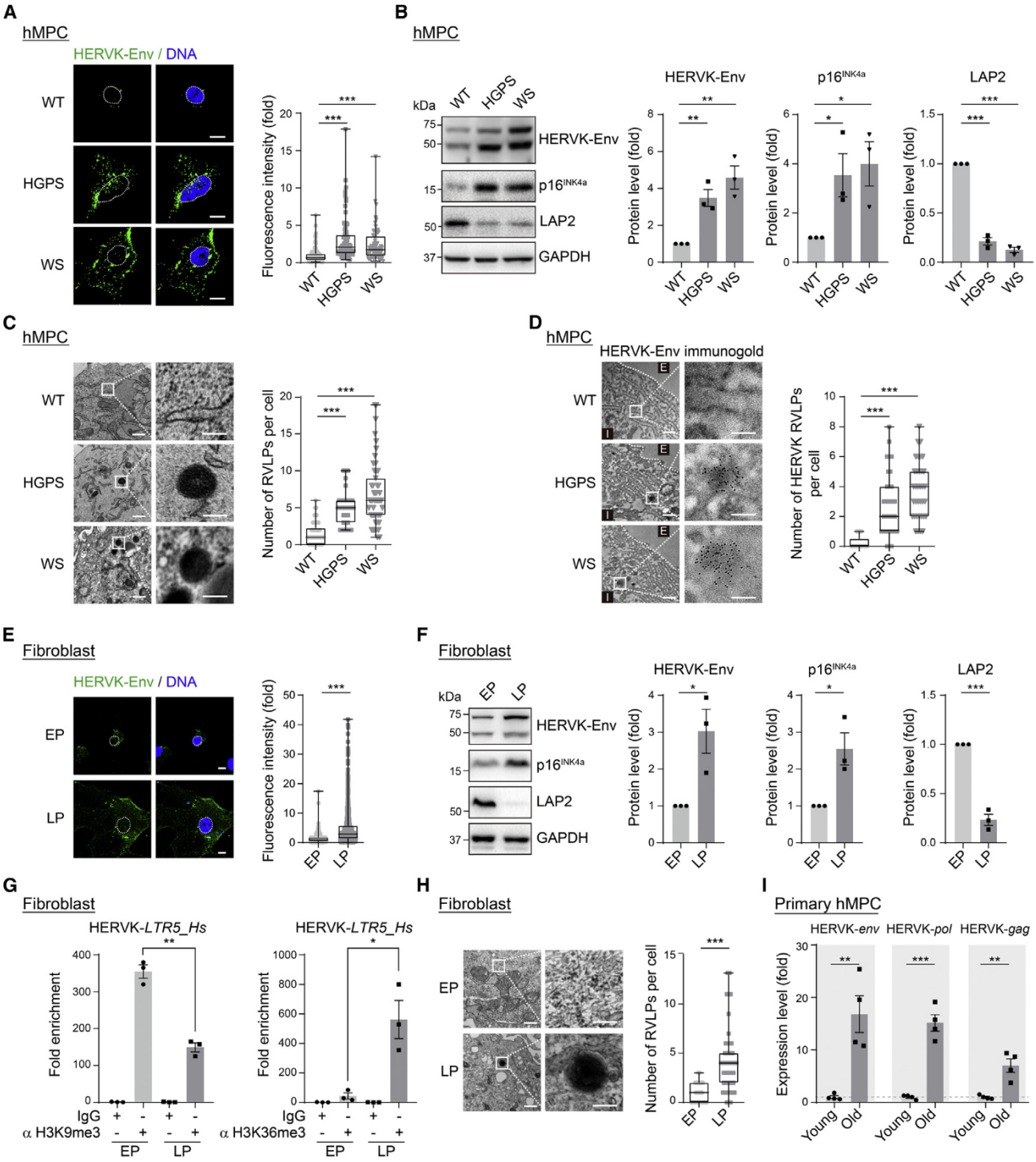

Figure 2. HERVK viral proteins and RVLPs are increased in senescent cells

(A) Immunofluorescence staining of HERVK-Env in WT, HGPS, and WS hMPCs.

(B) Western blotting of HERVK-Env, p16^{INK4a}, and LAP2 in WT, HGPS, and WS hMPCs.

(C) TEM analysis of WT, HGPS, and WS hMPCs.

(D) TEM analysis after immunogold labeling with anti-HERVK-Env antibody in WT, HGPS, and WS hMPCs. E, Extracellular; I, Intracellular.

(E) Immunofluorescence staining of HERVK-Env in primary human fibroblasts at EP and LP.

(F) Western blotting of HERVK-Env, p16^{INK4a}, and LAP2 in primary human fibroblasts at EP and LP.

(G) ChIP-qPCR analysis of H3K9me3 and H3K36me3 enrichment in HERVK-*LTR5_Hs* regions in primary human fibroblasts at EP and LP.

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alleviating cellular senescence in prematurely senescent hMPCs (Figure 4J). Moreover, we also blocked the downstream effect through Abacavir, a potent nucleoside reverse transcriptase inhibitor that can inhibit the activity of HERVK-encoded Pol (Figure 4K).[53] Senescent hMPCs treated with Abacavir demonstrated diminished HERVK DNA content (Figure 4L), along with substantial alleviation of a panel of senescence-associated phenotypes (Figures 4M–4O, S4J, and S4K). In contrast to the loss-of-function experiments described above, the activation of endogenous HERVK via the CRISPRa system or 5-AZA treatment led to an augmented innate immune response and upregulated expression of SASP cytokines in young WT hMPCs (Figures 4P–4S, S4L, and S4M; Table S4). These findings place the increased expression of HERVK as a contributing factor for cellular senescence, at least in part by triggering innate immune responses.

Extracellular HERVK RVLPs induce cellular senescence

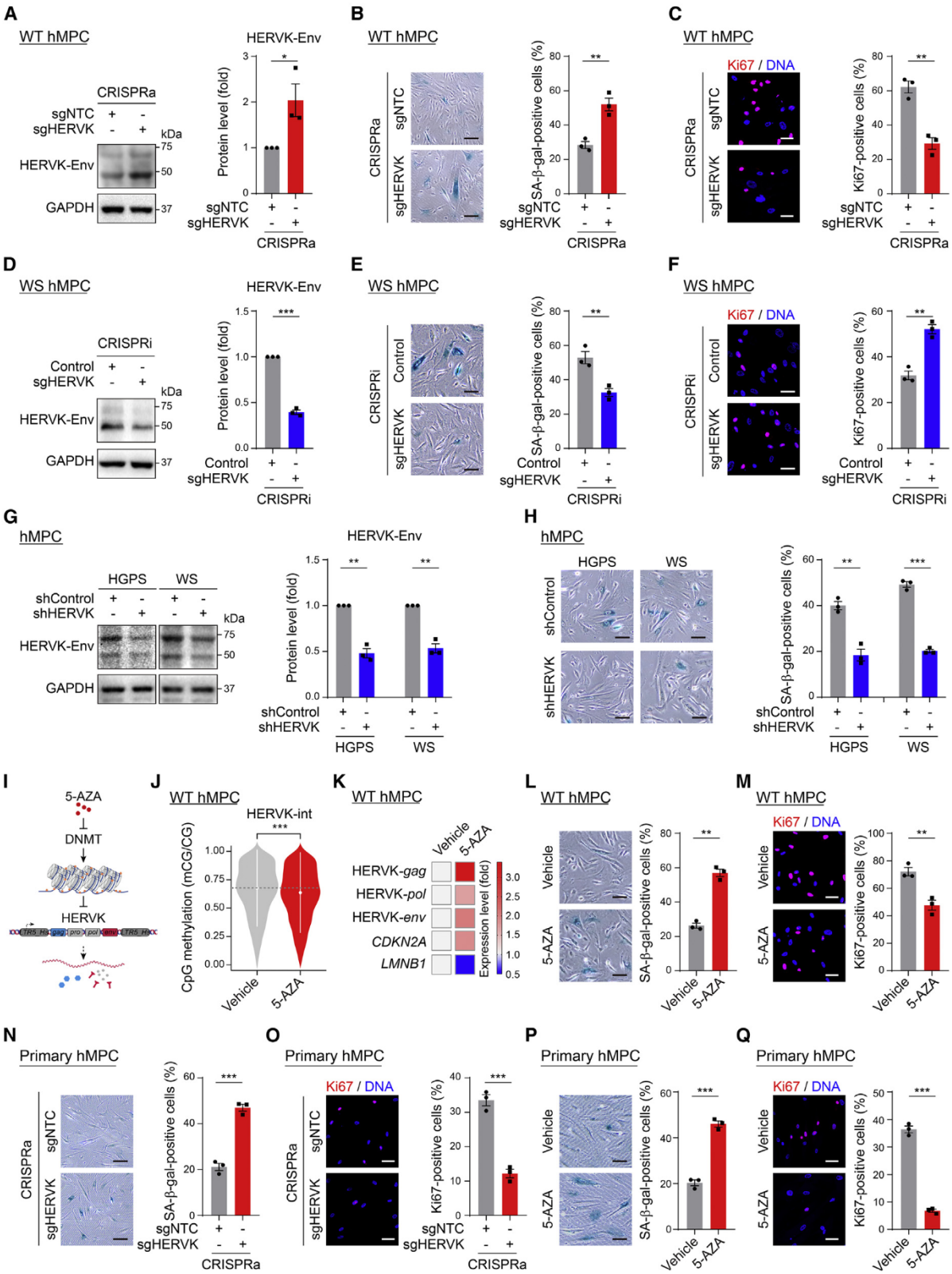
Previous studies indicated that tumor cell-derived HERVK RVLPs could be released into the culture medium and then taken up by other cells.[54] Given that the presence of HERVK RVLPs in senescent cells was observed in this study (Figures 2C, 2D, and S2D–S2F), we asked whether HERVK RVLPs produced by senescent cells could be released extracellularly and convey senescence signals to non-senescent cells (Figure 5A). To answer this question, we employed the sensitive droplet digital PCR (ddPCR) technology[55] to detect HERVK RNA (the genetic material that is supposed to be packaged in RVLPs) in conditioned medium (CM) harvested from WT and prematurely senescent hMPCs. In CM from prematurely senescent hMPCs, we found that the HERVK RNA level was 5 to 12 times higher than that in CM from young WT hMPCs (Figure 5B). In addition, both enzyme-linked immunosorbent assay (ELISA) and western blotting demonstrated increased HERVK-Env protein levels in CM from prematurely senescent hMPCs (Figures 5C and S5A), as well as in CM from RS hMPCs (Figures S5B and S5C). Furthermore, through TEM and immuno-TEM analyses, we detected RVLPs with diameters spanning from 80 to 120 nm, primarily on the outside of senescent hMPCs—some HERVK viral particles were detected budding from or adjacent to the cell surface (Figures 5D and S5D), whereas some particles were contained within coated vesicles in the extracellular environment (Figures S5D and S5E).

We next treated young hMPCs (WT) with CM collected from HGPS, WS, or RS hMPCs (referred to as senescent cell-conditional medium or SC-CM), using CM collected from young WT hMPCs as a control. With the TEM analysis, we found that more extracellular RVLPs adhered to the cell surface of young hMPCs or entered the young cells in the SC-CM-treated groups (Figure 5E). After SC-CM treatment, we observed an increased HERVK abundance in young hMPCs (Figure 5F), implying that HERVK in SC-CM may be transmitted into target cells. Moreover, we found that the invaded HERVK elements were associated with an ‘‘aging-promoting’’ effect; that is, young hMPCs that were incubated with SC-CM also underwent accelerated cellular senescence (Figures 5F, 5G, and S5F).

To further investigate whether HERVK present in SC-CM is one of the major factors that causes senescence in young hMPCs, we used an anti-HERVK-Env antibody[56] to immunodeplete HERVK. Western blotting confirmed the pull-down of both HERVK-Env and Gag proteins in SC-CM after incubation with the anti-HERVK-Env antibody but not in SC-CM incubated with the IgG control (Figure S5G). Accordingly, the ELISA data showed the depletion of HERVK-Env from SC-CM after incubation with the anti-HERVK-Env antibody (Figure S5H). As expected, after HERVK immunodepletion, we observed that fewer HERVK RVLPs adhered to the surface or were present in young hMPCs (Figure 5H). Furthermore, the immunodepletion of HERVK resulted in the reduction of HERVK abundance and alleviation of senescence phenotypes in young WT hMPCs compared with those treated with SC-CM without immunodepletion (Figures 5I, S5I, and S5J). Moreover, SC-CM activated the cGAS-STING pathway and induced the expression of SASP genes in young hMPCs (Figures 5F, 5J, and S5K). Importantly, the knockdown of STING rescued the senescent phenotypes induced by SC-CM (Figures 5J, 5K, and S5K), indicating that SC-CM, similar to endogenous HERVK expression, drove cellular senescence at least partially by activating the innate immunity pathway. Collectively, these data suggest that retroviral HERVK elements generated in senescent cells can be released in a paracrine manner and trigger cellular senescence in non-senescent cells.

Next, in order to verify that the infection of extracellular HERVK RVLPs is a direct driver of senescence, we employed our constructed expression vector containing synthesized full-length HERVK with a GFP cassette fused within Env to produce RVLPs (Figures S5L and S5M).[57,58] We then infected young hMPCs with purified HERVK RVLPs (Figures 5L and 5M) and found that both the RNA levels of HERVK and GFP fragments were upregulated in young hMPCs (Figure S5N). Young hMPCs infected with HERVK RVLPs, similar to those treated with SC-CM, showed typical premature senescence phenotypes (Figure 5N), and resembling SC-CM with immunodepletion, HERVK RVLP neutralization with the anti-HERVK-Env antibody abrogated HERVK RVLP-induced cellular senescence (Figures 5O, S5O, and S5P), such abrogation was also achieved by Abacavir treatment (Figures 5P, S5Q, and S5R). We further found an increased enrichment of cGAS on HERVK DNA in recipient hMPCs after infection with HERVK RVLPs (Figure 5Q). Additionally, we also detected the activation of cGAS-STING-mediated innate immune responses in infected hMPCs (Figures 5R, 5S, and S5S). Of note, these phenotypes were abrogated by neutralization with an anti-HERVK-Env antibody or treatment with Abacavir (Figures 5R and 5S), demonstrating that the cGAS-STING pathway drives the acquisition of senescence

(H) TEM analysis of primary human fibroblasts at EP and LP.
(I) qRT-PCR analysis showing the relative expression of HERVK in primary hMPCs from young and old donors.
Scale bars: 10 μm in (A) and (E); 100 and 200 nm (zoomed-in image) in (C), (D), and (H).
See also Figure S2 and Table S2.



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caused by HERVK RVLPs. Taken together, our data support that extracellular HERVK RVLPs transmit aging information to young cells.

Endogenous retrovirus can be used as a biomarker of aging

Next, we measured the levels of endogenous retroviral elements in aged primates and human individuals. ERVW is another endogenous retrovirus subfamily that persists in Old World monkeys due to infection of the ancestral germ line when apes and Old World monkeys diverged about 25 million years ago.[59,60] RT-qPCR results revealed that both ERVK and ERVW elements were increased in aged cynomolgus monkeys (Figure S6A), and with available antibodies,[61] the ERVW-Env protein levels were confirmed to increase in lung, liver, and skin tissues in physiologically aged cynomolgus monkeys relative to younger counterparts (Figures 6A–6C). Consistently, we also found that the innate immune responses in aged monkey tissues were increased, as evidenced by the upregulation of p-RelA (Figure 6B) and SASP factors (Figures S6B–S6D). Furthermore, when comparing lung, liver, and skin tissues isolated from both WT and HGPS cynomolgus monkeys that recapitulated the premature aging phenotypes of HGPS patients,[62] we found that the protein levels of ERVW-Env, along with p-RelA, were also increased in HGPS cynomolgus monkeys (Figures 6D–6F).

Finally, we sought to investigate whether elevated expression of HERVK is observed in human tissues during physiological aging. Likewise, in the skin and serum samples obtained from young and old donors,[63] HERVK-Env expression was markedly increased with age (Figures 6G–6I; Table S2). To ascertain whether HERVK in the serum from old individuals is a factor that drives aging, we cultured young primary hMPCs with a medium that contained serum from young or old individuals (Figure S6E). Strikingly, we found that serum from old individuals increased HERVK abundance, elicited innate immune responses, and accelerated cellular senescence in primary hMPCs, while this pro-senescence capability was abolished upon HERVK immunodepletion (Figures S6F–S6H). Taken together, these results indicate that human endogenous retrovirus HERVK may serve as a potential biomarker to assess human aging as well as a potential therapeutic target for alleviating tissue and cellular senescence.

Targeting endogenous retrovirus alleviates tissue aging

Next, we attempted to inhibit the expression of ERVs in aged mice. Unlike humans, mice harbor a range of active ERVs among which mouse mammary tumor virus (MMTV), is also a known beta retrovirus and is most closely related to that of HERVK (HML-2).[64,65] Similar to the increased levels of ERV elements as we observed in primate and human tissues during aging, MMTV-Env levels were increased in the lung, liver, and skin tissues of aged mice relative to young mice, alongside the activation of the innate immunity and inflammatory pathways therein (Figures S7A–S7C). Thus, with some evolutionary conservation, both primate and rodent ERVs are reactivated during aging.

Articular degeneration, or osteoarthritis (OA), is the most common joint pathology with aging and has been attributed to the senescence of mesenchymal progenitor cells.[66–72] Its pathology is demarcated by decreased numbers of Ki67-positive cells and reduced cartilage thickness (Figures S7D and S7E). In aged mice, we found that MMTV was substantially upregulated in the articular cartilage (Figure 7A), suggesting that increased levels of ERVs could be a potential driver for aging-associated articular degeneration. Consistently, when we performed intra-articular injections of lentivirus carrying CRISPRi-dCas9/sgMMTV (sgRNA targeting MMTV) to repress MMTV (Figures 7B, 7C, and S7F), we detected phenotypes indicative of the alleviation of tissue aging (Figures 7D, 7E, S7G, and S7H). We also observed structural and functional improvements in the joints of aged mice upon MMTV inhibition, as revealed by increased cartilage thickness and joint bone density, as well as enhanced grip strength (Figures 7F, 7G, and S7I).

Our findings that Abacavir treatment can inhibit the senescence-promoting effect of endogenous retrovirus (Figures 4N, 4O, 5P, 5S, S4K, and S5R) lay a foundation for further applying Abacavir to aging intervention *in vivo*. To this end, we performed weekly Abacavir injections into the articular cavities of 22-month-old mice (Figure 7H). Similar to the lentiviral knockdown of MMTV using a CRISPRi system, we found that Abacavir treatment reduced cartilage degeneration, as evidenced by mitigated senescence and attenuated aging-associated inflammation (Figures 7I, 7J, S7J, and S7K). We also detected alleviation of aging-related articular degeneration, including augmented cartilage thickness, bone density, and grip strength (Figures 7K, 7L, and S7L). Finally, we asked whether Abacavir treatment could more generally improve health in aged mice. To this end, we treated 18-month-old mice with Abacavir dissolved in daily drinking water for 6 months (Figure 7M). Strikingly, we detected increased grip strength, improved overall physical score, and improved short-term memory in aged mice that were treated with Abacavir relative to untreated mice (Figures 7N–7P). Taken together, these data indicate that repression of endogenous retrovirus *in vivo* alleviates tissue aging and, to some extent, organismal aging.

Figure 3. Increased HERVK drives senescence

(A–H) Western blotting of HERVK-Env, SA-β-gal, and Ki67 staining of WT hMPCs transduced with lentiviruses expressing sgNTC or sgHERVK using a CRISPRa system (A)–(C), those expressing control or sgHERVK using a CRISPRi system (D)–(F), and those delivering shControl or shHERVK (G) and (H).

(I) Schematic diagram showing the experimental design for activating HERVK with 5-AZA.

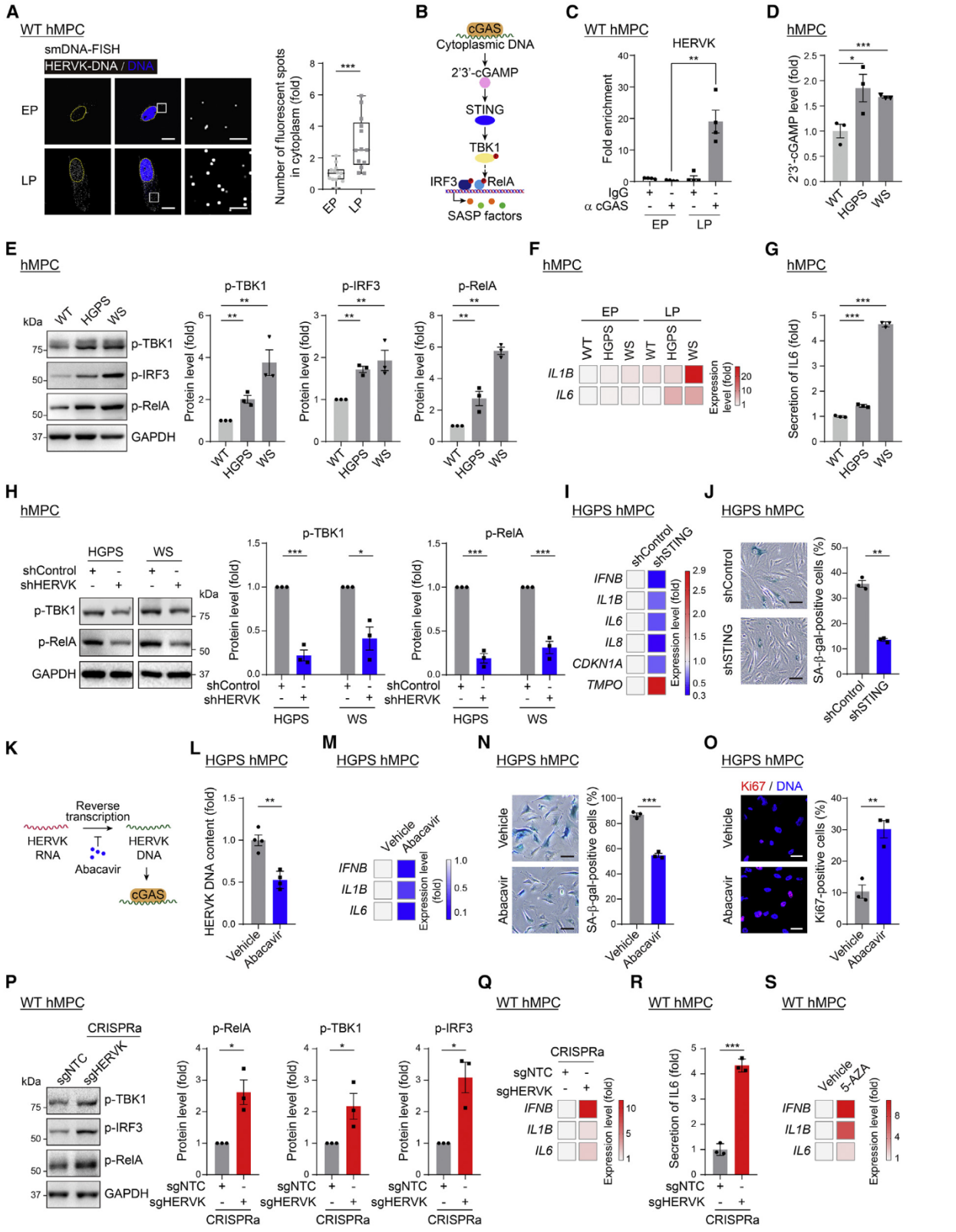
(J) Violin plot showing the relative CpG DNA methylation levels for HERVK in WT hMPCs treated with vehicle or 5-AZA.

(K) Heatmap showing the expression levels of HERVK-*gag/pol/env*, *CDKN2A*, and *LMNB1* in WT hMPCs treated with vehicle or 5-AZA by qRT-PCR analysis.

(L–Q) SA-β-gal and Ki67 staining of WT hMPCs treated with vehicle or 5-AZA (L) and (M), of primary hMPCs from a young individual transduced with lentiviruses expressing sgNTC or sgHERVK using the CRISPRa system (N) and (O), and of vehicle- or 5-AZA-treated primary hMPCs from a young individual (P) and (Q).

Scale bars, 20 μm (all panels).

See also Figure S3.



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DISCUSSION

In this study, using various primate and rodent aging models, we discovered a positive feedback loop between endogenous retrovirus activation and aging. Our comprehensive analysis unraveled the causal relationship between HERVK and aging in multiple species and was supported by pioneering studies showing increased expression of ERVs concomitant with aging in yeast, fly, and rodent models.[73–75] For instance, the activation of gypsy, a transposable element in *Drosophila* showing homology with vertebrate ERVs, was reported in aged flies.[76] In addition, emerging studies suggested a correlation between awakened ERVs and aging-related disorders, such as rheumatoid arthritis and neurodegenerative diseases.[42,77–81] More importantly, we successfully employed multiple strategies to block the pro-senescence effect of ERVs, demonstrating the alleviation of aging defects across cellular models and multiple tissues *in vivo*. In line with our results, attempts to alleviate neurodegenerative disorders including amyotrophic lateral sclerosis (ALS) via the inhibition of HERVK were reported.[82,83] Thus, ERVs represent druggable targets for alleviating aspects of aging and improving overall organismal health.

As another type of retrotransposon element, LINE1 can also be activated during senescence and age-associated degeneration, and exert certain pro-senescence effects.[8,10,12,84,85] Moreover, inhibition of LINE1 by reverse transcriptase inhibitors has been reported to alleviate aging-related phenotypes and extend the healthspan of mice.[9,11] However, unlike LINE1, which is incapable of producing viral particles and thus acts primarily in a cell-autonomous manner, our study provides evidence that the aging-induced resurrection of endogenous retrovirus (AIR-ERV) not only exerts a devastating cell-autonomous role but also triggers secondary senescence in a paracrine manner.

In summary, our research provides experimental evidence that the conserved activation of ERVs is a hallmark and driving force of cellular senescence and tissue aging. Our findings make fresh insights into understanding aging mechanisms and especially enrich the theory of programmed aging. As such, our work lays the foundation for understanding the contagiousness of aging, opening avenues for establishing a scientific method for evaluating aging and developing clinical strategies to alleviate aging.

Limitations of the study

Although we have provided evidence indicating the transmission process of HERVK RVLPs from senescent cells to young ones, more advanced technologies are required to monitor the exocytosis and endocytosis of viral particles by the donor and recipient cells in real time. It should be noted that a careful decision based on multiple indicators, beyond SA-β-gal activity alone,[86,87] is urgently needed when evaluating HERVK-mediated cellular senescence. In addition, as short-read sequencing cannot be utilized to pinpoint the full-length copy of HERVK, further investigations are necessary to address whether an intact HERVK provirus may exist and how the full life cycle of a HERVK provirus is completed during aging. As we demonstrated the effectiveness of aging alleviation via targeted inhibition of HERVK/MMTV in hMPCs and mice, it will be of great significance to perform preclinical trials in non-human primates for future translational applications. In addition, future studies will be needed to characterize endogenous retrovirus expression profiles across ages and species in large populations and examine the results with other aging clocks, such as DNA methylation and telomere length.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
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- EXPERIMENTAL MODEL AND SUBJECT DETAILS
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- METHOD DETAILS
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  - Grip strength test
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Figure 4. Increased HERVK activates the innate immunity pathway

(A) HERVK smDNA-FISH in RS WT hMPCs.

(B) Schematic diagram showing the innate immune response through the cGAS-STING pathway.

(C) Immunoprecipitation assay followed by qPCR analysis to assess cGAS enrichment on cytoplasmic HERVK DNA fragments in RS WT hMPCs.

(D–G) ELISA analysis of 2′3′-cGAMP levels (D), western blotting of p-TBK1, p-IRF3, and p-RelA (E), qRT-PCR analysis of the levels of SASP genes (F), and ELISA analysis of IL6 levels in the culture medium (G), in WT, HGPS, and WS hMPCs.

(H) Western blotting of p-TBK1 and p-RelA in HGPS or WS hMPCs after transduction with lentiviruses delivering shControl or shHERVK.

(I and J) qRT-PCR analysis of the levels of SASP and senescence marker genes (I), and SA-β-gal staining (J) of HGPS hMPCs after transduction with lentiviruses expressing shControl or shSTING.

(K) Schematic diagram showing the experimental design for repressing HERVK with Abacavir.

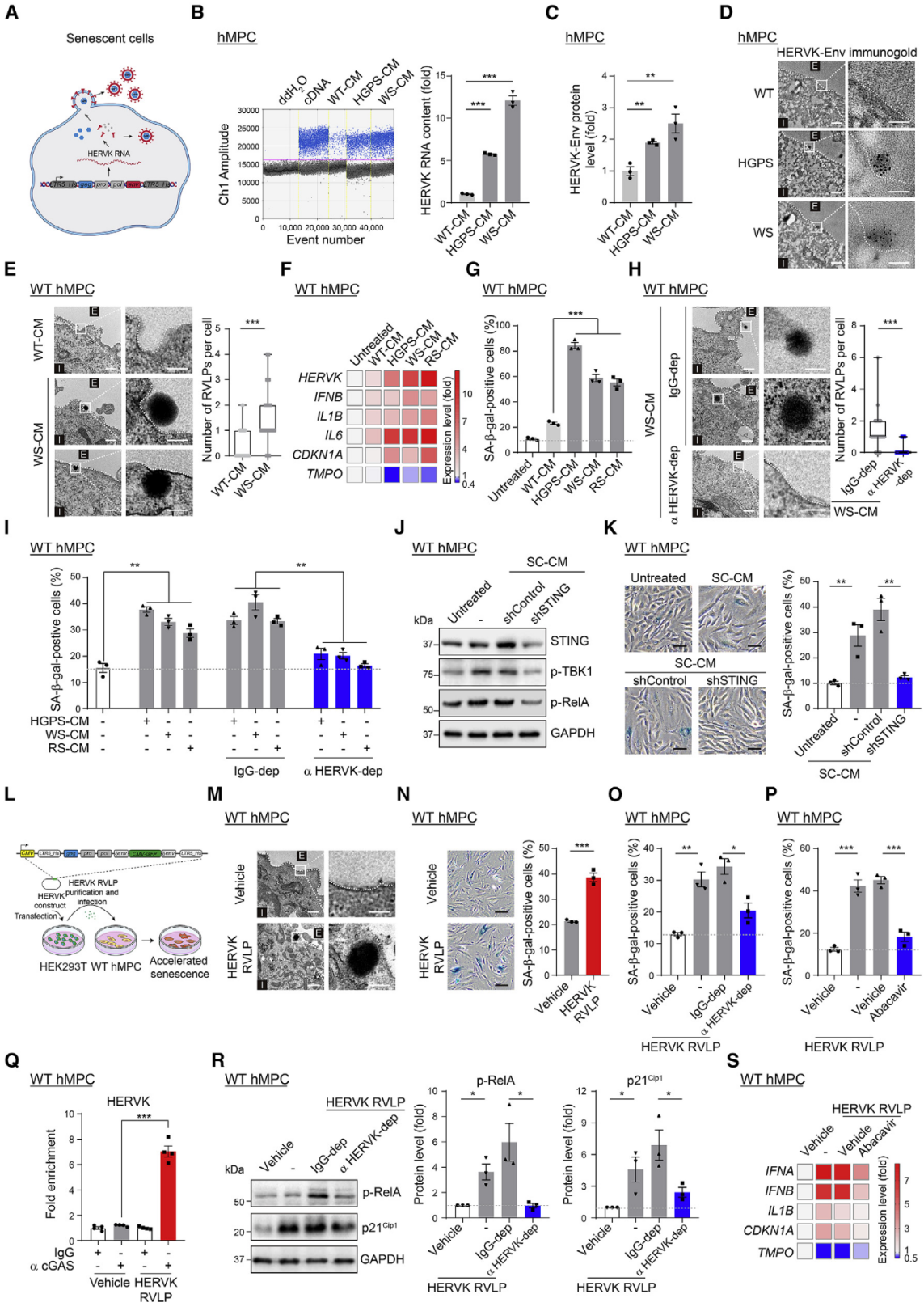
(L–O) qPCR analysis of the HERVK DNA contents (L) and qRT-PCR analysis of the expression of SASP genes (M), as well as SA-β-gal (N) and Ki67 (O) staining of HGPS hMPCs treated with vehicle or Abacavir.

(P–R) Western blotting showing the protein levels of p-TBK1, p-IRF3, and p-RelA (P), as well as qRT-PCR analysis of the expression of SASP genes (Q), and ELISA analysis of IL6 levels in the culture medium (R), in WT hMPCs transduced with lentiviruses expressing sgNTC or sgHERVK using a CRISPRa system.

(S) Heatmap showing the qRT-PCR analysis of levels of SASP genes in WT hMPCs treated with vehicle or 5-AZA.

Scale bars: 10 μm and 200 nm (zoomed-in images) in (A); and 20 μm in (J), (N), and (O).

See also Figure S4 and Tables S3 and S4.



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- *In vivo* bone histomorphometric analysis: Micro-Computed Tomography (Micro-CT)
- Western blotting
- Immunoprecipitation (IP) of HERVK from conditioned medium
- Purification of microvesicle particles from conditioned medium
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- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cell.2022.12.017.

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AUTHOR CONTRIBUTIONS

G.-H.L., J.Q., and W.Z. conceptualized the work and supervised the overall experiments. X.L. performed the cell culture and the experiments related to functional and mechanistic analyses, including western blotting, qRT-PCR, ddPCR, ChIP-qPCR, and immunofluorescence staining. Z.L. performed bioinformatic analyses. Z.W. performed cell culture and sequencing library construction. J.R. performed the manuscript writing. L.S. provided human serum samples. Y.F. performed immunohistochemistry staining in cynomolgus monkeys and humans. Y.N. provided HGPS cynomolgus monkey samples. G.C. and L.L. performed smRNA/DNA-FISH. X.J. performed fibroblast cell culture and functional analyses. Q.J., Y.E.Z., S.T., and Yingao Cai helped with the bioinformatic analyses. B.Z. performed statistical analysis of immunohistochemistry staining. C.W. performed the cell culture. Q.W. helped with the schematic diagram drawing. K.Y. helped with vector constructions. W.L. organized the animal

Figure 5. HERVK RVLPs released by senescent cells induce senescence in young cells

(A) Schematic diagram showing the proposed HERVK viral life cycle in senescent cells.

(B and C) Digital droplet PCR (ddPCR) analysis of HERVK RNA levels (B) and ELISA analysis of HERVK-Env protein levels (C) in the CM of WT, HGPS, and WS hMPCs.

(D) TEM analysis after immunogold labeling with anti-HERVK-Env antibody in WT, HGPS, and WS hMPCs.

(E) TEM analysis of young hMPCs treated with CM from young WT or senescent WS hMPCs.

(F and G) qRT-PCR analysis of the levels of *HERVK*, senescence marker genes and inflammatory cytokines (F), as well as statistical analysis of SA-β-gal-positive cells (G) in young hMPCs treated with SC-CM.

(H and I) TEM analysis (H) and statistical analysis of SA-β-gal-positive cells (I) in young hMPCs treated with SC-CM after immunodepletion with IgG or anti-HERVK-Env antibody.

(J and K) Western blotting of STING, p-TBK1, and p-RelA (J), as well as SA-β-gal staining (K) in young hMPCs treated with SC-CM after STING knockdown.

(L) Schematic diagram showing the experimental procedure for HERVK RVLP infection of WT hMPCs.

(M–P) TEM images (M) and SA-β-gal staining (N) of WT hMPCs infected with HERVK RVLPs, as well as SA-β-gal staining of WT hMPCs infected with HERVK RVLPs after pretreatment with IgG or anti-HERVK-Env antibody (O), or in the presence of Abacavir (P).

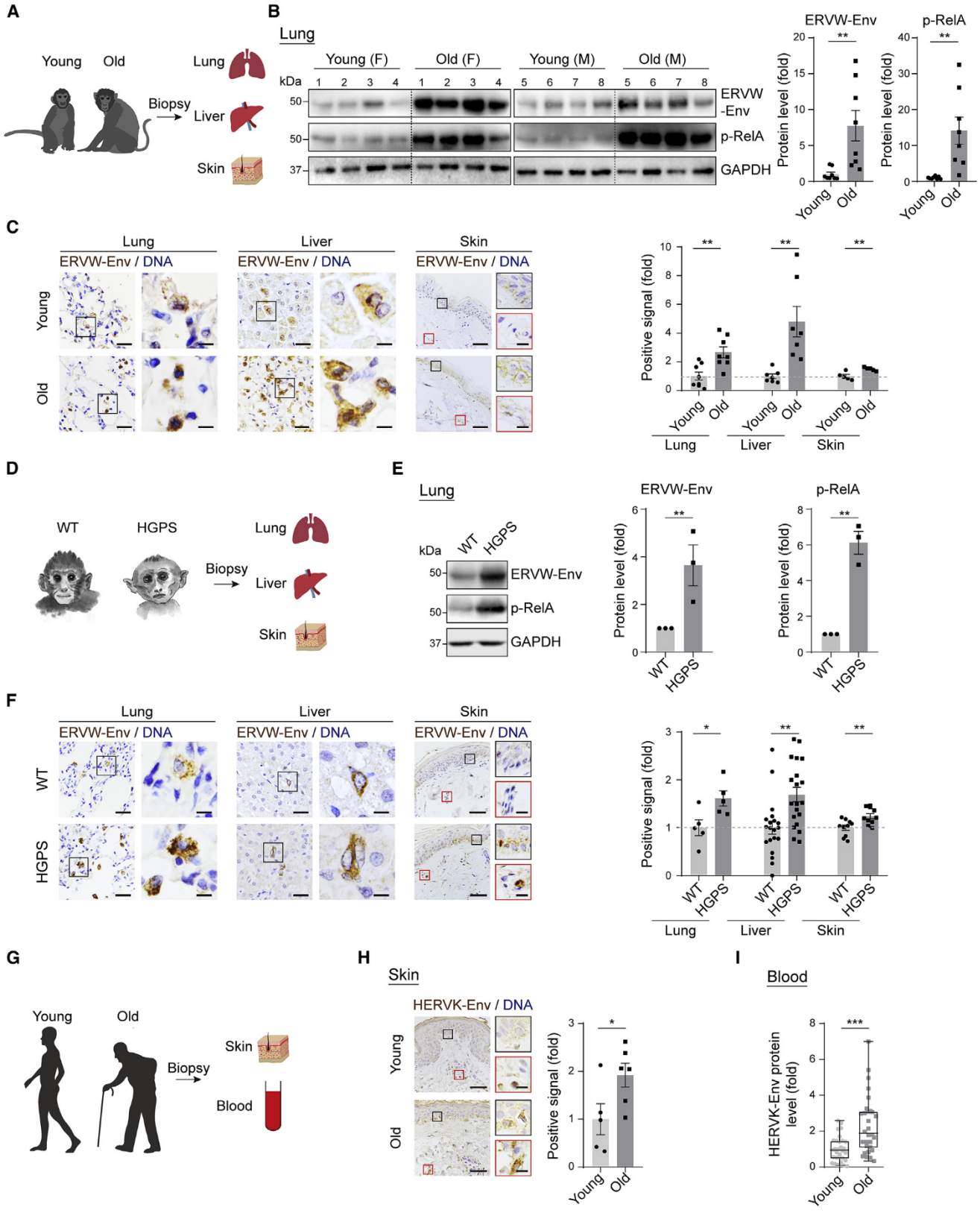
(Q) Immunoprecipitation assay followed by qPCR analysis to assess cGAS enrichment on HERVK DNA fragments in WT hMPCs infected with HERVK RVLPs.

(R) Western blotting of p-RelA and $p21^{Cip1}$ in WT hMPCs infected with HERVK RVLPs after pretreatment with IgG or anti-HERVK-Env antibody.

(S) Heatmap showing the qRT-PCR analysis of the expression levels of inflammatory cytokines and senescence marker genes in WT hMPCs infected with HERVK RVLPs in the presence of Abacavir.

Scale bars: 100 and 200 nm (zoomed-in images) in (D), (E), (H) and (M); and 20 μm in (K) and (N).

See also Figure S5.



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experiments. A.Z. helped with virus purification. W.J., Q.Z., J.C.I.B., C.R.E., F.L., and F.T. helped with supervision of the project. G.-H.L., J.Q., W.Z., J.R., X.L., Z.L., Z.W., Z.J., Yusheng Cai, S.W., and M.S. performed manuscript writing, reviewing, and editing. All authors reviewed the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Figure 6. Activation of endogenous retrovirus as a biomarker of aging

(A–F) Schematic diagram of samples (A), western blotting of ERVW-Env and p-RelA in the lungs (B), and immunohistochemistry analysis of ERVW-Env in the lungs, livers, and skin (C) from young and old cynomolgus monkeys or those from WT and HGPS cynomolgus monkeys (D)–(F).
(G–I) Schematic diagram of samples (G), immunohistochemistry analysis of HERVK-Env in the skins (H), and ELISA analysis of HERVK-Env levels in serum (I) from young and old human donors.
Scale bars, 50 and 10 μm (zoomed-in image) (all panels).
See also Figure S6 and Table S2.

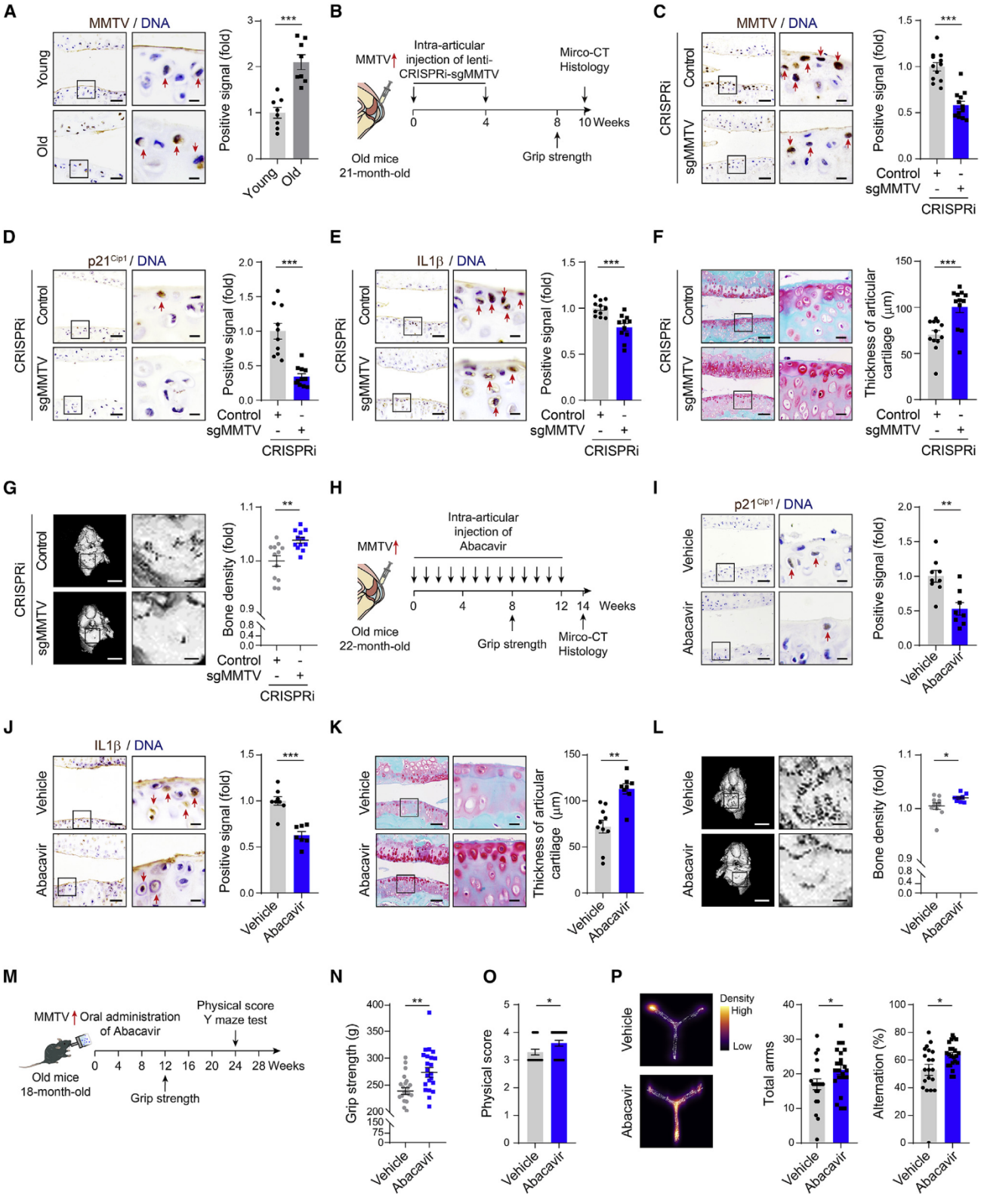

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Figure 7. Targeting endogenous retrovirus alleviates tissue aging

(A) Immunohistochemistry analysis of MMTV-Env in the articular cartilages of young and old mice.

(B–L) Schematic diagram showing the experimental procedure (B), immunohistochemistry analysis of MMTV-Env, $p21^{Cip1}$, IL1β, and Safranin-O/Fast Green staining of the articular cartilages (C)–(F), as well as micro-CT analysis showing the bone densities of the joints (G) of mice injected with lentiviruses expressing control or sgMMTV into articular cavities using a CRISPRi system, or those of mice intra-articularly injected with vehicle or Abacavir (H)–(L).

(M–P) Schematic diagram showing the experimental procedure (M), grip strength analysis (N), overall physical scores (O), and Y maze analysis (P) of old mice fed with vehicle or Abacavir in the drinking water.

Scale bars: 50 and 10 μm (zoomed-in images) in (A), (C)–(F), and (I)–(K); 500 and 100 μm (zoomed-in images) in (G) and (L).

See also Figure S7.

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