Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence

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Cellular senescence is triggered by various distinct stresses and characterized by a permanent cell cycle arrest. Senescent cells secrete a variety of inflammatory factors, collectively referred to as the senescence-associated secretory phenotype (SASP). The mechanism(s) underlying the regulation of the SASP remains incompletely understood. Here we define a role for innate DNA sensing in the regulation of senescence and the SASP. We find that cyclic GMP-AMP synthase (cGAS) recognizes cytosolic chromatin fragments in senescent cells. The activation of cGAS, in turn, triggers the production of SASP factors via stimulator of interferon genes (STING), thereby promoting paracrine senescence. We demonstrate that diverse stimuli of cellular senescence engage the cGAS–STING pathway in vitro and we show cGAS-dependent regulation of senescence following irradiation and oncogene activation in vivo. Our findings provide insights into the mechanisms underlying cellular senescence by establishing the cGAS–STING pathway as a crucial regulator of senescence and the SASP.

Senescence is a cellular program characterized by a permanent growth arrest of damaged or aged cells1,2. Cells undergoing senescence display profound phenotypic changes, which are driven by alterations in metabolism, chromatin organization and transcriptional activity. A prominent hallmark of senescent cells is the secretion of inflammatory mediators, including various cytokines, chemokines, extracellular matrix proteins and growth factors, collectively referred to as the senescence-associated secretory phenotype (SASP)3,4. Through the secretion of the SASP, senescent cells critically influence many biological processes, including wound healing5, tissue repair6, tumorigenesis7 or in vivo reprogramming8. Moreover, the inflammatory response linked to the SASP is considered to underlie many senescence-associated effects on ageing and age-related disorders9,10. Thus, understanding the regulation of the SASP is essential, both for deciphering the basis of senescent cell communication and also for discovering new targets controlling senescence effector responses. On a molecular level, several transcription factors have been implicated in the upregulation of SASP genes including NF-κB, C/EBP-β, p38 MAPK and GATA4 (refs 12–16). However, the upstream signalling pathway(s) that activate these transcriptional regulators within senescent cells remain incompletely characterized.

Inflammation is generally initiated through the activation of innate immune receptors, with the most mentionable being various pattern recognition receptors (PRRs)11. Despite being best known for the recognition of microbial products in the context of infection, some PRRs can also become activated by aberrant self-molecules. cGAS, which senses double-stranded DNA, represents a well-studied example of a PRR that responds equally to both microbial as well as endogenous DNA ligands12. Following activation, cGAS synthesizes the second messenger molecule 2’3’ cyclic GMP-AMP (cGAMP), which engages STING, thereby leading to the production of inflammatory cytokines, chemokines and type I interferons (IFNs)13,14. Although the localization within the cytosol usually prevents an unintended activation of the cGAS–STING signalling pathway, conditions wherein self-DNA gains access to the cytosol can trigger—occasionally detrimental—immunological responses. Genetic defects that compromise endogenous DNA metabolism or instances that trigger massive, synchronized accumulation of dead cells represent examples of this phenomenon15,16. However, whether self-DNA sensing by cGAS contributes to biological processes in a more ‘physiological’ manner remains unclear.

Here we report a function of innate DNA sensing through cGAS in the regulation of cellular senescence. Specifically, we
identified that senescent cells engage the cGAS–STING pathway, thereby regulating the SASP and facilitating paracrine senescence. We demonstrate that the activation of cGAS is based on its recognition of aberrant cytosolic chromatin fragments (CCFs), which arise in senescent cells as a consequence of nuclear lamin B1 degradation. We found that diverse triggers of cellular senescence, including oxidative stress, oncogene signalling, irradiation and pro-senescent drugs depend on cGAS–STING signalling to drive the production of inflammatory SASP components. Finally, we observed that cGAS-triggered senescence occurs following irradiation and oncogene activation in vivo. Together, these findings establish endogenous DNA sensing through the cGAS–STING pathway as an important regulator of senescence and the SASP.

**Deficiency of the cGAS–STING pathway impairs oxidative-stress-induced senescence**

While studying the function of cGAS, we repeatedly noticed that following serial passaging under standard culture conditions mouse embryonic fibroblasts (MEFs) deficient for cGAS (cGAS KO MEFs) exhibited an accelerated proliferation rate compared with wild-type (WT) MEFs. To explore this phenomenon in further detail, we cultured MEFs from an early passage on according to the 3T3 protocol and counted cells over several weeks. Comparing cell numbers obtained from the first few passages, we did not observe any difference in the proliferation rate of WT MEFs and cGAS KO MEFs (Fig. 1a). However, after serial passages WT MEFs proliferated less than cGAS KO MEFs (Fig. 1a). Quantifying the proliferation rate by 5-bromo-2’-deoxyuridine (BrdU) incorporation confirmed the increased replicative activity of cGAS KO MEFs compared with WT MEFs (Fig. 1b). In agreement with a senescent phenotype, WT cells became flat and enlarged and displayed high activity of senescence-associated β-galactosidase (SA-β-Gal) (Fig. 1c). In contrast, cGAS KO MEFs did not acquire these morphological changes and most of them did not become positive for SA-β-Gal (Fig. 1c). To systematically explore the altered phenotype of WT MEFs and cGAS KO MEFs, we performed gene expression profiling with RNA sequencing from cells cultured for 30–42 days. Notably, among the transcripts that we performed gene expression profiling with RNA sequencing from WT MEFs and cGAS KO MEFs (Fig. 1a). Afterward, by 5-bromo-2’-deoxyuridine (BrdU) incorporation confirmed the increased replicative activity of cGAS KO MEFs compared with WT MEFs (Fig. 1b). In agreement with a senescent phenotype, WT cells became flat and enlarged and displayed high activity of senescence-associated β-galactosidase (SA-β-Gal) (Fig. 1c). In contrast, cGAS KO MEFs did not acquire these morphological changes and most of them did not become positive for SA-β-Gal (Fig. 1c). To systematically explore the altered phenotype of WT MEFs and cGAS KO MEFs, we performed gene expression profiling with RNA sequencing from cells cultured for 30–42 days. Notably, among the transcripts that showed most increased expression in WT MEFs we found Cdkn2a (p16ink4a) and Cdkn2b (p15), crucial regulators of the senescent cell cycle arrest in eukaryotic cells. We found that addition of recombinant IFN-β to cultures of WT MEFs and cGAS KO MEFs relative to WT MEFs (Fig. 3c). Similarly, depletion of cGAS in WI-38 cells decreased the secretion of SASP components, which are controlled by cGAS, we analysed the CM from both WT MEFs and cGAS KO MEFs using a cytokine array. Remarkably, the secretion of several distinct SASP factors including IL-6, TNF-α and several chemokines depended on cGAS (Fig. 3c).

In the context of innate immunity the central function of the cGAS–STING pathway is the secretion of antiviral and inflammatory cytokines and chemokines. We therefore suspected that cGAS and STING might promote senescence indirectly through regulating the SASP. Indeed, we found that conditioned medium (CM) from WT MEFs exposed to oxidative stress promoted a senescence response (as indicated by decreased BrdU incorporation and increased SA-β-Gal activity) within both WT MEFs and cGAS KO MEFs. In contrast, CM from cGAS KO MEFs cultured under the same conditions failed to arrest MEFs. To better characterize the SASP components, which are controlled by cGAS, we analysed the CM from both WT MEFs and cGAS KO MEFs using a cytokine array. Remarkably, the secretion of several distinct SASP factors including IL-6, TNF-α and several chemokines depended on cGAS (Fig. 3c).

Moreover, the production of Cxcl10, a well-known cGAS-dependent interferon-stimulated gene (ISG), was reduced in cGAS KO MEFs relative to WT MEFs (Fig. 3c). Similarly, depletion of cGAS in WI-38 cells decreased the secretion of SASP components, which are controlled by cGAS, we analysed the CM from both WT MEFs and cGAS KO MEFs using a cytokine array. Remarkably, the secretion of several distinct SASP factors including IL-6, TNF-α and several chemokines depended on cGAS (Fig. 3c).

The cGAS–STING pathway promotes senescence in a paracrine manner

We next sought to determine how cGAS controls senescence. In the context of innate immunity the central function of the cGAS–STING pathway is the secretion of antiviral and inflammatory cytokines and chemokines. We therefore suspected that cGAS and STING might promote senescence indirectly through regulating the SASP. Indeed, we found that conditioned medium (CM) from WT MEFs exposed to oxidative stress promoted a senescence response (as indicated by decreased BrdU incorporation and increased SA-β-Gal activity) within both WT MEFs and cGAS KO MEFs. In contrast, CM from cGAS KO MEFs cultured under the same conditions failed to arrest MEFs. To better characterize the SASP components, which are controlled by cGAS, we analysed the CM from both WT MEFs and cGAS KO MEFs using a cytokine array. Remarkably, the secretion of several distinct SASP factors including IL-6, TNF-α and several chemokines depended on cGAS (Fig. 3c).

The induced expression of ISGs along with the reported role of type I IFNs in senescence prompted us to examine whether cGAS-mediated secretion of type I IFNs is involved in promoting oxidative-stress-induced senescence. We found that addition of recombinant IFN-β to cultures of WT MEFs and cGAS KO MEFs was sufficient to induce SA-β-Gal activity and p16ink4a expression and to promote cell cycle arrest (Fig. 3f,g and Supplementary Fig. 2f,g). Conversely, MEFs deficient for the type I IFN receptor (IFNAR KO MEFs) displayed a compromised oxidative-stress-induced senescence response (SA-β-Gal activity, BrdU incorporation, p16ink4a expression, IL-6 production, ISG induction) compared with WT MEFs (Fig. 3h–j and Supplementary Fig. 2h). Collectively, these data suggest a possible role for type I IFNs in relaying cGAS-mediated...
Figure 1 Absence of cGAS attenuates the senescence response. (a) Proliferation curve of primary wild-type (WT) and cGAS knockout (cGAS KO) mouse embryonic fibroblasts (MEFs) cultured under 20% O2. (b) Percentages of BrdU-positive WT or cGAS KO MEFs after 27 days in culture. (c) Left: images of SA-β-Gal-stained WT or cGAS KO MEFs are shown. Senescence markers are highlighted in green. (e,f) WT MEFs or cGAS KO MEFs were harvested after 3 weeks of culture and expression of depicted genes was measured via RT-qPCR or protein expression was analysed by immunoblotting. One representative experiment out of three (right) or mean and s.d. of n = 3 independent experiments (left) or n = 3 independent biological replicates (e) are shown. P values were calculated by unpaired t-test (∗∗ < 0.01, ∗∗∗ < 0.001, ∗∗∗∗ < 0.0001). Unprocessed original scans of blots are shown in Supplementary Fig. 7. Source data are available in Supplementary Table 1.

CCFs are recognized by cGAS in senescent cells

We next sought to characterize the DNA source responsible for the activation of cGAS in senescent cells. Degradation of the nuclear envelope component lamin B1 and the occurrence of CCFs are well-described features of senescent cells. In accordance with this, we found decreased expression of lamin B1 in senescent cells (Fig. 4a,b). Furthermore, using confocal fluorescence microscopy, we observed DNA-containing blebs budding off the nucleus as well as characteristic CCFs in cells undergoing oxidative-stress-induced senescence (Fig. 4c). We thus hypothesized that the herniation of nuclear DNA into the cytosol might represent the trigger for provoking cGAS-STING signalling in senescent cells. To investigate this, we reconstituted cGAS KO MEFs with a doxycycline (Dox)-inducible FLAG-tagged cGAS construct (MEFs Tet-cGAS-FLAG). Remarkably, in cells undergoing oxidative-stress-induced senescence, cGAS formed clusters that co-stained with DAPI-positive chromatin herniations (Fig. 4d). Likewise, endogenous cGAS co-localized with chromatin fragments at the nuclear-cytosolic border in senescent WI-38 cells (Supplementary Fig. 3). To assess whether the loss of...
Numerous stressors are able to induce senescence by signalling through various distinct pathways. We therefore sought to determine whether the activation of cGAS is a common feature of senescent cells. To this end, we exposed WT MEFs, cGAS KO MEFs and STING KO MEFs to distinct senescence-inducing stimuli and compared the expression of SASP components (IL-6, Cxcl10). We tested ionizing radiation (12 Gy) and palbociclib (PD-0332991), a CDK4 inhibitor, which mimics the effect of p16<sup>Ind44</sup>. Of note, the production of IL-6 or the induction of Cxcl10 was largely abolished in cGAS KO MEFs or STING KO MEFs (Fig. 5a). To complement the findings with human cells, we explored the impact of cGAS on the induction of IL-6 and Cxcl10 in WI-38 cells undergoing irradiation-induced (12 Gy) or oncogene-induced (HRasV12) senescence. For both triggers, and similarly to MEFs, the upregulation of IL-6 and Cxcl10 in WI-38 cells was dependent on cGAS (Fig. 5b). This shows that the cGAS signalling pathway contributes to the production of cytokines from senescent cells, whether induced by irradiation, by oncogene activation or pharmacologically. Despite severely affecting the production of SASP components, cGAS and STING only partially

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**Figure 2** cGAS facilitates oxidative-stress-induced senescence. (a–d) WT MEFs, cGAS KO MEFs or STING KO MEFs were cultured under hyperoxic conditions (40% O<sub>2</sub>) for 7 days and subsequently stained for SA-β-Gal activity (a), examined for percentages of Edu<sup>+</sup> cells by immunofluorescence (b) or assessed for expression of Cdkn2a by RT-qPCR (c) or p16<sup>Ind44</sup> by immunoblotting (d). (e) WI-38 cells were exposed to 40% O<sub>2</sub> treatment for 9 days. On day 7, cells were transfected with a non-targeting control siRNA (si Control) or with siRNAs against cGAS (si cGAS no. 1, si cGAS no. 2). Expression of cGAS was assessed by immunoblotting on day 9. (f–h) WI-38 cells were treated as in e. On day 9, SA-β-Gal activity was assessed by FACS (f) and expression of CDKN2A was examined by RT-qPCR (g) or p16<sup>Ind44</sup> was assessed by immunoblotting (h). Numbers in f indicate percentages of SA-β-Gal-positive cells. Mean and s.d. of n=5 independent experiments (a) or n=3 biological replicates (b,c,g) or one representative experiment out of 3 (f) or 2 (d,e,h) independent experiments are shown. P values were calculated by one-way (a) and two-way (b,c,g) ANOVA (*P<0.05, **P<0.01; NS, not significant). Source data are available in Supplementary Table 1. Unprocessed original scans of blots are shown in Supplementary Fig. 7.
influenced the induction of SA-β-Gal activity in MEFs undergoing irradiation- or palbociclib-dependent senescence, suggesting differences in the extent to which MEFs rely on paracrine senescence, at least in these in vitro contexts (Supplementary Fig. 4a). In contrast, depletion of cGAS in WI-38 cells impaired the propagation of senescence following irradiation or HRasV12 signalling, as revealed by diminished SA-β-Gal activity and impaired p16\(^{INK4a}\) induction in cells knocked down for cGAS (Supplementary Fig. 4b,c). Using fluorescence microscopy, we determined that all treatments from above triggered the degradation of lamin B1, suggesting that the loss of nuclear envelope integrity is a common substrate for the activation of cGAS in these contexts (Fig. 5c,d). To further substantiate the direct involvement of cGAS, we visualized the distribution patterns of nuclear DNA in relation to cGAS. Remarkably, both Dox-treated Tet-cGAS-FLAG MEFs and WI-38 cells displayed an overlay between herniated chromatin and cGAS after irradiation or following treatment with palbociclib or HRasV12 signalling (Fig. 5e and Supplementary Fig. 4d). Thus, a diverse range of distinct senescence inducers engages cGAS, which is required for the induction of SASP components.

cGAS regulates cellular senescence in vivo

Finally, we explored whether the cGAS-mediated regulation of senescence also occurs in vivo. To this end, we exposed mice to total body irradiation and examined the induced expression of IL-6, Cxcl10 and Cdkn2a in the lung\(^{44}\). Although, at 24 h post irradiation, the abundance of ISGs was less in irradiated cGAS KO mice or STING KO mice, Cdkn2a was induced to a similar extent in WT mice, cGAS KO mice or STING KO mice, indicating that cGAS and STING do not influence the in vivo DNA damage response (Supplementary Fig. 5a). As expected, at this early time point, Cdkn2a levels were not detectable. However, 16 weeks after irradiation WT mice expressed Cdkn2a in the lung (Fig. 6a). Strikingly, the upregulation of Cdkn2a was reduced in both cGAS KO and STING KO mice. We also noted decreased expression of IL-6 and Cxcl10 in cGAS KO and STING KO mice when compared with WT mice (Fig. 6a).

To corroborate the effect of cGAS on the senescence response in vivo, we employed a model of oncogene-induced senescence, wherein oncogenic Nras\(^{G12V}\) is stably delivered into mouse livers by hydrodynamic injection of transposable elements\(^7\). Both WT
mice and cGAS KO mice were injected with transposons encoding Nras\(^{G12V}\) and markers of senescence were analysed 6 days later. Delivery of transposable elements encoding for a signalling-defective Nras\(^{G12V}\) mutant \((\text{Nras}^{G12V}/\text{D38A})\) served as a control. Interestingly, despite similar expression of Nras\(^{G12V}\) in both genotypes (as assessed by immunostaining of Nras), livers of cGAS KO mice showed decreased levels of p21 and less activity of SA-Gal compared with livers of WT mice (Fig. 6b,c). Moreover, expression of several SASP factors including IL-6 and Cxcl10 was reduced in cGAS KO mice relative to WT mice (Fig. 6d). As expected, owing to immune-mediated clearance, WT mice showed a reduction of Nras-positive cells after 12 days (Supplementary Fig. 5b). In contrast, no significant decrease of Nras-positive cells was observed in cGAS KO mice, thus suggesting that absence of cGAS affects senescence induction and the subsequent immune-mediated clearance of oncogene-activated cells. Taken together, these data indicate that cGAS is important for the regulation of senescence in vivo, whether induced through irradiation or oncogenic signalling.

**DISCUSSION**

The secretion of the SASP is a key feature of senescent cells. Through the auto- and paracrine effects of the SASP, senescent cells on the one hand modulate their own cellular behaviour, while on the other hand they also influence their microenvironment. Thus, the SASP is considered as a pivotal effector branch of senescent cells in several physiological and pathological contexts. Here we provide evidence for a contribution of the innate DNA sensor cGAS and its downstream adaptor STING in the regulation of the SASP and show that signalling through this pathway acts to reinforce cellular senescence in a non-cell autonomous manner (Supplementary Fig. 6).

Previous work has demonstrated that the downregulation of lamin B1 and the subsequent partial loss of nuclear integrity with the occurrence of CCFs represents a characteristic feature of senescent cells\(^{15,31}\). Our data showing that these changes in the intracellular compartmentalization are connected—via cGAS and STING—to the induction of an inflammatory response illustrate its functional consequences for cellular senescence. Interestingly, despite...
on cGAS. Second, experiments using conditioned medium best revealed that the presence of cGAS was crucial to render a senescent cell’s secretome ‘pro-senescent’. Conversely, loss of cGAS did not influence the responsiveness of recipient cells in committing to paracrine senescence. Consistent with a SASP-mediated regulation of senescence, we found that stimulation of cells with palbociclib, which directly engages the p16 pathway, triggered SA-

Figure 5 Engagement of cGAS is a common feature of multiple senescence triggers. (a) MEFs with the indicated genotypes were exposed to 12 Gy ionizing irradiation or stimulated with palbociclib. Expression levels of Cxcl10 and IL-6 were quantified by RT-qPCR or ELISA, respectively. (b) WI-38 cells (left panel) were exposed to 12 Gy ionizing irradiation (IR) or WI-38 ER:RAS cells (right panel) were treated with 4-OHT (500 nM) for 7 days. At day 7 (irradiated cells) or day 3 (WI-38 ER:RAS cells) cells were transfected with a non-targeting control siRNA (si Control) or with siRNAs against cGAS (si cGAS no. 1, si cGAS no. 2). Expression of depicted genes was determined by RT-qPCR. (c) Expression of lamin B1 in individual WT MEFs exposed to irradiation (left panel) or treated with palbociclib (right panel) for 7 days was determined by confocal fluorescence microscopy (CTCF, corrected total cell fluorescence). (d) Expression of lamin B1 in individual WI-38 cells exposed to irradiation (left panel) or WI-38 ER:RAS cells treated with 4-OHT was assessed by confocal fluorescence microscopy (CTCF, corrected total cell fluorescence). (e) cGAS KO MEFs carrying a Dox-inducible lentiviral vector containing a FLAG-tagged murine cGAS construct were irradiated (12 Gy) or treated with palbociclib. Two days post treatment, cells were cultured in the presence of Dox overnight and then stained for FLAG (green) and nuclei (DAPI, grey). One representative experiment out of 2 experiments is shown (e) or mean and s.d. of n = 51 individual control MEFs compared with n = 48 individual irradiated MEFs (c, left); n = 50 individual control MEFs compared with n = 58 individual palbociclib-treated MEFs (c, right); n = 177 individual control WI-38 cells compared with n = 158 individual irradiated WI-38 cells (d, left); n = 151 individual control WI-38 cells compared with n = 87 individual 4-OHT-treated WI-38 cells (d, right) are shown. Data shown in a and b are from n = 2 independent experiments with the column representing the mean. P values were calculated by unpaired t-test (c,d) (** P < 0.0001; NS, not significant). Scale bar, 20 μm. Source data are available in Supplementary Table 1.

the immediate activation of multiple stress pathways, the emergence of the SASP is considered as a relatively late event in the development of senescence—usually requiring multiple days to arise45. Our finding that stimulation of the cGAS–STING pathway occurs through the recognition of CCFs in cells, which are already on the ‘senescence track’, provides one possible explanation of how a senescence-specific inflammatory SASP response may be initiated.

It is well established that the SASP functions as a strong amplifier of senescence. The findings presented here suggest that the cGAS–STING pathway regulates cellular senescence predominantly through the secretion of soluble factors and by involving autocrine and paracrine signalling. First, unbiased examination of the cGAS-controlled secretome showed that many inflammatory cytokines and chemokines associated with promoting senescence depended on cGAS. Second, experiments using conditioned medium best
Figure 6 cGAS contributes to cellular senescence in vivo. (a) Depicted mRNA levels of lungs from WT, cGAS KO and STING KO mice 16 weeks after irradiation. (b) Representative liver stainings of Nras, p21 and SA-β-Gal 6 days after intrahepatic delivery of Nras\(^{G12V}\) or Nras\(^{G12V}=D38A\) into WT or cGAS KO mice. Arrows indicate positive staining. (c, d) Quantification of Nras- or p21-positive cells (c) or expression of IL-6 protein levels or Cxcl10 mRNA levels (d) from b. Mean of \(n=2\) (WT mice untreated, cGAS KO mice) or mean and s.d. of \(n=3\) (WT mice irradiated, STING KO mice) (a), mean and s.d. of \(n=4\) (WT Nras\(^{G12V}\) and cGAS KO Nras\(^{G12V}\) or Nras\(^{G12V}=D38A\)) or \(n=5\) (WT Nras\(^{G12V}\) and cGAS KO Nras\(^{G12V}=D38A\)) (c,d) or representative images (b) are shown. P values were calculated by two-way ANOVA (comparing genotypes or treatments) (\(*P<0.05, \quad **P<0.01\); NS, not significant). Scale bars, 100\(\mu\)m. Source data are available in Supplementary Table 1.

it is the joint action of several mediators that synergize to promote cGAS-dependent senescence.

We also found that loss of cGAS–STING signalling compromised the senescence response in two distinct models of cellular senescence in vivo. Given that the decreased expression of senescence markers (SA-β-Gal or Cdkn2a) paralleled with reduced levels of inflammatory mediators, it is likely that the absence of cGAS–STING-dependent SASP factors accounts for the reduced in vivo senescence response.
In conclusion, our study reveals a key role of innate DNA sensing in controlling cellular senescence. On the basis of our work, we propose that beyond acting as a system to defend against infection, the cGAS–STING pathway may also serve as a cell-innate surveillance system that protects an organism against the deleterious consequences of (pre-) neoplastic cells. Indeed, senescence is well described to function as a potent anticancer mechanism. First, by imposing a permanent cell cycle arrest, cellular senescence prevents the proliferation of damaged and potentially mutated cells. Second, by secreting cytokines and chemokines, senescent cells recruit immune cells, thereby facilitating their own elimination secreting cytokines and chemokines, senescent cells recruit immune cells, thereby facilitating their own elimination thereby facilitating their own elimination.

Given its role in the immune-cell-mediated clearance of pre-malignant cells, as indicated by our study, we speculate that the cGAS–STING pathway may function to promote cell extrinsic antitumour effects.

In addition to these beneficial effects, however, cellular senescence is also implicated in ageing and various age-related pathological conditions, including, as recently reported, atherosclerosis or osteoarthritis. Because of its prominent role in stimulating an inflammatory response, cGAS and STING may participate in these and other adverse effects of senescence in vivo, a point that will need to be addressed by future studies.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

Note: Supplementary Information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

S.G., B.G., M.E.G., K.W., T.-W.K., L.Z. and A.A. designed experiments and analysed the data. S.G., B.G., M.E.G., T.-W.K. and A.A. performed experiments. N.A.S. assisted in the establishment of methods. A.B. and J.R. provided reagents, cells and mice. S.G., B.G. and A.A. wrote the manuscript with help from all authors. A.A. supervised the project.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.
39. Moiseeva, O., Mallette, F. A., Mukhopadhyay, U. K., Moores, A. & Ferbeyre, G. DNA damage signaling and p53-dependent senescence after prolonged IFN-γ stimulation. *Mol. Biol. Cell* **17**, 1583–1592 (2006).

40. Yu, Q. et al. DNA-damage-induced type I interferon promotes senescence and inhibits stem cell function. *Cell Rep.* **11**, 785–797 (2015).

41. Katlinskaya, Y. V. et al. Suppression of type I interferon signaling overcomes oncogene-induced senescence and mediates melanoma development and progression. *Cell Rep.* **15**, 171–180 (2016).

42. Braumüller, H. et al. T-helper-1-cell cytokines drive cancer into senescence. *Nature* **494**, 361–365 (2013).

43. Freund, A., Laberge, R. M., Demaria, M. & Campisi, J. Lamin B1 loss is a senescence-associated biomarker. *Mol. Biol. Cell* **23**, 2066–2075 (2012).

44. Le, O. N. et al. Ionizing radiation-induced long-term expression of senescence markers in mice is independent of p53 and immune status. *Aging Cell* **9**, 398–409 (2010).

45. van Deursen, J. M. The role of senescent cells in ageing. *Nature* **509**, 439–446 (2014).

46. Xue, W. et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **445**, 656–660 (2007).

47. Childs, B. G. et al. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. *Science* **354**, 472–477 (2016).

48. Jeon, O. H. et al. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nat. Med.* **23**, 775–781 (2017).
Glycerol, bromophenol blue and 4% Calbiochem. Protein concentration was measured using the BCA Pierce protease and phosphatase inhibitors (Protease Inhibitor Cocktail I, Animal-Free). Corrected total cell fluorescence. 

Click-iT EdU incorporation assay. The Click-iT EdU reaction was performed following the instructions of the Click-iT imaging kit (molecular probes by Life Technologies). Ten images per condition were acquired with a ×40 magnification objective. The number of EdU-positive cells and DAPI-positive cells was determined using ImageJ.

Senescence induction. Palbociclib (PD-0332991 HCl, Selleckchem) was added to the culture medium at a final concentration of 4 μM. Cells (MEFs, WI-38 cells) were irradiated with 12 Gy (RS-2000 X-Ray Irradiator). To induce senescence via oxidative stress, MEFs were irradiated at 20% and 40% O2 for 1 h. Reconstituted IFN-β was obtained from Biolegend and used at the indicated concentrations. Cells were treated with IFN-β every day for 2 weeks.

Lentiviral vector production and transduction. HEK 293T cells were transfected with pCMVDR8.74, pMD2.G plasmids and the puromycin-selectable lentiviral vector pTRIPZ containing the open reading frame of G4S-FLAG by the calcium phosphate precipitation method. The superantigen containing lentiviral particles was harvested at 48 and 72 h, pooled and concentrated by ultracentrifugation. WT MEFs were transduced with the lentiviral vectors by directly adding 10 μl to the culture medium.

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Transfection. MEFs were transfected with siRNAs using Lipofectamine 2000 reagent (Thermo Fisher Scientific) according to the manufacturer's instruction. Silencer select predesigned siRNAs were purchased from Life Technologies. Sequence Populations of primary MEFs (2 × 10^4 per 10 cm dish) were counted by trypsin blue exclusion every third day. SA-β-Gal staining was performed according to the manufacturer's instructions (Cell Signaling, SA-β-Gal staining kit (no. 9801)).

Antibodies. Detailed information on the antibodies used including their resources (company names, catalogue numbers) and dilutions are provided in Supplementary Table 2.

Mimoblotol. Cell pellets were lysed in a lysis buffer containing 0.5% Triton X-100, 20 mM HEPES (pH 7.4), 150 mM NaCl, 1.5 mM MgCl2, 2 mM EGTA, protease and phosphatase inhibitors (Protease Inhibitor Cocktail I, Animal-Free Calbiochem). Protein concentration was measured using the BCA Pierce Protein assay kit and normalized to the lowest concentration. Samples were diluted with a loading buffer (100 mM Tris HCl (pH6.8), 10% (v/v) SDS, 20% (v/v) glycerol, bromophenol blue and 4% 2-mercaptoethanol). The primary antibody was incubated in 0.1% NFDM for 16 h at 4 °C. The secondary antibodies anti-mouse or anti-rabbit HRP-conjugated antibodies were incubated for 1 h at room temperature. Proteins were visualized with the enhanced chemiluminescence substrate ECL (Pierce, Thermo Scientific) and imaged using the ChemiDoc XRS Biorad Imager and Image Lab Software. Imaging was performed in two channels: chemiluminescence and colorimetry, merged together in Supplementary Fig. 7 for uncropped images.

Immunochemistry. Cells were seed onto coverslips at a density of 75,000 cells per coverslip and fixed with 2% (v/v) paraformaldehyde for 10 min, permeabilized for 5 min in 0.1% (v/v) Triton X-100 and blocked with 2% BSA in PBS for 20 min at room temperature. Cells were incubated with the primary antibody in PBS containing 2% (v/v) BSA for 1 h at room temperature in a humid chamber. After washing, cells were incubated with the secondary antibody in PBS containing 5 μg ml^-1^ 4,6-diamino-2-phenylindole (DAPI) for 1 h at room temperature. Coverslips were mounted with Dako (fluorescent mounting medium). Images were acquired with a wide-field fluorescence microscope (Zeiss Axioplan) and processed in ImageJ software. Confocal sections were obtained with a confocal laser scanning microscope (Zeiss LSM700).

Corrected total cell fluorescence. Ten images from lamin B1-stained cells per condition were acquired using a Zeiss LSM 700 confocal with a 63× lens. The corrected total cell fluorescence was determined using ImageJ following the methods of ref. 52. Briefly, the integrated density of lamin B1, area and mean grey value from nuclei were measured by ImageJ. The corrected total cell fluorescence was calculated using the following formula: CTCF = integrated density – (area of selected cell × mean fluorescence of background readings).

BrdU incorporation assay. Cells plated in 10 cm dishes were incubated with 10 μM BrdU (Sigma-Aldrich) for 3 h. Cells were fixed with 70% (v/v) cold ethanol overnight. After washing, cells were resuspended in 2 M HCl for 30 min and then incubated in PBS-Tween 0.1% containing anti-BrdU antibodies conjugated to FITC (BD Biosciences, Clone B44) for 1 h. Samples were measured using a Gallios Flow Cytometer (Beckman Coulter). Data were analysed by the Kaluza software (Beckman Coulter).

Click-iT EdU incorporation assay. The Click-iT EdU reaction was performed following the instructions of the Click-iT imaging kit (molecular probes by Life Technologies). Ten images per condition were acquired with a ×40 magnification objective. The number of EdU-positive cells and DAPI-positive cells was determined using ImageJ.
5′-GACAAGCTTCCGTTCATGCAG-3′; hs CDKN2A forward 5′-AAATGAAAATCAGAAGGGG-3′; hs IL6 forward 5′-CCCTTGACCAACACAAAT-3′; hs IL6 reverse 5′-ATTGCCGAAGGAGCTTCCATG-3′; hs CXCL10 forward 5′-AGTTGGGCTTGGTTCTCTGGA-3′; hs IFI44 forward 5′-GATGGTGACCTGTGAGGTCC-3′; hs IFI44 reverse 5′-CTTACAGGTTCCA GCTCCC-3′.

ELISA. Supernatants were harvested and centrifuged at 1,000g to remove cell debris and dead cells. ELISA was performed following the instructions of the Mouse IL-6 ELISA Set from BD Biosciences (BD OptiELISA, Cat. No. 555240).

Cytokine array. Supernatants were harvested from indicated cells and centrifuged at 1,000g to remove dead cells. Cytokines from human cells were analysed following the instructions on Human Cytokine Antibody Array (Abcam, ab133997). Cytokines from MEFS were analysed following the instructions of the Proteome Profiler Array from R&D SYSTEMS. Dot blots were analysed using the plugin MicroArray Profile (OptiNav) on Fiji software.

RNA sequencing. RNA was isolated from the WT, cGAS KO MEFS exposed to 20% O2 after 33, 36 and 42 days in culture using the RNeasy Mini kit (Qiagen). RNA was further processed for sequencing by the Gene Expression Core Facility GECF (EPFL). mRNA-seq libraries were prepared using the TruSeq mRNA stranded LT (Illumina kit). Samples were sequenced by the NextSeq 500 system sequencing with 1 × 75 cycle (‘single read’). ‘High output’ mode (=expected minimal 400 mio reads), chemistry ‘v2’. Sequencing data were processed using HTStaion online software from the Bioinformatics and Biostatistics Core Facility (EPFL). Heat maps were produced from normalized expression data using Cluster 3.0 for computation and JTreeview for visualization.

In vivo total body irradiation and oncogene-induced senescence. WT, cGAS−/− or STING−/− mice (8–14 weeks of age) were mock-irradiated or exposed to ionizing radiation (4.25 Gy) and lung tissue was obtained after 24 h or 16 weeks. Mice from both gender were randomly allocated to different groups and experiments were conducted non-blinded. Mice were maintained under specific pathogen-free conditions at the University Hospital Tübingen. These animal experiments were approved by the local German authorities of the state of Baden-Württemberg (Regierungspräsidium Tübingen).

Immunohistochemistry. Immunohistochemistry stainings for Nras (clone F155, 1:50) and p21 (clone SXM30, 1:100, BD Pharmingen) were performed on paraffin-embedded liver sections. SA-β-Gal staining on frozen liver sections was performed at pH 5.5 as previously described48.

Statistics and reproducibility. All experiments were performed three or more times independently under similar conditions, except experiments shown in Figs 1f, 2d,e,h, 3b–j, 4a–d and 5 and Supplementary Figs 1b–d, 2a–f,h, 3, 4 and 5a, which were performed twice, and Figs 1d and 6 and Supplementary Figs 1a and 5b, which were performed once. Statistical significance was calculated by unpaired t-test (Figs 1b,c,e, 3b, 4b and 5c,d) or one-way (Figs 2a and 4e) and two-way (Figs 2b,c,g and 6a,c,d and Supplementary Figs 2e and 5a,b) ANOVA as described in the figure legends. Prism 6 software was used to generate graphs and to perform statistical analysis. P values of statistical significance are represented as ***P < 0.0001, **P < 0.001, *P < 0.01, *P < 0.05.

Data availability. RNA sequencing data have been deposited in the Gene Expression Omnibus (GEO) under the accession code (GSE100102). Source data from Figs 1–6 and Supplementary Figs 1, 2, 4 and 5 are shown in Supplementary Table 1 or available from the corresponding author on request.

49. Jin, L. et al. MPYS is required for IFN response factor 3 activation and type I IFN production in the response of cultured phagocytes to bacterial second messengers cyclic-di-AMP and cyclic-di-GMP. J. Immunol. 187, 2595–2601 (2011).
50. Bridgeman, A. et al. Viruses transfer the antiviral second messenger cGAMP between cells. Science 349, 1228–1232 (2015).
51. Mulher, U. et al. Functional role of type I and type II interferons in antiviral defense. Science 264, 1918–1921 (1994).
52. McCoy, R. A. et al. Partial inhibition of Cdk1 in G 2 phase overrides the SAC and decouples mitotic events. Cell Cycle 13, 1400–1412 (2014).
53. Kang, T. W. et al. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature 479, 547–551 (2011).
54. Krizhanovsky, V. et al. Senescence of activated stellate cells limits liver fibrosis. Cell 134, 657–667 (2008).
Supplementary Figure 1 Absence of cGAS attenuates the establishment of senescence. (a) Heat maps of RNA-seq analysis of WT MEFs and cGAS KO MEFs (Day 33, 36, 42). For each genotype one sample per day; MEFs collected at Day 33, 36, 42. Genes in cGAS KO MEFs exhibiting statistically significant (n=3 biological replicates; \( P < 0.05 \); student \( t \)-test) twofold or greater increases relative to WT MEFs are shown. E2F target genes are highlighted in green\(^1\). (b) Proliferation curves of primary WT MEFs, cGAS KO MEFs and STING KO MEFs cultured under 20% \( O_2 \). (c) WT MEFs, cGAS KO MEFs and STING KO MEFs were cultured under 20% \( O_2 \) for 27 days and expression of \( p16^{\text{ink4a}} \) was determined by immunoblotting. (d) WT MEFs, cGAS KO MEFs or STING KO MEFs were harvested after 3 weeks of culture and expression of depicted genes was measured via RT-qPCR. One representative experiment out of 2 independent experiments (b, c) independent experiments is shown. Data shown in (d) are from n=2 independent experiments with the column representing the mean. Source data are available in Supplementary Table 1. Unprocessed original blots are shown in Supplementary Fig. 7.
Supplementary Figure 2 cGAS-dependent regulation of cytokines. (a, b) Conditioned medium (CM) was collected from WT MEFs or cGAS KO MEFs exposed to 40% O₂ for 7 days. Percentages of proliferating cGAS KO MEFs were assessed by BrdU incorporation assay (a) or induction of SA-β-Gal activity was determined by microscopy (b). (c) Cytokine profile of the CM from WI-38 cells exposed to 40% O₂ for 7 days and treated with a control siRNA (si Control) or a cGAS-targeting siRNA (si cGAS #1) on day 3. Numbers indicate specific cytokines or chemokines and are highlighted in red rectangles. (d) WT MEFs, cGAS KO MEFs or STING KO MEFs were incubated in 40% O₂ for 7 days and Ifi44 expression was quantified by RT-qPCR. (e) WI-38 cells were exposed to 40% O₂ treatment for 9 days. On day 7 cells were transfected with a non-targeting control siRNA (si Control) or with siRNAs against cGAS (si cGAS #1, si cGAS #2). Expression of Ifi44 was determined by RT-qPCR. (f) WT MEFs and cGAS KO MEFs were treated with recombinant IFN-β as indicated every day and after 14 days expression levels of p16Ink4a were assessed by immunoblotting. (g) cGAS KO MEFs were treated with recombinant IFN-β as indicated and after 14 days expression levels of depicted genes was assessed by RT-qPCR. (h) WT MEFs and IFNAR KO MEFs were exposed to 40% O₂ for 7 days. Expression of mRNA levels of depicted genes was assessed by RT-qPCR. One representative experiment out of 2 independent experiments (c, f) mean of n=2 (a) or mean and s.d. of n=3 (e) independent experiments are shown. Data shown in (b, d, g, h) are from n=2 independent experiments with the column representing the mean. Source data are available in Supplementary Table 1. Unprocessed original blots are shown in Supplementary Fig. 7.
Supplementary Figure 3 Endogenous cGAS binds to chromatin at the nuclear-cytosolic border. WI-38 cells were cultured under 40% O₂ for 9 days and stained for cGAS (green) and DAPI (grey). Images are representative from 3 independent experiments. Scale bar: 20 µm.
Supplementary Figure 4 cGAS is a common regulator of senescence.

(a) MEFs with the indicated genotypes were exposed to 12 Gy ionizing irradiation or stimulated with Palbociclib. After 7 days cells were stained for SA-β-Gal activity.

(b) WI-38 cells (left panel) were exposed to 12 Gy ionizing irradiation or WI-38 ER:RAS cells (right panel) were treated with 4-OHT (500 nM) for 7 days. At day 3 cells were transfected with a non-targeting control siRNA (si Control) or with siRNAs against cGAS (si cGAS #1, si cGAS #2). At day 7 SA-β-Gal activity was determined by FACS. Data shown in (a) and (c) are from n=2 independent experiments with the column representing the mean. Scale bar: 20 µm. Source data are available in Supplementary Table 1.

(c) or expression of CDKN2A was quantified by RT-qPCR (c). (d) WI-38 cells (left panel) were exposed to 12 Gy ionizing irradiation or WI-38 ER:RAS cells (right panel) were treated with 4-OHT (500 nM) for 7 days. Cells were stained for cGAS (green) and DAPI (grey) and analysed by fluorescence microscopy. One representative experiment out of two (b, d) independent experiments is shown.
Supplementary Figure 5  Irradiation-induced activation of cGAS and STING in vivo. (a) Depicted mRNA levels of lungs from WT, cGAS KO and STING KO mice 24 h after irradiation are shown. Non-irradiated mice served as controls. (b) Quantification of Nras-positive cells 12 days after intrahepatic delivery of Nras$^{G12V}$ or Nras$^{G12V/D38A}$ into WT or cGAS KO mice are shown (n = 8-10 per group). Mean and s.d. of n=3 (a) or n=8 (WT G12V/D38A; cGAS KO G12V/D38A) or n=10 (WT G12V; cGAS KO G12V) (b) mice are shown. $P$ values were calculated by two-way ANOVA (** $P < 0.01$, *** $P < 0.001$, ns = not significant). Source data are available in Supplementary Table 1.
Supplementary Figure 6 Model of cGAS-mediated propagation of senescence. Within senescent cells cGAS senses herniated chromatin, which triggers the production of SASP factors. Cytokine signalling in turn induces the expression of cell-cycle inhibitors, including p16\(^{INK4a}\), which enforces cell-cycle arrest and senescence in an autocrine and paracrine manner.
Supplementary Figure 7  Uncropped images from western-blots.
Supplementary Table Legends

Supplementary Table 1  Statistics Source Data
Supplementary Table 2  Antibody sources
Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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### Experimental design

1. **Sample size**
   - Describe how sample size was determined.
   - No statistical test was used to determine sample size.

2. **Data exclusions**
   - Describe any data exclusions.
   - No data were excluded from the analysis.

3. **Replication**
   - Describe whether the experimental findings were reliably reproduced.
   - All experiments were performed three or more times independently under similar conditions, except experiments shown in Figs 1f, 2d, 2e, 2h, 3b-j, 4a-d, 5 and Supplementary Figs 1b-d, 2a-d, 2f-h, 3, 4, 5a which were performed twice and Fig 1d, Fig 6 and Supplementary Fig 1a and Fig 5b, which were performed once.

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - Samples were randomly allocated to different groups.

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - Investigators were not blinded to allocation during the experiment and outcome assessment.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| Item | Confirmed |
|------|-----------|
| The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) | ☒ |
| A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly | ☒ |
| A statement indicating how many times each experiment was replicated | ☒ |
| The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) | ☒ |
| A description of any assumptions or corrections, such as an adjustment for multiple comparisons | ☒ |
| The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted | ☒ |
| A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) | ☒ |
| Clearly defined error bars | ☒ |

See the web collection on statistics for biologists for further resources and guidance.
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Image Lab Software (Biorad), FlowJo, ImageJ, Kaluza (Beckman Coulter), Fiji, HTSstation, Bowtie2, Bioconductor limma, Gaph Pad Prism, Adobe Illustrator

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Full information about the antibodies used in this study is provided in Supplementary Table 2.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Wi-38 cells were purchased from ATCC CCL-75TM.

b. Describe the method of cell line authentication used.

No method of cell line authentication was used.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cells were repeatedly tested for mycoplasma using specific primers.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

WI-38 cells are not listed in ICLAC.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

MEFs were generated by standard methods from wild type, STING-deficient or cGAS-deficient mice. Detailed laboratory protocols are available upon request. All mice were on the C57Bl/6 background and obtained from both genders. Tmem173-/- (STING-deficient) mice were a gift from J Cambier 49. Mb21d1-/- (cGAS knockout first) mice were described in 50. Ifnar1-/- were a gift from C Reis e Sousa and were originally described in 51. This work was performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and institutional guidelines for animal care. This work was approved by a project license granted by the UK Home Office (PPL No. 40/3583) and was also approved by the Institutional Animal Ethics Committee Review Board at the University of Oxford.

In addition, WT, cGAS-/- or STING-/- mice (8-14 weeks of age) were mock-irradiated or exposed to IR (4.25 Gy) and lung tissue was obtained after 24 h or after 16 weeks. Mice from both gender were randomly allocated to different groups and experiments were conducted non-blinded. Mice were maintained under specific pathogen-free (SPF) conditions at Ecole Polytechnique Federale de Lausanne (EPFL), Switzerland. These animal experiments were approved by the Service de la consommation et des affaires vétérinaires (1066 Épalinges, Canton of Vaud, Switzerland).

The transposon system encoding for oncogenic NrasG12V or an effector loop mutant incapable of downstream signalling (NrasG12V/D38A) were previously described 53. For intrahepatic delivery of the transposon system, mice were hydrodynamically injected with a 5:1 molar ratio of transposon to transposase-encoding plasmid (30 μg total DNA) via the tail vein as previously described. Livers were obtained on day 6 and on day 12 after injection. No statistical method was used to predetermine sample size. The investigators were not blinded to allocation during experiments and outcome assessment. Mice were maintained under specific pathogen-free (SPF) conditions at the University Hospital Tübingen. These animal experiments were approved by the local German authorities of the state of Baden-Wuerttemberg (Regierungspräsidium Tübingen).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Not applicable
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

- **Data presentation**

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

- **Methodological details**

  5. Describe the sample preparation.

        BrdU incorporation assay: Cells plated in 10 cm dishes were incubated with 10 μM BrdU (Sigma-Aldrich) for 3h. Cells were fixed with 70% (v/v) cold ethanol overnight. After washing cells were resuspended in 2 M HCl for 30 min and then incubated in PBS-Tween 0.1% containing anti-BrdU antibodies conjugated to FITC (BD Biosciences, Clone B44) for 1 h. Samples were measured using a Gallios Flow Cytometer (Beckman Coulter). Data were analysed by the Kaluza software (Beckman Coulter).

        C12FDG SA-bGAL: For FACS-based determination of senescence, lysosomal alkalinization was induced by treating cells with 100 nM bafilomycin A1 for 1 h in fresh cell culture medium (2 ml per 35 mm dish) at 37 °C. 33 μl of 2 mM C12FDG (Thermofisher, ref. D2893) working solution was added to the cell culture medium to obtain a final concentration of 33 μM. After 1h of incubation, cells were washed, trypsinised and collected on a Gallios flow cytometer (Beckman Coulter) using Kaluza Acquisition software. Data were analysed with FlowJo software.

  6. Identify the instrument used for data collection.

        Beckman Coulter Gallios 10 Colors/3 Lasers

  7. Describe the software used to collect and analyze the flow cytometry data.

        Kaluza Acquisition Software is used to collect data.
        FlowJo Software is used to analyze cytometry data.

  8. Describe the abundance of the relevant cell populations within post-sort fractions.

        not applicable

  9. Describe the gating strategy used.

        Based on forward and side scatter properties a gate was set on the live cell population; dead cells were excluded. This gate had been applied to a single parameter histogram where two single distinct peaks were observed. The peak representing the negative cell population was determined by comparing their values to an unlabeled negative control or a biological negative control. In
addition, a positive control served to determine the positive cell population.

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