Cellular stress signaling activates type-I IFN response through FOXO3-regulated lamin posttranslational modification

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Neural stem/progenitor cells (NSPCs) persist over the lifespan while encountering constant challenges from age or injury related brain environmental changes like elevated oxidative stress. But how oxidative stress regulates NSPC and its neurogenic differentiation is less clear. Here we report that acutely elevated cellular oxidative stress in NSPCs modulates neurogenic differentiation through induction of Forkhead box protein O3 (FOXO3)-mediated cGAS/STING and type I interferon (IFN-I) responses. We show that oxidative stress activates FOXO3 and its transcriptional target glycine-N-methyltransferase (GNMT) whose upregulation triggers depletion of s-adenosylmethionine (SAM), a key co-substrate involved in methyl group transfer reactions. Mechanistically, we demonstrate that reduced intracellular SAM availability disrupts carboxymethylation and maturation of nuclear lamin, which induce cytosolic release of chromatin fragments and subsequent activation of the cGAS/STING-IFN-I cascade to suppress neurogenic differentiation. Together, our findings suggest the FOXO3-GNMT/SAM-lamin-cGAS/STING-IFN-I signaling cascade as a critical stress response program that regulates long-term regenerative potential.

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stem cells persist along the mammalian lifespan to maintain tissue homeostasis by replacing damaged or lost cells\(^1\). Meanwhile, elevation of stress response pathways, a hallmark of aging tissues, acts to promote adult stem cell depletion through induction of senescence or cell death\(^2\,3\,4\). Intrinsic and extrinsic cell-stressors such as oxidative stress, DNA damage, mitochondrial dysfunction, loss of proteostasis, and the inflammatory tissue milieu are the well-known factors that contribute to increased stress response. Among them, oxidative insults inflict damage to cellular macromolecules that lead to cytosostasis, cytotoxicity and ultimately, the functional decline of stem cells\(^5\). Studies in various model organisms have identified a range of key stress response molecules (i.e., Atm\(^6\), Foxo3\(^6\,10\), Prld\(^16\,17\)) that also function to protect stem cell reserves against physiological and pathological oxidative insults. However, the molecular pathway(s) that translate the stress signals to the cellular behaviors remain poorly understood. Forkhead box protein O (FOXO) transcription factors play evolutionarily conserved roles in a wide range of biological processes from aging to metabolism, not only by sensing stress but also through promoting stress resistance\(^18\). For example, previous studies indicated that FOXO is required for long-term regenerative potential of the hematopoietic stem cell by regulating the response to physiologic oxidative stress and quiescence\(^7\). Notably, among the many organs, the brain is particularly vulnerable to the oxidative stress due to its high oxygen consumption, unusual enrichment of polyunsaturated fatty acids as well as the presence of excitotoxic amino acids\(^13\). As a result, neural stem/progenitor cells (NSPCs) and their functional progenies in adult brains face constant stressful challenges that affect their neurogenic potential\(^4,14,15\). Indeed, previous studies found that FOXO expression in the central nervous system not only serves a key role in preserving neural stem cell pools\(^9,10\), it also protects neurons against age-related axonal degeneration across species\(^16\,18\). But despite these revelations, there still lacks a mechanistic understanding of how oxidative stress affects FOXO activation systematically and whether and how that contributes to the neuroprotective responses.

The type-I interferon (IFN-I) response is an innate immune response that can be induced by a number of pattern recognition receptors\(^19\). Among them, cytosolic DNA fragments are recognized by cyclic GMP-AMP synthetase (cGAS), which initiates reaction of GTP and ATP to form cyclic GMP-AMP (cGAMP), a ligand of the signaling adapter stimulator of interferon genes (STING, TMEFF173). The binding of cGAMP to STING activates TANK Binding Kinase 1 (TBK1) kinases-mediated phosphorylation of transcription factor IRF3 that triggers IFNα/β production and subsequent IFN response\(^20\,23\). Increased IFN-I response has been shown to promote NSPC quiescence and suppress neurogenic differentiation\(^14,24\). Interestingly, a recent study revealed that IFN-I signaling is elevated in the brain of aged humans and animals and correlates with increased oxidative stress\(^14\). But the connection between oxidative stress and IFN-I response is unclear.

Here, we report that oxidative stress-induced FOXO3 activation promotes transcriptional upregulation of glycine-N-methyltransferase (GNMT) that triggers intracellular depletion of S-adenosyl-L-methionine (SAM). Using NSPCs as a system, we further demonstrated that reduction of intracellular SAM availability compromises nuclear lamin maturation that would eventually lead to cytosolic DNA leakage, cGAS/STING activation, and IFN-I response to suppress neurogenic differentiation. These findings established FOXO3-GNMT/SAM-lamin-cGAS/STING-IFN-I signaling cascade as a critical stress response program that regulates NSPC differentiation.

### Results

#### High redox potential-mediated cellular stress activates IFN-I pathway

The neurogenic differentiation potential of NSPCs declines under iatrogenic insults, traumatic injuries, or inflammatory stress conditions\(^14,23\). To determine how oxidative stress signal impacts NSPC differentiation, we subjected the cultured murine NSPCs to either pro-oxidant agent paraquat (PQ) or antioxidant N-acetylcysteine (NAC). Measurement by a ratiometric Grx-roGFP2 sensor confirmed that PQ treatment induced a marked elevation of intracellular redox potential relative to the mock-treated NSPCs, whereas NAC treatment led to a significant reduction of redox potential (Fig. 1a, b). It is worthy to note that the PQ dosages used in the study were not cytotoxic in NSPCs (Supplementary Fig. 1a). Compared to the mock-treated control NSPCs, we found that NSPCs under PQ but not NAC treatment, exhibited marked reduction in their production of TUBB3-positive newly born neurons when induced to differentiate (Fig. 1c), suggesting a regulatory role of oxidative stress response on neurogenic differentiation.

To determine the signaling pathway that underlies the oxidative stress-induced neurogenic decline, we next performed gene expression profiling against mock-treated control and NSPCs following 48h redox preconditioning. Gene set enrichment analysis of differentially regulated genes revealed IFN-I signaling as one of the most enriched signature pathways following PQ treatment (Fig. 1d; Supplementary Data 1). Concordantly, heatmap visualization showed an overall increase of IFN-I pathway gene expression pattern in PQ-treated NSPCs, as compared to the control or NAC-treated cells (Fig. 1e). Quantitative real-time PCR (qRT-PCR) confirmed the transcriptional upregulation of major IFN-I signaling downstream surrogates, including Ifnb, Isg15, Sox3, and Mxl (Fig. 1f). Importantly, the PQ-induced IFN-I gene upregulation was completely blocked by co-treatment of anti-oxidant NAC, indicating that the response is redox potential dependent. Consistently, ELISA analysis of PQ-treated NSPCs revealed significantly elevated secretion of the key IFN-I response effector IFNβ as compared to the mock-treated control cells (Fig. 1g). Similar results were obtained in H2O2 treated NSPCs (Supplementary Fig. 1b, c).

To test whether activation of IFN-I signaling accounts for the redox stress-induced neurogenic decline, we next directly treated the NSPCs with IFNβ. Indeed, addition of IFNβ alone was sufficient to suppress neurogenic differentiation of NSPCs (Fig. 1h). Conversely, depletion of interferon-α/β receptor (IFNAR) β chain (IFNAR2), a key subunit of IFNAR dimer, blocked IFN-I response in PQ-treated NSPCs and restored their neurogenic potential (Supplementary Fig. 1d, e; Fig. 1i). These data collectively suggest that oxidative stress signaling regulates neurogenic differentiation of NSPCs through activation of IFN-I pathway.

#### FOXO3 is required for ROS-induced IFN-I response

FOXO proteins are known to regulate physiological oxidative stress response partly due to their role in modulating the transcriptional expression of ROS-scavenging enzymes\(^26,27\). Indeed, heatmap and GSEA analysis confirmed that transcriptional targets of FOXO3 were upregulated in PQ-treated NSPCs (Supplementary Fig. 2a, b). To determine the role of FOXO3 in ROS-induced IFN-I response, we next analyzed how FOXO3 depletion affects oxidative stress-induced IFN-I signaling activation. As expected, PQ treatment to the control NSPCs (non-targeted guide RNA, sg-N) elicited a robust IFN-I response, as evidenced by the markedly enhanced phosphorylation of IFN-I upstream and...
downstream signaling protein STAT1 and TBK1 (Fig. 2a, b), elevated IFNβ secretion (Fig. 2c), and the strongly upregulated mRNA expression of IFN-I stimulated genes (ISGs) (Isg15, Socs1, Usp18 and Nos2) (Fig. 2d). In comparison, the PQ treatment-induced IFN-I response and activation of its upstream and downstream signaling were evidently attenuated in FOXO3-depleted NSPCs (sg-Foxo3) (Fig. 2b–d), suggesting that FOXO3 plays a crucial role in regulation of ROS-induced IFN-I response. Interestingly, FOXO3 depletion in NSPCs also attenuated IFN-I response induced by other stressors (i.e., H2O2, oligomycin, zeocin) (Supplementary Fig. 2c–e), suggesting that FOXO3 may serve as a general cellular stress effector by activating IFN-I response.

FOXO3 integrates a variety of cellular signals that modulate its transcriptional activity. To examine whether activation of FOXO3 by itself was sufficient to trigger IFN-I response independently of oxidative stress, we transduced NSPCs with an adenoviral-encoded activated mutant form of FOXO3 (FOXO3TA, triple alanine form) that was known to localize to the nucleus (Fig. 2e, f). FOXO3TA expression markedly increased IFNβ secretion as well as enhanced expression of ISGs as compared to the control adenovirus-infected NSPCs (Fig. 2g, h),
indicating that FOXO3 is directly responsible for oxidation-stress-induced IFN-I activation.

**Oxidation of FOXO3 activates IFN-I response.** Previous reports suggest that ROS signaling activates FOXO by inducing its nuclear translocation. Similarly, we found that ROS treatment to NSPCs stimulated FOXO3 nuclear retention and activation that concomitantly led to an elevated FOXO3 protein expression (Fig. 3a–c). Conversely, treatment with the antioxidant NAC not only promoted FOXO3 cytoplasmic shuttling and reduced its expression as well as transcriptional activity, it also prevented the ROS-induced FOXO3 protein accumulation in PQ-treated NSPCs (Fig. 3a–c, Supplementary Fig. 3a).

The nucleo-cytoplasmic shuttling of FOXO is controlled through a combination of posttranslational modifications, particularly AKT-mediated phosphorylation that promotes its cytoplasmic sequestering. Notably, previous studies reported that ROS treatment could cause a significant reduction of FOXO phosphorylation at threonine 32/serine 253, suggesting that oxidative stress may induce FOXO3 nuclear translocation by impeding its phosphorylation. Since reversible cysteine thiol oxidation is a well-known mechanism that regulates signaling...
Fig. 3 Oxidation at Cys31 of FOXO3 activates IFN-I response. IF (a), DBE reporter (b, mean ± s.e.m., n = 3 independent experiments), and WB (c) for FOXO3 in NSPCs following 24 h of respective treatment. Scale bar = 10 μm. d WB for cysteine sulfenylation (Cys-SOH) following immunoprecipitation of FOXO3. e Conserved consensus sequence adjacent to AKT phosphorylation site of mouse FOXO proteins. f WB for indicated proteins from non-treated and PQ-treated (40 μM, 0.5 h) Foxo3 WT or C31A mutant transduced NSPCs. g Microscopic analysis of Foxo3 WT or C31A mutant tagged with c-terminal EGFP with or without PQ treatment (40 μM, 16 h). Scale bar = 20 μm. Percent of cells with nuclear FOXO3-EGFP is plotted on the right. Mean ± s.e.m. of three independent experiments. qRT-PCR analysis for transcriptional targets of FOXO3 (h) and ISGs (j). Foxo null NSPCs with WT or C31A mutant Foxo3 were analyzed following 4 days of PQ treatment. Mean ± s.e.m. of three independent experiments. i IFNβ secretion in the media following 48 h treatment. Mean ± s.e.m. of three independent experiments. k Schema for activation of FOXO3 by oxidation at Cys31 residue. For b, g, h, i, and j, statistical significance was determined by one-way ANOVA. Experiments for a, c, d, f, and g were repeated three times independently with similar results and representative images/blots are shown.

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FOXO3 significantly lowered that the ROS-induced nuclear translocation is lower for C31A mutant compared to the wild-type FOXO3 (Fig. 3g). Consistently, analysis of ROS-treated NSPCs transduced with C31A mutant revealed an attenuated induction of FOXO3 downstream genes (i.e., Sad2, Sesh3, pki3pi1), ISGs expression, and IFNβ secretion, as relative to the wild-type FOXO3-transduced cells (Fig. 3h–j). Notably, although the C31A mutant was defective of ROS-induced FOXO3 nuclear shuttling and activation, this mutation did not affect FOXO3 nuclear translocation upon treatment with either PI3K inhibitor (GDC0941) or AKT inhibitor (MK2206) (Supplementary Fig. 3c–e). These findings suggest that Cys thiol oxidation and its associated inhibitory function on FOXO3 phosphorylation is a key mechanism that underlies ROS-regulated FOXO3 and its downstream signaling activation (Fig. 3k).

**Compromised lamin processing upon oxidative stress invokes IFN-I response.** The IFN-I signaling is a cellular innate immune response and often triggered by the cytosolic DNA-sensing cGAS/STING pathway. To examine whether ROS-induced IFN-I activation is mediated by aberrant cytosolic DNA appearance, we treated the cGAS-GFP-expressing NSPCs with pro-oxidant PQ. Fluorescence microscopic analysis of the PQ-treated cells revealed an increased nuclear leakage as evidenced by the emergence of cytoplasmic H3K4m3-positive chromatin and the inappropriate formation of cytoplasmic cGAS-GFP-containing DNA foci (Fig. 4a), suggesting a compromised nuclear envelope integrity.

The nuclear lamina is essential for the maintenance of nuclear shape and mechanics, and its dysregulation causes nuclear envelopopathies and accumulation of cytosolic chromatin fragments. As an essential component of nuclear lamina, the maturation of functional lamin A/B from newly synthesized prelamin A/B follows a multistep process of posttranslational modification that involves farnesylation and methylation of its C-terminal cysteine before proteolytic cleavage of its C-terminal 15 amino acids (Fig. 4b). To test whether oxidative stress affects lamin distribution, we stably transduced NSPCs with a construct encoding either N-terminal GFP-tagged prelamin A (GFP-LMNA) or mCherry-tagged prelamin B1 (mCh-LMNB1). Strikingly, we found that compared to the control cells in which the tagged lamin proteins dispersed evenly along the nuclear envelopes, a large portion of PQ-treated NSPCs displayed an irregular lamin distribution, reminiscent of protein aggregation (Fig. 4c). To test whether the disruption of lamin processing could activate IFN-I response upon oxidative stress, we stably expressed in NSPCs a cysteine 585 to serine prelamin B1 mutant (LMNB1CS) that is defective of prelamin maturation—essential farnesylation and methylation. Immunofluorescence analysis of the cells indicated that the LMNB1CS mutant protein, recognizable by the mature lamin B1-specific 8D1 monoclonal antibody, formed the aggregate-like nucleoplasmic foci that were similar to the ones observed in wild-type lamin B1-transduced NSPCs under ROS treatment (Fig. 4d). qRT-PCR analysis further revealed that expression of LMNB1CS mutant alone was able to induce IFN-I response and downstream gene expression, and the effect could be further enhanced by ROS treatment (Fig. 4e). Conversely, expression of a C-terminus deletion form of mature lamin A mutant (LMNAΔC) strongly attenuated the ROS-induced IFN-I signaling activation and ISGs expression (Fig. 4f, g). These findings together suggest defective lamin processing as an underlying cause of IFN-I response under oxidative stress.

ROS-induced intracellular SAM depletion disrupts lamin maturation. Lamin maturation requires isoprenylation and methylation on the C-terminal cysteine residues. To determine how ROS regulates lamin posttranslational modification, we performed targeted quantitative polar metabolomics profiling by liquid chromatography-tandem mass spectrometry (LC–MS) on samples derived from control, pro- or anti-oxidant treated NSPCs. Among the 258 metabolites analyzed, we found that the turnover of SAM exhibited an inverse correlation with redox potential. As compared to the mock-treated control cells, treatment with the pro-oxidant PQ gave rise to a 1.5-fold reduction of cellular SAM and a 2.1-fold reduction of SAM to SAH ratio (Fig. 5a). By contrast, NSPCs treated with anti-oxidant NAC exhibited a 1.4-fold increase of cellular SAM level.

SAM is a principal methyl donor for a variety of biological processes including isoprenylcysteine carboxymethyl-transferase (ICMT)-mediated lamin methylation (Fig. 5b). Since prelamin methylation is a prerequisite step of lamin maturation, we next examined the effect of SAM depletion on lamin processing by treating NSPCs with cycloleucine (CL), a methionine adenosyl-transferase 2A (MAT2A) inhibitor. Immunofluorescence and immunoblot analyses revealed that inhibition of SAM production compromised lamin maturation, as indicated by the significantly reduced levels of 8D1-positive mature lamin B1 in CL- or PQ-treated NSPCs compared to their mock controls (Fig. 5c, d). In addition, CL treatment alone was sufficient to elicit IFNβ secretion and induction of ISGs (Fig. 5e, f). Consistently, disrupting lamin methylation by depletion of its methyltransferase ICMT (sg-Icmt) also inhibited lamin B1 maturation and provoked IFN-I response, as evidenced by the induction of TBK1 phosphorylation (Fig. 5g; Supplementary Fig.4), IFNβ secretion (Fig. 5h), and ISGs expression (Fig. 5i). Together, these findings suggest that SAM depletion and its dependent lamin methylation disruption are the underlying cause of ROS-induced cGAS/STING-IFN-I signaling activation.

ROS regulates intracellular SAM through GNMT. SAM is a universal co-substrate of methyl group transfer reactions. Intracellular SAM levels are balanced by MAT2A-catalyzed synthesis and its consumption through multiple catabolic processes. Since our metabolite profiling revealed little change of intracellular methionine—the precursor for SAM (Fig. 5a), we next examined the expression of the major enzymes involved in SAM metabolism. qRT-PCR analysis of control and ROS-treated NSPCs indicated that cellular expression of MAT2A, the enzyme that catalyzes the synthesis of SAM from methionine, remained relatively stable (Supplementary Fig. 5a). By contrast, the expression of GNMT, a key catabolic enzyme that catalyzes the SAM to SAH conversion, was markedly induced by PQ treatment but suppressed by anti-oxidant NAC (Fig. 6a–c).

GNMT catalyzes the reaction of glycine to sarcosine by using SAM as the methyl donor. Likewise, CRISPR/Cas9-mediated GNMT depletion by guide RNA (sg-Gnmt) led to two-fold enhancement of cellular SAM accumulation compared to the control sgRNA transduced cells (Fig. 6d, e). Conversely, doxycycline (DOX)-induced overexpression of exogenous GNMT conferred a rapid ~70% SAM depletion within 24 h and led to a reduction of global H3K4 methylation (Fig. 6f, g), consistent with its role as a principle methyl donor for histone methylation. Importantly, DOX-induced GNMT expression-induced IFNβ secretion and initiated a time-dependent IFN-I response by activating its downstream signaling and gene expression (Fig. 6h–j). These findings suggest that GNMT-regulated SAM depletion is a likely route to ROS-induced IFN-I activation.

To determine whether GNMT-regulated SAM depletion could also instigate nuclear leakage to activate cGAS/STING signaling, we transduced the DOX-inducible GNMT expressing NSPCs...
with a cytosolic DNA fragment-sensing cGAS-GFP construct. Compared to the mock-treated control cells, DOX-treated NSPCs exhibited a significant elevation of cGAS-GFP-containing foci that colocalized with cytosolic H3K4m3-positive DNA fragments (24.4 ± 1.275% vs 0.7 ± 0.45%) (Fig. 6k). Immunoblot analysis of the DOX-treated NSPCs further revealed a strong reduction of 8D1-positive mature lamin B1 protein level (Fig. 6l), suggesting that lamin maturation was compromised following GNMT induction. Consistently, transduction of a mature lamin mutant (GFP-LMNA\textsuperscript{CS}) in the DOX-inducible GNMT expressing NSPCs suppressed the GNMT induction-evoked IFN-β secretion as well as ISGs expression (Fig. 6m, n). Furthermore, co-treatment of NSPCs with cGAS inhibitor, RU.52145, largely suppressed PQ- or GNMT expression-induced IFN-I activation and restored their neuronal differentiation capacity (Supplementary Fig. 5b–d). Collectively, these data indicate that GNMT is a key regulator of IFN-I response under ROS treatment.

**Redox stress modulates NSPC neurogenic potential through FOXO3-regulated GNMT expression.** Our data indicate that FOXO3 is directly responsible for oxidative stress-induced IFN-I activation. Indeed, the frequency of PQ-induced cGAS-GFP foci was significantly reduced following FOXO3 depletion (Supplementary Fig. 6a, b). Conversely, enforced expression of the active FOXO3\textsuperscript{TA} mutant enhanced the percentage of irregular nuclei-containing NSPCs that displayed a reduced 8D1 staining...
Following 48 h of CL treatment. Mean ± s.e.m. of three independent experiments. Statistical significance was determined by one-way ANOVA for (f) and by two-sided unpaired t-test for (e, f, h, and i). Experiments for c, d, and g were repeated three times independently with similar results and representative images/blots are shown.

We next examined whether FOXO3-regulated ROS-initiated IFN-I response through GNMT. Treatment of NSPCs with pro-oxidant agent PQ promoted a marked increase of GNMT mRNA and protein expression relative to the mock-treated control cells (Fig. 7c, d). However, this ROS-induced GNMT up-regulation was significantly attenuated in the NSPCs depleted of FOXO3. As expected, the FOXO3-depleted NSPCs exhibited a steady increase of cellular SAM levels as relative to their respective controls before or after ROS treatment (Fig. 7e). Concordantly, knock-down of GNMT abolished FOXO3TA expression-induced IFN-I response and downstream gene expression (Fig. 7f).

We next went on to determine how FOXO3-GNMT/SAM-IFN-I signaling pathway regulates neurogenesis under oxidative stress condition. As expected, immunoblot and immunofluorescence analysis revealed that treatment of NSPCs with pro-oxidant PQ attenuated their neuronal differentiation capacity, as indicated by the reduction of both the expression level of neuronal marker TUBB3 and the percentage of TUBB3-positive cell population.
relative to the mock-treated control cells (Fig. 7g–j). Evidently, depletion of either FOXO3 or GNMT in the PQ-treated NSPCs reversed the ROS effect and was sufficient to restore their neurogenic potential (Fig. 7g–j). Notably, the FOXO3 depletion-promoted neuronal differentiation could be further blocked by DOX-induced exogenous GNMT expression, consistent with our finding that GNMT is a downstream effector of FOXO3 signaling (Fig. 7k).

To examine our findings under in vivo context, we next applied a well-established oxidative stress model by performing transient middle cerebral artery occlusion (tMCAO) followed by reperfusion in brain parenchyma46,47 (Supplementary Fig. 8a, b). Analysis of FOXO3 expression around SVZ regions showed a time-dependent increase of nuclear FOXO3 and loss of 8D1-positive mature lamin expression in NEStin + progenitor cell population following 1 h tMCAO (Supplementary Fig. 8c, d). Combined immunoblot and qRT-PCR analysis of the micro-dissected SVZ tissues further confirmed the TMCAO-induced increase of FOXO3, GNMT as well as other FOXO3 downstream and ISG gene expression (Supplementary Fig. 8e, f), suggesting ROS-FOXO3-GNMT/SAM-lamin-IFN-I signaling cascade is operative in vivo.

Lastly, considering the elevation of ROS in aging brain, we further examined type-I IFN stimulated gene expression in young old (≤60 year old) and aged (>60 year old) patient brain samples. Consistently, we observed a clear increase of ISGs along with GNMT mRNA expression in aged brains (Supplementary Fig. 9a, b). Altogether, our results suggest the FOXO3-GNMT/
Fig. 7 Redox stress impacts neurogenic potential of NSPCs through regulating SAM levels. a FOXO3 ChIP-qPCR analysis at Gnmt promoter in comparison to a gene desert region. Mean ± s.e.m. of four independent experiments. qRT-PCR (b, c) and WB (d) results for GNMT expression in NSPCs treated with PQ for 48 h. Mean ± s.e.m. of three independent experiments. e SAM levels in NSPCs expressing sg-NT or sg-Foxo3 treated for 48 h as indicated. Hundred percent of SAM = 50 μM. Mean ± s.e.m. of four independent experiments. f qRT-PCR results for Foxo3 and ISGs on either control adenovirus or FOXO3TA adenovirus-infected NSPC. Mean ± s.e.m. of three independent experiments. Statistical significance was determined by two-sided unpaired t-test for a and by one-way ANOVA for b, c, e, and f. WB (g, i, and k) and IF (h and j) for TUBB3 expressions of each NSPC line on 3 day of differentiation. Scale bar = 40 μm. Experiments for d, g, i, j, and k were repeated three times independently with similar results and representative images/bLOTS are shown. k. Model for the mechanism how cellular stress elicits IFN-I response and inhibits neurogenic differentiation potential of neural stem cells.

Discussion

Alterations of the redox state, as in many brain pathologies, regulate the fate of NSPCs18. Our study revealed that cellular stresses including a higher redox potential are translated into IFN-I response via FOXO3-GNMT/SAM-lamin changes (Fig. 7). In particular, we showed that redox potential controls NSPC function by altering IFN-I response through metabolic regulation of intracellular SAM availability. Mechanistically, our study uncovered a previously unidentified FOXO3 signaling cascade that functionally connects oxidative stress response with NSPC differentiation through SAM-depletion-induced IFN-I activation. Our findings of redox-dependent neurogenic regulation warrant future studies on the therapeutic rejuvenation of stress-impacted adult NSPCs.

FOXO transcription factors play a central role in a wide range of biological processes, including stress sensing and regulation of stress response12. Genetic studies from many organisms have repeatedly demonstrated the conserved insulin/IGF-P38-AKT-FOXO cascade as a major regulatory signaling pathway of aging and lifespan. In the central nervous system, expression of FOXO plays not only a key role in preserving neural stem cell pools9,10,
but also protects neurons against age-related axonal degeneration across species.\textsuperscript{16–18} Despite these advances, there still lacks a mechanistic understanding of how oxidative stress affects FOXO activation systematically and whether and how that contributes to the neuroprotective responses. In the current study, we identified FOXO3 oxidation at the evolutionarily conserved Cys31 residue as a regulatory mechanism that modulate redox-dependent FOXO3 nuclear-cytoplasmic shuttling and downstream signaling. Notably, a previous study reported that ROS-induced FOXO4 oxidation at Cys239 promotes its nuclear import by forming a disulfide-dependent protein complex with transportin-1.\textsuperscript{11} These findings suggest that redox-regulated nuclear shuttling is a conserved mechanism underlying FOXO-mediated oxidative stress response.

Our data indicate that FOXO3 mediates redox response through regulation of GNMT and downstream SAM catabolism. Enhanced SAM catabolism by GNMT extends the lifespan in Drosophila.\textsuperscript{19} In the nervous system, GNMT-mediated SAM metabolism is required for the proliferative signaling of NSPC and hippocampal neurogenesis.\textsuperscript{50} But the underlying mechanisms is unclear. Here, we found that treatment of NSPCs with oxidants led to upregulated GNMT expression and reduction of intracellular SAM availability. SAM is a metabolite generated via the one-carbon metabolism and is the main methyl donor in cellular methylation reactions.\textsuperscript{10,41} SAM depletion through dietary methionine restriction has been shown to modulate histone methylation and induce stem cell quiescence.\textsuperscript{43,44,51} Amongst the plethora of cellular SAM actions, our study found that not only SAM depletion in NSPCs confers a global reduction of H3K4 methylation, but is also sufficient to trigger cGAS/STING signaling and IFN-I response through regulation of nuclear lamin maturation. These findings support FOXO3-GNMT/SAM axis as a stress responsive program that protect tissue homeostasis by orchestrating anti-oxidative function, metabolic rewiring, and gene expression.

Defective lamin processing is known to cause various human pathologies, particularly those related to aging. A truncated lamin A causes a premature aging syndrome of Hutchinson–Gilford progeria. Consistent with our findings, expression of mutant lamin activated IFN-I response.\textsuperscript{52} In addition, recent reports suggest that lamins play important roles in both the inside-out and inside-out signaling processes. External mechanical forces trigger changes in nuclear envelope structure and composition, chromatin organization, and gene expression.\textsuperscript{53} Likewise, lamin A is stabilized upon external stress to protect the genome.\textsuperscript{54} These studies agree with our findings linking lamin and cellular stress response. We found that FOXO3 mediates oxidative stress response through regulation of intracellular SAM availability and nuclear lamin posttranslational modification. Introduction of a C-terminus deletion form of mature LMN\textsuperscript{m} blunts ROS-induced activation of cGAS/STING signaling and IFN-I response. These findings suggest a role of nuclear lamin as a signal transducer that mediates oxidative stress response.

Our study contributes to accumulating literature that links cytosolic DNA-sensing cGAS/STING-IFN-I program to physiologic and pathological responses to maintain CNS homeostasis. While initially recognized for its critical function in the innate immune response against viral infections, recent studies indicate that cGAS/STING-IFN-I pathway also mediates many other stress responses including signaling from DNA damage and oxidative stress.\textsuperscript{55–58} Notably, increased IFN-I response suppresses proliferation of NSPC and reduces their neuronal differentiation under oxidative stress.\textsuperscript{44,24,39} In this study, we demonstrated that oxidative stress response activates FOXO3-GNMT/SAM-cGAS/STING-IFN-I signaling cascade and regulates neurogenic potential of cultured NSPCs. Considering increased IFN-I response with declined neurogenesis is an indicative of aging brains,\textsuperscript{55–58} we propose cGAS/STING-IFN-I response as an intrinsic cellular surveillance system that protects NSPCs against the deleterious consequences of oxidative insults.

Consistent with our findings, previous aging studies reported that lowering systemic SAM levels by dietary restriction of its precursor methionine was effective toward extending lifespan and improving tissue functions in mammals.\textsuperscript{48} Engaging FOXO3-GNMT/SAM-lamin-IFN-I response to acute stress conditions is likely to protect organisms against losing long-term regenerative potential. This protective mechanism, nevertheless, may drive stem cell dysfunction by increasing quiescence and decreased differentiation potential at the face of chronic pathological stress stimuli. Altogether, our findings revealed molecular mechanisms that outline how oxidative stress may trigger IFN-I response-mediated cellular protective response and homeostasis under pathophysiological conditions.

**Methods**

**NSPC culture and differentiation.** Primary murine NSPCs isolated from subventricular zones (SVZ) and cultured as neurospheres are heterogeneous populations with limited repopulation potential. To avoid passage-dependent drift in NSPC populations, we utilized a neonate-derived immortalized Ink	extsuperscript{−/−} NSPC culture that maintains the multi-lineage differentiation capability.\textsuperscript{41} It contains a mixed population of relatively quiescent neural stem cells, activated NSCs and lineage-committed neuronal precursor cells, as well as oligodendroglial progenitors based on mRNA expression of lineage markers. NSPCs were cultured with N2 media including 20 ng/mL of EGF and BFGF in the presence or absence of 5 mM NAC, 10 μM PO, or 40 ng/mL Interferon-β. After 2 days, all growth factors and chemicals were removed and changed to N2 media including B27 supplement to induce differentiation. Cells were harvested at indicated time points for analysis. All the sources of materials are listed in Supplementary data 2.

**Generation of viral particles.** To generate lentivirus, 1.5 × 10\textsuperscript{7} 293T cells in 150 mm tissue culture dishes were transfected with 18 μg of each plasmid DNA along with 4.5 μg of pMD2.G and 9 μg of packaging vectors containing polyethyleneimine. The medium containing lentiviral particles were collected at 48 and 72 h posttransfection. The expression of GFP-FOXO3TA by adenoviral transduction was performed by incubating NSPC culture with pfu of purified adenoviral particles for 16 h. Empty adenoviral particles were used at the same pfu in control cultures. All the sources of plasmid DNAs, materials, viruses, and all oligo sequences are listed in Supplementary data 2.

**Measurement of ROS and redox potential.** Intracellular glutathione redox potential was determined by expressing pLPCX cyto Grx1-roGFP\textsuperscript{262}. Grx1-roGFP\textsuperscript{2} expressing NSPCs were treated with 10 mM N-ethylmaleimide for 5 min before fixing with 4% paraformaldehyde to prevent further oxidation. Cells from random fields were scanned by Olympus FLUOVIEW laser scanning confocal microscope using excitation at 405 nm/488 nm. Image analysis was performed using ImageJ (version1.52k) software to calculate 405/488 nm ratio.

**Measurement of IFNβ.** The medium was collected following 2 days of treatment. The cells were lysed with laemmli buffer and protein concentrations were determined by BCA assay. IFNβ was measured by using VeriKineTM Mouse IFNβ ELISA kit following the manufacturer’s instructions. All the sources of materials are listed in Supplementary data 2.

**SAM assay.** Metabolites were extracted by using cold 80% methanol from 3 million cells overnight at −80 °C. Relative SAM levels were determined by using MLL1 SAFacturer’s Assay kit following the manufacturer’s instructions. In brief, all standards and samples were incubated with MLL1 enzyme for 15 min and subsequently SAM-binding site probe for 15 min at room temperature. The levels of free probe for each well were determined by a plate reader (SpectraMax M4) with excitation and emission wavelengths of 575 nm and 620 nm, respectively. All the sources of materials are listed in Supplementary data 2.

**RNA extraction and qRT-PCR analysis.** Total RNAs were extracted from cells by using NucleoSpin RNA kit. Reverse transcription was carried out on 500 ng of total RNA using utilizing RevertAid RT kit. qRT-PCR was performed on CDNA samples using the PowerUp\textsuperscript{®} SYBR\textsuperscript{®} Green Master Mix on the 7500 Fast Real-time PCR system. All samples were run in duplicate and the mRNA level of each sample was normalized to that of ACTB mRNA. The relative mRNA level was presented as unit values of 2−ΔCt (∼Ct of ACTB−Ct of gene). All the sources of materials and primers are listed in Supplementary data 2.
RNA-seq and data analysis. Total RNAs were isolated from NSCs treated with 5 μM of PQ or 5 mM of NAC for 48 h and subjected to RNA sequencing at the Genomics Core facility of Well Cornell Medicine. RNA-seq libraries were prepared using the Illumina TruSeq stranded mRNA library preparation kit and sequenced on HiSeq4000 sequencer (Illumina). RNA-seq data were aligned to the mm9 reference genome using TopHat63, and Cufflinks was used to measure transcript abundances in fragments per kilobase of transcript per million mapped reads. GSEA analysis in this manuscript was generated from the GSEA preranked model65,66. The input of GSEA analysis is the gene expression level logFC (fold changes over control).

Immunofluorescence analysis. Cells were fixed with 4% paraformaldehyde for 10 min at room temperature followed by permeabilization with 0.2% Triton X-100 in PBS. The cells were subjected to immunofluorescence staining with antibodies against β-tubulin (TUBB3, 1:5000), H3K4m3 (1:10,000), Histone H3 (1:10,000), GNA11 (1:500), FOXO3 (1:1000), p-FOXO3/1/a (T24/T32, 1:1000), p-STAT1 (T701, 1:200), Akt (1:5000), p-PRAS40 (T246, 1:1000), ICMT (1:1000), lamin B1 (8D1, 1:500), lamin B1 (1:5000), or ICAM1 (1:1000) overnight at 4°C. Membranes were washed three times with PBS for 10 min and then incubated with HRP conjugated anti-mouse or anti-rabbit diluted in 3% skim milk for 1 h. Blots were developed with the SuperSignal™ West Pico Chemiluminescent substrate according to the manufacturer’s protocols. All the sources of materials and antibodies are listed in Supplementary data 2.

Immunoprecipitation for detecting Cys-sulfenylation. Cells were pretreated with 1 μM of dithiothreitol for 1 h and then treated with 100 μM of PQ or 200 μM of hydrogen peroxide for 6 h or 2 hr, respectively. Cell were lysed using 1% TNT buffer (135 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, and 1% Triton X-100) for 30 min on ice. After centrifugation to remove the debris, 1 μg of protein was incubated with 10 μL of Anti-DYKDDDDK Magnetic Agarose overnight at 4°C. Beads were washed three times with 1% TNT buffer and the proteins were eluted with 2× Laemmli buffer. The protein interaction was determined by western blot. All the sources of materials and antibodies are listed in Supplementary data 2.

Chromatin immunoprecipitation (ChIP). ChIP analysis was performed following the previous report66. In brief, 3 × 10^6 cells were crosslinked for 5 min with 1% paraformaldehyde and quenched with 125 mM glycine for 5 min at room temperature. After nuclei isolation, the chromatin was sheared in shearing buffer (50 mM Tris-HCl, 10 mM EDTA, and 0.1% SDS) using the Covaris M220 focused-ultrasonicator according to the manufacturer’s instructions. Immunoprecipitation was performed with 10 μg of anti-FOXO3 overnight at 4°C. Thirty microliters of pre-cleared Dynabeads® Protein G was added and incubated for 3 h at 4°C. The beads were washed by RIPA buffer (including LiCl) and eluted with elution buffer (50 mM Tris-HCl, 10 mM EDTA, and 1% SDS). After RNase and Proteinase K treatment, eluted DNA was reverse-crosslinked by 65°C incubation overnight. DNA was extracted using Nucleospin Gel and PCR clean-up DNA extraction kit and size-selection was carried out using a Agilent 2100 Bioanalyzer. qRT-PCR was performed using specific primers. All the sources of materials are listed in Supplementary data 2.

Metabolomics analysis. Ten million cells were homogenized in cold 80% methanol using homogenizer. Metabolites were extracted over 3 h at ~80°C. Samples were then centrifuged at 4°C for 10 min at 14,000 rpm. The supernatants were extracted and normalized based on tissue weight. Targeted LC/MS analyses were performed on a Q Exactive Orbitrap mass spectrometer coupled to a Vanquish UPLC system. The Q Exactive operated in polarity-switching mode. A Sequant ZIC-HILIC column (2.1 mm i.d. × 150 mm) was used for separation of metabolites. Flow rate was set at 150 μL/min. Buffers consisted of 100% acetonitrile for mobile A, and 0.1% NH4OH/20 mM CH3COONH4 in water for mobile B. Gradient ran from 85% to 30% A in 20 min followed by a wash with 30% A and re-equilibration at 85% A. Metabolites were identified on the basis of exact mass within 5 ppm and standard retention times. Relative metabolite quantitation was performed based on peak area for each metabolite.

Transient middle cerebral artery occlusion (tMCAO) model. Mice were group housed (up to 5 per cage) in individually ventilated cages with ad libitum access to food and acidified water (pH 2.5 to 2.8) in a temperature (22 ± 0.5°C) and humidity (30–70%) controlled facility with 12:12-h light-dark cycle. The animal care and use program is accredited AAALAC. All animal experiments were approved by the Well Cornell Institutional Animal Care and Use Committee. Eight- to ten-week-old mice (C57BL/6J) were subjected to the intraluminal suture-induced tMCAO model65,66. During the procedure, mice were deeply anesthetized with isoflurane and their body temperature was maintained at 37°C by a self-regulating heating pad. The intraluminal suture was used for the middle cerebral artery occlusion for 60 min followed by reperfusion. The success of the surgery was confirmed by measuring the blood flow in the territory of middle cerebral artery with laser speckle contrast analysis (PeriCam PSI HR). For sham surgery, animals were anesthetized and subjected to the same surgical procedures except insertion of the suture in the MCA. Brains were harvested for histological sectioning and protein/RNA extraction 3, 6, and 24 h after tMCAO.

Statistical analysis. We determined experimental sample sizes on the basis of preliminary data. All results are expressed as mean ± s.e.m. GraphPad Prism software (version 7.0e) was used for all statistical analysis. Normal distribution of the sample sets was determined before applying unpaired Student’s t-test for two group comparisons. One-way ANOVA was used to assess the differences between multiple groups. The mean values of each group were compared by the Bonferroni’s post-hoc procedure. Differences were considered significant when P < 0.05.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The data that support the findings of this study are available within the article and its Supplementary Information files. All the uncropped western blots and raw data are provided as a Source data file. RNA-seq data can be found on Gene Expression Omnibus (GEO) database with accession number GSE146243. Source data are provided with this paper.

Code availability. For specific requests, please contact the corresponding author.

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References.
1. Rossi, D. J., Jamieson, C. H. & Weissman, I. L. Stem cells and the pathways to aging and cancer. Cell 132, 681–698 (2008).
2. Molofsky, A. V. et al. Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during aging. Nature 443, 448–452 (2006).
3. Baker, D. J. & Petersen, R. C. Cellular senescence in brain aging and neurodegenerative diseases: evidence and perspectives. J. Clin. Investig. 128, 1208–1216 (2018).
4. Dong, C. M. et al. A stress-induced cellular aging model with postnatal neural stem cells. Cell Death Dis. 5, e1116 (2014).
5. Ito, K. et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature 431, 997–1002 (2004).
6. Miyamoto, K. et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell 1, 101–112 (2007).
7. Tothova, Z. & Gilliland, D. G. Foxo3 transcription factors and stem cell homeostasis: insights from the hematopoietic system. Cell Stem Cell 1, 140–152 (2007).
8. Yalcin, S. et al. Foxo3 is essential for the regulation of ataxia telangiectasia mutated and oxidative stress-mediated homeostasis of hematopoietic stem cells. J. Biol. Chem. 283, 25692–25705 (2008).
9. Renault, V. M. et al. Foxo3 regulates neural stem cell homeostasis. Cell Stem Cell 5, 527–539 (2009).
10. Paik, J. H. et al. Foxo3 cooperatively regulate diverse pathways governing neural stem cell homeostasis. Cell Stem Cell 5, 540–553 (2009).
11. Chukov, S., Levi, B. P., Smith, M. L. & Morrison, S. J. Pdmd16 promotes stem cell maintenance in multiple tissues, partly by regulating oxidative stress. Nat. Cell Biol. 12, 999–1006 (2010).
12. Martins, R., Lighthow, G. J. & Link, W. Long live FOXO: unraveling the role of FOXO proteins in aging and longevity. Aging Cell 15, 196–207 (2016).
13. Uttara, B., Singh, A. V., Zamboni, P. & Mahajan, R. T. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr. Neuropharmacol. 7, 65–74 (2009).
14. Kalamakis, G. et al. Quiescence modulates stem cell maintenance and regenerative capacity in the aging brain. Cell 176, 1407–1419.e1414 (2019).

15. Zipp, F. & Aktas, O. The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. Trends Neurosci. 29, 518–527 (2006).

16. Calixto, A., Jara, J. S. & Court, F. A. Diapause formation and downregulation of insulin-like signaling via DAF-16/FOXO delays axonal degeneration and extends lifespan in C. elegans. Aging Cell 18, 805–820 (2019).

17. Ishikawa, H. & Barber, G. N. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455, 674–678 (2008).

18. Kaletsky, R. et al. The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. Trends Neurosci. 29, 518–527 (2006).

19. Takesuchi, O. & Akira, S. Pattern recognition receptors and inflammation. Cell 153, 805–820 (2013).

20. Ishikawa, H. & Barber, G. N. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455, 674–678 (2008).

21. Okabe, Y., Sano, T. & Nagata, S. Regulation of the innate immune response by the threonine-phosphatase of eyes absent. Nat. Commun. 12, 640 (2021).

22. Hwang, I. et al. FOXO protects against age-progressive axonal degeneration. Aging Cell 17, e12701 (2018).

23. Kalamakis, G. et al. Quiescence modulates stem cell maintenance and regenerative capacity in the aging brain. Cell 176, 1407–1419.e1414 (2019).

24. Baruch, K. et al. Aging. Aging-induced type I interferon response at the transcriptome level. Cell Metab. 34, 533–544 (2021).

25. Nemono, S., Ostrove, J. & Samuels, C. F. Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. Science 295, 2450–2452 (2002).

26. Calnan, D. R. & Brunet, A. The FoxO code. Oncogene 32, 2457–2463 (2013).

27. Mentch, S. J. et al. Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism. Cell Metab. 22, 861–873 (2015).

28. Vincent, J. et al. Small molecule inhibition of cGAS reduces interferon expression in primary macrophages from autoimmune mice. Nat. Commun. 8, 750 (2017).

29. Murakami, K. et al. Mitochondrial susceptibility to oxidative stress exacerbates cerebral infarction that follows permanent focal cerebral ischemia in mutant mice with manganese superoxide dismutase deficiency. J. Neurosci. 18, 205–213 (1998).

30. Peters, O. et al. Increased formation of reactive oxygen species after permanent and reversible middle cerebral artery occlusion in the rat. J. Cereb. Blood Flow Metab. 18, 196–205 (1998).

31. Pozzorovski, T. V., Sirt1 contributes critically to the redox-dependent fate of neural progenitors. Nat. Cell Biol. 10, 385–394 (2008).

32. Obata, F. & Miura, M. Enhancing S-adenosyl-methionine catabolism extends Drosophila lifespan. Nat. Commun. 6, 8332 (2015).

33. Cai, X., Chiu, Y. H. & Chen, Z. J. The cGAS-cGAMP-STING pathway of immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatectomy MIF levels and stress resistance. Aging Cell 4, 119–125 (2005).

34. Eguchi, H., Fujiwara, N., Sakakura, Y., Yoshihara, D. & Suzuki, K. Hydrogen peroxide enhances LPS-induced nitric oxide production via the expression of interferon beta in BV-2 microglial cells. Neurosci. Lett. 494, 29–33 (2011).

35. Zheng, L. S. et al. Mechanisms for interferon-a-induced depression and neural stem cell dysfunction. Stem Cell Rep. 3, 73–84 (2014).

36. Miller, B. et al. Ase1p-tubulin is required for mitotic spindle assembly and cytokinesis. C. elegans. J. Cell Biol. 162, 1433–1443 (2003).

37. Bruggeman, S. W. et al. Inka and A if differentially affect cell proliferation and neural stem cell self-renewal in Bmi1-deficient mice. Genes Dev. 19, 1438–1443 (2005).

38. Gutsch, M. et al. Real-time imaging of the intracellular glutathione redox potential. Nat. Methods 5, 553–559 (2008).

39. Trappell, C., Pacher, L. & Salzberg, S. L. TopHat: discovering splice junctions with RNA-seq Bioinformatics 25, 1105–1111 (2009).

40. Trapnell, C. et al. Transcript assembly and quantification by RNA-Seq reveals unwarranted transcript annotations and韶 switching during cell differentiation. Nat. Biotechnol. 28, 511–515 (2010).

41. Subramanian, A. et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl Acad. Sci. USA 102, 15455–15505 (2005).

42. Mootha, V. K. et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat. Genet. 34, 267–273 (2005).

43. Wang, L. E. et al. CIC is a critical regulator of neuronal differentiation. JCI Insight 5, e135826 (2020).

44. Kim, G. S. et al. Critical role of sphinogmine-1-phosphate receptor-2 in the disruption of cerebrovascular integrity in experimental stroke. Nat. Commun. 6, 7193 (2015).

45. Lee, Y. K., Uchida, H., Smith, H., Ito, A. & Sanchez, T. The isolation and characterization of neural stem cell self-renewal in Bmi1-deficient mice. Nat. Genet. 34, 267–273 (2005).

46. Wang, L. E. et al. CIC is a critical regulator of neuronal differentiation. JCI Insight 5, e135826 (2020).

47. Kim, G. S. et al. Critical role of sphinogmine-1-phosphate receptor-2 in the disruption of cerebrovascular integrity in experimental stroke. Nat. Commun. 6, 7193 (2015).

48. Lee, Y. K., Uchida, H., Smith, H., Ito, A. & Sanchez, T. The isolation and characterization of neural stem cell self-renewal in Bmi1-deficient mice. Nat. Genet. 34, 267–273 (2005).
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Competing interests
L.C.C. is a founder and member of the BOD of Agios Pharmaceuticals and is a founder and receives research support from Petra Pharmaceuticals. These companies are developing novel therapies for cancer. Other authors have declared that no competing interests exists.

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