cGAS surveillance of micronuclei links genome instability to innate immunity

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DNA is strictly compartmentalized within the nucleus to prevent autoimmunity; despite this, cyclic GMP–AMP synthase (cGAS), a cytosolic sensor of double-stranded DNA, is activated in autoinflammatory disorders and by DNA damage. Precisely how cellular DNA gains access to the cytoplasm remains to be determined. Here, we report that cGAS localizes to micronuclei arising from genome instability in a mouse model of monogenic autoinflammation, after exogenous DNA damage and spontaneously in human cancer cells. Such micronuclei occur after mis-segregation of DNA during cell division and consist of chromatin surrounded by its own nuclear membrane. Breakdown of the micronuclear envelope, a process associated with chromothripsis, leads to rapid accumulation of cGAS, providing a mechanism by which self-DNA becomes exposed to the cytosol. cGAS is activated by chromatin, and consistent with a mitotic origin, micronuclei formation and the proinflammatory response following DNA damage are cell-cycle dependent. By combining live-cell laser microdissection with single cell transcriptomics, we establish that interferon-stimulated gene expression is induced in micronucleated cells. We therefore conclude that micronuclei represent an important source of immunostimulatory DNA. As micronuclei formed from lagging chromosomes also activate this pathway, recognition of micronuclei by cGAS may act as a cell-intrinsic immune surveillance mechanism that detects a range of neoplasia-inducing processes.

DNA is a key pathogen-associated molecular pattern that is sensed by innate immune receptors in the cytosol and endosomal compartments, so strict compartmentalization of cellular DNA in the nucleus and in mitochondria is necessary to avoid sensing of self-DNA. cGAS is an important cytosolic nucleic acid sensor, and double-stranded DNA (dsDNA) is its canonical ligand. cGAS activation generates the cyclic dinucleotide cyclic GMP–AMP (cGAMP), which in turn induces a type I interferon response via the adaptor STING (stimulator of interferon genes). Aberrant recognition of immunostimulatory cytosolic DNA has been implicated in neoplasia and systemic autoinflammatory diseases, with cGAS- or STING-dependent inflammation associated with mutations in multiple nucleases.

One such nuclease, RNase H2, maintains mammalian genome integrity through its role in ribonucleotide excision repair, suggesting that endogenous DNA damage may generate the nucleic acid ligands that are sensed by cGAS. Notably, micronuclei occur at a high frequency in Rnaseh2b−/− mice embryonic fibroblasts (MEFs) compared with Rnaseh2b+/+ or Trp53−/− MEFS (Fig. 1a; hereafter referred to as Rnaseh2b−/− and Rnaseh2b+/+, respectively). This led us to consider micronuclei as a potential source of immunostimulatory DNA. Such micronuclei, which have their own nuclear envelope (Fig. 1b), arise during mitosis from lagging chromosomal DNA and chromatin bridges, as a consequence of unresolved genome instability (Fig. 1c, Supplementary Video 1, Extended Data Fig. 1a, b and Supplementary Information). Increased micronuclei formation was also observed in Rnaseh2b+/−/− Trp53+/− or Rnaseh2b+/−/− Trp53+/− or Rnaseh2b+/−/− MEFs (Extended Data Fig. 1c, d), a model for the autoinflammatory disorder Aicardi-Goutières syndrome, confirming that micronuclei arise as a result of RNase H2 deficiency both in vitro and in vivo, irrespective of p53 status. As the interferon-stimulated gene (ISG) upregulation and proinflammatory response in both Rnaseh2b−/− MEFs and Rnaseh2b+/−/− MEFs is cGAS- and STING-dependent, accumulation of micronuclear DNA correlated with activation of the cGAS and STING pathway. Furthermore, investigation of the subcellular localization of cGAS in Rnaseh2b−/− MEFs stably expressing GFP–cGAS established that cGAS was strongly enriched in micronuclei (Fig. 1d; 83.3 ± 1.4% of micronuclei were GFP–cGAS-positive), whereas GFP alone showed no such localization (Extended Data Fig. 1e, f), consistent with cGAS binding to micronuclear DNA.

To determine whether localization of cGAS to micronuclei was a general phenomenon, exogenous DNA damage was induced in GFP–cGAS-expressing MEFs. After 1 Gy irradiation, we observed frequent localization of cGAS to micronuclei (Fig. 1e), along with a cGAS-dependent proinflammatory response. Increased secretion of CCL5 (a robust indicator of cGAS-dependent ISG responses in MEFs) correlated with increased frequency of micronuclei in both Trp53+/+ and Trp53−/− MEFs (Fig. 1f–h). Furthermore, consistent with an increased tendency to form micronuclei in Trp53−/− cells, following irradiation both micronucleus formation (P = 0.0078) and CCL5 production (P = 0.020) were significantly enhanced compared with Trp53+/+ cells. ISG transcripts were also induced (Extended Data Fig. 1g) at levels comparable to those found in previous studies of genotoxic damage.

In human U2OS osteosarcoma epithelial cells, endogenous cGAS was detected by immunofluorescence in spontaneously formed micronuclei (Extended Data Fig. 1h–j). Strong micronuclear enrichment of cGAS contrasted with weak diffuse cytoplasmic localization in cells without micronuclei, consistent with endogenous relocalization of cGAS to micronuclei. We therefore conclude that cGAS frequently localizes to micronuclei, irrespective of the source of DNA damage initiating their formation. However, given that a nuclear envelope normally encloses micronuclei, it was not clear how cGAS gains access to these structures.

Micronuclear DNA is particularly susceptible to DNA damage, leading to chromothripsis. This occurs as a consequence of irreversible nuclear envelope collapse, which arises frequently in micronuclei due to defective nuclear lamina organization. Given that this leads to partial loss of compartmentalization, we postulated that membrane rupture would also result in relocalization of cGAS to micronuclei, to induce a cGAMP-driven proinflammatory response (Fig. 2a). Consistent with this, we observed that micronuclei positive for γH2AX, 

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Figure 1 | cGAS localizes to micronuclei resulting from endogenous or exogenous DNA damage. a, Micronuclei form frequently in Rnaseh2b−/− MEFs and are associated with genome instability. Percentage of cells with micronuclei in two Rnaseh2b+/+ control and two Rnaseh2b−/− MEF lines. Mean ± s.e.m. of n = 3 independent experiments (≥500 cells counted per line). b, Micronuclear DNA is surrounded by its own nuclear envelope. Representative image with Lamin B1 (red) staining the nuclear envelope and DAPI (blue) staining DNA. c, Micronuclei form after mitosis as a consequence of impaired segregation of DNA during mitosis, originating from chromatin bridges and lagging chromosomes or chromatin fragments. d, GFP-cGAS localizes to micronuclei in Rnaseh2b−/− MEFs. Representative image of GFP-cGAS-expressing Rnaseh2b+/+ MEFs.

Figure 2 | cGAS localizes to micronuclei upon nuclear envelope rupture. a, Model: micronuclear membrane rupture leads to sensing of DNA by cGAS. Micronuclei are susceptible to nuclear envelope collapse, which permits cytosolic cGAS access to genomic dsDNA, initiating a cGAS–STING-dependent proinflammatory immune response through production of the second messenger cGAMP. b, c, Localization of cGAS to micronuclei in U2OS cells inversely correlates with localization of Rb, which is present only in micronuclei with an intact nuclear envelope. Representative images. c, Quantification (mean ± s.e.m. of n = 3 independent experiments; ≥250 micronuclei counted per experiment).

e–h, cGAS localizes to micronuclei induced by ionizing radiation and is associated with a cGAS-dependent proinflammatory response. e, Representative image of GFP-cGAS-positive micronuclei following 1 Gy irradiation (IR) in Trp53−/− MEFs. f, Trp53−/−, Trp53+/+ and cGAS−/− (also known as Mbx2d−/−) (cGAS null) MEFs were irradiated (1 Gy), and CCL5 production (g) and percentage of cells with micronuclei (h) assessed after 48 h. Mean ± s.e.m. of n = 2 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed t-test; NS, not significant. Scale bars, 10 μm. Rnaseh2b+/+ and Rnaseh2b−/− MEFs in this figure and subsequent figures are on a Trp53−/− C57BL/6 background (absence of p53 is a prerequisite for generation of Rnaseh2b−/− MEFs).
Integration, as measured by loss of micronuclear mCherry–NLS (Fig. 2e, Supplementary Video 2). We therefore conclude that nuclear envelope rupture results in exposure of DNA to the cytoplasmic compartment, leading to relocalization of cGAS to micronuclear chromatin.

Activation of cGAS requires dimerization, with each cGAS monomer binding to dsDNA molecules. Combined, these requirements might therefore preclude activation of cGAS that is bound to chromatin. In addition, a published model suggests that there would be steric clashes between bound DNA molecules and predicts that cGAS activation will therefore occur near the ends of dsDNA. However, we reasoned that the flexible nature of DNA could permit cGAS dimerization on continuous DNA and chromatin, given the accessibility of linker DNA between nucleosomes. To test whether cGAS can be activated by DNA that does not contain free ends, we measured production of cGAMP by recombinant cGAS in the presence of plasmid DNA using a chromatography-based assay and found that cGAMP production was similar in the presence of either circular DNA or fragmented plasmid DNA (Fig. 3a, b, Extended Data Fig. 4a, b), establishing that DNA ends are not required for activation of cGAS. Furthermore, supercoiled plasmid DNA induced strong cGAS-dependent CCL5 production in MEFs (Extended Data Fig. 4c). We then prepared synthetic chromatin and found that cGAS can also bind to DNA in the presence of nucleosomes (Extended Data Fig. 5a, b) and that this leads to substantial cGAMP production (Fig. 3c, Extended Data Fig. 5c). Chromatin isolated from cells also activated recombinant cGAS (Extended Data Fig. 5d–g). We therefore conclude that cGAS can bind to and be activated by chromatin.

Small DNA fragments detected by antibodies against dsDNA have been proposed to leak from sites of DNA damage through the interphase nuclear envelope into the cytoplasm and to activate the cGAS–STING pathway. In contrast, as micronuclei are generated at mitosis, a prediction of our model is that the immune response will be cell-cycle dependent. To test this prediction, we induced DNA damage in MEFs arrested in G0 by serum starvation. Such cell-cycle-arrested MEFs still displayed functional cGAS signalling, producing similar amounts of cytokine in response to transfected exogenous DNA compared with actively cycling cells (Extended Data Fig. 6a, b). However, cell-cycle-arrested MEFs did not form micronuclei, nor did they exhibit innate immune activation after exposure to equivalent levels of ionizing radiation, despite undergoing equal levels of DNA damage (Fig. 4a–d, Extended Data Fig. 6d, e). Therefore, DNA damage is not sufficient by itself to generate innate immune activation in response to ionizing radiation; with the dependence on cell-cycle progression consistent with mis-segregated DNA at mitosis being the origin of cGAS activation. Quantification of the levels of micronuclear DNA (Extended Data Fig. 7) indicates that it would be sufficient to generate a relevant cytokine response, also supporting micronuclei as an important source of cell-intrinsic immunostimulatory DNA.

We also investigated whether micronuclei initiated by a DNA damage-independent mechanism resulted in similar cGAS relocalization and an associated proinflammatory response. Micronuclei containing whole chromosomes were generated through pharmacological induction of lagging chromosomes by nocodazole treatment (Extended Data Fig. 8a, b). This resulted in a substantially increased frequency of micronuclei in both MEFs and U2OS cells (Extended Data Fig. 8c, d). The number of cGAS-positive micronucleated cells...
also increased significantly ($P = 0.0083$; Extended Data Fig. 8e), leading us to conclude that nocodazole-induced micronuclei could also be detected by endogenous cGAS. Induction of micronuclei by nocodazole also induced a proinflammatory response with significantly elevated CCL5 cytokine production in MEFs ($P = 0.0008$; Extended Data Fig. 8f). This response, but no increase in DNA damage, was detected from 16 h after nocodazole treatment, consistent with a post-mitotic origin (Extended Data Fig. 8h, i). We therefore conclude that micronuclei arising from mis-segregated chromosomes, as well as genome instability, can induce cGAS signalling.

To confirm a direct relationship between micronuclei and cGAS pathway activation, we assessed at the single-cell level whether the induction of ISGs was specific to micronucleated cells. Forty-eight hours after irradiation (1 Gy), we identified individual micronucleated cells (MN+) and control cells with normal nuclear morphology (MN−) microscopically (Extended Data Fig. 9) and isolated them using laser capture microdissection for subsequent single-cell mRNA sequencing (Fig. 5a). To avoid confounding biases, all cells were collected from the same culture dish and processed in parallel, with library preparation performed in a single 96-well plate with MN+ and MN− cells interdigitated, and sequencing datasets down-sampled to the same number of reads after mapping. For the thirty-five RNA sequencing libraries that passed quality control (Extended Data Fig. 9a), we first examined a high-confidence list of 11 ISGs that had been previously shown to be induced by endogenous genome instability in MEFs5. Six of the ISGs were represented in the RNA sequencing data, and strikingly we detected transcripts for five of these (Ccl5, Isg15, Ifi27l2a, Samd9l and Cxcl10) specifically in micronucleated cells ($P = 0.047$, Fisher’s exact test, Fig. 5b). Examination of pooled cells down-sampled to identical numbers of sequence reads aligned to these individual genes confirmed differential expression between MN+ and MN− cells (Fig. 5c).

We next assessed the MN+–specific upregulation of a large set of independently defined type I IFN-induced genes ($n = 336$ ISGs) on a transcriptome-wide basis. We performed a gene set enrichment analysis (GSEA) against genes ranked by $z$-scores for differential expression (MN+ versus MN−) calculated by the Single Cell Differential Expression (SCDE) analysis package. GSEA confirmed that expression of these ISGs was significantly enriched in the pool of micronucleated cells over control cells (Fig. 5d; normalized enrichment score 1.52, $P = 2.04 \times 10^{-4}$). These single-cell analyses were therefore consistent with micronuclei being the source of DNA damage-induced ISGs (Fig. 5e). In conclusion, while the formal possibility remains that other dsDNA fragments in cells with micronuclei also activate cGAS, this and our other experimental findings strongly implicate micronuclei as a substantial source of cell-intrinsic immunostimulatory DNA.

Micronuclear membrane breakdown provides a mechanism by which dsDNA is exposed to the cytoplasmic sensor cGAS, with spontaneous rupture being frequent and generally irreversible. As chromatin activates cGAS, physiological ruptures in the primary nuclear membrane when cells migrate through tight interstitial spaces
may also cause transient cGAS activation. Notably, mitotic chromosomal abnormalities are transiently exposed to the cytosol, prompting the important unanswered question of how cGAS–STING pathway activation is prevented during cell division. Although cGAS can localize to nuclear DNA during mitosis (Extended Data Fig. 10a, b), the transient nature of mitosis, hypercompaction of DNA and the peri-chromosomal layer of proteins may mitigate against cGAS binding and activation. In addition, post-translational regulation of cGAS or downstream pathway components in conjunction with transcriptional silencing of mitotic chromosomal abnormalities may also prevent ISG induction.

Conversely, nuclear membrane breakdown in disease states may be pathologically relevant. Additionally, other aberrant structures generated during cell division may lead to cytosolic DNA exposure. For instance, we infrequently observed cGAS on interphase chromatin bridges in Rnasch2β−/− cells and in U2OS cells (Extended Data Fig. 10c, d), such persistent chromatin bridges, such as those arising after telomere crisis, could also activate cGAS. Furthermore, while we observed enhanced ISG induction in micronucleated cells, free dsDNA fragments, perhaps released at mitosis, might also activate cGAS in specific contexts. Therefore, while micronuclei provide a substantial source of immunostimulatory DNA, in other pathological contexts additional mechanisms that impair nuclear compartment integrity may play a role.

Micronuclei frequently form in cancer cells, and chromosome and genome instability are key drivers of neoplasia. Hence, our work predicts that cGAS will often become activated by this route during neoplastic transformation, leading to cGAS- and STING-dependent tumour-suppressive immune responses. Consequently, there may be selection pressures during cancer evolution to inactivate cGAS–STING signalling, providing an additional explanation for its frequent inactivation in tumours, alongside oncogene-mediated silencing associated with virally induced neoplasia. In conclusion, sensing of ruptured micronuclei by cGAS represents a cell-intrinsic surveillance mechanism that links genome instability to innate immune responses, of relevance to both cancer and autoinflammation.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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The experiment was based on genotypes, no randomization was possible.

Propidium iodide (Sigma) was added before data acquisition, using an LSR Fortessa

Cells were grown on glass coverslips and fixed in 4% paraformaldehyde (PFA) in PBS for 20 min at room temperature. Cells were permeabilized in 0.5% Triton X-100 for 5 min before blocking for 30 min with 1% BSA in PBS. Coverslips were then incubated with primary antibody for 1 h, followed by incubation with a secondary antibody for 45 min. Primary and secondary antibodies were diluted in blocking buffer and all incubations were performed at room temperature. Coverslips were mounted using Vectashield AntiFade Mounting Medium with DAPI (Vector Laboratories) and imaged using a Photometrics CoolSnap HQ2 CCD camera and a Zeiss AxioPlan II fluorescence microscope with plan-neofluor objectives and images captured with iVision software (BioVision Systems). All scoring was performed under blinded conditions.

The following antibodies were used for immunofluorescence: cGAS (D1D3G, Cell Signalling, 1:200), phospho-histone H2A.X (Ser139) (2577, Cell Signalling, 1:1,000), Lamin B1 (ab16048, Abcam, 1:1,000) and Retinoblastoma (554136, BD Biosciences, 1:200). Secondary antibodies, anti-mouse–Alexa Fluor 488 (A11029), anti-rabbit–Alexa Fluor 488 (A11081) and anti-rabbit–Alexa568 (A11036) (all Invitrogen), were used at 1:500 or 1:1,000 dilution.

Determination of micronucleus frequency. After fixation and DAPI staining, the percentage of cells with micronuclei was determined by microscopy under blinded conditions. Micronuclei were defined as discrete DNA aggregates separate from the primary nucleus in cells where interphase primary nuclear morphology was normal. Cells with an apoptotic appearance were excluded.

Erythrocye micronuclear assay. The presence of micronuclei in erythrocytes was quantified using previously described methods21. 50 μl of blood from female Rnaseh2btm1d+/− mice (8–9 months old) and sex-matched control C57BL/6J mice was collected and expelled into 250 μl heparin solution (500 USP units per ml PBS) on ice. 180 μl of this suspension was expelled into 2 ml of pre-cooled (−80 °C) methanol and aggregates removed by robust tube tapping. The cell suspension was incubated at −80 °C for at least 24 h before staining for flow cytometry.

To stain the cells, 12 ml ice-cold saline solution (0.9% w/v NaCl, 5.3 mM NaHCO3, pH 7.3) was added to the cell suspension and the tube was inverted to mix and placed on ice. Cells were centrifuged at 600g for 5 min at 4°C, supernatant removed and resuspended by pipetting. 10 μl of cells per sample was stained with 90 μl of staining solution containing 20 μM Fluo-3/AM and 25 μM ionomycin A23187 at 10 μg/ml−1 (Sigma) and 1 μl rat anti-mouse CD71–FITC (Invitrogen RM5301) for 30 min on ice followed by 30 min at room temperature. 1 ml of 1.25 μg/ml−1 propidium iodide (Sigma) was added before data acquisition, using an LSR Fortessa (BD Biosciences). Data were analysed using FlowJo v7.6.5 software (Tree Star).

Assessment of immune responses. The concentration of CCL5 in supernatants was determined by ELISA (R&D Systems). The cellular response to dsDNA was determined using herring testes DNA (HT-DNA) (Sigma) at a final concentration of 1 μg/ml−1 (Extended Data Fig. 6) or interferon stimulatory DNA (ISD, naked, Invivogen) at a final concentration of 1.33 μg/ml−1 (Extended Data Fig. 8). Cells were incubated overnight and transfected the following day using Lipofectamine 2000/3000 in Opti-MEM reduced serum medium (both thermo Fisher Scientific). The immune response was assessed at 24 h by ELISA using collected supernatant.

RT–qPCR. RNA was extracted from adherent cells using the RNeasy kit (Qiagen) per the manufacturer’s instructions and using the included DNase I treatment. cDNA was prepared using iScript III RT and random oligomer primers (Thermo Fisher Scientific). qRT–PCR was performed using SYBR Select Master Mix (Thermo Fisher Scientific) on an LC480 Real-Time PCR machine (Roche). The expression of target genes was normalized to the housekeeping gene Hprt using the formula (2−ΔC). Supplementary Table 1 shows the primers used.

X-ray irradiation. 1.3 × 105 cells were seeded per well of a 6-well plate onto glass coverslips (Fig. 1). The following day, cells were irradiated for 1 min at a dose rate of 1 Gy per min using an X-ray irradiation system (Faxitron 43855D, Faxitron X-ray Corporation) operated at 130 kVp. After a further 48 h the culture medium was replaced and CCL5 concentration assessed by ELISA (R&D Systems). Cells on coverslips were fixed with 4% PFA in PBS for 20 min at room temperature for immunofluorescence imaging.

Note on imaging. For live cell imaging, Rnaseh2btm1d+/− MEFs transiently expressing mCherry–H2B or U2OS cells stably expressing GFP–cGAS and mCherry–NLS were seeded onto glass-bottomed plates (Greiner Bio One). pmCherry–H2B (gift from P. Vagnarelli, Brunel University, London) was transfected by electroporation with the Neon transfection system (Thermo Fisher Scientific) according to the manufacturer’s instructions. Prior to imaging, U2OS cells were treated with 200 ng/ml−1 nocodazole (Sigma) for 12 h and additionally stained with 0.5 μM SIR-DNA (Sirochrome). Cells were maintained at 37 °C in Leibovitz L-15 medium (Gibco). TRITC, FITC and Cy5 image datasets were collected using a 40× plan neofluor (0.75NA) objective on an Axiovert 200 fluorescence microscope (Zeiss) equipped with a Retiga6000 CCD camera (Qimaging) or an Axio-Observer Z1 fluorescence microscope (Zeiss) equipped with an evolve EMCCD camera (Photometrics). Images were recorded every 1.5 min over a 4-h period (U2OS cells) or every 5 min over a 16-h period (MEFs) using Micromanager (https://open-imaging.com/) and subsequently deconvolved using Velocity software (PerkinElmer).

cGAS recruitment following micronuclear envelope disruption was analysed by measuring the mean fluorescence intensity of mCherry–NLS or GFP–cGAS over time. The mean fluorescence intensity for a cytoplasmic background of matched area was subtracted from the micronuclear signal. The background subtracted fluorescence intensity values were then normalized so that the highest fluorescence intensity was 1 and the lowest fluorescence intensity 0. The time of rupture (t = 0) was set at the point of maximal decline in the mean mCherry–NLS fluorescence signal. Rupture was observed in n = 40 micronuclei during live imaging experiments, in 29 of which detectable levels of cGAS were observed subsequently, and time of entry was then quantified in 11 of these, where movie length was sufficient to follow cGAS accumulation to maximal intensity.

Serum starvation experiments. C57BL/6J (Trp53−/−) MEFS (1.5 × 104 for asynchronous and 6 × 104 for serum starvation per well) were seeded into a 6-well plate and serum-free 24 h before serum starvation was chosen to achieve similar cell numbers for both serum starvation and control conditions (assay endpoint). After 5 h the medium was replaced with low-serum medium (0.25% FBS) for serum starvation, and with fresh medium (10% FBS) for asynchronous/cycling cells. After a further 24 h, the medium was again replaced with 1.6 ml (0.25% FBS for serum starved cells; 10% FBS for control cells), and cells irradiated with 1 or 5 Gy (at 1 Gy per min) (Faxitron 43855D, Faxitron X-ray Corporation). After 48 h the supernatants and cells were harvested, CCL5 measured by ELISA and RT–qPCR performed.

To assess the immune response of serum-starved cells to HT-DNA, C57BL/6J (Trp53−/−) MEFS (1.5 × 104 per well) were seeded into a 6-well plate and grown in serum-containing medium for 24 h. Cells were then transfected with 1 μg per ml HT-DNA in Opti-MEM using Lipofectamine 3000 (Thermo Fisher Scientific). After 6 h the medium was replaced with 10% or 0.25% FBS-containing medium, respectively, and 24 h after transfection supernatants were taken for ELISA. To ensure differences in cell number (arising from G0 arrest or irradiation) were not confounders for ELISA results, adherent cells were
counted after trypsinization, with ELISA results corrected for final cell number per well.

To assess the frequency of micronuclei by immunofluorescence, cells were fixed with 4% PFA for 20 min at room temperature and permeabilized with 0.5% Triton X-100 for 5 min. Cover slips were mounted with Vectashield (with DAPI) and the percentage of cells with discrete micronuclei determined under blinded conditions. More than 500 cells per condition per experiment were analysed for micronucleus counts. To assess DNA damage by H2AX immunofluorescence in serum-starved and asynchronous cells post irradiation, 2 × 10^6 cells per well were seeded on coverslips and incubated in normal or 0.25% FBS medium. Cells were then irradiated with 1 or 5 Gy, and fixed 2 h later. n > 100 cells per condition per experiment (n = 2).

Chromosome mis-segregation assay. Micronuclei induced by chromosome mis-segregation were generated pharmacologically by nocodazole treatment as described previously. MEFs were plated at 8 × 10^5 cells per 100-mm plate, and 24 h later incubated with 100 ng/ml nocodazole (Sigma) for 6 h. Mitotic cells were then harvested by shake-off and washed three times with PBS before counting and plating. 1 × 10^5 mitotic cells were plated per well of a 12-well plate into 800 µl fresh medium. 1.5 × 10^5 mitotic cells were concurrently plated onto a coverslip in one well of a 12-well plate. After 48 h, culture medium was removed for ELISA and cells on coverslips fixed for imaging to determine micronucleus frequency.

To induce micronuclei in U2OS cells, 7 × 10^5 cells were seeded per 100-mm plate and incubated for 9 h. Cells were then synchronized with 2 µM thymidine (ACROS Organics) for 27 h and released by washing twice and replacing with fresh medium. After 14 h, U2OS cells were incubated with 100 ng/ml nocodazole (Sigma) for 6 h and mitotic cells harvested by shake-off. 3 × 10^5 mitotic cells were plated onto a coverslip in one well of a 12-well plate and incubated for 48 h before performing for microscopical analysis.

Chromosome mis-segregation time-course assay. C57BL/6J TgSp35 ^−− MEFs (8 × 10^5) were plated in 10-cm plates. The next day, cells were incubated with 100 ng/ml nocodazole (Sigma) for 6 h and mitotic cells harvested by shake-off. 3 × 10^5 mitotic cells were then plated into a 12-well plate (1 × 10^5 per well) or onto coverslips (7 × 10^5) for immunofluorescence analysis. Asynchronous cells were plated at the same number concurrently. Supernatants were taken and coverslips fixed after 6, 16 and 22 h. Immune response was analysed by ELISA and coverslips were pre-extracted on ice using 0.5% Triton X-100 for 5 min at room temperature. After blocking for 30 min with 3% BSA at room temperature, H2AX Ser139 antibody (05-636 Millipore) was added for 2 h at room temperature. Alexa Fluor 568 goat anti-mouse secondary antibody (Life technologies) was then applied and incubated for 1 h at room temperature. Coverslips were mounted using Vectashield antifade mounting medium with DAPI (Life technologies) and imaged at room temperature using a Coolsnap HQ CCD camera (Photometrics) and a Zeiss Axioplan II fluorescence microscope with a GFP filter, and LCM of individual cells performed using a Zeiss Palm Microbeam 4 (Laser Microdissection). The cGAS enzyme activity assay was performed as described previously, but an increased concentration of NP40 detergent (0.2%) was used in buffer NBB and buffer NBR was replaced by buffer NBR2 (buffer NBR modified to contain 1 mM MgCl₂ and 1 mM CaCl₂). Nuclei were resuspended at 20 A260 in buffer NBR2 and digested with MUnase (400 units per ml nuclei; NEB) for 10 min at room temperature. The reaction was stopped by adding EDTA to 10 mM, and nuclei were resuspended in 500 µl TEP20N (10 mM Tris, pH 8; 1 mM EDTA; 20 mM NaCl, 0.5 µM PMSE, 0.05% NP40) and incubated at 4°C overnight. Soluble chromatin was recovered by centrifugation (5 min, 20,000g) and purified on a 10–50% step gradient in TEP80 (10 mM Tris, pH 8; 1 mM EDTA; 80 mM NaCl, 0.5 µM PMSE) in a SW55 centrifuge tube (Beckman) and centrifuged at 50,000 r.p.m. for 10 min in a SW55 rotor as described. Chromatin was recovered by upward displacement while monitoring the absorbance at 254 nm in 10 samples of 0.5 ml. Aliquots of each fraction were analysed for DNA and protein to check sample integrity. Peak chromatin fractions were dialysed into TEP80 overnight and the concentration determined by measuring the absorbance at 260 nm in 2 M NaCl, 5 U/ml. Naked DNA was prepared from chromatin by proteinase K treatment in 0.25% SDS, 50 mM NaCl, 5 mM EDTA for 2 h at 55°C, followed by phenol/chloroform extraction and ethanol precipitation.

Synthetic chromatin was prepared using standard approaches adapted from refs 41, 42. Essentially, a 601 DNA template (25 × 197 bp 601 DNA) was reconstituted with purified chicken core histone octamers at a 1:1 molar ratio in TEP2000 (10 mM Tris, pH 7.5, and 0.2 mM EDTA, 2 M NaCl, 0.5 µM PMSE) and dialysed from 2 M to 400 mM over 6 h and then into 10 mM NaCl overnight, using a Thermofisher microdialysis cap (10,000 MWCO) in a linear gradient maker. Chromatin concentration was measured by measuring absorbance at 254 nm and reconstitution chromatin was analysed by band-shifts, sucrose gradient sedimentation and nucleosome digestion.

Single-cell laser capture microdissection. C57BL/6J MEFs were irradiated (1 Gy) and re-seeded onto 50 mm PEN membrane dishes (Zeiss) 32 h later. Pigcogene-containing medium (Quant-IT PicoGreen dsDNA reagent, 4 µl per ml) was added to the cells 48 h after irradiation to stain DNA, and laser capture microdissection (LCM) performed as follows. Micronucleated cells (n = 32) and control cells with normal nuclear morphology (n = 28) were identified from the same dish using an GFP filter, and LCM of individual cells performed using a Zeiss PicoSmear Microscope. Cells were collected into 50 µl 0.2% (v/v) Triton X-100, 2 µg/ml RNasin Ribonuclease Inhibitor (Promega), snap frozen on dry ice and stored at −80°C until library preparation.

cDNA and library preparation. All 60 cells obtained from LCM were processed until cDNA amplification (with approximately equal numbers of MN− and MN− cells in each batch, and cDNA from single cells was obtained using the Smart-seq2 protocol with minor modifications, as described previously). Cells from each batch were processed in LCM-compatible 0.5 ml Eppendorf tubes until cDNA amplification and both batches then transferred to a single 96-well plates for library generation using Illumina Nextera reagents. Libraries were assessed for size distribution on an Agilent Bioanalyzer (Agilent Technologies) with the DNA HS Kit, and then quantified using a Qubit 2.0 Flurometer (ThermoFisher). Sample and MN− screens and the FLA-5100 imaging system (Fujifilm). (11:7:2 v/v/v). The plate was air-dried and images collected using phosphorimaging screens and the FLA-5100 imaging system (Fujifilm).
and down-sampled to the same number of reads for every cell. Raw reads were counted over genes and genes with at least one read were scored as detected genes. Heatmaps were generated using Matrix2png. The upper part of the heatmap (Fig. 3b) shows detected ISGs from a high confidence list of genome instability induced ISGs, calculated as the overlap between genes identified in refs 52 and 5. Out of these 11 genes, transcripts for 5 were not detected in any cell. A Fisher's exact test was performed under the null hypothesis that there was no excess of transcribed ISGs in MN+ versus MN− cells. Genome browser shots were taken from pooled reads from all MN+ and MN− cells after pools were down-sampled to the same number of reads.

Statistics. All data are plotted as mean values, with variance as s.e.m. unless stated otherwise. Statistical analysis was performed using Prism (Graphpad Software Inc.). For all quantitative measurements, normal distribution was assumed, with t-tests performed, unpaired and two-sided unless otherwise stated. No statistical methods were used to predetermine sample sizes, which were determined empirically from previous experimental experience with similar assays, and/or from sizes generally employed in the field.

Data availability. Single-cell RNA sequencing data that support the findings of this study have been deposited in GEO with the accession code GSE100771. All other data are available upon reasonable request from the corresponding authors.

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Extended Data Figure 1 | See next page for caption.
Extended Data Figure 1 | Micronuclei form in RNase H2 deficiency, with cGAS localizing to these structures and inducing an ISG response.

a, Still images of live imaging in Rnaseh2b−/− MEFs, time in minutes; t = 0, prophase. Lagging DNA (blue arrowheads) and DNA bridges (orange arrowhead) at anaphase can result in interphase micronuclei (green arrowheads). b, Chromatin bridges and lagging chromosomal DNA (arrows) occur in Rnaseh2b−/− MEFs. Representative fixed cell images. c, d, Erythrocyte micronuclei assay. c, Representative flow cytometry plot with quadrants containing reticulocytes and micronucleated normochromatic erythrocytes indicated. d, Rnaseh2bA174T/A174T mice have a significantly increased frequency of micronucleated erythrocytes. Mean ± s.e.m., n = 3 mice per group; two-tailed t-test, *P < 0.01.

e, f, eGFP does not accumulate in micronuclei, whereas the majority of micronuclei show strong accumulation of GFP–cGAS. e, Representative image of micronucleus-containing Rnaseh2b−/− MEFs stably expressing eGFP. f, Quantification of GFP-positive micronuclei for GFP–cGAS-expressing and GFP-expressing Rnaseh2b−/− MEF lines. Mean ± s.e.m., n = 4 experiments (>500 cells counted per experiment). Scale bars, 10 μm.

g, Increased levels of ISG transcripts (Ifit1, Ifit3, Isg15, Cxcl10 and Oas1a) were detected in C57BL/6J (Trp53+/+) MEFs 48 h after irradiation. Transcript levels were normalized to Hprt. Mean ± s.e.m., n = 3 independent experiments. One-way ANOVA, 2 degrees of freedom, *P < 0.05. h, Endogenous cytosolic cGAS accumulates in micronuclei in U2OS cells. Representative images of endogenous cGAS distribution in cells with or without micronuclei. Images taken using different exposure times (200 vs 700 ms) to visualize weaker cytosolic cGAS signal. i, j, Verification of anti-cGAS antibody specificity in human cells. i, The percentage of cGAS-positive micronuclei, using anti-cGAS immunofluorescence, was determined microscopically after cGAS or luciferase siRNA knockdown. Mean ± s.e.m., n = 2 experiments (500 cells counted per experiment); two-tailed t-test. While several commercial cGAS antibodies were assessed, specific detection of mouse cGAS by immunofluorescence was not possible with these reagents (data not shown). j, Immunoblot after siRNA knockdown of cGAS in U2OS cells. siRNA targeting luciferase (siLUC) was used as a negative control. Probing with anti-actin antibody shows equal loading.
Extended Data Figure 2 | cGAS localization is associated with DNA damage in micronuclei. γH2AX foci in micronuclei correlate with GFP–cGAS localization in Rnaseh2b−/− MEFs and endogenous cGAS localization in U2OS cells. 

a, Representative immunofluorescence images: γH2AX, red; cGAS, green. 

b, Percentage of γH2AX-stained micronuclei (γH2AX +ve), either co-stained with cGAS (cGAS +ve), or in which cGAS was not detected (cGAS −ve). Rnaseh2b−/− MEFs; ≥500 cells counted per experiment. 

c, Quantification for U2OS cells, ≥250 micronuclei counted per experiment. Mean ± s.e.m., n = 3 experiments; *P < 0.05, **P < 0.001, two-tailed t-test. While our biochemical studies demonstrate that unbroken DNA and chromatin are sufficient to activate cGAS (Fig. 3, Extended Data Figs 4, 5), the increased accessibility of DNA after damage53 could further assist cGAS binding and activation. Scale bars, 10 μm.
Extended Data Figure 3 | cGAS localizes to micronuclei upon nuclear envelope rupture. a, cGAS localization to micronuclei in U2OS cells inversely correlates with localization of mCherry–NLS, which is present only in micronuclei with an intact nuclear envelope. a, Representative images of cells containing micronuclei with disrupted or intact nuclear envelopes. b, Percentage of intact and disrupted cGAS-positive micronuclei. Mean ± s.e.m., n = 3 independent experiments (≥250 micronuclei counted per experiment). NLS +ve and NLS −ve, *P < 0.001, two-tailed t-test. c, Single-channel image for representative stills shown in Fig. 2d from live imaging of U2OS cells expressing mCherry–NLS and GFP–cGAS. DNA visualized with Hoechst stain. Time (min) relative to loss of mCherry–NLS from micronucleus (t = 0, micronuclear membrane rupture). Arrows indicate micronuclei undergoing rupture. Scale bars, 10 μm.
Extended Data Figure 4 | cGAS is activated by circular plasmid DNA. 

**a**, Plasmid DNA (SC, supercoiled; OC, open circle; linear and fragmented) separated by agarose gel electrophoresis. pBluescript II SK(+) supercoiled plasmid DNA was treated with Nt.BspQI nicking endonuclease to generate open circle DNA; with EcoRI to generate a single 3-kb linear fragment; or with HpaII to generate 13 fragments between 710 and 26 bp in size.

**b**, Supercoiled, open circle, linear and fragmented pBluescript (pBS) DNA all activate recombinant cGAS to produce cGAMP. Representative images shown. Quantification of \( n = 3 \) experiments shown in Fig. 3b. c, Plasmid DNA induces cGAS-dependent CCL5 production in MEFs. Wild-type and cGAS\(^{-/-}\) (Mtb21d1\(^{-/-}\)) MEFs were transfected with 400 ng HT-DNA or supercoiled or linearized pBluescript, and CCL5 production after 24 h measured by ELISA. Mean ± s.e.m., \( n = 3 \) independent experiments.
Extended Data Figure 5 | cGAS is activated by chromatin. a, Agarose gel of micrococcal nuclease (MNase)-digested synthetic chromatin assembled onto a 601 DNA template indicates that it has a regular nucleosomal structure. b, Chromatin and DNA bind recombinant cGAS. DNA in wells could be the result of near charge neutrality of cGAS–DNA complexes or previously reported cGAS oligomerization. Chromatin is stable under cGAS assay conditions, remaining intact during incubation in cGAS reaction buffer, as evidenced by the bandshift compared to naked DNA. c, Representative TLC image demonstrating cGAMP generation by recombinant cGAS in the presence of chromatin. d, MNase treatment confirms a nucleosomal ladder pattern for chromatin isolated from mouse NIH3T3 cells. e, cGAS binds chromatin, and cellular chromatin is stable under cGAS assay conditions. f, g, Cellular chromatin activates recombinant cGAS, but at a slower rate than the same amount of deproteinized DNA. Representative images shown. Graphs shows quantification from n = 3 independent experiments, mean ± s.d. Reduced cGAS activation in vitro by chromatin isolated from cells is expected due to the presence of linker histones in addition to the nucleosomal core histones, which has been shown to bind part of the linker DNA, reducing the available sites for cGAS binding, and the use of MNase during the isolation of cellular chromatin. Whereas MNase treatment is needed to fragment the chromatin to allow its purification, it will preferentially cleave accessible non-protein-bound portions, which will further reduce the available sites to which cGAS can bind in the final chromatin preparation. However, such nucleosome-free regions are more likely to allow efficient binding and activation of cGAS in vivo.
Extended Data Figure 6 | ISG induction by ionizing radiation is abrogated in non-cycling cells. a, Experimental setup: to arrest cells in G0, serum was withdrawn 24 h before transfection with HT-DNA, and supernatant harvested 24 h later. b, CCL5 production in response to transfected HT-DNA was equivalent in cycling and serum starved MEFs. Mean ± s.e.m., n = 2 independent experiments. c, Schematic of experimental protocol. d, Cycling and G0-arrested cells exhibit the same level of DNA damage as measured by formation of γH2AX foci. Representative images; scale bar, 10 μm. Quantifications shown in Fig. 4d. e, There is no significant increase in ISG transcripts Ifit1, Ifit3, Isg15, Cxcl10 and Oas1a for cells arrested in G0 after serum starvation (experimental setup as in c). Transcript levels were normalized to Hprt. Mean ± s.e.m. One-way ANOVA, 2 degrees of freedom, n = 3 independent experiments; NS, not significant. Compare to Extended Data Fig. 1g, showing data for matched cycling cells assessed concurrently.
Extended Data Figure 7 | Micronuclear DNA is sufficient to account for the radiation-induced cytokine response. a, b, Measurement of micronuclear DNA content. a, Representative images. DAPI-stained primary nuclei and micronuclei surrounded by dotted lines. Scale bar, 10 μm. b, Quantification of surface areas of micronuclei and primary nuclei 48 h after 1 Gy irradiation. Micronuclear surface area per cell 9.72 ± 1.46 μm², primary nucleus surface area 303 ± 21 μm². Horizontal line and error bars: mean ± s.e.m., n = 54 cells. Hence, micronuclear content is ~3.2% of the total MEF genome after irradiation, equating to 190 Mbp of DNA. This corresponds to a total of 8.1 ng of micronuclear DNA in 10⁵ cells after 1 Gy irradiation (10⁵ diploid mouse cells contain a total of 650 ng of genomic DNA, with 39% of cells containing micronuclei, Fig. 1h). c, CCL5 response of wild-type C57/BL6 MEFs to ionizing radiation plotted in pg per 10⁵ cells. Reanalysis of this dataset (first depicted in Fig. 4b) confirms that the prior statistical analysis is robust to data normalization on the basis of cell counts at assay endpoint. 1 Gy of irradiation in cycling MEFs results in 38 ± 5 pg (mean ± s.d.) of CCL5 per 10⁵ cells. **P < 0.01, two-tailed t-test; NS, not significant. d, Dose–response curve of secreted CCL5 in wild-type C57BL/6 (Trp53⁺/⁺) MEFs transfected with serial dilutions of transfected HT-DNA. Therefore, around 4 ng of transfected DNA resulted in a similar level of cytokine production to c. Mean and 95% confidence interval indicated by black and grey dashed lines, respectively. Given the similarity of the two estimates, within the same order of magnitude, micronuclear DNA is likely to be sufficient to account for the immune response observed. Conversely, ionizing radiation would not be expected to generate this quantity of small DNA fragments as 1 Gy irradiation generates ~40 double strand breaks (DSBs)⁵⁴, and ~1,000 base lesions and single-stranded breaks. DSBs will have an average separation of 150 Mbp, and will therefore be too widely spaced to directly generate small dsDNA fragments. Repair of DNA lesions can generate small single-stranded DNA (ssDNA) fragments through endonuclease activity. The best characterized fragments are those generated by nucleotide excision repair, where endonucleolytic cleavage yields 24–32-nucleotide ssDNA fragments⁵⁵. As such these are not an ideal substrate for cGAS activation, and 5 million such lesions per cell would have to be generated to produce 4 ng of cytosolic DNA in 10⁵ cells. Hence, on the basis of our understanding of the current literature, such DNA fragments are likely to be generated at a level that is orders of magnitude lower than that of micronuclear DNA after radiation-induced damage.
Extended Data Figure 8 | Induction of micronuclei originating from lagging chromosomes leads to a proinflammatory response, but not increased DNA damage in the primary nucleus.

a, Model: micronucleus formation after nocodazole treatment. b, Schematic of experimental protocol. c, d, Percentage of micronucleated cells following nocodazole (noc) treatment of Trp53−/− MEFs (c) or U2OS cells (d). Mean ± s.e.m., n = 5 experiments for Trp53−/− MEFs, n = 3 for U2OS cells. e, Percentage of U2OS cells with cGAS-positive micronuclei following nocodazole treatment. Mean ± s.e.m. of n = 5 experiments. 

f, CCL5 secretion following nocodazole treatment of Trp53−/− MEFs. Mean ± s.e.m. of n = 5 experiments. **P < 0.01, ***P < 0.001, two-tailed t-test. g–i, Increased CCL5 production after nocodazole release is observed after 16 h and not associated with increased DNA damage in the primary nucleus. g, Experimental setup: Trp53−/− MEFs were arrested with nocodazole for 6 h and mitotic cells harvested by mitotic shake-off and re-plated in fresh medium with nocodazole omitted. Supernatants and cells were then collected at indicated time points after growth in medium. h, Increased CCL5 production was observed from 16 h after release from nocodazole block. Technical duplicate, mean ± s.d. Noc (−), asynchronously grown, plated at the same time as mitotic shake-off Noc (+) cells, arrested with nocodazole. i, No increase in the number of γH2AX foci in the primary nucleus was observed after release from nocodazole block. n ≥ 100 cells counted per condition. j, k, CCL5 response to interferon stimulatory DNA (ISD) is absent in U2OS cells (j) but present in MEFs (k). CCL5 measured by ELISA 8 h after transfection with ISD. n = 2 experiments for U2OS cells, n = 1 experiment for MEFs.
Extended Data Figure 9 | Single-cell RNA sequencing quality control and microscopy images of individual LCM-captured cells. a, Total gene feature counts (reads mapping to a protein coding gene) vs ERCC (RNA spike-in) percentage of total counts per cell. Cells with ERCC percentage counts $>10\%$ and/or with feature counts $<2,000$ were rejected, indicated by red shaded regions. b, Summary statistics for 21 micronucleated (MN+) cells and 14 non-micronucleated (MN−) cells that passed quality control. c, d, Microcopy images of cells captured by LCM that passed quality control after single-cell RNA sequencing. Fourteen live cells without micronuclei (c) and 21 live cells with micronuclei (d) were isolated from the same culture dish using LCM and used for single-cell mRNA sequencing. DNA was stained with picogreen dsDNA stain. Cells shown are those that passed quality control; numbers indicate the order in which cells were captured. Scale bars, 10 $\mu$m.
**Extended Data Figure 10 | cGAS localizes to telophase chromosomes and DNA bridges.**  

**a.** Endogenous cGAS was stained by immunofluorescence of U2OS cells in mitosis, showing a diffuse staining pattern without accumulation at the DAPI-stained condensed chromosomes at metaphase. Two representative images shown. During anaphase (and telophase), cGAS staining can be seen on DNA in some cells. Overexpressed GFP–cGAS also localizes more widely to mitotic DNA in U2OS cells and MEFs (data not shown).  

**b.** Quantification of cGAS staining during mitosis, by stage. *Rnaseh2b<sup>−/−</sup> Trp53<sup>−/−</sup> MEFs stably expressing GFP–cGAS show localization of cGAS at DNA bridges (orange arrowheads).  

**c.** Endogenous cGAS can also be seen to localize to DNA bridges that occasionally occur in U2OS cells. cGAS also localized to micronuclei in the same cells (green arrowheads). Interphase chromatin bridges with cGAS bound in *Rnaseh2b<sup>−/−</sup> Trp53<sup>−/−</sup> MEFs 0.08% of *n* = 1,223 cells; U2OS cells 0.06% of *n* = 1,632 cells. Scale bars, 10 μm.
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Experimental design

1. Sample size
   Describe how sample size was determined.
   Sample sizes used throughout were consistent with those used in previous studies.

2. Data exclusions
   Describe any data exclusions.
   No exclusion of data was made.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   Experimental findings were reliably reproduced. Number (n) of biological and experimental replicates is stated in the figure legends. All attempts at replication were successful for the experiments described in the manuscript.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   Mice/cell lines were allocated into groups according to genotype of interest.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   Investigators were blinded during data collection and analysis where possible. This included FACS data collection, enumeration of micronuclei numbers, and single RNAseq analysis up to the point of grouped analyses.

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).

   n/a  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.

FACSs data were collected using FACS DIVA 8.0.1 and analysed using FlowJo v.7.6.5 software (Tree Star). Graphical data was plotted and statistical analysis was performed using Prism (Graphpad Software Inc). Fixed and live images were captured with iVision or Micromanager and where stated in the methods, were deconvolved using Volocity software (PerkinElmer).

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 restrictions on availability of unique materials

9. Antibodies

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

The following previously validated antibodies were used for immunofluorescence: phospho-Histone H2A.X (Ser139) (2577, Cell signalling, validated www.cellsignal.com), Lamin B1 (ab16048, Abcam, validated Robijns et al, Sci Rep, 2016.) and Retinoblastoma (554136, BD Biosciences, validated Mittnacht and Weinberg, Cell, 1991). cGAS (D1D3G, Cell Signalling) was used for endogenous staining and was validated using siRNA of cGAS in human cells both by immunoblot and immunofluorescence.

10. Eukaryotic cell lines

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

C57BL/6J Rnaseh2b+/p53+/− and Rnaseh2b+/+ p53−/− MEFs were generated from individual E10.5 embryos. C57BL/6NTac cGas−/− MEFs and C57BL/6J (p53+/+) MEFs were generated from E13.5 embryos. U2OS cells were purchased from the European Collection of Authenticated Cell Cultures (ECACC, Cat no. 92022711).

b. Describe the method of cell line authentication used.

MEF lines were validated by PCR genotyping. U2OS cells were sourced from a well-recognised cell repository.

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

All cells were mycoplasma-free, with regular checks performed using the Lonza-Mycobacterial Mycoplasma Detection Kit.

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.

No commonly misidentified cell lines were used in this study.

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.

Cell lines used: C57BL/6j Rnaseh2b+/- (referred to as Rnaseh2b+/−), C57BL/6j Rnaseh2b+/-g-174T (referred to as Rnaseh2bA174T/A174T)(Mackenzie, K. J. et al, EMBO, 2016), Trp53tm1tyj/J (referred to as p53+/−)(Jacks, T. et al, Current biology, 1994) and 57BL/6NTac-Mb21d1tm1a(EUCOMM)Hmgu/IcsOrl (referred to as cGas−/−)(Bridgeman, A. et al. Science, 2015).

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.

N/A
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▶ 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.  See materials and methods section entitled: ‘Erythrocyte micronucleus assay’

6. Identify the instrument used for data collection.  LSR Fortessa (BD Biosciences) X20

7. Describe the software used to collect and analyze the flow cytometry data.  Data were collected using FACS DIVA 8.0.1 and analysed using FlowJo v.7.6.5 software (Tree Star).

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

9. Describe the gating strategy used.  Gating strategy was performed as in Reference 37

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