Mitotic progression following DNA damage enables pattern recognition within micronuclei

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Inflammatory gene expression following genotoxic cancer therapy is well documented, yet the events underlying its induction remain poorly understood. Inflammatory cytokines modify the tumour microenvironment by recruiting immune cells and are critical for both local and systemic (abscopal) tumour responses to radiotherapy1. A poorly understood feature of these responses is the delayed onset (days), in contrast to the acute DNA-damage responses that occur in minutes to hours. Such dichotomous kinetics implicate additional rate-limiting steps that are essential for DNA-damage-induced inflammation. Here we show that cell cycle progression through mitosis following double-stranded DNA breaks leads to the formation of micronuclei, which precede activation of inflammatory signalling and are a repository for the pattern-recognition receptor cyclic GMP–AMP synthase (cGAS). Inhibiting progression through mitosis or loss of pattern recognition by stimulator of interferon genes (STING)–cGAS impaired interferon signalling. Moreover, STING loss prevented the regression of abscopal tumours in the context of ionizing radiation and immune checkpoint blockade in vivo. These findings implicate temporal modulation of the cell cycle as an important consideration in the context of therapeutic strategies that combine genotoxic agents with immune checkpoint blockade.

Radiotherapy and many chemotherapeutics rely on DNA double-stranded break (DSB) formation to drive the killing of tumour cells over several cell division cycles2. Concomitant with this protracted cell death schedule, inflammatory cytokine production increases over days following the insult3,4. We used STAT1 phosphorylation at Y701 (pSTAT1 Y701) as a surrogate for inflammatory pathway activation after ionizing radiation (Fig. 1a, b). MCF10A mammary epithelial cells showed STAT1 activation between 3 and 6 days after ionizing radiation after doses ≥5 Gy. Total STAT1 protein and the mRNA levels of multiple inflammatory genes were also detected in a time-dependent manner (Fig. 1c, Extended Data Fig. 1a). Using inducible nucleases (Fig. 1d, Extended Data Fig. 1b, c), we observed a delayed accumulation of active STAT1 and inflammatory gene expression, confirming these signals are driven by DSBs.

We reasoned that if residual DSBs were driving inflammatory signals then failure of non-homologous end-joining (NHEJ) DSB repair should amplify the response. Paradoxically, we observed that inhibition of DNA-dependent protein kinase catalytic subunit (DNA-PK) or CRISPR–Cas9 knockout of multiple NHEJ components diminished STAT1 activation in MCF10A and prostate epithelial cells (Fig. 1e, Extended Data Fig. 1c, d). STAT1 activation through exogenous IFNγ was unaffected by DNA-PK (Extended Data Fig. 1e), ruling out a direct role in STAT1 phosphorylation. Inhibition of ATM kinase had little influence over the level of STAT1 activation (Extended Data Fig. 1f).

Figure 1 | Loss of NHEJ antagonizes DSB-induced inflammatory signalling. a, Representative western blots for activation of STAT1 signalling at indicated times after 20 Gy radiation. NIR, non-irradiated. (n = 3 biological replicates). b, Dose-dependency of STAT1 response at 6 days as in a (n = 3 biological replicates). c, RT–qPCR of inflammatory gene induction following ionizing radiation. Error bars represent s.e.m. of 3 biological replicates. d, STAT1 activation monitored as in a. Western blotting for STAT1 activation after 5 h induction of DSBs by AsiSI or I-PpoI nuclease with Shield/4-OHT and followed by washout and recovery for the indicated times. e, CRISPR–Cas9 knockout of NHEJ components were monitored for STAT1 activation as in a (n = 3 biological replicates). ‘cr’ indicates CRISPR–Cas9-mediated knockout of the protein; LIG4, DNA ligase 4; PKcs, protein kinase catalytic subunit. f, Representative immunofluorescence of 53BP1 and γH2AX indicating residual DSBs in crNHEJ cells in 3 independent experiments. Arrowhead indicates γH2AX positive micronucleus. Scale bar, 10 μm.

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DSB-induced STAT1 activation correlated with the appearance of aberrantly shaped nuclei and micronuclei (Figs 1f, 2a). The DSB marker γH2AX was increased in micronuclei, however, 53BP1 was absent, consistent with DSB signalling defects5,6 (Fig. 1f). Nuclei of NHEJ-knockout and DNA-PKi-treated cells were morphologically normal despite being replete with DSBs (Figs 1f, 2a). As micronuclei are by-products of mitotic progression, this suggested that STAT1 activation occurred following mitosis7. Flow cytometry showed progression from G2 into G1 between 24 and 48 h after irradiation, whereas NHEJ-knockouts were static over this time (Extended Data Fig. 2a). This corresponds to a wave of parental cells moving into mitosis as evidenced by phosphorylated histone H3 (pH3) staining that is absent in the NHEJ-knockout cells (Fig. 2b). Notably, the specific CDK1 inhibitor RO-3306 (CDK1i) or PLK1 inhibitor BI-2536 (PLK1i) blocked mitotic entry, micronuclei formation and STAT1 activation (Figs 2a–c, Extended Data Fig. 2b). Furthermore inhibition of cell cycle progression by other CDKi reduced the flux of cells through mitosis and STAT1 activation (Extended Data Fig. 2c, d). CDK1i also prevented STAT1 activation in PARPi-treated BRCA1-mutated ovarian cancer cells (Fig. 2d), suggesting that mitotic entry is a gateway to inflammatory signalling for diverse DSB-inducing stimuli. To further test the requirement for mitotic progression, we synchronized cells in G2 before irradiation. Release from CDK1i after irradiation resulted in progression from G2 into the following G1 over 72 h as determined by loss of the S/G2-phase and mitosis marker CENP-F (Fig. 2e). STAT1 phosphorylation levels were greater than those observed in asynchronous cells and absent in cells not released from G2 (Fig. 2f).

STAT1 phosphorylation can be elicited following activation of the DNA-dependent pattern recognition receptor cGAS and its downstream mediators STING and IRF3, which serve to transcriptionally activate type I interferon8–16. CRISPR–Cas9 knockout of either cytosolic cGAS or STING reduced activation of downstream IRF3 target genes ISG54, ISG56 and the NF-κB target CCL5 after ionizing radiation17,18 (Fig. 3a, Extended Data Fig. 3a). Attenuated STAT1 activation and IFITM1 induction was also evident in cGAS–STING knockouts (Fig. 3b, Extended Data Fig. 3b). Senescence-associated β-galactosidase staining was relatively unaffected by treatments that influence inflammatory activation (Extended Data Fig. 3c), suggesting that alterations in epithelial cell senescence were not contributory to the reduced interferon signalling observed upon inhibiting mitotic entry or pattern recognition receptor activation.

cGAS localization was prominent in lamin-B2-positive micronuclei of CENP-F-negative (G1) cells three days following ionizing radiation (Fig. 3c, d, Extended Data Fig. 3d, e). Synchronized cells showed cGAS localization to micronuclei concomitant with mitotic passage, G1 accumulation and STAT1 activation (Fig. 3e, f, Extended Data Fig. 4a). Delayed accumulation of mCherry–cGAS in micronuclei was also observed (Extended Data Fig. 4b) and live-cell microscopy showed dynamic and persistent relocation of cGAS to micronuclei following mitosis (Fig. 3e, Supplementary Video 1). Interestingly, cGAS localized to bulk chromatin during mitosis, but dissociated at completion of mitosis. cGAS concentrated in the micronuclei after a transient delay, consistent with reported changes in permeability owing to delayed micronuclear envelope rupture19. Live-cell imaging revealed that around 20% of daughter cells were undergoing rapid post-mitotic apoptosis (Supplementary Video 2). The remaining 80% of daughter cells all contained micronuclei, the majority of which showed strong and persistent cGAS concentration (Extended Data Fig. 4c).

The preceding data support a model whereby loss of micronuclear envelope integrity can provide access to damaged DNA. When MCF10A cells were subjected to migration through 3-μm pores, we observed cGAS localization to both micronuclei and nuclear blebs consistent with nuclear envelop disruption and transient DSB production20 (Extended Data Fig. 4d, e). cGAS positivity of these subcellular

Figure 2 | Progression through mitosis underlies inflammatory pathway activation. a, Representative images of DAPI-stained MCF10A nuclei. Quantification was as described in the Methods and error bars represent s.e.m. of three (DMSO and DNA-PKi) or two (CDK1i) independent experiments. Arrowheads highlight micronuclei. Scale bar, 10 μm. b, The mitotic fraction of cells was quantified using H3 (S10) phosphorylation measured by flow cytometry and expressed as a percentage of total single cells. ***P < 0.0001 with one-way ANOVA and Dunnett’s multiple comparisons for three independent biological replicates. Error bars represent s.e.m. c, Western blot of ionizing-radiation-induced STAT1 activation under the indicated conditions in MCF10A cells (n = 3 biological replicates). d, Western blot of STAT1 activation in UWB1.289 or UWB1.289 + BRCA1 reconstituted cells treated for 6 days with PARPi treatment with or without CDK1i (n = 3 biological replicates). e, Cells were synchronized in G2 with CDK1i before irradiation and released for the indicated times. CENP-F positive (S/G2), CENP-F negative (G0/G1) and mitotic cells were quantified for three independent biological replicates. Error bars represent s.e.m. f, Representative western blot for STAT1 activation following the release scheme described in e (n = 3 biological replicates). AS, asynchronous; Unrel., unreleased.
structures still occurred in the presence of CDK1i, demonstrating that mechanical nuclear rupture bypasses the need for mitotic progression. Furthermore, micronuclei generated using Aurora B kinase inhibition were cGAS-positive and elicited robust STAT1 activation without irradiation (Extended Data Fig. 4f, g).

Micronuclei harbouring accumulated cGAS may act to initiate inflammatory signalling. RNA fluorescent in situ hybridization (FISH) revealed coincident expression of interferon-stimulated gene ISG54 specifically in cells containing micronuclei ($P \leq 0.001$ at 6 days after 20 Gy by paired t-test; Fig. 3f). This effect was abrogated by knockout of cGAS,

Figure 3 | Relocalization of cGAS to micronuclei following mitotic progression triggers inflammatory signalling. a, RT–qPCR of ISG54 at 6 days following 20 Gy in parental MCF10A cells untreated or treated with CDK1i (RO-3306) or derivatives harbouring deletion of cGAS or STING. Error bars represent s.e.m. of three biological replicates. b, Representative western blots of STAT1 activation in CRISPR–Cas9 knockout MCF10A cells for cGAS or STING ($n$ = 3 biological replicates). c, Immunofluorescence of endogenous cGAS in MCF10A cells. Arrowhead indicates a representative cGAS-positive micronucleus. Scale bar, 10 μm. d, Quantification of micronuclei in c. Error bars represent s.e.m. of three biological replicates. e, Extracted frames from live-cell microscopy of irradiated MCF10A cells stably expressing GFP–H2B to mark chromatin and mCherry–cGAS. Inset depicts time from obvious mitotic induction in hours and minutes (hh:mm). f, Representative RNA FISH images (scale bar, 10 μm) and quantification of ISG54 status as described in the Methods. Error bars represent s.e.m. for three biological replicates. * * * $P < 0.0001$ by one-way ANOVA and Bonferroni multiple comparison of the fraction of micronucleated ISG54+ cells. Arrowhead indicates an example of a micronucleus. HT-DNA, herring testis DNA. g, Representative western blot of STAT1 activation of naive cells treated with indicated conditioned media for 24 h ($n$ = 2 biological replicates).
STING, or XRCC4, and by CDK1i. In an independent test of this association, expression of an IFNβ1 promoter controlled eGFP transgene increased specifically in micronucleated cells at 6 days following 10 Gy (P ≤ 0.01 by 1-way ANOVA; Extended Data Fig. 5a). These findings firmly establish an important relationship between the presence of micronuclei and inflammatory signalling. We cannot ascribe the inflammatory response solely to micronuclei as small DNA fragments in the cytoplasm could also contribute to cGAS–STING activation. However, our data show robust relocation of cGAS to the micronucleus and activation of inflammatory responses within these micronucleated cells.

Only subtle induction of nuclear phosphorylated IRF3 (pIRF3) was detected by immunofluorescence in damaged cells (Extended Data Fig. 5b). This suggests that chronic DNA-damage-induced inflammatory activation emanating from cGAS-positive micronuclei may differ in magnitude from the acute signalling that occurs within hours following transfection of micromanipulated DNA (Extended Data Fig. 5c). To determine whether paracrine signals could amplify cGAS–STING-mediated signalling, we transferred media from irradiated cells to naive parental cells and assayed for STAT1 activation (Fig. 3g). These experiments reveal ionizing radiation induced paracrine signalling that required mitotic progression and cGAS–STING.

Several case reports have described an abscopal effect defined as tumour regression outside of the irradiated field. This is most often induced by checkpoint blockades (that is, anti-CTLA4 antibody)22–24, suggesting that local radiation can be immunomodulatory. We have extensively characterized how irradiation of one tumour along with immune checkpoint blockade can produce T-cell-dependent responses in the contralateral unirradiated tumour using the B16 syngeneic mouse model of melanoma24,25. To examine the dependence of abscopal responses on the cGAS–STING pathway, we used this B16 model and irradiated cells ex vivo (Fig. 4a). Irradiation of B16 melanoma cells before injection caused significant reduction in the growth of abscopal tumours after anti-CTLA4 treatment (Fig. 4b).

In contrast, implanting irradiated B16 cells harbouring STING deletion eliminated the radiation-mediated growth delay of the abscopal tumour after anti-CTLA4 (Fig. 4b, Extended Data Fig. 6a, b). Radiation in the absence of anti-CTLA4 was insufficient to induce the abscopal effect (Extended Data Fig. 6c). The abscopal tumour volume reduction as measured at day 15 was not observed when the implanted cells were STING deficient (Fig. 4c). Loss of STING in the irradiated tumour also significantly reduced overall survival in the mice (Extended Data Fig. 6d). A similar effect on tumour size with STING loss was noted in abscopal tumours when the contralateral tumour was irradiated directly in the mice (Extended Data Fig. 6f, f). Consistent with a requirement for T cell responses, STING knockout prevented the enrichment of intratumoral CD8 T cells in the abscopal tumour24,26 (Fig. 4d). Thus, STING signalling is a critical component of host immune activation that drives regression of distal tumours in radiotherapy and anti-CTLA4 combination therapy.

Checkpoint adaptation and insensitivity has been described in a wide range of eukaryotic organisms27,28. Our data support a model in which imperfect cell cycle checkpoints allow passage through mitosis and accumulation of micronuclei where pattern recognition occurs (Fig. 4e). This represents a situation in which actively cycling cells contribute to delayed onset inflammatory signalling in the context of DSB-inducing therapies. These findings suggest possibilities to modulate the host immune system and ultimately the success of genotoxic therapies.

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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**Author Contributions** S.M.H. and R.A.G. designed the study and wrote the manuscript. S.M.H. performed most of the experiments. J.L.B. designed and performed in vivo experiments described in Fig. 4 and Extended Data Fig. 6. J.I. designed and performed experiments described in Extended Data Fig. 4d, e. A.J.M. and D.E.D. provided guidance for the design of experiments and edited the manuscript.

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were blocked in 5% non-fat milk powder in PBS (Sigma) for 20 min on ice and cleared by centrifugation. Protein was quantified with 1 mM phenylmethansulfonyl fluoride, 10 mM NaF, 2 mM imidazole, 1.15 mM benzamidine HCl, 1 μM β-galactosidase (Invitrogen) for around 1 week and knockouts were created by infecting cells with lentivirus created using lentiGuide-Puro (a gift from F. Zhang, Addgene #52963)36 according to standard procedures, digested with XhoI/NotI and isolated from the pHFUW-mCherry-TRF-FOKI plasmid31 with Q5 polymerase and primers (5′-ccatcttcatctcgagCTTGTACAGCTCGTCCATGC-3′, 5′-atggcggccgcTCAAAATTCATCAAAAACTGGAAACTCATTGTTTC-3′) for pH3 positive colonies. Micronucleated cells were infected with cGAS-positive micronuclei these structures were counted manually for each field and expressed as a percentage of total cells within the field. Total nuclear intensity was calculated on a per cell basis using FIJI (NIH). Graphs and statistics were prepared using Prism Graphpad.

Flow cytometry. Cells were collected from a single well of a six-well plate using trypsin and washed 1× in PBS before fixation with drop-wise addition of ice-cold 70% ethanol. After fixation on ice (or storage at −20°C) cells were washed 1× in PBS and 1× in 1X-100PBS solution on ice for 15 min. Cells were washed 1× in PBS and resuspended in 1% bovine serum albumin/ PBS solution containing 1 μl per sample of pH3 (serine 10) antibody conjugated to Alexa Fluor 488 (DC28, Cell Signaling). After washing 1× in PBS, cells were resuspended in a solution of PBS containing 90 μg/ml propidium iodide (Santa Cruz Biotechnology) and 100 μg/ml RNase A (Roche). Flow cytometry was performed on a FACS Calibur (BD Biosciences) and analysed using FlowJo software. Single cells and G1/S/G2 peaks were manually gated. The gates for pH3-positive cells were chosen by comparison to a population in which the antibody was omitted during processing.

Plasmid construction. The plasmid backbone was isolated from pLVX-pPTUNER-N using Q5 polymerase and primers (5′-ggatccGTTTAAAAATAATGCTCCTCTAGTAGAGTCGGTGTC-3′, 5′-acaggtcgccgcgcatgGCGGCCGCCCCCTCTCC-3′) for Gibson assembly. The mCherry fragment was isolated from the pHHFw-mCherry-TRF-FOKI plasmid34 with Q5 polymerase and primers (5′-agaggtcatatttaataacgagaccGGTAGTGACAGGGCG-3′, 5′-ccatctcatctttgGTAGCTGACGCTATGTC-3′). These two fragments were joined by Gibson assembly (New England Biolabs) and digested with XhoI and NotI (New England Biolabs). qGAS was amplified by PCR from Human Lung Fibroblast DNA (Biosetta) using primers: 5′-catccgagACGACGCTTACATCAAAACTCTGAAACTCATTGGTTTC-3′. After digestion with XhoI and NotI the fragment was inserted using a Quick Ligation Kit (New England Biolabs). MCF10A cells were infected with virus as described above.

Live-cell microscopy. The pBBBE-H2B-GFP plasmid was a gift from F. Dick (Addgene plasmid #26790)35 and retrovirus was created using standard protocols and introduced into MCF10A cells expressing mCherry–gGAS. Dual mCherry/GFP-positive cells were isolated by cell sorting and seeded onto 35 mm Mattek dishes (Mattek). Cells were synchronized with Cdk1i and released according to the procedure described above. Imaging was started immediately after release. Imaging was carried out with a DeltaVision microscope enclosed in a 37°C humidified chamber equilibrated with 5% CO2. Images were captured using a Photometrics CoolSnapHQ camera using a 40× air objective. Single plane images were acquired every 15 min for 20–24 h. Focus was stable throughout with minor manual adjustments over the imaging time-course. This procedure was repeated three times during which around 100 daughter nuclei could be analysed, corresponding to 30 mitotic events. The mobility of the cells limited the ability to follow all mitotic events to completion. H2B–GFP bodies separate from the main nuclei were identified as micronuclei and apoptotic cells were identified by rapid and severe H2B–GFP condensation before complete dissociation and loss from the cell surface (Supplementary Video 2).

Senescence-associated β-galactosidase assay. Cells were seeded at around 2×104 cells per well of a six-well plate, treated 24 h after seeding with ionizing radiation and the indicated agents, and 6 days later were fixed and stained for SA-33GAL as described33. Three technical replicates were performed within at least two independent biological experiments.
Conditioned media experiments. Cells for all treatments were plated at a constant density and irradiated as described. Conditioned media was obtained by changing to fresh media 24 h before collection and filtering through a 0.22 μm filter. This conditioned media was transferred to naive cells and protein lysates for western blotting were collected 24 h later.

RNA FISH. RNA FISH was performed as described with modifications29. Cells were fixed on coverslips with 2% formaldehyde in PBS and permeabilized at least 24 h and up to 1 week at 4 °C in 70% ethanol. After a brief rinse in RNA Wash buffer (10% formalamide/2× SSC), cells were inverted into hybridization buffer containing Custom Stellaris FISH Probes designed against ISG54 (NM_001547) by using the Stellaris RNA FISH Probe Designer (Biosearch Technologies, Inc.) available online at http://www.biosearchtech.com/stellarisdesigner. After overnight hybridization cells were washed twice for 30 min at room temperature in RNA wash buffer, once for 2 min in 2× SSC and mounted in Vectashield. Imaging was performed using a 60× objective as described above. Using ImageJ, all images from a given treatment were combined in a montage and background subtracted with a rolling ball radius of 10. A threshold was chosen based on HT-DNA transfected cells to eliminate any remaining background that was not a clear diffraction limited RNA FISH spot. Cells with at least 10 FISH spots were counted as positive for ISG54. P values to compare between treatments were computed using Graphpad, using a one-way ANOVA with a Bonferroni correction. To compare ISG54+ normal versus micronucleated cells within the treatment group a paired t-test was used.

IFN3–GFP reporter assay. The IFN3–luciferase reporter plasmid was a gift of R. Vance34. Using Gibson assembly the luciferase reporter gene was swapped with eGFP (from pBABE-H2B described above) to form the IFN3–GFP cassette. A fragment derived from the pLKO.1-Hygro plasmid (a gift from B. Weinberg, Addgene #24150) that omitted the U6 promoter sequence was combined with the IFN3–GFP cassette to generate the pLKO.1-Hygro–IFN3–GFP reporter. A stable MCF10A cell line was derived as described above by viral infection and selection with 100 μg ml−1 hygromycin (Invivogen). Activation of GFP was monitored by microscopy using a one-way ANOVA with a Bonferroni correction.

Transwell migration. For migration through Transwells (Corning Inc.), cells were seeded at 300,000 cells cm−2 onto the top side of the filter membrane and left to migrate in normal culture condition for either 9 or 24 h. For experiments involved CDK11i, cells were exposed to 9 μM CDK11i and DMSO for 1 h before the seeding onto Transwells. Transwell membrane was fixed in 4% formaldehyde (Sigma) for 15 min, followed by permeabilization by 0.25% Triton X-100 (Sigma) for 10 min, blocked by 5% BSA (Sigma) and overnight incubation in various primary antibodies: lamin-A/C (Santa Cruz), lamin-B (Santa Cruz) and cGAS (Cell Signalling). Finally, the primary antibodies were tagged with the corresponding secondary antibodies for 1.5 h (ThermoFisher). DNA was stained with 8 μM Hoechst 33342 (ThermoFisher) for 15 min. Confocal imaging was performed on a Leica TCS SP8 system using a 63×/1.4 NA oil-immersion lens.

Modified RadVax procedure. Five- to seven-week-old female C57BL/6 mice were obtained from Charles River and maintained under pathogen-free conditions. Mice were divided randomly into cages upon arrival and were randomly injected and measured without blinding. All animal experiments were performed according to protocols approved by the Institute of Animal Care and Use Committee of the University of Pennsylvania (IACUC). The minimal number of animals was used based on prior experience to yield consistent measurements. On the day before the experiment was to begin B16-F10 cells were treated as necessary in vitro with 10 Gy of ionizing radiation with or without CDK11i. As necessary the CDK11i was added 1 h before irradiation and maintained until cell isolation and injection on day 2. On day 0 untreated B16-F10 cells (5×106) in 50 μl of PBS were mixed with an equal volume of Matrigel (BD Biosciences) and injected into the right flank. On day 2, 5×105 B16-F10 parental or STING-knockout cells treated as described above were mixed with Matrigel and injected on the opposite flank. On days 5, 8 and 11 anti-CTLA4 antibody (9H10; BioXCell) was administered interperitoneally at 200 μg per mouse. Volumes were measured using calipers starting at day 11 and calculated using the formula l × w2 × 0.52, where l is the longest dimension and w is perpendicular to l. Animals were killed when either tumour reached 1.5 cm in the largest dimension according to IACUC guidelines. Overall survival and flow cytometry for tumour infiltrating CD8+ T cells was performed as described35. For the in situ irradiation experiments of Extended Data Fig. 4c, f experiments were performed using the Small Animal Radiation Research Platform (SARAP) as previously described34.

Data availability. The authors declare that the data that support the findings of this study are available within the article and Supplementary Information.

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Extended Data Figure 1 | Non-homologous end-joining inhibition impedes DSB-induced inflammatory signalling. a, Total STAT1 protein levels are increased in a time-dependent manner after ionizing radiation (representative of three biological replicates). b, U2OS cells were monitored for STAT1 activation during recovery from I-PpoI nuclease (representative of two biological replicates). c, Gene expression changes were monitored for indicated genes during recovery from I-PpoI damage in MCF10A cells (see Fig. 1d). Error bars represent s.e.m. of 2–3 biological replicates. d, Prostate epithelial cells (PREC) were analysed for STAT1 phosphorylation at 6 days following 10 Gy (n = 2 biological replicates). e, STAT1 phosphorylation following IFNβ treatment is not DNA-PK or CDK1 dependent (n = 2 biological replicates). f, pSTAT1 activation measured by western blot is unchanged by ATMi. DNA-PKi and ATMi block phosphorylation of DNA-PKcs S2056 and pKAP1 S824, respectively, as measured by western blot.
Extended Data Figure 2 | Prevention of mitotic entry and cell cycle progression impairs ionizing-radiation-induced STAT1 activation.

a, Cell cycle profiles of MCF10A cells were monitored by flow cytometry of propidium-iodide-stained cells treated as indicated (n = 3 biological replicates). Error bars represent s.e.m.

b, U2OS cells were monitored for STAT1 activation in the presence of the indicated inhibitors (n = 2 biological replicates).

c, H3 (Ser10) phosphorylation in MCF10A cells was measured by flow cytometry and expressed as a percentage of total single cells. Error bars represent s.e.m. of at least two biological replicates. Western blots show loss of STAT1 activation in treatments corresponding to the flow cytometry data.

d, Example dot plots of pH3 (H3P) flow cytometry showing the gating strategy using a sample with and without the conjugated pH3 antibody. An example propidium-iodide-staining gating strategy is also shown.
Extended Data Figure 3 | Loss of cGAS–STING signalling in micronuclei impedes ionizing-radiation-induced inflammatory gene activation with minimal effect on senescence. a, RT–qPCR of CCL5 and ISG56 at 6 days following 20 Gy in parental MCF10A cells untreated or treated with CDK1i (RO-3306) or derivatives harbouring deletion of cGAS or STING. Error bars represent s.e.m. of three biological replicates.
b, Knockouts for two separate CRISPR–Cas9 sgRNA for cGAS or STING cause similar reductions in STAT1 signalling (representative of two biological replicates). c, Senesence-associated β-galactosidase staining at 6 days following indicated treatments. Error bars represent s.e.m. of at least two biological replicates. d, Representative immunofluorescent staining of cGAS shows loss of staining in cGAS-knockout cells. Scale bar, 10 μm. e, Immunofluorescent staining shows co-staining of lamin B2 in cGAS positive micronuclei. Scale bar, 10 μm.
Extended Data Figure 4 | cGAS dynamically relocates to micronuclei following mitosis, during migration and after Aurora B inhibition. 

**a**, Cells in Fig. 2e were analysed for the fraction of cGAS-positive micronuclei following release from CDK1i. Error bars represent s.e.m. of three biological replicates. 

**b**, mCherry–cGAS-expressing cells were monitored by immunofluorescence following 10 Gy. Arrowhead indicates mCherry–cGAS-positive micronucleus (representative of three independent experiments). 

**c**, Histogram represents the fraction of daughter cells with cGAS-positive micronuclei or that underwent apoptosis during live-cell imaging. All non-apoptotic daughter cells were micronucleated after division. Error bars are s.e.m. of three biological replicates (n = 99 total daughters). 

**d**, Schematic of nuclear migration transwell system. Blue, DAPI; green, lamin A; red, lamin B. cGAS is shown in grayscale. 

**e**, Quantification of aberrant nuclei (nuclear blebs and micronuclei) that are cGAS-positive in the transwell migration assay. 

**f**, Immunofluorescent staining in an untreated and two representative cells after six-day treatment with Aurora B kinase inhibition (AuroraBi). Scale bar is 10 μm and similar patterns were observed in two independent experiments. 

**g**, Representative (n = 3) western blot of STAT1 activation in non-irradiated cells treated with Aurora B inhibitor for 3 or 6 days.
Extended Data Figure 5 | cGAS–STING-driven inflammatory signalling occurs in micronucleated cells. a, Representative images of pIFNβ–GFP reporter cells treated as indicated. Scale bar, 10 μm. Quantification is as described in the Methods and error bars represent s.e.m. of two biological replicates. b, Mean nuclear intensity of pIRF3 staining was quantified. P values are based on pooled data from three independent experiments and calculated by one-way ANOVA. c, Representative (n = 2) western blot of STAT1 activation following transfection of herring testis DNA (HT-DNA) in indicated CRISPR–Cas9 knockout MCF10A cell lines shows STAT1 activation in DNA-PKcs- and all NHEJ-deficient cells, but not in cGAS STING knockouts.
Extended Data Figure 6 | STING knockout in B16-F10 impairs ionizing-radiation-induced inflammatory gene expression in vitro and the abscopal response after in vivo irradiation of contralateral tumour. a, b, Radiotherapy- (a) and cGAMP-induced (b) gene induction is absent in STING knockout B16 cells. c, Injection of B16 parental or STING knockout cells (with or without irradiation) without combination anti-CTLA4 treatment is insufficient to induce an abscopal effect. d, Overall survival of mice when B16 parental or STING knockout were injected after indicated treatment. All mice received anti-CTLA4 antibody. P value calculated by log-rank test. e, B16 tumours are injected into opposite flanks of mice and treated as indicated. The index tumour is irradiated with 20 Gy and both the index and abscopal tumours are measured starting on day 11 after injection. f, Irradiation of the index tumour leads to an abscopal response that is dependent on STING (left panel). This response is not seen in unirradiated mice (right panel).
Experimental design

1. Sample size
   Describe how sample size was determined.
   Sample sizes were selected based on previous experience to obtain statistical significance and reproducibility.

2. Data exclusions
   Describe any data exclusions.
   For abscopal experiments, mice would be excluded if tumors ulcerated at <1cm in the largest dimensions. This is in agreement with our mouse protocol for humane treatment of mice in tumor studies.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   All experimental data was reliably reproduced in multiple independent experiments as indicated throughout the manuscript.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   For all animal studies, Mice were divided randomly into cages upon arrival and are randomly injected and measured. This procedure is described in detail in the Methods Section.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   The investigators were not blinded during data collection and analysis.
   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.

GraphPad, ImageJ, and Licor odyssey programs were used to analyze data.

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

9. Antibodies

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

All antibodies are listed in Supplementary table 2. STING and cGAS antibodies showed no detectable signal in knockout cells. pSTAT1 was increased by interferon-b treatment and reduced by Jak kinase inhibitors as previously described. Antibodies to pKAP1 were validated by disappearance following ATMi. pDNA-PKcs antibody signal was eliminated by DNA-PKcs inhibitor treatment.

10. Eukaryotic cell lines

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

MCF10A, B16 melanoma and other Cell lines were purchased from ATCC.

b. Describe the method of cell line authentication used.

Cell line authentication was not performed as cells were not listed in the commonly misidentified category.

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

Yes. They tested negative for mycoplasma.

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.

These cells are not in the database

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.

Five to seven week old female C57BL/6 mice were obtained from Charles River and maintained under pathogen free conditions. Mice were divided randomly into cages upon arrival and are randomly injected and measured. All animal experiments were performed according to protocols approved by the Institute of Animal Care and Use Committee of the University of Pennsylvania.

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