The molecular basis of tight nuclear tethering and inactivation of cGAS

Nucleic acids derived from pathogens induce potent innate immune responses. Cyclic GMP–AMP synthase (cGAS) is a double-stranded DNA sensor that catalyses the synthesis of the cyclic dinucleotide cyclic GMP–AMP, which mediates the induction of type I interferons through the STING–TBK1–IRF3 signalling axis. cGAS was previously thought to not react with self DNA owing to its cytosolic localization, however, recent studies have shown that cGAS is localized mostly in the nucleus and has low activity as a result of tight nuclear tethering. Here we show that cGAS binds to nucleosomes with nanomolar affinity and that nucleosome binding potently inhibits its catalytic activity. To elucidate the molecular basis of cGAS inactivation by nuclear tethering, we determined the structure of mouse cGAS bound to human nucleosome by cryo-electron microscopy. The structure shows that cGAS binds to a negatively charged acidic patch formed by histones H2A and H2B via its second DNA-binding site. High-affinity nucleosome binding blocks double-stranded DNA binding and maintains cGAS in an inactive conformation. Mutations of cGAS that disrupt nucleosome binding alter cGAS-mediated signalling in cells.

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The presence of billions of base pairs of DNA in the mammalian genome raises the question of how cGAS activity towards self DNA is inhibited. The prevalent explanation for this is that cGAS is sequestered in the cytosol and does not have access to host DNA. However, this idea has been challenged by several recent studies, which have demonstrated that cGAS is also localized in the nucleus and is tightly tethered to chromatin. The catalytic activity of cGAS is suppressed when it is tethered in the nucleus. A patch of conserved, positively charged residues of cGAS within its second DNA-binding site was identified to be critical for its interaction with chromatin. In addition, it has been suggested that intact chromatin structure is essential for cGAS nuclear tethering. However, the molecular basis of cGAS nuclear tethering remains largely unknown.

cGAS binds tightly to individual nucleosomes

We first confirmed that cGAS is predominantly localized in the nucleus of both human (HeLa) cells and mouse embryonic fibroblasts (MEFs) (Fig. 1a). Quantitative fluorescent microscopy revealed that 70–80% of cGAS is localized in the nucleus (Fig. 1b, c). To test whether cGAS binds to nucleosomes directly, we prepared cell-derived nucleosomes using histone proteins and expressed biotin-labelled full-length human cGAS and conducted binding studies by surface plasmon resonance (SPR) (Fig. 1d, Extended Data Figs. 1i, 2a). We observed that both reconstituted and cell-derived nucleosomes bind to human cGAS with high affinity (dissociation constant of 8.6 and 6.3 nM, respectively; Fig. 1d, Extended Data Fig. 2a). The catalytic domain of human cGAS binds to the nucleosome with similar affinity (K of 2.9 nM; Extended Data Fig. 2b). Similar results were obtained by bio-layer interferometry (Extended Data Fig. 2c, d). In addition, binding studies showed that full-length mouse cGAS and its catalytic domain also bind to the nucleosome with nanomolar affinities (Extended Data Fig. 2e–h). We also analysed a mixture of nucleosome, 45-bp double-stranded DNA (dsDNA) and mouse cGAS by gel-filtration chromatography, which revealed that the nucleosome competes with dsDNA to bind cGAS (Extended Data Fig. 2i).

To determine the binding affinity between the nucleosome and cGAS, we expressed biotin-labelled full-length human cGAS and conducted binding studies by surface plasmon resonance (SPR) (Fig. 1d, Extended Data Figs. 1i, 2a). We observed that both reconstituted and cell-derived nucleosomes bind to human cGAS with high affinity (dissociation constant of 8.6 and 6.3 nM, respectively; Fig. 1d, Extended Data Fig. 2a). The catalytic domain of human cGAS binds to the nucleosome with similar affinity (K of 2.9 nM; Extended Data Fig. 2b). Similar results were obtained by bio-layer interferometry (Extended Data Fig. 2c, d). In addition, binding studies showed that full-length mouse cGAS and its catalytic domain also bind to the nucleosome with nanomolar affinities (Extended Data Fig. 2e–h). We also analysed a mixture of nucleosome, 45-bp double-stranded DNA (dsDNA) and mouse cGAS by gel-filtration chromatography, which revealed that the nucleosome competes with dsDNA to bind cGAS (Extended Data Fig. 2i).

Nucleosome binding inhibits the activity of cGAS

Next, we conducted cGAS activity assays to determine whether the nucleosome-bound cGAS is active. First, we conducted cGAS activity assays using purified mouse cGAS–nucleosome complexes, and detected no cGAS activity (Fig. 1e). Next, we added mouse cGAS to the mouse cGAS–nucleosome complex, and observed that a small amount of 2′,3′-cyclic GMP-AMP (cGAMP) was formed (Fig. 1e), indicating that the dsDNA associated with the nucleosome cannot effectively activate cGAS that was not bound to the nucleosome. We then added 45-bp dsDNA and salmon sperm DNA (Extended Data Fig. 2j) to the mouse cGAS–nucleosome complex. We observed very low activity from the nucleosome-bound cGAS towards the 45-bp dsDNA (Fig. 1e). However, salmon sperm DNA weakly
Fig. 1 | Tight nucleosome binding inactivates cGAS. a, Western blot analyses of cGAS expression in 1% SDS whole-cell lysates (WCL), 0.2% NP-40 cytosolic fractions, 0.5 M NaCl nuclear extracts and 1% SDS nuclear lysates of HeLa cells and Cgas−/− MEF40V1 cells expressing haemagglutinin (HA)-tagged mouse cGAS. b, Immunofluorescence of cells as in a stained with cGAS, haemagglutinin and α-tubulin antibodies and DAPI. Scale bars, 50 μm. c, The ratio of nucleosome to whole-cell cGAS quantified by microscopy. Data are mean ± s.e.m. d, SPR binding studies show that full-length human cGAS binds to in vitro reconstituted human nucleosome with nanomolar affinity. Kd values were determined by fitting the binding data to a simple one-to-one binding model. Nucleosome concentration is indicated. RU, resonance units. e, Enzyme activity assays by ion-exchange chromatography show that nucleosome binding potently inhibits the catalytic activity of mouse cGAS. Abs, absorbance; AU, absorbance units; complex, mouse cGAS–nucleosome. Activated cGAS bound to the nucleosome (Fig. 1e). As a positive control, ligand-free cGAS could be robustly activated by the 45-bp dsDNA or salmon sperm DNA (Fig. 1e). Consistent with these results, very low activity was detected for cGAS bound to oligonucleosomes (Extended Data Fig. 2k). The cGAS–oligonucleosome complex was weakly activated by salmon sperm DNA (Extended Data Fig. 2k). Surprisingly, ligand-free cGAS was activated by the cGAS–oligonucleosome complex, probably through the naked linker dsDNA (Extended Data Fig. 2k).

Structure of cGAS bound to the nucleosome

The structure of mouse cGAS bound to the nucleosome reveals that cGAS binds to the top of the nucleosome, interacting with the exposed histone proteins (Fig. 3a). Mouse cGAS in the DNA-free conformation (PDB 4K8V) fits better into the density map than mouse cGAS in the DNA-bound conformation (PDB 4LEZ) (Extended Data Fig. 6a). A few loops of cGAS have slightly altered conformations compared with ligand-free cGAS, maximizing its interaction with the nucleosome (Extended Data Fig. 6a). Consistent with previous studies15, the structure shows that cGAS interacts with the nucleosome mainly through histone H2A and H2B (Fig. 3b). The buried surfaces have areas of about 1,000 Å² at the cGAS–H2A interface and about 620 Å² at the cGAS–H2B interface. cGAS does not interact with histones H3 and H4 or the dsDNA associated with the nucleosome.

cGAS interacts with the nucleosome mainly through electrostatic interactions via a surface that overlaps with its second DNA-binding site, the B site (Fig. 3b, c). The side chains of Arg222, Lys240 and Arg241 interact with the carbohydrate groups of Glu61, Glu64, Asp90 and Glu92 of histone H2A through electrostatic interactions, anchoring cGAS to the nucleosome. Arg342 forms two hydrogen bonds with the carbonyl group of Pro50 of histone H2B. Arg342 forms two hydrogen bonds with the carbonyl group of Lys323. The buried surfaces have areas of about 1,000 Å² at the cGAS–H2A interface and about 620 Å² at the cGAS–H2B interface. cGAS does not interact with histones H3 and H4 or the dsDNA associated with the nucleosome.

To confirm that cGAS binds to the nucleosome by interacting with H2A and H2B, we generated wild-type and mutant H2A–H2B dimers and conducted cGAS–nucleosome binding studies. We observed that cGAS bound to the wild-type H2A–H2B, but did not bind to the acidic patch mutants (Extended Data Fig. 6d). Moreover, we conducted a nucleosome-binding study using the H2A.Bdb nucleosome, which contains mutations at the acidic patch (Extended Data Fig. 6e) and observed that the H2A.Bdb nucleosome did not bind cGAS (Extended Data Fig. 6f).

Structural determination by cryo-EM

To generate samples for cryo-electron microscopy (cryo-EM), we purified mouse cGAS–nucleosome complex using both reconstituted nucleosomes and nucleosomes purified from HEK293T cells (Extended Data Fig. 3a, b). Particle classification yielded a 3D reconstruction at a nominal resolution of 2.96 Å for mouse cGAS bound to the reconstituted nucleosome (Fig. 2a, Extended Data Fig. 3, Extended Data Table 1). In addition, we observed that a small fraction of the particles contained two bound cGAS molecules (Extended Data Fig. 3e, i, k–m). Structural models of the catalytic domain of inactive mouse cGAS (Protein Data Bank (PDB) 4K8V) and human nucleosome (PDB 3AFA) were docked into the 2.96 Å resolution map to generate the initial model of the cGAS–nucleosome complex. The model was rebuilt manually and refined against the cryo-EM density map using Phenix22. The nucleosome was well defined in the cryo-EM map (Fig. 2b, Extended Data Fig. 4). A majority of the cGAS residues interacting with the nucleosome were also well defined (Extended Data Fig. 4). Reconstruction of the complex using nucleosomes purified from cells resulted in a 4.36 Å map and a structural model with similar conformation (Extended Data Fig. 5, Extended Data Table 1). Hereafter, discussions focus on the 2.96 Å structure of the 1:1 mouse cGAS–nucleosome complex.
cGAS mutations affect nucleosome binding

To determine how the positively charged residues at the cGAS–nucleosome interface contribute to nucleosome binding, we generated mouse cGAS mutants and performed nucleosome-binding studies (Fig. 4a, Extended Data Fig. 7a, b). The mutation of key residues mediating cGAS nuclear tethering, R222E, K240E, R241E and R341E, abolished nucleosome binding; R337E and R342E markedly reduced nucleosome binding; and K315E, K323E, K335E and K382E slightly reduced nucleosome binding (Fig. 4a); whereas K238E and R244E did not affect nucleosome binding (Fig. 4a). Similar results were obtained using oligonucleosomes in the binding studies (Extended Data Fig. 7c). Circular dichroism spectroscopy confirmed that these mouse cGAS mutants folded properly (Extended Data Fig. 7d). Next, we expressed human cGAS mutants and performed nucleosome-binding studies using SPR (Fig. 4b, Extended Data Fig. 8). The mutation of four conserved residues that mediate cGAS nuclear tethering, R236E, K254E, R255E and R353E, abolished nucleosome binding (Fig. 4b, Extended Data Fig. 8); and K347E, R349E and K350E substantially reduced the binding affinity (Fig. 4b, Extended Data Fig. 8); whereas R246E, K252E, K258E, K327E and K355E had almost no effect on nucleosome binding (Fig. 4b, Extended Data Fig. 8). The mutation of a residue that mediates cGAS dimerization, K394E, did not affect nucleosome binding (Fig. 4b, Extended Data Fig. 8).

DNA binding and activity of cGAS mutants

Next, we tested how these mutations affect dsDNA binding by cGAS. Mutation in mouse cGAS of residues at the DNA-binding B site, R222E, K240E, R241E, R244E, R341E and R342E, almost completely abolished dsDNA binding; and K238E, K315E, K323E, K335E and R337E substantially reduced DNA binding (Fig. 4c), By contrast, a mutation at the cGAS dimer interface, K382E, only slightly reduced DNA binding (Fig. 4c). Consistent with these results, gel-shift assays showed that human cGAS mutations R236E and K327E markedly reduced DNA binding; K254E, R255E, K258E, K350E and R353E substantially reduced DNA binding; and K347E, R349E and K355E slightly reduced DNA binding. By contrast,

Fig. 3 | Interactions between cGAS and the nucleosome. a, cGAS binds to the acidic patch of the nucleosome. cGAS is shown by the green ribbons. The histone core is shown by the surface representation coloured according to surface electrostatic potential. Positively charged surface is coloured in blue and negatively charged surface is in red. b, Interactions between cGAS (green), histone H2A (cyan) and histone H2B (slate). c, Superposition of the cGAS–dsDNA and the cGAS–nucleosome complex structures shows that the second DNA-binding site (site B) of cGAS mediates its interactions with the nucleosome. d, Two overlapping sets of residues are involved in nucleosome and DNA binding by cGAS.
mutation of R246E, K252E and K394E had only minor effects on DNA binding (Extended Data Fig. 9a).

We also analysed the catalytic activities of these cGAS mutants. Mouse cGAS mutation K238E slightly reduced the catalytic activity; R244E and K315E reduced the activity by nearly 70%; and K240E, R241E and K323E reduced the activity by almost 90%; by contrast, R222E, K335E, and K315E reduced the activity by nearly 70%; and K240E, R241E and K323E reduced the activity by almost 90%; whereas the K382E mutation, which abolishes cGAS enzymatic activity but does not affect nucleosome binding (Extended Data Fig. 9a), greatly affected the activity (Fig. 4d) even though its catalytic activity was reduced by more than 90% (Fig. 4b). The R241E mutant showed slightly higher activity compared with the wild type, probably owing to the disruption of nuclear tethering (Fig. 4d). The R241E mutant showed slightly higher activity compared with the wild type, probably owing to the disruption of nuclear tethering (Fig. 4d) in agreement with the severely compromised catalytic activities of these mutants (Fig. 4b, Extended Data Fig. 9b). The effects of mutations K238E, R244E, K315E, K323E and K335E on signalling (Fig. 4d) roughly correlated with their catalytic activities (Fig. 4b, Extended Data Fig. 9b). The K382E mutation, which abolishes cGAS enzymatic activity but does not affect nucleosome binding (Fig. 4a, b), also abolished signalling in cells (Fig. 4d). Similar results were obtained when human cGAS mutants were expressed in the cells (Extended Data Fig. 9d). The R236E, K254E and R255E mutants of human cGAS, which do not bind to the nucleosome (Fig. 4b), showed similar or slightly higher activity compared with the wild type (Extended Data Fig. 9d). Mutations K350E and R335E, which markedly reduced nucleosome binding and catalytic activity (Fig. 4b), also reduced the reporter signal (Extended Data Fig. 9d). To validate the results from the reporter assays, we measured cGAMP levels in cells transfected with cGAS mutants that disrupt nucleosome binding. Consistent with results from previous studies, cells expressing mouse cGAS(R222E) or cGAS(R241E), or human cGAS(R236E) or cGAS(R255E) produced significantly higher levels of cGAMP compared with those expressing wild-type cGAS (Fig. 4e, f). Transfection of cells with the mouse cGAS(K240E) or human cGAS(K254E) also produced slightly higher
levels of cGAMP (Fig. 4e, f). Moreover, the production of cGAMP in these cells was inducible by transfection with dsDNA (Fig. 4e, f).

**Discussion**

These structural and functional studies reveal the molecular basis of nuclear tethering that inactivates cGAS. We propose that high affinity nucleosome binding maintains cGAS in an inactive conformation and inhibits cGAS activation by dsDNA to avoid autoimmune responses to genomic DNA. Although the naked dsDNA in the linker region between nucleosomes can still activate cGAS, most of the nucleosome-bound dsDNA does not activate cGAS efficiently. By contrast, un tethered cGAS in the cytosol is able to engage naked dsDNA and induce the expression of type I interferons. The delicate balance between nuclear-tethered and free cytosolic cGAS is probably critical for prevention of autoreactivity, while also providing a mechanism for host defence. It has been suggested that the sequestration of cGAS in an inactive state in the nucleus could be an important mechanism for optimization of the dynamic range of the immune responses mediated by cGAS and to limit the ‘noise’ of the system. Mutations that disrupt the balance between the free and nuclear-tethered protein render cGAS constitutively active in cells and abolish its response to dsDNA transfection[14,15]. Perturbation of nuclear tethering of cGAS has the potential to boost antiviral and antitumour immune responses, but may also increase the probability of causing autoimmunity. Our data do not definitively show that cGAS mutants unable to bind nucleosomes are more active against nuclear or mitochondrial DNA, and thus evidence for autoreactivity on endogenous DNA is indirect at this stage. Further studies are required to determine the cellular biological basis governing how distinct pools of cGAS are regulated to limit autoreactivity while also maintaining responsiveness to pathogen-derived DNA and endogenous DNA damage. Since cGAS binds to nucleosomes with high affinity by interacting with the acidic patch, this interaction probably blocks access by other nucleosome-binding proteins that are involved in DNA damage repair, epigenetic regulation, cancer and cell death. However, the exact roles of cGAS in these processes remain to be determined.

**Online content**

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Article
Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Antibodies
Commercially obtained antibodies include the following primary antibodies: rabbit anti-human cGAS (15102 s, Cell Signaling Technology; western blot (WB) 1:1,000, immunofluorescence (IF) 1:200), rabbit anti-mouse Cgas (31659 s, Cell Signaling Technology; WB 1:1,000), rabbit anti-human STING (13647 s, Cell Signaling Technology; WB 1:1,000), rat anti-PARP1 (MAB600, R&D Systems; WB 1:1,000), rabbit anti-histone H3 (17168-1, Protienecht; WB 1:2,000), rat anti-haemagglutinin (1186743001, Roche; WB 1:2,000, IF 1:600), mouse anti-α-tubulin (AB_1157911, DSHB; IF 1:1,000), mouse anti-GAPDH (600004-1, Protienecht; WB 1:2,000) and mouse anti-actin (MAS-11869, Thermo Fisher Scientific; WB 1:4,000).

Secondary antibodies: Peroxidase-conjugated AffiniPure Donkey Anti-Mouse IgG (H+L) (715-055-150, Jackson ImmunoResearch; IF 1:1,000), Alexa Fluor 488-conjugated AffiniPure Donkey Anti-Rabbit IgG (H+) (712-020-153, Jackson ImmunoResearch; WB 1:20,000 for cGAS, WB 1:200,000 for histone H3), Peroxidase-conjugated AffiniPure Donkey Anti-Rat IgG (H+) (712-035-153, Jackson ImmunoResearch; WB 1:20,000 for HA, WB 1:20,000 for PARP1), Anti-rabbit IgG HRP-linked Antibody (7074 s, Cell Signaling: WB 1:1,000 for cGAS and STING in HEK293T cells), Anti-mouse IgG, HRP-linked Antibody (7076 s, Cell Signaling: WB 1:1,000 for actin in HEK293T cells), IgG (H+L) Highly Cross-Adsorbed Donkey anti-Mouse, Alexa Fluor 555 (A31570, Invitrogen; STING in HEK293T cells), Anti-mouse IgG, HRP-linked Antibody (7076 s, Cell Signaling: WB 1:1,000 for actin in HEK293T cells), IgG (H+L) Highly Cross-Adsorbed Donkey anti-Mouse, Alexa Fluor 555 (A31570, Invitrogen; IF 1:1,000), Alexa Fluor 488-conjugated AffiniPure Donkey Anti-Rabbit IgG (H+) (711-545-152, Jackson ImmunoResearch; IF 1:600) and Alexa Fluor 488-conjugated AffiniPure Donkey Anti-Rat IgG (H+) (712-545-153, Jackson ImmunoResearch; IF 1:800).

Cell culture
HeLa cells were purchased from ATCC, maintained in DMEM (D5796, Sigma) supplemented with 10% FBS (97068-085, VWR) and kept at low passage to minimize drift. Primary Cgas−/−MEFs were generated from embryonic day 13.5 embryos in accordance with animal use protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University, maintained in DMEM supplemented with 10% FBS, then transduced with retroviruses encoding SV40 large T antigen (pBABE: puro SV40 LT, Addgene) plasmid to obtain immortalized Cgas−/−MEF (Cgas−/−MEF SV40I). Cgas−/−SV40I cells were then reconstituted with mouse cGAS construct (pMXs-blasc-mcGAS-HA cloned from plmuno-mcGAS-HA3x, InvivoGen) to generate Cgas−/−MEF SV40I + mcGAS-HA cells. HEK293T cells (ATCC, CRL-3216) were cultured in DMEM (1×) + GlutaMAX medium (Gibco, 10569-010) supplemented with 10% FBS (Gibco, 21160-049) and washed with 1× PBS twice. After centrifugation at 300 × g for 5 min, the cells were resuspended in 200 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 7.5 and 1% Nonidet P-40 supplemented with EDTA-free protease inhibitor cocktail (Roche, 1183617001) and boiled with denaturing SDS–PAGE sample buffer at 95 °C for 10 min. The proteins were loaded into 10% SDS–PAGE gel and transferred to nitrocellulose membrane (Bio-Rad, 1620215). After incubation with the primary antibodies overnight at 4 °C, the membrane was further incubated with the corresponding HRP-conjugated secondary antibodies at room temperature for 2 h. Detection of the target proteins was performed with a ChemiDoc Imager and Image lab touch software 2.3.0.07 (Bio-Rad).

Immunofluorescence microscopy
Cells were grown on coverslips overnight before staining. After washing in PBS, cells were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.1% Triton X-100 in PBS for 5 min, blocked with PBS containing 5% FBS for 30 min, stained with primary antibodies for 1 h and washed with secondary antibodies for 1 h. Cells were washed with PBS containing 5% FBS between each step. Coverslips were mounted with ProLong Diamond Antifade Mountant with DAPI (Molecular Probes). Cells were imaged on a Lionheart FX (BioTek) with a 10× or 40× objective. At least 100 cells were used to obtain statistical significance for nuclear cGAS intensity analysis. Gen5 v.3.08 software was used to define the cell and nuclear region, and calculate the ratio of nuclear to whole cell cGAS fluorescent intensity.

Protein expression and purification
The cDNA of cGAS full length and catalytic domain were cloned into a modified pET-28a vector with an N-terminal Avi-His6-SUMO tag. Mouse cGAS catalytic domain (residues 142–507) was expressed in Esherichia coli BL21 (DE3) with 0.4 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) induction overnight at 16 °C and purified as described previously19. Biotin–Avi–His6–SUMO human and mouse cGAS full length and catalytic domains (human cGAS domain residues 157–522) were expressed in E. coli BL21 (DE3) cells co-transformed with the plasmids coding for cGAS and the pBiRAsc plasmid coding for BirA. Protein expression was induced with 0.4 mM IPTG in the presence of 0.5 μg ml−1 biotin (Sigma-Aldrich, B4501). The proteins were first purified using a Ni2⁺-NTA column (Qiagen) and were further purified over a Superdex200 column (GE Healthcare Life Sciences). Biotin–Avi–His6–SUMO fusion with full-length human or mouse cGAS or human cGAS catalytic domain were eluted with buffer containing 20 mM Tris, 500 mM NaCl and pH 7.5. Biotin–Avi–His6–SUMO human or mouse cGAS catalytic domain was eluted with buffer containing 20 mM Tris, 150 mM NaCl and pH 7.5. All mutants were generated using a PCR-based technique with appropriate primers and confirmed by DNA sequencing. The mutant cGAS proteins were expressed and purified in the same way as the wild-type cGAS.

Nucleosome purification from HEK293T cells
Nucleosomes were extracted and purified from HEK293T cells using the nucleosome preparation kit (Active Motif, 53504) according to

Salt fractionations and immunofluorescence
Cellular fractionation was performed largely as described14. In brief, cells were divided into two aliquots, and one aliquot (10%) was reuspended in 50 μl of 1% SDS lysis buffer, boiled for 5 min, sonicated for 1 min and saved as whole-cell lysate. The second equal aliquot (90%) was reuspended in 400 μl extraction buffer containing 10 mM HEPES, 10 mM KCl, 1.5 mM MgCl2, 0.34 mM sucrose, 10% glycerol, 0.2% NP-40, pH 7–7.6 and protease inhibitors. The homogenates were incubated on ice for 10 min with occasional vortexing, centrifuged at 6,500g for 5 min three times, and the supernatant was saved as the cytosol fraction. The nuclei were then washed for 1 min on ice in extraction buffer without NP-40 and centrifuged at 6,500g for 5 min at 4 °C twice, and

resuspended in 150 μl salt buffer (50 mM Tris, 0.05% NP-40, 500 mM NaCl, pH 8.0). Incubated on ice for 15 min with vortexing for 15 s every 5 min. Lysates were centrifuged at 15,000 rpm at 4 °C for 5 min, and the supernatants were collected as the 0.5 M NaCl fraction. The final pellet was then lysed in 50 μl of 1% SDS lysis buffer, boiled at 95 °C for 5 min and sonicated for 1 min. All samples were supplemented with denaturing SDS–PAGE sample buffer, loaded into 10–20% SDS–PAGE gradient gel, and transferred onto 0.22 μm PVDF membranes. After air drying to return to a hydrophobic state, membranes were incubated in primary antibodies at 4 °C overnight in 1× PBS containing 1% casein, HRP-conjugated secondary antibody at room temperature for 1 h, and then developed with Luminata Crescendo Western HRP Substrate (Millipore). For HEK293T cells, the cells were collected 24 h after transfection and washed with 1× PBS twice. After centrifugation at 300g for 5 min, the cells were lysed in 200 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 7.5 and 1% Nonidet P-40 supplemented with EDTA-free protease inhibitor cocktail (Roche, 1183617001) and boiled with denaturing SDS–PAGE sample buffer at 95 °C for 10 min. The proteins were loaded into 10% SDS–PAGE gel and transferred to nitrocellulose membrane (Bio-Rad, 1620215). After incubation with the primary antibodies overnight at 4 °C, the membrane was further incubated with the corresponding HRP-conjugated secondary antibodies at room temperature for 2 h. Detection of the target proteins was performed with a ChemiDoc Imager and Image lab touch software 2.3.0.07 (Bio-Rad).

Nucleosome purification from HEK293T cells
Nucleosomes were extracted and purified from HEK293T cells using the nucleosome preparation kit (Active Motif, 53504) according to
the manual. In brief, HEK293T cells were cultured in a 10-cm tissue culture dish to 70–80% confluency and were collected with a cell scraper. Twenty-million cells were washed twice with 10 ml 1× PBS. Then the cells were resuspended in 1 ml ice-cold lysis buffer supplemented with 5 μl protease inhibitor cocktail and 5 μl 100 mM PMSF. After incubation on ice for 30 min, the lysed cells were centrifuged at 2,400g for 10 min at 4 °C to pellet the nuclei. The nuclei pellet was resuspended in 350 μl digestion buffer supplemented with 1.75 μl protease inhibitor cocktail and 1.75 μl 100 mM PMSF. After incubation at 37 °C for 5 min, the suspended nuclei were mixed with 17 μl diluted enzymatic shearing cocktail and incubated at 37 °C for 50 min. To generate oligonucleosomes, the mixture was incubated at 37 °C for 15 min. During the incubation, the mixture was vortexed approximately every 2 min. To stop the reaction, 7 μl ice-cold 0.5 M EDTA was added into the mixture for 10 min on ice. Then, the mixture was centrifuged at 21,000g for 10 min. Nucleosomes or oligonucleosomes in the supernatant were purified using a Superose 6 increase 10/300 GL column (GE Healthcare Life Sciences) with the running buffer 20 mM Tris, 150 mM NaCl, pH 7.5.

Bio-layer interferometry

Bio-layer interferometry-based assays using the Octet RED96 instrument and ForteBio Data Acquisition 11.1 software (ForteBio) were performed to detect the interaction between cGAS and nucleosome. Specifically, streptavidin biosensors were loaded with 5 μg ml⁻¹ biotin-labelled cGAS proteins. The cGAS-immobilized tips were dipped into twofold serial dilutions of nucleosome (0.25, 0.5, 1, 2, 4 and 8 nM) in 1× HBS-EP buffer (GE healthcare life science, BR-1008-26) supplemented with 5 mM MgCl₂. The association and dissociation phases were measured for 100 s and 150 s respectively. The buffer control was subtracted from raw data and curves were aligned to the baseline. All data were analysed using Octet Data Analysis 11.1 software (ForteBio) and the binding affinities (K₈) were determined by fitting the data to a steady-state 1:1 binding model.

cGAS activity assay

All cGAS activity assays were performed in 20 mM HEPES, 5 mM MgCl₂, 5 mM DTT, 150 mM NaCl, 2 mM ATP (Sigma Aldrich, A2383) and 2 mM GTP (Sigma Aldrich, G8877), pH 7.5. For mouse cGAS catalytic domain, 2.5 μM protein was incubated with 0.2 mM salmon sperm DNA (Invitrogen, 15632-011) for 1 h at 37 °C. For mouse cGAS–nucleosome complex or mouse cGAS–oligonucleosome complex, nucleosomes or oligonucleosomes purified from HEK293T cells were incubated with excess mouse cGAS catalytic domain (in the mixture, the molar ratio of mouse cGAS:nucleosome is 10:1 and mouse cGAS:oligonucleosome is 20:1) for 1 h on ice. The complexes were purified by Superdex 200 10/300 GL column (GE Healthcare Life Sciences) with the running buffer 20 mM Tris, 150 mM NaCl, pH 7.5. 2.5 μM mouse cGAS nucleosome complex or 1.25 μM mouse cGAS–oligonucleosome complex was incubated with or without 2.5 μM mouse cGAS catalytic domain, 0.2 mg ml⁻¹ 45-bp interferon stimulatory dsDNA (ISD) or 0.2 mg ml⁻¹ salmon sperm DNA for 1 h at 37 °C. For full-length human cGAS, 2.5 μM protein was incubated with 0.2 mg ml⁻¹ salmon sperm DNA for 4 h at 37 °C. The product in the supernatant was separated from cGAS, DNA and nucleosome by ultrafiltration and was analysed on a Mono Q S/50 GL ion exchange column (GE Healthcare Life Sciences). The relative enzymatic activities of the mouse and human cGAS mutants were calculated by dividing the cGAMP peak height of the cGAS mutants by the cGAMP peak height of wild-type cGAS.
dsDNA was mixed with wild-type and mutant mouse cGAS catalytic domain at a molar ratio of 1:20. For full-length human cGAS proteins, the molar ratio of DNA and protein is 1:4. The mixtures were incubated on ice for 30 min and then analysed on a 1% agarose gel as described previously.

Cryo-EM data acquisition
Reconstituted nucleosomes or nucleosome complexes were purified from HEK293T cells were incubated with excess mouse cGAS catalytic domain for 1 h on ice. Excess cGAS was removed with a Superdex 200 10/300 GL column (GE Healthcare Life Sciences). The cGAS–nucleosome complex fraction was collected and concentrated to 0.4 mg ml⁻¹. Aliquots of 3 μl nucleosome–cGAS complexes were loaded onto glow-discharged holey carbon grids (Electron Microscopy Sciences, CF312-SO, CFlat, Cu, R2/1, 300 mesh). Grids were blotted for 8 s and plunged frozen in liquid ethane using a Vitrobot at 4 °C and with 100% humidity. Grids were transferred to a Titan Krios electron microscope (Thermo fisher) operating at 300 kV equipped with a Gatan Gif Quantum energy filter (Slit width 20 eV). Micrographs were recorded by EPU through a Gatan K2 Summit detector in counting mode at a nominal magnification of 130,000× (yielding a pixel size of 1.07 Å). The dose rate on the camera was set to be 6 electrons per physical pixel per second. Exposure of 8 s was dose-fractionated into 40 movie frames, leading to a total accumulated dose of 42 electrons per Å² on the specimen. Sample for the reconstituted nucleosome bound to mouse cGAS were recorded with a defocus in the range from 0.7 to 1.8 μm for a total of 4,959 micrographs. Nucleosomes purified from HEK293T cells bound to cGAS were recorded with a defocus in the range from 0.8 to 2.0 μm for a total of 2,979 micrographs.

Cryo-EM data processing
Imaging data for the in-vitro reconstituted and HEK293T cells purified nucleosomes in complex with cGAS were processed identically using Relion-3.0b. The collected movies were subjected to MotionCor2 for drift correction and in-house 3D classification with ab initio models generated in Relion. The 3D classes with cGAS densities were selected, yielding 1,563,321 and 502,961 particles for reconstituted and HEK293T cells purified nucleosome–cGAS complexes, respectively. Multiple rounds of reference-free 2D classification were run to remove aggregates, ice contamination and carbon edges by Relion, yielding 1,563,321 and 502,961 particles for reconstituted and HEK293T cells purified nucleosome–cGAS complexes, respectively. Particles were selected using Relion 3D classification with ab initio models generated in Relion. The 3D classes with cGAS densities were selected, yielding 1,563,321 and 502,961 particles for reconstituted and HEK293T cells purified nucleosome–cGAS complexes, respectively. The particles were subjected to 3D auto refine in Relion, followed by additional cGAS focused skip-align 3D classification. The 3D classes with clear cGAS densities were selected again, yielding 182,358 and 23,463 particles for reconstituted and HEK293T cells purified nucleosome–cGAS complexes, respectively. The particles were then re-centred and re-extracted to 3D auto refinement in Relion. The HEK293T cells purified nucleosome–cGAS complex was reconstructed to 4.36 Å. The reconstituted nucleosome–cGAS complex was reconstructed to 3.21 Å and further subjected to skip-align 3D classification. Class of two cGAS bound nucleosome contained 9,454 particles and was refined to 6.79 Å. The cryo-EM map of one cGAS bound nucleosome was refined to 2.96 Å by contrast transfer function refinement and Bayesian polishing. The reported resolutions are based on the gold-standard Fourier shell correlation 0.143 criterion. Local resolution variations were estimated using Relion.

Cryo-EM model building and refinement
Human nucleosome model containing the 601 DNA was generated using a published nucleosome structure (PDB 3AZA). The nucleosome and mouse cGAS (PDB 4KSV) models were docked in the cryo-EM map in Chimera and fine-tuned by manual adjustment with Coot. This model was refined against the cryo-EM map in Phenix. Several loop regions of mouse cGAS, tails of the histone proteins, and the 601 dsDNA was manually adjusted to fit into the map using Coot. The model was refined again in Phenix and the crystal structure of mouse cGAS was used as reference. This refined model was docked into the cryo-EM map derived from nucleosome purified from HEK293T cells bound to mouse cGAS and the model was refined in Phenix to improve the fitting without further remodelling owing to the lower resolution of the map. All structure figures were made by UCSF Chimera, UCSF ChimeraX, and PyMOL.

Ni-NTA pull-down assay
The H2A–H2B acidic patch residues were mutated to alanine (H2A: E61A, E64A, D90A, E91A, E92A; H2B: D51A; referred as H2A–H2B 6A) or Lysine (H2A: E61K, E64K, D90K, E91K, E92K; H2B: D51K; referred as H2A–H2B 6K). Forty micrograms of 6× His-tagged wild-type H2A–H2B, H2A–H2B 6A or H2A–H2B 6K dimer was incubated with 20 μg Ni-NTA beads in pull-down buffer (20 mM Tris, 150 mM NaCl, 5 mM DTT, pH 7.5) for 5 min at 4 °C. Beads were washed by pull-down buffer for three times and mixed with 60 μg mouse cGAS domain, then incubated for 5 min at 4 °C. Excess proteins were washed off the beads using 300 μl pull-down buffer six times. Twenty microlitres of 5× SDS loading buffer was added to the resin and boiled for 5 min, thereafter, the samples were centrifuged briefly. Fifty microlitres of supernatant was analysed by SDS–PAGE. The protein bands were visualized by Coomassie blue staining.

Circular dichroism spectroscopy
Wild-type mouse cGAS catalytic domain and its mutants were buffer exchanged into 10 mM phosphate buffer containing 50 mM K2SO4 at pH 7.5. The CD spectra were measured using a Chirascan spectrometer and Chirascan Spectrometer Control Panel software (v.4.5.1848.0) with each protein samples at 3 μM concentration. The spectra from 280 nm to 190 nm wavelength were recorded at room temperature using a 2-mm path length quartz cuvette. The data were processed with Pro-Data Viewer (v.4.5.1848.0).

IFN-β luciferase reporter assay
The cDNAs encoding human and mouse cGAS, human STING were cloned into pcDNA3.1 (-) vector, respectively. All mutations were generated by site-directed mutagenesis and confirmed by DNA sequencing. We optimized the amount of STING plasmid used for transfection to ensure that the reporter signal results from cGAS transfection instead of the overexpression of STING. We also conducted the assays at two different cGAS concentrations to test how cGAS expression level affects signalling. HEK293T cells were seeded in CoStar White 96-well plate (Corning, 3917) at 4 × 10⁴ cells per well and cultured at 37 °C with 5% CO₂. After 24 h, the cells were transfected with indicated amounts of pcDNA3.1-human or mouse cGAS plasmids, pcDNA3.1-human STING plasmid (0.4 ng per transfection), IFN-β firefly luciferase reporter plasmid (20 ng per transfection) and pRL-TK–Renilla luciferase plasmid (2 ng per transfection) using the transfection reagent Lipofectamine 2000 (Invitrogen, 11668-019) according to the manufacture’s manual. Empty pcDNA3.1 (-) plasmid was added to normalize the amount of DNA in each transfection. After 24 h, the luminescent signals were detected using the Dual-Glo Luciferase report assay kit (Promega, E2940) according to its manual with a BioTek Synergy HTX Multi-Mode microplate reader and Gen5 2.06 software. The relative firefly luciferase activity was normalized using the Renilla luciferase activity. The relative IFN-β reporter fold of induction represents the ratio normalized to the values from the cells transfected with the empty pcDNA3.1 (-) plasmid with the same treatment. Microsoft Excel was used to analyse the data. All data are presented as mean ± s.e.m. Two groups were compared using a two-tailed Student’s t-test assuming equal variances. The statistical significance between the indicated samples is designated as *P < 0.05, **P < 0.01, ***P < 0.001 or not significant (NS) (P > 0.05).
cGAMP assay
HEK293T cells were seeded in Costar 24-well plate (Corning, 3526) at 2 x 10^5 cells per well and cultured at 37 °C with 5% CO_2. After 24 h, the cells were transfected with 1.5 ng pcDNA3.1-human cGAS plasmids or 0.05 ng pcDNA3.1-mouse cGAS plasmids using the Lipofectamine 2000 according to the manufacturer’s manual. Twenty-four hours later, the cells were further transfected with 10 μg ml⁻¹ salmon sperm DNA using Lipofectamine 2000 or with Lipofectamine 2000 alone. After 4 h, the cells were washed with 1× PBS once and lysed in 200 μl M-PER buffer (Thermo Fisher Scientific, 78501). After centrifugation at 15,000 rpm for 10 min at 4 °C, cGAMP in the supernatant was quantified using cGAMP ELISA kit (Cayman Chemical, 501700) according to the manufacturer’s manual.

Statistics and reproducibility
All data presented in this paper are representative of 2–4 independent experiments.

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

Data availability
The three-dimensional cryo-EM density maps are deposited into the Electron Microscopy Data Bank (EMDB) under accession numbers EMD-22046, EMD-22206 and EMD-22047. The coordinates were deposited in the Protein Data Bank (PDB) with accession numbers 6X59, 6XJD and 6XSA. Source data are provided with this paper.

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Author contributions
P.L. conceived the study. B.Z. and P.X. expressed proteins, conducted binding studies and determined the structures. C.M.R. prepared the reconstituted nucleosome. T.J. conducted the binding studies and generated and purified the cGAS mutants. O.S. purified the cGAS mutants. Y.L. and A.P.W. studied the nuclear localization of cGAS. B.Z., P.X., W.R.L. and P.L. wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information
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Correspondence and requests for materials should be addressed to W.R.L. or P.L.

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Extended Data Fig. 1 | cGAS binds tightly with nucleosome. **a**, Gel-filtration chromatography and SDS–PAGE analyses of nucleosomes purified from HEK293T cells. **b**, Gel-filtration chromatography and SDS–PAGE analyses of the in vitro reconstituted nucleosomes. **c**, Gel-filtration chromatography showing that mouse cGAS catalytic domain binds to nucleosomes. **d**, SDS–PAGE analysis of nucleosome and mouse cGAS catalytic domain complex purified by gel-filtration chromatography. **e**, Polyacrylamide gel EMSA showing the interactions between mouse cGAS catalytic domain with reconstituted nucleosomes and nucleosomes purified from HEK293T cells. cGAS is mixed with nucleosomes at a molar ratio of 6:1. **f**, Agarose gel electrophoresis of the dsDNA from purified mononucleosomes and oligonucleosomes. The histones have been digested with proteinase K. **g**, SDS–PAGE analyses of purified mononucleosomes and oligonucleosomes. **h**, Polyacrylamide gel EMSA showing that mouse cGAS catalytic domain binds to mononucleosomes and oligonucleosomes (left panel). In the mixtures, the molar ratio of cGAS/mononucleosome is 3:1 and cGAS/oligonucleosome is 6:1. SDS–PAGE analyses of the input samples for EMSA were shown in the right panel. **i**, SDS–PAGE analyses of biotin-Avi-His6-SUMO fusion of human and mouse cGAS full length and catalytic domain proteins used for nucleosome binding studies.
Extended Data Fig. 2 | cGAS-nucleosome binding studies and activity assays of cGAS-nucleosome complex. a, SPR binding studies show that full-length human cGAS binds to nucleosomes purified from HEK293T cells with nanomolar affinity. b, SPR binding studies show that human cGAS catalytic domain binds to reconstituted nucleosomes with nanomolar affinity. c, Bio-layer interferometry binding studies of full-length human cGAS and its catalytic domain with nucleosomes (HEK293T). d, SPR binding studies show that full-length mouse cGAS and its catalytic domain bind nucleosomes (HEK293T) with nanomolar affinities. e, Bio-layer interferometry binding studies of full-length mouse cGAS and its catalytic domain with nucleosomes (HEK293T). f, SPR binding studies show that full-length mouse cGAS and its catalytic domain bind nucleosomes (HEK293T) with nanomolar affinities. g, Bio-layer interferometry binding studies of full-length mouse cGAS and its catalytic domain with nucleosomes (HEK293T). h, Bio-layer interferometry binding studies of full-length mouse cGAS and its catalytic domain with nucleosomes (HEK293T). i, Gel-filtration chromatography (top) and SDS–PAGE (bottom) analyses of 45-bp ISD dsDNA, nucleosome and cGAS mixture show that the nucleosome efficiently competes with dsDNA to bind cGAS. j, Agarose gel electrophoresis of the salmon sperm DNA used in cGAS activity assays. k, cGAS activity assays by ion exchange chromatography show that oligonucleosome binding potently inhibits the activity of cGAS and ligand-free cGAS can be activated by the cGAS-oligonucleosome complex.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Cryo-EM analysis of mouse cGAS catalytic domain in complex with the reconstituted nucleosome. 

a, Purification and SDS–PAGE analysis of nucleosomes from HEK293T cells in complex with mouse cGAS catalytic domain. 
b, Purification and SDS–PAGE analysis of reconstituted nucleosomes in complex with mouse cGAS catalytic domain. 
c, Representative micrograph of mouse cGAS-nucleosome complex in vitrified ice. Scale bar, 50 nm. 
d, 2D class averages of mouse cGAS-nucleosome complex particles. Scale bar, 10 nm. 
e, Flowchart of data processing; see Methods for details. 
f, Angular distribution of the mouse cGAS-nucleosome (1:1) particles included in the final reconstruction. 
g, Angular distribution of the mouse cGAS-nucleosome (2:1) particles included in the final reconstruction. 
h, Final 3D reconstruction of the mouse cGAS-nucleosome (1:1) complex, coloured according to the local resolution. 
i, Final 3D reconstruction of the mouse cGAS-nucleosome (2:1) complex, coloured according to the local resolution. 
j, Corrected Gold-standard Fourier shell correlation curves of the mouse cGAS-nucleosome (1:1) complex for the 3D electron microscopy reconstruction. 
k, Corrected Gold-standard Fourier shell correlation curves of the mouse cGAS-nucleosome (2:1) complex for the 3D electron microscopy reconstruction. 
l, Polyacrylamide gel shift assay (left) showing that one nucleosome can bind to two molecules of mouse cGAS catalytic domain. Nucleosome is mixed with mouse cGAS at molar ratio of 1:1, 1:2 and 1:3. SDS–PAGE analysis of the samples used for the gel shift assays was shown on right panel. 
m, Density map (contoured at 3σ) and structural model of mouse cGAS-nucleosome (2:1) complex at 6.8 Å resolution.
Extended Data Fig. 4 | Density maps and structural models of cGAS-nucleosome (reconstituted, 1:1) complex. a–f, The density maps (grey mesh) of histones H2A, H2B, H3, H4, part of mouse cGAS catalytic domain, and the Widom 601 nucleosome positioning sequence DNA contoured at 3σ. The protein and DNA structures fitted into the density map are shown by the stick models.
Extended Data Fig. 5 | Cryo-EM analysis of mouse cGAS domain in complex with nucleosome purified from HEK293T cells. **a,** Representative micrograph of mouse cGAS-nucleosome complex in vitrified ice. Scale bar, 50 nm. **b,** 2D class averages of mouse cGAS-nucleosome complex particles. Scale bar, 10 nm. **c,** Flowchart of data processing; see Methods for details. **d,** Angular distribution of particles included in the final reconstruction. **e,** Final 3D reconstruction, coloured according to the local resolution. **f,** Corrected Gold-standard Fourier shell correlation curves for the 3D electron microscopy reconstruction. **g,** Density map (contoured at 3σ) and structural model of cGAS-nucleosome (1:1) complex.
Extended Data Fig. 6 | Mutations in the acidic patch of the nucleosome abolished cGAS binding. a, Superposition of structures of ligand-free mouse cGAS (PDB, 4K8V), mouse cGAS in complex with dsDNA (PDB, 4LEY), and mouse cGAS bound to the nucleosome. b, Sequence alignment of human and mouse cGAS around the nucleosome binding site. The conserved basic residues around the nucleosome binding site are coloured red. Residues that abolish nucleosome binding when mutated are highlighted yellow. c, Superposition for the structures of ligand-free human cGAS (PDB, 4LEV), ligand-free mouse cGAS (PDB, 4K8V) and mouse cGAS bound to the nucleosome. d, Ni-NTA agarose pull-down assays of mouse cGAS catalytic domain by His-tagged H2A-H2B dimer. The 6A dimer contains mutations E61A, E64A, D90A, E91A, E92A of H2A and D51A of H2B. The 6K dimer contains mutations E61K, E64K, D90K, E91K, E92K of H2A and D51K of H2B. e, Sequence alignment of wild-type (WT) human H2A and human H2A_Bbd. The acidic patch residues of WT H2A are coloured red. f, Polyacrylamide gel shift assay (left) showing that the recombinant nucleosome variant (H2A_Bbd) does not bind to mouse cGAS catalytic domain. In this assay, mouse cGAS was mixed with nucleosomes at a molar ratio of 3:1. SDS–PAGE analysis of the samples for gel shift assay were shown on the right panel.
**Extended Data Fig. 7 | Characterization of mouse cGAS catalytic domain mutants and oligonucleosome binding studies.**

**a**, SDS–PAGE analysis of mouse cGAS catalytic domain mutants used for the gel shift assays and enzyme activity assays.  
**b**, SDS–PAGE analysis of mouse cGAS and nucleosome mixture samples used for the gel shift assay.  
**c**, Polyacrylamide gel EMSA shows that mutations at the cGAS-nucleosome interface affect oligonucleosome binding by cGAS. In these samples, mouse cGAS was mixed with oligonucleosomes at a molar ratio of 6:1. The samples used for the binding studies were analysed by SDS–PAGE (right panel).  
**d**, Circular dichroism of mouse cGAS catalytic domain and its mutants used for gel shift assays and enzyme activity assays. cGAS mutants that have strong binding to nucleosomes are shown by the spectra on the left. cGAS mutants that have weak or no binding to nucleosomes are shown by the spectra on the right.
Extended Data Fig. 8 | Mutations at the cGAS-nucleosome interface affect nucleosome binding by human cGAS. 

a, SDS–PAGE analysis of biotin-labelled full-length human cGAS mutants used for the SPR binding studies. 
b–n, SPR binding studies of full-length human cGAS mutants with reconstituted nucleosomes.
Extended Data Fig. 9 | Mutations at the cGAS-nucleosome interface affect dsDNA binding, cGAS activity and cGAS-mediated signalling. a, Agarose gel shift assay shows that mutations at the nucleosome binding surface of human cGAS affect the binding of a 45-bp dsDNA. b, Enzyme activity assays of mouse cGAS catalytic domain mutants by ion exchange chromatography. In this assay, 2.5 μM mouse cGAS was incubated with 0.2 mg ml⁻¹ salmon sperm DNA. Wild-type mouse cGAS and negative control without DNA are coloured in green and blue. Mutations that abolish nucleosome binding are coloured red. The negative control mutation K382E is coloured orange. c, Enzyme activity assays of full-length human cGAS mutants by ion exchange chromatography. In this assay, 2.5 μM human cGAS was incubated with 0.2 mg ml⁻¹ salmon sperm DNA. WT human cGAS and negative control without DNA are coloured in green and blue. Mutations that abolish nucleosome binding are coloured red.

The negative control mutation K394E is coloured orange. d, IFN-β luciferase reporter assays show that mutations of human cGAS affect signalling in HEK293T cells. Luciferase reporter signals from the cells transfected with 0.025 ng cGAS and 0.4 ng STING are indicated by the orange bars, from cells transfected with 0.00625 ng cGAS and 0.4 ng STING by the cyan bars, from cells transfected with 0.025 ng cGAS, 0.4 ng STING, or the vector control by the green, brown and purple bars, respectively. The data (mean ± s.e.m.) are representative of three independent experiments. Each dot represents a biological replicate (n = 3). Two-tailed Student’s t-test: *P < 0.05, **P < 0.01, ***P < 0.001; NS, not significant. e, Western blot shows that WT mouse cGAS and its mutants have similar expression level in the transfected HEK293T cells. f, Western blot shows WT human cGAS and its mutants have similar expression level in the transfected HEK293T cells.
## Extended Data Table 1 | Cryo-EM data collection, refinement and validation statistics

### Cryo-EM data collection, refinement and validation statistics

|                         | mcGAS/nucleosome (reconstituted, 1:1) (EMDB-22046) (PDB 6X59) | mcGAS/nucleosome (reconstituted, 2:1) (EMDB-22026) (PDB 6XJD) | mcGAS/nucleosome (HEK 293T, 1:1) (EMDB-22047) (PDB 6X5A) |
|-------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| **Data collection and processing** |                                                               |                                                               |                                                               |
| Magnification           | 130,000                                                       | 130,000                                                       | 130,000                                                       |
| Voltage (kV)            | 300                                                           | 300                                                           | 300                                                           |
| Electron exposure (e-/Å²) | 42                                                            | 42                                                            | 42                                                            |
| Defocus range (μm)      | -1.8 ~ -0.7                                                   | -1.8 ~ -0.7                                                   | -2.0 ~ -0.8                                                   |
| Pixel size (Å)          | 1.07                                                          | 1.07                                                          | 1.07                                                          |
| Symmetry imposed        | C1                                                            | C1                                                            | C1                                                            |
| Initial particle images (no.) | 2,202,680                                             | 2,202,680                                                     | 578,302                                                       |
| Final particle images (no.) | 165,092                                                        | 9,454                                                         | 23,463                                                       |
| Map resolution (Å)      | 2.96                                                          | 6.79                                                          | 4.36                                                          |
| FSC threshold 0.143     |                                                               |                                                               |                                                               |
| Map resolution range (Å) | 6.0 ~ 2.8                                                    | 10.7 ~ 4.1                                                    | 9.1 ~ 3.9                                                     |
| **Refinement**          |                                                               |                                                               |                                                               |
| Initial model used (PDB code) | 3AFA 4K8V                                                | 3AFA 4K8V                                                     | 3AFA 4K8V                                                     |
| Model resolution (Å)    | 3.00                                                          | 7.16                                                          | 4.54                                                          |
| FSC threshold 0.5       |                                                               |                                                               |                                                               |
| Model resolution range (Å) | 32.5 ~ 2.8                                                 | 39.8 ~ 6.8                                                    | 35.3 ~ 4.4                                                    |
| Map sharpening B factor (Å²) | -15                                                          | -48                                                           | N/A                                                           |
| Model composition       |                                                               |                                                               |                                                               |
| Non-hydrogen atoms      | 14,963                                                        | 17,957                                                        | 14,963                                                        |
| Protein residues        | 1,123                                                         | 1,485                                                         | 1,123                                                         |
| Nucleotide              | 290                                                           | 290                                                           | 290                                                           |
| Ligands                 | 1                                                             | 2                                                             | 1                                                             |
| B factors (Å²)          |                                                               |                                                               |                                                               |
| Protein                 | 83.69                                                         | 104.67                                                        | 405.53                                                        |
| Nucleotide              | 74.19                                                         | 74.19                                                         | 278.45                                                        |
| Ligand                  | 194.81                                                        | 194.81                                                        | 997.41                                                        |
| R.m.s. deviations       |                                                               |                                                               |                                                               |
| Bond lengths (Å)        | 0.006                                                         | 0.008                                                         | 0.007                                                         |
| Bond angles (°)         | 0.834                                                         | 1.533                                                         | 0.887                                                         |
| **Validation**          |                                                               |                                                               |                                                               |
| MolProbity score        | 2.64                                                          | 2.73                                                          | 2.23                                                          |
| Clashscore              | 10.75                                                         | 10.82                                                         | 13.71                                                         |
| Poor rotamers (%)       | 7.57                                                          | 8.16                                                          | 1.66                                                          |
| Ramachandran plot       |                                                               |                                                               |                                                               |
| Favored (%)             | 93.67                                                         | 91.95                                                         | 93.67                                                         |
| Allowed (%)             | 6.15                                                          | 8.05                                                          | 6.15                                                          |
| Disallowed (%)          | 0.18                                                          | 0.00                                                          | 0.18                                                          |
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
|-----|-----------|
| ☐   | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐   | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐   | The statistical test(s) used AND whether they are one- or two-sided |
| ☐   | Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| ☐   | A description of all covariates tested |
| ☐   | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐   | A full description of the statistical parameters including central tendency (e.g. mean) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐   | For null hypothesis testing, the test statistic [e.g. F, t, r] with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values wherever suitable. |
| ☐   | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☐   | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐   | Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated |

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | Biacore X100 control software [v. 2.0], EPU Software, Gen5 2.06 [BioTek Synergy HTX plate reader], Image Lab Touch Software [v. 2.3.0.07, Bio-rad], ForteBio Data Acquisition [v. 11.1.2.24], Chirascan Spectrometer Control Panel [v. 4.5.1848.0] |
| Data analysis   | Phenix [v. 1.12, 1.13], PyMol [v. 1.2], Biacore X100 evaluation software [v. 2.0], Gen5 v3.08 software, Reilon-3.0b, UCSF Chimera [v. 1.14], UCSF ChimeraX [v. 1.1], Coot [v. 0.8.9], Image Lab [v. 6.0.0.0], GraphPad Prism 8.0, Microsoft Excel 2016, ForteBio Data Analysis [v. 11.1.2.9], Pro-Data Viewer [v. 4.5.1848.0] |

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The three-dimensional cryo-EM density maps are deposited into the Electron Microscopy Data Bank (https://www.emdb.org/), under accession numbers EMD-22046, EMD-22206, and EMD-22047. The coordinates were deposited in the Protein Data Bank (https://www.rcsb.org/) with accession numbers 6X39, 5XID, and 5XSA.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to predetermine sample size. 3 biologically replicates (n=3) were performed as indicated in the figure legends. cryoEM datasets consist of thousands of independent images, as described in Methods. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | CryoEM single particles are included or excluded following common image processing, as described in Methods. The damaged or false-picked particles are excluded and this function is implemented in Relion 3.0b. |
| Replication | Experimental findings were performed with 2-4 independent experiments and were reliably reproduced. |
| Randomization | No randomization was used in this study. Each experiment was performed with identified controls and mutants. Randomization was not relevant to the study as the study does not involve participant groups. |
| Blinding | No blinding was performed during data collection and/or analysis as the investigator need to exclude damaged or false-picked particles in data collection and processing. |

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**Materials & experimental systems**

| n/a | Involved in the study |
|-----|-----------------------|
| [ ] | Antibodies |
| [x] | Eukaryotic cell lines |
| [ ] | Palaeontology and archaeology |
| [ ] | Animals and other organisms |
| [x] | Human research participants |
| [x] | Clinical data |
| [x] | Dual use research of concern |

**Methods**

| n/a | Involved in the study |
|-----|-----------------------|
| [x] | ChIP-seq |
| [x] | Flow cytometry |
| [x] | MRI-based neuroimaging |

**Antibodies**

| Antibodies used | rabbit anti-human cGAS [15102s, Cell Signaling Technology], rabbit anti-mouse cGAS [31655s, Cell Signaling Technology], rabbit anti-human STING [13647s, Cell Signaling Technology], rat anti-PARP1 [MA600, R&D Systems], rabbit anti-Histone H3 [17168-1, Proteintech], rat anti-HA [11857423001, Roche], mouse anti-a-tubulin [AB_1157911, DSHB], mouse anti-GAPDH [600004-1, Proteintech], mouse anti-actin [MA5-11869, Thermo Fisher Scientif], Peroxidase-conjugated AffiniPure Donkey Anti-Mouse IgG (H + L) [715-035-151, Jackson Immunoresearch], Peroxidase-conjugated AffiniPure Donkey Anti-Rabbit IgG (H + L) [712-035-152, Jackson Immunoresearch], Peroxidase-conjugated AffiniPure Donkey Anti-Rabbit IgG (H + L) [712-035-153, Jackson Immunoresearch], IgG (H + L) Highly Cross-Adsorbed Donkey Anti-Mouse, Alexa Fluor 555 [A31570, Invitrogen], Alexa Fluor 488-conjugated AffiniPure Donkey Anti-Rabbit IgG (H + L) [712-545-152, Jackson Immunoresearch], and Alexa Fluor 488-conjugated AffiniPure Donkey Anti-Rat IgG (H + L) [712-545-153, Jackson Immunoresearch] |

**Validation**

All antibodies used in this study are commercial. Most of them were evaluated in Western Blots of cell lysates containing endogenous or overexpressed target proteins or immunofluorescence.

| rabbit anti-human cGAS [15102s, Cell Signaling Technology], Species [Human], Application [WB]. As noted on the manufacturer’s website, the citation is 34. https://www.cellsignal.com/products/primary-antibodies/cgas-d1d3-rabbit-mab/15102 |
| rabbit anti-mouse cGAS [31655s, Cell Signaling Technology], Species [Mouse], Application [WB, IP]. As noted on the manufacturer’s website, the citation is 19. https://www.cellsignal.com/products/primary-antibodies/cgas-d3b0-rabbit-mab-mouse-specific/31655 |
| rabbit anti-human STING [13647s, Cell Signaling Technology], Species [Human, Mouse], Application [WB, IP, IHC]. As noted on the manufacturer’s website, the citation is 93. https://www.cellsignal.com/products/primary-antibodies/sting-d2o2f-rabbit-mab/13647 |
| rat anti-PARP1 [MA600, R&D Systems], Species [Human, Mouse], Application [WB]. As noted on the manufacturer’s website, the citation is 4. https://www.rndsystems.com/products/human-mouse-parp-antibody-s3035_mab600 |
Eukaryotic cell lines

Policy information about: cell lines

Cell line source(s)  HEK293T cells were purchased from ATCC [Cat# CRU-3218]. HeLa cells were purchased from ATCC. Primary cGAS-/I- MEFs were generated from E13.5 embryos.

Authentication  All cell lines were kept at low passages in order to maintain their identity. The cells have been authenticated by validating cGAS, STING, or actin by western blotting or immunofluorescence.

Mycoplasma contamination  All cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines  [See CLAC register]  No commonly misidentified cell lines were used in this work.

Animals and other organisms

Policy information about: studies involving animals, ARRIVE guidelines, recommended for reporting animal research

Laboratory animals  Timed matings of cGAS-/I- male and female breeding pairs [Jackson Labs mouse strain 026554] were established when the mice were between 8-9 months of age. MEFs were generated from pregnant females 13.5 days post fertilization.

Wild animals  Did not involve wild animals.

Field-collected samples  Did not involve field collected specimens or samples.

Ethics oversight  Animal experiments and protocols were fully reviewed and approved by the Institutional Animal Care and Use Committee of Texas AM University in accordance with regulations established by the National Research Council, DLAW, and AAALAC.

Note that full information on the approval of the study protocol must also be provided in the manuscript.