

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. means) 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 <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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, Relion-3.0b, UCSF Chimera (v.1.14), UCSF ChimeraX (v.1.0), Coot (v.0.8.9), Image Lab (v.6.0.0), GraphPad Prism 8.0, Microsoft Excel 2016, ForteBio Data Analysis (v. 11.1.2.9), Pro-Data Viewer (v. 4.5.1848.0)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

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.ebi.ac.uk/pdbe/emdb/>) under accession numbers EMD-22046, EMD-2206, and EMD-22047. The coordinates were deposited in the Protein Data Bank (<https://www.rcsb.org/>) with accession numbers 6X59, 6XJD, and 6X5A.

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.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.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 includes 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.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	rabbit anti-human cGAS (15102s, Cell Signaling Technology), rabbit anti-mouse cGAS (31659s, Cell Signaling Technology), rabbit anti-human STING (13647s, Cell Signaling Technology), rat anti-PARP1 (MAB600, R&D Systems), rabbit anti-Histone H3 (17168-1, Proteintech), rat anti-HA (11867423001, Roche), mouse anti- α -tubulin (AB_1157911, DSHB), mouse anti-GAPDH (600004-1, Proteintech), mouse anti-actin (MA5-11869, Thermo Fisher Scientific), Peroxidase-conjugated AffiniPure Donkey Anti-Mouse IgG (H+L) (715-035-151, Jackson ImmunoResearch), Peroxidaseconjugated AffiniPure Donkey Anti-Rabbit IgG (H+L) (711-035-152, Jackson ImmunoResearch), Peroxidase-conjugated AffiniPure Donkey Anti-Rat 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) (711-545-152, 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-d1d3g-rabbit-mab/15102 rabbit anti-mouse cGAS (31659s, 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-d3o8o-rabbit-mab-mouse-specific/31659 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-d2p2f-rabbit-mab/13647 rat anti-PARP1 (MAB600, 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-53015_mab600

rabbit anti-Histone H3 (17168-1, proteintech), Species (Human, Mouse, Rat), Application (WB, IHC, IF). As noted on the manufacturer's website, the citation is 212. <https://www.ptglab.com/products/Histone-H3-Antibody-17168-1-AP.htm#product-information>

rat anti-HA (11867423001, Roche), Application (WB). As noted on the manufacturer's website, the citation is 3. <https://www.sigmaaldrich.com/catalog/product/roche/roahaha?lang=en®ion=US>

mouse anti- α -tubulin (AB_1157911, DSHB), Species (Human, Mouse), Application (WB, IF, IHC). As noted on the manufacturer's website, no citation information is found. <https://dshb.biology.uiowa.edu/12G10-anti-alpha-tubulin>

mouse anti-GAPDH (600004-1, Proteintech), Species (Human, Mouse), Application (WB, IF, IP). As noted on the manufacturer's website, the citation is 2405. <https://www.ptglab.com/products/GAPDH-Antibody-60004-1-Ig.htm>

mouse anti-actin (MA5-11869, Thermo Fisher Scientific), Species (Human, Mouse), Application (WB, IF, IP, IHC). As noted on the manufacturer's website, the citation is 289. <https://www.thermofisher.com/antibody/product/Actin-Antibody-clone-ACTN05-C4-Monoclonal/MA5-11869>

Peroxidase-conjugated AffiniPure Donkey Anti-Mouse IgG (H+L) (715-035-151, Jackson ImmunoResearch), Species (Mouse), Application (WB). As noted on the manufacturer's website, the citation is 139. <https://www.jacksonimmuno.com/catalog/products/715-035-151>

Peroxidase-conjugated AffiniPure Donkey Anti-Rabbit IgG (H+L) (711-035-152, Jackson ImmunoResearch), Species (Rabbit), Application (WB). As noted on the manufacturer's website, the citation is 499. <https://www.jacksonimmuno.com/catalog/products/711-035-152>

Peroxidase-conjugated AffiniPure Donkey Anti-Rat IgG (H+L) (712-035-153, Jackson ImmunoResearch), Species (Donkey), Application (WB). As noted on the manufacturer's website, the citation is 39. <https://www.jacksonimmuno.com/catalog/products/712-035-153>

IgG (H+L) Highly Cross-Adsorbed Donkey anti-Mouse, Alexa Fluor 555 (A31570, Invitrogen), Species (Mouse), Application (ICC, IF, IHC). As noted on the manufacturer's website, the citation is 23. <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31570>

Anti-rabbit IgG, HRP-linked Antibody (7074s, Cell Signaling), Species (Rabbit), Application (WB). As noted on the manufacturer's website, the citation is 3422. <https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074>

Anti-mouse IgG, HRP-linked Antibody (7076s, Cell Signaling), Species (Mouse), Application (WB). As noted on the manufacturer's website, the citation is 1820. <https://www.cellsignal.com/products/secondary-antibodies/anti-mouse-igg-hrp-linked-antibody/7076>

Alexa Fluor 488-conjugated AffiniPure Donkey Anti-Rabbit IgG (H+L) (711-545-152, Jackson ImmunoResearch), Species (Rabbit), Application (IF). As noted on the manufacturer's website, the citation is 393. <https://www.jacksonimmuno.com/catalog/products/711-545-152>

Alexa Fluor 488-conjugated AffiniPure Donkey Anti-Rat IgG (H+L) (712-545-153, Jackson ImmunoResearch), Species (Rat), Application (IF). As noted on the manufacturer's website, the citation is 36. <https://www.jacksonimmuno.com/catalog/products/712-545-153>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HEK293T cells were purchased from ATCC (Cat# CRL-3216). HeLa cells were purchased from ATCC. Primary cGAS ^{-/-} 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 ICLAC 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 ^{-/-} male and female breeding pairs (Jackson Labs mouse strain 026554) were established when the mice were between 4-8 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 A&M University in accordance with regulations established by the National Research Council, OLAW, and AAALAC.

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