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Corresponding author(s): Hua Cai
Jean-Luc Imler
Rune Hartmann

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# 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<br>*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. 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<br>*Give P values as exact values whenever 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 | Analyst Software 1.7 (Sciex) for LC-MS/MS<br>CFX Maestro Software (Bio-Rad) for qPCR<br>Wallac 1420 Workstation (PerkinElmer) for luciferase assays<br>Unicorn 5.10 (Cytiva) for ion exchange chromatography |
| Data analysis | MultiQuant 3.0.3 (Sciex) for analysis of LC-MS/MS data<br>GraphPad Prism 9.0.1 (GraphPad Software) for statistical analyses |

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 authors declare that the data supporting the findings of this study are available within the paper.

Accession codes for sequences and structures used in this study are as follows
CG12970: A1ZA55 (UniProt)

CG30424: A8DYP7 (UniProt)
CG4746: Q9U3W6 (UniProt)
CG4766: Q9Y106 (UniProt)
CG7194: Q9VSH0 (UniProt)
Porcine OAS1: Q29599 (UniProt)
Human OAS1: P00973-1 (UniProt)
Human cGAS: Q8N884-1 (UniProt)
Porcine cGAS: I3LM39 (UniProt)
Murine cGAS: Q8BSY1 (UniProt)
Murine cGAS in complex with DNA and a cGAMP intermediate analog: 4K98 (PDB code)

Extended Data Fig. 2b, Extended Data Fig. 4b, Extended Data Fig. 10a, Extended Data Fig. 10c and Extended Data Fig. 11c have associated raw data (gel source data)

# 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

# Life sciences study design

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

| | |
|---|---|
| Sample size | Sample sizes for studies in flies was based on Cai et al. (PMID: 33262294) using three independent experiments (addressing variation pertaining to the day of the experiment), each involving three independent groups (addressing homogeneity of the data and the genetic population studied) with 6-10 flies per group (addressing individual variability).<br>Samples sizes for studies in cell cultures were determined based on consistency and variability of the data. |
| Data exclusions | No data were excluded from the analyses |
| Replication | All experiments were successfully replicated. The exact number of experimental repeats shown in the figures is stated in the figure legends. |
| Randomization | For all experiments, samples of the same type (e.g. flies of the same genotype or cells of the same cell line) were randomly allocated into experimental groups. |
| Blinding | For fly experiments, investigators were blinded to group allocation during data analysis. For all other experiments, investigators were not blinded as the read-outs could be evaluated objectively. |

# 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 | The Anti-Sting antibody used in this study was custom-made by the company BioGenes. The antibody was raised in rabbit against the peptide HMQNKTKTIDEISN. After bleeding the rabbit, the antibody was affinity purified from serum using the peptide.<br><br>The remaining antibodies used in this study are commercially available:<br>Anti-V5 Antibody (Invitrogen; R960-25)(RRID: AB_2556564)<br>Anti-Actin, clone 4 (EMD Millipore; MAB1501)(RRID: AB_2223041)<br>Monoclonal ANTI-FLAG® clone M2 (Sigma-Aldrich; F3165)(RRID: AB_259529)<br>STING (clone D2P2F) Rabbit mAb (Cell Signaling Technology; #13647)(RRID: AB_2799947) |

Peroxidase-conjugated AffiniPure F(ab')2 Fragment Donkey Anti-Mouse IgG (JacksonImmunoResearch; 715-036-150)(RRID: AB_2340773)
Anti-Rabbit IgG HRP-linked Antibody (Cell Signalling Technology; #7074)(RRID: AB_2099233)

Validation

The Anti-Sting antibody was validated by immunoblot using recombinant Sting produced in Escherichia coli.

The commercially available antibodies have been validated by the manufacturer as follows:
Anti-V5 Antibody: https://www.thermofisher.com/antibody/product/V5-Tag-Antibody-Monoclonal/R960-25
Anti-Actin, clone 4: https://www.merckmillipore.com/DK/en/product/Anti-Actin-Antibodyclone-C4,MM_NF-MAB1501R?ReferrerURL=https%3A%2F%2Fwww.google.com%2F&bd=1
Monoclonal ANTI-FLAG® M2: https://www.sigmaaldrich.com/DK/en/product/sigma/f3165
STING (D2P2F) Rabbit mAb: https://www.cellsignal.com/products/primary-antibodies/sting-d2p2f-rabbit-mab/13647
Peroxidase-conjugated AffiniPure F(ab')2 Fragment Donkey Anti-Mouse IgG: https://www.jacksonimmuno.com/catalog/products/715-036-150
Anti-Rabbit IgG HRP-linked Antibody: https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074

## Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK293T cells (ATTC; CRL3216)
HEK293T/MAVS KO cells (kindly provided by Veit Hornung, Gene Center Munich, and described in PMID: 26216125)
HT-1080 cells (German Collection of Microorganisms and Cell Cultures GmbH; ACC 315)
Schneider 2 (S2) cells (German Collection of Microorganisms and Cell Cultures GmbH; ACC 130)

Authentication

The cell lines were authenticated by the companies from which they were purchased as follows
HEK293T cells: STR fingerprinting
HT-1080 cells: STR fingerprinting
Schneider 2 (S2) cells: COI DNA barcoding

The HEK293T/MAVS KO cells were authenticated as described previously in Andersen et al. (PMID: 26216125)

Mycoplasma contamination

All cell lines have regularly been tested negative for mycoplasma using Mycoplasmacheck (Eurofins Genomics)

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study

## Animals and other organisms

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

Laboratory animals

Adult Drosophila melanogaster flies 3-5 days old (For the experiments with transgenic flies oxerexpressing cGLRs, only males were used. In all other experiments, equal numbers of male and female flies were used)

Wild animals

No wild animals were involved in this study

Field-collected samples

This study did not involve field-collected samples

Ethics oversight

No ethical approval or guidance is required for Drosophila melanogaster

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