

Reporting Summary

Nature Portfolio 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 Portfolio 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 (<i>n</i>) 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), 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	BD Biosciences FACSDiva™ v9.7 & FACSCorus™ v3.0; Biorad Image Lab v6.1; Tecan SPARKCONTROL v3.1SP1; Zeiss Zen Blue v2.3SP1; Nikon NIS Elements AR v5.21.01; Biorad CFX Manager v3.1
Data analysis	Synthego CRISPR ICE v3.0; FlowJo v10.8.2 and v11; CRISPick; ImageJ/Fiji v2.14.0; Biorad CFX Manager v3.1 ; Comet r2022.01; KNIME Analytics platform v4.6.0; Cellpose v4.0; Prism v10.6.1 ; StarDist v0.7.0; Trackmate v7.12.1; fastp v0.24.0; STAR aligner v2.7.11; samtools v1.22.1.; featureCounts (subread package v2.1.1); DeSeq2 (Galaxy Version 2.11.40.2); edgeR v4.0.16

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data supporting the findings of this study are available within the paper and its Supplementary Information, except for bulk RNA sequencing data, which were deposited to NIH GEO with identifiers GSE321692 and GSE321707.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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 pre-determine sample sizes, but sample sizes are similar to those used in the field. For biochemical and cellular experiments, no information was derived about a population based on sampling, and therefore sample size determination was not necessary. For imaging experiments, >20 fields of view including at least 150 cells (typically >500 cells) were imaged per condition and repeat. For live cell imaging assays 5-10 fields of view including at least 100 cells (typically >250 cells) were imaged per condition and repeat.
Data exclusions	No data were excluded from the analysis.
Replication	All data were replicated independently multiple times (at least two to three times) with consistent results as described in the figure legends. For assays using microtiter plates (ELISA, LDH, Griess, H2DCFDA fluorescence assays) and RT-qPCR assays each repeat usually included 2-4 technical replicates.
Randomization	For assays using microtiter plates (ELISA, LDH, Griess, H2DCFDA fluorescence assays), the position of samples was randomized between repeats to reduce plate/edge effects. For qPCR assays using 384-well plates, the position of samples was randomized between repeats to reduce plate/edge effects. For acquisition of quantitative imaging data, fields of view were randomly selected to minimize observer bias. No other randomization was performed, as this does not apply to the biochemical and cell-based experiments described in this study.
Blinding	Blinding was not performed as subjective analysis was not needed. Each experiment was analyzed using consistent methods. Random allocation and quantitative measurements using various approaches and kits as described in the Methods minimized biased assessments. Image analysis used automated analysis pipelines for quantification to improve consistency and remove observer bias.

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

Methods

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

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

Antibodies

Antibodies used

Primary antibodies (also see Supplementary Table 4-5):
 rat IgG2a, κ PE anti-mouse CD284 (TLR4), 145404, Biolegend; rat IgG2a, κ PE anti-mouse CD282 (TLR2), 148604, Biolegend; rat IgG2b, κ PE anti-mouse CD317 (BST2), 127009, Biolegend; rat IgG2a, κ PE Rat IgG2a, κ; isotype Ctrl, 400508, Biolegend; rat IgG1, κ APC Rat IgG1, κ; Isotype Ctrl, 400412, Biolegend; rat IgG1, κ PE Rat IgG1, κ; Isotype Ctrl, 400408, Biolegend; mouse IgG2a, κ PE Mouse IgG2a, κ; isotype Ctrl, 400212, Biolegend; rat IgG1, κ PE anti-mouse TNF-α, 506306, Biolegend; rat IgG1, κ APC anti-mouse IL-6, 504508, Biolegend; rat anti-β-Actin, 664802, Biolegend; rabbit anti-spCas9, #65832, CST; rabbit anti-pERK1/2 (Thr202/Tyr204), #4370, CST; rabbit anti-ERK1/2, #9102, CST; mouse anti-Flag(M2), F3165, Sigma-Aldrich; rabbit anti-HA, #3724, CST; rabbit anti-pIKKα/β (Ser176/180), #2697, CST; rabbit anti-IKKα, #2682, CST; rabbit anti-IKKβ, #8943, CST; rabbit anti-pIKKε (Ser172), #8766, CST; rabbit anti-IKKe, #3416, CST; rabbit anti-IL-1β, GTX130021, Genetex; rabbit anti-pJNK (Thr183/Tyr185), #9251, CST; rabbit anti-IRAK2, 3595, ProSci; mouse anti-IRAK4, ab119942, abcam; rabbit anti-IκBα, #9242, CST; rabbit anti-Myc-Tag, #2278, CST; goat anti-MyD88 (mouse), AF3109, R&D Systems; rabbit anti-p-p38α (Thr180/Tyr182), #4511, CST; rabbit anti-p38α, #9212, CST; mouse anti-p62, ab56416, abcam; rabbit anti-p-p65 (Ser536), #3033, CST; rabbit anti-p65, #8242, CST; rabbit anti-pSTAT1 (Ser727), #9177, CST; rabbit anti-STAT1, #9172, CST; rabbit anti-pTBK1 (Ser172), #5483, CST; mouse anti-TBK1, #51872, CST; rabbit anti-TIRAP, ab17218, abcam; rabbit anti-TLR4 (mouse), Jonathan Kagan laboratory; mouse anti-TRAF6, 654501, Biolegend; rat anti-TRAM (757706), MAB4348, R&D Systems; rabbit anti-TRAM (TIR-specific), ab17221, abcam; rabbit anti-Viperin, #13996, CST

Secondary antibodies (also see Supplementary Table 6):
 goat anti-mouse IgG (H+L), HRP, 115-035-003, Jackson ImmunoResearch; goat anti-rabbit IgG (H+L), HRP, 111-035-003, Jackson ImmunoResearch; donkey anti-goat IgG (H+L), HRP, 705-035-003, Jackson ImmunoResearch; donkey anti-rat IgG (H+L), HRP, 712-035-150, Jackson ImmunoResearch; donkey anti-rabbit IgG (H+L), Alexa Fluor™ 488, A21206, Invitrogen; goat anti-rat IgG (H+L), Alexa Fluor™ 488, A11006, Invitrogen; goat anti-rabbit IgG (H+L), Alexa Fluor™ 647, A21245, Invitrogen

Validation

All primary antibodies used in this study were previously validated for specificity in immunoblot and immunofluorescence analysis, see <https://doi.org/10.1038/s41586-024-07614-7>.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Parental cell lines: HEK293T (CRL-11268, ATCC), iBMDM (Jonathan Kagan laboratory, Boston Children's Hospital). All other cell lines were derived from these cell as described in this study.

Primary BMDMs were differentiated from bone marrow from tibiae and femurs of C57BL/6J, WT or Ticam2-/- mice (10-week old female, provided by Dr. Liwu Li, Virginia Tech).

Authentication

Cells were not authenticated as they were directly purchased from ATCC. The iBMDM cells were used in previously published, peer-reviewed studies.

Mycoplasma contamination

Cell lines were regularly tested and found negative for Mycoplasma contamination using PCR.

Commonly misidentified lines (See [ICLAC](#) register)

None of the used lines is listed as commonly misidentified.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

This study used 12-week old female C57BL/6N WT mice (Mus musculus) purchased from Envigo and 10-week old female C57BL/6J, WT or Ticam2-/- mice (Li lab, Virginia Tech).

Wild animals

The study did not involve wild animals.

Reporting on sex	All experiments were conducted using female mice only. Sex was not included as an experimental variable; a sex-based analysis was not performed because only one sex was used to minimize variability and maintain consistency across experiments.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal studies were approved by the Boston Children’s Hospital Institutional Animal Care and Use Committee (IACUC).

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A