

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 [Authors & Referees](#) 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

Illumina HiSeq 2500 was used for RNA sequencing;
Novelcyto system was used to capture the transcriptomic information of the single cells;
ACEA NovoCyte flow cytometer was used for flow cytometry.

Data analysis

HISAT2(v2.1.0) was used to map reads to the genome;
NovelBrain Cloud Analysis Platform was used for single-cell RNA sequencing analysis;
Vazyme Cloud Analysis Platform was used for CUT-Tag analysis;
NovoExpress(v1.6.2) was used for analysis of flow cytometry data;
DNBC4tools(v2.1.0) was used to process raw single-cell RNA-Seq data;
Seura R package(v5.0) and DoubletFinder(v2.0.4) were used to perform single-cell RNA-Seq data analysis;
GraphPad Prism 10 was used to perform data and statistics analysis.

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/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 RNA-Seq data reported in this paper have been deposited in the Sequence Read Archive database, <http://www.ncbi.nlm.nih.gov/sra> (accession no. PRJNA1345184).

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	Sample sizes were determined based on pilot data and variance estimates from previous studies, carefully balancing statistical power with ethical and resource considerations. All key experiments included at least three independent biological replicates. Animal group sizes were established in accordance with institutional ethical approvals. For sequencing experiments, sample numbers and sequencing depths adhered to community standards and yielded consistent effect sizes across replicates.
Data exclusions	No data were excluded from this study.
Replication	All key experiments were independently repeated at least three times with consistent results, and representative results are presented in the figures.
Randomization	For cell culture experiments, all cells within each biological replicate were derived from the same cell batch and randomly assigned to treatment groups. In mouse experiments, animals of the same sex were randomly allocated to cages to ensure consistent housing conditions.
Blinding	Investigators were blinded to group allocation during both the experimental procedure and outcome assessments to minimize 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	n/a
☐ ☒ Antibodies	☒ ☐ ChIP-seq
☐ ☒ Eukaryotic cell lines	☐ ☒ Flow cytometry
☒ ☐ Palaeontology	☒ ☐ MRI-based neuroimaging
☐ ☒ Animals and other organisms	
☒ ☐ Human research participants	
☒ ☐ Clinical data	

Antibodies

Antibodies used	anti-IRF7(1:1000,AF6909, Affinity Biosciences), anti-Phospho-IRF(Ser477)(1:1000,AF8486,Cell Signaling Technology), anti-Phospho-IRF7(Ser437/Ser438) (1:500, AF3886, Affinity Biosciences), anti-Phospho-IRF7(Ser471/Ser472) (1:500, AF3885, Affinity Biosciences), anti-TBK1(1:500, DF7026, Affinity Biosciences), anti-Phospho-TBK1(Ser172) (1:500, AF8190, Affinity Biosciences), anti-IKK epsilon (1:500, DF6047,Affinity Biosciences), anti-Phospho-IKK epsilon(Ser172) (1:500, AF8156, Affinity Biosciences), anti-STAT5B(1:500, sc-1656, Santa Cruz), anti-Phospho-STAT5B(Ser731) (1:500, AF3340, Affinity Biosciences), anti-c-MYC(1:2000,2272, Cell Signaling Technology), anti-Flag(1:2000,TB146, Cell Signaling Technology), anti-HA(1:2000,2367,Cell Signaling Technology), anti-GAPDH (1:10000,T2146, Cell Signaling Technology), anti-GST (1:2000. T0007.Affinity Biosciences).
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Validation

anti-IRF7(Human;WB;IHC;CUT-Tag), tested by the manufacturer;
 anti-Phospho-IRF7(Ser477)(Human;WB), tested by the manufacturer;
 anti-Phospho-IRF7(Ser437/Ser438)(Human;WB), tested by the manufacturer;
 anti-Phospho-IRF7(Ser471/Ser472)(Human;WB), tested by the manufacturer;
 anti-TBK1(Human;WB), tested by the manufacturer;
 anti-Phospho-TBK1(Ser172)(Human;WB), tested by the manufacturer;
 anti-IKK epsilon (Human;WB), tested by the manufacturer;
 anti-Phospho-IKK epsilon(Ser172) (Human;WB), tested by the manufacturer;
 anti-STAT5B(Human;WB;IP), tested by the manufacturer;
 anti-Phospho-STAT5B(Ser731)(Human;WB), tested by the manufacturer;
 anti-c-MYC(Human;WB;IHC;IP;CUT-Tag), tested by the manufacturer;
 anti-Flag(Human;WB;IP), tested by the manufacturer;
 anti-HA(Human;WB;IP), tested by the manufacturer;
 anti-GAPDH(Human;WB), tested by the manufacturer;
 anti-GST(Human;WB). tested by the manufacturer.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

HeLa (ATCC,CRM-CCL-2); Jurkat(ATCC,TIB-152); ED and Tlom-1 were provided by Tiejun Zhao Laboratory,mESC-J1 cell line was provided by Xiaomin Liu Laboratory.

Authentication

Cell lines were authenticated by DNA profiling assays (STR).

Mycoplasma contamination

Cell lines were confirmed negative for mycoplasma contamination.

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

NSG mice, male, 4 to 5 weeks, Hangzhou Ziyuan Experimental Animal Technology Co.,Ltd.
 Zebrafish, AB background, male and female, China Zebrafish Resource Center.

Wild animals

No wild animals were used in this study.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All animal studies were performed in compliance with the Guide for the Care and Use of Laboratory Animals by the Medical Experiment Animal Care Commission of China Pharmaceutical University.

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

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

The 5×10^5 cells were washed three times with PBS and resuspended in 100uL of 1X Annexin V binding buffer, supplemented with 2.5uL of Annexin V-FITC and 2.5uL of PI. The cells were incubated at room temperature for 15 minutes in the dark. 400uL of Annexin V Binding Buffer was added, and the cells were immediately analyzed using the NovoCyte Flow Cytometer.

Instrument

ACEA NovoCyte flow cytometer

Software

NovoExpress(v1.6.2)

Cell population abundance

No sorting in this study.

Gating strategy

Initial gating of the cell population (FCS-A vs. FSC-H and SSC-A vs. SSC-H) was performed to exclude doublets and cell aggregates, ensuring that only single cells were included in the analysis. The same gating strategy was consistently applied across all samples analyzed simultaneously.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.