nature portfolio

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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 (*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. 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 *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	Whole exome sequencing and bulk RNA sequencing were performed using the Illumina Novaseq system. Whole genome libraries were constructed and sequenced by Novogene (Beijing, China) on the Illumina NovaSeq 6000 platform with 150 bp paired-end reads. Single-cell RNA sequencing was conducted using the 10x Genomics Chromium platform for single-cell capture and cDNA preparation, followed by sequencing on the Illumina Novaseq system. Luciferase activity was measured using a multimode plate reader (BioTek). Cytometric Bead Array were acquired on CytoFLEX LX flow ctyometer (Beckman Coulter). Flow cytometry data for p-IRF3 were acquired on CytoFLEX LX flow cytometer (Beckman Coulter). Viable single cells were sorted using a Beckman Moflo Astrios EQ sorter. CyTOF data were acquired on a Helios mass cytometer (Fluidigm). Quantitative real-time PCR was performed on ROCHE LightCycler 480II system. Immunoblot images were detected and analyzed with the Odyssey infrared imaging system (LI-COR Biosciences) and the ImageQuant 800 (Cytiva). LC-MS analysis was performed using nanoElute system (Bruker) and timsTOF Pro mass spectrometer (Bruker).
Data analysis	UCSF ChimeraX (version 1.11.0) (protein structure illustration); FlowJo v10.8 Software (flow cytometry); FCAP Array V3 software (Cytometric Bead Array); GraphPad Prism (version 9.5.0) for Mac OS X and Microsoft Excel (graphs, statistics); ANNOVAR (2019Oct24) (variants annotation); R Software (version.4.4.0) (data analysis and graph); GATK4 (CNVs detection pipeline); HISAT2 (reads mapping); DESeq2 R package (version 1.44.0) (bulk RNA sequencing analysis); CellRanger (version 7.1.0) (10× Genomics) and R package Seurat (version 4.4.0) (scRNA-seq analysis); R package CytoTRACE (version 0.3.3) (cellular differentiation status prediction); Slingshot (version 2.12.0), Diffusion Map (Python package scanpy, version 1.10.3) and SCORPIUS (version 1.0.9) (pseudotime inference). R package AUCell (version 1.26.0) (signature score calculation); NolanLab Beads Normalizer package (version 0.3) (CyTOF); MZmine (version 4.9.14) (Mass spectrometry). The code for this paper is available on the GitHub [https://github.com/shuangyuema95-glitch/IRAK2025] and Zenodo [https://doi.org/10.5281/zenodo.17136147].

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

The raw bulk RNA sequencing and scRNA-seq data, together with the raw WES/WGS data generated in this study, have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human) under accession code: HRA018055 [https://ngdc.cncb.ac.cn/gsa-human/browse/HRA018055] and HRA018317 [https://ngdc.cncb.ac.cn/gsa-human/browse/HRA018317], respectively. All other data within the article or the Supplementary Information are available from the corresponding author on request. Source data are provided with this paper.

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	Sex was recorded for all participants. Gender identity was not collected. Sex was determined through participants' self-reports during data collection. The cohort included 4 biological males and 8 biological females (Supplementary Table 1). The human samples used in this study were derived from isolated peripheral blood mononuclear cells (PBMCs).
Reporting on race, ethnicity, or other socially relevant groupings	As all participants in this study were of Asian descent, specifically from China, no individuals from other racial or ethnic backgrounds were included. Therefore, no comparison across different racial or ethnic categories was performed.
Population characteristics	The patients characteristics were described in Supplementary Patient Information and Supplementary Table 1.
Recruitment	Patient samples were obtained from individuals with immune dysregulation disorders who did not have a clear genetic diagnosis. Multiple age- and sex-matched healthy controls were randomly selected for the experiment.
Ethics oversight	All patients enrolled in the study were evaluated under protocols approved by the Institutional Review Boards of the Children's Hospital, Zhejiang University School of Medicine (2021-IRB-0172-Y-02), and Jinling Hospital (2022DZKY-061-01). Written informed consents were obtained from all participants or their legal guardians. This study was conducted in accordance with relevant ethical regulations and guidelines. Given the mechanistic focus of this study, sex and/or gender was not included as variables in the study design or analysis. Routine clinical data were obtained through retrospective chart review.

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

Life sciences study design

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

Sample size	No statistical method was applied to determine the sample size. Sample size was chosen based on previous experience and established protocols. Patient sample size was chosen using all available patients. Mice sample size was chosen by the availability of animals with the correct genotypes or numbers used in previous publications.
Data exclusions	No data were excluded from analysis.
Replication	The number of independent replicates to similar results is indicated in the respective figure legends or the Statistics and Reproducibility section. All replication attempts yielded similar results. For in vitro experiments, each experiment was independently repeated at least three times. For all animal experiments, biological replication was performed with at least five subjects. Bulk RNA sequencing was carried out with two technical replicates per sample. Single-cell RNA sequencing of patients and healthy controls was performed once. CyTOF analysis of patients and healthy controls was also performed once.
Randomization	Randomization was not applicable, as this study did not involve a clinical trial or cohort study and no samples were assigned to experimental groups. For animal experiments, mice were age- and sex-matched within each experiment, with littermates used.
Blinding	Blinding was not applicable for experiments in which results were quantified using automated instruments and no manual assessment was involved. Mouse genotypes were blinded in the liver and renal histology analyses.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

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

Antibodies

Antibodies used

1. Western Blot:

The following antibodies were purchased from Cell Signaling Technology: p-p65 (Ser536) (#3033; 1:1000), p65 (#8242; 1:1000), p-p38 (Thr180/Tyr182) (#4511; 1:1000), p38 (#8690; 1:1000), HA-tag (#3724S; 1:1000), p-p105 (Ser932) (#4806; 1:1000), p105 (#4717; 1:1000), p-JNK (Thr183/Tyr185) (#4668; 1:1000), JNK (#9252; 1:1000), IRF3 (#4302; 1:1000), p-STAT1 (Tyr701) (#9167; 1:1000), STAT1 (#14994; 1:1000), p-STAT2 (Tyr690) (#88410; 1:1000), STAT2 (#72604; 1:1000), β-ACTIN (#4970; 1:1000), p-IKKε (Ser172) (#8766; 1:1000), IKKε (#2905; 1:1000), p-TBK1 (Ser172) (#5483; 1:1000), TBK1 (#3504; 1:1000), TRIF (#16809; 1:1000).

Flag-tag (MA1-91878; 1:3000) was purchased from Sigma-Aldrich.

Myc-tag (60003-2-Ig; 1:3000) and HRP-conjugated GAPDH (HRP-60004; 1:3000) were purchased from Proteintech.

p-IRF3 (Ser386) (AP0995; 1:1000) was purchased from Abclonal.

Myc-tag (S0B0383; 1:3000) was purchased from STARTER.

2. Flow cytometry

p-IRF3 (Ser386) (#37829) was purchased from CST.

Alexa Fluor 647-conjugated anti-rabbit IgG (H+L) was purchased from Thermo Fisher.

Validation	All antibodies have been verified by the manufacturers 1. Western Blot: p-p65 (Ser536): https://www.cellsignal.jp/products/primary-antibodies/phospho-nf-kb-p65-ser536-93h1-rabbit-mab/3033 p65: https://www.cellsignal.jp/products/primary-antibodies/nf-kb-p65-d14e12-xp-rabbit-mab/8242 p-p38 (Thr180/Tyr182): https://www.cellsignal.jp/products/primary-antibodies/phospho-p38-mapk-thr180-tyr182-d3f9-xp-rabbit-mab/4511 p38: https://www.cellsignal.jp/products/primary-antibodies/p38-mapk-d13e1-xp-rabbit-mab/8690 HA-tag: https://www.cellsignal.jp/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724 p-p105 (Ser932): https://www.cellsignal.jp/products/primary-antibodies/phospho-nf-kb-p105-ser932-18e6-rabbit-mab/4806 p105: https://www.cellsignal.jp/products/primary-antibodies/nf-kb1-p105-antibody/4717 p-JNK (Thr183/Tyr185): https://www.cellsignal.jp/products/primary-antibodies/phospho-sapk-jnk-thr183-tyr185-81e11-rabbit-mab/4668 JNK: https://www.cellsignal.jp/products/primary-antibodies/sapk-jnk-antibody/9252 IRF3: https://www.cellsignal.jp/products/primary-antibodies/irf-3-d83b9-rabbit-mab/4302 p-STAT1 (Tyr701): https://www.cellsignal.jp/products/primary-antibodies/phospho-stat1-tyr701-58d6-rabbit-mab/9167 STAT1: https://www.cellsignal.jp/products/primary-antibodies/stat1-d1k9y-rabbit-mab/14994 p-STAT2 (Tyr690): https://www.cellsignal.jp/products/primary-antibodies/phospho-stat2-tyr690-d3p2p-rabbit-mab/88410 STAT2: https://www.cellsignal.jp/products/primary-antibodies/stat2-d9j7l-rabbit-mab/72604 β-ACTIN: https://www.cellsignal.jp/products/primary-antibodies/b-actin-13e5-rabbit-mab/4970 p-IKKε (Ser172): https://www.cellsignal.jp/products/primary-antibodies/phospho-ikke-ser172-d1b7-rabbit-mab/8766 IKKε: https://www.cellsignal.jp/products/primary-antibodies/ikke-d20g4-rabbit-mab/2905 p-TBK1 (Ser172): https://www.cellsignal.jp/products/primary-antibodies/phospho-tbk1-nak-ser172-d52c2-xp-rabbit-mab/5483 TBK1: https://www.cellsignal.jp/products/primary-antibodies/tbk1-nak-d1b4-rabbit-mab/3504 TRIF: https://www.cellsignal.com/products/primary-antibodies/trif-f6k4e-rabbit-monoclonal-antibody/16809 Flag-tag: https://www.thermofisher.com/antibody/product/DYKDDDDK-Tag-Antibody-clone-FG4R-Monoclonal/MA1-91878 Myc-tag: https://www.ptglab.com/products/MYC-Antibody-60003-2-Ig.htm HRP-conjugated GAPDH: https://www.ptglab.com/products/GAPDH-Antibody-HRP-60004.htm p-IRF3 (Ser386): https://www.abclonal.com/catalog-antibodies/PhosphoIRF3S386RabbitmAb/AP0995 Myc-tag: https://www.starter-bio.com/s0b0383_s_rmab_myc_tag_recombinant_rabbit_mab_s_114_13.html 2. Flow cytometry p-IRF3 (Ser386): https://www.cellsignal.jp/products/primary-antibodies/phospho-irf-3-ser386-e7j8g-xp-rabbit-mab/37829 Alexa Fluor 647-conjugated anti-rabbit IgG (H+L): https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T and THP-1 cell lines were from the American Type Culture Collection.
Authentication	Cell lines from ATCC have been authenticated by ATCC.
Mycoplasma contamination	The cell lines were tested and confirmed to be free of mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	Irak2Δex2/Δex2 mice were generated on a C57BL/6JGpt background by GemPharmatech Co.,Ltd. (Nanjing, China) and maintained under specific-pathogen-free (SPF) conditions with a 12-hour light/12-hour dark cycle, controlled ambient temperature (20-26 ℃), and relative humidity (40-70%). The age, sex, generation, and number of mice used in each experiment are indicated in the corresponding figure legends.
Wild animals	The study did not involve any wild animals.
Reporting on sex	For mechanistic and disease-phenotyping experiments (BMDMs stimulation, intraperitoneal LPS challenge, E. coli infection, and lupus-like phenotyping of 50-week-old mice), only female mice were used with age-matched wild-type littermate controls to maximize sensitivity for detecting disease-relevant phenotypes, as lupus-like manifestations are female biased in established models. Both male and female mice were included in LPS-challenge survival analyses.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All experimental protocols involving animals were approved by Zhejiang University's Institutional Animal Care and Use Committee (AP CODE: ZJU20240500).

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

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	EDTA-anticoagulated peripheral whole blood was collected from patients and healthy controls. Peripheral blood mononuclear cells (PBMCs) were isolated using lymphocyte separation medium (LSM) according to the manufacturer's instructions. For detection of phosphorylated protein, freshly isolated PBMCs were permeabilized using Perm Buffer III (558050, BD Biosciences). Cells were then stained with an antibody against p-IRF3 (Ser386) (#37829, CST) followed by Alexa Fluor 647-conjugated anti-rabbit IgG (H+L) (a-31573, ThermoFisher).
Instrument	All events were acquired on CytoFLEX LX Flow Cytometer (Beckman Coulter).
Software	All events were analyzed with FlowJo™ v10.8 Software
Cell population abundance	A total of 1.0E6 PBMCs were used for each assay. Cell viability and counts were determined using a Countess II FL automated cell counter (Thermo Fisher) with 0.4% Trypan Blue. The viability of PBMCs in all samples exceeded 90%.
Gating strategy	Flow cytometry data were analyzed using a standardized gating strategy. Single cells were first identified by forward scatter (FSC) and side scatter (SSC) parameters to exclude debris and doublets. The phosphorylated IRF3 (p-IRF3) was measured in the APC-A channel by CytoFLEX LX and quantified in the total PBMCs population.

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