

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                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                                   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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 | Real-time PCR: QuantStudio 6 Flex system (Thermo Fisher Scientific).<br>Luciferase activity: Luminoskan Ascent luminometer (Thermo Fisher Scientific).<br>Confocal immunofluorescence and PLA images: ZEISS ZEN3.9.<br>Immunoblot: Image Lab 6.0.1.                                                                                                                                                                                                                                                                           |
| Data analysis   | Data analysis and statistical tests were done using Prism (GraphPad Prism 9.4.1) or Excell (Microsoft 2021).<br>Confocal immunofluorescence and PLA images were analyzed with ImageJ (ImageJ 1.8.0).<br>Luciferase activity were analyzed with SkanIt Software 5.0<br>The intensity of immunoblotting bands was quantified by the Image Lab software (Image Lab 6.0.1).<br>Structural analyses were conducted using PyMOL version 4.0.<br>RNA-seq data were analyzed by DESeq2 (Version 1.42.0) and goatools (Version 1.4.4). |

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 RNA-seq datasets generated in this study are available in the NCBI Sequence Read Archive (SRA) under the BioProject accession PRJNA1288125. 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

This study does not involve human participants so no such information is collected.

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

Sample sizes for each experiment are provided in figure legends. For in vitro experiments, three biological replicates were achieved. Such sample sizes are typical for the in vitro experiments and sufficient for a statistical analysis. For in vivo assay, n=6 mice were used. Sample sizes were determined based on published studies in the field or our previous experiences. No statistics was used to predetermine the sample size.

Data exclusions

No data were excluded from analysis.

Replication

For most of the experiments, at least three independent biological experiments were performed. For each experiment, detailed description of number of replicates, sample size and statistics is provided in figure legend. Similar parameters were evaluated by multiple methods.

Randomization

The samples for each experiment were randomized to be examined ( No specific methods were used).

Blinding

The investigator was not blinded. However, the selection and detection of the variables was in most cases automated (e.g. measuring Immunofluorescence, Western blotting and QPCR), to prevent any bias by the investigator.

## 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 &amp; experimental systems

|                                     |                                                                 |
|-------------------------------------|-----------------------------------------------------------------|
| n/a                                 | Involved in the study                                           |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines       |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                                 |

## Methods

|                                     |                                                 |
|-------------------------------------|-------------------------------------------------|
| n/a                                 | Involved in the study                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Antibodies

## Antibodies used

Anti-APT-2 (Cat#ab151578, 1:1000, polyclonal), anti-rabbit immunoglobulin G (IgG) H&L horseradish peroxidase (Cat#ab6721, 1:10000, polyclonal), goat anti-mouse IgG H&L GRP (Cat#ab6789, 1:10000, polyclonal), goat anti-mouse IgG H&L AF594 (Cat#ab150116, 1:200, polyclonal), goat anti-rat IgG H&L AF488 (Cat#ab150165, 1:200, polyclonal) were purchased from Abcam. Anti-phosphor-TBK1/NAK (Ser172) (Cat#5483, 1:1000, clone name D52C2), anti-phosphor-IRF3 (Ser396) (Cat#4947, 1:1000, clone name 4D4G), anti-IRF3 (Cat#11904, 1:1000, clone name D6I4C), anti-hemagglutinin (HA) (Cat#5017, 1:1000, clone name C29F4), anti-MYC (Cat#2276, 1:1000, clone name 9B11), anti-p62 (Cat#88588, 1:1000, clone name D5L7G), anti-K48-Ub (Cat#8081, 1:1000, clone name D9D5), anti-phosphor-STING (Ser365) (Cat#72971, 1:1000, clone name D8F4W) were purchased from Cell Signaling Technology. Anti-NDP52 (Cat#12229-1-AP, 1:1000, polyclonal), anti-TOLLIP (Cat#11315-1-AP, 1:1000, polyclonal), anti-TBK1 (Cat#83686-3-RR, 1:10000, clone name 240769G5), anti-STING (Cat# 19851-1-AP, 1:20000, polyclonal), anti-p65 (Cat#80979-1-RR, 1:1000, clone name 4C7), anti-ZDHHC9 (Cat#82739-1-RR, 1:1000, clone name 9G5) were purchased from Proteintech. Anti-FLAG (Cat#A8592, 1:1000, clone name M2) was purchased from Sigma-Aldrich. Anti-TRIM27 (Cat#823342, 1:1000, polyclonal), anti-GAPDH (Cat#200306-7E4, 1:10000, clone name 7E4-H6-H6), anti-MAVS (Cat#161894, 1:1000, polyclonal), anti-phosphor-p65 (Ser536) (Cat#340830, 1:1000, polyclonal), anti-Histone H3 (Cat#R24572, 1:1000, clone name R06-4I8), anti-GFP (Cat#250065, 1:1000, clone name 6A2-1H8-5G7), anti-K63-Ub (Cat#R381564, 1:1000, clone name R06-2A6), anti-Ub (Cat#R26025, 1:1000, clone name R02-4I6) were purchased from Zen-Bio.

## Validation

All antibodies were validated by the suppliers for the applications in their data sheets or manufacturers' websites that we used in this study, and were checked in our lab by comparing to vendors' results.

APT-2 (1:1000, ab151578, Abcam), <https://www.abcam.cn/products/primary-antibodies/apt-2-antibody-ab151578.html>

anti-rabbit immunoglobulin G (IgG) H&L horseradish peroxidase (1:10000, ab6721, Abcam), <https://www.abcam.cn/products/secondary-antibodies/goat-rabbit-igg-hl-hrp-ab6721.html>

goat anti-mouse IgG H&L GRP (1:10000, ab6789, Abcam), <https://www.abcam.cn/products/secondary-antibodies/goat-mouse-igg-hl-hrp-ab6789.html>

goat anti-mouse IgG H&L AF594 (1:200, ab150116, Abcam), <https://www.abcam.cn/products/secondary-antibodies/goat-mouse-igg-hl-alex-a-fluor-594-ab150116.html>

goat anti-rat IgG H&L AF488 (1:200, ab150165, Abcam), <https://www.abcam.cn/products/secondary-antibodies/goat-rat-igg-hl-alex-a-fluor-488-preadsorbed-ab150165.html>

Phosphor-TBK1/NAK (Ser172) (1:1000, #5483, Cell Signaling Technology, U.S.A), <https://www.cellsignal.cn/products/primary-antibodies/phospho-tbk1-nak-ser172-d52c2-xp-rabbit-mab/5483>

Phosphor-IRF3 (Ser396) (1:1000, #4947, Cell Signaling Technology, U.S.A), <https://www.cellsignal.cn/products/primary-antibodies/phospho-irf-3-ser396-4d4g-rabbit-mab/4947>

IRF3 (1:1000, #11904, Cell Signaling Technology, U.S.A), <https://www.cellsignal.cn/products/primary-antibodies/irf-3-d6i4c-xp-rabbit-mab/11904>

HA (1:1000, #5017, Cell Signaling Technology, U.S.A), <https://www.cellsignal.cn/products/antibody-conjugates/ha-tag-c29f4-rabbit-mab-biotinylated/5017>

MYC (1:1000, #2276, Cell Signaling Technology, U.S.A), <https://www.cellsignal.cn/products/primary-antibodies/myc-tag-9b11-mouse-mab/2276>

p62 (1:1000, #88588, Cell Signaling Technology, U.S.A), <https://www.cellsignal.cn/products/primary-antibodies/sqstm1-p62-d5l7g-mouse-mab/88588>

K48-Ub (1:1000, #8081, Cell Signaling Technology, U.S.A), <https://www.cellsignal.cn/products/primary-antibodies/k48-linkage-specific-polyubiquitin-d9d5-rabbit-mab/8081>

P-STING (1:1000, #72971, Cell Signaling Technology, U.S.A), <https://www.cellsignal.cn/products/primary-antibodies/phospho-sting-ser365-d8f4w-rabbit-mab/72971>

NDP52 (1:1000, 12229-1-AP, Proteintech), <https://www.ptgcn.com/products/CALCOCO2-Antibody-12229-1-AP.htm>

TOLLIP (1:1000, 11315-1-AP, Proteintech), <https://www.ptgcn.com/products/TOLLIP-Antibody-11315-1-AP.htm>

TBK1 (1:10000, 83686-3-RR, Proteintech), <https://www.ptgcn.com/products/TBK1-Antibody-83686-3-RR.htm>

STING (1:20000, 19851-1-AP, Proteintech), <https://www.ptgcn.com/products/TMEM173-Antibody-19851-1-AP.htm>

p65 (1:1000, 80979-1-RR, Proteintech), <https://www.ptgcn.com/products/NF-B-p65-Antibody-80979-1-RR.htm>

ZDHHC9 (1:1000, 82739-1-RR, Proteintech), <https://www.ptgcn.com/products/ZDHHC9-Antibody-82739-1-RR.htm>

FLAG (1:1000, A8592, Sigma-Aldrich), <https://www.sigmaaldrich.cn/CN/zh/product/sigma/a8592>

TRIM27 (1:1000, 823342, Zen-Bio, China), <https://www.zen-bio.cn/goodsDetailproductNo=823342&type=0&sort=0&pageNum=1&pageSize=5>

GAPDH (1:10000, 200306-7E4, Zen-Bio, China), <https://www.zen-bio.cn/goodsDetail?productNo=200306-7E4&type=0&sort=0&pageNum=1&pageSize=5>

MAVS (1:1000, 161894, Zen-Bio, China), <https://www.zen-bio.cn/goodsDetail?>

productNo=161894&type=0&sort=0&pageNum=1&pageSize=5  
 Phosphor-p65 (1:1000, 340830, Zen-Bio, China), <https://www.zen-bio.cn/goodsDetail?productNo=340830&type=0&sort=0&pageNum=1&pageSize=5>  
 Histone H3 (1:1000, R24572, Zen-Bio, China), <https://www.zen-bio.cn/goodsDetail?productNo=R24572&type=0&sort=0&pageNum=1&pageSize=5>  
 GFP (1:1000, 250065, Zen-Bio, China), <https://www.zen-bio.cn/goodsDetail?productNo=250065&type=0&sort=0&pageNum=1&pageSize=5>  
 K63-Ub (1:1000, R381564, Zen-Bio, China), <https://www.zen-bio.cn/goodsDetail?productNo=R381564&type=0&sort=0&pageNum=1&pageSize=5>  
 Ub (1:1000, R26025, Zen-Bio, China), <https://www.zen-bio.cn/goodsDetail?productNo=R26025&type=0&sort=0&pageNum=1&pageSize=5>

## Eukaryotic cell lines

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

|                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|-------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                               | Human embryonic kidney (HEK) 293T (Cat#CRL-321) and murine macrophage RAW 264.7 cells (Cat#TIB-7) were obtained from the American Type Culture Collection (ATCC).<br>Lypla2-KO RAW 264.7、Tbk1-KO RAW 264.7、Mavs-KO RAW 264.7、BECN1-KO HEK293T、ATG5-KO HEK293T、NDP52-KO HEK293T、p62-KO HEK293T、TOLLIP-KO HEK293T、TBK1-KO HEK293T were generated by using the CRISPR/Cas9 system.<br>BMDMs were obtained from the rear-leg bone marrow of two-month-old littermate and female C57BL/6J mice.<br>Mouse PEMs were acquired from ascites of female C57BL/6J mice. |
| Authentication                                                    | Cell lines were verified by manufacturer's website or published papers and Identity of these cell lines were frequently checked by their morphological features.                                                                                                                                                                                                                                                                                                                                                                                             |
| Mycoplasma contamination                                          | All cell lines were tested negative for mycoplasma contamination.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Commonly misidentified lines (See <a href="#">ICLAC</a> register) | No cell lines used in this study were found in the database of commonly misidentified cell lines maintained by ICLAC and NCBI Biosample.                                                                                                                                                                                                                                                                                                                                                                                                                     |

## 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      | All mice were in C57BL6/J (Guangdong Medical Laboratory Animal Center, Guangzhou, Guangdong, China) background. All animal experiments were approved by the Southern Medical University Animal Care and Use Committee (SMUL2019243). All animal experiments were performed in specific pathogen-free levels using 6- to 8-week-old mice. Mice were kept with 50% to 60% humidity and daily cycles of 12 h of light at an ambient temperature of 21°C to 23°C. |
| Wild animals            | No wild animals were used in this study.                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Reporting on sex        | All animals used in this study were female mice. Females were selected because male mice are known to be more resistant to infection with Plasmodium species, exhibiting lower infection rates and highly variable parasitemia compared to females [Duncombe, C.J. et al, Nature Communications, 2023]. The use of female mice ensured the establishment of a robust, consistent, and reproducible infection model, thereby minimizing sex-based variability. |
| Field-collected samples | No field-collected samples were used.                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Ethics oversight        | All of the experiments were conducted in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (China) and were approved by the Southern Medical University Animal Ethics Committee.                                                                                                                                                                                                                              |

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 |