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<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 | Optical density of bacterial suspension: SoftMax Pro 6 software (version 6.5.1)<br>Fuorescence images: Zeiss ZEN blue software (version 3.8), Olympus BX63 CellSens Dimension software (version 1.18)<br>H&E staining: TEKSQRAY SlideScan software (version 1.1.10)<br>Scanning electron microscopy (SEM): Phenom Pro Suite software (version 3.2)<br>Micro-computed tomography (micro-CT): SkyScan1276 software (version 1.1)<br>Flow cytometry: CytExpert software (version 2.5.0. 77)<br>RT-qPCR: QuantStudio Design and Analysis software (version 1.3)<br>Western blot: GelView 6000 Pro software (version 1.0.0.5)<br>In vivo/ex vivo fluorescence imaging: IVIS Spectrum imaging system<br>Transmission electron microscopy (TEM) for cellular ultrastructure: Tecnai G2 20 TWIN (FEI)<br>Transmission electron microscopy (TEM) for nanoparticle morphology: JEM-F200 (JEOL)<br>Seahorse extracellular flux assay data acquisition: Wave software<br>Proteomics data acquisition: Orbitrap Astral mass spectrometer<br>DLS and zeta potential measurement: Zetasizer Nano ZS<br>XRD acquisition: XRDynamic 500 diffractometer<br>FTIR acquisition: Nicolet iS20 spectrophotometer<br>XPS acquisition: Axis Ultra DLD spectrometer |
| Data analysis | Micro-CT analysis: NRecon software (version 1.7.5.0), CTAn software (version 1.15.4.0), DataViewer software (version 1.5.6.2), |

CTvox software (version 3.3.0)
Flow cytometry analysis: FlowJo software (version 10.4), Java software (version 1.8.0)
RNA-sequencing data analysis: DESeq2 package (version 1.16.1), clusterProfiler package (version 4.8.1), ggplot2 package (version 4.3.0), VennDiagram package (version 1.6.20), GseaVis package (version 0.0.8), enrichplot package (version 1.20.0)
Proteomics (DIA) raw data processing: DIA-NN software (version 1.8.1)
Proteomics downstream bioinformatics/statistics: R software (version 4.3.1)
PPI network analysis/visualization: STRING database; Cytoscape software (version 3.9.1)
Online visualization: OmicShare Tools (GENE DENOVO, online)
Image analysis: Image J (FIJI)
Data representation and Statistical analysis: GraphPad Prism software (version 9.4.0)

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

Previously published RNA-sequencing data analyzed in this study are publicly available in the NCBI Gene Expression Omnibus under accession number GSE227521 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE227521], GSE166522[https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166522], and GSE102444[https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102444]. The mass spectrometry proteomics data generated in this study have been deposited to the ProteomeXchange Consortium [https://proteomecentral.proteomexchange.org/?pxid=PXD076182] via the iProx partner repository with the dataset identifier PXD076182. All remaining data generated in this study are provided in the Source Data files and Supplementary Information. Source data are provided with this paper. No access restrictions apply.

## 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

# Life sciences study design

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

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| Sample size | No specific statistical tests were performed to predetermine the sample size. Sample size was chosen based on our experience and common practice in previous experiments (Bonilla et al. Science 2012; Winkler et al. Nature Immunology 2019). Sample size for each independent experiment is indicated in the figure legend. |
| Data exclusions | No data was excluded from analysis. |
| Replication | All experiments were performed at least 3 independent experiments. The sample size and number of independent experiments are indicated in the figure legend. The experimental findings were reliably reproduced. |
| Randomization | All mice were randomly assigned to each group after establishment of model. |

| | |
|---|---|
| Blinding | Blinding was not relevant for this study, because subjective measurement was not involved. All data were quantitative. |

# 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 |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

## Methods

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

## Antibodies

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|---|---|
| Antibodies used | Rabbit anti-S. aureus antibody — Abcam — Ab20920 — 1:100<br>Rat anti-F4/80 antibody — BIO-RAD — MCA497GA — 1:100<br>Rabbit anti-Sirt3 antibody — ABclonal — A5419 — 1:100<br>Rabbit anti-PINK1 antibody — Proteintech — 23274-1-AP — 1:100<br>Goat anti-rat IgG Dylight 594 — Abbkine — A23440 — 1:500<br>Goat anti-rabbit IgG Dylight 488 — Abbkine — A23220 — 1:500<br>DAPI — BBI Life Science — E607303-0002 — 1:100<br>Rabbit anti-S. aureus antibody — Abcam — Ab20920 — 1:100<br>Rabbit anti-Sirt3 antibody — ABclonal — A5419 — 1:100<br>Anti-rabbit IgG Dylight 594 — Abbkine — A23420 — 1:500<br>Goat anti-rabbit IgG Dylight 488 — Abbkine — A23220 — 1:500<br>Rabbit anti-Pink1 antibody — Proteintech — 23274-1-AP — 1:500<br>Rabbit anti-Parkin antibody — Proteintech — 14060-1-AP — 1:1000<br>Rabbit anti-phospho-Parkin antibody — Bioss — bs-19882R — 1:500<br>Rabbit anti-LC3B antibody — Affinity Biosciences — AF5384 — 1:500<br>Rabbit anti-p62 antibody — Proteintech — 18420-1-AP — 1:500<br>Rabbit anti-Sirt3 antibody — ABclonal — A5419 — 1:500<br>Mouse anti-beta actin antibody — Proteintech — 66009-1-Ig — 1:5000<br>Rabbit anti-HA-tag antibody — Proteintech — 51064-2-AP — 1:5000<br>Anti-CD16/32 (Fc block) — BioLegend — 101320 — 1:50<br>Anti-CD11b (BV605) — BioLegend — 101257 — 1:80<br>Anti-F4/80 (APC-Cy7) — BioLegend — 157315 — 1:80<br>Live/dead viability dye (eFluor 450) — Thermo — L34963 — 1:100<br>Anti-CD11b (PerCp/Cy5.5) — Elabscience — E-AB-F1081f — 1:20<br>Anti-Ly6G (APC) — Elabscience — E-AB-F1108E — 1:20<br>Anti-F4/80 (APC) — BioLegend — 123115 — 1:80<br>Anti-CD3 (PerCp/Cy5.5) — Elabscience — E-AB-F1013J — 1:20<br>Anti-CD4 (PE) — Elabscience — E-AB-F1097D — 1:20<br>Anti-CD8 (APC) — Elabscience — AN00576E — 1:20<br>Live/dead viability dye (Zombie Yellow) — BioLegend — 423103 — 1:100 |
| Validation | All antibodies used are well-established clones, commercially available, and validated by both the manufacturer and peer-reviewed literature. Detailed validation data for each antibody are available on the respective manufacturer's websites. |

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | RAW264.7 mouse macrophage cell line (serial: SCSP-5036, Cell Bank of the Chinese Academy of Sciences ,Shanghai, China) |
| Authentication | The cell line was obtained directly from commercial vendor but was not independently authenticated in our laboratory. Cell morphology was routinely monitored throughout the experiment. |

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| Mycoplasma contamination | The cell line was tested negative for Mycoplasma contamination before usage. |
| Commonly misidentified lines (See ICLAC register) | Not listed in ICLAC. |

## Animals and other research organisms

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

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| Laboratory animals | The study did not involve laboratory animals |
| Wild animals | Wild-type mice with a C57BL/6 genetic background were obtained from the Experimental Animal Center of Southern Medical University. For the S. aureus osteomyelitis model, we used 10-12-week-old mice. Bone marrow stromal cells were isolated from 4-5-week-old mice and differentiated into preadipocytes, while bone marrow-derived macrophages were prepared from 6-8-week-old mice. |
| Reporting on sex | Findings apply to both sexes. |
| Field-collected samples | This study did not involve field-sample collections. |
| Ethics oversight | All animal experiments and procedures were conducted in accordance with the ARRIVE Guidelines 2.0 and approved by the Animal Welfare and Use Committee of Nanfang Hospital, Southern Medical University. |

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 | Flow cytometry sample preparation (mitophagy detection in SIOM model)<br>Bone marrow cells were isolated from femurs (as described above). Red blood cells were lysed using ACK lysis buffer on ice, and the remaining cells were resuspended in 0.1% BSA-PBS and adjusted to $1 \times 10^6$ cells per 100 µL. Fc receptors were blocked with anti-CD16/32. Cells were then stained with fluorophore-conjugated surface antibodies (anti-CD11b and anti-F4/80) together with a live/dead viability dye (eFluor 450). For mitophagy detection, cells were further incubated with the Mitophagy Detection Kit (PerCP-Cy5.5) combined with a Lysosome Tracker (FITC) at 37 °C for 30 min in the dark. After washing, cells were resuspended in PBS for flow cytometric acquisition.<br>Flow cytometry sample preparation (immune cell composition in infected femoral cavity)<br>To characterize the immune microenvironment in the infected medullary cavity, bone marrow cells were isolated from femurs as described above, followed by red blood cell lysis and Fc blocking. Cells were stained with fluorophore-conjugated antibodies against CD11b, Ly6G, F4/80, CD3, CD4 and CD8 to identify myeloid and lymphoid populations. A live/dead viability dye (Zombie Yellow) was included to exclude dead cells. After staining and washing, cells were subjected to flow cytometric acquisition. |

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| Instrument | CytoFLEX Flow Cytometer (Beckman Coulter) |
| Software | CytExpert software (version 2.5.0.77), FlowJo software (version 10.4), Java software (version 1.8.0) |
| Cell population abundance | The abundance of cell populations is indicated in representative FACS plots |
| Gating strategy | Cells were gated using FSC-H vs. SSC-H to identify events corresponding to lymphocytes. Doublets were excluded by gating on single cells using FSC-H vs. FSC-A. Living cells were selected by negativity for the viability dye (Zombie Aqua dye). Subsequent gatings were established according to the bimodal expression patterns of surface markers or intracellular antigens. The complete gating strategies are presented in Supplementary Figures along with their corresponding legends. Positive populations were defined using unstained and single-stained controls, with a minimum of 20000 cells analyzed per sample |

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