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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics

For	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			
	X	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement		
	X	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly		
	x	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		
	X	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		
X		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated		
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.		

Software and code

 Policy information about availability of computer code

 Data collection
 Flow cytometry data: BD LSRFortessa™. Real-Time PCR data: Roche LightCycler 480 II. Confocal imaging data: Leica TCS SP8 MP; Opera Phenix High content analysis system. Transmission electron microscopy data: Hitachi HT-7800 transmission electron microscopy. Calcium and ROS measurement data: SpectraMax M5 microplate reader. FRET measurement data: EnVision 2105 Multi-label microplate inspection system. RNA sequencing data: Illumina NovaSeq6000. Mass Spectrometry data: Thermo ScientificTM Q Exactive Plus. Metabolomic data: Agilent 1290 Infinity.

 Data analysis
 GraphPad Prism 8.2.1

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.

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. 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 are included in the Supplementary Information or available from the authors, as are unique reagents used in this Article. The raw numbers for charts and graphs are available in the Source Data file whenever possible. The mass spectrometry proteomics data genetated in this study have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository under accession code PXD056047.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race</u>, <u>ethnicity and racism</u>.

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.

x 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	For experiments involving quantification of puncta, and numbers of RFP-L. monocytogenes in cells, n=3 was chosen as the minimal replicate number, and sample size was determined by the number positive cells or puncta within the replicates. We determined this to be sufficient owing to internal control (specific staining of positionally defined cell types using known markers) and low observed variability between stained samples. For experiments involving animals, sample size was determined to obtain a significant difference between groups, with a power of 80%, when the statistical significance was set at less than a p value of 0.05. The sample size chosen withstands the standard deviation within groups to give us information about the true mean of the group and takes into account expected attrition (due to death of animals) based on our previous studies. All experiments were started with 6 animals per group to account for the expected attrition.
Data exclusions	No data were excluded.
Replication	All the reported experiments were reproducible. Data reproducibility was confirmed by more than three independent experiments.
Randomization	WT, Pdcd6-deficient, Rubcn-deficient, Cybb-deficient, Pdcd6 and Rubcn double-deficient, and Pdcd6 and Cybb double-deficient mice were randomized into different groups, matched for gender and similar body weight.
Blinding	The studies were not blinded, as they did not involve subjective measurements. All samples were processed uniformly using standard, and in certain cases, automated procedures (e.g., flow cytometry, mass spectrometry), which are intended to minimize any potential bias in the outcomes.

Behavioural & social sciences study design

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

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

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

Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.
Research sample	Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.
Did the study involve field	d work? Yes No

April 2023

Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
Access & import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

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	n/a Involved in the study	
Antibodies	ChIP-seq	
Eukaryotic cell lines	Flow cytometry	
🗶 🗌 Palaeontology and archaeology	MRI-based neuroimaging	
Animals and other organisms		
🗶 🗌 Clinical data		
🗶 🔲 Dual use research of concern		
X Plants		
•		

Antibodies

Antibodies used

Rabbit polyclonal anti-phospho-p40phox (Thr154) Cell Signaling Technology Cat# 4311; 1:1000 IB Rabbit monoclonal anti-phospho-AMPKα (Thr172) Cell Signaling Technology Cat# 2535S; 1:1000 IB Rabbit monoclonal anti-AMPKa (D63G4) Cell Signaling Technology Cat# 5832S; 1:1000 IB Rabbit monoclonal anti-phospho-S6 Ribosomal Protein (Ser235/236) Cell Signaling Technology Cat# 4856; 1:1000 IB Rabbit monoclonal anti-S6 Ribosomal Protein Cell Signaling Technology Cat# 2217; 1:1000 IB Rabbit monoclonal anti-phospho-p70 S6 Kinase (Thr389) Cell Signaling Technology Cat# 9234; 1:1000 IB Rabbit polyclonal anti-p70 S6 Kinase Cell Signaling Technology Cat# 9202; 1:1000 IB Rabbit monoclonal anti-Atg5 Cell Signaling Technology Cat# 12994; 1:1000 IB Rabbit polyclonal anti-Beclin-1 Cell Signaling Technology Cat# 3738; 1:1000 IB Rabbit monoclonal anti-Atg7 Cell Signaling Technology Cat# 8558; 1:1000 IB Rabbit monoclonal anti-Atg16L1 Cell Signaling Technology Cat# 8089; 1:1000 IB Rabbit polyclonal anti-LC3B Cell Signaling Technology Cat# 2775; 1:1000 IB Rabbit polyclonal anti-SQSTM1/p62 Cell Signaling Technology Cat# 5114; 1:1000 IB Rabbit monoclonal anti-PI3K (D9A5) (4263) Cell Signaling Technology Cat# 4263; 1:1000 IB Rabbit monoclonal anti-Rubicon Cell Signaling Technology Cat# 8465; 1:1000 IB Rabbit monoclonal anti-UVRAG (13115) Cell Signaling Technology Cat# 13115; 1:1000 IB Rabbit monoclonal anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Cell Signaling Technology Cat# 4370; 1:1000 IB Rabbit monoclonal anti-p44/42 MAPK (Erk1/2) Cell Signaling Technology Cat# 4695; 1:1000 IB Rabbit monoclonal anti-phospho-JNK (Thr183/Tyr185) Cell Signaling Technology Cat# 4668; 1:1000 IB Rabbit monoclonal anti-JNK2 Cell Signaling Technology Cat# 9258; 1:1000 IB Rabbit polyclonal anti-phospho-p38 MAPK (Thr180/Tyr182) Cell Signaling Technology Cat# 9211; 1:1000 IB Rabbit monoclonal anti-p38 MAPK Cell Signaling Technology Cat# 8690; 1:1000 IB Rabbit monoclonal anti-phospho-IKK α/β (Ser176/180) Cell Signaling Technology Cat# 2697; 1:1000 IB Rabbit polyclonal anti-IKKβ Cell Signaling Technology Cat# 2678; 1:1000 IB Rabbit monoclonal anti-phospho-NF-KB p65 (Ser536) Cell Signaling Technology Cat# 3033; 1:1000 IB Rabbit monoclonal anti-NF-KB p65 Cell Signaling Technology Cat# 8242; 1:1000 IB Rabbit monoclonal anti-phospho-IκBα (Ser32) Cell Signaling Technology Cat# 2859; 1:1000 IB Rabbit polyclonal anti-IκBα Cell Signaling Technology Cat# 9242; 1:1000 IB Rabbit polyclonal anti-phospho-ULK1 (Ser757) Cell Signaling Technology Cat# 6888; 1:1000 IB Rabbit polyclonal anti-ULK1 (R600) Cell Signaling Technology Cat# 4773; 1:1000 IB Mouse monoclonal anti-Caspase-1 (p20) Adipogen Cat# AG-20B-0042; 1:2000 IB Mouse monoclonal anti-LC3 (M152-3) MBL Cat# M152-3; 1:200 IF

isotypic antibodies. The information about host specificity, reactivity and applications are freely available on the

vendors web under the indicated catalog number of each antibody.

Eukaryotic cell lines

Validation

Policy information about <u>cell lines and Sex and Gender in Research</u>			
Cell line source(s)	THP-1 cells, L929 cells and HEK293T cells were obtained from American Type Culture Collection (ATCC).		
Authentication	Cell lines were purchased from ATCC and no other authentication method was performed.		
Mycoplasma contamination	All cell lines were tested for mycoplasma contamination and were not contaminated.		
Commonly misidentified lines (See <u>ICLAC</u> register)	No commonly misidentified cell lines were used.		

Palaeontology and Archaeology

Specimen provenance	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.	
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.	
Dating methods	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.	
Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.		
Ethics oversight	Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.	

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

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 used for this study were on a C57BL/6J background. All mice were maintained at the standard temperature of 22 °C. The experimental mice were bred in house in standard specific pathogen-free animal living conditions of relative humidity (60% ± 10%) and photoperiod (12h light/dark cycle). Animals were fed on a standard pelleted diet, and sterilized water was provided ad libitum.

Wild animals	No wild animals were used in the study.
Reporting on sex	No sex- or gender-based analyses were performed. In each group of animal experiments, both female and male animals were randomly selected, and sex did not influence the experimental outcomes.
Field-collected samples	No field-collected samples were employed in this study.
Ethics oversight	All mice were housed in SPF facilities and all in vivo experiments were conducted in accordance with the National Institute of
	Health Guide for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee (IACUC). The
	study was approved by the Ethics Committee of Shandong Normal University, and all procedures were conducted in accordance
	with the experimental animal guidelines of Shandong Normal University.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.		
Clinical trial registration	Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.	
Study protocol	Note where the full trial protocol can be accessed OR if not available, explain why.	
Data collection	Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.	
Outcomes	Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.	

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
	Public health
	National security
	Crops and/or livestock
	Ecosystems
	Any other significant area

Experiments of concern

L

Does the work involve any of these experiments of concern:

No	Yes
	Demonstrate how to render a vaccine ineffective
	Confer resistance to therapeutically useful antibiotics or antiviral agents
	Enhance the virulence of a pathogen or render a nonpathogen virulent
	Increase transmissibility of a pathogen
	Alter the host range of a pathogen
	Enable evasion of diagnostic/detection modalities
	Enable the weaponization of a biological agent or toxin
	Any other potentially harmful combination of experiments and agents

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Plants

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

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO.

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.
Files in database submission	Provide a list of all files available in the database submission.
Genome browser session (e.g. <u>UCSC</u>)	Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

x The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

x 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).

X All plots are contour plots with outliers or pseudocolor plots.

X A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Macrophages single cell suspensions were perfused with PBS.
Instrument	BD LSRFortessa™ was used for flow cytometry data collection.
Software	FlowJo (v10) was used for flow cytometric data analysis.

Gating strategy

Cell populations were sorted to >95% purity as determined by flow cytometry.

Live cells were gated based on forward scatter area (FSC-A) and side scatter area (SSC-A), establishing the P1 gate. A subset of P1 gates was identified using FITC-A, with subset P2 serving as the blank control gate and subset P3 representing the positive signal gate.

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

Magnetic resonance imaging

Experimental design		
Design type	Indicate task or resting state; event-related or block design.	
Design specifications	Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.	
Behavioral performance measure	S State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).	
Acquisition		
Imaging type(s)	Specify: functional, structural, diffusion, perfusion.	
Field strength	Specify in Tesla	
Sequence & imaging parameters	Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.	
Area of acquisition	State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.	
Diffusion MRI Used	□ Not used	
Preprocessing		
Preprocessing software	Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).	
Normalization	If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.	
Normalization template	Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.	
Noise and artifact removal	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).	
Volume censoring	Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.	
Statistical modeling & inferer	nce	
Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).	
Effect(s) tested	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.	
Specify type of analysis: 🗌 Wh	iole brain 🗌 ROI-based 🔲 Both	
Statistic type for inference	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.	
(See <u>Eklund et al. 2016</u>)		
Correction	Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).	

Models & analysis

n/a Involved in the study Image: State of the study Functional and/or effective connectivity Image: State of the study Graph analysis Image: State of the study Multivariate modeling or predictive analysis				
Functional and/or effective connectivity	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).			
Graph analysis	Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).			
Multivariate modeling and predictive analysis	Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.			