# 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>.

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For a	II statistical an	alyses, 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 stateme	nt on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	The statist  Only comm	ical test(s) used AND whether they are one- or two-sided on tests should be described solely by name; describe more complex techniques in the Methods section.
$\boxtimes$	A descripti	on of all covariates tested
	🔀 A descripti	on of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full desc AND variat	ription of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) cion (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hy  Give P value	pothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted as as exact values whenever suitable.
$\boxtimes$	For Bayesi	an analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$	For hierard	chical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$	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.
Sof	tware and	d code
Policy information about <u>availability of computer code</u>		
Da	ta collection	MestRec 4996, Living Image(64-bit), Accuri C6 Plus Software v1.0.23.1, LAS X, NIS-Elements AR 4.51.00

Data analysis FlowJo.v10.9.0, Graphpad Prism 10, LAS X, OriginPro 9.1 64Bit, Excel, Image J

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 authors declare that the data supporting the findings of this study are available in the article and its Supplementary Information and Source data files. All devices and reagents were commercially available and are described in the Methods section.

Non-participation

Randomization

# nature portfolio | reporting summary

# Ecological, evolutionary & environmental sciences study design All studies must disclose on these points even when the disclosure is negative. Study description Research sample Sampling strategy Data collection Timing and spatial scale Data exclusions Reproducibility Randomization Blinding Did the study involve field work? Field work, collection and transport Field conditions Location Access & import/export Disturbance 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 Involved in the study Involved in the study Mntibodies ChIP-seq Eukaryotic cell lines | Flow cytometry Palaeontology and archaeology MRI-based neuroimaging Animals and other organisms Clinical data Dual use research of concern Plants **Antibodies** Antibodies used The following antibodies were used for flow cytometry. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.

PE-anti-mouse-CD11c antibody, Biolegend, cat.no. 117308, Clone: N418, 1:400 dilution.

PE-anti-mouse-CD3 antibody, Biolegend, cat. no. 100205, Clone: 17A2, 1:400 dilution.

APC-anti-mouse-CD8a antibody, Biolegend, cat. no. 100712, Clone: 53-6.7, 1:400 dilution.

APC-anti-mouse-CD86 antibody, Biolegend, cat. no. 105012, Clone: GL-1, 1:400 dilution.

PerCP/Cyanine5.5 anti-mouse-CD4 antibody, Biolegend, cat. no. 100434, Clone: GK1.5, 1:400 dilution.

PerCP/Cyanine5.5 anti-mouse-CD80 antibody, Biolegend, cat. no. 104722, Clone: 16-10A1, 1:400 dilution.

FITC-anti-mouse-CD206 antibody, Biolegend, cat. no. 141704, Clone: C068C2, 1:400 dilution.

PE-anti-mouse-F4/80 antibody, Biolegend, cat. no. 111704, Clone: W20065D, 1:400 dilution.

FITC-anti-mouse-CD279(PD-1) antibody, Biolegend, cat. no. 135214, Clone: 29F.1A12, 1:400 dilution.

FITC-anti-mouse-CD47 antibody, Biolegend, cat. no. 127504, Clone: miap301, 1:400 dilution.

Alexa Fluor-anti-mouse-CD45 antibody, Biolegend, cat. no. 103127, Clone: 30-F11, 1:400 dilution.

FITC-anti-mouse-CD25 antibody, Proteintech, cat.no. FITC-65137, Clone: PC61.5, 1:400 dilution.

 $APC\text{-anti-mouse-Foxp3} \ antibody, \ Proteintech, \ cat. \ no. \ APC\text{-}65089, \ Clone: \ 3G3, \ 1:400 \ dilution.$ 

APC-anti-mouse-CD11b antibody, Cell Signaling Technology, cat. no. 41249S, 1:400 dilution.

Alexa Fluor 488-anti-mouse-Calreticulin antibody, Abcam, cat. no. ab196158, 1:400 dilution.

FITC-Anti-CD8(Mouse)mAb antibody, MBL, K0227-4, Clone: KT15.

PE-Tetramer-SIINFEKL antibody, MBL, TS-5001-1C.

The following antibodies were used for immunofluorescence. They are listed as antigen first, followed by supplier, catalog number and clone/ lot number as applicable.

Alexa Fluor 488-anti-mouse-Calreticulin antibody, Abcam, cat. no. ab196158, 1:200 dilution.

Alexa Fluor 488-anti-mouse-HMGB1 antibody, Biolegend, cat. no. 651410.Clone: 3E8, 1:200 dilution.

The following antibodies were used forimmunohistochemistry. They are listed as antigen first, followed by supplier, catalog number and clone/ lot number as applicable.

Rabbit Anti-CD8 antibody, Bioss, cat. no. bs-0648R, 1:500 dilution.

Rabbit Anti-CD4 antibody, bioss, cat. no. bs-52469R, 1:500 dilution.

CD3 antibody, Santa Cruz Biotechnology, cat. no. sc-18843, 1:500 dilution.

HMGB1 antibody, Abmart, cat. no. T55060F, 1:500 dilution.

Rabbit Anti-Foxp3 antibody, Bioss, cat. no. bs-10211R, 1:500 dilution.

The following antibodies were used for western blot. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.

Anti-DDIT3 antibody, abcam, cat.no. ab11419, 1:1000 dilution.

Anti-MLKL antibody, abcam, cat.no.ab183770, 1:1000 dilution.

 $Anti-p-PERK\ antibody,\ Cell\ Signaling\ Technology,\ cat.\#3179S,\ 1:1000\ dilution.$ 

Anti-p-elf2α antibody, Cell Signaling Technology, cat.#9721S, 1:1000 dilution.

Anti-ATF4 antibody, Santa Cruz Biotech, cat.no.sc-390063, 1:1000 dilution.

Anti-cleaved caspase-1 antibody, Affinity, cat.no. Ala317, 1:1000 dilution. anti-N-GSDMD antibody, Biolegend, cat.no. 939701, 1:1000 dilution.

The following antibodies were used for in vivo. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.

Purified anti-mouse-CD47 antibody, Biolegend, cat. no.127501. Clone: miap301.

Purified anti-mouse-CD279(PD-1) antibody, Biolegend, cat. no.114101. Clone: RMP1-14.

### Validation

The species and application of the following antibodies used for flow cytometry were validated by the manufacturer. PE-anti-mouse-CD11c antibody, Biolegend, https://www.biolegend.com/en-us/products/pe-anti-mouse-cd11c-antibody-1816 PE-anti-mouse-CD3 antibody, Biolegend, https://www.biolegend.com/en-us/products/pe-anti-mouse-cd3-antibody-47 APC-anti-mouse-CD8a antibody, Biolegend, https://www.biolegend.com/en-us/products/apc-anti-mouse-cd8a-antibody-150 APC-anti-mouse-CD86 antibody, Biolegend, https://www.biolegend.com/en-us/search-results?Keywords=APC+anti-mouse+CD86 PerCP/Cyanine5.5 anti-mouse-CD4 antibody, Biolegend, https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd4-antibody-4220

PerCP/Cyanine5.5 anti-mouse-CD80 antibody, Biolegend, https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd80-antibody-4275

FITC-anti-mouse-CD206 antibody, Biolegend, https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd206-mmr-antibody-7318

PE-anti-mouse-F4/80 antibody, Biolegend, https://www.biolegend.com/en-us/products/pe-anti-mouse-f4-80-antibody-22815 FITC-anti-mouse-CD279(PD-1) antibody, Biolegend, https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd279-pd-1-antibody-7004

FITC-anti-mouse-CD47 antibody, Biolegend, https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd47-antibody-4753 Alexa Fluor-anti-mouse-CD45 antibody, Biolegend, https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd45-antibody-3407

FITC-anti-mouse-CD25 antibody, Proteintech, https://www.ptgcn.com/products/CD25-Antibody-FITC-65137.htm
APC-anti-mouse-Foxp3 antibody, Proteintech, https://www.ptgcn.com/products/Foxp3-Antibody-APC-65089.htm
APC-anti-mouse-CD11b antibody, Cell Signaling Technology, https://www.cellsignal.cn/products/antibody-conjugates/cd11b-itgam-m1-70-rat-mab-apc-conjugate/41249

Alexa Fluor 488-anti-mouse-Calreticulin antibody, Abcam, https://www.abcam.cn/products/primary-antibodies/alexa-fluor-488-calreticulin-antibody-epr3924-er-marker-ab196158.html

The species and application of the following antibodies used for immunofluorescence staining were validated by the manufacturer. Alexa Fluor 488-anti-mouse-Calreticulin antibody, Abcam, https://www.abcam.cn/products/primary-antibodies/alexa-fluor-488-calreticulin-antibody-epr3924-er-marker-ab196158.html

 $A lexa Fluor\ 488-anti-mouse-HMGB1\ antibody,\ Biolegend,\ https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-hmgb1-antibody-12706$ 

The species and application of the following antibodies used for immunohistochemistry assay were validated by the manufacturer. Rabbit Anti-CD8 antibody, Bioss, http://www.bioss.com.cn/prolook\_03.asp?id=AF08169606001059&pro37=1

Rabbit Anti-CD4 antibody, bioss, http://www.bioss.com.cn/prolook\_03.asp?id=Al10230950273742&pro37=1 CD3 antibody, Santa Cruz Biotechnology, https://www.scbt.com/zh/p/cd3-antibody-17a2?requestFrom=search HMGB1 antibody, Abmart, http://www.ab-mart.com.cn/page.aspx?node=%2077%20&id=%201363 Rabbit Anti-Foxp3 antibody, Bioss, http://www.bioss.com.cn/prolook\_03.asp?id=AF08169606017707&pro37=1

The species and application of the following antibodies used for western blot was validated by the manufacturer. Anti-DDIT3 antibody, abcam, https://www.abcam.cn/products/primary-antibodies/ddit3-antibody-9c8-ab11419.html

The species and application of the following antibodies used for in vivo were validated by the manufacturer. Purified anti-mouse-CD47 antibody, Biolegend, https://www.biolegend.com/en-us/products/purified-anti-mouse-cd47-antibody-4752

# Eukaryotic cell lines

Reporting on sex

Ethics oversight

Field-collected samples

Policy information about <u>ce</u>	Il lines and Sex and Gender in Research		
Cell line source(s)	B16F10, RAW264.7, DC, LLC, Pan02, U87-MG, 4T1, MC38 cells were obtained from American Type Culture Collection (ATCC).		
Authentication	These cell lines were authenticated by Wuhan Pricella Biotechnology (Wuhan, China) using STR analysis. Specifically, 20 STR loci were amplified using Microreader 21 ID System. The PCR products were detected by GenReader 7010, and the results were analyzed by GeneMapper Software6 and compared with ExPASy database.		
Mycoplasma contaminati	All cell lines were tested negative by using Myco-Lumi Luminescent Mycoplasma Detection Kit for mycoplasma contamination.		
Commonly misidentified l (See <u>ICLAC</u> register)	ines No misidentified cell lines used in the study.		
Palaeontology and	d Archaeology		
Specimen provenance			
Specimen deposition			
Dating methods			
Tick this box to confirm	n that the raw and calibrated dates are available in the paper or in Supplementary Information.		
Ethics oversight			
Note that full information on th	ne approval of the study protocol must also be provided in the manuscript.		
Animals and othe	r research organisms		
Policy information about <u>studies involving animals</u> ; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in</u> Research			
Laboratory animals	C57BL/6J mice (Six-eight-week-old, female) were purchased from SPF (Beijing) Biotechnology Co, and maintained at the animal facility of ZhengZhou University in a pathogen-free facility in a standard environmentally controlled room with 30% to 70% humidity and 22°C temperature under a 12 h light-dark cycle. Standard water and diet were offered for the mice.		
Wild animals	N/A		

This research complies with all relevant ethical regulations. All animal experiments were executed according to the Institute of Drug

Discovery & Development of Zhengzhou University (syxk (yu) 2018-0004) approved protocol.

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

No field collected samples were used in the study.

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Authentication

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 publicat	ion.			
Files in database submission				
Genome browser session (e.g. <u>UCSC</u> )				
Methodology				
Replicates				
Sequencing depth				
Antibodies				
Peak calling parameters				
Data quality				
Software				
Flow Cytometry				
Plots				
Confirm that:				
The axis labels state the	marker and fluorochrome used (e.g. CD4-FITC).			
The axis scales are clear	ly 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 nu	umber of cells or percentage (with statistics) is provided.			
Methodology				
Sample preparation	For cell samples, the specific experimental methods and procedures are given in the article For tissue sample, the tissue was first mechanically disrupted from mice and divided into small pieces and homogenized in cold staining buffer to form single cell suspensions in the presence of digestive enzyme.			
Instrument	Flow cytometer (BD Accuri C6 Plus, USA)			
Software	Data was analyzed by FlowJo 10.9.0			
Cell population abundance	No sorting was performed.			
Gating strategy	Generally, cells were first gated on FSC/SSC. Singlet cells were usually gated using FSC-H and FSC-A. Surface antigen gating was performed on the live cell population.			
Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.				
Magnetic resonanc	e imaging			
Experimental design				
Design type				
Design specifications				

Behavioral performance measures

Acquisition
Imaging type(s)
Field strength
Sequence & imaging parameters
Area of acquisition
Diffusion MRI Used Not used
Preprocessing
Preprocessing software
Normalization
Normalization template
Noise and artifact removal
Volume censoring
Statistical modeling & inference
Model type and settings
Effect(s) tested
Specify type of analysis: Whole brain ROI-based Both
Statistic type for inference
(See Eklund et al. 2016)
Correction
Models & analysis
n/a Involved in the study
Functional and/or effective connectivity  Graph analysis
Multivariate modeling or predictive analysis
Functional and/or effective connectivity
Graph analysis
Multivariate modeling and predictive analysis