nature portfolio

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Reporting Summary

Statistics

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>.

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	nfirmed
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	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.
X		A description of all covariates tested
	\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	\boxtimes	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)
	\boxtimes	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 <i>Give P values as exact values whenever suitable.</i>
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

Our web collection on $\underline{statistics\ for\ biologists}$ contains articles on many of the points above.

For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

Software and code

Policy information about availability of computer code

Data collection no software was used

Data analysis All statistical analyses were performed using GraphPad Prism 9.0.0 and R version 4.0.2

Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

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

We have provided a full data availability statement in the manuscript.

Human rese	arch parti	cipants	
Policy information	about <u>studies i</u>	nvolving human research participants and Sex and Gender in Research.	
Reporting on sex	and gender	The study did not involve human research participants.	
Population characteristics		The study did not involve human research participants.	
Recruitment		The study did not involve human research participants.	
Ethics oversight		The study did not involve human research participants.	
Note that full informa	ation on the appr	oval of the study protocol must also be provided in the manuscript.	
Field-spe	ecific re	porting	
Please select the o	ne below that is	the best fit for your research. If you are not sure, read the appropriate sections before making your selection.	
Life sciences	В	ehavioural & social sciences	
For a reference copy of t	the document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>	
Life scier	nces stu	ıdy design	
All studies must dis	sclose on these	points even when the disclosure is negative.	
Sample size		imulative results from at least three to six independent replicates. One-way and two-way ANOVA were used to analyze cance, followed by Tukey's multiple comparisons tests.	
Data exclusions	No data were e	xcluded from the analyses.	
Replication	All attempts at	replication were successful.	
Randomization	The mice were	randomly allocated into experimental findings.	
Blinding	Two independe	nt pathologists quantified lung and intestine injury scoring in a double-blind manner in the study.	
		Decific materials, systems and methods about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material,	
		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 th			
Eukaryotic			
	ogy and archaeol		
	nd other organism	———	

Antibodies

Antibodies used

Clinical data

Dual use research of concern

- 1. HRP conjugated Rabbit Anti-mouse IgG H&L, Supplier name: Abcam, Catalog numer: ab6728, Clone name: no, Lot number: GR3289920-6, Application: Dot blot, ELISA, IHC-P, IHC-Fr, Immunomicroscopy, ICC/IF.
- 2. HRP conjugated Goat Anti-mouse IgM mu chain, Supplier name: Abcam, Catalog numer: ab97230, Clone name: no, Lot number: GR3336726-4, Application: ELISA, WB, ICC, IHC;
- 3. HRP conjugated Goat Anti-mouse IgG1, Supplier name: Abcam, Catalog numer: ab97240, Clone name: no, Lot number: GR3320187-8, Application: ICC, ELISA, IHC-P, WB.
- 4. HRP conjugated Goat Anti-mouse IgG2a heavy chain, Supplier name: Abcam, Catalog numer: ab97245, Clone name: no, Lot

number: GR3324477-7, Application: IHC-P, ELISA, WB, ICC.

- 5. PE-conjugated CD4 Monoclonal Antibody, Supplier name: ThermoFisher, Catalog numer: 12-0041-82, Clone name: GK1.5, lot number: 2376147, Application: WB, IHC, ICC/IF, Flow, FN. Species: Fish, Human, Mouse
- 6. PE-conjugated CD8 alpha Monoclonal AntibodySupplier name: ThermoFisher, Catalog numer: MCD0804, Clone name: 5H10, Lot number: 2170194, Application: Flow. Species: Mouse.
- 7. PE/Cy5-conjugated Anti-IFN-γ, Supplier name: Abcam, Catalog numer: ab272255, Clone name: XMG1.2, Lot number: GR3394901-3, Application: Flow. Species: Mouse.
- 8. Alexa Fluor 647-conjugated Rat anti-Mouse IL-17A, Supplier name: BD Biosciences, Catalog numer: 560184, Clone name: TC11-18H10, Lot number: 9352425, Application: Intracellular staining (flow cytometry). Species: Mouse.
- 9. eFluor 450-conjugated anti-Mouse TNF-α, Supplier name: ThermoFisher, Catalog numer: 48-7321-82, Clone name: MP6-XT22, Lot number: 2373837, Application: Flow, IHC, Neu. Species: Mouse.
- 10. PE/Cy5-conjugated Rat IgG1 Kappa Light Chain Isotype, Supplier name: NOVUS Biologicals, Catalog numer: NBP1-43076, Clone name: RG1, Lot number: 2082873, Application: Flow-Isotype Control.
- 11. Alexa Fluor 647-conjugated Rat IgG1, Supplier name: SouthernBiotech, Catalog numer: 0116-31, Clone name: KLH/G1-2-2, Lot number: K1312-P9748, Application: Flow Cytometry, ELISA, FLISA, Immunocytochemistry, Blocking, In vitro Control, In vivo Control. 12. eFluor 450-conjugated Rat IgG1 kappa Isotype Control Supplier name: ThermoFisher, Catalog numer: 48-4301-82, Clone name: eBRG1, Lot number: 2082873, Application: Flow-Isotype Control.

Validation

- $1.\ PE-conjugated\ CD4\ Monoclonal\ Antibody,\ Application:\ WB,\ IHC,\ ICC/IF,\ Flow,\ FN.\ Species:\ Fish,\ Human,\ Mouse$
- 2. PE-conjugated CD8 alpha Monoclonal Antibody, Application: Flow. Species: Mouse.
- 3. PE/Cy5-conjugated Anti-IFN-γ, Application: Flow. Species: Mouse.
- 4. Alexa Fluor 647-conjugated Rat anti-Mouse IL-17A, Application: Intracellular staining (flow cytometry). Species: Mouse.
- 5. eFluor 450-conjugated anti-Mouse TNF- α , Application: Flow, IHC, Neu. Species: Mouse.

Animals and other 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 Research</u>

Laboratory animals

Six-week-old female C57BL/6 mice.

Wild animals

The study did not involve wild animals.

Reporting on sex

The findings apply to only one sex, and the sex was not considered in the study.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

This study was approved by the Animal Ethics committee of Harbin Veterinary Research Institute, China (Ethical Committee Approval number HVRI-IACUC-200723-01).

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

Flow Cytometry

Plots

Confirm	that:
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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

The lymphocytes were isolated from the spleen of mice using the Mouse Spleen Lymphocyte Separation Medium Kit (TBD Science, Tianjin, China) according to the manufacturer's instructions. The details of the procedure are as follows. The mouse spleen was aseptically separated, cut into small pieces, and ground in the homogenate rinse fluid (TBD Science). The single-cell suspension was obtained through a 70-mesh screen, and then centrifuged at 350 × g for 10 min and the supernatants were discarded. All tissue cells were resuspended in tissue sample diluent (TBD Science) and then added onto the surface of lymphocyte separation medium (TBD Science). The spleen lymphocytes were obtained by centrifugation at 400 × g for 30 min and then washed with a wash solution (TBD Science).

Instrument

Apogee A50-Micro Nanoscale Flow Cytometry

Software

Apogee Histogram software, PE-labeled CD4 and CD8 cells were detected in the Org channel (488 nm). PE/Cy5-labeled cells secreting IFN- γ were detected in the red channel (488 nm). Alexa Fluor 647-labeled cells secreting IL-17A cells were detected in the red channel (638 nm). eFluor 450-labeled cells secreting TNF- α cells were detected in the Blu channel (405 nm).

Cell population abundance

The lymphocytes were isolated from the spleen of mice using the Mouse Spleen Lymphocyte Separation Medium Kit (TBD Science, Tianjin, China). The lymphocytes were purified by removing the adhering cells.

Gating strategy

The gating strategies were as follows: CD4+ or CD8+ T cells was the first gated and then other markers were detected within the gates. CD4-positive or CD8-positive T cells were defined as positive T cell population and CD4-negative or CD8-negative T cells were defined as negative T cell population.

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