nature portfolio

Corresponding author(s): COMMSBIO-25-12370A

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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 *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 *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	NovoExpress software (version 1.6.2), Nikon NIS-Elements software (version5.22.00), Zetasizer software (version7.13)
Data analysis	Image J software (version 1.8.0), FlowJo software (version X10), NovoExpress software (version 1.6.2), Graphpad Prism software (version 9.0.0), Excel (version 2511)

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

All raw and processed data will be made available upon request.

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	Not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable.
Population characteristics	Not applicable.
Recruitment	Not applicable.
Ethics oversight	Not applicable.

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.

Sample size	The sample size was not predetermined using statistical methods. Instead, it was selected according to standard practices in the field (with at least three replicates per condition), thereby yielding a sufficiently large dataset.
Data exclusions	No data were excluded from the analyses.
Replication	Reported results were consistently replicated across multiple experiments with all replicates generating similar results. Experiments were repeated at least three times.
Randomization	The animals were allocated into experimental group in random.
Blinding	Blinding was not possible as experimental conditions were mostly evident from the image data. Blinding is unnecessary during data collection because experimental conditions are well controlled. Furthermore, as the results are quantitative and require no subjective judgment or interpretation, blinding is also not essential.

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

Antibodies used	anti PD-L1 (1:1000, polyclonal, Proteintech, cat:17952-1-AP), anti-CD44 (1:100, clone:JE64-01, Huabio, cat:HA722845), anti CD16/32

Antibodies used	antibody (1:200, clone:93, eBioscience, cat:14016182), anti CD3-FITC (1:100, clone:17A2, eBioscience, cat:11003282), anti CD4-PE-Cy7 (1:100, clone:GK1.5, eBioscience, cat:25004182), anti CD8a-APC (1:200, clone:53-6.7, eBioscience, cat:17008182), anti CD11c-PE (1:50,clone:N418, eBioscience, cat:12011482), anti F4/80-FITC (1:50, clone:BM8, eBioscience, cat:11480182), ant MHC II-FITC (1:100, clone:M5/114.15.2, eBioscience, cat:25532182), anti CD11b-APC (1:200, clone:M1/70, eBioscience, cat:17011282), anti PD-L1-PE (1:400,clone:MIH5, eBioscience, cat:12598282), anti IFN-γ-FITC (1:50, clone:XMG1.2, eBioscience, cat:11731182), anti Granzyme B-PE-Cy7 (1:200, clone:NGZB, eBioscience, cat:25889882), anti cGAS (1:1000, polyclonal, Huabio, cat:HA500023), anti p-IRF3 (1:1000, clone:SU03-28, Huabio, cat:ET1608-22), anti IRF3 (1:2000, clone:SD2062, Huabio, cat:ET1612-14), anti STING (1:1000, clone:JM03-47, Huabio, cat:ET1705-68)
Validation	See supplier pages for validation. anti PD-L1:https://www.ptgcn.com/products/CD274-Antibody-17952-1-AP.htm, anti CD44: https://huabio.cn/products/CD44-antibody-HA722845, anti CD16/32: https://www.thermofisher.cn/cn/zh/antibody/product/CD16-CD32-Antibody-clone-93-Monoclonal/14-0161-82, anti CD3: https://www.thermofisher.cn/cn/zh/antibody/product/CD3-Antibody-clone-17A2-Monoclonal/11-0032-82, anti CD4: https://www.thermofisher.cn/cn/zh/antibody/product/CD4-Antibody-clone-GK1-5-Monoclonal/25-0041-82, anti CD8a: https://www.thermofisher.cn/cn/zh/antibody/product/CD8a-Antibody-clone-53-6-7-Monoclonal/17-0081-82, anti-CD11c: https://www.thermofisher.cn/cn/zh/antibody/product/CD11c-Antibody-clone-N418-Monoclonal/12-0114-82, anti F4/80: https://www.thermofisher.cn/cn/zh/antibody/product/F4-80-Antibody-clone-BM8-Monoclonal/11-4801-82, anti MHC II: https://www.thermofisher.cn/cn/zh/antibody/product/MHC-Class-II-I-A-I-E-Antibody-clone-M5-114-15-2-Monoclonal/25-5321-82, anti CD11b: https://www.thermofisher.cn/cn/zh/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/17-0112-82, anti PD-L1: https://www.thermofisher.cn/cn/zh/antibody/product/CD274-PD-L1-B7-H1-Antibody-clone-MIH5-Monoclonal/12-5982-82, anti IFN-γ: https://www.thermofisher.cn/cn/zh/antibody/product/IFN-gamma-Antibody-clone-XMG1-2-Monoclonal/11-7311-82, anti Granzyme B: https://www.thermofisher.cn/cn/zh/antibody/product/Granzyme-B-Antibody-clone-NGZB-Monoclonal/25-8898-82, anti cGAS: https://huabio.cn/products/cGAS-antibody-HA500023, anti p-IRF3: https://huabio.cn/products/Phospho-IRF3-S386-antibody-ET1608-22, anti IRF3: https://huabio.cn/products/IRF3-antibody-ET1612-14, anti STING: https://huabio.cn/products/TMEM173-antibody-ET1705-68

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HCT116 (human, National Collection of Authenticated Cell Cultures, cat:SCSP-5076); CT26 (mouse, National Collection of Authenticated Cell Cultures, cat:SCSP-523)
Authentication	Cell lines were authenticated.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	Female Balb/c mice (5-7 weeks)
Wild animals	No wild animals were used in this study.
Reporting on sex	Since only female animals were used, sex-disaggregated data is not applicable, and no sex-based comparative analyses were performed.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal handling procedures were performed according to protocols approved by the Ethics Committee of the Animal Protection Institution of Shanxi University (2024054), and the care and handling procedures strictly complied with "Guide for the Care and Use of Laboratory Animals" of China (D.N. 55, 2001)

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

Plants

Seed stocks	No plant seed stocks or plant materials were used in this study.
Novel plant genotypes	No novel plant genotypes were generated or used in this study.
Authentication	No plant materials or genotypes were involved in this study, so no authentication procedures were performed.

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	Collect tumor tissue and mince it to prepare a single-cell suspension. Briefly, the tissue fragments were digested with collagenase I (1 mg/mL) and DNase I (0.2 mg/mL) at 37 °C for 45 min. The digested suspension was filtered through 70 μm cell strainers and treated with red blood cell lysis buffer. The cells were collected by centrifugation and washed. Prior to staining, cells were incubated with anti-CD16/32 antibody for 30 min to block non-specific receptor binding, and then cells were stained for 45 min at 4 °C in the dark with fluorescein-labeled antibodies by turn.
Instrument	Novocyte Flow Cytometer (Model: NovoCyte; Regulatory Model Number: RMNNC3000); MoniSight620
Software	NovoExpress software (version 1.6.2); FlowJo software (version X10)
Cell population abundance	Immune cell subset abundance was calculated as the percentage of each target subset in total viable immune cells. Data are shown as mean ± SD (n=3 independent experiments), with gating boundaries determined by FMO controls.
Gating strategy	The gating strategy was as follows:Initial gating: A live cell gate was defined based on forward scatter (FSC) and side scatter (SSC) to exclude debris and dead cells. Single-cell discrimination: A FSC-H vs FSC-A gate was applied to eliminate cell doublets. Population-specific gating: Target cell populations were identified using fluorescence markers, with positive/negative boundaries set based on fluorescence minus one controls.

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