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 | Living Image software (v4.7.2), FlowJo software (v10; BD Biosciences, USA), No custom code was used for data collection. |
| Data analysis | All statistical analyses were performed on Graphpad Prism (v6.0). All flow-cytometry data were analyzed on FlowJo software (v10; BD Biosciences, USA). Living image software (v4.7.2, Perkin Elmer) was used to analyse bioluminescent and fluorescent images. |

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 main data supporting the results in this study are available within the paper and its Supplementary Information. Additional data that support the findings of this study are available from the corresponding authors upon reasonable request. Source data are provided with this paper.

## 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 | *Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.*<br>*Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.*<br>*Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.* |
| Reporting on race, ethnicity, or other socially relevant groupings | *Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).*<br>*Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)*<br>*Please provide details about how you controlled for confounding variables in your analyses.* |
| Population characteristics | *Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."* |
| Recruitment | *Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.* |
| Ethics oversight | *Identify the organization(s) that approved the study protocol.* |

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 | No statistical method was used to predetermine the sample size for each study. For property measurement experiments, samples were prepared and tested at least twice. For in vivo studies, each group contains at least 3 (n ≥3) for evaluating the statistical significance. Sample size was chosen to ensure reproducibility of the experiments in accordance with the replacement, reduction and refinement principles of animal ethics regulation. |
| Data exclusions | No data was excluded from the analysis. |
| Replication | Experiments were repeated at least twice, unless otherwise stated in the respective figure legend. |
| Randomization | Randomization was used to divide up the animals for in vivo treatment study. |
| Blinding | No blinding was employed as the researcher performing the treatment was also responsible for the analysis. The researchers should keep careful track of protocols because that most of the experiments needed multiple treatments. |

# 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 |
|---|---|
| [ ] | [x] Antibodies |
| [ ] | [x] Eukaryotic cell lines |
| [x] | [ ] Palaeontology and archaeology |
| [ ] | [x] Animals and other organisms |
| [x] | [ ] Clinical data |
| [x] | [ ] Dual use research of concern |
| [x] | [ ] Plants |

## Methods

| n/a | Involved in the study |
|---|---|
| [x] | [ ] ChIP-seq |
| [ ] | [x] Flow cytometry |
| [x] | [ ] MRI-based neuroimaging |

# Antibodies

Antibodies used

The following primary antibodies were used for flow cytometry:
FITC anti-mouse CD3 (Biolegend, cat#: 100204, clone: 17A2), dilution 1:200;
PE anti-mouse CD4 (Biolegend, cat#: 100407, clone: GK1.5), dilution 1:200;
PE anti-mouse CD8a (Biolegend, cat#: 100731, clone: 53-6.7), dilution 1:200;
FITC anti-mouse CD45 (Biolegend, cat#: 103107, clone: 30-F11), dilution 1:200;
PE anti-mouse CD11b (Biolegend, cat#: 101207, clone: M1/70), dilution 1:200;
APC anti-mouse F4/80 (Biolegend, cat#: 123115, clone: BM8), dilution 1:200;
APC anti-mouse Ly-6G/Ly-6C (Biolegend, cat#: 108411, clone: RB6-BC5), dilution 1:200;
APC anti-mouse CD62L (Biolegend, cat#: 104412, clone: MEL-14), dilution 1:200;
FITC anti-mouse CD80 (MULTISCIENCES, cat#: F2108001, clone: 16-10A1), dilution 1:20;
APC anti-mouse CD11c (MULTISCIENCES, cat#: F21011C03, clone: N418), dilution 1:20;
PE anti-mouse CD86 (MULTISCIENCES, cat#: F2108602, clone: GL-1), dilution 1:20;
The following primary antibodies were used for immunofluorescence:
Anti-mouse calreticulin (ZEN-BIOSCIENCE, cat#: R380959, clone: R03-9K4), dilution 1:100;
Anti-mouse α-SMA (ZEN-BIOSCIENCE, cat#: R380653, clone: R09-6B3), dilution 1:100;
Anti-mouse Collagen (ZEN-BIOSCIENCE, cat#: R26615, clone: R02-5E5), dilution 1:100;
Anti-mouse tumor necrosis factor-α (TNF-α) (Abbkine, cat#: ABP0127), dilution 1:100;
All the antibodies were diluted and used following the suppliers' protocols.

Validation

All antibodies were verified by the supplier, and each lot has been quality-tested. These antibodies are used without additional validation.

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Cell line sources were provided under "Method: cell culture" section. 4T1 (mouse breast cancer cell line), Luc-4T1 (mouse breast cancer cell line expressing firefly luciferase) and B16F10 (mouse melanoma cancer cell line) were obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences.

Authentication

The cell lines were certified by the manufacturers (surface markers, morphology). Engineered cell line was verified using negative control and maintained with selection medium according to manufacturer's suggestion.

Mycoplasma contamination

All cell lines were tested for mycoplasma contamination. No mycoplasma contamination was found.

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

As reported in the "Method: Animals" section. Six-weeks old female BALB/c mice (18-22 g) and C57BL/6 mice (18-22 g) were ordered from Ziyuan Laboratory Animal Technology Co. Ltd (Hangzhou, China).

Wild animals

This study did not involve wild animals.

Reporting on sex

Female BALB/c mice and female C57BL/6 mice were used.

Field-collected samples

The study did not involve samples collected from the field

| | |
|---|---|
| Ethics oversight | All animal experiments were performed according to animal care regulations and the Ethics Committee of the Anhui University of Chinese Medicine (approval ID: AHUCM-mouse-2023065). |

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

# 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 | As described in the Methods section. The single cell suspension was washed with FACS buffer, incubated with CD16/32, and then stained with the indicated antibodies. |
| Instrument | BD Fortessa or FACSCalibur flow cytometers (USA). |
| Software | Flow cytometry data were analyzed using FlowJo (v10.6.2) for windows. |
| Cell population abundance | Sorting was not done. Due to the poor infiltration of lymphocytes in tumors, cell populations of interest were collected as much as possible in the intratumoural immunology assay. |
| Gating strategy | Generally, cells were first gated on FSC/SSC. Single cells were usually gated using FSC-H and FSC-A. Cells were gated on the basis of positive and fluorescence minus one controls, and the frequencies of cells staining positive for each marker was recorded. |

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