nature portfolio

Corresponding author(s): Xian-Zheng Zhang

Last updated by author(s): Jul 21, 2024

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	SEM images were obtained on Zeiss Sigma FESEM. TEM images were obtained by a JEM-2100 (JEOL) transmission electron microscope. Zeta potential was measured on a Nano-ZS ZEN3600 particle sizer (Malvern Instruments). UV-vis absorbance was measured by UV-vis spectroscopy (Lambda Bio40). Confocal microscopy images were obtained from a confocal laser scanning microscope (CLSM, Nikon C1-si TE2000). Cell viability was evaluated through the MTT assay using a microplate reader (Bio-Rad, Model 550, USA). Blood routine was measured by an Auto Hematology Analyzer (MC-6200VET), and blood biochemistry was analyzed by a biochemical auto analyzer (MNCHIP, Tianjin, China). In vivo imaging was performed by a Spectrum Pre-clinical In vivo Imaging System (Pekin Emer). μ-CT scanning was conducted on a Quantum FX microCT system (PerkinElmer). MRI imaging was obtained from an Aspect M3 MRI imaging system (Israel). PAI imaging was conducted by a Vevo® LAZR system (visualSonics, Canada). Immune cell fluorescence intensity was analyzed by flow cytometry (BD FACS Aria III, USA).
Data analysis	All statistical analyses were performed on Graphpad Prism 8 or Excel 2016. Living Image software (Perkin Elmer) was used to analyze bioluminescent and fluorescent images. Omics data were analyzed online with I-Sanger Cloud Platform.

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 RNA sequencing data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under accession code PRJNA1042652. The 16 s rDNA sequencing data generated in this study have been deposited in the NCBI SRA database under accession code PRJNA1041940 and PRJNA1041943. The remaining data are available within the Article, Supplementary Information, or Source Data file. 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	Research did not involve human participants.
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	No statistical methods were used to predetermine sample sizes. Instead, sample sizes were established based on prior experimental experience, which suggested they were sufficient to enable robust statistical analysis. These sample sizes are detailed in the legends of each figure.
Data exclusions	No data were excluded.
Replication	All in vitro and in vivo experiments were carried out with at least 3 biological replicates for each experimental group. All attempts to replicate results are successful.
Randomization	All samples/organisms were randomly allocated into experimental groups.
Blinding	Blinding and randomization were applied to all experiments.

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

The antibodies used for FCM were as follows: anti-mouse CD3 (FITC, Catalog number: 100203, Clone: 17A2), anti-mouse CD4 (APC, Catalog number: 100411, Clone: GK1.5), anti-mouse CD8a (PE, Catalog number: 100707, Clone: 53-6.7), anti-mouse CD11c (FITC, Catalog number: 117305, Clone: N418), anti-mouse CD80 (PE, Catalog number: 104707, Clone: 16-10A1), anti-mouse CD86 (APC, Catalog number: 105011, Clone: GL1), anti-mouse CD11b (APC, Catalog number: 101211, Clone: M1/70), anti-mouse Ly-6G (Gr-1) (FITC, Catalog number: 108405, Clone: RB6-8C5), anti-mouse F4/80 (FITC, Catalog number: 123107, Clone: BM8), anti-mouse CD206 (PE, Catalog number: 141705, Clone: C068C2), anti-mouse CD44 (PE, Catalog number: 103023, Clone: IM7), anti-mouse CD62L (APC, Catalog number: 104411, Clone: MEL-14), anti-mouse CD4 (PE, Catalog number: 116006, Clone: RM4-4), anti-mouse CD8a (APC, Catalog number: 100711, Clone: 53-6.7), anti-mouse Foxp3 (PE, Catalog number: 126403, Clone: MF-14), anti-mouse IFNγ (PE, Catalog number: 163503, Clone: W18272D), anti-mouse GzmB (PE, Catalog number: 372207, Clone: QA16A02). All antibodies used for FCM and anti-PD-L1 antibody was purchased from BioLegend.

Validation

Validations were conducted by the respective manufacturer as noted on the antibody specification sheet. A validation statement for each antibody is available on the manufacturer's website.
Biolegend (https://www.biolegend.com/)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Panc-01 (Catalog no. GDC0309), HPSC (Catalog no. GPC0058), and RAW264.7 (Catalog no. GDC0143) cell lines were obtained from China Center for Type Culture Collection (CCTCC). Panc02 (CL-0736) were kindly provided by Wuhan Pricella Biotechnology Co.,Ltd.

Authentication

Panc02 cell line was authenticated by STR profiling, and other cell lines we used were morphologically confirmed according to the information provided by CCTCC.

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

Six-week-old C57BL/6 female mice (18 ± 2g) were obtained from the Animal Biosafety Level III Lab at Wuhan University. Six-week-old KPC female mice, expressing mutant intrapancreatic Kras and Trp53, were obtained from Cyagen Biosciences Inc. (Suzhou, China). All mice were housed at 12-h light-dark cycle within 25-27°C and 45-55% humidity.

Wild animals

The study did not involve wild animals.

Reporting on sex

The study did not intend to focus on a single sex. To the best of our knowledge, there is no established evidence suggesting that sex differences in mice influence the impact of intratumoral flora on PDAC immunotherapy.

Field-collected samples

The study did not involve filed-collection samples.

Ethics oversight

Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Animal Experiment Center of Wuhan University (Wuhan, China). All animal experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of People's Republic of China.

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

Plants

Seed stocks	Research involves no plants.
Novel plant genotypes	Research involves no plants.
Authentication	Research involves no plants.

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	Tumor tissues were processed into single-cell suspensions using collagenase IV (Roche), DNAase I (BioFroxx), and hyaluronidase (bioSharp) at 37°C for 2 hours. The resulting cell suspension was subjected to red blood cell lysis and dispersed in PBS (1 mL). The cell suspension was then filtered through a 70 μm cell strainer and separated using percoll (Stem Cell). The single-cell suspensions were subsequently stained with antibody cocktails, and fluorescently labeled cells were counted using a BD Accuri™ C6 flow cytometer.
Instrument	BD Accuri™ C6 flow cytometer, model:Facscalibur, manufacturers :BD
Software	Data were analysed on Flowjo V10 (Treestar, 139 Ashland, USA).
Cell population abundance	Flow cytometry was used for quantification only. No post-sort fractions were collected.
Gating strategy	Cells were first gated on FSC/SSC. The specific gating strategy was shown in the Supporting Information.

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