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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	SOFTWARES used for data collection include: Tanon MP for western blot images; ABI 7900 system for RT-PCR ; Illumina HiSeq™ 2500 system for microarray; LC MS analysis was performed using UHPLC (1290 Infinity) coupled to a QTRAP 6500+ MS (Sciex)
Data analysis	GraphPad Prism 8 and Microsoft Office Excel were used for statistic analysis; Western Blot images were analyzed using Tanon Gis; Microarray analysis was performed at I-Sanger Cloud Platform (www.oebiotech.com; OE Biotech Co., Ltd. Shanghai, China); Targeted metabolomics data were processed and analyzed with SIMCA P (v14.1).No custom code was used.

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 accession number for the sequencing data reported in this paper is SRA: PRJNA1196505 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1196505/]. Targeted

metabolomics data used in this publication have been deposited to the EMBL-EBI MetaboLights database with the identifier MTBLS14989 [https://www.ebi.ac.uk/metabolights/MTBLS14989]. The full targeted metabolomics dataset is available in the source data. The remaining data that support the findings of this study are available within the article, its supporting information and source data and/or from the corresponding author upon 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	The study included 33 male and 27 female pancreatic cancer patients, as well as 26 healthy male and 24 healthy female controls. Sex and gender were considered in the study design to ensure representation across both sexes.
Reporting on race, ethnicity, or other socially relevant groupings	The study included a random sample of participants from diverse occupational, income, and educational backgrounds. The inclusion criteria were designed to capture a wide range of socioeconomic statuses without relying on racial or ethnic classifications.
Population characteristics	• Age: The average age of pancreatic cancer patients was 67.4 years, while the average age of healthy controls was 41.3 years. • Sex Ratio: The gender ratio was balanced, with an approximately equal number of males and females in both groups. • Ethnicity: All participants were of Han Chinese ethnicity. • Health Status: Healthy individuals were excluded if they had chronic diseases or a history of cancer. • Diagnosis: Pancreatic cancer patients were pathologically diagnosed as pancreatic ductal adenocarcinoma (PDAC) and had not undergone any treatment prior to the study.
Recruitment	Recruitment Process: Participants were rigorously recruited at random according to the predefined inclusion and exclusion criteria. This systematic approach ensured that selection bias was minimized, enhancing the generalizability of our findings. Bias Control: To control for potential biases, we implemented strict recruitment protocols that adhered to the established inclusion and exclusion criteria. Additionally, we employed various strategies, such as stratified random sampling, to ensure a representative sample of the target population. Informed Consent: Prior to participation, all individuals were provided with detailed information about the study's purpose, procedures, potential risks, and benefits. Informed consent was obtained from each participant, confirming their voluntary agreement to take part in the study.
Ethics oversight	PDAC or healthy human stool samples and blood were collected from patients diagnosed with PDAC following ethical guidelines and with the approval of the Institutional Review Board of Fudan University Affiliated Zhongshan Hospital (B2023-282R).

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 sample size calculation was performed, but sample sizes were sufficient to carry out the required experiments with sufficient statistics and standard using t-test for such kind of experiments. To minimize any potential bias, we randomly assigned mice of same genotype to different treatments. In addition, each figure legend describes the number of technical and biological replicates.
Data exclusions	No data were excluded.
Replication	The data reported were generated using at least three different biological replicates in most required experiments. All experimental findings were reproduced for at least twice with similar results.
Randomization	Mice were allocated randomly.
Blinding	Microarray were performed blinded. Other experiments were not blinded, however, we followed standard laboratory procedures of randomization. Each experiment was associated with proper controls, and compared samples were collected and analyzed under the same conditions.

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 following primary antibodies were used for Western blotting, FCM, and IF/IHC:
anti- GRP78 (Cat. No. A0241, 1:1000, Abclonal, Wuhan, China);
anti- XBP1 (Cat. No. A1731, 1:1000, Abclonal, Wuhan, China);
anti-mTOR (Cat. No. 66888-1-Ig 1:1000, Proteintech, Chicago, IL, USA);
anti-pmTOR (Cat. No. 80596-1-RR, 1:1000, Proteintech, Chicago, IL, USA);
anti- AKT (Cat. No. 9272, 1:1000, Cell Signaling Technology, MA, USA);
anti- pAKT (Cat. No. 4060, 1:2000, Cell Signaling Technology, MA, USA);
anti- RARβ (Cat. No. 14013-1-AP, 1:1000, Proteintech, Chicago, IL, USA);
anti- TC45 (Cat. No. 58935, 1:1000, Cell Signaling Technology, MA, USA);
anti- STAT1 (Cat. No. 14994, 1:1000, Cell Signaling Technology, MA, USA);
anti- pSTAT1 (Cat. No. 9267, 1:1000, Cell Signaling Technology, MA, USA);
anti- P2Y14R (Cat. No. A8178, 1:1000, Abclonal, Wuhan, China);
anti- -GAPDH (Cat. No. 60004-1-Ig, 1: 200000, Proteintech, Chicago, IL, USA);
anti-βactin (Cat. No. 66009-1-Ig, 1:10000, Proteintech, Chicago, IL, USA).
anti-mouse CD45 BV510(Cat. No. 103138, 1:100, BioLegend, San Diego, USA);
anti-mouse CD11b SUV387(Cat. No. 101292, 1:100, BioLegend, San Diego, USA);
anti-mouse MHC-II FITC(Cat. No. 107605, 1:200, BioLegend,San Diego, USA);
anti-mouse CD274 PDL1 PE(Cat. No. 558091, 1:100, BD,New Jersey, USA);
anti-mouse F480 APC(Cat. No. 17-4801-82, 1:200, Thermo, Massachusetts, USA);
anti-mouse CD86 BV421(Cat. No. 105032, 1:100, BioLegend,San Diego, USA);
anti-mouse CD206 PE-Cy7(Cat. No. 25-2061-82, 1:100, Thermo, Massachusetts, USA);
anti-mouse CD3 APC(Cat. No. 17-0031-82, 1:100, eBioscience,San Diego, USA);
anti-mouse CD4 FITC(Cat. No. 11-0042-85, 1:100, Thermo, Massachusetts, USA);
anti-mouse CD8 BV421(Cat. No. 100753, 1:100, BioLegend,San Diego, USA);
anti-mouse IFN-r PE-Cy7(Cat. No. 12-5321-82, 1:100, eBioscience,San Diego, USA);
anti-mouse CD279 PD1 BV421(Cat. No. 135218, 1:100, BioLegend,San Diego, USA);
anti-mouse CD11C BUV395(Cat. No. 363-0114-82, 1:100, Thermo, Massachusetts, USA);
anti-mouse Gr1 PerCP-eFluor710(Cat. No. 46-5931-82, 1:100, Thermo, Massachusetts, USA);
anti-mouse CD19 APC(Cat. No. 115512, 1:100, BioLegend,San Diego, USA);
anti-mouse CD45.1 APC(Cat. No. 110713, 1:100, BioLegend,San Diego, USA);
anti-mouse CD45.2 FITC(Cat. No. 109805, 1:100, BioLegend,San Diego, USA);
Anti-F4/80(Cat. No. 70076, 1:200, CST,Massachusetts, USA);
Anti-CD68(Cat. No. ab955, 1:100, Abcam, Cambridge,UK);
Anti-Ki67(Cat. No. GB121141, 1:300, Servicebio, Wuhan,China);
anti-Histone H3 (phospho S10) (Cat. No. ab14955, 1:500, Abcam, Cambridge,UK);
Anti- Cytokeratin 19(Cat. No. ab52625, 1:500, Abcam, Cambridge,UK);

Validation

Antibodies sourced from commercial corporation are well-validated by the manufacturer and are widely used in the scientific community for Western blotting, FCM, and IF/IHC.
anti- GRP78 (Cat. No. A0241, 1:1000, Abclonal, Wuhan, China) was validated by other users and cited 61 times;
anti- XBP1 (Cat. No. A1731, 1:1000, Abclonal, Wuhan, China) was validated by other users and cited 30 times;
anti-mTOR (Cat. No. 66888-1-Ig, 1:1000, Proteintech, Chicago, IL, USA) was validated by other users and cited 3387 times;
anti-pmTOR (Cat. No. 80596-1-RR, 1:1000, Proteintech, Chicago, IL, USA) was validated by other users and cited 22 times;
anti- AKT (Cat. No. 9272, 1:1000, Cell Signaling Technology, MA, USA) was validated by other users and cited 10931 times;
anti- pAKT (Cat. No. 4060, 1:2000, Cell Signaling Technology, MA, USA) was validated by other users and cited 11758 times;
anti- RARβ (Cat. No. 14013-1-AP, 1:1000, Proteintech, Chicago, IL, USA) was validated by other users and cited twice;
anti- TC45 (Cat. No. 58935, 1:1000, Cell Signaling Technology, MA, USA) was validated by other users and cited 17 times;
anti- STAT1 (Cat. No. 14994, 1:1000, Cell Signaling Technology, MA, USA) was validated by other users and cited 544 times;
anti- pSTAT1 (Cat. No. 9267, 1:1000, Cell Signaling Technology, MA, USA) was validated by other users and cited 45 times;
anti- P2Y14R (Cat. No. A8178, 1:1000, Abclonal, Wuhan, China) was validated by other users and cited once;

anti- -GAPDH (Cat. No. 60004-1-Ig, 1: 200000, Proteintech, Chicago, IL, USA) was validated by other users and cited 12226 times;
anti-βactin (Cat. No. 66009-1-Ig, 1:10000, Proteintech, Chicago, IL, USA) was validated by other users and cited 6928 times;
anti-mouse CD45 BV510(Cat. No. 103138, 1:100, BioLegend, San Diego, USA)was validated by other users and cited 162 times;
anti-mouse CD11b SUV387(Cat. No. 101292, 1:100, BioLegend, San Diego, USA)was validated by other users and cited 1 time;
anti-mouse MHC-II FITC(Cat. No. 107605 1:200, BioLegend,San Diego, USA)was validated by other users and cited 133 time;
anti-mouse CD274 PDL1 PE(Cat. No. 558091, 1:100, BD,New Jersey, USA)was validated by other users and cited 89 time;
anti-mouse F480 APC(Cat. No. 17-4801-82, 1:200, Thermo, Massachusetts, USA)was validated by other users and cited 463 time;
anti-mouse CD86 BV421(Cat. No. 105032, 1:100, BioLegend,San Diego, USA)was validated by other users and cited 23 time;
anti-mouse CD206 PE-Cy7(Cat. No. 25-2061-82, 1:100, Thermo, Massachusetts, USA)was validated by other users and cited 62 time;
anti-mouse CD3 APC(Cat. No. 17-0031-82, 1:100, eBioscience,San Diego, USA)was validated by other users and cited 253 time;
anti-mouse CD4 FITC(Cat. No. 11-0042-85, 1:100, Thermo, Massachusetts, USA)was validated by other users and cited 366 time;
anti-mouse CD8 BV421(Cat. No. 100753, 1:100, BioLegend,San Diego, USA)was validated by other users and cited 105 time;
anti-mouse IFN-r PE-Cy7(Cat. No. 25-7311-82, 1:100, eBioscience,San Diego, USA)was validated by other users and cited 245 time;
anti-mouse CD279 PD1 BV421(Cat. No. 135218, 1:100, BioLegend,San Diego, USA)was validated by other users and cited 57 time;
anti-mouse CD11C BUV395(Cat. No. 363-0114-82, 1:100, Thermo, Massachusetts, USA)was validated by other users and cited 1time;
anti-mouse Gr1 PerCP-eFluor710(Cat. No. 46-5931-82, 1:100, Thermo, Massachusetts, USA)was validated by other users and cited 86 time;
anti-mouse CD19 APC(Cat. No. 115512, 1:100, BioLegend,San Diego, USA)was validated by other users and cited 78 time;
anti-mouse CD45.1 APC(Cat. No. 110713, 1:100, BioLegend,San Diego, USA)was validated by other users and cited 90 time;
anti-mouse CD45.2 FITC(Cat. No. 109805, 1:100, BioLegend,San Diego, USA)was validated by other users and cited 115 time;
Anti-F4/80(Cat. No. 70076, 1:200, CST,Massachusetts, USA)was validated by other users and cited 861 time;
Anti-CD68(Cat. No. ab955, 1:100, Abcam, Cambridge,UK)was validated by other users and cited 871 time;
Anti-Ki67(Cat. No. GB121141, 1:300, Servicebio, Wuhan,China)was validated by other users and cited 72 time;
anti-Histone H3 (phospho S10) (Cat. No. ab14955, 1:500, Abcam, Cambridge,UK)was validated by other users and cited 273 time;
Anti- Cytokeratin 19(Cat. No. ab52625, 1:500, Abcam, Cambridge,UK)was validated by other users and cited 380 time;

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	KPC cell line were purchased from Shanghai model organisms center(NM-YD04-TG01) ; Panc02 cell line were purchased from National Collection of Authenticated Cell Cultures, Chinese Academy of Sciences(SCSP-5468) THP-1 cell lines were purchased from Cell Bank of Shanghai Institute of Cell Biology, CAS.
Authentication	The cell lines have been authenticated by Cell Bank of Shanghai Institute of Cell Biology, CAS.
Mycoplasma contamination	The cell culture incubator was treated with mycoplasma scavenger; all cell lines were tested negative for mycoplasma contamination by Cell Bank of Shanghai Institute of Cell Biology, CAS.
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	Male C57BL/6J mice and NSG mice aged 8-12 weeks were purchased from Shanghai Biomodel Organism Science & Technology Development Co.,Ltd., Shanghai, China. NSG mice
Wild animals	The study did not involve wild animals.
Reporting on sex	Referring to multiple pancreatic cancer-related studies that have used male mice as research subjects, and based on our preliminary experiments showing higher tumor consistency among male mice compared to female mice, we ultimately chose male mice as the research subjects in this study.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experiments were approved by the Ethics Committee of Zhongshan Hospital, Fudan University (B2023-074).

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

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

Excise and mince the tumor tissue, spleen or bone marrow, then digest the tissue with Type IV collagenase and DNase I. The digestion solution consists of 1640 medium containing 2% fetal bovine serum, 0.5 mg/ml Type IV collagenase, and 200 U/ml DNase I. Add 500 µL of digestion solution per 300 mg of tissue and place it in a 37°C shaker for digestion for 1-2 hours. Terminate the digestion with FACS buffer (containing 1% fetal bovine serum, 2 mM EDTA, 50 mL PBS), then filter through a 70 µm mesh to collect the single cell suspension for later use. Prepare the blocking solution by mixing CD16/32 antibody with FACS buffer at a ratio of 1:200, and incubate at room temperature for 10 minutes to block the Fc region of the antibody and its receptors on the cells. After washing the cells, incubate with specific flow antibodies and live/dead dye at 4°C in the dark for 30 minutes. To evaluate the cytokine profile of immune cells, stimulate T cells with 100 ng/ml PMA, 1 µg/ml ionomycin, and 10 µg/ml brefeldin A at 37°C in the dark for 4 hours. After surface staining, add 200 µL of Fixation and Permeabilization Solution to each sample to fix and permeabilize the cells, incubate at room temperature in the dark for 30 minutes, then stain with intracellular or nuclear fluorescent antibodies. Incubate at room temperature for 30 minutes. After filtering through a 70 µm mesh, proceed to flow cytometry analysis.

Instrument

BD Fortessa X20 flow cytometer

Software

FlowJo_v10.8.1_CL

Cell population abundance

- CD4+ T cells: percentage of the CD3 positive lymphocyte population.
- CD8+ T cells: percentage of the CD3 positive lymphocyte population.
- B cells: percentage of the total lymphocyte population.
- Macrophages: percentage of the total leukocyte population.
- Dendritic cells (DCs): percentage of the total leukocyte population.
- Myeloid-Derived Suppressor Cells (MDSCs): percentage of the total leukocyte population.

Gating strategy

Gating strategy:
- Initial Gating: Cells were first gated based on forward scatter (FSC) and side scatter (SSC) to exclude debris and aggregate cells.
- Viability Staining: Dead cells were excluded by staining with Fixable Viability Dye eFluorTM 780 (1:1,000, Thermo Fisher Scientific) according to the manufacturer's instructions.

Specific Cell Population Gating:
- Immune cells: Gated as CD45+ cells in live cells.
- T cells: Gated as CD3+ cells in CD45+ cells.
- CD4+ T cells: Gated as CD4+ cells in CD3+ cells.
- CD8+ T cells: Gated as CD8+ cells in CD3+ cells.
- B cells: Gated as CD19+ cells in CD45+ cells..
- Macrophages: Gated as F4/80+ and CD11B+ cells in CD45+ cells.
- Dendritic cells (DCs): Gated as MHC II+ and CD11C+ cells in CD45+ cells.
- MDSCs: Gated as Gr1+ and CD11B+ cells in F4/80- and CD11C- cells.

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