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Reporting Summary

Nature Research 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 Research 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	MicroPET/CT device (Siemens Inveon PET/CT), gamma counter (Wizard 2480, Perkin-Elmer), Laser scanning confocal microscope (Olympus FV1200), Leica DM4 B, Beckman coulter CytoFLEX (flow cytometry).
Data analysis	GraphPad Prism 7.0 (statistical analysis of the majority of in vitro and in vivo experiments). FlowJo10. (flow cytometry). ImageJ 2X. V2.1.4.6. (histology). Inveon Research Workplace (PET/CT). IBM SPSS statistics 26.

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that the data supporting the findings of this study are available within the article and its Supplementary Information Files or from the corresponding author on reasonable request.

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	Sample sizes were chosen based on estimates from previous studies to enable significant statistical analysis. For example, in vitro studies were repeated three times independently with triplicate or quintuplicate samples, and in the treatment experiments with 8 mice per group were performed. Statistics such as error bars, significance and p values can be derived from n≥3.
Data exclusions	No data exclusion for this study.
Replication	All experiments were performed with biological replicates, and technical replicates were performed for studies as described in the Methods section, Figure Legends, and Main text. Experimental findings were reliably reproduced.
Randomization	In the case of in vivo studies, once tumors were established, mice were randomly assigned to the different experimental groups and control group.
Blinding	All the investigators were blinded to group assignment in the course of data collection and analysis.

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Anti-PD-L1 antibody (clone EPR19759, abcam, ab213524)
Anti-CD4 antibody (clone EPR19514, abcam, ab183685)
Anti-CD8 antibody (polyclone, abcam, ab203035)
Anti-FOXP3 antibody (clone: 2A11G9, abcam, ab36607)
Anti-IFN-γ antibody (clone: R4-6A2, santa cruz biotechnology, sc-53700)
Anti-NF-kB p65 antibody (clone: E379, abcam, ab32536)
Phospho-NF-κB p65 (Ser536) Rabbit mAb (clone: 93H1, Cell Signaling Technology, 3033)
Anti-IRF3 antibody (clone: EPR2418Y, abcam, ab68481)
Phospho-IRF-3 (Ser396) Rabbit mAb (clone: D6O1M, Cell Signaling Technology, 29047)
β-Actin Mouse mAb (clone: 8H10D10, Cell Signaling Technology, 3700)
APC anti-mouse/human CD11b antibody (clone: M1/70, Biolegend, 101211)
FITC anti-mouse CD80 antibody (clone: 16-10A1, Biolegend, 104705)
PE anti-mouse CD45 antibody (clone: 30-F11, Biolegend, 103106)
PE anti-mouse CD86 antibody (clone: GL-1, Biolegend, 105007)
FITC anti-mouse Ly-6G/Ly-6C (Gr-1) antibody (clone: RB6-8C5, Biolegend, 108406)
PE anti-mouse CD206 (MMR) antibody (clone: C068C2, Biolegend, 141706)
Alexa Fluor 488 anti-mouse F4/80 antibody (clone: BM8, Biolegend, 123120)
APC anti-mouse/human CD44 antibody (clone: IM7, Biolegend, 103012)
FITC anti-mouse CD62L antibody (clone: MEL-14, Biolegend, 104405)

iNOS Monoclonal Antibody, PE (clone: CXNFT, eBioscience, 12-5920-82)
InVivo Plus anti-mouse PD-L1 antibody (clone 10F.9G2, BioXcell, BP0101)
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488. (Invitrogen, Catalog #A-11008)
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488. (Invitrogen, Catalog #A-11006)
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, FITC. (Invitrogen, Catalog #F-2761)
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, PE-Alexa Fluor 647. (Invitrogen, Catalog #A-20991)
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647. (Invitrogen, Catalog #A-21236)
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Cyanine3 (Invitrogen, Catalog #A10521)
Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP. (Invitrogen, Catalog #31460)
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP. (Invitrogen, Catalog #G-21040)

Validation

Anti-PD-L1 antibody (clone EPR19759, abcam, ab213524); Suitable for: Flow Cyt, WB, IHC-P, ICC/IF, IP; manufacturer's website: https://www.abcam.com/pd-l1-antibody-epr19759-ab213524.html
Anti-CD4 antibody (clone EPR19514, abcam, ab183685); Suitable for: IP, IHC-P, IHC-Fr, WB; manufacturer's website: https://www.abcam.com/cd4-antibody-epr19514-ab183685.html
Anti-CD8 antibody (polyclone, abcam, ab203035);Suitable for: IP, IHC-P, Flow Cyt; manufacturer's website: https://www.abcam.com/cd8-antibody-ab203035.html
Anti-FOXP3 antibody (clone: 2A11G9, abcam, ab36607); Suitable for: ICC/IF, WB, Flow Cyt; manufacturer's website: https://www.abcam.com/foxp3-antibody-2a11g9-ab36607.html
Anti-IFN-γ antibody (clone: R4-6A2, santa cruz biotechnology, sc-53700); Suitable for: ICC/IF, Flow Cyt; manufacturer's website: https://datasheets.scbt.com/sc-53700.pdf
Anti-NF-kB p65 antibody (clone: E379, abcam, ab32536); Suitable for: WB, IHC-P, ICC/IF, IP; manufacturer's website: https://www.abcam.com/nf-kb-p65-antibody-e379-ab32536.html
Phospho-NF-κB p65 (Ser536) Rabbit mAb (clone: 93H1, Cell Signaling Technology, 3033); Suitable for: ICC/IF, WB, Flow Cyt; manufacturer's website: https://www.cellsignal.cn/products/primary-antibodies/phospho-nf-kb-p65-ser536-93h1-rabbit-mab/3033
Anti-IRF3 antibody (clone: EPR2418Y, abcam, ab68481); Suitable for: ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://www.abcam.com/irf3-antibody-epr2418y-ab68481.html
Phospho-IRF-3 (Ser396) Rabbit mAb (clone: D6O1M, Cell Signaling Technology, 29047); Suitable for: ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://www.cellsignal.cn/products/primary-antibodies/phospho-irf-3-ser396-d6o1m-rabbit-mab/29047?site-search-type=Products&N=4294956287&Ntt=29047&fromPage=plp&_requestid=118099
β-Actin Mouse mAb (clone: 8H10D10, Cell Signaling Technology, 3700); Suitable for: ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://www.cellsignal.cn/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700?site-search-type=Products&N=4294956287&Ntt=3700&fromPage=plp&_requestid=118334
APC anti-mouse/human CD11b antibody (clone: M1/70, Biolegend, 101211); Suitable for: Flow Cyt; manufacturer's website: https://www.biolegend.com/en-us/products/apc-anti-mouse-human-cd11b-antibody-345?GroupID=GROUP20
FITC anti-mouse CD80 antibody (clone: 16-10A1, Biolegend, 104705); Suitable for: Flow Cyt; manufacturer's website: https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd80-antibody-41
PE anti-mouse CD45 antibody (clone: 30-F11, Biolegend, 103106); Suitable for: Flow Cyt; manufacturer's website: https://www.biolegend.com/en-us/products/pe-anti-mouse-cd45-antibody-100
PE anti-mouse CD86 antibody (clone: GL-1, Biolegend, 105007); Suitable for: Flow Cyt; manufacturer's website: https://www.biolegend.com/en-us/products/pe-anti-mouse-cd86-antibody-256
FITC anti-mouse Ly-6G/Ly-6C (Gr-1) antibody (clone: RB6-8C5, Biolegend, 108406); Suitable for: Flow Cyt; manufacturer's website: https://www.biolegend.com/en-us/products/fitc-anti-mouse-ly-6g-ly-6c-gr-1-antibody-458
PE anti-mouse CD206 (MMR) antibody (clone: C068C2, Biolegend, 141706); Suitable for: Flow Cyt; manufacturer's website: https://www.biolegend.com/en-us/products/pe-anti-mouse-cd206-mmr-antibody-7424
Alexa Fluor 488 anti-mouse F4/80 antibody (clone: BM8, Biolegend, 123120); Suitable for: Flow Cyt; manufacturer's website: https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-f4-80-antibody-4073
APC anti-mouse/human CD44 antibody (clone: IM7, Biolegend, 103012); Suitable for: Flow Cyt; manufacturer's website: https://www.biolegend.com/en-us/products/apc-anti-mouse-human-cd44-antibody-312
FITC anti-mouse CD62L antibody (clone: MEL-14, Biolegend, 104405); Suitable for: Flow Cyt; manufacturer's website: https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd62l-antibody-384
iNOS Monoclonal Antibody, PE (clone: CXNFT, eBioscience, 12-5920-82); Suitable for: Flow Cyt; manufacturer's website: https://www.thermofisher.com/cn/zh/antibody/product/iNOS-Antibody-clone-CXNFT-Monoclonal/12-5920-82
InVivo Plus anti-mouse PD-L1 antibody (clone 10F.9G2, BioXcell, BP0101); Suitable for: in vivo PDL1 block, ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://bxcell.com/product/m-pdl-1/
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488. (Invitrogen, Catalog #A-11008); Suitable for: ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488. (Invitrogen, Catalog #A-11006); Suitable for: ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11006
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, FITC. (Invitrogen, Catalog #F-2761); Suitable for: ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/F-2761
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, PE-Alexa Fluor 647. (Invitrogen, Catalog #A-20991); Suitable for: ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-20991
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647. (Invitrogen, Catalog #A-21236); Suitable for: ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21236
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Cyanine3, (Invitrogen, Catalog #A10521); Suitable for: ICC/IF, WB, IHC-P, Flow Cyt; manufacturer's website: https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-

Adsorbed-Secondary-Antibody-Polyclonal/A10521
Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP. (Invitrogen, Catalog #31460); Suitable for: ICC/IF, WB, IHC-P; manufacturer's website: https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Rabbit-IgG-H-L-Secondary-Antibody-Polyclonal/31460
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP. (Invitrogen, Catalog #G-21040); Suitable for: WB, IHC-P, ELISA; manufacturer's website: https://www.thermofisher.com/cn/zh/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/G-21040

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The murine colorectal cancer cell lines (MC38 and CT26) were purchased from the China National Infrastructure of Cell Line Resource. The murine breast cancer cell (4T1) and melanoma cell lines (B16F10) were purchased from the American Type Culture Collection (ATCC).
Authentication	Cell lines have been authenticated by short tandem repeat profiling, and the results were compared with reference database.
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 organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	BALB/c mice, BALB/c nude mice and C57BL/6 mice (female, 6-8 weeks age, 16-18 g body weight) were used in our studies. All animals were randomly assigned to the experimental groups, obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and housed with a 12 h light-dark cycle at 22 °C and food and water ad libitum.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal protocols were approved by the Institutional Animal Care and Use Committee of National Center for Xiamen University (ID XMULAC20190150).

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	A 66-Year-old man who diagnosed as lung adenocarcinoma(T2N1M0,IIA).
Recruitment	Tumor specimens from this NSCLC patient who underwent presurgical 18F-FDG PET/CT were selected.
Ethics oversight	The Internal Review and the Ethics Boards of First Affiliated Hospital of Xiamen University (Xiamen, China) approved this study, and written informed consent was obtained from this participant.

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	For flow cytometry analysis of spleens, tumors were harvested and cut into small fragments, and placed in DMEM containing Collagenase IV (1 mg/mL; Gibco, USA), trypsin inhibitor (1 mg/mL; EMD Millipore), and DNase I (2 U/mL; Promega). The fragments were then incubated at 37 °C for 60 min with gentle shaking every 10 min. Specimens were passed through a 70 µm mesh and centrifuged at 350 g for 5 min. Red blood cells were eliminated from the samples with a hypo-osmotic red blood cell lysis buffer (Solarbio). Each sample was fixed with 100 µL of 4% paraformaldehyde for 10 minutes. After that, the

	cells were collected and washed three times with PBS and incubated with 10% goat serum to reduce nonspecific binding. For detecting PD-L1 expression and T cell alteration, preprocessed cells were stained with the corresponding first antibodies.
Instrument	Flow cytometry data was collected on Beckman coulter CytoFLEX (Beckman coulter, USA).
Software	Data was analyzed using FlowJo software version 10.
Cell population abundance	No cell sorting was used.
Gating strategy	Gating strategy for all relevant experiments is indicated in the corresponding main text and Supplementary information.

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