

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input checked="" type="checkbox"/>	<input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input type="checkbox"/>	<input checked="" type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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	TEM and high-resolution TEM (HR-TEM): FEI Talos F200X G2 (Thermo Fisher); Ultraviolet-visible (UV-Vis) absorption spectra: UV-vis spectrophotometer (Lambda 950, Perkin Elmer); AFM: Dimension XR Fast Scan (Bruker); DLS and zeta potential: particle size analyzer (Nano-ZS); Circular dichroism: Chirascan VX (Applied Photophysics); Thermal imaging camera: FLuke Ti27; In-vivo Imaging: IVIS Spectrum System (Perkin Elmer); Flow cytometry: Cytex NL-CLC3000; Immunohistochemical and immunofluorescence imaging: 3DHISTECH; Western blotting imaging: ImageQuant LAS 500 (GE)
Data analysis	Statistical analyses were performed using GraphPad Prism software. FlowJo was used to process all the flow cytometry data. LivingImage software was used to process mouse images. SmartView was used to process photothermal images. ImageJ was used to process all the western blotting, immunohistochemical and immunofluorescence 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](#)

Data Availability. The authors declare that all the data supporting the findings of this study are available within the article and its Supplementary Information files or from the corresponding author upon reasonable 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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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](https://www.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

Data exclusions

Replication

Randomization

Blinding

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

The following antibody was used for in vivo PD-L1 blockade and treatment. It is listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.

1) InVivo anti-mouse PD-L1 (B7-H1) (BioXCell, mAb, #BE0101)

The following antibodies were used for western blotting. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.

- 1) Anti-HSP70 antibody (Servicebio, Rabbit pAb, GB11241, 1:1000 dilution)
- 2) Anti-p-mTOR antibody (Abclonal, Rabbit mAb, AP0115, 1:1000 dilution)
- 3) Anti-mTOR antibody (Abclonal, Rabbit pAb, A23168, 1:1000 dilution)
- 4) Anti-p-P70S6K antibody (Abclonal, Rabbit mAb, AP1389, 1:1000 dilution)
- 5) Anti-P70S6K antibody (Abclonal, Rabbit mAb, A4898, 1:1000 dilution)
- 6) Anti-p-S6 antibody (Abclonal, Rabbit pAb, AP0538, 1:1000 dilution)
- 7) Anti-S6 antibody (Abclonal, Rabbit mAb, A11874, 1:1000 dilution)
- 8) Anti-p-4EBP1 antibody (Abclonal, Rabbit mAb, AP1363, 1:1000 dilution)
- 9) Anti-4EBP1 antibody (Abclonal, Rabbit mAb, A24691, 1:1000 dilution)
- 10) Anti-p-AKT antibody (Abclonal, Rabbit mAb, AP0637, 1:1000 dilution)
- 11) Anti-AKT antibody (Servicebio, Rabbit pAb, GB13427, 1:1000 dilution)
- 12) Anti-Actin antibody (Servicebio, Mouse mAb, GB15001, 1:3000 dilution)
- 13) HRP conjugated Goat Anti-Rabbit IgG (Servicebio, GB23303, 1:10000 dilution)
- 14) HRP conjugated Goat Anti-Mouse IgG (Servicebio, GB23301, 1:10000 dilution)

The following antibodies were used for immunohistochemical staining and immunofluorescence staining. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.

- 1) Anti-Calreticulin (CRT) antibody (Servicebio, Rabbit pAb, GB112134, 1:250 dilution)
- 2) Anti-HSP70 antibody (Servicebio, Rabbit pAb, GB11241, 1:500 dilution)
- 3) Anti-HMGB1 antibody (Servicebio, Rabbit pAb, GB11103, 1:300 dilution)
- 4) Anti-Cleaved caspase-3 (Servicebio, Rabbit pAb, GB11532, 1:500 dilution)
- 5) Anti-Ki67 (Servicebio, Mouse mAb, GB121141, 1:300 dilution)
- 6) HRP conjugated Goat Anti-Rabbit IgG (Servicebio, GB23303, 1:1000 dilution)
- 7) HRP conjugated Goat Anti-Mouse IgG (Servicebio, GB23301, 1:1000 dilution)
- 8) Cy3-conjugated goat anti-rabbit IgG (Servicebio, GB21303, 1:300 dilution)
- 9) Alexa Fluor® 488-conjugated goat anti-rabbit IgG (Servicebio, GB25303, 1:300 dilution)

The following antibodies were used for flow cytometry. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.

- 1) BB515 rat anti-mouse CD45 antibody (BD, 564590, 1:100 dilution)
- 2) APC/Fire™ 750 anti-mouse CD3 antibody (BioLegend, 100248, 1:100 dilution)
- 3) APC anti-mouse CD4 antibody (BioLegend, 100412, 1:100 dilution)
- 4) PE/Cyanine7 anti-mouse CD8a antibody (BioLegend, 100722, 1:100 dilution)
- 5) APC/Cyanine7 anti-mouse CD11c antibody (BioLegend, 117324, 1:100 dilution)
- 6) PE anti-mouse CD80 antibody (BioLegend, 104708, 1:100 dilution)
- 7) Brilliant Violet 421™ anti-mouse CD86 antibody (BioLegend, 105032, 1:100 dilution)
- 8) APC anti-mouse I-A/I-E antibody (BioLegend, 107614, 1:100 dilution)
- 9) PE anti-mouse/human CD11b antibody (BioLegend, 101208, 1:100 dilution)
- 10) PE/Cyanine7 anti-mouse Ly-6G/Ly-6C (Gr-1) antibody (BioLegend, 108416, 1:100 dilution)
- 11) PE anti-mouse CD25 antibody (BioLegend, 102007, 1:100 dilution)
- 12) FOXP3 monoclonal antibody (FJK-16s), PerCP-Cyanine5.5 (Thermo Fisher Scientific, 45-5773-82, 1:100 dilution)
- 13) PE/Cyanine7 anti-mouse/human CD44 antibody (BioLegend, 103030, 1:100 dilution)
- 14) APC anti-mouse CD62L antibody (BioLegend, 104412, 1:100 dilution)
- 15) PE anti-mouse CD4 antibody (BioLegend, 116005, 1:100 dilution)
- 16) TruStain FcX™ (anti-mouse CD16/32) antibody (BioLegend, 101320, 1:100 dilution)

Validation

All antibodies were verified by the supplier and have been quality tested. All validation statements can be found in the respective antibody website:

1) InVivo anti-mouse PD-L1 (B7-H1): <https://bioxcell.com/invivomab-anti-mouse-pd-l1-b7-h1-be0101>

- 2) Anti-HSP70 antibody: <https://www.servicebio.cn/goodsdetail?id=1465>
- 3) Anti-p-mTOR antibody: <https://abclonal.com.cn/catalog/AP0115>
- 4) Anti-mTOR antibody: A23168 is currently discontinued.
- 5) Anti-p-P70S6K antibody: <https://abclonal.com.cn/catalog/AP1389>
- 6) Anti-P70S6K antibody: <https://abclonal.com.cn/catalog/A4898>
- 7) Anti-p-S6 antibody: <https://abclonal.com.cn/catalog/AP0538>
- 8) Anti-S6 antibody: <https://abclonal.com.cn/catalog/A11874>
- 9) Anti-p-4EBP1 antibody: <https://abclonal.com.cn/catalog/AP1363>
- 10) Anti-4EBP1 antibody: <https://abclonal.com.cn/catalog/A24691>
- 11) Anti-p-AKT antibody: <https://abclonal.com.cn/catalog/AP0637>
- 12) Anti-AKT antibody: <https://www.servicebio.cn/goodsdetail?id=5144>
- 13) Anti-Actin antibody: <https://www.servicebio.cn/goodsdetail?id=6718>
- 14) HRP conjugated Goat Anti-Rabbit IgG: <https://www.servicebio.cn/goodsdetail?id=266>
- 15) HRP conjugated Goat Anti-Mouse IgG: <https://www.servicebio.cn/goodsdetail?id=264>
- 16) Anti-Calreticulin (CRT) antibody: <https://www.servicebio.cn/goodsdetail?id=5108>
- 17) Anti-HMGB1 antibody: <https://www.servicebio.cn/goodsdetail?id=1383>
- 18) Anti-Cleaved caspase-3 antibody: <https://www.servicebio.cn/goodsdetail?id=1271>
- 19) Anti-Ki67 antibody: <https://www.servicebio.cn/goodsdetail?id=6801>
- 20) Cy3-conjugated goat anti-rabbit IgG: <https://www.servicebio.cn/goodsdetail?id=253>
- 21) Alexa Fluor® 488-conjugated goat anti-rabbit IgG: <https://www.servicebio.cn/goodsdetail?id=273>
- 22) BB515 rat anti-mouse CD45 antibody: <https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bb515-rat-anti-mouse-cd45.564590>
- 23) APC/Fire™ 750 anti-mouse CD3 antibody: <https://www.biolegend.com/en-gb/products/apc-fire-750-anti-mouse-cd3-antibody-13052>
- 24) APC anti-mouse CD4 antibody: <https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd4-antibody-245>
- 25) PE/Cyanine7 anti-mouse CD8a antibody: <https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-mouse-cd8a-antibody-1906>
- 26) APC/Cyanine7 anti-mouse CD11c antibody: <https://www.biolegend.com/en-gb/products/apc-cyanine7-anti-mouse-cd11c-antibody-3931>
- 27) PE anti-mouse CD80 antibody: <https://www.biolegend.com/en-gb/products/pe-anti-mouse-cd80-antibody-43>
- 28) Brilliant Violet 421™ anti-mouse CD86 antibody: <https://www.biolegend.com/en-gb/products/brilliant-violet-421-anti-mouse-cd86-antibody-7282>
- 29) APC anti-mouse I-A/I-E antibody: <https://www.biolegend.com/en-gb/products/apc-anti-mouse-i-a-i-e-antibody-2488>
- 30) PE anti-mouse/human CD11b antibody: <https://www.biolegend.com/en-gb/products/pe-anti-mouse-human-cd11b-antibody-349>
- 31) PE/Cyanine7 anti-mouse Ly-6G/Ly-6C (Gr-1) antibody: <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-ly-6g-ly-6c-gr-1-antibody-1931>
- 32) PE anti-mouse CD25 antibody: <https://www.biolegend.com/en-gb/products/pe-anti-mouse-cd25-antibody-242>
- 33) FOXP3 monoclonal antibody (FJK-16s), PerCP-Cyanine5.5: <https://www.thermofisher.cn/cn/zh/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/45-5773-82>
- 34) PE/Cyanine7 anti-mouse/human CD44 antibody: <https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-mouse-human-cd44-antibody-3932>
- 35) APC anti-mouse CD62L antibody: <https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd62l-antibody-381>
- 36) PE anti-mouse CD4 antibody: <https://www.biolegend.com/en-gb/products/pe-anti-mouse-cd4-antibody-2499>
- 37) TruStain FcX™ (anti-mouse CD16/32) antibody: <https://www.biolegend.com/en-gb/products/trustain-fcx-anti-mouse-cd16-32-antibody-5683>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	CT26-Luc cells (luciferase-expressing mouse colorectal cancer cells) were sourced from the Key Laboratory of Molecular Imaging, Institute of Automation, Chinese Academy of Sciences (Beijing, China).
Authentication	The cell lines used in this study have been authenticated by STR (Short Tandem Repeat) profiling, with certification reports from accredited institutions.
Mycoplasma contamination	Cells were routinely screened for free of Mycoplasma contaminations. All cell lines are Mycoplasma negative with this study.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

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	BALB/c mice and NTG mice (NOD-scid IL2Rγ ^{-/-}), both 6 weeks old, were obtained from SPF Biotechnology Co. Ltd. (Beijing, China). Animals were kept in a specific pathogen-free (SPF) environment at 20 ± 3 °C with a 12-hour light/dark cycle and free access to food and water. Animal procedures followed the guidelines for ethical research use set by Peking Union Medical College Hospital.
Wild animals	The study did not involve wild animals.

Reporting on sex	This study did not involve sex.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	Animal procedures followed the guidelines for ethical research use set by Peking Union Medical College Hospital (permit number: XHDW-2022-016).

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

Plants

Seed stocks	This study did not involve seed stocks.
Novel plant genotypes	This study did not involve novel plant genotypes.
Authentication	This study did not involve the authentication of 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-infiltrating immune cells and spleen single cells were isolated and prepared as follows: After anesthetizing and euthanizing the mice, tumor tissues were excised and placed in sterile PBS containing 5% FBS. The tissues were weighed, minced with surgical scissors, and digested with a digestion solution (5 times the tissue volume) containing 10% FBS, 0.5 mg/mL Collagenase IV, 0.15 mg/mL DNase I, and RPMI 1640 at 37°C for 1 h, with gentle shaking every 10 min. After digestion, 10 mL of RPMI 1640 medium with 10% FBS was added to terminate the digestion. The mixture was centrifuged at 300g for 5 min, and the resulting pellet was resuspended in RPMI 1640 containing 10% FBS and filtered through a 70 µm cell strainer to remove large tissue fragments. The suspension was then centrifuged again at 300g for 5 min, and the pellet was resuspended in PBS containing 1% FBS, with the final cell concentration adjusted to 10 ⁷ cells/mL for subsequent analysis. For spleen single-cell preparation, the spleen was excised under sterile conditions, and any surrounding fat tissue was removed. The spleen was placed in sterile PBS containing 5% FBS and minced in a Petri dish. The tissue was transferred to a 70 µm cell strainer and homogenized using a syringe plunger in 4 mL of PBS (containing 10% FBS) until completely filtered through the nylon mesh. The resulting cell suspension was centrifuged at 300g for 5 min, and the supernatant was discarded. To lyse red blood cells, 5 mL of 1x RBC lysis buffer was added and incubated at room temperature for 10 min. The suspension was then centrifuged again at 300g for 5 min, and the pellet was resuspended in 5 mL of sterile PBS containing 10% FBS. After a final centrifugation step, the pellet was resuspended in PBS containing 1% FBS, and the cell concentration was adjusted to 10 ⁷ cells/mL for further use.
Instrument	Cytek NL-CLC3000
Software	Flowjo_V10.8.1
Cell population abundance	No sorting was performed.
Gating strategy	Gating was first based on FSC/SSC and singlet cells were gated for further analysis. The cell populations were then analyzed based on expression of markers. Gating was then based on positive levels. The detailed gating strategies are shown in Supplementary Fig. 18.

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