

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <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>
☒	☐ 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 <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	Zeiss Zen 2012 Black Edition for confocal microscopy; BD FACSAria v6.1.3 or Beckman CytoFLEX S for flow cytometry and cell sorting; BGISEQ-500 for bulk RNA-seq; 10x Genomics 3' v3.1 and Illumina NovaSeq 6000 platform for scRNA-seq.
Data analysis	GraphPad Prism (v8.0.2) for data analysis and statistics; FlowJo (v10.8.1, Treestar) and CytExpert (v2.4, Beckman) for flow cytometry; Olympus VS2000 for HE images; Olympus VS2000, Zeiss Zen 2012 Blue Edition (Zeiss) and CaseViewer (v2.1, 3D Histech) for confocal microscopy images; ImageStudio (v5.2, Li-Cor) for the Odyssey infrared images; Fiji Image J (v2.1.0/1.53c, Freeware/NIH) for fluorescent image quantification; Nano measurer (v1.2, Fudan University) for measurement of the average size of colonies; Cell Ranger (v3.0.1, 10x Genomics), Seurat R (v4.0), Scrublet (v0.2.3), TopGO (v2.44.0, Bioconductor), CytoTRACE (v0.3.3) and R (v3.6.3, The R Project for Statistical Computing) for scRNA-seq data.

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 bulk RNA-seq and scRNA-seq data generated in this study have been deposited in the GEO database under accession code GSE285529 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE285529>] and GSE282386 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE282386>], respectively. 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	Sex or gender was not considered in the study design.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, or other socially relevant groupings was not used in the study.
Population characteristics	This study included specimens of primary and metastatic melanoma.
Recruitment	Specimens with diagnosis of melanoma were randomly selected from the hospital biobank. There was no self-selection bias in this study.
Ethics oversight	The protocol was approved by the Institutional Review Board of Renji Hospital, Shanghai Jiao Tong University School of Medicine.

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	No statistical method was used to predetermine sample size. All sample sizes were rendered sufficient statistical power for comparisons.
Data exclusions	In single-cell RNA-seq analysis, to ensure quality control metrics, cells detected with fewer than 200 genes or more than 10% mitochondrial reads or more than 10% red cell reads were removed as described in Methods section.
Replication	Data supporting the conclusions of the study are presented as means $\pm$ s.e.m., with at least three independent biological replicates per experimental group. All attempts at replication were successful. This was indicated in the Figure legends.
Randomization	Human specimens with diagnosis of melanoma were randomly selected from the hospital biobank. The samples in other experiments were divided into different groups randomly.
Blinding	The investigators were blinded to group allocation during 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
☒	☐ 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

Anti-mouse CD11c PE (clone N418, Invitrogen, cat. 12-0114-82, lot. E01079-1633, 1/80 dilution)
 Anti-mouse Ly-6G/Ly-6C PE (clone RB6-8C5, Invitrogen, cat. 12-5931-81, lot. 4335548, 1/600 dilution)
 Anti-mouse CD11b FITC (clone M1/70, Invitrogen, cat. 11-0112-82, lot. 2172513, 1/200 dilution)
 Anti-mouse F4/80 FITC (clone BM8, Invitrogen, cat. 11-4801-85, lot. 4318484, 1/200 dilution)
 Anti-mouse CD3 PerCP-Cyanine5.5 (clone 145-2C11, Invitrogen, cat. 45-0031-80, lot. 4304568, 1/40 dilution)
 Anti-mouse CD4 PE (clone GK1.5, Invitrogen, cat. 12-0041-83, lot. E01010-1636, 1/300 dilution)
 Anti-mouse CD8 FITC (clone 53-6.7, Invitrogen, cat. 11-0081-85, lot. 4271604, 1/200 dilution)
 Anti-mouse CD19 PE (clone eBio1D3, Invitrogen, cat. 12-0193-81, lot. 1946584, 1/300 dilution)
 Anti-mouse NK1.1 FITC (clone PK136, Invitrogen, cat.11-5941-82, lot. E00742-1632, 1/200 dilution)
 Anti-mouse PD-L1 PE (clone MIH5, Invitrogen, cat. 12-5982-82, lot. 4276912, 1/600 dilution)
 Anti-mouse CD16/32 antibody (clone 93, Invitrogen, cat. 14-0161-82, lot. E03557-1634, 1/100 dilution)
 Anti-mouse CD133 APC (clone 315-2C11, BioLegend, cat. 141207, lot. B232568, 1/80 dilution)
 Anti-mouse CD133 PE (clone 315-2C11, BioLegend, cat. 141203, lot. B262124, 1/20 dilution)
 Anti-mouse PD-L1 PE-Cy7 (clone 10F.9G2, BioLegend, cat. 124314, lot. B261299, 1/80 dilution)
 Anti-mouse α SMA PE (clone 1A4/asm-1, Novus Biologicals, cat. NBP2-34522PE, lot. 59-1PABX170905-021519-PE, 1/150 dilution)
 Anti-mouse TGF- β BV421 (clone TW7-16B4, BD Biosciences, cat. 565638, lot. 8085548, 1/20 dilution)
 Anti-mouse IL-10 PerCP-Cy5.5 (clone JES5-16E3, Invitrogen, cat. 45-7101-82, lot. 2791188, 1/80 dilution)
 Anti-mouse VEGF- α APC (clone VG1, Novus Biologicals, cat. NB100-664APC, lot. F-2-080218-APC, 1/100 dilution)
 Rabbit anti-CRT antibody (clone EPR3924, Abcam, cat. ab92516, lot. GR3252549-1, 1/50 dilution)
 Anti-mouse CD45 APC (clone 30-F11, Invitrogen, cat. 17-0451-82, lot. 2504481, 1/300 dilution)
 Anti-mouse CD45 PE (clone 30-F11, Invitrogen, cat. 12-0451-82, lot. E01244-1635, 1/1200 dilution)
 Anti-mouse CD4 FITC (clone RM4-4, Invitrogen, cat. 11-0043-82, lot. 2407411, 1/400 dilution)
 Anti-mouse CD25 APC (clone PC61.5, Invitrogen, cat. 17-0251-81, lot. E07105-1640, 1/300 dilution)
 Anti-mouse CD45 PE-Cyanine5 (clone 30-F11, Invitrogen, cat. 15-0451-82, lot. 2785857, 1/600 dilution)
 Anti-mouse CD11b PerCP-Cyanine5.5 (clone M1/70, Invitrogen, cat. 45-0112-82, lot. 2563995, 1/160 dilution)
 Anti-mouse Gr-1 APC (clone 1A8-Ly6g, Invitrogen, cat. 17-9668-82, lot. 4286053, 1/80 dilution)
 Anti-mouse CD44 PerCP-Cyanine5.5 (clone IM7, Invitrogen, cat. 45-0441-82, lot. 1984139, 1/160 dilution)
 Anti-mouse CD62L APC (clone MEL-14, Invitrogen, cat. 17-0621-81, lot. 4300018, 1/600 dilution)
 Anti-mouse Granzyme B PE (clone NGZB, Invitrogen, cat. 12-8898-80, lot. 2295595, 1/300 dilution)
 Anti-mouse IFN- γ PerCP-Cyanine5.5 (clone XMG1.2, Invitrogen, cat. 45-7311-80, lot. 4329723, 1/160 dilution)
 Anti-mouse Foxp3 PerCP-Cyanine5.5 (clone FJK-16s, Invitrogen, cat. 45-5773-82, lot. 4295813, 1/160 dilution)
 Anti-mouse CD271 PE (clone ME20.4, cat. 12-9400-42, Invitrogen, lot. 2618940, 1/20 dilution)
 Anti-mouse ABCG2 APC (clone S17006D, cat. 164303, BioLegend, lot. B393993, 1/20 dilution)
 Anti-human CD68 FITC (clone Y1/82A, BioLegend, cat. 333806, lot. B281870, 1/20 dilution)
 Anti-human CD86 PE/Cyanine5 (clone IT2.2, BioLegend, cat. 305408, lot. B284508, 1/20 dilution)
 Anti-human CD163 APC (clone GHI/61, BioLegend, cat. 333610, lot. B311341, 1/20 dilution)
 Anti-human HLA-DR PE (clone L243, BioLegend, cat.307606, lot. B340727, 1/20 dilution)
 Anti-human HLA-A,B,C FITC (clone W6/32, BioLegend, cat. 311404, lot. B281620, 1/20 dilution)
 Anti-human SIRP α APC (clone 15-414, BioLegend, cat. 372105, lot. B316544, 1/20 dilution)
 Anti-human CD133 PE (clone TMP4, Invitrogen, cat. 12-1338-42, lot. 2633386, 1/20 dilution)
 Anti-human PD-L1 APC (clone MIH1, Invitrogen, cat. 17-5983-42, lot. 2764014, 1/20 dilution)
 Anti-human CD8 FITC (clone RPA-T8, Invitrogen, cat. 11-0088-42, lot. 2247551, 1/20 dilution)
 Anti-human CD69 PE (clone FN50, Invitrogen, cat. 12-0699-42, lot. 2264967, 1/20 dilution)
 Anti-human TGF- β PE-Cyanine7 (clone S20006A, BioLegend, cat. 300007, lot. B404652, 1/20 dilution)
 Mouse anti-alpha-Smooth Muscle Actin (clone 1A4, Boster, cat. BM0002, lot. 23254K14, 1/1000 dilution)
 Rabbit anti-Collagen II (Boster, cat. BA0533, lot. 13B121, 1/200 dilution)
 Rabbit anti-Collagen IV (Boster, cat. BA3626, lot. 11D891, 1/200 dilution)
 Mouse anti-CD133 (clone CMab-43, Abcam, cat. ab264538, lot. GR3344038-2, 1/2000 dilution)
 Rat anti-PD-L1 (clone CAL10, Abcam, cat. ab279294, lot. GR3383390-1, 1/800 dilution)
 Anti-mouse PD-L1 (clone 10F.9G2, BioXCell, cat. BE0101, lot. 665717A2, 200 μ g/mice)
 Anti-mouse PD-1 (clone RMP1-14, BioXCell, cat. BE0146, lot. 861023J2, 200 μ g/mice)
 Anti-mouse CD8 (clone 2.43, BioXCell, cat. BE0061, lot. 86882301, 200 μ g/mice)

Validation

All antibodies used were commercially available and were validated by the manufacturers. Validation statements are available on the manufacturer's website.
 Anti-mouse CD11c PE (clone N418, Invitrogen, cat. 12-0114-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD11c-Antibody-clone-N418-Monoclonal/12-0114-82>
 Anti-mouse Ly-6G/Ly-6C PE (clone RB6-8C5, Invitrogen, cat. 12-5931-81)

<https://www.thermofisher.cn/cn/zh/antibody/product/Ly-6G-Ly-6C-Antibody-clone-RB6-8C5-Monoclonal/12-5931-81>
 Anti-mouse CD11b FITC (clone M1/70, Invitrogen, cat. 11-0112-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/11-0112-82>
 Anti-mouse F4/80 FITC (clone BM8, Invitrogen, cat. 11-4801-85)
<https://www.thermofisher.cn/cn/zh/antibody/product/F4-80-Antibody-clone-BM8-Monoclonal/11-4801-85>
 Anti-mouse CD3 PerCP-Cyanine5.5 (clone 145-2C11, Invitrogen, cat. 45-0031-80)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD3e-Antibody-clone-145-2C11-Monoclonal/45-0031-80>
 Anti-mouse CD4 PE (clone GK1.5, Invitrogen, cat. 12-0041-83)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD4-Antibody-clone-GK1-5-Monoclonal/12-0041-83>
 Anti-mouse CD8 FITC (clone 53-6.7, Invitrogen, cat. 11-0081-85)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD8a-Antibody-clone-53-6-7-Monoclonal/11-0081-85>
 Anti-mouse CD19 PE (clone eBio1D3, Invitrogen, cat. 12-0193-81)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD19-Antibody-clone-eBio1D3-1D3-Monoclonal/12-0193-81>
 Anti-mouse NK1.1 FITC (clone PK136, Invitrogen, cat. 11-5941-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/NK1-1-Antibody-clone-PK136-Monoclonal/11-5941-82>
 Anti-mouse PD-L1 PE (clone MIH5, Invitrogen, cat. 12-5982-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD274-PD-L1-B7-H1-Antibody-clone-MIH5-Monoclonal/12-5982-82>
 Anti-mouse CD16/32 antibody (clone 93, Invitrogen, cat. 14-0161-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD16-CD32-Antibody-clone-93-Monoclonal/14-0161-82>
 Anti-mouse CD133 APC (clone 315-2C11, BioLegend, cat. 141207)
<https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd133-antibody-7243>
 Anti-mouse CD133 PE (clone 315-2C11, BioLegend, cat. 141203)
<https://www.biolegend.com/en-gb/products/pe-anti-mouse-cd133-antibody-7066>
 Anti-mouse PD-L1 PE-Cy7 (clone 10F.9G2, BioLegend, cat. 124314)
<https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-mouse-cd274-b7-h1-pd-l1-antibody-6721>
 Anti-mouse α SMA PE (clone 1A4/asm-1, Novus Biologicals, cat. NBP2-34522PE)
https://www.novusbio.com/products/alpha-smooth-muscle-actin-antibody-1a4-asm-1_nbp2-34522pe#datasheet
 Anti-mouse TGF- β BV421 (clone TW7-16B4, BD Biosciences, cat. 565638)
<https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv421-mouse-anti-mouse-lap.565638>
 Anti-mouse IL-10 PerCP-Cy5.5 (clone JES5-16E3, Invitrogen, cat. 45-7101-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/IL-10-Antibody-clone-JES5-16E3-Monoclonal/45-7101-82>
 Anti-mouse VEGF- α APC (clone VG1, Novus Biologicals, cat. NB100-664APC)
https://www.novusbio.com/products/vegf-antibody-vg1_nb100-664apc
 Rabbit anti-CRT antibody (clone EPR3924, Abcam, cat. ab92516)
<https://www.abcam.cn/products/primary-antibodies/calreticulin-antibody-epr3924-er-marker-ab92516.html>
 Anti-mouse CD45 APC (clone 30-F11, Invitrogen, cat. 17-0451-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/17-0451-82>
 Anti-mouse CD45 PE (clone 30-F11, Invitrogen, cat. 12-0451-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/12-0451-82>
 Anti-mouse CD4 FITC (clone RM4-4, Invitrogen, cat. 11-0043-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD4-Antibody-clone-RM4-4-Monoclonal/11-0043-82>
 Anti-mouse CD25 APC (clone PC61.5, Invitrogen, cat. 17-0251-81)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD25-Antibody-clone-PC61-5-Monoclonal/17-0251-81>
 Anti-mouse CD45 PE-Cyanine5 (clone 30-F11, Invitrogen, cat. 15-0451-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/15-0451-82>
 Anti-mouse CD11b PerCP-Cyanine5.5 (clone M1/70, Invitrogen, cat. 45-0112-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/45-0112-82>
 Anti-mouse Gr-1 APC (clone 1A8-Ly6g, Invitrogen, cat. 17-9668-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/Ly-6G-Antibody-clone-1A8-Ly6g-Monoclonal/17-9668-82>
 Anti-mouse CD44 PerCP-Cyanine5.5 (clone IM7, Invitrogen, cat. 45-0441-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD44-Antibody-clone-IM7-Monoclonal/45-0441-82>
 Anti-mouse CD62L APC (clone MEL-14, Invitrogen, cat. 17-0621-81)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD62L-L-Selectin-Antibody-clone-MEL-14-Monoclonal/17-0621-81>
 Anti-mouse Granzyme B PE (clone NGZB, Invitrogen, cat. 12-8898-80)
<https://www.thermofisher.cn/cn/zh/antibody/product/Granzyme-B-Antibody-clone-NGZB-Monoclonal/12-8898-80>
 Anti-mouse IFN- γ PerCP-Cyanine5.5 (clone XMG1.2, Invitrogen, cat. 45-7311-80)
<https://www.thermofisher.cn/cn/zh/antibody/product/IFN-gamma-Antibody-clone-XMG1-2-Monoclonal/45-7311-80>
 Anti-mouse Foxp3 PerCP-Cyanine5.5 (clone FJK-16s, Invitrogen, cat. 45-5773-82)
<https://www.thermofisher.cn/cn/zh/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/45-5773-82>
 Anti-mouse CD271 PE (clone ME20.4, cat. 12-9400-42, Invitrogen)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD271-NGF-Receptor-Antibody-clone-ME20-4-Monoclonal/12-9400-42>
 Anti-mouse ABCG2 APC (clone S17006D, cat. 164303, BioLegend)
<https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd338-abcg2-antibody-23398>
 Anti-human CD68 FITC (clone Y1/82A, BioLegend, cat. 333806)
<https://www.biolegend.com/en-gb/products/fitc-anti-human-cd68-antibody-4844>
 Anti-human CD86 PE/Cyanine5 (clone IT2.2, BioLegend, cat. 305408)
<https://www.biolegend.com/en-gb/products/pe-cyanine5-anti-human-cd86-antibody-779>
 Anti-human CD163 APC (clone GHI/61, BioLegend, cat. 333610)
<https://www.biolegend.com/en-gb/products/apc-anti-human-cd163-antibody-6276>
 Anti-human HLA-DR PE (clone L243, BioLegend, cat. 307606)
<https://www.biolegend.com/en-gb/products/pe-anti-human-hla-dr-antibody-790>
 Anti-human HLA-A,B,C FITC (clone W6/32, BioLegend, cat. 311404)
<https://www.biolegend.com/en-gb/products/fitc-anti-human-hla-a-b-c-antibody-1871>
 Anti-human SIRP α APC (clone 15-414, BioLegend, cat. 372105)
<https://www.biolegend.com/en-gb/products/apc-anti-human-cd172a-sirpalph-antibody-14165>

Anti-human CD133 PE (clone TMP4, Invitrogen, cat. 12-1338-42)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD133-Prominin-1-Antibody-clone-TMP4-Monoclonal/12-1338-42>
 Anti-human PD-L1 APC (clone MIH1, Invitrogen, cat. 17-5983-42)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD274-PD-L1-B7-H1-Antibody-clone-MIH1-Monoclonal/17-5983-42>
 Anti-human CD8 FITC (clone RPA-T8, Invitrogen, cat. 11-0088-42)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD8a-Antibody-clone-RPA-T8-Monoclonal/11-0088-42>
 Anti-human CD69 PE (clone FN50, Invitrogen, cat. 12-0699-42)
<https://www.thermofisher.cn/cn/zh/antibody/product/CD69-Antibody-clone-FN50-Monoclonal/12-0699-42>
 Anti-human TGF- β PE-Cyanine7 (clone S20006A, BioLegend, cat. 300007)
<https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-human-lap-tgf-beta1-antibody-21121>
 Mouse anti-alpha-Smooth Muscle Actin (clone 1A4, Boster, cat. BM0002)
http://boster.com/index/products/productsDetail?goods_sn=BM0002
 Rabbit anti-Collagen II (Boster, cat. BA0533)
http://boster.com/index/products/productsDetail?goods_sn=BA0533
 Rabbit anti-Collagen IV (Boster, cat. BA3626)
http://boster.com/index/products/productsDetail?goods_sn=BA3626
 Mouse anti-CD133 (clone CMab-43, Abcam, cat. ab264538)
<https://www.abcam.cn/products/primary-antibodies/cd133-antibody-cmab-43-ab264538.html>
 Rat anti-PD-L1 (clone CAL10, Abcam, cat. ab279294)
<https://www.abcam.cn/products/primary-antibodies/pd-l1-antibody-cal10-rat-igg2a-chimeric-ab279294.html>
 Anti-mouse PD-L1 (clone 10F.9G2, BioXCell, cat. BE0101)
<https://bioxccl.com/invivomab-anti-mouse-pd-l1-b7-h1-be0101>
 Anti-mouse PD-1 (clone RMP1-14, BioXCell, cat. BE0146)
<https://bioxccl.com/invivomab-anti-mouse-pd-1-cd279-be0146>
 Anti-mouse CD8 (clone 2.43, BioXCell, cat. BE0061)
<https://bioxccl.com/invivomab-anti-mouse-cd8-alpha-be0061>

Eukaryotic cell lines

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

Cell line source(s)	Mouse melanoma cell line B16F10 (cat. CRL-6475), mouse embryonic fibroblast cell line NIH3T3 (cat. CRL-1658) and human melanoma cell line A375 (cat. CRL-1619) were obtained from American Type Culture Collection (ATCC). CT26-Luc (CT26.WT-Fluc-Neo) cell line was obtained from Imanis Life Sciences.
Authentication	Cell lines were authenticated by the supplier. Morphology check by microscope and growth curve analysis were performed periodically.
Mycoplasma contamination	The cells in the experiment were free of mycoplasma contamination.
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	Female C57BL/6 mice (6-8 weeks old) were ordered from Sino-British Sippr/BK Lab Animal Inc. (Shanghai, China). BALB/c mice (male, 18-20 g) were purchased from Shanghai Linchang Biological Co. Ltd. (Shanghai, China). Male and female B-NDG Il2rg ^{-/-} /hIL3-hGMCSF-hCSF mice (6-8 weeks old) were purchased from Biocytogen Inc. (Beijing, China). The mice were allowed to adapt to the environment for one week before the experiments, and housed under specific pathogen free conditions with free access to food and water.
Wild animals	The study did not involve wild animals.
Reporting on sex	The findings was not sex-specific and no sex-based analysis was performed.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	All the animal experiments were performed in compliance with the guidelines established by Institutional Animal Care and Use Committee (IACUC) of School of Pharmacy, Fudan University.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	The tumors of the mice were mechanically dissociated and digested in the Accutax Cell Detachment Solution (Merck, SCR006) at room temperature for 45 min in the shaking incubator (150 r.p.m.). The spleens of the mice were directly homogenized by mechanical disaggregation, filtered through a 40 µm nylon cell strainer, centrifuged, and lysed by the red blood cell lysis buffer. Before staining, the obtained single cell suspension was incubated with an Fc blocker, anti-mouse CD16/32 antibody (Invitrogen, cat. 14-0161-82) for 20 min at 4 °C. For the cellular membrane staining, the cells were incubated with the indicated fluorochrome-conjugated antibodies at the manufacturer's recommended dilutions for 30 min on ice in dark. For the intracellular cytokine staining, the cells were incubated with Cell Stimulation Cocktail plus protein transport inhibitors overnight, and then fixed with IC Fixation Buffer (Invitrogen, cat. 00-8222-49) for 20 min at room temperature. The cells were washed twice with permeabilization buffer (Invitrogen, cat. 00-8333-56), and incubated with the intracellular antibodies in the permeabilization buffer for 30 min at room temperature in the dark. For the nuclear staining, the cells were fixed and permeabilized with Foxp3 Fixation/Permeabilization working solution (Invitrogen, cat. 00-5521-00) for 60 min on ice in dark. Washed twice with the permeabilization buffer, the cells were incubated with the nuclear antibody in the permeabilization buffer for 30 min at room temperature in dark.
Instrument	BD FACSAria and Beckman CytoFLEX S.
Software	FlowJo (v10.8.1, Treestar) and CytExpert (v2.4, Beckman).
Cell population abundance	The purity of the sorted tumor cells was evaluated by acquisition of the sorted fractions after the sorting. The purity was generally above 90%.
Gating strategy	Gating strategy started with FSC-A/SSC-A to exclude cell debris, followed by FSC-H/FSC-W and SSC-H/SSC-W (BD FACSAria) or FSC-A/FSC-H (Beckman CytoFLEX S) to confirm singlets. Live cells were selected by gating Live/Dead dye negative cells. Then gating was dependent on clearly distinguished cell population, or comparison with the isotype staining.
☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.	