

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<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                                |
| <input type="checkbox"/>            | <input checked="" 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 checked="" type="checkbox"/> | <input 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<br><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 checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input type="checkbox"/>            | <input checked="" 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 | Roche LightCycler 480 System (Roche), CytoFLEX LX (Beckman Coulter), ZEISS LSM 880 LSM880 confocal microscope equipped with fast airyscan (Carl Zeiss AG), ChemiDoc Touch Imaging System (Bio-Rad) and Spark 10M Multimode Microplate Reader (TECAN).                                                                                                                                                                                                                                                                                                                                                                                                 |
| Data analysis   | Image Lab version 4.1 (Bio-Rad) was used to acquire Immunoblots. Acquired raw images were analyzed using Image J 1.8.0 ( <a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a> ), ZEISS ZEN 2.6 blue edition software (Carl Zeiss AG) and HALO v3.6.4134.193 (India Labs). Flow Cytometry data was analyzed using CytExpert software (Beckman Coulter). JASPAR software was used to predict the STAT1 binding sequence. MaxQuant 2.0.1.0 was used as the mass spectrometry database search software for analyzing the LC-MS/MS results. Statistical analysis was performed with GraphPad Prism version 8.0.2 (GraphPad Software, Inc.). |

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 processed bulk RNA-seq and single-cell RNA-seq data reported in this study have been deposited in the Gene Expression Omnibus (GEO) database with accession number GSE262868 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE262868>) and GSE263524 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE263524>), and the protein mass spectrometry raw data have been uploaded to the PRIDE website (<https://www.ebi.ac.uk/pride/>, project accession: PXD053978). RNA-sequencing expression (level 3) profiles and corresponding clinical information from 33 types of cancer samples and corresponding normal samples were downloaded from the TCGA dataset (<https://portal.gdc.com>). The selection of membrane proteins and secreted proteins is based on the protein annotations provided by The Human Protein Atlas data (<https://www.proteinatlas.org/humanproteome/tissue/secretome>). Three immunotherapy cohorts were collected, including Liu (120 samples), Gide (73 samples) and Van Allen (40 samples). Code used for all processing and analysis is available upon request. All data are included in the Supplementary Information or available from the authors, as are unique reagents used in this Article. The raw numbers for charts and graphs are available in the Source Data file whenever possible.

## 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 patients included males and females, and the information on sex and gender was not relevant in our study.                                                                                                                                                                                                                                                                                                                          |
| Reporting on race, ethnicity, or other socially relevant groupings | N/A                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Population characteristics                                         | A total of 100 pairs of normal versus CRC tissue specimens (40 female, 60 male, average age: $56 \pm 12$ years) and additional 81 CRC tissue specimens (34 female, 47 male, average age: $55 \pm 13$ years) were obtained from Sun Yat-sen University Cancer Center (SYSUCC, Guangzhou, China) between 2002 and 2012.                                                                                                                  |
| Recruitment                                                        | All CRC patient samples were obtained from Sun Yat-sen University Cancer Center (SYSUCC, Guangzhou, China) after appropriate review of our research project to ensure that it was covered under their ethics approval (G2023-172-01). All patient samples were collected by SUSYCC based on availability and informed consent provided by the individuals. Baseline characteristic of samples were balanced among groups without bias. |
| Ethics oversight                                                   | The study protocol was approved by the Institutional Review Board of Sun Yat-sen University Cancer Center (G2023-172-01). Written informed consent was obtained from the patients who provided samples.                                                                                                                                                                                                                                |

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     | We determined sample sizes based on established practices in similar studies and preliminary experiments demonstrating consistent effect sizes. For animal studies, $n=5-10$ per group was selected to ensure statistical power to detect biologically relevant differences while complying with the 3R principles (Reduction). In vitro experiments used $n = 3-5$ biologically independent replicates and were performed in three independent batches with similar results. No formal a priori power analysis was performed, but our sample sizes align with widely align with published standards in the field. |
| Data exclusions | No data were excluded for analysis.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Replication     | For each representative image/data, experiments were performed at least three times with similar results unless otherwise noted in the manuscript.                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Randomization   | All the samples, organisms and participants were randomly allocated into different experimental groups.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

# 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    |                                                                 | Methods                             |                                                    |
|-------------------------------------|-----------------------------------------------------------------|-------------------------------------|----------------------------------------------------|
| n/a                                 | Involved in the study                                           | n/a                                 | Involved in the study                              |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  | <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines       | <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          | <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |
| <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                                 |                                     |                                                    |

## Antibodies

Antibodies used

Immunoblotting  
Anti-MBTPS1 antibody (Invitrogen, PA5-39883), Anti-STAT1 antibody (Abcam, Ab239360), Anti-Phospho-Stat1 (Tyr701) antibody (CST 7649T), Anti-Phospho-Stat1 (Tyr701) antibody (CST 9167T), Anti-USP13 antibody (Proteintech, 16840-1-AP), Anti-Ubiquitin antibody (CST, 3936S), Anti-Tri-Methyl-Histone H3 (Lys27) antibody (CST, 9733), Anti-Di-Methyl-Histone H3 (Lys79) antibody (CST, 5427), Anti-Di-Methyl-Histone H3 (Lys4) antibody (CST, 9725), Anti-Flag-Tag antibody (Sigma, F1804), Anti-HA-Tag antibody (CST, 3724T), Anti-β-Actin antibody (CST, 4967S), Anti-Lamin B1 antibody (Proteintech, 12987-1-AP), Anti-Histone H3 antibody (Proteintech, 17168-1-AP), Anti-α-Tubulin antibody (Abcam, ab7291), Anti-Mouse IgG, HRP-linked antibody (CST, 7076S), Anti-Rabbit IgG, HRP-linked antibody (CST, 7074S).

Immunohistochemistry  
Anti-MBTPS1 antibody (Invitrogen, PA5-101662), Anti-STAT1 antibody (Abcam, Ab239360), Anti-Phospho-Stat1 (Tyr701) antibody (CST 9167T), Anti-human CD8 antibody (Abcam, Ab245118), Anti-mouse CD8 antibody (Abcam, Ab209775).

Immunofluorescence  
Anti-STAT1 antibody (Abcam, Ab239360), Anti-mouse CD8 antibody (Servicebio, GB114196).

Immunoprecipitation  
Anti-STAT1 antibody (Abcam, Ab239360), Anti-Normal rabbit IgG (CST, 2729S).

Chromatin Immunoprecipitation  
Anti-STAT1 antibody (Abcam, Ab239360), Anti-Normal rabbit IgG (CST, 2729S).

Proximity Ligation Assay  
Anti-STAT1 antibody (Abcam, Ab239360), Anti-Flag-Tag antibody (Sigma, F1804), Anti-Normal rabbit IgG (CST, 2729S), Anti-Normal mouse IgG (Santa Cruz, sc2025).

Flow cytometry  
Zombie Aqua (Biolegend, 423102), Zombie UV™ (Biolegend, 423107), PerCP/Cyanine5.5 anti-mouse CD45 (Biolegend, 103132), APC/Fire™ 750 anti-mouse CD45 (Biolegend, 103153), Brilliant Violet 785™ anti-mouse CD3 (Biolegend, 100232), Alexa Fluor® 700 anti-mouse CD8a (Biolegend, 100730), FITC anti-mouse CD4 (Biolegend, 100510), PE/Cyanine7 anti-mouse NK-1.1 Antibody (Biolegend, 108713), PE anti-mouse IFN-γ (Biolegend, 505808), Brilliant Violet 605™ anti-mouse CD183 (CXCR3) (Biolegend, 126523), Brilliant Violet 605™ anti-mouse Ki-67 (Biolegend, 652413), Alexa Fluor® 647 anti-mouse/rat/human FOXP3 (Biolegend, 320013), FITC anti-mouse/human CD11b (Biolegend, 101205), Brilliant Violet 510™ anti-mouse Ly-6C (Biolegend, 128033), Alexa Fluor® 700 anti-mouse Ly-6G (Biolegend, 127621), PE anti-mouse F4/80 Recombinant (Biolegend, 157303), Brilliant Violet 421™ anti-mouse CD206 (MMR) (Biolegend, 141717), Brilliant Violet 650™ anti-mouse CD80 (Biolegend, 104732), APC anti-mouse I-A/I-E (Biolegend, 107613), Brilliant Violet 785™ anti-mouse CD11c (Biolegend, 117336), Brilliant Violet 421™ anti-human/mouse Granzyme B Recombinant (Biolegend, 396413), PE/Dazzle™ 594 anti-mouse CD19 (Biolegend, 115553), PE/Cyanine7 anti-T-bet (Biolegend, 644823), Brilliant Violet 421™ anti-GATA3 (Biolegend, 653813), TruStain FcXTM anti-mouse CD16/32 (Biolegend, 101319), APC anti-mouse H-2Kb (Biolegend, 116517).

Activation  
Anti-mouse CD3ε antibody, functional grade, Clone 145-2C11, Biolegend 100340,  
Anti-mouse CD28 antibody, functional grade, Clone 37.51, Biolegend, 102116.

Neutralization

Mouse-CD8 $\alpha$  antibody (Bioxcell, BE0061), Rat IgG2b isotype control, anti-keyhole limpet hemocyanin (Bioxcell, BE0090), Mouse-CXCR3 antibody (Bioxcell, BE0249), IPolyclonal Armenian hamster IgG (Bioxcell, BE0091), Mouse-PD-1 antibody (Bioxcell, BE0146), Rat IgG2a isotype control, anti-trinitrophenol (Bioxcell, BE0089).

## Validation

All antibodies used in this study have been validated and detailed information was presented on the following web pages:

Anti-MBTPS1 antibody for immunoblotting: <https://www.thermofisher.cn/cn/zh/antibody/product/Phospho-SREBP1-Ser439-Antibody-Polyclonal/PAS-39813>

Anti-STAT1 antibody: <https://www.abcam.cn/products/primary-antibodies/stat1-antibody-epr23049-111-chip-grade-ab239360.html>

Anti-Phospho-Stat1 (Tyr701) antibody: <https://www.cellsignal.cn/products/primary-antibodies/phospho-stat1-tyr701-d4a7-rabbit-mab/7649>

Anti-Phospho-Stat1 (Tyr701) antibody: <https://www.cellsignal.cn/products/primary-antibodies/phospho-stat1-tyr701-58d6-rabbit-mab/9167>

Anti-USP13 antibody: <https://www.ptgcn.com/products/USP13-Antibody-16840-1-AP.htm>

Anti-Ubiquitin antibody: <https://www.cellsignal.cn/products/primary-antibodies/ubiquitin-p4d1-mouse-mab/3936>

Anti-Tri-Methyl-Histone H3 (Lys27) antibody: <https://www.cellsignal.cn/products/primary-antibodies/tri-methyl-histone-h3-lys27-c36b11-rabbit-mab/9733>

Anti-Di-Methyl-Histone H3 (Lys79) antibody: <https://www.cellsignal.cn/products/primary-antibodies/di-methyl-histone-h3-lys79-d15e8-xp-rabbit-mab/5427>

Anti-Di-Methyl-Histone H3 (Lys4) antibody: <https://www.cellsignal.cn/products/primary-antibodies/di-methyl-histone-h3-lys4-c64g9-rabbit-mab/9725>

Anti-Flag-Tag antibody: <https://www.sigmaaldrich.cn/CN/zh/product/sigma/f1804>

Anti-HA-Tag antibody: <https://www.cellsignal.cn/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724>

Anti- $\beta$ -Actin antibody: <https://www.cellsignal.cn/products/primary-antibodies/b-actin-antibody/4967>

Anti-Lamin B1 antibody: <https://www.ptgcn.com/products/LMN1-Antibody-12987-1-AP.htm>

Anti-Histone H3 antibody: <https://www.ptgcn.com/products/Histone-H3-Antibody-17168-1-AP.htm>

Anti- $\alpha$ -Tubulin antibody: <https://www.abcam.cn/products/primary-antibodies/alpha-tubulin-antibody-dm1a-loading-control-ab7291.html>

Anti-Mouse IgG, HRP-linked antibody: <https://www.cellsignal.cn/products/secondary-antibodies/anti-mouse-igg-hrp-linked-antibody/7076>

Anti-Rabbit IgG, HRP-linked antibody: <https://www.cellsignal.cn/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074>

Anti-MBTPS1 antibody for immunohistochemistry: <https://www.thermofisher.cn/cn/zh/antibody/product/MBTPS1-Antibody-Polyclonal/PAS-101662>

Anti-human CD8 antibody: <https://www.abcam.cn/products/primary-antibodies/cd8-alpha-antibody-epr22483-288-ab245118.html>

Anti-mouse CD8 antibody: <https://www.abcam.cn/products/primary-antibodies/cd8-alpha-antibody-epr20305-ab209775.html>

Anti-mouse CD8 antibody: <https://www.servicebio.com/goodsdetail?id=94943>

Anti-Normal rabbit IgG: <https://www.cellsignal.cn/products/primary-antibodies/normal-rabbit-igg/2729>

Anti-Normal mouse IgG: <https://www.scbt.com/zh/p/normal-mouse-igg>

Zombie Aqua: <https://www.biolegend.com/en-us/products/zombie-aqua-fixable-viability-kit-8444>

Zombie UV™: <https://www.biolegend.com/en-us/products/zombie-uv-fixable-viability-kit-9336>

PerCP/Cyanine5.5 anti-mouse CD45: <https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd45-antibody-4264>

APC/Fire™ 750 anti-mouse CD45: <https://www.biolegend.com/en-us/products/apc-fire-750-anti-mouse-cd45-antibody-13049>

Brilliant Violet 785™ anti-mouse CD3: <https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-cd3-antibody-7953>

Alexa Fluor® 700 anti-mouse CD8a: <https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd8a-antibody-3387>

FITC anti-mouse CD4: <https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd4-antibody-480>

PE/Cyanine7 anti-mouse NK-1.1 Antibody: <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-nk-1-1-antibody-2840>

PE anti-mouse IFN- $\gamma$ : <https://www.biolegend.com/en-us/products/pe-anti-mouse-ifn-gamma-antibody-997>

Brilliant Violet 605™ anti-mouse CD183 (CXCR3): <https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd183-cxcr3-antibody-7647>

Brilliant Violet 605™ anti-mouse Ki-67: <https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-ki-67-antibody-8983>

Alexa Fluor® 647 anti-mouse/rat/human FOXP3: <https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-rat-human-foxp3-antibody-2892>

FITC anti-mouse/human CD11b: <https://www.biolegend.com/en-us/products/fitc-anti-mouse-human-cd11b-antibody-347>

Brilliant Violet 510™ anti-mouse Ly-6C: <https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-ly-6c-antibody-8726>

Alexa Fluor® 700 anti-mouse Ly-6G: <https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-ly-6g-antibody-6754>

PE anti-mouse F4/80 Recombinant: <https://www.biolegend.com/en-us/products/pe-anti-mouse-f4-80-recombinant-antibody-18755>

Brilliant Violet 421™ anti-mouse CD206 (MMR): <https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd206-mmr-antibody-8638>

Brilliant Violet 650™ anti-mouse CD80: <https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-mouse-cd80-antibody-7642>

APC anti-mouse I-A/I-E: <https://www.biolegend.com/en-us/products/apc-anti-mouse-i-a-i-e-antibody-2488>

Brilliant Violet 785™ anti-mouse CD11c: <https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-cd11c-antibody-7963>

Brilliant Violet 421™ anti-human/mouse Granzyme B Recombinant: <https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-humanmouse-granzyme-b-recombinant-antibody-17577>

PE/Dazzle™ 594 anti-mouse CD19: <https://www.biolegend.com/en-us/products/pe-dazzle-594-anti-mouse-cd19-antibody-10071>  
 PE/Cyanine7 anti-T-bet: <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-t-bet-antibody-8328>  
 Brilliant Violet 421™ anti-GATA3: <https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-gata3-antibody-9401>  
 TruStain FcXTM anti-mouse CD16/32: <https://www.biolegend.com/en-us/products/trustain-fcx-anti-mouse-cd16-32-antibody-5683>  
 APC anti-mouse H-2Kb: <https://www.biolegend.com/en-us/products/apc-anti-mouse-h-2kb-antibody-6573>  
 Anti-mouse CD3ε antibody, functional grade, Clone 145-2C11: <https://www.biolegend.com/en-us/products/ultra-leaf-purified-anti-mouse-cd3epsilon-antibody-7722>  
 Anti-mouse CD28 antibody, functional grade, Clone 37.51: <https://www.biolegend.com/en-us/products/ultra-leaf-purified-anti-mouse-cd28-antibody-7733>  
 Mouse-CD8α antibody: <https://www.bioxcell.com.cn/in-vivo-antibodies/invivomab-anti-mouse-cd8%CE%B1-be0061.html>  
 Rat IgG2b isotype control, anti-keyhole limpet hemocyanin: <https://bioxcell.com/catalogsearch/result/?q=BE0090>  
 Mouse-CXCR3 antibody: <https://www.bioxcell.com.cn/in-vivo-antibodies/invivomab-anti-mouse-cxcr3-cd183-be0249.html>  
 Polyclonal Armenian hamster IgG: <https://bioxcell.com/catalogsearch/result/?q=BE0091>  
 Mouse-PD-1 antibody: <https://www.bioxcell.com.cn/in-vivo-antibodies/invivomab-anti-mouse-pd-1-cd279-be0146.html>  
 Rat IgG2a isotype control, anti-trinitrophenol: <https://bioxcell.com/catalogsearch/result/?q=BE0089>

## Eukaryotic cell lines

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

|                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                                  | The human embryonic kidney (HEK) 293T cell line, mouse CT26 CRC cell line, and E0771 mouse breast cancer cell line was purchased from the American Type Culture Collection (ATCC, Rockville, USA). The MC38 mouse CRC cell line was purchased from iCell (Shanghai, China). The B16 mouse melanoma cell line was purchased from Pricella Life Science & Technology (Wuhan, China). The mouse pancreatic cell line was a kind gift from Prof. Jian Zheng (Sun Yat-sen University Cancer Center, Guangzhou). Cells were cultured in RPMI-1640 or DMEM (Gibco, Grand Island, USA) supplemented with 10% FBS (Wisent Inc., Quebec, Canada) and 1% penicillin/streptomycin (Gibco). Cells were characterized before use by short tandem repeat DNA fingerprinting (STR)-PCR DNA profiling and were determined to be free of mycoplasma contamination. |
| Authentication                                                       | All cell lines were authenticated based on STR fingerprinting before use.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Mycoplasma contamination                                             | All cell lines were tested negative for mycoplasma contamination. Stated in the Methods section.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> 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      | BALB/c mice (female, 6-8 weeks, Strain NO. 211), BALB/c Nude mice (female, 5-6 weeks, Strain NO. 401), and C57BL/6J mice (female, 6-8 weeks, Strain NO. 213) were purchased from Beijing Vital River Laboratory (Beijing, China). B6-Rag1-KO mice (Rag1-/-, female, 5-6 weeks, Strain NO. T004753), NCG mice (female, 5-6 weeks, Strain NO. T001475) and APC-min mice (male and female, 8-12 weeks, Strain NO. T001457) were purchased from Gempharmatech (Jiangsu, China). Intestine-specific Mbtps1 knockout mice on the C57BL/6J background (female, 6-10 weeks) were generated by crossing mice carrying a Mbtps1 exon 4-7 floxed allele (male and female, 8-12 weeks, Strain NO. T025387) with Villin-Cre-ERT2 mice (male and female, 8-12 weeks, Strain NO. T004829) purchased from Gempharmatech. Given that our experimental design excluded gender-related factors and preliminary findings indicated no difference in Mbtps1 knockdown phenotypes between male and female mice, we opted to use only female mice in subsequent experiments to maintain control over variables. All mice were housed in a temperature-controlled, specific pathogen-free (SPF) facility with a regular 12-h light-dark cycle, and euthanasia was carried out using carbon dioxide. The allocation of animals for the study was randomized through computer-generated random numbers and they were co-housed in the same environment. The number of animals per group for each animal experiment ranges from 5 to 10. |
| Wild animals            | The study did not involve wild animals.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Reporting on sex        | Given that our experimental design excluded gender-related factors and preliminary findings indicated no difference in Mbtps1 knockdown phenotypes between male and female mice, we opted to use only female mice in subsequent experiments to maintain control over variables.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Field-collected samples | This study did not include field-collected samples.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Ethics oversight        | All patient samples used in the present study were obtained from the Sun Yat-sen University Cancer Center (SYSUCC) in Guangzhou, China. Prior to sample collection, written informed consent was obtained from each participant, ensuring compliance with the strict guidelines set forth by the Medical Ethics Committee of SYSUCC, as well as the principles enshrined in the Declaration of Helsinki. Additionally, the present study received approval from the Ethical Committee of SYSUCC (G2023-172-01). All animal studies were performed in strict accordance with the protocol approved by the Institutional Ethics Committee for Clinical Research and Animal Trials of SYSUCC (L025501202112016). The tumors that were transplanted were required to have a volume not exceeding 2,000 mm <sup>3</sup> or to constitute no more than 10% of the body weight as permitted by the Institutional Ethics Committee for Clinical Research and Animal Trials of the SYSUCC.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

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, mosaicism, 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                                                                                                                                        | For mouse tumor samples, subcutaneous tumors were collected on Day 14 after tumor cell injection. Immediately following euthanasia, freshly isolated tumor samples were surgically dissected into fine fragments and transferred to 5-mL Eppendorf tubes containing enzymes D, R, and A, which were obtained from the Mouse Tumor Dissociation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Subsequently, the samples were incubated at 37 °C with gentle rotation for 40 min. Following incubation, the samples were passed through 70-µm cell strainers (Biosharp, Beijing, China) and washed twice with MACS buffer to remove any residual debris. The supernatant was then aspirated, and the cell pellet was gently resuspended in 40% Percoll (Cytiva, Shanghai, China) for purification via gradient centrifugation. For tumor-draining lymph node samples, the DLNs were crushed in 6-cm dishes, passed through 40-µm cell strainers, and washed twice with MACS buffer. Similarly, for mouse spleen samples, the spleens were crushed in 6-cm dishes, passed through 40-µm cell strainers, and washed twice with MACS buffer. Subsequently, the cell pellets were resuspended in red blood cell lysis buffer (Biosharp, Beijing, China) and incubated for 5 min. For mouse blood dissociation, whole blood was collected into tubes containing heparin sodium. The blood was then lysed with red blood cell lysis buffer for 15-20 min at room temperature with gentle shaking, followed by neutralization with PBS. The samples were passed through 40-µm cell strainers and washed with MACS buffer. Finally, dissociated single-cell pellets from these samples were resuspended in sorting buffer (PBS containing 2% FBS) for further flow cytometry analysis. |
| Instrument                                                                                                                                                | CytoFLEX LX (Beckman Coulter)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Software                                                                                                                                                  | CytExpert software (Beckman Coulter)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Cell population abundance                                                                                                                                 | Reported in Supplementary information and Methods section.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Gating strategy                                                                                                                                           | Reported in Supplementary information.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <input checked="" type="checkbox"/> Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |