

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	No custom software was used for data collection in this study other than the softwares in instruments indicated in the manuscript. Biacore T200 instrument(GE Healthcare)for SPR experiments; PyMOL version 3.1.3 for molecular docking; Q Exactive HF-X; Beckman CytoFlex flow cytometer version 2.3.0.84 for flow cytometry; Leica TCS SP5 microscope for immunofluorescence confocal imaging version 4.4; IVIS Lumina Series 3 In Vivo Imaging System; Tecan Infinite 200Pro microplate reader; Zeiss Axio Vert.A1 flurescence microscope;
Data analysis	Biacore T200 Evaluation Software, FlowJo version 10.4, Leica Application Suite X version 3.7.2.22383, Living Image Software version 4.4, Biopharma Finder software version 5.1, Image J software, GraphPad Prism version 9.2.0, ModFit version 3.2.1, Zeiss Zen version 3.7

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 main data supporting the results in this study are available within the paper, its supplementary file, 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	Study does not involve human participants. Commercial human specimens were purchased/procured without sex, race, ethnicity considerations.
Reporting on race, ethnicity, or other socially relevant groupings	Please see above.
Population characteristics	Please see above.
Recruitment	Please see above.
Ethics oversight	All fresh human tumor tissue samples were appropriately consented and the studies were approved by the Ethics Committee of the Drum Tower Hospital.

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	Sample size was determined based on previous publications containing similar procedure to maintain balance between reaching the statistical significance and minimizing the number of animal/reagents use (for example, Nat Commun 13, 7466 (2022), Nat. Commun 9, 2512 (2018), and Mol Cancer Ther 17, 1494–1503 (2018)). These and other similar reports demonstrate the sample sizes are adequate to assure statistical power.
Data exclusions	No data were excluded from the analyses.
Replication	All experiments and in vitro assays were repeated in at least two independent experiments with consistent observations.
Randomization	Samples/animals in all experiments were randomized to each group.
Blinding	No blinding. Blinding was not needed in the study because conditions were well controlled with syngenic mice. Blinding is not typically used in the field.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	
Research sample	

Sampling strategy	
Data collection	
Timing	
Data exclusions	
Non-participation	
Randomization	

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing and spatial scale	
Data exclusions	
Reproducibility	
Randomization	
Blinding	

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	
Location	
Access & import/export	
Disturbance	

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

Antibodies used in this study were purchased from commercial vendors as follows: anti-MSLN antibody (Clone EPR19025-42, Cat#ab196235), Goat Anti-Rabbit IgG H&L (Alexa Fluor 488) (Cat#ab150077), DyLight 650 Mouse monoclonal to 6X His tag (Cat#ab117504) and Rabbit monoclonal to LAMP1 (Clone EPR21026, Cat#ab208943) from Abcam; rabbit anti-HMGB1 antibody (Cat#AG2167) and rabbit anti-CRT antibody (Cat#AF1666) from Beyotime; CD3-APC/Cy7 (Clone 145-2C11, Cat#557596), CD8-PE/Cy5.5 (Clone 53-6.7, Cat#551162), CD4-PE/Cy7 (Clone RM4-5, Cat#552775) and Fixable Viability stain 780 (Cat#565388) from BD Biosciences; CD44-PE (Clone IM7, Cat#103008), CD62L-APC (Clone MEL-14, Cat#104412), PD-1-BV421 (Clone 29F.1A12, Cat#135221), CD11c-FITC (Clone N418, Cat#117306), CD80-APC (Clone 16-10A1, Cat#104714), CD86-PE (Clone GL-1, Cat#105008) and Zombie Aqua Fixable Viability Kit (Cat#423102) from Biolegend. The dilutions used for each antibody are described in the Methods section of the manuscript.

For treatment, anti-PD1 (clone 29F.1A12, BioXCell) were used at 200 ug per i.p. dose.

Validation

All antibodies are from commercial companies and are well validated by manufacturer and widely used in researches. Their validation data are available on the manufacturer's websites (traceable from catalog numbers).

Eukaryotic cell lines

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

Cell line source(s)

Cell lines MKN45(SCSP-5473), NCI-N87(TCHu130), SW480(TCHu172), Huh7(TCHu182), CFPAC1(TCHu112), PANC1(TCHu98) and CT26(SCSP-523) cell lines were purchased from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, and cultured following vendor instructions. CT26-GFP-hMSLN cells were generated by transfection of the CT26 cells with gcGFP lentiviral vector (GeneChem, China).

Authentication

Each cell line was maintained separately and stocked in early passages, to minimize contamination and to preserve cell identity. No further authentication was performed before use.

Mycoplasma contamination

Our laboratory performed mycoplasma testing on all cells cultured every month and all cell lines used tested negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No misidentified cell lines listed by ICLAC were used.

Palaeontology and Archaeology

Specimen provenance

Specimen deposition

Dating methods

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

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

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 nude male/female mice and BALB/c male/female mice aged 5-6 weeks were purchased from Shanghai Sippr-BK laboratory

Laboratory animals	animal Co.Ltd. (Shanghai,China) and kept in the specific pathogen-free (SPF) Laboratory Animal Center of Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School.
Wild animals	The study did not involve wild animals.
Reporting on sex	Both male and female mice were used in the immunodeficiency mouse model, while only female mice were used in the immunocompetent mouse model for practical reasons (to reduce the chance of fighting when housed in groups).
Field-collected samples	The study did not involve samples collected from the field
Ethics oversight	All animal experimental procedures were approved by the Laboratory Animal Care and Use Committee of the Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School.

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	
Study protocol	
Data collection	
Outcomes	

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks

Novel plant genotypes

Authentication

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

Files in database submission

Genome browser session

(e.g. [UCSC](#))

Methodology

Replicates

Sequencing depth

Antibodies

Peak calling parameters

Data quality

Software

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

Single-cell suspensions of TDLNs and spleens were prepared using the mechanical trituration method. Spleens were resuspended in erythrocyte lysate for 5 min. Tumors were cut into small pieces (2 to 4 mm), digested with collagenase type IV (1 mg/mL, Sigma) for 2 h at 37 °C and 5% CO₂ with gentle shaking, and filtered through cell strainers. All samples were then resuspended in ice-cold NS. Cell surface markers were stained with specific antibodies for 30 min at 4 °C.

Instrument

Flow cytometric analysis was performed using a Beckman CytoFlex flow cytometer (Beckman Coulter, USA) and data was analyzed by FlowJo software.

Software

FlowJo v10

Cell population abundance

For in vitro analysis, at least 6000 live tumor cells were collected for analysis. For in vivo analysis, at least 20000 live lymphocytes were collected for analysis.

Gating strategy

Gating strategy was described in the relevant figures.

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

Magnetic resonance imaging

Experimental design

Design type

Design specifications

Behavioral performance measures

Acquisition

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI

☐ Used☐ Not used

Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

(See [Eklund et al. 2016](#))

Correction

Models & analysis

n/a | Involved in the study

☐

Functional and/or effective connectivity

☐

Graph analysis

☐

Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis