nature portfolio

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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 (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Nanoparticle tracking analyzer: Malvern NTA5330; Microplate reader: EnVision; Confocal laser scanning microscopy:Zeiss 880; Flow cytometer: Attune Nxt; Living image: Living image: IVIS lumina III; Inverted fluorescence microscope: Olympus IX73; qPCR: Bio-Rad CFX96 optical reaction; Digital Slide Scanners: PANNORAMIC MIDI II; Transmission electron microscope: JEM-1400 Electron Microscope; Western blot imaging system: ImageQuant LAS 500.
Data analysis	GraphPad prism 8.0; Flowjo V10; CaseViewer 2.4; Living image software (Perkin Elmer); NanoSight NTA software: NTA 3.0 analysis software (Malvern).

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

All data supporting the findings of this study are available within the Article, Supplementary Information or Source Data file. Source data are provided with this

paper and are also available in Figshare: https://doi.org/10.6084/m9.figshare.27901938.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

Life sciences study design

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

Sample size	In vitro experiments were performed using three technical replicates and each experiment was repeated at least three times. Sample sizes for in vitro experiments were chosen based on the standard in the field. For in vivo studies, samples size were anticipated for an effect size of >0.8 with an alpha error probability of 0.05 at a power of 80%. Thus the recommended sample size in all the mice experiments performed were kept to at least 3 animals per group. For the survival rate analysis, we calculated the required sample size using formulas appropriate for proportions. By combining these calculations with the resource equation approach and considering experimental designs from previous literature, we set the sample size at 10 mice per group for the survival analyses. Sample size was chosen in consideration of animal individual differences and the 4R principle for the credibility of the results.
Data exclusions	No data was excluded from studies.
Replication	We confirmed that all repeated attempts were successful. Experiment repeat numbers are reported in Figure Legends.
Randomization	Samples were randomly allocated into experimental groups.
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-CD3-APC (BioLegend, Cat#100236, clone: 17A2, 1: 200 dilution)
anti-mouse CD8-Pacific Blue (BioLegend, Cat#100725, clone: 53-6.7, 1: 200 dilution),
anti-mouse CD8-PE (BioLegend, Cat#100708, clone: 53-6.7, 1: 200 dilution)
anti-mouse CD4-FITC (BioLegend, Cat#100510, clone: GK1.5, 1: 200 dilution)
anti-mouse CD45-PE/Cyanine7 (BioLegend, Cat#103114, clone: 30-F11, 1: 200 dilution)
anti-mouse Granzyme B-PE (BioLegend, Cat#372207, clone: QA16A02, 1: 200 dilution)
anti-mouse CD80-APC (BioLegend, Cat#104713, clone: 16-10A1, 1: 200 dilution)
anti-mouse CD86-PE (BioLegend, Cat#105007, clone: GL-1, 1: 200 dilution)
anti-mouse Foxp3-PE (BioLegend, Cat#126404, clone: MF14, 1: 200 dilution)
anti-mouse CD25-PerCP/Cyanine5.5 (BioLegend, Cat#102030, clone: PC61, 1: 200 dilution)
anti-mouse CD11b-Pacific Blue (BioLegend, Cat#101224, clone: M1/70, 1: 200 dilution)
anti-mouse Gr1-APC/Cyanine 7 (BioLegend, Cat#108424, clone: RB6-8C5, 1: 200 dilution)
anti-mouse F4/80-PerCP/Cyanine5.5 (BioLegend, Cat#123127, clone: BM8, 1: 200 dilution).
anti-mouse CD274(PDL1)-PEcy7 (BioLegend, Cat#124313, clone: 3-C6, 1: 200 dilution)
anti-human CD274(PDL1)-PE (BioLegend, Cat#329705, clone: 29E.2A3, 1: 200 dilution)
anti-mouse CD16/32 (Biolegend, Cat#101302, Clone: 93, 1: 200 dilution)
anti-mouse CD3 (PeproTech, Cat#05112-25-500, Clone: 17A2, 5 µg/mL)
anti-mouse CD28 (PeproTech, Cat#10312-25-500, Clone: 37.51, 2 µg/mL)
anti-mouse CD31-Alexa Fluor® 488 (Abcam, Cat#ab307133, clone: MEC 7.46, 1: 50 dilution)
anti-mouse CD44-FITC (BioLegend, Cat#103021, Clone: IM7, 1: 200 dilution)
anti-mouse CD62L-APC (BioLegend, Cat#104411,Clone: MEL-14, 1: 200 dilution)
PE-conjugated H-2Kb/OVA (SIINFEKL) tetramer (MBL, Cat#TC-5001-1, 1: 200 dilution)
anti-mouse iNOS (Proteintech, Cat#80517-1-RR, clone: 6O22, dilution 1:1000)
anti-mouse COX-2 (Proteintech, Cat#66351-1-Ig, clone: 3G2B9, dilution 1:1000)
anti-mouse PD-L1 (Proteintech, Cat#66248-1-Ig, clone: 2B11D11, dilution 1:200)
anti-mouse CD31 (Abcam, Cat#ab182981, clone: EPR17259, dilution 1:200)
anti-mouse PD-1-FITC (BioLegend, Cat#135213, clone: 29F.1A12, 1: 200 dilution),
anti-mouse LAG-3-Brilliant Violet (BioLegend, Cat#125219, clone: C9B7W, 1: 200 dilution),
anti-mouse TIGIT-PE (BioLegend, Cat#142103, clone: 1G9, 1: 200 dilution),
anti-mouse TIM-3-APC (BioLegend, Cat#134007, clone: B8.2C12, 1: 200 dilution)
Biotin-Protein L(genscript, Cat#M00097, 1: 2000 dilution)
FITC-Protein L (genscript, Cat#M00920, 1: 1000 dilution)
FITC anti-GFP antibody (Abcam, Cat#ab6662, 1: 200 dilution)
anti-Myc tag (Abcam, Cat#ab32, clone: 9E10, 1: 500 dilution)

Validation

All antibodies were verified by the supplier and each lot has been quality tested.
1)anti-mouse-CD3-APC, BioLegend, https://www.biolegend.com/en-ie/products/apc-anti-mouse-cd3-antibody-8055?GroupID=BLG242;
2)anti-mouse CD8-Pacific Blue, BioLegend, https://www.biolegend.com/en-ie/products/pacific-blue-anti-mouse-cd8a-antibody-2856;
3)anti-mouse CD8-PE, BioLegend, https://www.biolegend.com/en-ie/products/pe-anti-mouse-cd8a-antibody-155;
4)anti-mouse CD4-FITC, BioLegend, https://www.biolegend.com/en-ie/products/fitc-anti-mouse-cd4-antibody-480;
5)anti-mouse CD45-PE/Cyanine7, BioLegend, https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-cd45-antibody-1903;
6)anti-mouse Granzyme B-PE, BioLegend, https://www.biolegend.com/en-ie/products/pe-anti-human-mouse-granzyme-b-recombinant-antibody-14431;
7)anti-mouse CD80-APC, BioLegend, https://www.biolegend.com/en-ie/products/apc-anti-mouse-cd80-antibody-2340;
8)anti-mouse CD86-PE, BioLegend, https://www.biolegend.com/en-ie/products/pe-anti-mouse-cd86-antibody-256;
9)anti-mouse Foxp3-PE, BioLegend, https://www.biolegend.com/en-ie/products/pe-anti-mouse-foxp3-antibody-4660;
10)anti-mouse CD25-PerCP/Cyanine5.5, BioLegend, https://www.biolegend.com/en-ie/products/percp-cyanine5-5-anti-mouse-cd25-antibody-4262;
11)anti-mouse CD11b-Pacific Blue, BioLegend, https://www.biolegend.com/en-ie/products/pacific-blue-anti-mouse-human-cd11b-antibody-3863;
12)anti-mouse Gr1-APC/Cyanine 7, BioLegend, https://www.biolegend.com/en-ie/products/apc-cyanine7-anti-mouse-ly-6gly-6c-gr-1-

antibody-3935;
13)anti-mouse F4/80-PerCP/Cyanine5.5, BioLegend, https://www.biolegend.com/en-ie/products/percp-cyanine5-5-anti-mouse-f480-antibody-4303;
14)anti-mouse CD274(PDL1)-PEcy7, BioLegend, https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-cd274-b7-h1-pd-l1-antibody-6721;
15)anti-human CD274(PDL1)-PE, BioLegend, https://www.biolegend.com/en-ie/products/pe-anti-human-cd274-b7-h1-pd-l1-antibody-4375;
16)anti-mouse CD16/32, Biolegend, https://www.biolegend.com/en-ie/products/purified-anti-mouse-cd16-32-antibody-190;
17)anti-mouse CD3, PeproTech, https://www.bio-gems.com/anti-mouse-cd3-safire-purified.html;
18)anti-mouse CD28, PeproTech, https://www.bio-gems.com/anti-mouse-cd28-safire-purified.html;
19)anti-mouse CD31-Alexa Fluor® 488, Abcam, https://www.abcam.com/en-us/products/primary-antibodies/alexa-fluor-488-cd31-antibody-mec-746-ab307133;
20)anti-mouse CD44-FITC, BioLegend, https://www.biolegend.com/en-ie/products/fitc-anti-mouse-human-cd44-antibody-314;
21)anti-mouse CD62L-APC, BioLegend, https://www.biolegend.com/en-ie/products/apc-anti-mouse-cd62l-antibody-381;
22)PE-conjugated H-2Kb/OVA, SIINFEKL tetramer, MBL, https://www.mblbio.com/bio/g/dtl/T/?pcd=TS-5001-1C;
23)anti-mouse iNOS, Proteintech, https://www.ptgcn.com/products/iNOS-Antibody-80517-1-RR.htm;
24)anti-mouse COX-2, Proteintech, https://www.ptgcn.com/products/COX2--Cyclooxygenase-2-Antibody-66351-1-Ig.htm
25)anti-mouse PD-L1, Proteintech, https://www.ptgcn.com/products/PD-L1-CD274-Antibody-66248-1-Ig.htm;
26)anti-mouse CD31, Abcam, https://www.abcam.com/en-us/products/primary-antibodies/cd31-antibody-epr17259-ab182981;
27)anti-mouse PD-1-FITC, BioLegend, https://www.biolegend.com/en-ie/products/fitc-anti-mouse-cd279-pd-1-antibody-7004;
28)anti-mouse LAG-3-Brilliant Violet, BioLegend, https://www.biolegend.com/en-ie/products/brilliant-violet-785-anti-mouse-cd223-lag-3-antibody-12532;
29)anti-mouse TIGIT-PE, BioLegend, https://www.biolegend.com/en-ie/products/pe-anti-mouse-tigit-vstm3-antibody-7429;
30)anti-mouse TIM-3-APC, BioLegend, https://www.biolegend.com/en-ie/products/apc-anti-mouse-cd366-tim-3-antibody-9227;
31)Biotin-Protein L genscript, https://www.genscript.com/molecule/M00097-Biotin_Protein_L.html;
32)FITC-Protein L genscript, https://www.genscript.com/protein/M00920-FITC_Protein_L.html;
33)FITC anti-GFP antibody Abcam, https://www.abcam.com/en-us/products/primary-antibodies/fitc-gfp-antibody-ab6662;
34)anti-Myc tag Abcam, https://www.abcam.com/en-us/products/primary-antibodies/myc-tag-antibody-9e10-ab32.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293 (human embryonic kidney cell line, CRL-3216), LLC (Lewis lung carcinoma, CRL-1642) and B16-F10 (mouse melanoma, CRL-6475) cells were supplied by American Type Culture Collection (ATCC). PC-9 (lung adenocarcinoma)were supplied by the Chinese Academy of Sciences (Cat: SCSP-5085). LLC and B16-F10 cell lines overexpressing MSLN/Luciferase or OVA were produced by our laboratory
Authentication	Cell line validation with short tandem repeat (STR) markers was conducted via Shanghai Biowing Applied Biotechnology Co.,Ltd (Shanghai, China).
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cells lines were used in the study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Female C57BL/6J mice (4-6 weeks old) were supplied by the Guangdong Medical Laboratory Animal Center. The living environment of animals were maintained at a temperature of ~25 °C and a humidity of 50 ± 5% with a 12 h light/dark cycle, with free access to standard food and water.
Wild animals	The study did not involve wild animals.
Reporting on sex	Although we have used single-sex animals in our research, we think that the research results were not only applicable to single-sex.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experiments were performed in accordance with the rules of the Animal Care and Use Occasion of the Fifth Affiliated Hospital of Sun Yat-sen University.

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, mosiacism, 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 tissue sample, the tissue was first mechanically disrupted from mice and divided into small pieces and homogenized in cold staining buffer to form single cell suspensions in the presence of digestive enzyme.
Instrument	Attune Nxt
Software	Flowjo_V10
Cell population abundance	No sorting was performed.
Gating strategy	Generally, cells were first gated on FSC/SSC. Singlet cells were usually gated using FSC-H and FSC-A. Surface antigen gating was performed on the live cell population.

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