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	Illumina sequencer NovaSeq for RNAseq data.
Data analysis	FlowJo v10.8.1 for flow cytometry data. Graphpad Prism v10.0 for statistical analysis. CellRanger (10x Genomics) v6.1.2 for read alignment. Seurat R package v4.0.6 for single-cell RNAseq data integration and analysis. Kallisto v0.46.0 for gene alignment in bulk RNAsee analysis. tximport v1.30.0 and EdgeR v3.14.0 R package for bulk RNAseq data analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data Availability Statement
Single-cell and bulk RNA-seq data have been deposited in NCBI's Gene Expression Omnibus with accession GSE246970 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE246970] and GSE246969 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE246969], respectively.
All remaining data are available within the Article and its Supplementary Files, or available from the authors upon request. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Healthy volunteers with all sexes and genders participated this studies.
Reporting on race, ethnicity, or other socially relevant groupings	Healthy volunteers participated this studies, regardless of their race, ethnicity, or other socially relevant groupings.
Population characteristics	Human peripheral blood mononuclear cell (PBMC)-derived monocytes were provided by Human Immunology Core of the University of Pennsylvania, isolated from the of healthy volunteers, aged 16 to 64.
Recruitment	The healthy donors were recruited in the Hosptical of the University of Pennsylvania. The written informed consent was obtained from each participant.
Ethics oversight	The collection of human tissues (blood) in compliance with the tissue banking protocol was approved by the University of Pennsylvania Institutional Review Board.

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	The sample size was chosen in advance based on common practice of the described experiments in the literature and our previous work (PMID: 32102932 and 27043280). Sample sizes for comparisons also followed Mead's recommendations. At least 3 of samples per group for all in vitro and in vivo experiments.
Data exclusions	No data was excluded.
Replication	Technical replicates and independent experiments were performed to verify reproducibility of the assays. The replication information is provided in the figure legends.
Randomization	In cell culture experiments, cell dishes were randomly assigned to different study group. In animal studies, all mice used were age- and gender-matched, and were randomly selected.
Blinding	Blinding was not carried out in cell culture experiments or in data collection, in which all experiments had to be carried out by the same researchers. In animal studies, investigators were not blinded, as all primary tissues from which subsequent samples were derived were labeled by genotypes or treatment ways. Blinding was not necessary since measurements did not involve bias from the experiments. All data were processed using the same software.

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-CD3ε (final concentration 1 µg/ml, BioLegend, 100302, Clone 145-2C11) for T cell stimulation
anti-CD28 (final concentration 1 µg/ml, BioLegend, 102102, Clone 37.51) for T cell stimulation
anti-CD3 (1:100, BioLegend,100203/100233, Clone 17A2) for flow cytometry
anti-CD4 (1:100, BioLegend, 100540, Clone RM4-5) for flow cytometry
anti-CD8a (1:100, BioLegend, 100706/100708/100733, Clone 53-6.7) for flow cytometry
anti-CD45 (1:200, BioLegend, 103134/103133, Clone 30-F11) for flow cytometry
anti-F4/80 (1:200, BioLegend, 123107, Clone BM8) for flow cytometry
anti-CD206 (1:100, Biolegend, 141719, Clone C068C2) for flow cytometry
anti-CD80 (1:100, BioLegend, 104713, Clone 16-10A1) for flow cytometry
anti-CD86 (1:100, Biolegend, 105005, Clone GL-1) for flow cytometry
anti-CD86 (1:100, Miltenyi Biotec, 130-102-604, Clone:PO3.3) for flow cytometry
anti-IFN-g (1:100, Biolegend, 505825, Clone XMG1.2) for flow cytometry
anti-Granzyme B (1:100, Biolegend, 372211, Clone QA16A02) for flow cytometry
anti-Ki67 (1:100, Thermo Fisher, 17-5698-80, Clone SolA15) for flow cytometry
anti-LAG3 (1:100, eBioscience, 11-2231-80, Clone C9B7W) for flow cytometry
anti-LAG3 (1:100, Biolegend, 125225/125209, Clone C9B7W) for flow cytometry
anti-PD-1 (1:100, Biolegend, 135223, Clone 29F.1A12) for flow cytometry
anti-Tim-3 (1:100, Biolegend, 134009, Clone B8.2C12) for flow cytometry
anti-CD11b (1:100, Biolegend, 101206/101212, Clone M1/70) for flow cytometry
anti-CD206 (1:100, Biolegend, 321109, Clone 15-2) for flow cytometry
anti-NK-1.1 (1:100, Biolegend, 156505, Clone S17016D) for flow cytometry
anti-TMEM119 (1:100, Thomas Fisher, 25-6119-80, Clone V3RT1GOsz) for flow cytometry
anti-CD25 (1:100, Biolegend, 101915, Clone 3C7) for flow cytometry
anti-CD25 Antibody (1:100, Biolegend, 302610, clone: BC96) for flow cytometry
anti-GD2 (1:100, Biolgend, 357324, Clone 14G2a) for flow cytometry
anti-arginase-1 (1:500, Santa Cruz, sc-20150, Clone H-52) for immunoblot
anti-GAPDH (1:3,000, Cell Signaling, 5174, Clone D16H11) for immunoblot
anti-GD2 (1:200, BioLegend, 357302, Clone 14G2a) for immunofluorescence

Validation

All antibodies were purchased from commercial sources and have been validated by the vendors. Additional validation has also been given in previous publication with PubMed IDs listed:
anti-CD3ε (BioLegend, 100302) PMID: 32901001, 34822775, 36630913 https://www.biolegend.com/nl-be/products/purified-anti-mouse-cd3epsilon-antibody-28
anti-CD28 (BioLegend, 102102) PMID: 32901001, 33893298, 32434937 https://www.biolegend.com/nl-be/products/purified-anti-mouse-cd28-antibody-117
anti-CD3 (BioLegend,100203) PMID: 28560793, 29456159,30393066, 8293463, 2197981 https://www.biolegend.com/nl-be/products/fitc-anti-mouse-cd3-antibody-45
anti-CD4 (BioLegend, 100540) PMID: 29429633, 30446387, 30076101 https://www.biolegend.com/nl-be/products/percp-cyanine5-5-anti-mouse-cd4-antibody-4230
anti-CD8a (BioLegend, 100706/100733) PMID: 29129787, 29363160, 29777108 https://www.biolegend.com/nl-be/products/fitc-anti-mouse-cd8a-antibody-153
anti-CD45 (1:200, BioLegend, 103134/103133) PMID: 30796225, 28008921, 35046097 https://www.biolegend.com/nl-be/products/brilliant-violet-421-anti-mouse-cd45-antibody-7253
anti-F4/80 (BioLegend, 123107) PMID: 28939843, 29664018, 29070674 https://www.biolegend.com/nl-be/products/fitc-anti-mouse-f4-80-antibody-4067
anti-CD206 (1:100, Biolegend, 141719) PMID: 33278339, 35105806, 33571109 https://www.biolegend.com/nl-be/products/pe-cyanine7-anti-mouse-cd206-mmr-antibody-8631
anti-CD80 (1:100, BioLegend, 104713) PMID: 30595553, 30650377, 22308386 https://www.biolegend.com/nl-be/products/apc-anti-mouse-cd80-antibody-2340
anti-CD86 (1:100, Biolegend, 105005) PMID: 33535045, 26644347, 26880763 https://www.biolegend.com/nl-be/products/fitc-anti-mouse-cd86-antibody-254
anti-CD86 (1:100, Miltenyi Biotec, 130-102-604) PMID: 16709832, 15456701 https://www.miltenyibiotec.com/US-en/products/cd86-antibody-anti-mouse-po3-3.html#conjugate=pe:size=30-ug-in-200-ul
anti-IFN-γ (Biolegend, 505825) PMID: 33271118, 30005826, 33711270 https://www.biolegend.com/nl-be/products/pe-cyanine7-

anti-mouse-ifn-gamma-antibody-5865
anti-Granzyme B (1:100, Biolegend, 372211) PMID: 32195350, 36280674, 36054938 https://www.biolegend.com/nl-be/products/percp-cyanine5-5-anti-humanmouse-granzyme-b-recombinant-antibody-15597
anti-Ki67 (1:100, Thermo Fisher, 17-5698-80) PMID: 35045305, 35241842, 36417858 https://www.thermofisher.com/antibody/product/Ki-67-Antibody-clone-SolA15-Monoclonal/17-5698-80
anti-LAG3 (1:100, eBioscience, 11-2231-80) PMID: 36731891, 35915084, 34446716 https://www.thermofisher.com/antibody/product/CD223-LAG-3-Antibody-clone-eBioC9B7W-C9B7W-Monoclonal/11-2231-82
anti-LAG3 (1:100, Biolegend, 125225/125209) PMID: 30580966, 31031094, 33838102 https://www.biolegend.com/nl-be/products/pe-cyanine7-anti-mouse-cd223-lag-3-antibody-14782
anti-PD-1 (1:100, Biolegend, 135223) PMID: 31350404, 32619407, 29958801 https://www.biolegend.com/nl-be/products/apc-cyanine7-anti-mouse-cd279-pd-1-antibody-9742
anti-Tim-3 (1:100, Biolegend, 134009) PMID: 32860752, 34642245, 31722203 https://www.biolegend.com/nl-be/products/pe-cyanine7-anti-mouse-cd366-tim-3-antibody-13929
anti-CD11b (1:100, Biolegend, 101206/101212) PMID: 31488882, 29777220, 29777220 https://www.biolegend.com/nl-be/products/fitc-anti-mouse-human-cd11b-antibody-347
anti-CD206 (1:100, Biolegend, 321109) PMID: 32210962, 32210962, 30995475 https://www.biolegend.com/nl-be/products/apc-anti-human-cd206-mmr-antibody-2996
anti-NK-1.1 (1:100, Biolegend, 156505) PMID: 34852237 https://www.biolegend.com/nl-be/products/apc-anti-mouse-nk-11-antibody-19843
anti-TMEM119 (1:100, Thomas Fisher, 25-6119-80) PMID: 35705056, 33261619 https://www.thermofisher.com/antibody/product/Tmem119-Antibody-clone-V3RT1GOsz-Monoclonal/25-6119-80
anti-CD25 (1:100, Biolegend, 101915) PMID: 29894690, 33106640, 35040435 https://www.biolegend.com/nl-be/products/pe-cyanine7-anti-mouse-cd25-antibody-13235
anti-CD25 Antibody (1:100, Biolegend, 302610) PMID: 35690062, 36261015, 25994968 https://www.biolegend.com/nl-be/products/apc-anti-human-cd25-antibody-614
anti-GD2 (1:100, Biolgend, 357324) PMID: 22585577, 19339105 https://www.biolegend.com/nl-be/products/apccyanine7-anti-human-ganglioside-gd2-antibody-19352
anti-arginase-1 (1:500, Santa Cruz, sc-20150) PMID: 18824264, 22972923, 17577033 https://datasheets.scbt.com/sc-20150.pdf
anti-GAPDH (1:3,000, Cell Signaling, 5174) PMID: 38168040, 37935976, 38095297 https://www.cellsignal.com/products/primary-antibodies/gapdh-d16h11-xp-rabbit-mab/5174
anti-GD2 (1:200, BioLegend, 357302) PMID: 22585577, 19339105 https://www.biolegend.com/nl-be/products/purified-anti-human-ganglioside-gd2-antibody-8407

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	GL261 cells were from PerkinElmer.
Authentication	GL261 cells were authenticated by the supplying company. No further authentication was performed.
Mycoplasma contamination	All cell lines have been tested and shown negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	There were no commonly misidentified cell lines used in this 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	Math1-Cre;SmoM2fl/fl (Rosa-LSL-SmoW539L/YFP) mice were generated by breeding SmoM2fl/fl mice with Math1-Cre mice. Wild-type C57/B6 mice were purchased from Jackson Lab. All animals were housed at room temperature with a 12-hour-light/12-hour-dark cycle in the Association for the Assessment and Accreditation of Laboratory Animal Care-accredited animal facility of the University of Pennsylvania. Relative humidity and temperature were maintained at 30-70% and 68-79 oF. For glioma tumor induction experiments, both female and male eight-week-old mice were used.
Wild animals	The study did not involve wild animals.
Reporting on sex	Half male and half female mice were used.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All experiments with mice were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania.

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

Plants

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

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Tumors were isolated and subjected to mechanical dissociation with a gentleMACS Dissociator (Miltenyi) and enzymatic digestion with collagenase II and dispase II to obtain single cell suspensions. Macrophages were isolated from mouse bone marrow and human blood, followed by different treatments. Single-cell suspensions were stained with control isotype IgG or fluoresence-conjugated antibody, followed by flow cytometry analysis.
Instrument	Canto II (BD Biosciences)
Software	FlowJo v10 software
Cell population abundance	More than 200 thousand cells were sorted.
Gating strategy	All cells were gated. The gating strategy was shown in Extended Data Figure 1.

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