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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	1. Flow cytometry data collection was performed with an Attune NxT Flow Cytometer with Attune NxT Software v.3 (Invitrogen / Thermal Fischer Scientific). 2. The generated gene expression libraries were sequenced using an Illumina HiSeq 4000 with a sequencing depth of 50,000 paired-end reads per cell. 3. The gRNAs were designed using the publicly available online gRNA design tool CRISPick (https://portals.broadinstitute.org/gppx/crispick/public). 4. Single-cell co-profiling of epigenomic landscape and gene expression in the same single nuclei was performed using the Chromium Next GEM Single Cell Multiome ATAC + Gene Expression kit (10×Genomics, Cat# PN-1000283). 5. In vivo Bioluminescence data was obtained through IVIS Spectrum In Vivo Imaging System (PerkinElmer). 6. Metabolomic data was collected using Agilent Quantitative analysis software (version B.07.00, MassHunter Agilent technologies).
Data analysis	1. Flow cytometry data were analyzed using FlowJo (version 10.6.1,Tree Star, Oregon, USA). 2. Statistical analysis was performed using GraphPad Prism (version 10.1.2, GraphPad software, Inc, La Jolla, CA, USA). 3. The raw fastq files were generated and demultiplexed by CeleScope rna from Singleron (version 3.0.1) and primary data analysis was performed with CeleScope (version 1.10.0) using a custom reference package based on reference genome (Mus_musculus_ensembl_92). Downstream data analysis was performed with the Seurat v4 pipeline. Cells were first filtered based on two metrics: 1) the number of detected genes per cell must be between 200 to 5000; 2) the proportion of mitochondrial gene counts (UMIs from mitochondrial genes / total UMIs) must be less than 10%. Then, the gene expression data was normalized using Seurat sctransform. No major batch effects were observed between the two samples. Finally, the "SCT" data assay was reduced to two dimensions using UMAP for visualization, with 30 computed PCs as input. Differentially expressed genes (DEGs) were identified using the function "FindMarkers" for pairwise comparison between two conditions. A log fold-change threshold of 0.25 was applied to select genes as differentially expressed. The function

"AddModuleScore" was used to calculate the module scores of each cluster based on the aggregated expression of defined gene sets.
4. Raw LC-MS/MS data was processed using the Agilent Quantitative analysis software (version B.07.00, MassHunter Agilent technologies). Relative quantification of metabolites was based on EIC (Extracted Ion Chromatogram) areas for the monitored MRM transitions. The obtained tables (containing peak areas of detected metabolites across all samples) were exported to "R" software (version 4.2.1, http://cran.r-project.org/). Signal intensity drift correction and noise filtering was done within the MRM PROBS software. The preprocessed data with peak areas were imported into Metaboanalyst 5.0 for further data analysis.
5. Single-cell ATAC+Gene co-profiling data processing and analysis: The Cell Ranger ARC v2.0.2 (10x Genomics) was utilized to perform sample demultiplexing, barcode processing, identification of open chromatin regions, and simultaneous counting of transcripts and peak accessibility in single cells from the sequenced data. The output per barcode matrices underwent joint RNA and ATAC analysis using Signac v1.12.0 and Seurat v4. Quality filtering criteria adhered to default settings. Specifically, cells were retained if they exhibited an ATAC peak count ranging from 1,000 to 100,000, a gene count ranging from 1,000 to 25,000, a nucleosome_signal below 2, and a TSS enrichment score exceeding 1. To enhance the accuracy of peak identification, we employed MACS2 v2.2.9.1 with the "CallPeaks" function.

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 generated and supporting the findings of this study are available within the paper. Raw and processed single-cell sequencing data for this study can be accessed in the NCBI Gene Expression Omnibus (GEO) database under the accession number GSE259409. Additional information and materials will be made available 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	Sex and/or gender was not considered in this study.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, or other socially relevant groupings were not considered in this study.
Population characteristics	Population characteristics were not considered as a variable in this study.
Recruitment	Primary T lymphocytes from healthy donors were provided by the Cleveland Clinic's BioRepository Core.
Ethics oversight	Cleveland Clinic's BioRepository Review Committee provided insight.

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	Group sizes for in vivo validation experiments were selected empirically based on previous results of the intra-group variation of tumor growth upon similar treatments. For in vitro experiments, group sizes were selected on the basis of previous publication and prior knowledge of variation and were replicated at least for 3 individual and independent experiments. Ref: Nat Immunol 2021 Jun; 22 (6): 746-756
Data exclusions	Rout outlier tests were run with default parameters (Q = 1%) in Prism on all mouse experimental data due to inherent variability within the model system.
Replication	All presented results were repeatable. Replicates were used in all experiments as noted in figure legend or methods.
Randomization	Age and sex-matched animals were used for each experiment. Mice were randomized prior to treatment. For the experiments without mice,

Randomization	there is no randomization for samples because these in vitro experiments were observational and replicated at least for 3 individual, independent experiments.
Blinding	In vivo tumor engraftment and grouping was conducted randomly before treatment. Fully blinded experiments were not performed due to insufficient personnel availability to accommodate such situation and requirements for cage labeling and staffing needs.

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

The following antibodies or staining reagents were purchased from BioLegend:
CD16/32 (93, 101302), Thy1.1 (OX-7, 202529), Thy1.2 (30-H12, 105343), CD45.1 (A20, 110707), CD45.2 (104, 109814), CD8α (53-6.7, 100714), CD8β (YTS256.7.7, 126606), CD4 (RM4-5, 100526), NK1.1 (PK136, 108740), F4/80 (BM8,123108), CD3ε (17A2, 100306), CD19 (6D5,115520), CD44 (IM7, 103006), CD11c (N418, 117348), I-A/I-E (MHC-II, M5/114.15.2, 107643), Siglec-F (S17007L, 155508), CD80 (16-10A1, 104734), CD86 (GL-1, 105006), Foxp3 (MF-14, 126406), CD11b (M1/70, 101228), Granzyme B (GB11, 515403), IFN-γ (XMG1.2, 505826), TNF-α (MP6-XT22, 506308), IL-2 (JES6-5H4, 503822), IL-4Rα (I015F8, 144806), CD69 (H1.2F3, 104512), Gr-1 (RB6-8C5, 108423), CD107a (1D4B, 121626), CD95 (SA367H8, 152608), CD178 (MFL3, 106605), PD-1 (29F.1A12, 135216), TIM-3 (RMT3-23, 119706), TIM-3 (RMT3-23, 119737), HRP-Actin (2F1-1, 643808), GATA3 (16E10A23, 653805), Tbet (4B10, 644827), anti-rabbit IgG (minimal x-reactivity) Antibody (Poly4064, 406414), anti-mouse IgG1 Antibody (RMG1-1, 406617), STAT6 (16G12A08, 657902), Zombie Aqua™ Fixable Viability Kit (423102), human CD3 (OKT3, 317306), human CD4 (OKT4, 317416), human CD8 (SK1, 344724), human IFN-γ (B27, 506516), and human TNF-α (MAb11, 502940).

The following antibodies or staining reagents were purchased from BD Biosciences:
Anti-TCF-7/TCF-1(S33-966, 566693), anti-phospho-Akt (pT308) (J1-223.371, 558275), anti- phospho-Akt (pS473) (M89-61, 560404), anti-Akt (7/Akt/PKBα, 610836), anti-active Caspase-3 (C92-605.rMAb, 570334)

The following antibodies or staining reagents were purchased from BioXcell:
Anti-CD8 (YTS 169.4, BE0117), anti-CD4 (YTS 177, BE0288), anti-NK1.1 (PK136, BP0036), anti-Ly6G (NIMP-R14, BE0320), anti-lgG (LTF-2, BP0090) and anti-IL-4 (11B11, BE0045), anti-mouse CD3 (17A2, BE0002), anti-mouse CD28 (PV-1, BE0015-5), anti-mouse PD-1 (RMP1-14, BE0146), and anti-mouse CTLA-4 (9H10, BE0131)

The following antibodies or staining reagents were purchased from Invitrogen:
Goat anti-Rat IgG Fc Secondary Antibody (31226) and eBioscience™ Cell Stimulation Cocktail (00-4970-03), and anti-phospho-STAT6 (Tyr641) (46H1L12, 700247)

The following antibodies or staining reagents were purchased from Cell Signaling Technology: Anti-rabbit HRP-IgG (7074), and anti-Glut-1 (73015)

The following antibodies or staining reagents were purchased from Proteintech: Anti-P70S6K (14485-1-AP), Anti-LDHA (19987-1-AP)

The following antibodies or staining reagents were purchased from Antibodies: Anti-phospho-P70S6K (pThr389) (ABIN7265266)

The following antibodies or staining reagents were purchased from ACRO Biosystems: Anti-FMC63 scFv (CAR19) (FM3-HPY53)

Antibodies for surface staining were used at a 1:100 dilution, for intracellular staining at a 1:50 dilution, and for WB at a 1:1000 staining.

Validation

For antibodies used in flow cytometry, each antibody has been validated by the manufacturer for use to detect mouse or human species targets. These antibodies are further validated and routinely used in our lab with good reproducibility. Detailed validation information for each antibody is available at the following websites:

1. CD16/32 (93, 101302), https://www.biolegend.com/en-us/products/purified-anti-mouse-cd16-32-antibody-190
2. Thy1.1 (OX-7, 202529), https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-rat-cd90-mouse-cd90-1-thy-1-1-antibody-7307
3. Thy1.2 (30-H12, 105343), https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd90-2-thy1-2-

antibody-13864
4. CD45.1 (A20, 110707), https://www.biolegend.com/en-ie/products/pe-anti-mouse-cd45-1-antibody-199?GroupID=BLG1933
5. CD45.2 (104, 109814), https://www.biolegend.com/en-ie/products/apc-anti-mouse-cd45-2-antibody-2759
6. CD8α (53-6.7, 100714), https://www.biolegend.com/en-ie/products/apc-cyanine7-anti-mouse-cd8a-antibody-2269
7. CD8β (YTS256.7.7, 126606), https://www.biolegend.com/en-ie/products/fitc-anti-mouse-cd8b-antibody-4475
8. CD4 (RM4-5, 100526), https://www.biolegend.com/en-ie/products/apc-cyanine7-anti-mouse-cd4-antibody-1937
9. NK1.1 (PK136, 108740), https://www.biolegend.com/en-ie/products/brilliant-violet-605-anti-mouse-nk-1-1-antibody-8665
10. F4/80 (BM8,123108), https://www.biolegend.com/en-ie/products/fitc-anti-mouse-f4-80-antibody-4067
11. CD3ε (17A2, 100306), https://www.biolegend.com/en-ie/products/fitc-anti-mouse-cd3epsilon-antibody-23
12. CD19 (6D5,115520), https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-cd19-antibody-1907
13. CD44 (IM7, 103006), https://www.biolegend.com/en-ie/products/fitc-anti-mouse-human-cd44-antibody-314
14.CD11c (N418, 117348), https://www.biolegend.com/en-ie/products/pe-dazzle-594-anti-mouse-cd11c-antibody-9846
15. I-A/I-E (MHC-II, M5/114.15.2, 107643), https://www.biolegend.com/en-ie/products/brilliant-violet-711-anti-mouse-i-a-i-e-antibody-12086
16. Siglec-F (S17007L, 155508), https://www.biolegend.com/en-ie/products/apc-anti-mouse-cd170-siglec-f-antibody-16373
17. CD80 (16-10A1, 104734), https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-cd80-antibody-9320
18. CD86 (GL-1, 105006), https://www.biolegend.com/en-ie/products/fitc-anti-mouse-cd86-antibody-254
19. Foxp3 (MF-14, 126406), https://www.biolegend.com/en-ie/products/alexa-fluor-488-anti-mouse-foxp3-antibody-4661
20. CD11b (M1/70, 101228), https://www.biolegend.com/en-ie/products/percp-cyanine5-5-anti-mouse-human-cd11b-antibody-4257
21. Granzyme B (GB11, 515403), https://www.biolegend.com/en-ie/products/fitc-anti-human-mouse-granzyme-b-antibody-6066
22. IFN-γ (XMG1.2, 505826), https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-ifn-gamma-antibody-5865
23. TNF-α (MP6-XT22, 506308), https://www.biolegend.com/en-ie/products/apc-anti-mouse-tnf-alpha-antibody-975
24. IL-2 (JES6-5H4, 503822), https://www.biolegend.com/en-ie/products/percp-cyanine5-5-anti-mouse-il-2-antibody-4441
25. IL-4Rα (I015F8, 144806), https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-cd124-il-4ralpha-11769
26. CD69 (H1.2F3, 104512), https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-cd69-antibody-3168
27. Gr-1 (RB6-8C5, 108423), https://www.biolegend.com/en-ie/products/apc-cyanine7-anti-mouse-ly-6gly-6c-gr-1-antibody-3935
28. CD107a (1D4B, 121626), https://www.biolegend.com/en-ie/products/percp-cyanine5-5-anti-mouse-cd107a-lamp-1-antibody-13079
29. CD95 (SA367H8, 152608), https://www.biolegend.com/en-ie/products/pe-anti-mouse-cd95-fas-antibody-13907
30. CD178 (MFL3, 106605), https://www.biolegend.com/en-ie/products/pe-anti-mouse-cd178-fasl-antibody-391
31. PD-1 (29F.1A12, 135216), https://www.biolegend.com/en-ie/products/pe-cyanine7-anti-mouse-cd279-pd-1-antibody-7005
32. TIM-3 (RMT3-23, 119706), https://www.biolegend.com/en-ie/products/apc-anti-mouse-cd366-tim-3-antibody-8238
33. TIM-3 (RMT3-23, 119737), https://www.biolegend.com/en-ie/products/apcfire-750-anti-mouse-cd366-tim-3-antibody-17871
34. HRP-Actin (2F1-1, 643808), https://www.biolegend.com/en-ie/products/direct-blot-hrp-anti-beta-actin-antibody-12776
35. GATA3 (16E10A23, 653805), https://www.biolegend.com/en-ie/products/apc-anti-gata3-antibody-9104
36. Tbet (4B10, 644827), https://www.biolegend.com/en-ie/products/pe-dazzle-594-anti-t-bet-antibody-11891
37. anti-rabbit IgG (minimal x-reactivity) Antibody (Poly4064, 406414), https://www.biolegend.com/en-ie/products/alexa-fluor-647-donkey-anti-rabbit-igg-minimal-x-reactivity-9379
38. anti-mouse IgG1 Antibody (RMG1-1, 406617), https://www.biolegend.com/en-ie/products/alexa-fluor-647-anti-mouse-igg1-9685
39. STAT6 (16G12A08, 657902), https://www.biolegend.com/en-ie/products/purified-anti-stat6-antibody-9066
40. Zombie Aqua™ Fixable Viability Kit (423102), https://www.biolegend.com/en-ie/products/zombie-aqua-fixable-viability-kit-8444
41. human CD3 (OKT3, 317306), https://www.biolegend.com/en-ie/products/fitc-anti-human-cd3-antibody-3644
42. human CD4 (OKT4, 317416),https://www.biolegend.com/en-us/products/apc-anti-human-cd4-antibody-3657
43. human CD8 (SK1, 344724), https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-human-cd8-antibody-9062
44. human IFN-γ (B27, 506516), https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-human-ifn-gamma-antibody-3445
45. human TNF-α (MAb11, 502940),https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-human-tnf-alpha-antibody-9034

Anti-mouse CD3 (17A2, BE0002) and anti-mouse CD28 (PV-1, BE0015-5) were used to stimulate the proliferation and cytokine production of mouse T cells. Detailed validation information for each antibody is available at the following sites:
1. Anti-mouse CD3 (17A2, BE0002), https://bioxcell.com/invivomab-anti-mouse-cd3-be0002
2. anti-mouse CD28 (PV-1, BE0015-5), https://bioxcell.com/invivomab-anti-mouse-cd28-be0015-5

Anti-CD8 (YTS 169.4, BE0117), anti-CD4 (YTS 177, BE0288), anti-NK1.1 (PK136, BP0036), anti-Ly6G (NIMP-R14, BE0320), anti-lgG (LTF-2, BP0090) and anti-IL-4 (11B11, BE0045), anti-mouse PD1 (RMP1-14, BE0146), and anti-mouse CTLA4 (9H10, BE0131) were used in vivo to deplete corresponding cells or blockade signals. Detailed validation information for each antibody is available at the following sites:
1. Anti-CD8 (YTS 169.4, BE0117), https://bioxcell.com/invivomab-anti-mouse-cd8-alpha-be0117
2. anti-CD4 (YTS 177, BE0288), https://bioxcell.com/invivomab-anti-human-cd4-be0288
3. anti-NK1.1 (PK136, BP0036), https://bioxcell.com/invivoplus-anti-mouse-nk1-1-bp0036
4. anti-Ly6G (NIMP-R14, BE0320), https://bioxcell.com/invivomab-anti-mouse-ly6g-ly6c-be0320
5. anti-lgG (LTF-2, BP0090), https://bioxcell.com/invivoplus-rat-igg2b-isotype-control-anti-keyhole-limpet-hemocyanin
6. anti-IL-4 (11B11, BE0045), https://bioxcell.com/invivomab-anti-mouse-il-4-be0045
7. anti-mouse PD1 (RMP1-14, BE0146), https://bioxcell.com/invivomab-anti-mouse-pd-1-cd279-be0146
8. anti-mouse CTLA4 (9H10, BE0131), https://bioxcell.com/invivomab-anti-mouse-ctla-4-cd152-be0131

Anti-phospho-STAT6 (Tyr641) (46H1L12, 700247), anti-Glut-1 (73015) , Anti-P70S6K (14485-1-AP), Anti-LDHA (19987-1-AP) , and Anti-phospho-P70S6K (pThr389) (ABIN7265266) were used for WB. Detailed validation information for each antibody is available at the following sites:
1. Anti-phospho-STAT6 (Tyr641) (46H1L12, 700247), https://www.thermofisher.com/antibody/product/Phospho-STAT6-Tyr641-Antibody-clone-46H1L12-Recombinant-Monoclonal/700247
2. anti-Glut-1 (73015) , https://www.cellsignal.com/products/primary-antibodies/glut1-e4s6i-rabbit-mab/73015
3. Anti-P70S6K (14485-1-AP), https://www.ptglab.com/products/p70(S6K)-Antibody-14485-1-AP.htm
4. Anti-LDHA (19987-1-AP) , https://www.ptglab.com/products/LDHA-Specific-Antibody-19987-1-AP.htm

5. Anti-phospho-P70S6K (pThr389) (ABIN7265266), https://www.biocompare.com/Search-Antibodies/?search=ABIN7265266

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	B16F10, YUMM1.7 melanoma cells, Raji lymphoma cells, MC38 colon cancer cells,Nalm6 cells, CTLL-2 cells, K562 cells, HEK293T cells, and Phoenix-Eco cells were originally purchased from the American Type Culture Collection. OVA-transduced B16F10 (B16F10-OVA) mouse melanoma cell lines were provided by Prof. Darell J. Irvine (Massachusetts Institute of Technology). gp33-transduced B16F10 (B16F10-gp33) and OVA-transduced YUMM1.7 (YUMM1.7-OVA) mouse melanoma cell lines were provided by Werner Held (University of Lausanne). HER2-transduced MC38 mouse colon cancer cell lines (MC38-HER2) were provided by Prof. Pedro Romero (University of Lausanne). Luciferase positive Nalm6 cell lines (Nalm6-Luciferase) were provided by Prof. Sidi Chen (Yale University).
Authentication	None of the cell lines were authenticated in these studies. In all related studies, cell lines with low passage number were used.
Mycoplasma contamination	All cell lines were confirmed mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other research organisms

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

Laboratory animals	Six-to-eight-week-old female CD45.2+Thy1.2+ C57BL/6 (C57BL/6J) mice, CD45.1+ mice (B6.SJL-Ptprca Pepcb/BoyCrl), and NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice were purchased from Charles River Laboratories (Lyon, France). CD45.1+CD45.2+ mice were generated by crossing CD45.1+ mice with CD45.2+ C57BL/6 mice. TCR-transgenic Thy1.1+ pmel-1 (PMEL) mice (B6.Cg-Thy1a/Cy Tg(TcraTcrb)8Rest/J) and TCR-transgenic OT-I mice (C57BL/6-Tg(TcraTcrb)1100Mjb/J), CD45.2+ background Rosa26-Cas9 knock-in mice (B6J.129(Cg)-Gt(ROSA)26Sortm1.1(CAG-cas9*,-EGFP)Fezh/J) were originally purchased from the Jackson Laboratory and maintained at the EPFL's pathogen-free facility. OT1 mice were crossed with CD45.1+ mice to generate CD45.1+ OT1 mice. CRISPR–Cas9 knock-in CD45.2+ mice were crossed with CD45.1+ OT1 mice to generate CRISPR–Cas9 knock-in OT1 TCR-transgenic mice. Tcf7DTR-GFP P14 mice on a CD45.2 background were generated as described before (Ref: Immunity. 2019 Jan 15;50(1):195-211). All mice were housed in the EPFL Center of PhenoGenomics or a conventional animal facility of the University of Lausanne and were kept in individually ventilated cages, at 19-23 °C, with 45-65% humidity, and with a 12 h dark/light cycle.
Wild animals	Study did not involve wild animals.
Reporting on sex	Sex is not relevant in this study, and most animals used were female mice.
Field-collected samples	Study did not involve field-collected samples.
Ethics oversight	Experimental procedures in mouse studies were approved by the Swiss authorities (Canton of Vaud, animal protocol IDs VD3206, VD3533, VD3902, VD3912, VD3915, and VD3040x2d) and performed in accordance with the guidelines from the Center of PhenoGenomics of the EPFL and the animal facility of the University of Lausanne. Mice were euthanized when body weight loss was beyond 15%, or the tumor area reached 150 mm2 (as a predetermined endpoint), or other ending points reach the requirements of the animal licenses.

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

Plants

Seed stocks	Plants or seed stocks were not used in this study.
Novel plant genotypes	Plant genotypes are not relevant in this study
Authentication	Plant authentication is not performed in this study

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 collected, weighed, mechanically minced, and stirred at 1,000 r.p.m. in RPMI-1640 medium with collagenase Type IV (1 mg ml−1, Thermo Fisher Scientific), dispase 2 (100 μg ml−1, Sigma-Aldrich), hyaluronidase (100 μg ml−1, Sigma-Aldrich) and DNase I (100 μg ml−1, Sigma-Aldrich) for 60 min at 37 °C for digestion. Red blood cells (RBC) in the digested tumor samples were lysed with ACK lysing buffer for 3 min at room temperature. Tumor-infiltrating leukocytes were then enriched by density gradient centrifugation against Percoll (GE Healthcare), resuspended in PBS with BSA albumin (0.2%, wt/v, Sigma-Aldrich), stained with indicated antibodies, and analyzed with flow cytometry. Similarly, spleens and lymph nodes were collected and mechanically minced through strainers (70 μm) and then RBC inside were lysed with ACK lysing buffer for 5 min at room temperature before antibody staining and flow cytometry analysis. Blood samples were collected and resuspended immediately in the EDTA-PBS buffer (10 mM, EDTA) and RBC inside were lysed with ACK lysing buffer for 5 min at room temperature and then the leukocytes were enriched by density gradient centrifugation against Percoll (GE Healthcare) as above. Bones were crushed in EDTA-PBS buffer (10 mM, EDTA) and the bone marrow cells were collected after filtering through the strainer, remaining RBC was lysed with ACK lysing buffer as above.
Instrument	Data was collected using Attune NxT Flow Cytometer (Invitrogen / Thermal Fischer Scientific). T cell sorting was performed with FACS Aria II (BD Biosciences) or SH800S Cell Sorter (SONY).
Software	Flow cytometry data were analyzed using FlowJo 10.6.1 (Tree Star, Oregon, USA). Flow cytometry data collection was performed using Attune NxT Software version 3 (Invitrogen / Thermal Fischer Scientific).
Cell population abundance	For a typical analysis, >50k leukocytes were collected for further gating. Purity was determined by flow cytometry for PD1+TIM-3+ T cells (> 95%) and CAR-T cells (> 95%).
Gating strategy	We used standard gating strategies: gating on the typical lymphocyte population based on FSC-SSC signals, doublet exclusion based on FSC-H and FSC-A comparison, Live / Dead discrimination based on DAPI or fixable Aqua dye signals. Cell populations were identified based on the expression markers listed below. CD4 T cells: CD45+/CD3+/CD4+; CD8 T cells: CD45+/CD3+/CD8+; Tregs: CD45+/CD3+/CD4+/FoxP3+; B cells: CD45+/CD3-/CD19+; NK cells: CD45+/CD3-/NK1.1+; DC: CD45+/Gr-1-/CD11b+/CD11c+/MHCII+; Tumor-associated macrophages: CD45+/Gr-1-/CD11b+/F4/80+/MHCII+; MDSC: CD45+/Gr-1+/CD11b+; Eosinophils: CD45+/CD11b+/Siglec-F+.

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