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	CytoFLEX 2.3, BD FACSMelody, NanoDrop, SpectroSTAR nano plate reader, ChemiDoc™ Touch Gel Imaging System, CYTEK SpectroFlo® Software 4.0, IncuCyte S3 2018B, PhenoImager HT 2.0
Data analysis	GraphPad Prism software 10.0, FlowJo software v10.10.0, Cell Ranger (version 7.1.0, 10x Genomics), Seurat package (version 4.4.0), CytExpert 2.3, CYTEK SpectroFlo® Software 4.0, IncuCyte S3 2018B, QuPath 5.1

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 are available in the main text or the supplementary materials. Source data are provided with this paper. The single cell RNA sequencing data generated in this study can be accessed through the Gene Expression Omnibus under accession no. GSE342584. Additional data that support the findings of this study are available from the corresponding author on 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	*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	Sterile blood was obtained at the time of cesarean section from de-identified human umbilical cords that are normally discarded. Head and Neck Squamous Cell Carcinoma (HNSCC) patient tumor derived fragments (PDTFs) were obtained at the time of tumor tissue removal surgery. To maintain anonymity, links between the donor's medical and social histories including fetal sex are not maintained.
Recruitment	Human cord blood samples were provided by UT Southwestern Parkland Hospital. The HNSCC patient PDTFs assay was performed without knowing the patients' detailed information in advance. No potential self-selection bias.
Ethics oversight	The procedure was conducted under a protocol approved by the Institutional Review Board (IRB) of the University of Texas Southwestern Medical CenterUTSW (IRB study number: STU 092013-032), and written informed consent was obtained prior to the procedure.

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	Sample size were determined from the similar experiments in the former publications of the group (Z Sun et al., Nat Commun 2019, PMID: 31462678; J Qiao et al., Cancer Cell 2019, PMID: 31185213).
Data exclusions	No data were excluded.
Replication	At least 2 independent experiments were performed. the performed replications were successful.
Randomization	Mice in this study were matched with age and tumor size, and were randomly allocated to the experimental groups. For invitro experiments, all samples were randomly allocated to experimental groups.
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.

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

InVivoMAb anti-mouse CD4 (GK1.5) - BioXcell Cat# BE0003-1
InVivoMAb anti-mouse CD8 (YTS 169.4) - BioXcell Cat# BE0117
InVivoMAb anti-mouse TNFα (XT3.11) - BioXcell Cat# BE0058
Pacific Blue™ anti-mouse CD45 Antibody BioLegend Cat# 103126
FITC anti-mouse CD45.2 Antibody BioLegend Cat# 109806
APC anti-mouse CD45.1 Antibody BioLegend Cat# 110714
Brilliant Violet 785™ anti-mouse CD3ε Antibody BioLegend Cat# 100355
Alexa Fluor® 700 anti-mouse CD8a Antibody BioLegend Cat# 100730
Brilliant Violet 605™ anti-mouse CD4 Antibody BioLegend Cat# 100548
APC/Cyanine7 anti-mouse CD279 (PD-1) Antibody BioLegend Cat# 135224
PE/Cyanine7 anti-mouse CD366 (TIM-3) Antibody BioLegend Cat# 119716
Anti-mouse IFN gamma Monoclonal Antibody (XMG1.2), APC Thermo Fisher Cat# 17-7311-82
FITC anti-human/mouse Granzyme B Recombinant Antibody BioLegend Cat# 372206
PerCP/Cyanine5.5 anti-mouse TNF-α Antibody BioLegend Cat# 506322
PE/Cyanine7 anti-mouse CD39 Antibody Biolegend Cat# 143806
APC/Cyanine7 anti-mouse CD11c Antibody BioLegend Cat# 117324
CD11c Monoclonal Antibody (N418), eFluor™ 506 Thermo Fisher Cat# 69-0114-82
Brilliant Violet 605™ anti-mouse/rat XCR1 Antibody BioLegend Cat# 148222
PE/Cyanine7 anti-mouse CD172a (SIRPα) Antibody BioLegend Cat# 144008
PE/Cyanine7 anti-mouse/human CD11b Antibody BioLegend Cat# 101216
PerCP-Cy™5.5 Rat Anti-CD11b Antibody BD Biosciences Cat# 550993
Alexa Fluor® 700 anti-mouse I-A/I-E Antibody BioLegend Cat# 107622
Brilliant Violet 785™ anti-mouse F4/80 Antibody BioLegend Cat# 123141
PerCP/Cyanine5.5 anti-mouse CD40 Antibody BioLegend Cat# 124623
PE/Cyanine7 anti-mouse CD80 Antibody BioLegend Cat# 104734
FITC anti-mouse CD86 Antibody BioLegend Cat# 105110
MHC Class I (H-2Kb) Monoclonal Antibody (AF6-88.5.5.3), APC Thermo Fisher Cat# 17-5958-82
PE anti-mouse H-2Kb Antibody BioLegend Cat# 116508
MCL-1 Monoclonal Antibody (LVUBKM), PE Thermo Fisher Cat# 12-9047-41
Pacific Blue™ anti-human CD45 Antibody BioLegend Cat# 304022
PerCP/Cyanine5.5 anti-human CD3 Antibody BioLegend Cat# 300328
FITC anti-human CD8a Antibody BioLegend Cat# 300906
PE/Cyanine7 anti-human CD69 Antibody BioLegend Cat# 310912
APC anti-human CD11c Antibody BioLegend Cat# 301614
Brilliant Violet 785™ anti-human CD11b Antibody BioLegend Cat# 301346
iTAg Tetramer/PE - H-2 Kb OVA (SIINFEKL) MBL Cat# TB-5001-1
H-2 Kb (SIINFEKL), PE, 50 tests immudex Cat# JD02163-PE
Anti-FcγIII/II receptor (clone 2.4G2) BD Biosciences Cat# 553141
Anti-mouse CD8a (IHC, D4W2Z) Cell Signaling Technology Cat# 98941

Validation

Antibody validations were performed by suppliers and have been published by our group and others

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

American Type CultureCollection (ATCC).

Authentication

The cell lines obtained from ATCC with responsive authentication and characterization. No further authentication was performed before use.

Mycoplasma contamination	All cell line used were tested Mycoplasma-free before use.
Commonly misidentified lines (See ICLAC register)	None

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	6-10 weeks. Wild-type (WT) female C57BL/6J and Balb/c mice were purchased from the Jackson Laboratory. Il10−/−, Il10r−/−, Il10rFl/Fl, Lyz2Cre, Cd8Cre, Zbtb46Cre, Xcr1Cre, Rag1−/−, Batf3−/−, NSG-SGM3, IFNγ-eYFP, OT-1 mice were purchased from the Jackson Laboratory and maintained in the laboratories of J.Q. For the conditional knockout mice, the Il10rFl/Fl were first crossed with cre-specific expressing mice to generate F1 mice; then, the F1 mice were inbred to generate the cre+ mice with homozygous Il10rFl/Fl; the insertion of cre and flox elements were confirmed by PCR. All mice were maintained in the Animal Resource Center (ARC) facility at UT Southwestern Medical Center. Animals were housed at 21±2°C; humidity was 50–60%. The light cycle consisted of light for 12h and darkness for 12h. All animal procedures were performed in accordance with the animal experimental guidelines set by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center. The detailed information about all mice strains are listed: C57BL/6J Jackson Laboratory Cat# 000664 BALB/cJ Jackson Laboratory Cat# 000651 B6.129S7-Rag1tm1Mom/J Jackson Laboratory Cat# 002216 NOD.Cg-Prkdcscid Il2rgtm1Wjl Tg(CMV-IL3, CSF2, KITLG) 1Eav/MloySzJ Jackson Laboratory Cat# 013062 B6.129S(C)-Batf3tm1Kmm/J Jackson Laboratory Cat# 013755 B6.129S2-Il10rbtm1Agt/J Jackson Laboratory Cat# 005027 B6.129P2-Il10tm1Cgn/J Jackson Laboratory Cat# 002251 B6(SJL)-Il10ratm1.1Tlg/J Jackson Laboratory Cat# 028146 B6.Cg-Zbtb46tm3.1(cre)Mnz/J Jackson Laboratory Cat# 028538 C57BL/6-Tg(Cd8a-cre)1Itan/J Jackson Laboratory Cat# 008766 B6.129P2-Lyz2tm1(cre)Ifo/J Jackson Laboratory Cat# 004781 B6(129S4)-Xcr1tm1.1(cre)Kmm/J Jackson Laboratory Cat# 035435 B6.129S4-Ifngtm3.1Lky/J Jackson Laboratory Cat# 017581 B6.SJL-Ptprca Pepcb/BoyJ Jackson Laboratory Cat# 002014
Wild animals	No wild animal was used
Reporting on sex	Sex was not considered in study design
Field-collected samples	No field-collected sample was used.
Ethics oversight	All animal experiments were performed with ethical compliance and approval from Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center (animal protocol number [APN] 2015-101350, [APN] 2018-102474).

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	Single cell suspensions were obtained from cell culture, mouse tissues and human tumor fragments. Mouse tumors and human tumor fragments were dissociated by Collagenase (1mg/mL) and DNAse I (0.2 mg/mL) and lymph nodes were dissociated with 70 µm cell strainer. For each single cell suspension,Fc receptor was blocked with anti-FcγIII/II (clone 2.4G2) for 20 minutes, followed by staining with selective antibodies of cell surface markers and live/dead dyes. Intracellular markers were stained after cell permeabilization with True-Nuclear transcription factor buffer set (BioLegend).
Instrument	CytoFLEX (Backman coulter), BD Melondy, Cytek® Northern Lights™
Software	CytExpert 2.3, FlowJo software v10.10, CYTEK SpectroFlo® Software 4.0
Cell population abundance	For in vitro analysis, at least 2000 live interested cells were collected for analysis. For analysis of tissues, at least 20000 live CD45 Cell were collected for analysis.
Gating strategy	Physical parameter and LIVE/DEAD™ fixable dye (ThermoFisher) was used to exclude dead cells. Positive populations were defined using not stained cells as reference. Isotype controls were used to confirm the specificity of the staining.

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