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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	Custom code was used to collect data is available at https://github.com/tanaylab/BarboyBercovich
Data analysis	For FACS analysis, we used the following sofrware: FACSDiva 7 FlowJo 10.4.2 Data analysis was performed in R (version 4.0.3) and python (version 3.7.5). Data analysis was done with the published Metacells package (version 0.9.0-dev.1) and custom algorithms available at https://github.com/tanaylab/.

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

scRNA-seq data that support the findings of this will be deposited in the Gene Expression Omnibus under an accession code GSE249630. Previously published human breast cancer scRNA-seq data that were reanalyzed here are available under accession code EGAS00001004809, and the previously published human pan-cancer atlas data are available under accession code GSE156728.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	We did not employ any statistical methods for pre-determining sample sizes, but our sample sizes align closely with those detailed in prior publications and standard in the field.
Data exclusions	For tumor growth measurements, tumor size measurements were log-transformed, and outliers were identified via the identify_outliers method of the rstatix package and removed. In addition, tumor samples were not further processed in cases were they represented extreme outliers in their respective treatment group. Processed samples were excluded in cases were they did not meet technical quality control criteria accepted in the field (and hence not informative).
Replication	Number of replicates per experiment was sufficient to establish statistical significance, as presented. Experiments were not further replicated. Single-cell RNA-Seq and animal treatment experiments performed 2 times, unless otherwise stated in the legend. Biological and technical replications of the collected cells are further described in the manuscript and table S1.
Randomization	All animal experiments were randomized before any experimental intervention.
Blinding	Tumor sizes were measured blindly to the conditions of the experiments. For treatment purposes, blinding is not possible due to treatment schedule. For sample processing, blinding is not relevant as samples from all conditions are jointly processed.

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

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

For flow cytometry, murine cells were stained with 1ug/100ul unless stated otherwise.

Anti-mouse Pe/Cy7-conjugated CD8, clone 53-6.7, Biolegend, Cat#100721
Anti-mouse FITC-conjugated CD8, clone 53-6.7, Biolegend, Cat#100705
Anti-mouse APC/Cy7-conjugated CD45.1, clone A20, Biolegend Cat#110715
Anti-mouse PE-conjugated CD45.2, clone 104, Biolegend Cat#109808
Anti-mouse APC-conjugated CD279 (PD1), clone 29F.1A12, Biolegend Cat#135209
Anti-mouse BV421-conjugated CD366, clone RMT3-23, Biolegend, Cat#119723
Anti-mouse Pe/Cy7-conjugated CD366, clone RMT3-23, Biolegend, Cat#119715
Anti-mouse PerCP/Cy5.5-conjugated CD366, clone B8.2C12,Biolegend, Cat#134011
Anti-mouse APC/Cy7-conjugated CD45, clone 30-F11, Biolegend, Cat#103115
Purified anti-mouse CD16/32 Antibody, clone 93, Biolegend, Cat#101301
Anti-mouse AF700-conjugated CD62L, clone MEL-14, Biolegend, Cat#104426
Anti-mouse BV510-conjugated PD1, clone 29F.1A12, Biolegend, Cat#135241
Anti-mouse FITC-conjugated TCRb, clone H57-597, Biolegend Cat#109205
Anti-mouse superbright702-conjugated CD8a, clone L138D7, ThermoFisher Cat#67-0087-42
Anti-mouse APC-conjugated Cxcr5, clone ,Biolegend Cat#145505,1:50
Anti-mouse Pacific blue-conjugated Ly108, clone 330-AJ, Biolegend, Cat#134608,1:50
PE-conjugated Vb5.1, 5.2 (OT1),clone MR9-4, Biolegend, Cat#139503
DAPI ,Biolegend, Cat#422801, 1:500

Therapeutic antibodies:
InVivoMAb anti-mouse PD-1 (CD279), clone RMP1-14, BioXcell, #BE0146
InVivoMAb anti-mouse 4-1BB (CD137),clone LOB12.3, BioXcell, #BE0169

Validation

All antibodies utilized in this study underwent validation by the supplier, ensuring their suitability for the intended applications. Detailed validation statements and pertinent references for these antibodies can be accessed on the manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

B16 melanoma cells (H2b), which stably express chicken ovalbumin (OVA) and the fluorescent protein mCherry, were kindly provided by Prof. Lea Eisenbach's laboratory. MC-38 murine colon adenocarcinoma cell line was kindly provided by Dr. Rony Dahan's laboratory. LLC tumor cells were obtained from Prof. Evan Newell laboratory.

Authentication

scRNAseq data was used for authentication of the tumor cells

Mycoplasma contamination

all cell lines were tested negative for Mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell 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

C57BL/6 WT , TCR-transgenic OT-I (harboring OVA-specific CD8+ T cells), and Xcr1-iDTR mice were used in the study. In all experiments, 8-to-12-week-old female mice were used.

Wild animals

No wild animals were used in the study

Reporting on sex

Female mice were used for all experiments

Field-collected samples

No field collected samples were used in the study

Ethics oversight | The research complies with all relevant ethical regulations. The experimental mouse protocol was approved by the Weizmann Institutional Animal Care and Use Committee (10250119-2 and 04450520-2,00580121-2).

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

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	Mice were sacrificed at various time points by cervical dislocation. Tumor, spleen, and tumor draining lymph nodes (tdLN) were collected and put through a process of mechanical and enzymatic digestion. To achieve single-cell suspensions, tumors were chopped into small pieces and suspended in RPMI supplemented with DNase (2mg/ml, Sigma-Aldrich) and collagenase IV (100mg/ml, Worthington). Tumors were homogenized by GentleMacs tissue homogenizer (Miltenyi Biotec) and incubated at 37oC for 30min, with frequent agitation. Following dissociation procedures, cells were washed with cold PBS, filtered through a 70-µm cell strainer, and centrifuged at 300g for 5 min at 4°C. Spleen and tdLN were mashed through a 100uM cell strainer and washed with ice-cold MACS buffer (PBS supplemented with 0.2mM EDTA, pH 8, and 0.5% BSA). Cells were centrifuged at 300g, 5 min, 4°C. Spleen was suspended in red blood lysis buffer (Sigma-Aldrich #R7757) and DNase (0.33U/ml, Sigma-Aldrich), incubated for 5min at room temperature, washed with cold PBS, passed through a 70µm mesh strainer, centrifuged at 300g, 5 min, 4°C and then resuspended in ice-cold MACS buffer.
Instrument	Cell populations were sorted using either SORP-aria (BD Biosciences), ARIA-III instrument (BD Biosciences) or Symphony S6 (BD Biosciences).
Software	Analyzed using BD FACSDIVA software (BD Biosciences) and FlowJo software.
Cell population abundance	All sorted populations were processed and analyzed with scRNA-seq methods (MARS-seq) and their cell abundances were calculated and reported in the manuscript, Tables S1 and S2 .
Gating strategy	B16-OVA models-> SC/SSC -> DAPI neg -> CD8+CD45.1+ for OT1 CD8+ T cells, CD8+GFP+ for bystanders CD8+ T cells, and CD8 +CD45.2+ to sort endogenous CD8+ T cells. MC-38 tumor model:FSC/SSC -> DAPI neg ->CD45/CD8+. LLC model: SC/SSC -> DAPI neg-> Sorting of the mRiok1-tet+CD8 T cell was performed by gating for CD8a+ which are positive for the streptavidin-peptide–MHC-complex.

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