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	Attune NxT control software (version 4.2) was used to collect flow cytometry data. Glomax Explorer software v3 was used in the cancer cell killing assays. Living Image software v4.7.0 (Xenogen) was used for acquisition of bioluminescence imaging datasets.
Data analysis	Flow cytometric analysis was done using FlowJo v10 software. Graphs were produced with Graphpad Prism v9 software. Figures were compiled with Adobe Illustrator v25. Amplicon sequencing data were processed with CRISPResso2. RNA-Seq reads were processed with kallisto. ATAC-seq reads were trimmed using cutadapt v1.18 to remove Nextera transposase sequences, then aligned to hg19 using Bowtie2 v2.3.4.3. Low-quality reads were removed using samtools v1.9 view function (samtools view -F 1804 -f 2 -q 30 -h -b). Duplicates were removed using picard v2.18.26, then reads were converted to BED format using bedtools bamtobed function and normalized to reads per million. ATAC-seq reads mapping within a 1kb window surrounding CRISPR cut sites were counted using the bedtools intersect 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

RNA sequencing data been deposited in the Gene Expression Omnibus (GEO) under the accession code GSE202909. ATAC sequencing data been deposited in the

GEO under the accession code GSE130089. Amplicon sequencing data been deposited in the GEO under the accession codes GSE202596 and GSE203349. Other relevant data are available from the corresponding authors upon reasonable request.

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	No statistical method was used to predetermine sample size. Sample sizes are similar to those reported in previous publications (PMID: 35027758, PMID: 31819258, PMID: 29995861).
Data exclusions	For mouse tumor studies, a second cohort of mice treated with cells from a second donor was excluded because tumor failed to efficiently engraft in control group.
Replication	Replicate experiments were performed as described in figure legends and methods
Randomization	Mice were randomized to achieve similar distributions of tumor load measured with bioluminescence the day before T cells injection. Controls and treatments for studies with primary human cells were performed in matched cells from the same donors.
Blinding	The investigators were not blinded to allocation during experiments and outcome assessment.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	The following antibodies were used. Biolegend: FoxP3-Alexa647 (206D), Helios-PE (22F6), Streptavidin-APC, CD19-PacBlue (HIB19), CD25-BV421 (BC96), CD271-FITC (ME20.4), CD3-APC (OKT3), CD3-PECy7 (OKT3), CD34-BV421 (561), CD4-PECy7 (OKT4), CD4-FITC (SK3), CD45RA-APC (HI100), CD56-BV421 (5.1H11), CD8-BV421 (RPA-T8), CD95-BV711 (DX2), TCRα/β-BV421 (IP26). Invitrogen: TCR gamma/delta-FITC (5A6.E9). Tonbo: CD45RO-FITC (UCHL1). Cell Signaling: HA-Tag-Alexa647 (6E2) and Myc-Tag-Alexa647 (9B11). BD: Streptavidin-PE, CTLA4-BV421 (BNI3), CD4-PE (RPA-T4), CD45RA-BV650 (HI100), CD62L-BV421 (DREG-56). Miltenyi: CD197-PE (REA108). Beckman Coulter: CD5-PacBlue (BL1a). Additional details are provided in Supplementary Table 1.
Validation	*Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	31 8- to 12-week-old NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) male mice (in-house breeding) were used for these studies. Mice were housed with standard 12:12 light:dark cycle, temperature 68-79 degrees F, and humidity between 30-70%.
Wild animals	No wild animals were used in this study.

Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	Mice were used in accordance with ethical guidelines approved by the UCSF Institutional Animal Care and Use Committee.

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	Primary adult blood cells were obtained from anonymous healthy human donors as a leukopheresis pack purchased from StemCell Technologies, Inc. or Allcells Inc, as a Trima residual from Vitalant, or from fresh whole blood under a protocol approved by the UCSF Committee on Human Research (CHR #13-11950). If needed, peripheral blood mononuclear cells were isolated by Ficoll-Paque (GE Healthcare) centrifugation. Primary human cell types were then further isolated by positive and/or negative selection using EasySep magnetic cell isolation kits purchased from StemCell for CD3+ T cells (Cat #17951), CD4+ T cells (Cat #17952), CD8+ T cells (Cat #17953), B cells (Cat #17954), NK cells (Cat #17955), or CD4+CD127lowCD25+ regulatory T cells (Cat #18063) per manufacturer instructions. Primary human γδ T cells were isolated using a custom γδ T cell negative isolation kit without CD16 and CD25 depletion obtained from StemCell. Primary adult peripheral blood G-CSF-mobilized CD34+ hematopoietic stem cells were purchased from StemExpress, LLC.
Instrument	All flow cytometry was performed on an Attune NxT flow cytometer with a 96-well autosampler (Thermo Fisher Scientific).
Software	Flow cytometric analysis was done using FlowJo v10 software. Graphs were produced with Graphpad Prism v9 software. Figures were compiled with Adobe Illustrator v25.
Cell population abundance	All sorts were end-point sorts and not for subsequent culture.
Gating strategy	A representative example of the gating strategy used in these studies is demonstrated in Supplementary Figure 3b. As shown, cells are sequentially gated on FSC/SSC, single cells by FCS-A vs FSC-HA, live cells by viability dye, and then for the indicated surface or intracellular markers.

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