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Corresponding author(s): Nicholas Arpaia and Tal Danino

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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	FACS Diva v8.0 and SpectroFlo v3.0 were used for flow cytometry data collection.
Data analysis	Software used for sequencing data analysis: Illumina bcl2fastq v2.17, Trimmomatic v0.39, bcftools v1.13, Illumina Dragen Bio-IT platform v1.0, Broad Institute GATK v4.2.0.0, pVacTools v2.0.3, STAR aligner v2.5.2b and Subread Package v1.5.2, Bam-readcount v1.0, DESeq2 v1.20.0, VAtools v5.0.0, Ensembl Variant Effect Predictor v104, NetMHCpan v4.1, NetMHC v4.0, NetMHCIIpan v4.1, and NNAlign v2.0. Software used for flow cytometry data anlysis: FlowJo v10. Software used for statistical analysis: GraphPad Prism v9.0 and v10.0.

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

Whole exome sequencing and RNA-Seq data generated for this study are deposited under umbrella BioProject accession number PRJNA1025007. Whole exome

data are available on the Short Read Archive with BioProject ID PRJNA1024050. RNA-seq data are available on NCBI Gene Expression Omnibus with accession number GSE244808. All other data are available within the article or its Supplementary Information. Source data are provided with this paper. Datasets utilized for analyses in this study: Ensembl release 102 M. musculus GRCm38 gene annotations (GRCm38, https://useast.ensembl.org/Mus_musculus/Info/Index, accession: GCA_000001635.8), and dbSNP build 142 (ftp.ncbi.nih.gov/snp).

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 base our sample size on standards in the field, as well as our prior experience and work involving bacterial and mammalian cell studies, and animal trials (PMCIDs: PMC6688650, PMC7685004, PMC10915968). These previous studies, along with small pilot studies, served as the basis for determination of sample size. Sample size is explicitly stated for each experimental group for individual experiments in figure captions and data descriptions.
Data exclusions	No data were excluded.
Replication	All results in the manuscript were replicated at least 2-3 times in independent experiments.
Randomization	For subcutaneous tumor models: treatment groups were created by selecting from a pool of animals with comparable tumor volume, such that the average tumor between each group was approximately equal at the beginning of all experiments. For metastastic tumor models: animals were randomly distributed between groups after intravenous injection of tumor cells prior to treatment and imaging. For in vitro experiments, equal numbers of cells were seeded or utilized, and randomly allocated between experimental groups.
Blinding	Investigators were not blinded to groups in in vitro or in vivo studies as this was necessary information to carry out studies.

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 antibodies used in the study are listed below. They are listed in the following format: antigen target, clone name, supplier, catalog number, manufacturer link.

For immunofluorescence:
1. Anti-chicken ovalbumin, EPR27117-90, Abcam, ab306591, https://www.abcam.com/en-us/products/primary-antibodies/ovalbumin-antibody-epr27117-90-ab306591#
2. Anti-CD11b (murine), M1/70, Abcam, ab8878, https://www.abcam.com/en-us/products/primary-antibodies/cd11b-antibody-m1-70-ab8878

For immunoblotting:
1. Anti-6xHis, αTHE, Genscript, A00186S, https://www.genscript.com/antibody/A00186S-THE_His_Tag_Antibody_mAb_Mouse.html
2. Anti-DnaK, 8E2/2, Abcam, ab69617, https://www.abcam.com/en-us/products/primary-antibodies/dnak-antibody-8e2-2-ab69617

For flow cytometry, the fluorochrome-conjugated anti-mouse antibodies used were:
1. CD4-PEDazzle594, RM4-5, Biolegend, 100565, https://www.biolegend.com/en-us/products/pe-dazzle-594-anti-mouse-cd4-antibody-9845
2. NKp46-BV605, 29A1.4, BD Biosciences, 564069, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv605-rat-anti-mouse-cd335-nkp46.564069
3. NK1.1-BUV395, PK136, BD Biosciences, 564144, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-mouse-anti-mouse-nk-1-1.564144
4. CD45-BV650, 30-F11, Biolegend, 103151, https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-mouse-cd45-antibody-11987
5. CD45-BUV395, 30-F11, BD Biosciences, 564279, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-rat-anti-mouse-cd45.564279
6. B220-BUV496, RA3-6B2, BD Biosciences, 612950, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv496-rat-anti-mouse-cd45r-b220.612950
7. CD19-APC/Fire810, 6D5, Biolegend, 115577, https://www.biolegend.com/en-us/products/apc-fire-810-anti-mouse-cd19-antibody-20595?GroupID=BLG10556
8. CD8a-AF700, 53-6.7, Biolegend, 100729, https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd8a-antibody-3387
9. TIM1-BV421, RMT1-4, BD Biosciences, 566336, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/BV421-Rat-Anti-Mouse-CD365-(TIM-1).566336
10. CD69-BUV563, H1.2F3, BD Biosciences, 741234, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv563-hamster-anti-mouse-cd69.741234
11. Foxp3-FITC, FJK-16s, Thermo, 11-5773-82, https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/11-5773-82
12. CD3ε-BV785, 145-2C11, Biolegend, https://www.biolegend.com/en-us/clone-search/brilliant-violet-785-anti-mouse-cd3epsilon-antibody-12081
13. CD3ε-PerCP/Cy5.5, 145-2C11, Biolegend, 100327, https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd3epsilon-antibody-4191
14. TCRβ-BV711, H57-507, BD Biosciences, 563135, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv711-hamster-anti-mouse-tcr-chain.563135
15. Ki67-PE, SolA15, Thermo, 12-5698-80, https://www.thermofisher.com/antibody/product/Ki-67-Antibody-clone-SolA15-Monoclonal/12-5698-80
16. GranzymeB-PE-Cy7, QA16A02, Biolegend, 372213, https://www.biolegend.com/en-us/products/pe-cyanine7-anti-humanmouse-granzyme-b-recombinant-antibody-15582
17. TNFα-AF647, MP6-XT22, Biolegend, 506314, https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-tnf-alpha-antibody-2724
18. IFN -PE, XMG1.2, Biolegend, 505807, https://www.biolegend.com/en-us/products/pe-anti-mouse-ifn-gamma-antibody-997
19. Ly6C-FITC, HK1.4, Biolegend, 128005, https://www.biolegend.com/en-us/products/fitc-anti-mouse-ly-6c-antibody-4896
20. I-A/I-E-BV480, M5/114.15.2, BD Biosciences, 566088, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv480-rat-anti-mouse-i-a-i-e.566088
21. I-A/I-E-PerCP/Cy5.5, M5/114.15.2, BD Biosciences, 562363, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/percp-cy-5-5-rat-anti-mouse-i-a-i-e.562363
22. XCR1-BV605, ZET, Biolegend, 148222, https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-rat-xcr1-antibody-22025
23. CD11b-AF700, M1/70, Biolegend, 101222, https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-human-cd11b-antibody-3388

24. CD11b-BV650, M1/70, Biolegend, 101239, https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-mouse-human-cd11b-antibody-7638?GroupID=BLG10599
25. CD103-BV785, 2E7, Biolegend, 121439, https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-cd103-amtibody-17353
26. F4/80-PE-Cy5, BM8, Biolegend, 123111, https://www.biolegend.com/en-us/products/pe-cyanine5-anti-mouse-f4-80-antibody-4069
27. F4/80-APC, BM8, Biolegend, 123115, https://www.biolegend.com/en-us/products/apc-anti-mouse-f4-80-antibody-4071
28. CD11c-BUV737, HL3, BD Biosciences, 612796, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv737-hamster-anti-mouse-cd11c.612796
29. CD172a-BUV563, P84, Biolegend, 741349, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv563-rat-anti-mouse-cd172a.741349
30. Ly6G-APC/Fire810, 1A8, Biolegend, 127669, https://www.biolegend.com/en-us/products/apc-fire-810-anti-mouse-ly-6g-antibody-21380
31. Ly6G-PE-CF594, 1A8, BD Biosciences, 562700, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cf594-rat-anti-mouse-ly-6g.562700
32. PDL1-PE-Cy7, 10F.9G2, Biolegend, 124313, https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-cd274-b7-h1-pd-l1-antibody-6721
33. CD301b-AF647, URA-1, Biolegend, 146805, https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-cd301b-mgl2-antibody-9657
34. CD19-PerCP/Cy5.5, 1D3, Biolegend, 152405, https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd19-antibody-13640
35. NK1.1-PerCP/Cy5.5, PK136, Biolegend, 108727, https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-nk-11-antibody-4289
36. NKp46-BV510, 29A1.4, Biolegend, 137623, https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-cd335-nkp46-antibody-9578
37. CD64-PEDazzle594, X54-5/7.1, Biolegend, 139319, https://www.biolegend.com/en-us/products/pe-dazzle-594-anti-mouse-cd64-fcgammari-antibody-12424
38. CD80-PE, 16-10A1, Biolegend, 104707, https://www.biolegend.com/en-us/products/pe-anti-mouse-cd80-antibody-43
39. CD86-BUV805, GL-1, BD Biosciences, 741946, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv805-rat-anti-mouse-cd86.741946
40. H2Kb-SIINFEKL-PE, 25-D1.16, Biolegend, 141603, https://www.biolegend.com/en-us/products/pe-anti-mouse-h-2kb-bound-to-siinfekl-antibody-7247
41. CD3-PE, 17A2, Biolegend, 100205, https://www.biolegend.com/en-us/products/pe-anti-mouse-cd3-antibody-47

For in-vivo antibody depletion:
1. Anti-mouse CD4, clone GK1.5, BioXcell, #BE0003-1, https://bioxcell.com/invivomab-anti-mouse-cd4-be0003-1
2. Anti-mouse CD8b, clone Ly-3.2, BioXcell, #BE0223, https://bioxcell.com/invivomab-anti-mouse-cd8-beta-lyt-3-2-be0223?queryID=968b7720c10d16b0d5bb810a89b7bd1f&objectID=30662&indexName=bioxcell_live_default_products
3. IgG1 isotype control, clone HRPN, BioXcell, #BE0088, https://bioxcell.com/invivomab-rat-igg1-isotype-control-anti-horseradish-peroxidase-be0088?queryID=c68a9872d06a3d920db5f601de05fe61&objectID=30559&indexName=bioxcell_live_default_products

Validation

All antibodies used in this study are commercially available, and were validated by the manufactures, which can be accessed through the link provided for each antibody listed.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	B16F10 (ATCC CRL-6475), CT26 (ATCC CRL-2638), 4T1 (ATCC CRL-2539)
Authentication	Cells were frozen down at early passages after receipt from ATCC and thus did not require re-authetication.
Mycoplasma contamination	Cells were confirmed mycoplasma free.
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	Mice were housed in a facility with a 12-hour light-dark cycle, and provided unrestricted access to both food and water. The housing facility was maintained at 21–24°C, and kept at 40-60% humidity. Animals used included female 6-7 week old BALB/c, C57BL/6, and B6(Cg)-Tyrc-2J/J mice.
Wild animals	No wild animals were used in this study.
Reporting on sex	Female mice were used in animal studies, as male mice exhibit aggressive behaviors and fight which we noted to cause wounding to the tumors and one another, which can affect experimental outcomes (PMCIDs: PMC9817818, PMC7538892).
Field-collected samples	No field-collected samples were used in this study.

Ethics oversight	Institutional Animal Care and Use Committee (IACUC) of Columbia University (Protocol: AABQ5551)

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	Tumors and TDLN were extracted either 2 or 8 days post intravenous treatment for flow cytometry immunophenotyping. Lymphoid and myeloid immune subsets were isolated from tumor tissue by medechanical homogenization of tumor or TDLN tissue, followed by digestion with collagenase A (1mg/ml, Roche) and DNase I (0.5 µg/ml, Roche) in isolation buffer (RPMI 1640 with 5% FBS, 1% L-glutamine, 1% penicillin-streptomycin, and 10mM HEPES) for 1 hour at 37°C for tumors or 30 minutes at 37°C for TDLNs, on a shaker platform at 150rpm. For ex vivo lymphocyte stimulation with PMA and ionomycin, TDLNs were not digested prior. Tumor and TDLN homogenates were filtered through 100 µm cell strainers and washed in isolation buffer. To measure cytokine production by T cells, cells were stimulated for 3-hours with PMA (50ng/ml, Sigma-Aldrich) and ionomycin (1nM, Calbiochem) in the presence of brefeldin A (1µg/ml). To measure neoantigen-specific cytokine production by T cells, cells were stimulated for 5 hours with pools of peptides (2µg/ml) representing the neoantigens encoded in therapeutic strains in the presence of brefeldin A (1µg/ml). Cells were stained in FACS buffer. Ghost Dye cell viability reagent was used to exclude dead cells (diluted 1:1000 in PBS), followed by extracellular staining. After extracellular staining, cells were washed with FACS buffer, and either directly used for flow cytometry analysis, or fixed using the FOXP3/ transcription factor staining buffer set (Tonbo), as per manufacturer's instructions. Cells were then stained intracellularly for lymphoid immunophenotyping panels. After staining, cells were washed and resuspended with FACS buffer for flow cytometry analysis.
Instrument	BD LSRFortessa, Cytek Aurora cell analyzer.
Software	Collection: FACS Diva v8.0. or SpectroFlo v3.0. Analysis: Flowjo v10.0.
Cell population abundance	Cell population abundance depends on the particular cell type, tissue type, and treatment conditions. All cell populations were identifiable by flow cytometry following digestion of either tumor or TDLN.
Gating strategy	Pre-processed FSC files were initially gated on FSC-A and SSC-A to exclude large and small debris or clumps. FSC-H vs. FSC-A, followed by SSC-H vs. SSC-A were used to exclude non-singlet events. CD45 vs. Viability dye were gated to include all live lymphocytes. For myeloid cell analysis: PMNs were defined as CD45+CD11b+Ly6G+ cells. Macrophages were defined as CD45+CD11b +Ly6G-Ly6C-F4/80+ cells. Monocytes were defined as CD45+CD11b+Ly6G-Ly6C+F4/80-Lin-CD64+MHCII+. M-MDSC cells were defined as CD45+CD11b+Ly6G-Ly6c+F4/80-MHCII-CD64- cells. cDC1 were defined as CD45+Ly6G-Ly6c-F4/80-CD11c+MHCII +CD11b-CD172a-Lin-XCR1+ and/or CD103+ cells. cDC2 were defined as CD45+Ly6G-CD11b+CD172a+/-Ly6C-F4/80+/-CD11c +MHCII+Lin-CD103-, and also CD301b+ in the B16 model. Expression markers (PD-L1, CD80, CD86) were gated on major lineage cells ex vivo. For lymphoid cell analysis: B cells were defined as CD45+CD19/B220+NK1.1-CD3-TCRb-. NKp46 was used in place of NK1.1 for BALB/c mice. CD8+ T cells were defined as CD45+CD19/B220-NK1.1-CD3+TCRb+CD4-CD8+. Conventional Foxp3-CD4+ T cells were defined as CD45+CD19/B220-NK1.1-CD3+TCRb+CD8-Foxp3-CD4+ cells. NK cells were defined as CD45+CD19/B220-CD3-TCRb-NK1.1+ cells. Expression markers (CD69, Granzyme-B, Ki67) were gated on major lineage cells ex vivo, while cytokines (IFNy, TNFa) were gated post stimulation.

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