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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 data was acquired on LSR-Fortessa or BD Symphony instruments using FACSDiva version 8.0.1 or Cytek Aurora using SpectroFlo version v3.0.3. Processing for single-cell gene expression (scRNA-seq) and T cell receptor V(D)J clonotypes (scTCR-seq) was done using the Chromium Single Cell 5' Library and Gel Bead Kit (10X Genomics), following manufacturer's instructions. Sequencing files from Illumina assays were run through CellRanger version 6.1.1 against a transcriptome derived from ENSEMBL version 2.2.0 for the mouse genome GRCm38. The combined matrix files from the filtered_feature_bc_matrix directory for the RNA and ADT libraries were divided into separate submatrices for each sample, based on 52,636 genes for expression, 6 tetramer barcodes for ADT counts, 24 antibody measurements for CITE-seq, and 10 barcodes for multiplexing of the blood samplees. Measurements corresponding to various alleles of T cell receptor genes (e.g., Trbv1 through Trbv31) were combined into a single gene measurement (Trbv). Single cells from pooled blood samples were demultiplexed as described in the Methods section.

Data analysis

FACS analysis was performed using FlowJo version 10 or higher, and figures were generated using GraphPad Prism version 9.4.1. Mouse single-cell analyses were performed in R version 4.2.0 and with scripts written for Perl version 5.16.3. Single-cell UMI count matrix for each tissue sample was processed using scDblFinder version 1.12.0. Remaining singlet count matrices were processed using Seurat version 4.1.1. using the SCTransform function. Additional analysis was performed using Seurat procedures NormalizedData, FindVariableFeatures, ScaleData,RunPCA, RunUMAP, FindNeighbors, FindClusters. Batch correction was performed using Harmony package 0.1.1. PCA cell embeddings were calculated following the procedure in https://cran.r-project.org/web/packages/harmony/vignettes/Seurat.html. Additional analysis was performed using Matrix 1.4-1, sparseMatrixStats version 1.8.0, SingleR version 1.10.0, superheat version 1.0.0, and RColorBrewer version 1.1-3. RNA velocity analysis was performed using kallisto bustools (version 0.46.1), with mapping to a transcriptome index from Ensembl version 90 on genome GRCm38. The transcriptome index was generated using kallisto with a read length of 90 nucleotides and intronic sequences from BUSpaRse (https://github.com/BUStools/BUSpaRse). Objects were processed by scvelo package 0.2.4 within Python version 3.7.3 using the commands "pp.filter_and_normalize", "pp.moments", "Tl.recover_dynamics" and "Tl.velocity" with mode="dynamical."

Velocity graphs were generated using the command "tl.velocity_graph" and "pl.velocity_embedding_stream."Additional figures were generated using Adobe Illustrator (version 27.0 or higher) and BioRender. External datasets were obtained from NCBI GEO and ArrayExpress, with metadata provided directly from authors by direct request or cell assignments from the Seurat object provided online, as described in the Methods section. Analysis of clinical trial data was performed using R version 4.2.0. Human genes were converted to their mouse orthologs using babelgene version 22.9. Human CD8+ T cells were then separated by patient and normalized with SCTransform in Seurat version 4.2 using default parameters. MapQuery function in Seurat was used to transfer cell type labels, integrate embeddings and to project the query data onto the reference UMAP. , R version 4.2.0 packages survminer version 0.4.9 and survival 3.4-0 were used to generate Kaplan-Meier plots. Computer code used to generate the single-cell analyses and figures in this paper are provided as a Supplementary File to the NCBI GEO accession GSE220901.

Computer code used to generate the single-cell analyses and figures in this paper are provided as a Supplementary File to the NCBI GEO accession GSE220901. Code for reanalyzed datasets from GO30103 (NCT02794571) (Ref 4,31), CITYSCAPE (NCT03563716) (Ref 3,4), and OAK (NCT02008227) (Ref 4,32) clinical trials are accessible at https://github.com/cwtran/nutsch_nature_cancer_2024/.

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

FASTQ files containing raw sequencing reads for the scRNA-seq, scTCR-seq, ADT-seq, and CITE-seq analyses have been deposited with the NCBI Short Read Archive (SRA) under accession PRJNA911822. Processed output files from Cell Ranger and metadata with cluster assignments, clonotypes, and ADT assignments have been deposited with the NCBI Gene Expression Omnibus (GEO) under accession GSE220901. Raw counts and metadata for reanalyzed scRNAseq data from GO30103 (NCT02794571) (Refs (4,31) are available from the European Genome-Phenome Archive (EGA) under accession EGAD50000000367 and https://github.com/cwtran/nutsch_nature_cancer_2024/. Reanalyzed datasets from CITYSCAPE (NCT03563716) (Refs 3,4) and OAK (NCT02008227) (Refs 4,32) clinical trials can be found under EGA accession EGAD50000000251 and EGAD50000000368, respectively. All data relating to mouse sequencing data at NCBI SRA and NCBI GEO and pseudoanonymized clinical trial data are available without restrictions. Due to legal requirements for data sharing, users must agree to the Data Access Agreement detailed in the EGA entries above before they can access these human datasets. Data access requests are reviewed by the Genentech DevSci Data Access Committee (devsci-dac-d@gene.com).

Reanalysis of previously published datasets generated by others and publicly available was as follows: for Huang et al., 2022 (Ref 16), we used metadata provided by the authors to us and scRNA-seq count data for the six samples referenced in the metadata from NCBI GEO for GSE180095, GSE122712, GSE152628, and GSE182509; for Daniel et al., 2022 (Ref 24), we used metadata from supplementary files and the scRNA-seq count data, both available at NCBI GEO for GSE188666; for Deak et al., 2022 (Ref 25), we used metadata provided by the authors to us, and scRNA-seq count data available at ArrayExpress for E-MTAB-11773; for Giles et al., 2022 (Ref 26), we used metadata from the Seurat object through the link provided in their paper under Data Availability and scRNA-seq count data available at NCBI GEO for GSE199563; for Li et al., 2022 (Ref 28), we used metadata (taking cluster assignments for celltype_cluster-2) and scRNA-seq normalized data from the Scanpy object available at ArrayExpress for E-MTAB-10176.

Source data for Fig. 1, 2a, 2d, 2e, 3b, 3c, 4, 5c, 6a, 6b, 6c and Extended Data Fig. 1b, 1c, 3a-q, 6a, 6e, 7a, 8, 9b, 9c have been provided as Source Data files. All other data supporting 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	Sex and gender for subjects for the CITYSCAPE phase 2 clinical trial are described in the publication Cho et al., Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study, The Lancet 23, 781-792, 202 (Ref 3). The placebo plus atezolizumab group had a slightly higher proportion of male patients (48 [71%] versus 39 [58%]).
Reporting on race, ethnicity, or other socially relevant groupings	Population characteristics of subjects for the CITYSCAPE phase 2 clinical trial have been previously described (Ref 3).
Population characteristics	Population characteristics of subjects for the CITYSCAPE phase 2 clinical trial are also described in the publication above (Ref 3). Patients, irrespective of treatment arm, were separated on the basis of clinical response (complete response/partial response vs. stable disease/progressive disease). Baseline characteristics of the intention-to-treat (ITT) population include a median age of 68 years in both the tiragolumab + atezolizumab (T+A) or placebo + atezolizumab (P+A) treatment groups. The distribution by sex was 58% male to 42% female in the T+A arm and 71% male to 29% female in the P+A arm. ECOG status was 0 for approximately 30% of patients and 1 for 70% of patients for both treatment arms. 81% of NSCLC patients were stage IV in the T+A arm and 72% stage IV in the P+A arm. PD-L1 positivity by 22C3 was 57% at the 1-49% cutoff and 43% at the PD-L1 ≥ 50% cutoff for both treatment arms. Tumor histology was approximately 60% non-squamous and 40% squamous for both treatment arms. Approximately 10% of patients were never smokers, 65% previous smokers, and 25% current smokers for both arms. Patients with EGFR or ALK mutations were excluded from study.

Recruitment	Patients were recruited between Aug 10, 2018, and March 20, 2019. Of 275 patients assessed, 135 eligible patients were included in the study and randomly assigned to tiragolumab plus atezolizumab (n=67) or placebo plus atezolizumab (n=68), from across Europe (42%, n-57), Asia (31%, n=42) and the USA (27%, n=36).
Ethics oversight	Study design, patient cohort and response assessment for clinical trials GO30103 (NCT02794571) (Ref 31) and CITYSCAPE (NCT03563716) (Ref 3) have been previously described, with trial protocols approved by the institutional review board or ethics committee at each participating center and complied with good clinical practice guidelines, and studies performed in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonization guidelines for Good Clinical Practice, and country-specific laws and regulations, as noted in the originally published clinical trials (Ref 3,31). GO30103 was conducted at 13 sites in 6 countries (Australia, Canada, France, South Korea, Spain, and the USA. CITYSCAPE was conducted in 41 clinical centers across France, Serbia, South Korea, Spain, Taiwan, and the USA. All patients provided written informed consent. An internal monitoring committee reviewed available safety data periodically to make recommendations regarding study conduct to ensure the safety of patients enrolled in the study, as noted in the originally published clinical trials (Ref 3,31).

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	No statistical methods were used to predetermine sample size. Sample sizes were based on previous experiences (Johnston, R. J. et al. The Immunoreceptor TIGIT Regulates Antitumor and Antiviral CD8+ T Cell Effector Function. Cancer Cell 26, 923-937, doi:10.1016/j.ccell.2014.10.018 (2014).), balancing animal welfare and statistical robustness.
Data exclusions	Animals whose tumors became ulcerated prior to progression or complete response or at time of allocation of experiment groups were euthanized and removed from the study. Single cells from blood and tumor were sorted by FACS to be CD90+. Single cells from draining lymph nodes were sorted to be CD90+CD44+. Single cells were further separated computationally to analyze CD8+ T cells in detail, as described in the Methods section.
Replication	The number of repeats and sample sizes are provided in each figure legend where applicable. All data were reliably reproducible.
Randomization	Tumor injected mice were randomly assigned to experimental groups. All UMAP figures plot cells in random order. Random jitter is added to scatterplots to visually display all points. For analysis of human clinical trial data, patients were separated on the basis of clinical responses, irrespective of treatment arm.
Blinding	Blinding was not relevant to this study and the data analysis of clinical trial biomarker data. Patients, irrespective of treatment arm, were separated on the basis of clinical response. The Phase 2 CITYSCAPE trial was a randomized, double-blind, placebo-controlled trial.

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	All antibodies and dyes used for Flow analysis and/or FACS sorting for single cell analysis are provided as supplementary information.

Antibodies used

Fluorophore/Type; Target; Catalog Number; Supplier; Clone Number; Lot Number; Species
APC-Cy7 B220 103224 BioLegend RA3-6B2 B308558 Rat
PerCP-Cy5.5 B220 103235 BioLegend RA3-6B2 B308915 Rat
Dye Calcein blue C1429 Invitrogen n/a 2326042 n/a
APC-Cy7 CD11b 561039 BioLegend DX5 7278811 Rat
PerCP-Cy5.5 CD11b 101227 BioLegend M1/70 B308467 Rat
APC-Cy7 CD11c 117323 BioLegend N418 B197821 Armenian Hamster
PerCP-Cy5.5 CD226 133624 BioLegend TX42.1 B316769 Rat
BV785 CD226 133611 BioLegend TX42.1 B317733
BUV737 CD4 612843 BD Biosciences RM4-5 1198910 Rat
Qdot 605 CD4 50-113-7562 Invitrogen RM4-5 2366139 Rat
PE-Cy7 CD44 103030 BioLegend IM7 B308091 Rat
AF700 CD45 56-0441-82 Invitrogen IM7 1980496 Rat
BUV395 CD45 564279 BD Biosciences 30-F11 1145827 Rat
BV605 CD62L 104437 BioLegend MEL-14 B336181 Rat
BV421 CD69 104545 BioLegend H1.2F3 B291490 Armenian Hamster
AF700 CD8 100730 BioLegend 53-6.7 B285812 Rat
BUV737 CD8 564297 BD Biosciences 53.6-7 6294901 Rat
FITC CD8 100706 BioLegend 53.6-7 B318296 Rat
BV785 CD90.2 105331 BioLegend 30-H12 B289707 Rat
BUV395 CD90.2 565257 BD Biosciences 53-2.1 9311233 Rat
BUV805 CD90.2 741908 BD Biosciences 30-H12 2075373 Rat
eFluor 780 Fixable Viability Dye 65-0856-14 Invitrogen n/a 2450571 n/a
eFluor 506 FoxP3 69-5773-82 Invitrogen FJK-16s 2246975 Rat
pMHC Monomer gp70 n/a in house n/a n/a n/a
pMHC Monomor p15e n/a in house n/a n/a n/a
PE H3K27Me 40724S Cell Signaling C36B11 1 Rabbit
BV650 IFNγ 563854 BD Biosciences XMG1.2 7096621 Rat
PE IL10 505008 BioLegend JES5-16E3 B249530 Rat
BV711 Ki67 350516 BioLegend Ki-67 B345424 Mouse
BUV737 Lag3 741820 BD Biosciences C9B7W 1333079 Rat
BV650 Lag3 125227 BioLegend C9B7W B309510 Rat
BUV496 Ly108/SlamF6 750046 BD Biosciences 13G3 2126118 Mouse
BV605 Ly108/SlamF6 745250 BD Biosciences 13G3 1068515 Mouse
PE-Cy7 PD-1 135216 BioLegend 29F.1A12 B355884 Rat
Dye Propidium Iodide 50-66211E Invitrogen n/a 9352710 n/a
BV650 Streptavidin 405231 BioLegend n/a B196153 n/a
PE TCF1/TCF7 14456S Cell Signaling C63D9 6147819 Rabbit
FITC TIGIT 11-9501-82 Invitrogen GIGD7 2318622 Rat
APC TIM3 134008 BioLegend B8.2C12 B198857 Rat
BV421 TIM3 134019 BioLegend B8.2C12 B321967 Rat
BV711 TIM3 119727 BioLegend RMT3-23 B284683 Rat
BV421 TNFa 506328 BioLegend MP6-XT22 B333754 Rat
APC Tox 130-118-474 Miltenyi Biotec REA473 5211104954 Human
Total-Seq C CD4 100571 BioLegend RM4-5 B318116
Total-Seq C CD8a 100785 BioLegend 53-6.7 B310956
Total-Seq C CD122 (IL-2Rβ) 105915 BioLegend 5H4 B315383
Total-Seq C CD127 (IL-7Rα) 135047 BioLegend A7R34 B300524
Total-Seq C CD137 (41BB) 106119 BioLegend 17B5 B305895
Total-Seq C CD183 (CXCR3) 126545 BioLegend CXCR3-173 B302524
Total-Seq C CD223 (LAG-3) 125237 BioLegend C9B7W B305125
Total-Seq C CD226 (DNAM-1) 128825 BioLegend 1.00E+06 B331517
Total-Seq C CD279 (PD-1) 109127 BioLegend RMP1-30 B300527
Total-Seq C CD28 102133 BioLegend 37.51 B304136
Total-Seq C CD366 (Tim-3) 119739 BioLegend RMT3-23 B302525
Total-Seq C CD38 102735 BioLegend 90 B299602
Total-Seq C CD39 143815 BioLegend DUHA59 B297080
Total-Seq C CD69 104551 BioLegend H1.2F3 B319428
Total-Seq C CD73 127237 BioLegend TY/11.8 B310991
Total-Seq C CX3CR1 149043 BioLegend SA011F11 B296692
Total-Seq C Ly108 134613 BioLegend 330-AJ B328193
Total-Seq C TIGIT (Vstm3) 142119 BioLegend IG9 B299596
Total-Seq C mouse IgG1 400187 BioLegend MOPC-21 B333559
Total-Seq C mouse IgG2 400293 BioLegend MOPC-173 B319350
Total-Seq C rat IgG1 400467 BioLegend RTK2071 B313972
Total-Seq C rat IgG2a 400577 BioLegend RTK2758 B307173
Total-Seq C rat IgG2b 400677 BioLegend RTK4530 B320446
Total-Seq C Arm Hamster 400977 BioLegend HTK888 B313973
Hashtags TotalSeq™-C0301 anti-mouse 155861 BioLegend M1/42; 30-F11 B325958
Hashtags TotalSeq™-C0302 anti-mouse 155863 BioLegend M1/42; 30-F12 B331515
Hashtags TotalSeq™-C0303 anti-mouse 155865 BioLegend M1/42; 30-F13 B332386
Hashtags TotalSeq™-C0304 anti-mouse 155867 BioLegend M1/42; 30-F14 B296942
Hashtags TotalSeq™-C0305 anti-mouse 155869 BioLegend M1/42; 30-F15 B339941
Hashtags TotalSeq™-C0306 anti-mouse 155871 BioLegend M1/42; 30-F16 B322559
Hashtags TotalSeq™-C0307 anti-mouse 155873 BioLegend M1/42; 30-F17 B323978
Hashtags TotalSeq™-C0308 anti-mouse 155875 BioLegend M1/42; 30-F18 B322555

Hashtags TotalSeq™-C0309 anti-mouse 155877 BioLegend M1/42; 30-F19 B328421
Hashtags TotalSeq™-C0310 anti-mouse 155879 BioLegend M1/42; 30-F20 B322104

Antibodies used for in vivo injection
Target; Catalog Number; Supplier; Clone Number; Lot Number; Species, Amount
anti-PD-L1 n/a GNE-in house 6E11 n/a n/a 10 mg/kg
anti-TIGIT (IgG2a; LALAPG) n/a GNE-inhouse 10A7 n/a n/a 10mg/kg
anti-gp120 n/a GNE-in house 3E5 n/a n/a 10mg/kg

Validation

For Flow analysis and FACS sorting, all the commercial antibodies were validated by the manufactures and prior studies of others. BD Biosciences antibody validation was performed as described by the manufacturer: "The specificity is confirmed using multiple methodologies that may include a combination of flow cytometry, immunofluorescence, immunohistochemistry or western blot to test staining of a combination of primary cells, cell lines or transfectant models" (https://bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/quality-and-reproducibility). For BioLegend flow cytometry reagents, validation was performed as described by the manufacturer: "Specificity testing of 1-3 target cell types with either single- or multi-color analysis (including positive and negative cell types)" (https://www.biolegend.com/en-us/quality/quality-control). Certificates of analysis for Biolegend lots are available at http://biolegend.com/en-us/quality/quality-assurance-certificates. Quality certificate for BD Biosciences lots are available at http://regdocs.bd.com/regdocs/searchCOAAction.do. Quality certificates for Invitrogen lots are available at https://www.thermofisher.com/search/results?docTypes=COA&persona=DocSupport&linkIn=true. Additionally, changes in biological patterns of cellular markers of interest were monitored by using FMO or an isotype control. Selected titration of murine antibodies were determined on spleens or TILs of tumor bearing mice.

In-vivo antibodies (anti-gp120, anti-TIGIT, anti-PD-L1) were validated from prior publications (Johnston, R. J. et al. The Immunoreceptor TIGIT Regulates Antitumor and Antiviral CD8+ T Cell Effector Function. Cancer Cell 26, 923-937, doi:10.1016/j.ccell.2014.10.018 (2014).)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	CT26 (ATCC - CRL2638), EO771 (ATCC - CRL3461)
Authentication	CT26 or EO771 cells were purchased from ATCC with corresponding certificates of analysis
Mycoplasma contamination	Cells were maintained at a dedicated internal cell line facility and tested to be mycoplasma-free.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this 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	BALB/c or C57BL/6 mice were purchased from the Charles River or Jackson Laboratories. Mice were housed under specific-pathogen-free conditions at the Genentech animal facility. Mice were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Genentech is an American Association of Laboratory Animal Care (AAALAC)-accredited facility and all animal activities in this research study were conducted under protocols approved by the Genentech Institutional Animal Care and Use Committee (IACUC). Mice were housed in individually ventilated cages within animal rooms maintained under a 14h–10h light–dark cycle. Animal rooms were temperature and humidity controlled between 68 and 79°F (20.0 to 26.1°C) and from 30% to 70%, respectively, with 10 to 15 room air exchanges per hour. Female mice (aged 6-8 weeks) that appeared healthy and free of obvious abnormalities were used for the study. The maximum tumor size approved by IACUC was 2,000 mm3. Animals bearing tumors exceeding 2,000 mm3 or showing ulceration were euthanized following protocols approved by IACUC. Tumors were measured 3 times per week. In the case of tumors exceeding 2,000 mm3, tumor measurement was recorded prior to euthanasia. To minimize the number of mice with tumors exceeding 2,000 mm3, mice were euthanized if tumors were measured at greater than 1,700 mm3 on any given day, as tumor growth rate would make it highly likely for the tumor to exceed 2,000 mm3 by the next measurement. However, despite these measures, some tumors grew in excess of 2.000 mm3 between two measurements, as outlined here. In Fig. 1a, 8 mice were euthanized with tumors >1,700 mm3, 8 mice with tumors >2,000 mm3, 44 mice with ulcerations, and 1 mouse for other reason, across the entire study. In Fig. 1c, 5 mice were euthanized with tumors >2,000 mm3, 11 mice before tumors reached 2,000 mm3, and 1 mouse with ulceration, across the entire study. Details for experimental groups and individual mice are provided in Fig. 1 Source Data. In Extended Data Fig. 1b, 34 mice were euthanized with tumors >1,700 mm3, 26 mice with tumors >2,000 mm3, and 8 mice with ulcerations, across the entire study. Details for experimental groups and individual mice are provided in Extended Data Fig. 1 Source Data.
Wild animals	No wild animals were used.
Reporting on sex	C57BL/6 female mice were used wtih EO771 tumor studies as this line is derived and isolated from the mammary gland of a mouse with breast carcinoma. This study used genetically identical female mice to minimize biological variability, especially since androgen has been shown to affect gene expression in T cells.
Field-collected samples	No samples were collected from the field.

Ethics oversight	All experimental animal studies were conducted under the approval of the Institutional Animal Care and Use Committees of Genentech Lab Animal Research and were performed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

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	For pharmacodynamic analyses by FACS, mice were euthanized at day 7 after initial treatment. Tumors were dissociated into single cell suspensions by using gentleMACSTM dissociator (Miltenyi Biotec) and enzymatically digested in a buffer containing collagenase D (2 mg/mL) and DNAse (40 U/mL, Roche). Single cell suspensions of draining lymph nodes were obtained by mechanical dissociation through 40 µm cell strainers and performing red blood cell lysis as needed. Blood was obtained by terminal cardiac puncture and collected in lavender Microtainer Blood Collection Tubes (BD Biosciences, 365974) and subjected to red blood cell lysis. For detection of intracellular or nuclear staining by FACS, the FoxP3 nuclear staining buffer set (Invitrogen) was performed using recommended manufacturer's instructions. For intracellular cytokine detection, cells were stimulated for 4 hours with Cell Stimulation Cocktail (Invitrogen, 00-4870-93) at 37℃. For obtaining cells for single cell analysis, tumors and dLNs were processed into single cell suspensions as described elsewhere, and subjected to first tetramer staining, then surface markers and CITE-seq antibodies together. Processing of blood samples at day 0 before any treatment or at day 7 were first stained with hashed-tagged antibodies, then stained with surface markers.
Instrument	Cells were purified by fluorescence-activated cell sorting (FACS) on a Becton Dickinson FACSAria Fusion cell sorter equipped with four lasers (405 nm, 488 nm, 561 nm and 638nm). A 70-µm nozzle running at 70 psi and 90 kHz was used as the setup for each sort session. For flow analysis, All samples were acquired on LSR-Fortessa, BD Symphony Instruments (BD

	Biosciences) or Cytek Aurora.
Software	FACSDiva (V8.0.1), Flowjo (V10.8.1), SpectroFlo (V3.0.3)
Cell population abundance	Sorted cell purities were more than 95%.
Gating strategy	Examples of gating boundaries are provided as supplementary information. Boundaries were set against control samples (i.e. isotype or FMO samples) or based on density distribution.

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