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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Human multiplex IF was collected using Vectra multispectral imaging platform (Vectra 3). Flow collection was performed using FACSDiva 8.0.1 (BD Pharmingen). All sequencing data was collected on a NextSeq500 (Illumina).
Data analysis	All flow data was analyzed using FlowJo version 10.4.1. All statistical tests were run using Graphpad Prism 7 or R 3.3.1. Human multiplex IF was analyzed using Inform 2.3 software with survival analyses performed in R using the Survminer (0.4.1) and Survival (2.43.3) packages. RNA-seq and ATAC-seq sequence processing was performed with Bcl2fastq (2.19.1), Trimmomatic (0.33), Bowtie2 (2.2.4), Samtools (1.3), and HTSeq (0.6.1p1). ATAC-seq peaks were called using MACS (2.1.1) and analysis was performed using GREAT (3.0.0) and HOMER (4.10). Tracks were visualized with Integrative Genomics Viewer (2.3.77). Statistics for RNA-seq and ATAC-seq were performed using DESeq2 (1.18.1). GSEA (3.0) was performed using gene-sets from the MSigDB database. Single-cell RNA-seq sequence data was aligned using CellRanger (1.2) and analyzed using Seurat (2.1.0) and FastProject (0.9.2). TCR sequencing data was analyzed using Immunoseq Analyzer (3.0). K-means clustering, heatmap visualization, and similarity matrix calculations were performed using GENE-E (3.0.215)

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Data Availability

All sequencing data from this study have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) and are accessible through the GEO Series accession number GSE122713. All other relevant data are available from the corresponding author on 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	Group sizes for in vivo validation experiments were selected empirically based upon prior knowledge of the intragroup variation of tumor challenges and immunotherapy treatment. Similarly, group sizes in vitro were selected on the basis of prior knowledge of variation.
Data exclusions	Rout outlier tests were run with default parameters in Prism on all mouse experimental data due to inherent variability within the model system. This was pre-determined based on standard lab procedure.
Replication	Replicates were used in all experiments as noted in text, figure legends and methods. All experiments presented for which replication was attempted were successfully replicated.
Randomization	Age and sex-matched animals were used for each experiment. Animals were also co-housed when possible. Mice were randomized prior to treatment.
Blinding	No blinding was performed due to requirements for cage labeling and staffing needs.

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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	All antibodies used with relevant information summarized in supplemental table 8. In brief (target, clone, source, fluorophore, dilution): Mouse antibodies for flow: Annexin V, Fisher Scientific, FITC, 1:20; BrdU, BU20A, Invitrogen, FITC, 1:50; CD44, IM7, BioLegend, PerCP-Cy5.5, 1:100; CD45, 30-F11, BioLegend, BV510, 1:200; CD45.1, A20, BioLegend, BV510, 1:200; CD45.2, 104, BioLegend, FITC, 1:200; CD8a, 53-6.7, BioLegend, BV786, 1:200; GP33 H-2Db Tetramer , NIH Tetramer Core Facility, APC, 1:600; Granzyme B, GB11, BioLegend, PE, 1:50; H-2Kb, AF6-88.5, BioLegend, APC or PE, 1:100; IFN-?, XMG1.2, BioLegend, APC, 1:50; IL-2, JES6-5H4, Biolegend, PE-Cy7, 1:50; Ki-67, B56, BioSciences, PerCP-Cy5.5, 1:50; live/dead stain, Invitrogen, NIR, 1:1,000; PD-1, RMP1-30, BioLegend, PE-Cy7, 1:100; SIINFEKL H-2Kb Tetramer, NIH Tetramer Core Facility, APC, 1:400; Slamf6, 13G3, BD Pharmingen, PE, 1:100; Tcf1, C63D9, Cell Signaling, AF488, 1:50; Tim-3, RMT3-23, BioLegend, BV421, 1:100; TNF, MP6-XT22, BioLegend, PerCP-Cy5.5, 1:50 Human antibodies for IF: CD8, C8/144B, Dako, 1:5,000; PD-1, EH33, Dr. Gordon Freeman, 1:11,000; TCF1, C63D9, Cell Signaling, 1:2,000; SOX10, EP268, Cell Marque, 1:20,000
Validation	Representative flow panels are shown in Supplementary Figure 2. Further validation is present on the manufacturer's website as noted in the Methods section and Supplementary Table 8.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	B16.F10 cells and GVAX (B16 cells secreting GM-CSF) were a gift from G. Dranoff. D4M.3A cells were a gift from Dr. David Fisher.
Authentication	B16.F10, B16-ova, and D4M.3A cells have been validated through whole exome sequencing.
Mycoplasma contamination	All cell lines were confirmed mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals	Female C57BL/6J mice and CD45.1+ congenic mice (strain B6.SJL-PtprcaPepcb/BoyJ) were purchased from The Jackson Laboratory and used at age 7-10 weeks.
Wild animals	Study did not involve wild animals.
Field-collected samples	Study did not involve field-collected samples.
Ethics oversight	All animal procedures were performed in accordance with ethical regulations and pre-approved by the Dana-Farber Cancer Institute Institutional Animal Care and Use Committee.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Human studies were performed on biopsies from adult patients (male and female, age 27-83) with stage III-IV melanoma who received combination Nivolumab + Ipilimumab therapy. Biopsies were taken from primary, primary recurrent, or metastatic tumor sites. Please see Supplementary Table 6 for additional details.
Recruitment	Through the Center for Immuno-Oncology at Dana-Farber Cancer Institute, IRB-approved consent was obtained from patients with advanced melanoma to collect and analyze tumor samples as companion to the combination treatment of Ipilimumab and Nivolumab they received. This recruitment method may lead to self-selection bias, potentially enhancing for more severe cases. This is a single-center cohort, which could limit generalizability to other populations.
Ethics oversight	All human studies were performed in accordance with ethical regulations and pre-approved by the Dana-Farber Cancer Institute IRB (2016P002770).

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 were dissected from the surrounding fascia, mechanically minced, and treated with collagenase P (2mg/mL, Sigma) and DNAse I (50µg/mL, Sigma) for 10 minutes at 37°C. Tumor-infiltrating leukocytes were enriched using an Optiprep (Sigma) density gradient followed by CD45+ or CD8+ MACS positive selection (Miltenyi). When necessary, cells were sorted on a FACS Aria II (BD Biosciences) to obtain greater than 95% purity. Spleen and lymph nodes were dissected from mice, mechanically minced, and filtered to single-cell suspension. RBC lysis was performed on all spleen samples.
Instrument	BD LSR Fortessa SORP was used to collect data for analysis. BD FACSAria II was used for cell sorting.

Software	All flow data was collected using FACSDIVA 8.0.1 (BD Pharmingen) and analyzed using FlowJo version 10.4.1.
Cell population abundance	All sorts had a purity > 95%, checked by post-sort re-sampling.
Gating strategy	Gating strategy summarized in Supplementary Figure 2, with gates drawn based on single-stain and full-minus-one (FMO) controls.

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