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	The samples for flow cytometry were collected on a FACS LSR Fortessa (BD Biosciences). In vivo bioluminescence signal was assessed with the IVIS Spectrum In Vivo Imaging System (PerkinElmer). Immunoblotting images were obtained using X-ray Film, LI-COR Odessey CLx (LI-COR Biosciences) and ChemiDoc Imaging System (Bio-Rad). Immunohistochemistry (IHC) was performed by the University of Michigan Pathology Core.
Data analysis	FACS data were analyzed with FACSDiva 9.0 (BD Biosciences). Western Blotting data were processed and analyzed by Image Lab 6.1 (BIO-RAD), and Image J 1.51n (NIH). IHC data were analyzed by QuPath 0.5.1. Statistical analysis and data presentation were performed by R packages, and Graphpad Prism 10.2.2.

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

Clinical sequencing data are publicly available with raw data available upon request from dbGaP phs000673.v5.p1.
RNA-seq data newly generated in this study for in vitro analysis have been deposited in the GEO repository at NCBI under accession GSE289764.
Single-cell RNA sequencing data that support the findings of this study was downloaded from Gene Expression Omnibus (GEO) under the accession number GSE169246.
The two original melanoma RNA-seq datasets were deposited in the European Nucleotide Archive (ENA) under accession number PRJEB23709 and in dbGaP under accession number phs000452.v2.p1. The processed data for these two melanoma datasets can be found at https://ngdc.cncb.ac.cn/icb/resources.
The remaining data are available within the Article, Supplementary Information or Source Data file. Source data are provided with this paper.

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	The biological sex of all patients undergoing ICB therapy was documented.
Reporting on race, ethnicity, or other socially relevant groupings	The race and cancer type of all patients who underwent ICB therapy can be found in Extended data Table 1.
Population characteristics	The relevant characteristics of all patients who underwent ICB therapy can be found in Extended data Table 1.
Recruitment	Patients who underwent ICB therapy were recruited from the University of Michigan Hospital in Ann Arbor, MI, USA. No potential self-selection biases were identified.
Ethics oversight	All human studies were conducted under the oversight and approval of the Institutional Review Board at the School of Medicine, University of Michigan.

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 sample size calculation were performed. For in vivo studies, 5-11 mice per group are sufficient to detect meaningful biological differences with good reproducibility. For in vitro studies, all the experiments were replicated at least for 2 individual, independent experiments. Group sizes of experiments were selected empirically based upon previous published literatures and prior knowledge (Wang et al, 2019; Yu et al, 2021; Zhou et al, 2021). All samples sizes for various assays are listed in the Methods section or the figure legends. References: Wang, W. et al. CD8+ T cells regulate tumour ferroptosis during cancer immunotherapy. Nature 569, 270–274 (2019). Yu, J. et al. Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T cell elimination. Nat Med 27, 152–164 (2021). Zhou, J. et al. The ubiquitin ligase MDM2 sustains STAT5 stability to control T cell-mediated antitumor immunity. Nat Immunol 22, 460-470 (2021).
Data exclusions	No data were excluded from the manuscript.
Replication	As reported in the figure legends, the findings were reliably reproduced.
Randomization	For all in vivo experiments, animals were randomly assigned into a treatment group after tumor inoculation. The starting tumor burden in the treatment and control groups were similar before treatment. All groups were age and sex matched. In experiments not involving mice, we did not randomize because in vitro studies were observational and replicated at least for 2 independent experiment.

Blinding

Experiments were not performed in a blinded manner. The investigator needed to know the treatment groups in order to perform the study. Blinding was not possible as most of the data acquisition and analysis were done by a single person. Blinding would have required at least two individuals for each experiment which was not feasible during the course of the study. The data were observational, not at risk of bias in interpretation. All data were acquired and analyzed by software with objective standard, thus blinding was not relevant to the study.

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

For Western blots, primary antibodies were used at a dilution of 1:1000 unless otherwise specified.
anti-STAT1 (D1K9Y, #14994), anti-STAT2 (D9J7L, #72604), anti-STAT3 (Rabbit, 79D7, #4904; Mouse, 124H6, #9139), anti-STAT4 (C46B10, #2653), anti-STAT5 (D2O6Y, #94205), anti-STAT6 (D3H4, #5397), anti-JAK1 (6G4, #3344), anti-JAK2 (D2E12, #3230), anti-JAK3 (D7B12, #8863), anti-TYK2 (E9H4T, #35615), anti-p65 (D14E12, #8242), anti-CD80 (E6J6N, #54521), anti-CD86 (E5W6H, #19589), anti-IL-6Rα (E7H4J, #39837, Lot 1) , anti-GP130 (#3732, Lot 6), anti-pSTAT1 (58D6, #9167), anti-pSTAT3 (D3A7, #9145), anti-pSTAT5 (D47E7, #4322), anti-pSTAT6 (D8S9Y, #56554), anti-pJAK1 (D7N4Z, #74129), anti-pJAK2 (C80C3, #3776), anti-pJAK3 (D44E3, #5031), anti-pp65 (93H1, #3033), anti-β-actin (#4967) were from Cell Signaling Technology. Anti-JAK2 (C-10, sc-390539), Anti-GMRβ (F-12, sc-393281), anti-β-actin (C4, sc-47778) were from Santa Cruz Biotechnology. Anti-pTYK2 (PA5-37762) was from Invitrogen. For secondary antibodies, HRP horse anti-mouse IgG antibody (PI-2000, 1:5000), HRP goat anti-rabbit IgG (PI-1000, 1:5000) were from Vector Laboratories.
For IHC, Anti-CD31 (D8V9E, #77699, Lot 6, 1:100) was from Cell Signaling Technology.
For Flow cytometry, antibodies were used at a dilution of 1:50 unless otherwise specified.
PE-CY7 Anti-Mouse CD11c (clone HL3, #558079, Lot 3334101),FITC Anti-mouse-CD90 (clone 30-H12, #553013, Lot 4176004), FITC anti-mouse CD3 (clone 17A2, #561798, Lot 1286349), FITC anti-mouse CD45R/B220 (clone RA3-6B2, #553088, Lot 3110705), PE anti-mouse CD80 (Clone 16-10A1, 553769, Lot 3298935), BV650 anti-mouse CD80 (Clone 16-10A1, 563687, Lot 0015825), APC-R700 anti-mouse CD8 (clone 53-6.7, #564983, Lot 4249800), V500 anti-mouse CD8 (clone 53-6.7, #560776, Lot 1116792), PerCP anti-mouse CD45 (clone 30-F11, #557235, Lot 8179968), APC-CY7 anti-mouse CD4 (clone GK1.5, #552051, Lot 4144628), BV786 anti-mouse-IFNγ (clone XMG1.2, #563773, Lot 4008777), APC anti-mouse CD4 (clone RM4.5, #553051, Lot 0231898), BV605 anti-mouse H-2 class I (clone M1/42, #749709), PE anti-granzyme B (clone GB11, #561142, Lot 4149375), APC anti-mouse-IL-2 (clone JES6-5H4, #554429, Lot 3242915), Alexa Fluor 700 Mouse anti-Ki-67 (Clone B56, #561277, Lot 1154059), PE-CY7 Anti-mouse-TNFα (clone MP6-XT22, #557644, Lot 4256222), APC-Cy7 Anti-mouse-CD90 (clone 53-2.1, # 561641), FITC Anti-Human IFN-γ (Clone B27, #554700, Lot 2283301), PE-Cy7 Anti-Human TNF (Clone MAb11, #557647, Lot 8143874), R718 Anti-Human CD8 (Clone RPA-T8, #567056) were purchased from BD Biosciences. BV421 anti-mouse I-A/I-E (Clone M5/114.15.2, #404-5321-82, Lot 3037246), PE anti-mouse CD86 (Clone GL1, #12-0862-82, Lot 2863198), APC anti-mouse MHC class I (clone AF6-88.5.5.3, #17-5958-82, Lot 2961236), Pacific Orange anti-mouse CD45 (clone HI30, #MHCD4530, Lot 2668640), PE-Cyanine7 phospho-STAT5 (clone SRBCZX, #25-9010-42, Lot 2442266, 1:20) were purchased from Thermo Fisher Scientific. PE Anti- Phospho-STAT3 (clone 13A3-1, #651004, Lot B341628, 1:20), APC/Cyanine7 anti-mouse XCR1 (clone ZET, #148224, Lot B388581) were purchased from BioLegend.

Validation

All antibodies used were commercial and validated for the appropriate application.
anti-STAT1 (D1K9Y, #14994, Lot 8)
https://www.cellsignal.com/products/primary-antibodies/stat1-d1k9y-rabbit-mab/14994
anti-STAT2 (D9J7L, #72604, Lot 2)
https://www.cellsignal.com/products/primary-antibodies/stat2-d9j7l-rabbit-mab/72604
anti-STAT3 (Rabbit, 79D7, #4904, Lot7) https://www.cellsignal.com/products/primary-antibodies/stat3-79d7-rabbit-mab/4904
anti-STAT3 (mouse, 124H6, #9139, Lot 16) https://www.cellsignal.com/products/primary-antibodies/stat3-124h6-mouse-mab/9139
anti-STAT4 (C46B10, #2653, Lot 4)
https://www.cellsignal.com/products/primary-antibodies/stat4-c46b10-rabbit-mab/2653
anti-STAT5 (D2O6Y, #94205, Lot 5)
https://www.cellsignal.com/products/primary-antibodies/stat5-d2o6y-rabbit-mab/94205
anti-STAT6 (D3H4, #5397, Lot 5)
https://www.cellsignal.com/products/primary-antibodies/stat6-d3h4-rabbit-mab/5397
anti-JAK1 (6G4, #3344, Lot 6)

https://www.cellsignal.com/products/primary-antibodies/jak1-6g4-rabbit-mab/3344
anti-JAK2 (D2E12, #3230, Lot 13)
https://www.cellsignal.com/products/primary-antibodies/jak2-d2e12-xp-rabbit-mab/3230
anti-JAK3 (D7B12, #8863, Lot 3)
https://www.cellsignal.com/products/primary-antibodies/jak3-d7b12-rabbit-mab/8863
anti-TYK2 (E9H4T, #35615, Lot 1)
https://www.cellsignal.com/products/primary-antibodies/tyk2-e9h4t-rabbit-mab/35615
anti-p65 (D14E12, #8242, Lot 16)
https://www.cellsignal.com/products/primary-antibodies/nf-kb-p65-d14e12-xp-rabbit-mab/8242
anti-CD80 (E6J6N, #54521, Lot 1)
https://www.cellsignal.com/products/primary-antibodies/cd80-e6j6n-rabbit-mab/54521
anti-CD86 (E5W6H, #19589, Lot 5)
https://www.cellsignal.com/products/primary-antibodies/cd86-e5w6h-rabbit-mab/19589
anti-IL-6Rα (E7H4J, #39837, Lot 1)
https://www.cellsignal.com/products/primary-antibodies/il-6ra-cd126-e7h4j-rabbit-mab/39837
anti-GP130 (#3732, Lot 6)
https://www.cellsignal.com/products/primary-antibodies/gp130-antibody/3732
anti-pSTAT1 (58D6, #9167, Lot 18)
https://www.cellsignal.com/products/primary-antibodies/phospho-stat1-tyr701-58d6-rabbit-mab/9167
anti-pSTAT3 (D3A7, #9145, Lot 43)
https://www.cellsignal.com/products/primary-antibodies/phospho-stat3-tyr705-d3a7-xp-rabbit-mab/9145
anti-pSTAT5 (D47E7, #4322, Lot 5)
https://www.cellsignal.com/products/primary-antibodies/phospho-stat5-tyr694-d47e7-xp-rabbit-mab/4322
anti-pSTAT6 (D8S9Y, #56554, Lot 1)
https://www.cellsignal.com/products/primary-antibodies/phospho-stat6-tyr641-d8s9y-rabbit-mab/56554
anti-pJAK1 (D7N4Z, #74129, Lot 2)
https://www.cellsignal.com/products/primary-antibodies/phospho-jak1-tyr1034-1035-d7n4z-rabbit-mab/74129
anti-pJAK2 (C80C3, #3776, Lot 13)
https://www.cellsignal.com/products/primary-antibodies/phospho-jak2-tyr1007-1008-c80c3-rabbit-mab/3776
anti-pJAK3 (D44E3, #5031, Lot 7)
https://www.cellsignal.com/products/primary-antibodies/phospho-jak3-tyr980-981-d44e3-rabbit-mab/5031
anti-pp65 (93H1, #3033, Lot 19)
https://www.cellsignal.com/products/primary-antibodies/phospho-nf-kb-p65-ser536-93h1-rabbit-mab/3033
anti-β-actin (Rabbit, #4967, Lot 15)
https://www.cellsignal.com/products/primary-antibodies/b-actin-antibody/4967
anti-β-actin (Mouse, C4, sc-47778, Lot F1323)
https://www.scbt.com/p/beta-actin-antibody-c4
Anti-JAK2 (C-10, sc-390539, Lot #K0822)
https://www.scbt.com/p/jak2-antibody-c-10
Anti-GMRβ (F-12, sc-393281, Lot #K1921)
https://www.scbt.com/p/il-3-il-5-gm-csfrbeta-antibody-f-12
Anti-pTYK2 (PA5-37762, Lot ZC4271911) https://www.thermofisher.com/antibody/product/Phospho-TYK2-Tyr1054-Antibody-Polyclonal/PA5-37762
HRP horse anti-mouse IgG antibody (PI-2000, Lot ZK040)
https://vectorlabs.com/products/peroxidase-horse-anti-mouse-igg/
HRP goat anti-rabbit IgG (PI-1000, Lot ZK0623), validated by manufacturer and citations at https://vectorlabs.com/products/peroxidase-goat-anti-rabbit-igg/
Anti-CD31 (D8V9E, #77699, Lot 6)
https://www.cellsignal.com/products/primary-antibodies/cd31-pecam-1-d8v9e-xp-rabbit-mab/77699
PE-CY7 Anti-Mouse CD11c (clone HL3, #558079, Lot 3334101),
https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-hamster-anti-mouse-cd11c.558079?tab=product_details
FITC Anti-mouse-CD90 (clone 30-H12, #553013, Lot 4176004), https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-rat-anti-mouse-cd90-2.553013?tab=product_details
FITC anti-mouse CD3 (clone 17A2, #561798, Lot 1286349), https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-rat-anti-mouse-cd3-molecular-complex.561798?tab=product_details
APC-Cy7 Anti-mouse-CD90 (clone 53-2.1, # 561641), https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-cy-7-rat-anti-mouse-cd90-2.561641?tab=product_details
FITC anti-mouse CD45R/B220 (clone RA3-6B2, #553088, Lot 3110705),
https://www.bdbiosciences.com/en-eu/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/fitc-rat-anti-mouse-cd45r-b220.553088?tab=product_details
PE anti-mouse CD80 (Clone 16-10A1, 553769, Lot 3298935),
https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-hamster-anti-mouse-cd80.553769?tab=product_details
BV650 anti-mouse CD80 (Clone 16-10A1, 563687, Lot 0015825),
https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv650-hamster-anti-mouse-cd80.563687?tab=product_details
APC-R700 anti-mouse CD8 (clone 53-6.7, #564983, Lot 4249800),
https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-r700-rat-anti-mouse-cd8a.564983?tab=product_details
V500 anti-mouse CD8 (clone 53-6.7, #560776, Lot 1116792),
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/v500-rat-anti-mouse-cd8a.560776?tab=product_details
PerCP anti-mouse CD45 (clone 30-F11, #557235, Lot 8179968),
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/

percp-rat-anti-mouse-cd45.557235?tab=product_details
APC-CY7 anti-mouse CD4 (clone GK1.5, #552051, Lot 4144628),
https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-cy-7-rat-anti-mouse-cd4.552051?tab=product_details
BV786 anti-mouse-IFNγ (clone XMG1.2, #563773, Lot 4008777), https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv786-rat-anti-mouse-ifn.563773?tab=product_details
PE anti-granzyme B (clone GB11, #561142, Lot 4149375),
https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-mouse-anti-human-granzyme-b.561142?tab=product_details
APC anti-mouse-IL-2 (clone JES6-5H4, #554429, Lot 3242915),
https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-rat-anti-mouse-il-2.554429?tab=product_details
PE-CY7 Anti-mouse-TNFα (clone MP6-XT22, #557644, 4256222),
https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-rat-anti-mouse-tnf.557644?tab=product_details
Brilliant Violet 421 anti-mouse I-A/I-E (Clone M5/114.15.2, #404-5321-82, Lot 3037246), https://www.thermofisher.com/antibody/product/MHC-Class-II-I-A-I-E-Antibody-clone-M5-114-15-2-Monoclonal/404-5321-82
PE anti-mouse CD86 (Clone GL1, #12-0862-82, Lot 2863198)
https://www.thermofisher.com/antibody/product/CD86-B7-2-Antibody-clone-GL1-Monoclonal/12-0862-82
APC anti-mouse MHC class I (H-2Kb) (clone AF6-88.5.5.3, #17-5958-82, Lot 2961236),
https://www.thermofisher.com/antibody/product/MHC-Class-I-H-2Kb-Antibody-clone-AF6-88-5-5-3-Monoclonal/17-5958-82?imageId=91186
Pacific Orange anti-mouse CD45 (clone HI30, #MHCD4530, Lot 2668640),
https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-HI30-Monoclonal/MHCD4530
PE-Cyanine7 phospho-STAT5 monoclonal antibody (clone SRBCZX, #25-9010-42, Lot 2442266)
https://www.thermofisher.com/antibody/product/Phospho-STAT5-Tyr694-Antibody-clone-SRBCZX-Monoclonal/25-9010-42
PE Anti- Phospho-STAT3 (Tyr705) (clone 13A3-1, #651004, Lot B341628)
https://www.biolegend.com/de-de/products/pe-anti-stat3-phospho-tyr705-antibody-12914
APC/Cyanine7 anti-mouse XCR1 (clone ZET, #148224, Lot B388581)
https://www.biolegend.com/de-de/products/apc-cyanine7-anti-mouse-rat-xcr1-antibody-16709
FITC Anti-Human IFN-γ (Clone B27, #554700, Lot 2283301)
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-mouse-anti-human-ifn.554700?tab=product_details
PE-Cy7 Anti-Human TNF (Clone MAb11, #557647, Lot 8143874)
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-mouse-anti-human-tnf.557647?tab=product_details
R718 Anti-Human CD8 (Clone RPA-T8, #567056)
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/r718-mouse-anti-human-cd8.567056?tab=product_details
APC anti-mouse CD4 (clone RM4.5, #553051, Lot 0231898)
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-rat-anti-mouse-cd4.553051?tab=product_details
BV605 anti-mouse H-2 class I (clone M1/42, #749709),
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv605-rat-anti-mouse-h-2-class-i.749709?tab=product_details
Alexa Fluor 700 Mouse anti-Ki-67 (Clone B56, #561277, Lot 1154059),
https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-700-mouse-anti-ki-67.561277?tab=product_details

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	JAWSII, 293T, B16F10, CT26, LLC, EMT-6, and 4T1 were purchased from the American Type Culture Collection (ATCC). MC38 was obtained from the University of Texas Southwestern Medical Center (Dr. Yang-Xin Fu). Ovarian cancer cell line luciferase-ID8 was cited.
Authentication	Cell lines were not authenticated.
Mycoplasma contamination	All cell lines in our laboratory are routinely tested for mycoplasma contamination and cells used in this study are negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No misidentified cell 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	All mice were maintained under specific pathogen-free housing (~22°C with ~40% humidity) on a 12-hour dark/12-hour light cycle. The following mice (at 6-8 week of age) (The Jackson laboratory) were used for this study: C57BL/6J, BALB/cJ, NOD-scid IL2Rγ null

(NSG), Rag1tm1Mom (Rag1–/–), C57BL/6-Tg(TcraTcrb)1100Mjb/J (OT-I), B6.129S(C)-Batf3tm1Kmm/J (Batf3-/-), B6.129S(Cg)-Stat1tm1Dlv/J (Stat1-/-), B6(129S4)-Xcr1tm1.1(cre) Kmm/J (Xcr1-Cre), B6.129S1-Stat3tm1Xyfu/J (Stat3flox/flox) mice, CD-1 mice were obtained from Charles River Laboratories. Stat5b–/– C57BL/6J mice were from the National Institutes of Health (Warren J. Leonard). Stat3flox/flox mice were crossed with Xcr1-Cre mice to obtain specific STAT3 deficiency in DC1s (Stat3-/- mice). In-house littermates were used in the control arm when specific mouse strains were generated in-house.

Wild animals	The study did not involve wild animals.
Reporting on sex	No specific gender.
Field-collected samples	The study did not involve samples collected from field.
Ethics oversight	All mouse experiments were performed in accordance with protocol approved by the University of Michigan 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	Single-cell suspensions were prepared from fresh mouse tumor tissues or spleen, and lymphocytes were enriched by density gradient centrifugation. For intracellular cytokine staining, lymphocytes were incubated in culture medium containing PMA (phorbol 12-myristate-13-acetate, 5 ng/mL, Sigma Aldrich), Ionomycin (500 ng/mL, Sigma Aldrich), Brefeldin A (1: 1000, BD Biosciences). and Monensin (1: 1000, BD Biosciences) at 37°C for 4 hours. Antibodies were added for 20 minutes for surface staining. The cells were then washed and resuspended in 1 mL of freshly prepared Fix/Perm solution (BD Biosciences) at 4°C for overnight. After being washed with Perm/Wash buffer (BD Biosciences), the cells were stained with antibodies against intracellular proteins for 30 minutes, washed, and fixed in 4% formaldehyde (Sigma Aldrich). For DC1 function detection, lymphocytes were stained with antibodies for 20 minutes at room temperature in the dark, and then cells were analyzed by flow cytometry. For phosphor-protein detection, the cells were initially stained with antibodies for surface staining. After staining, the cells were then washed and resuspended in IC Fixation Buffer (BD Biosciences) for 30 minutes. The samples were centrifuged at 600 x g for 5 minutes at room temperature, discard the supernatant. Resuspended the cell pellet in residual volume and added 1 mL of ice-cold 90-100% methanol. Vortexed to mix and incubated for 30 minutes at 4°C. After being washed with staining buffer, the cells were stained with antibodies against phospho-proteins for 30 minutes, washed, and fixed in 4% formaldehyde (Sigma Aldrich).
Instrument	All samples were read on an BD LSRFortessa cytometer (BD Biosciences).
Software	All data were analyzed with FACS DIVA software v. 9.0 (BD Biosciences).
Cell population abundance	When cells were sorted or enriched, the purity was confirmed by flow cytometry and in each case was above 90% purity.
Gating strategy	The cells were gated based on FSC-A/SSC-A in regions known to contain lymphoid cells and tumor cells. For T cell functional analysis, CD90+CD3+ cells were selected, followed by gating for CD8+ and CD4+ populations. Subsequently, the percentages of IFNγ, granzyme B, IL-2, Ki67, and TNFα cells were analyzed within the CD8+ gate. For dendritic cell (DC) functional analysis, the CD45+ population was initially gated. The CD90+CD3+CD45R+ population was excluded, and then the XCR1+ and CD11c+ populations were gated. Subsequently, the mean fluorescence intensity (MFI) of MHC-I, MHC-II, CD80, and CD86 was analyzed within the CD11c+XCR1+ gate.

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