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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

Default softwares in the data collection instruments were used.
Flow cytometry data was collected usingFACS BD Aria II, BD Symphony S6, or Attune NxT Software v3.1;
RT-PCR Quant studio 3;
All deep sequencing data were collected using Illumina Sequencers at Yale Center for Genome Analysis (YCGA).

Data analysis

Data analysis was performed using the following software / code:
FlowJo v.10.7;
Prism 9;
Python v3.8.6;
FastQC v0.11.9;
fastp v0.23.2;
cutadapt v4.9;
bowtie v1.3.1;
SAMTools v1.16;
pigz v2.7;
STAR v2.7.10b;
Blast v2.15.0;
Biopython 1.83;
cellranger v5.0.1;
Seurat 4.0.1;
harmony 0.1.0;
ggplot2 3.3.1;
pheatmap 1.0.10;

DESeq2 1.2.10;
dplyr 1.1.0;
cowplot 1.1.2;
clusterProfiler 4.10.0.
Codes that support the findings of this research are available from the corresponding author upon reasonable request.

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

All data generated or analyzed during this study are included in this article and its supplementary information files. Specifically, source data and statistics for regular experiments are provided in an excel file of Source data and statistics. NGS data are being deposited to Gene Expression Omnibus (GEO), with GSE269516. Other materials and data are available either through public repositories, or via reasonable requests by the academic community to the corresponding authors.

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	Sample size was determined according to the lab's prior work or similar approaches in the field. For most cases, biological triplicate experiments were performed unless otherwise noted. Details on sample size for experiments were indicated in methods and figure legends. No statistical methods were used to predetermine the sample size. Sample sizes for experiments were estimated based on previous experience with similar setups that showed significance.
Data exclusions	No data was excluded.
Replication	Number of biological replicates (usually n >= 3) are indicated in the figure legends. Key findings (non-NGS) were replicated in at least two independent experiments. NGS experiments were performed with biological replications as indicated in the manuscript.
Randomization	Regular in vitro experiments were not randomized. For in-vitro studies, the allocation was not random, which was not relevant to the experiments due to the experimental settings described in methods and legends. Mouse experiments were randomized by using littermates, and blinded using generic cage barcodes and eartags where applicable. High-throughput experiments and analyses were blinded by barcoded metadata.
Blinding	Investigators were blinded to the identity and treatment groups of animals when measuring tumor burden. Investigators were not blinded in in vitro experiments. In certain NGS data analysis, investigators were blinded for initial processing of the original data using key-coded metadata.

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

InVivoMAb anti-mouse CD8α [Clone: 2.43] BioXcell BE0061
InVivoMAb anti-mouse Ly6G/Ly6C (Gr-1) [Clone: RB6-8C5] BioXcell BE0075
InVivo MAb rat IgG2b isotype control [Clone: LTF-2] BioXcell BE0090
InVivoMAb anti-mouse CTLA4 (CD152) [Clone: 9D9] BioXcell BE0164
InVivoMAb anti-mouse PD-1 (CD279) [Clone: RMP1-14] BioXcell BE0146
PerCP/Cyanine5.5 anti-mouse CD45.2 (Clone: 104) Biolegend 109828
Brilliant Violet 605™ anti-mouse CD8a Antibody [Clone: 53-6.7] Biolegend 100744
PE anti-mouse CD4 Antibody [Clone: GK1.5] Biolegend 100408
FITC anti-mouse/human CD11b [Clone:M1/70] Biolegend 101205
PE anti-mouse F4/80 Antibody [Clone:BM8] Biolegend 123109
PE/Dazzle™ 594 anti-mouse CD11c Antibody [Clone: N418] Biolegend 117348
APC anti-mouse Ly-6C Antibody [Clone: HK1.4] Biolegend 128016
Brilliant Violet 605™ anti-mouse Ly-6G Antibody [Clone: 1A8] Biolegend 127639
PE/Cyanine7 anti-mouse I-A/I-E Antibody [Clone: M5/114.15.2] Biolegend 107630
PE/Cyanine7 anti-mouse CD3 Antibody [Clone: 17A2] Biolegend 100220
Brilliant Violet 605™ anti-mouse I-A/I-E Antibody [Clone: M5/114.15.2] Biolegend 107639
Brilliant Violet 421™ anti-mouse CD8a Antibody [Clone:53-6.7] Biolegend 100753
APC anti-HA.11 Epitope Tag Antibody [Clone: 16B12] Biolegend 901523
APC Mouse IgG1, κ Isotype Ctrl (ICFC) Antibody [MOPC-21] Biolegend 400142
APC anti-mouse Galectin-9 [Clone: 108A2] Biolegend 141404
PE anti-mouse Galectin-9 [Clone: 108A2] Biolegend 137904
PE anti-mouse LAP (TGF-1) [Clone: TW7-16B4] Biolegend 505007
PE anti-mouse IL-10 [Clone: JES5-16E3] Biolegend 696803
Alexa Fluor® 594 anti-Nos2 (iNOS) [Clone: W16030C] Biolegend 127206
PE anti-mouse CD73 [Clone: TY/11.8] Biolegend 143803
PE anti-mouse CD39 [Clone: Duha59] Biolegend 126706
PE anti-mouse/human Mac-2 (Galectin-3) [Clone: Gal397] Biolegend 134505
APC anti-mouse/human Mac-2 (Galectin-3) [Clone: M3/38] Biolegend 125420
PE anti-mouse CD66a (CEACAM1a) [Clone: MAb-CC1] Biolegend 107205
PE anti-mouse CD273 (B7-DC, PD-L2) [Clone: TY25] Biolegend 654004
PE anti-mouse CD274 (B7-H1, PDL1) Antibody [Clone: 10F.9G2] Biolegend 124308
APC anti-mouse CD274 (B7-H1, PDL1) Antibody [Clone: 10F.9G2] Biolegend 124312
PE Rat IgG2b, κ Isotype Ctrl Antibody [Clone: RTK4530] Biolegend 400636
PE Mouse IgG1, κ Isotype Ctrl Antibody [Clone: MOPC-21] Biolegend 400112
APC Mouse IgG2b, κ Isotype Ctrl Antibody [Clone: MG2b-57] Biolegend 401210
APC Rat IgG2a, κ Isotype Ctrl Antibody [Clone: RTK2758] Biolegend 400512
APC/Cyanine7 anti-mouse CD47 Antibody [Clone: miap301] Biolegend 127526
APC anti-mouse CD47 Antibody [Clone: miap301] Biolegend 127514
APC anti-mouse IFN-γ Antibody [Clone: XMG1.2] Biolegend 505810
PE anti-mouse H-2Kb bound to SIINFEKL Antibody [Clone: 25-D1.16] Biolegend 141604
APC anti-mouse H-2Kb bound to SIINFEKL Antibody [Clone: 25-D1.16] Biolegend 141605
APC anti-mouse H-2Kd Antibody [Clone: SF1-1.1] Biolegend 116620
PE anti-mouse H-2Kb Antibody [Clone: AF6-88.5] Biolegend 116508

Validation

Commercial antibodies were validated for reactivity in mice and their application in flow cytometry or in vivo cell depletion by the vendors. They were re-validated in-house as appropriate through antigen-specific experiments.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Commercial cell lines were acquired from commercial vendors.
HEK293FT, from ThermoFisher
E0771, from CH3
B16F10, Hepa1-6, A20, LLC, from ATCC

	Colon26, Pan02, GL261, from Charles River MB49, from Millipore-Sigma MC38, from Kerafast
Authentication	Cell lines were authenticated by original vendors, and re-validated in lab as appropriate by morphology.
Mycoplasma contamination	All the cell lines used here tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in the study.

Animals and other organisms

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

Laboratory animals	C57BL/6Ncr (B6) mice, strain #556, were purchased from Charles River. BALB/c mice, strain# JAX000651, were purchased from Jackson lab. NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG), strain#005557, were purchased from Jackson Lab. OT1 mice, strain#003831, were purchased from Jackson Lab. Mice, both female and male, aged 8-12 weeks were used for experiments.
Wild animals	no wild animals were used in the study.
Field-collected samples	no field collected samples were used in the study
Ethics oversight	This study has received institutional regulatory approval. All recombinant DNA and biosafety work were performed under the guidelines of Yale Environment, Health and Safety (EHS) Committee with an approved protocol (Chen-rDNA 15-45; 18-45; 21-45). All human sample work was performed under the guidelines of Yale University Institutional Review Board (IRB) with an approved protocol (HIC#2000020784). All animal work was performed under the guidelines of Yale University Institutional Animal Care and Use Committee (IACUC) with approved protocols (Chen 2018-20068; 2021-20068).

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	Various sample prep details are provided in the Methods section.
Instrument	Flow cytometric was performed on an BD Symphony S6, BD FACSAria II or thermo Attune™ NxT.
Software	FlowJo v.10.7.1 was used for flow cytometry data analysis.
Cell population abundance	N/A.
Gating strategy	A lymphocyte gate was defined first from FSC-A v SSC-A. Singlet gates were then defined on FSC-H v FSC-W. Additional gating was performed as described in figure and extended data legends for individual experiments.

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