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 codes used for bulk and single-cell RNA-seq analysis followed typical pipelines from public R packages and software. All codes are available upon request.
Data analysis	Graph pad Prism V7 and VS, FlowJo Vl0.6.2, Microsoft Office 2016, l0X Genomics Cellranger (v2.0.2), Uniform Manifold Approximation and Projection (UMAP), Seurat function AddModuleScore, Velocyto.R package, Cuffdiff2, EdgeR, DESeq2, Galaxy, Homer, ngs.plot, Genepattern, GSEA V4 were used.

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 described RNA-seq, and scRNA-seq data were submitted under the GEO accession number: GSE163084 (It has been made public);
The colorectal cancer (CRC) patient data is sourced from GSE108989.

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 chosen according to standard practices in the field, RNA-seq and scRNA-seq samples were prepared in triplicates and duplicates, respectively. All biochemical experiments were preformed 3+ times.
Data exclusions	None excluded
Replication	All in vivo, in vitro, and flow experiments were performed at least 2+ times
Randomization	no clinical studies
Blinding	IHC scoring was done blinded. FACS and molecular analyses by nature required grouping of data and could not use blinding.

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

Invitrogen LIVE/DEAD Fixable Blue Stain (dilution: 1/750; L34962)
BioLegend
anti-CD4-PerCP/Cyanine5.5 (dilution: 1/300; clone: RM4-5; Cat: 116012)
anti-CD4-AF700 (dilution: 1/200; clone: RM4-5; cat: 116022)
anti-CD25-Brilliant Violet 650 (dilution: 1/200; clone: PC61; cat: 102038)
anti-CD44-Brilliant Violet 785 (dilution: 1/500; clone: IM7; cat: 103059)
anti-CD278 (ICOS)-PE-Cy7 (dilution: 1/200; clone: C398.4A; cat: 313520)
anti-CD279 (PD-1)-Brilliant Violet 421 (dilution: 1/200; clone: 29F.1A12; cat: 135218)
anti-CD45.1-PE/Cy7 (dilution: 1/500; clone: A20; cat: 110730)
anti-Helios-Brilliant Violet 421 (dilution: 1/200; clone: 22F6; cat: 137234)
anti-Helios-PerCP/Cyanine5.5 (dilution: 1/200; clone: 22F6; cat: 137230)
anti-CD45.2-APC (dilution: 1/500; clone: 104; cat: 109814) BD Biosciences
anti-CD8a-V500 (dilution: 1/200; clone: 53-6.7; cat: 560776)
anti-CD62L-FITC (dilution: 1/200; clone: MEL-14; cat: 553150)
anti-CD69-Brilliant Violet 785 (dilution: 1/200; clone: H1.2F3; cat: 564683)
anti-RORγT-Brilliant Violet 421 (dilution: 1/200; clone: Q31-378; cat: 562894)
anti-RORγT-PE (dilution: 1/200; clone: Q31-378; cat: 562607)
anti-IL-17A-PE, (clone: TC11-18H10; cat: 559502)
Purified mouse β-catenin (dilution: 1/200; clone: 14/ β-catenin (RUO); cat: 610154)
National Institutes of Health Tetramer Core Facility
50 µl of a 1:50 dilution of APC-conjugated Db:VP2121-130 tetramer
R & D Systems
TGFβ RI-PE (dilution: 10µl/test; Cat: FAB5871P)
Rat IgG2A-PE (dilution: 10µl/test; IC006P)

TGFβ RII-PE (10µl/test; cat: FAB532P)
Goat IgG-PE (dilution: 10µl/test; IC108P) eBioscience
anti-Foxp3-APC or anti-Foxp3-FITC (dilution: 1/200; clone: FJK-16s Cat: 17-5773-82)
anti-IFNγ-APC (dilution: 1/200; clone: XMG1.2; cat: 17-7311-82)
FITC-conjugated STAT5 phosphorylated at Tyr694 (dilution: 1µg/test; clone: SRBCZX; cat: 11-9010-42)
Mouse IgG1 kappa-FITC (dilution: 1µg/test; clone: P3.6.2.8.1; cat: 11-4714-81)
Cell Signaling Technology
anti-TCF1/TCF7-Alexa Fluor 647 (dilution: 1/300; clone: C63D9; cat: 6709S)
PE-conjugated S6 phosphorylated at Ser235 and Ser236 (dilution: 1/100; clone: D57.2.2E; cat: 5316S)
rabbit IgG-PE (dilution: 1/200; clone: DA1E; cat: 5742S),
PE-conjugated Smad2/Smad3 phosphorylated at Ser465/467 and Ser423/425 (dilution: 1/50; clone: D27F4; cat: 11979S)
rabbit IgG-PE (dilution: 1/100; clone: DA1E; cat: 5742S).
Novus Biologicals
anti-Gr-1 (dilution: 1/50; clone: cat: NIMP-R14; NB600-1387)

Validation

Invitrogen
LIVE/DEAD Fixable Blue Stain
BioLegend
Anti-CD4-PerCP/Cyanine5.5
Reactivity: Mouse
Application: Flow Cytometry
Anti-CD4-AF700
Reactivity: Mouse
Application: Flow Cytometry
Anti-CD25-Brilliant Violet 650
Reactivity: Mouse
Application: Flow Cytometry
Anti-CD44-Brilliant Violet 785
Reactivity: Mouse, Human
Application: Flow Cytometry
Anti-CD278 (ICOS)-PE-Cy7
Reactivity: Human, African Green, Baboon, Cynomolgus, Mouse, Rat, Rhesus, Swine (Pig, Porcine)
Application: Flow Cytometry
Anti-CD279 (PD-1)-Brilliant Violet 421
Reactivity: Mouse
Application: Flow Cytometry
Anti-CD45.1-PE/Cy7
Reactivity: Mouse
Application: Flow Cytometry
Anti-Helios-Brilliant Violet 421
Antibody discontinued
Anti-Helios-PerCP/Cyanine5.5
Reactivity: Mouse, Human
Application: Fl-Gr-1 (dilution: 1/50; clone: cat: NIMP-R14; NB600-1387)
Anti-CD45.2-APC
Reactivity: Mouse
Application: Flow Cytometry

BD Biosciences
Anti-CD8a-V500
Reactivity: Mouse (QC Testing)
Application: Flow cytometry (Routinely Tested)
Anti-CD62L-FITC
Reactivity: Mouse (QC Testing)
Application: Flow cytometry (Routinely Tested)
Anti-CD69-Brilliant Violet 785
Reactivity: Mouse (QC Testing)
Application: Flow cytometry (Routinely Tested)
Anti-RORγT-Brilliant Violet 421 or Anti-RORγT-PE
Mouse (QC Testing)
Application: Intracellular staining (flow cytometry) (Routinely Tested)
Anti-IL-17A-PE
Reactivity: Mouse (QC Testing)
Application: Intracellular staining (flow cytometry) (Routinely Tested)
Purified mouse β-catenin
Reactivity: Human (QC Testing), Mouse, Rat, Dog, Chicken (Tested in Development)
Application: Western blot (Routinely Tested), Immunofluorescence, Immunohistochemistry, Immunoprecipitation (Tested During Development)

National Institutes of Health Tetramer Core Facility
50 µl of a 1:50 dilution of APC-conjugated Db:VP2121-130 tetramer
R & D Systems
TGFβ RI-PE
Specificity: Detect mouse TGF-beta RI/ALK-5 in flow cytometry.
Application: Flow Cytometry

Rat IgG2A-PE
Specificity: Selected for use as rat IgG2A isotype control for flow cytometry applications.
from hybridoma culture supernatant
Applications: Negative control for use in conjunction with R&D Systems antibodies.
TGFβ RII-PE
Specificity: Detects mouse TGF-beta RII in direct ELISAs and Western blots.
Applications: Flow Cytometry
Goat IgG-PE
Specificity: Serum was obtained from naive (non-immunized) goats.
Applications: Negative control for use in conjunction with R&D Systems antibodies for flow cytometry applications.
eBioscience
Anti-Foxp3-APC or anti-Foxp3-FITC
Species Reactivity: Bovine, Dog, Cat, Mouse, Pig, Rat
Applications Reported: This FJK-16s antibody has been reported for use in intracellular staining followed by flow cytometric analysis./
Anti-IFNγ-APC
Species Reactivity: Mouse
Applications Reported: This XMG1.2 antibody has been reported for use in intracellular staining followed by flow cytometric analysis.
FITC-conjugated STAT5 phosphorylated at Tyr694
Species Reactivity: Human, Mouse
Applications Tested: This SRBCZX antibody has been pre-titrated and tested by intracellular staining followed by flow cytometric analysis of stimulated U937 cells.
Mouse IgG1 kappa-FITC
Description: The monoclonal mouse IgG1 K immunoglobulin is useful as an isotype control.
Applications Reported: FITC Mouse IgG1 K Isotype Control has been reported for use in immunocytochemistry, immunohistochemistry, and flow cytometric analysis.

Cell Signaling Technology
Anti-TCF1/TCF7-Alexa Fluor 647
Reactivity: H M
Sensitivity: Endogenous
Application Key:
WB-Western Blot IP-Immunoprecipitation IHC-Immunohistochemistry ChIP-Chromatin Immunoprecipitation IF-Immunofluorescence F-Flow Cytometry E-P-ELISA-Peptide
PE-conjugated S6 phosphorylated at Ser235 and Ser236
Reactivity: H M R Mk Mi Sc
Sensitivity: Endogenous
Application Key:
WB-Western Blot IP-Immunoprecipitation IHC-Immunohistochemistry ChIP-Chromatin Immunoprecipitation IF-Immunofluorescence F-Flow Cytometry E-P-ELISA-Peptide
rabbit IgG-PE
Sensitivity: Endogenous
Application Key:
WB-Western Blot IP-Immunoprecipitation IHC-Immunohistochemistry ChIP-Chromatin Immunoprecipitation IF-Immunofluorescence F-Flow Cytometry E-P-ELISA-Peptide
PE-conjugated Smad2/Smad3 phosphorylated at Ser465/467 and Ser423/425
Reactivity: H M R Mk
Sensitivity: Endogenous
Application Key: WB-Western Blot IP-Immunoprecipitation IHC-Immunohistochemistry ChIP-Chromatin Immunoprecipitation IF-Immunofluorescence F-Flow Cytometry E-P-ELISA-Peptide
Novus Biologicals
Anti-Gr-1
Specificity: mouse Ly-6G6C
Clonality: monoclonal
Applications Notes: The monoclonal antibody NIMP-R14 has been successfully used to stain neutrophils for fluorescent activated cell sorting and in frozen and paraffin sections. Immunocytochemistry/Immunofluorescence was reported in scientific literature

Animals and other organisms

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

Laboratory animals	C57BL/6 mice, male unless otherwise stated, up to 5 mice per cage, 12/12 light dark cycle, tumor bearing mice and controls were at 5.5 months of age, otherwise ages as indicated
Wild animals	none
Field-collected samples	none
Ethics oversight	Protocol #A73113, approved by the Mayo Clinic Institutional 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	Mesenteric lymph nodes, and spleens were resected. Single cell suspensions were generated by mashing the organs through 40-100 µm cells strainers while flushing the strainers with ice-cold 1 x PBS. Erythrocytes in the spleen single cell suspensions were lysed by resuspending the cell pellets in 1 ml ACK buffer (Lonza), for 1 min on ice. The reaction was stopped by adding 15 ml of PBS + 2% FCS, cells were spun down immediately after and washed in 10 ml ice cold PBS. Dead cells were removed by filtering the lysed cells suspensions through 40 µm cell strainers.
Instrument	BD Fortessa X20, BD LSR II, BD FACSAriall
Software	BD Diva, FlowJo Versionl0.6.2
Cell population abundance	90-97 % purity
Gating strategy	% purity MLNs and spleen sells were gated for lymphocytes by FSC,SSC, doublets were discriminated, and live cells were analyzed for CD4+ expression. CD4+ T cells were further analyzed for Foxp3+ expression. Foxp3+ and Foxp3-CD4+ were further divided into RORyt+, CD69+, ICOS+, PD-1+ and CD25 subpopulations. Foxp3+ were also divided into RORyt+ and Helios-suppopulations. Mononuclear cells from gut tissues were gated for lymphocytes by FSC,SSC, doublets were discriminated, and live cells were analyzed for CD3+ CD4+expression. CD3+CD4+ T cells were further analyzed as described above.

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