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	Software used for data collection includes BioRad CFX Manager v3.1 CytExpert v2.5 Leica LAS X v3.6.0.20104 FACSDiva v8.0 FACSDiva v Living Image v4.7.2
Data analysis	Data Analysis software includes: GraphPad Prism v10 FlowJo v10.8.1 ImageJ-win64

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

Data set that includes the limited T-cell focused microRNA PCR based microarray from Qiagen (first generation) is provided in the form of a spreadsheet as Source Supplementary Data file. Any additional requests for information can be directed to, and will be fulfilled by, the corresponding authors.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Patient data set has been de-identified prior to analysis and did not include sex or gender information.
Population characteristics	Patient data was de-identified and did not include race, ethnicity or social groupings characteristics. The median age of all groups was 47.0yrs. Disease duration varied between groups from the man of 5y to 8y and for inactive disease meas were 22y and 16.8 y. Current IBD medication was recorded for each group. No other characteristics were provided with the de-identified patient samples.
Recruitment	CD and UC patients were diagnosed using established criteria and CD phenotype per the Montreal criteria and were offered to participate in the study which included endoscopic evaluation, during which intestinal mucosal biopsies were obtained. Informed, written consent was obtained from all patients prior to inclusion in the study. Patient data and samples were completely de-identified prior to being given to the researches for analysis. As a result, the researchers are unaware of any potential self-selection bias or other biases that would have the potential to impact the analysis.
Ethics oversight	Institutional Review Board of the University of Colorado

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	Sample size: Our samples in this study included colons, mesenteric lymph nodes and spleens from mice subjected to experimental colitis. We used 3R Principle to reduce number of animals in accordance with the Guidelines of the Institutional Animal Care and Use Committee (IACUC) at UT Health Science Center. Sample size for mouse experiments was determined by feasibility and the magnitude of the interventions impact. Power calculations were informed by relevant in-house pilot experiments to determine the minimum number of animals that were needed to see the effect of treatment or gene deletion. Similar published studies were consulted, for example Wirtz, S., Neufert, C., Weigmann, B. et al. Nat Protoc 2, 541–546 (2007) and Patricia Kiesler, Ivan J. Fuss, Warren Strober, Cellular and Molecular Gastroenterology and Hepatology, Volume 1, Issue 2, 2015, Pages 154-170 for selection of appropriate number of animals in colitis/ileitis models. For RT Q-PCR experiments at least 3 samples were included per experiment. Validation of experimental observations was achieved by performing at least 2 independent replicates. Statistical analyses between 2 groups included t-tests and ANOVA (parametric data).
Data exclusions	Transfer colitis: animals that showed no evidence of T cell reconstitution upon completion of the study (as evidenced by spleen and lymph node analysis) were excluded form further analysis. Our reconstitution experiments were 90-95% effective per each experimental group. TNBS colitis: animals that lost les than 5% of their body weight in the 24h following rectal enema with TNBS solution were excluded from further analysis.
Replication	Whenever it was feasible, we took effort to replicate the different experimental analysis. Animal models were replicated at least twice including a follow up analysis. All qPCR experiments included triplicate samples and biological replicates when feasible (for example, at least 3 mice) for each condition, with all assays replicated two times at minimum. Histological scoring included at least 5 animals per each group. Whenever feasible, data from replicate experiments was combined into a single panel, as indicated in the figure legends.
Randomization	Within age -matched cohorts all animals were randomly allocated into experimental groups and both female and male mice were used. In

Randomization	adoptive transfer experiments only males were used as recipients and both male and female mice were used as donors. For all other experiments samples were allocated at random with no bias into control or treatment groups.
Blinding	Investigators were blinded to sample identity during histological scoring and to group allocation in disease and disease outcomes scoring. In all other experiments the investigators were further blinded to the sample identity and group allocation during any data collection (for example, gene expression level measurements, Flow Cytometric population analysis, IVIS image analysis etc.) and blinded during subsequent data analysis.

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

Methods

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

Antibodies

Antibodies used

Anti-mouse antibodies:
anti-EF5-Cy5 conjugate (Clone: ELK3-51, Cat#: EF5012-250UG, Sigma-Aldrich)
IgG1 CY5, Clone: 15H6, Isotype Control, Southern Biotech
anti-CD3ε (Clone: 145-2C11, Cat#: 100359, Biolegend), 1ug/ml or as specified in text
anti-CD28 (Clone: 37.51, Cat#: 102121, Biolegend), 2ug/ml or as specified in text
anti-CD4-FITC (Clone: GK1.5, Cat#: 11-0041-82, Invitrogen), 1:400
anti-CD45RB-PE (Clone: C363.16A, Cat#: 12-0455-82, Invitrogen), 1:600
anti-CD4-eFluor450 (Clone: GK1.5, Cat#: 48-0041-82, Invitrogen), 1:400
anti-CD8a-PE (Clone: 53-6.7, Cat#: 100707, Biolegend), 1:400
anti-CD8a-Pacific Blue (Clone: 53-6.7, Cat#: 100728, Biolegend), 1:400
anti-CD62L-APC (Clone: MEL-14, Cat#: 17-0621-82, Invitrogen), 1:500
anti-CD44-FITC (Clone: IM7, Cat#: 11-0441-82, Invitrogen), 1:200
anti-CD25-PB (Clone PC61.5 Cat#: 404-0251-82, Invitrogen), 1:400
anti-IFNg-APC (Clone: XMG1.2, Cat#: MABF1515, Sigma-Aldrich), 1:400
anti-IL-17a-PE (Clone:TC11-18H10.1, Cat#: 506903, Biolegend), 1:400
anti-TNFa-PE (Clone: TN3-19.12, Cat#: 506104, Biolegend), 1:400
anti-Tbet-PE (Clone: O4-46, Cat#: 561268, BD Bioscience), 1:400
anti-Tbet-BV421 (Clone: O4-46, Cat#: 563318, BD Bioscience), 1:400
anti-HIF2A (Cat#: NB100-122, Novus Biologicals), 2ug/reaction
anti-control isotype Rabbit IgG (Cat#: 2729s, Cell Signaling Technologies), 2ug/reaction
anti-IFNg (Clone: XMG1.2, Cat#: 505847, Biolegend), 10ug/ml
anti-IL-4 (Clone: 11B11, Cat#: 504136, Biolegend), 5ug/ml
anti-IL-2 (Clone: JES6-1A12, Cat#: 503707, Biolegend), 5ug/ml
anti-IFNg (Clone XMG1.2, Catalog #BE0055, BioXcell) 10µg/ml
anti-IL-4 (11B11, Catalog #BE0045, BioXCell) 10µg/ml
anti-IL-12 (C17.8, Catalog #BE0051 BioXCell) 10µg/ml

Validation

Validation:
anti-EF5: validated by using IgG1 CY5, Clone: 15H6
anti-HIF2A: validated in assay by using anti-control isotype Rabbit IgG.
All Invitrogen, Biolegend, BioXcell and Sigma-Aldrich antibodies: validation is readily provided on manufacturer's website for each product and contains an extensive (21-560) list of peer-reviewed references for each antibody, including a PubMed link for each reference.

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

We used floxed mouse lines crossed to Lck Cre+ lines to generate our T cell specific mutants: Lck-Cre+, Hif-2αloxP/loxP Lck Cre +, miR-29aloxP/loxP Lck Cre+, Lck-Cre- or LSL-HIF2dPA Lck Cre. Wild type mice used were C57BL/6J. 129S7(B6)-Rag1tm1Mom/J mice

	were used as recipients in the transfer colitis model. The B6.129S-Tnftm2GKl/Jarn strain (TNFΔARE/+; MGI:3720980) was generated by continuous backcrosses between heterozygous TNFΔARE/+ on a mixed background 12 to C57BL6/J mice. Experimental animals were heterozygous for the ΔARE mutation (TNFΔARE/+) or homozygous wildtype (WT), which served as controls.
Wild animals	Our study did not involve wild animals
Reporting on sex	we used both male and female mice in our studies with the exception of adoptive transfer experiments where only male mice were used. This allowed us to use both male and female mice as cell donors in the adoptive transfers.
Field-collected samples	We did not use field collected samples
Ethics oversight	Animal procedures were approved by the institutional Animal Care and Use Committee at the UT Health Science Center in Houston and at the University of Colorado

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	Cell samples for flow cytometry were prepared by producing a single-cell suspensions of mesenteric lymph node or spleen cells. Lamina propria lymphocytes were prepared Lamina Propria Dissociation Kit, mouse (130-097-410, Miltenyi Biotec) per manufacturer's instruction and followed by Percol gradient centrifugation.
Instrument	BD FACSCantoII (BD Biosciences), CytoFlex LX (Beckman Coulter Lifesciences). Sorting was performed with BD FACSAria II (BD Biosciences).
Software	Analyses were performed with FLOWJo (Tree Star, Ashland, OR)
Cell population abundance	Live cells represented more than 90% of the cell identified as targets with the exception of hypoxia experiments where cell death was specifically scored as an experimental parameter.
Gating strategy	Cells were first selected on FSC area/SSC area, then singlets were selected on FSC area/FSC height. Then CD4+ T cells were gated based live exclusion marker and on surface staining and those were subsequently analyzed for additional surface or intracellular markers (on area).

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