

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Flow cytometry data were collected using FACSDiva software (version 8.0, BD Biosciences). Images of immunofluorescence were acquired using a Nikon Eclipse 90i upright microscope equipped with a Hamamatsu Orca-ER CCD camera, and APC line 1200 light source.
Data analysis	Flow cytometry data were analyzed using FlowJo software (version 10, TreeStar). Statistical analysis was performed by GraphPad Prism 9.3.1. ImageJ v2.1.0/1.53c was used for the quantification of fluorescent area in the images

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](#)

Bulk RNA-seq data, ATAC-seq data, and single-cell RNA-seq data have been deposited at GEO (accession number: GSE222713)

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	Resected human colonic sections that were diagnosed as adenomas were obtained from 8 patients with familial adenomatous polyposis (FAP) and 11 patients without FAP. Resected human colonic sections that were diagnosed as CRCs at different pathologic tumour stages (pT1-pT4) were obtained from 39 patients. Inclusion criteria were male or female adults aged 20–90 years.
Population characteristics	Inclusion criteria were male or female adults aged 20–90 years.
Recruitment	Resected human colonic sections that were diagnosed as adenomas were obtained from 8 patients with familial adenomatous polyposis (FAP) and 11 patients without FAP. Resected human colonic sections that were diagnosed as CRCs at different pathologic tumour stages (pT1-pT4) were obtained from 39 patients.
Ethics oversight	The Massachusetts General Hospital (MGH) Institutional Review Board committee approved the study protocol.

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](https://www.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 statistical methods were used to predetermine sample size. Sample sizes were based on those used in previous and preliminary studies from our lab.
Data exclusions	For the single-cell RNA-seq data, doublets and cells with low mRNA quality were discarded as described in the method section.
Replication	All experiments presented in this study were performed using at least 3 biological replicates. All presented results were repeatable.
Randomization	Mice were allocated to study groups randomly.
Blinding	Blinding was not performed in the experiments.

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

IHC:
 goat polyclonal anti-SOX17 (1:200, R&D, AF1924)
 rabbit monoclonal anti-SOX17 (1:2000, abcam, ab224637)
 rabbit monoclonal anti-CD4 (1:400, abcam, ab183685)
 rabbit monoclonal anti-CD8 (1:1000, abcam, ab217344).
 mouse monoclonal anti- β -catenin (1:50, BD Biosciences, 610154)
 rat monoclonal anti-BrdU (1:2000, abcam, ab6326)
 mouse monoclonal anti-Ki67 (1:400, BD, 550609)
 mouse monoclonal anti-CD8 (1/100, Abcam, ab17147)

IF:
 rabbit monoclonal anti-Phospho-Stat1 (1:400, Cell signaling, #9167)
 rabbit monoclonal anti-SOX17 (1:2000, abcam, ab224637)
 goat polyclonal anti-GFP (1:500, abcam, ab6673)

Flow cytometry:
 CD45 APC (1:200, Biolegend, 103112)
 EpCAM efluor450 (1:50, Thermo Fisher Scientific, 48-5791-82)
 MHC-I efluor450 (1:100, Thermo Fisher Scientific, 48-5958-82)
 CD8a BUV395 (1:400, BD, 563786)
 IFN γ FITC (1:200, Biolegend, 505806)
 TNF α PE (1:400, Biolegend, 506306)
 IFNAR-1 (1:100, Biolegend, 127305)
 CD119 (IFN- γ R α chain) (1:200, Biolegend, 112803)
 APC streptavidin (1:200, Biolegend, 405207)

In vivo dosing:
 CD8 α antibody (BioXcell, BE0061)
 CD4 antibody (BioXcell, BE0003-1)
 IgG isotype antibody (BioXcell, BE0090)
 IFNAR1 antibody (BioXcell, BE0241)
 IFN γ antibody (BioXcell, BE0055)
 IgG1 isotype antibody (BioXcell, BE0083)

Validation

All antibodies for IHC, IF, flow cytometry, and in vivo dosing were well-recognized clones in the field and validated by the manufacturers.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Organoid lines were developed by the authors from normal colon crypts from mice and human.

Authentication

Authentication of genetic events were confirmed as described in the manuscript.

Mycoplasma contamination

Organoid lines have been routinely tested and confirmed negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

None

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

Both male and female mice were used at the ages of 8-12 wks.

Wild animals	Wild animals were not used in this study.
Reporting on sex	Both male and female mice were used at the ages of 8-12 wks.
Field-collected samples	This study did not involve samples collected in the field
Ethics oversight	Mice were under the husbandry care of the Department of Comparative Medicine in the Koch Institute for Integrative Cancer Research. All procedures were conducted in accordance with the American Association for Accreditation of Laboratory Animal Care and approved by MIT's Committee on Animal Care.

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	Details are described in the method section. Briefly, colon tumours were collected, minced, and digested in a digestion buffer. Tumours were then further dissociated with a gentleMACS Octo Dissociator, filtered through a 100-µM filter, and centrifuged for 5 min at 2000 rpm. To isolate lymphocytes, cells were resuspended in 5 ml 44% percoll, underlaid with 2ml 67% percoll, centrifuged at 2000 rpm for 20 min with no brake. Buffy coat at the interface was isolated, resuspended in culture media, and centrifuged at 2000 rpm for 10 min.
Instrument	BD LSRFortessa four-laser, 18-color flow cytometer and FACS Aria II were used.
Software	FACSDiva software (version 8.0, BD Biosciences) and FlowJo software (version 10, TreeStar).
Cell population abundance	When CD45+ cells were sorted for scRNA-seq, the purity was confirmed by UMAP plot with Ptprc expression.
Gating strategy	Single lymphocytes were gated on FSC-A versus SSC-A, FSC-H versus FSC-W, and SSC-H versus SSC-W. Live CD8+ T cells were gated on positive CD8α and negative Fixable Viability Dye eFluor™ 780 staining. For scRNA-seq, CD45+ immune cells were gated on negative 7-AAD, negative EpCAM, and positive CD45 staining.

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