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# Reporting Summary

Nature Research 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 Research 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<br>*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<br>*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: Mass cytometry data in this study was collected with a Helios mass cytometer using the Fluidigm CyTOF Software (Version 6.7).

Data analysis: Data analysis was performed with Cytobank (Version 7.2.0) and publicly available R and python packages. Specifically:

Python >3.6 with packages:
fcsparser
fcswrite
numpy
pandas
plotly
rpy2
scprep
sklearn
umap-learn

R >3.6 with packages:
DT
factoextra
FactoMineR
flowCore
Ggally
ggplot2
Hmisc

MASS
matrixStats
plotly
psych
RColorBrewer
shiny
tidyverse

MATLAB (R2020b):
Single Cell Debarcoder (https://github.com/zunderlab/single-cell-debarcoder)

Statistical tests were performed using Graphpad Prism (Version 7.0).
Immunofluorescence staining images were processed using FIJI (Version 2.0.0).

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All raw data, processed data, and working illustrations are available as a Community Cytobank project (https://community.cytobank.org/cytobank/experiments#project-id=1334).

# 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 was performed as this is a method development and proof-of-concept project. Sample size was determined based on the experience and expertise of the investigators. |
| Data exclusions | All cells were gated for Gaussian discrimination parameters (Event length, Centre, Residual, and Width values) as recommended by Fluidigm to remove non-events such as debris and doublets. The cell-gating strategy was described in Figure 6. |
| Replication | Multiple cohorts of murine and patient-derive organoids were used in this project, yielding comparable results and thereby validated the robustness of our methods. Details on technical/biological replications of experiments are included in relevant figure legends. |
| Randomization | Animals and organoid samples were randomly chosen for inclusion in this study. |
| Blinding | No conditions presented in this study required 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

All antibody information, including clone name and supplier, is provided in Supplementary Table 2 with the addition of the following antibodies:
CEACAM1, Clone CC1, Thermo Fisher
Podoplanin, Clone 8.1.1, BioLegend
RFP, Clone 8E5.G7, 2BScientific
F4/80, Clone BM8, BioLegend
CD68, Clone FA-11, BioLegend

Validation

Cell-type identification antibodies are validated in our previous publication Qin, X., Sufi, J., Vlckova, P. et al. Cell-type-specific signaling networks in heterocellular organoids. Nat Methods 17, 335–342 (2020). https://doi.org/10.1038/s41592-020-0737-8.
PTM antibodies selected for this study are widely used by the mass cytometry community and well-validated.
Antibody panels were carefully designed and titrated in accordance with known monoisotopic impurities and antigen abundance to ensure minimal cross-channel contamination.

# Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

- Wild-type, Apc knockdown (shApc), and shApc / KrasG12D/+ murine colon organoids were a kind gift from Prof. Lukas Dow (Cornell University).
- Colorectal cancer patient-derived organoids were a kind gift from Dr. Mathew Garnett (Sanger Institute).
- Wild-type murine small intestinal organoids were a kind gift from Dr. Vivian Li (Crick Institute).
- Murine colonic fibroblasts were isolated and immortalised at UCL Cancer Institute as described in Qin, X., Sufi, J., Vlckova, P. et al. Cell-type-specific signaling networks in heterocellular organoids. Nat Methods 17, 335–342 (2020). https://doi.org/10.1038/s41592-020-0737-8.

Authentication

Cell lines used in this study have not been authenticated during the development of the project.

Mycoplasma contamination

Cells were checked for mycoplasma infection monthly using the MycoAlertTM PLUS Mycoplasma Detection Kit (Lonza LT07-701) and remained negative throughout this project.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

# Animals and other organisms

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

Laboratory animals

- Murine bone marrow-derived macrophages were isolated from 10- to 12-week-old C57BL/6 mice provided by Dr. Sophie Acton (University College London).

Wild animals

This study did not involve the use of wild animals.

Field-collected samples

This study did not involve the use of field-collected samples.

Ethics oversight

All animal work carried out was approved by local ethical review and licensed by the UK Home Office.

Note that full information on the approval of the study protocol must also be provided in the manuscript.