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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	All sequencing data was enabled through 10X Chromium with initial collection and processing performed through CellRanger (10X Genomics) as described in the methods section. Flow cytometry data was collected on the BD Fortessa X-20 using BD FACSDiva Software (v9.7). Microscopy data was collected on the Operetta CLS using Harmony (v4.9), the Leica STELLARIS 8 using Las X (v4.7.0.28176), and the Vectra Polaris using PhenoChart (v1.0.12) and inForm (v2.4). Supernatant analysis of cytokines was collected on the Bio-Rad Bioplex-200 using the accompanying BioManager (v6.2) software. Further details are described in the methods.
Data analysis	Analysis of single-cell transcriptomics was performed with R Statistical Framework (v4.2.1) and Python (v3.7) on an x86_64-pc-linux-gnu (64-bit) platform running under Red Hat Enterprise Linux. The following programs were also used in the bioinformatic analysis of the sequencing data: CellRanger (v6.0.2), Celda (v1.16.1) Seurat (v3), DoubletFinder (v2.3.0), Presto (v1.0.0), Destiny (v3.14.0), NicheNet (v02.01.2000) Flow cytometry data was analysed using Flowjo v10. Imaging was analysed using Harmony (v4.9), PhenoChart (v1.0.12), inForm (v2.4), Halo AI (v3.2.1851.328), KNIME (v5.2.4), CellProfiler (v4.2.5) and ImageJ (v1.54i). Non-sequencing data was plotted and analysed statistically using GraphPad Prism (v10.2.0). Sequencing data was plotted and assessed statistically in R Statistical Framework (v4.2.1).

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

Sequencing files in FASTQ format supporting single cell transcriptomic analysis of homeostasis conditions as well as EpCAM TCB treatment have been deposited in ArrayExpress and are accessible at E-MTAB-14171 and E-MTAB-14170 respectively. Raw count matrices, together with metadata information, for all scRNA-seq samples discussed in this study have been deposited in Mendeley and are available at https://data.mendeley.com/datasets/5h3ym82bnb/1. Source data underlying all graphical representations used in the figures is provided as supplementary information.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Biological material was derived from both sexes, 16 males and 11 females ranging from ages 40 - 89. Gender-based analysis was not performed - researchers were blinded to the sex and age of the donating patients at the point of experimentation. All experiments contained intra-donor controls such that donor-specific effects could be considered in the context of each biological replicate. Our findings were consistent across a diverse range of age and sex and strictly governed by variables independent of sex, namely drug treatment or location of immune cell source (blood v intestine).
Reporting on race, ethnicity, or other socially relevant groupings	Researchers were blinded to the race, ethnicity and other socially relevant groupings of the donating patients. All experiments contained intra-donor controls such that donor-specific effects could be considered in the context of each biological replicate. Our findings were consistent across all biological replicates tested.
Population characteristics	Patients underwent visceral surgery with partial resection of intestines for various oncologic indications, e.g. pancreaticojejunostomy due to pancreatic adenocarcinoma. We used micro- and/or macroscopically tumor-free regions of resectates for experiments in this study.
Recruitment	Patients undergoing surgery (e.g. pancreatic or colorectal) in academic centers donated resected tissues within the HTCR framework. The authors were not involved in recruitment, and sample selection was opportunistic for availability, amount of tissue and blood.
Ethics oversight	Human intestinal tissue samples and annotated data were obtained and experimental procedures performed within the framework of the non-profit foundation HTCR (Munich, Germany) including informed patient's consent. The framework of the HTCR Foundation, has been approved by the ethics commission of the Faculty of Medicine in the LMU (number 025-12) and the Bavarian State Medical Association (number 11142).

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	We considered one organoid to represent an intestinal unit. Each organoid experiment in the manuscript included tens to hundreds of individual organoids. Analysis of immune cell behaviour from intestinal tissue or IIO cultures was repeated on two to six biological replicates, each with two to six technical replicates (information on individual experiments is clearly stated in the figure legends). No statistical method was used to determine sample size. Instead sample sizes were determined empirically, taking into account both opportunistic availability of primary tissue and variability of results between technical experimental and biological replicates.
Data exclusions	For scRNA-seq, all data exclusion criteria were pre-established and based on standard sequencing analysis practises. Low quality cells with a low number of expressed genes or high proportion of mitochondrial gene counts or abnormal library sizes were excluded from the dataset. No additional data from any experiment was excluded.
Replication	All non-sequencing experiments presented in this study were repeated in at least three biologically-independent experiments, with the exception of figure 3c (1 biological donor in technical duplicates) and Extended Data Figure 7 (2 biological donors in technical duplicates or triplicates). Where only representative data is shown, replicated data were successful and showed similar results. This information is clearly

stated in the figure legends.
The first scRNA-sequencing experiment (figure 2) was performed after pooling at least 5 technical replicates for each experimental condition from three biological donors. The second scRNA-sequencing experiment (figure 3-4) was performed after pooling at least 5 technical replicates for each experimental condition from one biological donor. The main observations were then confirmed via flow cytometry analysis in five independent biological donors.

Randomization

Randomization was not performed. Potential covariates were controlled for by running intra-donor comparisons, using donor-matched experimental conditions treated with control molecules (non-targeting TCBs, isotype antibodies or vehicle solutions), to compare to the test conditions (EpCAM TCB, ROCKi, TNFi, Immune checkpoint inhibitors)

Blinding

Blinding was not performed. All experimental analysis contained donor-matched controls and was based on machine-derived empirical quantification (scRNA-seq, flow cytometry, cytokine quantification), not amenable to subjective operator bias.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

All antibodies, dilutions and vendor information are described in Supplementary Information Table 3.
BUV395 -CD4 BD Biosciences RPA-T4 564724 1/100,
BUV737-CD69 BD Biosciences FN50 612817 1/200,
BUV737-TCR gd BD Biosciences 11F2 748533 1/100,
BUV805-CD8 BD Biosciences SK1 612889 1/100,
BV421-Ki67 Biolegend Ki67 350506 1/100,
BV510-CD45 Biolegend 2D1 368526 1/200,
BV605-CD19 Biolegend HIB19 302244 1/100,
BV605-CD103 Biolegend Ber-ACT8 350218 1/200,
BV650-HLA-DR Biolegend L243 307650 1/100,
BV711-CD117 Biolegend 104D2 313230 1/100,
BV786-CD45RA Biolegend HI1/100 304140 1/100,
BV786-CD107a Biolegend HP-3G10 339930 1/100,
FITC-CD45RO Biolegend UCHL1 304204 1/100,
PE-TNFa Biolegend MAb11 502909 1/100,
PE-CD45RO Biolegend UCHL1 304204 1/100,
PE-CD49a Biolegend TS2/7 328304 1/100,
PE-TCR Vα7.2 Biolegend 3C10 351706 1/100,
PE-Dazzle-594-GzmB Biolegend QA16A02 372215 1/100,
PE-Dazzle-594-CD25 Biolegend BC96 302646 1/100,
PE-Dazzle-594-CD27 Biolegend M-T271 356422 1/100,
PE-Dazzle-594-TCR Vd2 Biolegend B6 3314226 1/100,
PE-Cy7-CD56 Biolegend 5.1H11 362510 1/100,
PE-Cy7-CD38 Biolegend HB-7 356608 1/100,
APC-CD39 Biolegend A15153G 328210 1/100,
APC-IFNg Biolegend 4S.B3 502511 1/100,
APC-Perforin Biolegend B-D48 353312 1/100,
Alexa Fluor 700 -CD3 Biolegend HIT3a 300324 1/100,
APC-H7 Efluor780 Fixable L/D Thermo Fisher N/A 65-0865-14 1/1000
FABP1 Life Technologies PAS28945 1/9
CD103 Abcam 224202 1/100
CD3 Ventana 790-4341 prediluted
CD4 Ventana 790-4423 prediluted
CD8 Ventana 790-4460 prediluted

CD69 Abcam ab233396 1/10
Cleaved Caspase-3 Cell Signaling Technology 9661 1/9
E-Cadherin Ventana 760-4497 prediluted

Validation

For flow cytometry, expected staining patterns of lineage-defining surface proteins were confirmed using pre-qualified control samples. Gates were set against FMO (fluoresence minus one) or unstimulated negative controls. For imaging, secondary only and isotypes controls were applied during establishments of antibody staining panels. Here, all antibodies were tested on human native tissue as validation, before staining the cultures.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Organoid lines and tissue-derived immune cells were derived from the healthy margin of primary tumor surgical resections. Information on the sex of the patients is listed in Supplementary Information Table 4.
Authentication	All samples were derived from primary patient tissue samples. Absolute validation was confirmed by expression of cell lineage-defining markers and transcriptomic analysis.
Mycoplasma contamination	All lines used in the studies were verified to be negative for mycoplasma before experimentation.
Commonly misidentified lines (See ICLAC register)	None.

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	Egressed immune cells from primary tissue were harvested, stained and characterized using standard flow cytometry staining protocols (full details in the methods section). Immune cell-organoid cocultures were first digested into a single cell suspension and filtered, before being subjected to flow cytometry staining (as detailed in the methods). Samples were always acquired immediately after staining.
Instrument	BD LSRFortessa X20.
Software	FowJo v10.8.1.
Cell population abundance	No flow cytometry based sorting was performed.
Gating strategy	Viable immune cells were selected based on expression of CD45 and a viability dye. Debris and doublets were subsequently excluded based on scatter signal. Immune cell subpopulations were defined on the basis of lineage-defining markers which clearly separated populations into positive and negative expressors. For analysis of intracellular cytokines, gating was compared to and based on background expression levels in matched unstimulated control samples.

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