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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	No software was used
Data analysis	Analysis performed in R version 4.2, Seurat v4.1.0, dropEst v0.8.6 (https://github.com/kharchenkolab/dropEst), STAR v2.7.9a (https://github.com/alexdobin/STAR), scRNABatchQC v0.10.3 (https://github.com/liuqivandy/scRNABatchQC), scMRMA v1.0 (https://github.com/JiaLiVUMC/scMRMA), scUnifrac v0.9.6 (https://github.com/liuqivandy/scUnifrac), CIBERSORTv1.05 (https://cibersortx.stanford.edu/), CellChat v1.4.0 (https://github.com/sqjin/CellChat), SeuratWrapper v0.3.0, velocyto v0.6 (http://velocyto.org/), CytoTRACE v0.3.3 (https://cytotrace.stanford.edu), PAGA v1.3.3 (https://github.com/theislab/paga), SNPsea v1.0.3 (https://github.com/slowkow/snpsea), spacexr v2.2.1 (https://github.com/dmcable/spacexr), SpaGene v0.1.0 (https://github.com/liuqivandy/SpaGene), GEOquery v2.64.2. Multiplex immunofluorescence imaging analysis was performed using Ilastik v1.4.1 (https://github.com/ilastik/ilastik) and Fiji v2.9.0. For more details the R scripts used are available on Github (https://github.com/JiaLiVUMC/GCA_LND).

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 raw and processed single-cell RNA sequencing data have been uploaded to GEO accession GSE266546.

Publicly available data were downloaded from the following accession numbers:
(1) six bulk RNA sequencing data for validation
Figure 3. The normalized matrix was downloaded from GEO, including GSE179285, GSE75214, GSE186582, GSE12366, GSE20881 and GSE66207
(2) one bulk RNA sequencing data for treatment comparison
Figure 6. The normalized matrix was downloaded from GEO GSE16879.

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	The summary of biological sex of the patients is reported in the manuscript.
Reporting on race, ethnicity, or other socially relevant groupings	The ethnic background and family history of the patients is reported in the manuscript.
Population characteristics	Age range is reported in the manuscript, histology and treatments are reported in the manuscript and Table S1. Patient age is range from 18 to 75. Disease severity of each specimen was classified as active CD (including mild, moderate and severe), inactive CD (including normal and quiescent) and controls. Five treatments (Immunomodulator, Anti_TNF, Anti_Integrin, Stelara and Corticosteroid) were recorded for each patient.
Recruitment	Written informed consent was obtained from non-IBD control and CD subjects to obtain terminal ileum (TI) and ascending colon (AC) tissue at the time of scheduled endoscopic procedures. TI and AC tissues from non-IBD control and CD subjects undergoing surgical resection were also obtained from under a separate IRB protocol in coordination with the Comparative Human Tissue Network (CHTN).
Ethics oversight	The study protocol was approved by the Institutional Review Board at Vanderbilt University Medical Center.

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 for single-cell RNA sequencing was determined by the availability of collected patient samples. For all participants, tissue biopsies were examined in a blinded manner by a gastrointestinal pathologist and graded accordingly as : control, inactive CD (normal_CD, quiescent) and active CD (mild, moderate, severe). The exact number of samples are listed below: (1) single cell RNA sequencing 82 terminal Ileum (TI) and 88 ascending colon (AC) specimens from either endoscopic biopsies or surgical resection across 83 unique individuals (65 Crohn's disease patients and 18 non-Inflammatory bowel disease controls). (2) multiplex immunofluorescence imaging 55 tissues, of which 38 have single cell RNA sequencing profiling (17 Crohn's disease and 3 controls in TI, 15 Crohn's disease and 3 controls in AC). (3) spatial transcriptomics 4 Crohn's disease samples (2 TI and 2 AC).

Data exclusions	Cells with >40% mitochondria reads, or <500 UMI counts, or <200 or >6,000 genes expressed were considered as low quality and excluded. After this rough quality control, each sample was manually checked to remove those clusters of empty droplets (low number of UMI and genes, and no distinct markers) and clusters of doublets (high number of UMI and genes, and markers from two different cell types).
Replication	Technical replicates of scRNAseq were performed on several samples and the consistent cellular compositions were obtained. scRNAseq experiments were performed on >=14 biological replicates in both TI (15 control, 30 inactive CD and 17 active CD) and AC (14 control, 32 inactive CD and 18 active CD). The results of the specialized epithelial cell type were validated in six independent cohorts.
Randomization	Not applicable.
Blinding	Patients were de-identified and assigned an identifier. Researchers were aware of basic demographic information and relevant medical history. Clinicians were aware of each patient's full history.

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Multiplex immunofluorescence antibodies:
CD11B (Thermo Fisher, C67F154, 53-0196-82)
https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-C67F154-Monoclonal/53-0196-82
CD20 (Santa Cruz, D-10, sc-393894)
https://www.scbt.com/p/cd20-antibody-d-10
CD3D (Abcam, EP4426, ab208514)
https://www.abcam.com/products/primary-antibodies/alexa-fluor-555-cd3d-antibody-ep4426-ab208514.html
CD4 (Abcam, EPR6855, ab196147)
https://www.abcam.com/products/primary-antibodies/alexa-fluor-647-cd4-antibody-epr6855-ab196147.html
CD45 (Santa Cruz, 2D-1, sc-1187)
https://www.scbt.com/p/cd45-antibody-2d-1
CD68 (Santa Cruz, KP1, sc-20060)
https://www.scbt.com/p/cd68-antibody-kp1
CD8 (Biolegend, C8/114B, 372906)
https://www.biolegend.com/en-gb/products/alexa-fluor-647-anti-human-cd8a-antibody-14127?GroupID=BLG15860
CGA (Santa Cruz, C-12, sc-393941)
https://www.scbt.com/p/chr-a-antibody-c-12
COLLAGEN (3Helix, R-CHP, RED300)
https://www.3helix.com/product/red300/
COX2 (Cell Signaling, D5H5, 13596S)
https://www.cellsignal.com/products/antibody-conjugates/cox2-d5h5-xp-rabbit-mab-alexa-fluor-488-conjugate/13596?_requestid=775627
ERBB2 (Abcam, EPR19547, ab225510)
https://www.abcam.com/products/primary-antibodies/alexa-fluor-647-erbb2--her2-antibody-epr19547-12-ab225510.html
FOXP3 (Biolegend, 206D, 320114)
https://www.biolegend.com/en-gb/products/alexa-fluor-647-anti-human-foxp3-antibody-2945?GroupID=BLG4131
HLAA (Abcam, EP1395Y, ab199837)
https://www.abcam.com/products/primary-antibodies/alexa-fluor-647-hla-a-antibody-ep1395y-ab199837.html
LYSOZYME (Santa Cruz, E-5, sc-518012)
https://www.scbt.com/p/lysozyme-c-antibody-e-5
MUC2 (Santa Cruz, F-2, sc-515032)

https://www.scbt.com/p/mucin-2-antibody-f-2
NAKATPASE (Abcam, EP1845Y, ab198367)
https://www.abcam.com/products/primary-antibodies/alexa-fluor-647-sodium-potassium-atpase-antibody-ep1845y-plasma-membrane-marker-ab198367.html
OLFM4 (Cell Signaling, D1E4M, 14369S)
https://www.cellsignal.com/products/primary-antibodies/olfm4-d1e4m-xp-rabbit-mab/14369
PSTAT3 (Cell Signaling, D3A7, 4324S)
https://www.cellsignal.com/products/antibody-conjugates/phospho-stat3-tyr705-d3a7-xp-rabbit-mab-alexa-fluor-647-conjugate/4324
PANCK (Thermo Fisher, AE1/AE3, 53-9003-82)
https://www.thermofisher.com/antibody/product/Pan-Cytokeratin-Antibody-clone-AE1-AE3-Monoclonal/53-9003-82
PCNA (Cell Signaling, PC-10, 8580S)
https://www.cellsignal.com/products/antibody-conjugates/pcna-pc10-mouse-mab-alexa-fluor-488-conjugate/8580
PEGFR (Abcam, EP774Y, ab205827)
https://www.abcam.com/products/primary-antibodies/alexa-fluor-488-egfr-phospho-y1068-antibody-ep774y-ab205827.html
SMA (Millipore Sigma, 1A4, C6198-100UL)
https://www.sigmaaldrich.com/US/en/product/sigma/c6198
SOX9 (Abcam, EPR14335, ab202516)
https://www.abcam.com/products/primary-antibodies/alexa-fluor-555-sox9-antibody-epr14335-ab202516.html
VIMENTIN (Santa Cruz, E-5, sc-373717)
https://www.scbt.com/p/vimentin-antibody-e-5
LCN2 (Novus Biologicals, Cat. number NB100-1503)
https://www.novusbio.com/products/lipocalin-2-ngal-antibody_nb100-1503
NOS2 (Novus Biologicals, Cat. number NBP2-22119AF750)
https://www.novusbio.com/products/inos-antibody-4e5_nbp2-22119f
DUOX2 (Novus Biologicals, Cat. Number NB110-61576AF647)
https://www.novusbio.com/products/duox2-antibody_nb110-61576

Immunohistochemistry antibodies:LCN2 (Sigma, Cat. #HPA002695)
https://www.sigmaaldrich.com/US/en/product/sigma/hpa002695
NOS2 (Novus Biologicals, Cat. Number NBP2-99091)
https://www.novusbio.com/products/inos-antibody_nbp2-99091
DUOX2 (Novus Biologicals, Cat. Number NB110-61576)
https://www.novusbio.com/products/duox2-antibody_nb110-61576

Validation

Validations are available for all antibodies from the manufacturer. Please refer to references contained in the provided links.