

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 acquired using FACSDiva (v9.1, v9.0, BD Biosciences) on FACSsymphony A3 (BD Biosciences) and LSRFortessa (BD Biosciences), and cell sorting was performed on FACSaria and FACS Fusion (BD Biosciences). Chemiluminescence data were collected using Azure C500 imaging system (v1.7.6.1202). Immunofluorescence images were captured on ZEISS Axio Vert.A1 TL/FL-LED using ZEN (v3.10, ZEISS), Leica SP8 WLL FLIM, or PhenolImager (Akoya Biosciences). Whole-exome sequencing was performed on Illumina HiSeq 2500, and single-cell CITE-seq and TCR V(D)J libraries were generated using 10x Genomics Chromium platform for single-cell capture and cDNA preparation and Illumina NovaSeq Xplus 10B or DNBSEQ-T7 PE100 (BGI) for sequencing.
Data analysis	Flow cytometry data were analyzed using FlowJo (Tree Star, v10.9.0). Images were processed with ImageJ (v2.14.0), Imaris (v10.2.0), QuPath (v0.5.1), LAS X(Leica, v3.5.7), and SimpleITK (PMID: 29181613). Single-cell RNA-seq, CITE-seq, and TCR V(D)J analyses were processed with 10x Genomics CellRanger multi (v9.0) and CellRanger count (v6.1.2), and analyzed in R (v4.4.0) using Seurat (v5.0.3), SingleR (v2.6.0), and scRepertoire (v2.4.0). Functional enrichment analyses were performed with SIGNAL (PMID: 33894945) and DAVID (PMID: 19131956). Heatmaps were generated using the pheatmap package (v1.0.13). Gene set enrichment analysis (GSEA) was performed using the fgsea package (v1.36.2). Volcano plots were generated using the ggplot2 package (v4.0.2). Additional analyses were conducted in Python using Scanpy (v1.9.1). Bulk exome and genome sequencing reads were aligned using the Burrows–Wheeler Aligner (BWA, v0.7.17), and variants were identified using the Genome Analysis Toolkit (GATK, v3.5). Genetic association and population structure analyses were performed using PLINK (v1.9), scikit-learn (v1.3.2), generalized linear mixed-effects models implemented with lme4 (v1.1-38) in R(v4.5.1), and multivariable logistic regression in R (v4.5.1) or with statsmodels (v0.14.4) in Python (v3.10.16). General statistical analyses and data visualization were conducted using GraphPad Prism (v10.2.0).

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 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](#)

Human whole exome and genome sequencing data from the four families evaluated at NIH have been deposited in dbGaP (phs003950.v1.p1). Raw WES data for the Turkish/Qatari and Iranian cohorts are available under controlled access due to ethical and legal restrictions related to patient privacy and the terms of informed consent. Requests for access should be directed to Bernice Lo (blo@sidra.org) for the Turkish and Qatari cohorts and Hassan Abolhassani (Seyed.Abolhassani@ukbonn.de) for the Iranian cohort and are subject to institutional ethics approval and data use agreements. Human and mouse single-cell RNA-Seq datasets generated in this study have been deposited in Gene Expression Omnibus (GEO) (GSE314446). Raw human scRNA-seq reads are available through dbGaP (phs003950.v1.p1). Single-cell RNA-seq data from healthy adult and pediatric guts were obtained from the Gut Cell Atlas (<https://www.gutcellatlas.org/>), with raw data available at ArrayExpress (E-MTAB-9543, E-MTAB-9536, E-MTAB-9532, E-MTAB-9533 and E-MTAB-10386) (PMID: 34497389). Single-cell RNA-seq data from the UC patients were obtained from GEO (Accession GSE125527) (PMID: 32826341). Single-cell RNA-seq data from the CD patients were obtained from the Broad Institute Single Cell Portal (project SCP1884; https://singlecell.broadinstitute.org/single_cell/), with raw data available at Broad DUOS (Accession DUOS-000146 CD_Atlas_2021_GIDER; DUOS-000145 CD_Atlas_2021_PRISM) (PMID: 38091954). Single-cell transcriptomic data of blood KIR+CD8+ T cells was obtained from GEO (Accession GSE193770) (PMID: 35258337). AoU dataset were obtained from the All of Us Research Program (Controlled Tier Dataset v8, <https://www.researchallofus.org/>) (PMID: 31412182). UK Biobank analysis were assessed through GeneBass (<https://app.genebass.org/>) (PMID: 36778668). Population allele frequencies were obtained from gnomAD (<https://gnomad.broadinstitute.org/>) (PMID: 32461654). Variant deleteriousness scores were derived from CADD (<https://cadd.gs.washington.edu/>) (PMID: 24487276). Protein structural predictions were obtained from AlphaFold (<https://alphafold.ebi.ac.uk/>) (PMID: 34265844).

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	Patients and relatives were enrolled for evaluation without regard to sex or gender.
Reporting on race, ethnicity, or other socially relevant groupings	Patients and relatives were enrolled for evaluation without regard to race, ethnicity, or other socially relevant groupings.
Population characteristics	Participants included individuals with and without GPR15 mutations, healthy relatives, individuals with unsolved genetic diseases, and patients with IBD. Covariate-relevant characteristics, including age, sex, race, or ethnicity, disease status, and GPR15 genotype, were recorded and considered in analyses.
Recruitment	Participants were recruited during clinical care or derived from public datasets, including the All of Us Research Program. Clinical recruitment may introduce selection bias toward affected individuals, while population datasets may reflect self-selection bias; however, genotype- and disease-based grouping is unlikely to materially affect the primary conclusions.
Ethics oversight	Study subjects, including patients, relatives, and healthy controls, provided written informed consent under National Institutes of Health (NIH) protocol 06-I-0015 (ClinicalTrials.gov NCT00246857), protocol 1500768 approved by the Sidra Medicine and Marmara Medical Center Institutional Review Board (IRBs), and protocol IR.TUMS.CHMC.REC.1398.030 approved by the Tehran University of Medical Sciences Ethics Committee, in accordance with local ethics and IRB regulations. Collection of blood and leftover biopsy samples (collected when medically indicated) are covered by these protocols. Blood samples from anonymized healthy donors were obtained at the NIH Clinical Center under approved protocols.

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 Sample size was not pre-determined by statistical calculation. For in vitro and in vivo experiments, at least n = 3 biological replicates per group were used. For single-cell RNA-seq analyses, some groups included data from two individuals, with large cell numbers supporting robust

analysis. Sample sizes were determined based on similar previously published studies (e.g., PMID: 32647003, PMID: 24165795, PMID: 26206937, PMID: 38172257, PMID: 35303419, PMID: 33771991).

Data exclusions	No data were excluded.
Replication	All experiments were reproducible and often performed independently by different members of the research team. Where applicable, independent experiments were repeated 3–5 times. Exceptions include single-cell RNA-seq analyses and histological/endoscopic assessments in IBD patients, in which two independent endoscopic examination results during active IBD were collected per patient. Reproducibility of histological findings was confirmed by adjacent-section staining.
Randomization	For animal experiments, WT and mutant mice were randomly allocated to untreated or treated conditions. For human samples, randomization was not performed, as comparisons were based on biological groupings, including GPR15 mutation status and disease status. For population-based analyses, samples were similarly grouped by clinical diagnosis or GPR15 genotype rather than random assignment. Potential covariates, including age, sex, and race or ethnicity, were accounted for in the analyses.
Blinding	Blinding was not applied during group allocation. In both animal and human studies, investigators were not blinded to genotype, as group assignment was based on GPR15 genotype and required accurate sample identification. For histological image analysis, genotypes were blinded to ensure unbiased quantification.

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

Immunoblotting:
 mouse anti-human GPR15 367902 R&D Systems MAB3654 1:1000
 mouse anti-HSP90 K3720A BioLegend 661802 1:5000
 rabbit anti-β-Arrestin 1/2 D24H9 Cell Signaling 4674 1:1000
 rabbit anti-14-3-3 (pan) E9S9M Cell Signaling 95422 1:1000
 rabbit anti-Flag SIG1-25 Sigma F2555 1:1000
 goat anti-mouse IgG HRP SouthernBiotech 1030-05 1:2000
 goat anti-rabbit IgG HRP SouthernBiotech 4050-05 1:2000
 mouse anti-rabbit IgG HRP (TrueBlot) eB182 Rockland 18-8816-31 1:1000

Co-immunoprecipitation:
 Rat anti-Flag L5 BioLegend 637303 Co-IP: 5 µg antibody/1.5 mg Dynabeads Protein G per sample

Cell immunofluorescence staining and confocal microscopy:
 rabbit anti-calnexin C5C9 Cell Signaling 2679 1:200
 rabbit anti-giantin Poly23A01 BioLegend 621352 1:1000
 Alexa Fluor Plus 594 Goat anti-Rabbit IgG (H+L) Invitrogen A32740 1:1000
 Alexa Fluor 488 Donkey anti-Mouse IgG (H+L) Invitrogen A21202 1:1000

Immunohistochemistry (IHC) and multiplex immunofluorescence staining:
 rabbit anti-CD11c AF647 EP1347Y Abcam ab275338 1:50
 mouse anti-CD68 iFluor 594 KP-1 Caprico Biotechnologies 164136 1:50
 mouse anti-HLA-DR AF532 TAL1B5 Novus Biologicals NB600-989AF532 1:500
 rabbit anti-CD3 SP7 Abcam ab16669 1:50
 Goat anti-Rabbit IgG (H+L) AF532 ThermoFisher A11009 1:250
 rabbit anti-CD4 EPR6855 Abcam ab133616 1:1000
 rabbit anti-FOXP3 SP97 ThermoFisher MA5-16365 1:200
 rabbit anti-CD8 EPR10640(2) Abcam ab189926 1:1000
 mouse anti-CD3 LN10 Leica PA0553 Ready-to-use
 rabbit anti-human GPR15 Sigma HPA013775 1:50
 rat anti-F4/80 BM8 ThermoFisher 14-4801-82 1:150
 rabbit anti-CD206 Abcam ab64693 1:1000

rabbit anti-NOS2 Abcam ab15323 1:100

Macrophage killing blocking antibodies:

anti-mouse TNF α QA19A26 BioLegend 935903 10 μ g/mL
 anti-mouse IFN γ R4-6A2 BioLegend 505708 10 μ g/mL
 anti-mouse FasL MFL3 BioLegend 106611 10 μ g/mL
 anti-mouse TWEAK MTW-1 BioLegend 120009 10 μ g/mL
 anti-mouse TRAIL N2B2 BioLegend 109315 10 μ g/mL
 anti-mouse H-2 M1/42 BioLegend 125502 10 μ g/mL

Human flow antibodies:

APC/Cyanine7 anti-human CD3 344818 BioLegend SK7 1:100
 APC anti-human GPR15 373006 BioLegend SA302A10 1:100
 PE anti-human CD197 (CCR7) 353204 BioLegend G043H7 1:100
 PerCP/Cyanine5.5 anti-human CD4 300530 BioLegend RPA-T4 1:100
 PE/Cyanine7 anti-human CD45RA 983006 BioLegend HI100 1:100
 Alexa Fluor 700 anti-human CD8 344724 BioLegend SK1 1:100
 PE anti-human CD183 (CXCR3) 989502 BioLegend G025H7 1:100
 Alexa Fluor 488 anti-human CD38 303512 BioLegend HIT2 1:100
 Brilliant Violet 421 anti-human HLA-DR 307636 BioLegend L243 1:100
 Alexa Fluor 488 anti-human CD196 (CCR6) 353414 BioLegend G034E3 1:100
 PE/Cyanine7 anti-human CD27 302838 BioLegend O323 1:100
 APC/Cyanine7 anti-human CD19 363009 BioLegend SJ25C1 1:100
 Brilliant Violet 510 anti-human IgD 348220 BioLegend IA6-2 1:100
 Alexa Fluor 700 anti-human CD20 302322 BioLegend 2H7 1:100
 FITC anti-human CD14 325604 BioLegend HCD14 1:100
 PE anti-human CD56 362508 BioLegend 5.1H11 1:100
 PerCP/Cyanine5.5 anti-human CD123 306016 BioLegend H6 1:100
 PE/Cyanine7 anti-human CD11c 371504 BioLegend S-HCL-3 1:100
 Brilliant Violet 510 anti-human CD16 302048 BioLegend 3G8 1:100
 Alexa Fluor 488 anti-human CD127 351314 BioLegend A019D5 1:100
 PE anti-human FOXP3 320008 BioLegend 150D 1:100
 Brilliant Violet 605 anti-human CD25 302631 BioLegend M-A251 1:100
 APC/Cyanine7 anti-human CD45RO 304227 BioLegend UCHL1 1:100
 Brilliant Violet 711 anti-human CD3 344838 BioLegend SK7 1:100
 Alexa Fluor 488 anti-human CD56 (NCAM) 362518 BioLegend 5.1H11 1:100
 FITC anti-human CD69 310904 BioLegend FN50 1:100
 PE anti-human CD25 985802 BioLegend M-A251 1:400

Mouse flow antibodies:

PE anti-mouse GPR15 154604 BioLegend S15042I 1:150
 PerCP/Cyanine5.5 anti-mouse TCR γ/δ 118118 BioLegend GL3 1:200
 Brilliant Violet 650 anti-mouse CD8 α 100742 BioLegend 53-6.7 1:200
 PE/Cyanine7 anti-mouse TCR β chain 109222 BioLegend H57-597 1:150
 APC/Cyanine7 anti-mouse CD3 100222 BioLegend 17A2 1:150
 Brilliant Violet 421 anti-mouse CD8 β 126629 BioLegend Ly-3 1:200
 Brilliant Violet 711 anti-mouse CD4 100557 BioLegend RM4-5 1:200
 Alexa Fluor 700 anti-mouse CD45 103128 BioLegend 30-F11 1:200
 Brilliant Violet 605 anti-mouse CD19 115540 BioLegend 6D5 1:100
 Alexa Fluor 647 anti-mouse CD45.1 110720 BioLegend A20 1:200
 Alexa Fluor 700 anti-mouse CD45.2 109822 BioLegend 104 1:200
 PE anti-mouse F4/80 157303 BioLegend QA17A29 1:100
 PE-Cy7 Hamster Anti-Mouse CD11c 558079 BD Biosciences HL3 1:100
 BUV737 Rat Anti-CD11b 612800 BD Biosciences M1/70 1:100
 BUV395 Rat Anti-Mouse I-A, I-E (MHC-II) 569244 BD Biosciences 2G9 1:400
 APC anti-mouse CD103 156914 BioLegend QA17A24 1:150
 BUV737 anti-mouse CD4 612761 BD Biosciences GK1.5 1:200
 APC-eFluor 780 anti-mouse CD8 α 47-0081-82 eBioscience 53-6.7 1:200
 PerCP/Cyanine5.5 anti-mouse CD5 100624 BioLegend 53-7.3 1:200
 BV711 anti-mouse TCR β chain 563135 BD Biosciences H57-597 1:200
 APC anti-mouse CD122 (IL-2R β) 123213 BioLegend TM- β 1 1:100
 PE-Cy7 anti-mouse MHC Class I (H-2Kb) 25-5958-82 eBioscience AF6-88.5.5.3 1:200
 Alexa Fluor 700 anti-mouse CD44 56-0441-82 eBioscience IM7 1:200
 eFluor 450 anti-mouse CD279 (PD-1) 48-9985-82 eBioscience J43 1:200

Flow blocking:

Human TruStain FcX 422302 BioLegend RUO 1:100
 TruStain FcX PLUS (anti-mouse CD16/32) Antibody 156604 BioLegend S17011E 1:100

Mouse Total SeqA antibodies:

CD4 RM4-5 AACCAAGACCCTTGAG BioLegend CD4 1:200
 CD8 α 53-6.7 TACCCGTAATAGCGT BioLegend CD8 α 1:200
 TCR β H57-597 TCCTATGGGACTCAG BioLegend TCR β chain 1:200
 CD44 IM7 TGGCTTCAGGTCCTA BioLegend CD44 1:100
 CD62L MEL-14 TGGGCCTAAGTCATC BioLegend CD62L 1:100
 TCR γ GL3 AACCCAAATAGCTGA BioLegend TCR γ/δ 1:100

CD8b YTS156.7.7 TTCCTCTATGGAGC BioLegend CD8b 1:100
 GPR56 CG4 GCCTAGTTCCGTTT BioLegend GPR56 1:100
 CD94 18d3 CACAGTTGTCCTGT BioLegend CD94 1:100
 Ly49D 4E5 TATATCCCTCAACGC BioLegend Ly49D 1:100
 Ly49G AT8 CGTATCTGTATTAG BioLegend Ly49G 1:100
 Ly49H 3D10 CCAGTAGGCTTATTA BioLegend Ly49H 1:100
 CD103 2E7 TTCATTAGCCCGCTG BioLegend CD103 1:50
 CCR7 4B12 TTATTAACAGCCAC BioLegend CCR7 1:50
 PD1 RMP1-30 GAAAGTCAAAGCACT BioLegend PD1 1:50
 CD69 H1.2F3 TTGTATTCCGCCATT BioLegend CD69 1:50
 CXCR4 L276F12 GTCGTGGTGTGTTC BioLegend CXCR4 1:50
 Mouse IgG1, κ isotype Ctrl Antibody MOPC-21 GCCGGACGACATTAA BioLegend Mouse IgG1, κ isotype Ctrl 1:200
 Rat IgG2a, κ Isotype Ctrl Antibody RTK2758 AAGTCAGGTTCGTTT BioLegend Rat IgG2a, κ Isotype Ctrl 1:200
 Rat IgG2b, κ Isotype Ctrl Antibody RTK4530 GATTCTTGACGACCT BioLegend Rat IgG2b, κ isotype Ctrl 1:200
 Armenian Hamster IgG Isotype Ctrl Antibody HTK888 CCTGTCATTAAGACT BioLegend Armenian Hamster IgG Isotype Ctrl 1:200

Mouse Hashtag antibodies:

TotalSeq™-A0301 anti-mouse Hashtag 1 Antibody M1/42 ACCCACCAGTAAGAC BioLegend 155801 1:50
 TotalSeq™-A0302 anti-mouse Hashtag 2 Antibody M1/42 GGTCGAGAGCATTCA BioLegend 155803 1:50
 TotalSeq™-A0303 anti-mouse Hashtag 3 Antibody M1/42 CTTGCCGATGTCAT BioLegend 155805 1:50
 TotalSeq™-A0304 anti-mouse Hashtag 4 Antibody M1/42 AAAGCATTCTTCACG BioLegend 155807 1:50

Human Total SeqC antibodies:

Hu.GPR15 SA302A10 ATGATCTGTGCCGGA BioLegend 373021 1:50
 TotalSeq™-C Human Universal Cocktail V1.0 BioLegend 399905 Read-to-use

Human Hashtag antibodies:

HTO-1 LNH-94; 2M2 GTCAACTCTTTAGCG BioLegend 394661 1:50
 HTO-2 LNH-94; 2M2 TGATGGCCTATTGGG BioLegend 394663 1:50
 HTO-3 LNH-94; 2M2 TTCCGCCTCTCTTG BioLegend 394665 1:50
 HTO-4 LNH-94; 2M2 AGTAAGTTCAGCGTA BioLegend 394667 1:50
 HTO-5 LNH-94; 2M2 AAGTATCGTTTCGCA BioLegend 394669 1:50
 HTO-6 LNH-94; 2M2 GGTGCGCAGATGTCA BioLegend 394671 1:50
 HTO-7 LNH-94; 2M2 TGTCTTCTGCGCAG BioLegend 394673 1:50
 HTO-8 LNH-94; 2M2 CTCCTCTGCAATTAC BioLegend 394675 1:50
 HTO-9 LNH-94; 2M2 CAGTAGTCACGGTCA BioLegend 394677 1:50

Validation

All antibodies used came from commercial vendors, and we based specificity on their provided description and data sheets.

Immunoblotting:

mouse anti-human GPR15 https://www.rndsystems.com/products/human-gpr15-antibody-367902_mab3654
 mouse anti-HSP90 <https://www.biolegend.com/en-us/products/purified-anti-hsp90alpha-beta-antibody-10275>
 rabbit anti- β -Arrestin 1/2 <https://www.cellsignal.com/products/primary-antibodies/beta-arrestin-1-2-d24h9-rabbit-monoclonal-antibody/4674>
 rabbit anti-14-3-3 (pan) <https://www.cellsignal.com/products/primary-antibodies/14-3-3-pan-e9s9m-rabbit-monoclonal-antibody/95422>
 rabbit anti-Flag <https://www.sigmaaldrich.com/US/en/product/sigma/f2555>
 goat anti-mouse IgG HRP <https://www.southernbiotech.com/goat-anti-mouse-igg-human-ads-hrp-1030-05>
 goat anti-rabbit IgG HRP <https://www.southernbiotech.com/goat-anti-rabbit-igg-h-l-mouse-human-ads-hrp-4050-05>
 mouse anti-rabbit IgG HRP (TrueBlot) <https://www.rockland.com/categories/trueblot/rabbit-trueblot-ultra-anti-rabbit-igg-hrp-18-8816-31/>

Co-immunoprecipitation:

Rat anti-Flag <https://www.biolegend.com/en-us/products/purified-anti-dykdddk-tag-antibody-4905>

Cell immunofluorescence staining and confocal microscopy:

rabbit anti-calnexin <https://www.cellsignal.com/products/primary-antibodies/calnexin-c5c9-rabbit-monoclonal-antibody/2679>
 rabbit anti-giantin <https://www.biolegend.com/en-us/products/purified-anti-giantin-antibody-23907>
 Alexa Fluor Plus 594 Goat anti-Rabbit IgG (H+L) <https://www.fishersci.com/shop/products/goat-anti-rabbit-igg-h-l-highly-cross-adsorbed-secondary-antibody-alex-a-fluor-plus-594/PIA32740#?keyword=A32740>
 Alexa Fluor 488 Donkey anti-Mouse IgG (H+L) <https://www.fishersci.com/shop/products/donkey-anti-mouse-igg-h-l-highly-cross-adsorbed-secondary-antibody-alex-a-fluor-488/A21202#?keyword=A21202>

Immunohistochemistry (IHC) and multiplex immunofluorescence staining:

rabbit anti-CD11c AF647 <https://www.abcam.com/en-us/products/primary-antibodies/alex-a-fluor-647-cd11c-antibody-ep1347y-ab275338>
 mouse anti-CD68 iFluor 594 <https://www.capricobio.com/product/cd68-kp1-ifluor-594/>
 mouse anti-HLA-DR AF532 https://www.rndsystems.com/products/hla-dr-antibody-tal-1b5_nb600-989af532
 rabbit anti-CD3 <https://www.abcam.com/en-us/products/primary-antibodies/cd3-epsilon-antibody-sp7-ab16669>
 Goat anti-Rabbit IgG (H+L) AF532 <https://www.fishersci.com/shop/products/goat-anti-rabbit-igg-h-l-cross-adsorbed-secondary-antibody-alex-a-fluor-532/A11009#?keyword=A11009>
 rabbit anti-CD4 <https://www.abcam.com/en-us/products/primary-antibodies/cd4-antibody-epr6855-ab133616>
 rabbit anti-FOXP3 <https://www.fishersci.com/shop/products/foxp3-monoclonal-antibody-sp97/PIMA516365?searchHijack=true&searchTerm=MA5-16365&searchType=RAPID&matchedCatNo=MA5-16365>
 rabbit anti-CD8 <https://www.abcam.com/en-us/products/primary-antibodies/cd8-alpha-antibody-epr106402-n-terminal-ab189926>
 mouse anti-CD3 <https://shop.leicabiosystems.com/us/ihc-ish/ihc-primary-antibodies/pid-cd3?>

rsId=AfmB0orPCL3JXJeQalHyk02vyq2b33zqpSeUFXDr8IJZM2eWoDRsAdX
 rabbit anti-human GPR15 <https://www.sigmaaldrich.com/US/en/product/sigma/hpa013775>
 rat anti-F4/80 <https://www.fishersci.com/shop/products/f4-80-monoclonal-antibody-bm8-ebioscience-invitrogen/501129661?searchHijack=true&searchTerm=14-4801-82&searchType=RAPID&matchedCatNo=14-4801-82>
 rabbit anti-CD206 <https://www.abcam.com/en-us/products/primary-antibodies/primary-antibodies/mannose-receptor-antibody-ab64693>
 rabbit anti-NOS2 <https://www.abcam.com/en-us/products/primary-antibodies/inos-antibody-ab15323>

Macrophage killing blocking antibodies:

anti-mouse TNF α <https://www.biolegend.com/en-us/products/ultra-leaf-purified-anti-mouse-tnf-alpha-recombinant-antibody-20426>
 anti-mouse IFN γ <https://www.biolegend.com/en-us/products/ultra-leaf-purified-anti-mouse-ifn-gamma-antibody-18657>
 anti-mouse FasL <https://www.biolegend.com/en-us/products/ultra-leaf-purified-anti-mouse-cd178-fasl-antibody-18343>
 anti-mouse TWEAK <https://www.biolegend.com/en-us/products/ultra-leaf-purified-anti-mouse-cd255-tweak-antibody-19384>
 anti-mouse TRAIL <https://www.biolegend.com/en-us/products/ultra-leaf-purified-anti-mouse-cd253-trail-antibody-18254>
 anti-mouse H-2 <https://www.biolegend.com/en-us/products/purified-anti-mouse-h-2-antibody-5344>

Human flow antibodies

APC/Cyanine7 anti-human CD3 <https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-cd3-antibody-6940>
 APC anti-human GPR15 <https://www.biolegend.com/en-us/products/apc-anti-human-gpr15-antibody-13952>
 PE anti-human CD197 (CCR7) <https://www.biolegend.com/en-us/products/pe-anti-human-cd197-ccr7-antibody-7498>
 PerCP/Cyanine5.5 anti-human CD4 <https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-human-cd4-antibody-4216>
 PE/Cyanine7 anti-human CD45RA <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd45ra-21929>
 Alexa Fluor 700 anti-human CD8 <https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-human-cd8-antibody-9062>
 PE anti-human CD183 (CXCR3) <https://www.biolegend.com/en-us/products/pe-anti-human-cd183-25983>
 Alexa Fluor 488 anti-human CD38 <https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-human-cd38-antibody-3263>
 Brilliant Violet 421 anti-human HLA-DR <https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-human-hla-dr-antibody-7226>
 Alexa Fluor 488 anti-human CD196 (CCR6) <https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-human-cd196-ccr6-antibody-7562>
 PE/Cyanine7 anti-human CD27 <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd27-antibody-8434>
 APC/Cyanine7 anti-human CD19 <https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-cd19-antibody-10302>
 Brilliant Violet 510 anti-human IgD <https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-human-igd-antibody-8012>
 Alexa Fluor 700 anti-human CD20 <https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-human-cd20-antibody-3400>
 FITC anti-human CD14 <https://www.biolegend.com/en-us/products/fitc-anti-human-cd14-antibody-3951>
 PE anti-human CD56 <https://www.biolegend.com/en-us/products/pe-anti-human-cd56-ncam-antibody-9958>
 PerCP/Cyanine5.5 anti-human CD123 <https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-human-cd123-antibody-4244>
 PE/Cyanine7 anti-human CD11c <https://www.biolegend.com/en-us/products/pe-anti-human-cd11c-antibody-13285>
 Brilliant Violet 510 anti-human CD16 <https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-human-cd16-antibody-8003>
 Alexa Fluor 488 anti-human CD127 <https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-human-cd127-il-7ralpha-antibody-7171>
 PE anti-human FOXP3 <https://www.biolegend.com/en-us/products/pe-anti-mouse-rat-human-foxp3-antibody-2889>
 Brilliant Violet 605 anti-human CD25 <https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-human-cd25-antibody-7658>
 APC/Cyanine7 anti-human CD45RO <https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-cd45ro-antibody-7372>
 Brilliant Violet 711 anti-human CD3 <https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-human-cd3-antibody-12007>
 Alexa Fluor 488 anti-human CD56 (NCAM) <https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-human-cd56-ncam-antibody-10245>
 FITC anti-human CD69 <https://www.biolegend.com/en-us/products/fitc-anti-human-cd69-antibody-1671>
 PE anti-human CD25 <https://www.biolegend.com/en-us/products/pe-anti-human-cd25-antibody-20955>

Mouse flow antibodies:

PE anti-mouse GPR15 <https://www.biolegend.com/en-us/products/pe-anti-mouse-gpr15-bob-antibody-15609>
 PerCP/Cyanine5.5 anti-mouse TCR γ/δ <https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-tcr-gamma-delta-antibody-6702>
 Brilliant Violet 650 anti-mouse CD8a <https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-mouse-cd8a-antibody-7635>
 PE/Cyanine7 anti-mouse TCR β chain <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-tcr-beta-chain-antibody-4144>
 APC/Cyanine7 anti-mouse CD3 <https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd3-antibody-6068>
 Brilliant Violet 421 anti-mouse CD8 β <https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd8b-ly-3-antibody-17374>
 Brilliant Violet 711 anti-mouse CD4 <https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-cd4-antibody-7925>
 Alexa Fluor 700 anti-mouse CD45 <https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd45-antibody-3407>
 Brilliant Violet 605 anti-mouse CD19 <https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd19-antibody-7645>
 Alexa Fluor 647 anti-mouse CD45.1 <https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-cd45-1-antibody-3104>
 Alexa Fluor 700 anti-mouse CD45.2 <https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd45-2-antibody-3393>
 PE anti-mouse F4/80 <https://www.biolegend.com/en-us/products/pe-anti-mouse-f4-80-recombinant-antibody-18755>
 PE-Cy7 Hamster Anti-Mouse CD11c https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-hamster-anti-mouse-cd11c.558079?tab=product_details
 BUV737 Rat Anti-CD11b https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv737-rat-anti-cd11b.612800?tab=product_details
 BUV395 Rat Anti-Mouse I-A, I-E (MHC-II) https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-rat-anti-mouse-i-a-i-e.569244?tab=product_details
 APC anti-mouse CD103 <https://www.biolegend.com/en-us/products/apc-anti-mouse-cd103-recombinant-antibody-21155>

BUV737 anti-mouse CD4 https://wwwbdbiosciences.com/en-pt/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv737-rat-anti-mouse-cd4.612761?utm_source=chatgpt.com&tab=product_details
 APC-eFluor 780 anti-mouse CD8a https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-53-6-7-Monoclonal/47-0081-82?utm_source=chatgpt.com
 PerCP/Cyanine5.5 anti-mouse CD5 <https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd5-antibody-9760>
 BV711 anti-mouse TCR β chain https://wwwbdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv711-hamster-anti-mouse-tcr-chain.563135?tab=product_details
 APC anti-mouse CD122 (IL-2R β) <https://www.biolegend.com/en-us/products/apc-anti-mouse-cd122-il-2rbeta-antibody-8908>
 PE-Cy7 anti-mouse MHC Class I (H-2Kb) https://www.thermofisher.com/antibody/product/MHC-Class-I-H-2Kb-Antibody-clone-AF6-88-5-5-3-Monoclonal/25-5958-82?utm_source=chatgpt.com
 Alexa Fluor 700 anti-mouse CD44 https://www.thermofisher.com/antibody/product/CD44-Antibody-clone-IM7-Monoclonal/56-0441-82?utm_source=chatgpt.com
 eFluor 450 anti-mouse CD279 (PD-1) https://www.thermofisher.com/antibody/product/PD-1-CD279-Antibody-clone-J43-Monoclonal/48-9985-82?utm_source=chatgpt.com

Flow blocking:

Human TruStain FcX <https://www.biolegend.com/en-us/products/human-trustain-fcx-fc-receptor-blocking-solution-6462>
 TruStain FcX PLUS (anti-mouse CD16/32) Antibody <https://www.biolegend.com/en-us/products/trustain-fcx-plus-anti-mouse-cd16-32-antibody-17085>

Mouse Total SeqA antibodies:

CD4 <https://www.biolegend.com/en-gb/products/totalseq-a0001-anti-mouse-cd4-antibody-16176>
 CD8a <https://www.biolegend.com/en-gb/products/totalseq-a0002-anti-mouse-cd8a-antibody-15920>
 TCRb <https://www.biolegend.com/en-gb/products/totalseq-a0120-anti-mouse-tcr-%CE%B2-chain-antibody-16424>
 CD44 <https://www.biolegend.com/en-gb/products/totalseq-a0073-anti-mouse-human-cd44-antibody-15923>
 CD62L <https://www.biolegend.com/en-gb/products/totalseq-a0112-anti-mouse-cd62l-antibody-16419>
 TCRgd <https://www.biolegend.com/en-gb/products/totalseq-a0121-anti-mouse-tcr-gamma-sigma-antibody-16151>
 CD8b <https://www.biolegend.com/en-gb/products/totalseq-a0230-anti-mouse-cd8b-ly-3-antibody-17124>
 GPR56 <https://www.biolegend.com/en-gb/products/totalseq-a0912-anti-human-gpr56-antibody-18966>
 CD94 <https://www.biolegend.com/en-gb/products/totalseq-a1009-anti-mouse-cd94-antibody-18686>
 Ly49D <https://www.biolegend.com/en-gb/products/totalseq-a0841-anti-mouse-ly49d-antibody-17508>
 Ly49G <https://www.biolegend.com/en-gb/products/totalseq-a0840-anti-mouse-ly49g-antibody-20699>
 Ly49H <https://www.biolegend.com/en-gb/products/totalseq-a0839-anti-mouse-ly49h-antibody-18102>
 CD103 <https://www.biolegend.com/en-gb/products/totalseq-a0201-anti-mouse-cd103-antibody-16456>
 CCR7 <https://www.biolegend.com/en-gb/products/totalseq-a0377-anti-mouse-cd197-ccr7-antibody-17283>
 PD1 <https://www.biolegend.com/en-gb/products/totalseq-a0004-anti-mouse-cd279-pd-1-antibody-15917>
 CD69 <https://www.biolegend.com/en-gb/products/totalseq-a0197-anti-mouse-cd69-antibody-16422>
 CXCR4 <https://www.biolegend.com/en-gb/products/totalseq-a0444-anti-mouse-cd184-cxcr4-antibody-17270>
 Mouse IgG1, κ isotype Ctrl Antibody <https://www.biolegend.com/en-gb/products/totalseq-a0090-mouse-igg1-kappa-isotype-control-15774>
 Rat IgG2a, κ Isotype Ctrl Antibody <https://www.biolegend.com/en-gb/products/totalseq-a0238-rat-igg2a-kappa-isotype-ctrl-antibody-16185>
 Rat IgG2b, κ Isotype Ctrl Antibody <https://www.biolegend.com/en-gb/products/totalseq-a0095-rat-igg2b-kappa-isotype-ctrl-16228>
 Armenian Hamster IgG Isotype Ctrl Antibody <https://www.biolegend.com/en-gb/products/totalseq-a0241-armenian-hamster-igg-isotype-ctrl-17278>

Mouse Hashtag antibodies:

TotalSeq™-A0301 anti-mouse Hashtag 1 Antibody <https://www.biolegend.com/en-gb/products/totalseq-a0301-anti-mouse-hashtag-1-antibody-16103>
 TotalSeq™-A0302 anti-mouse Hashtag 2 Antibody <https://www.biolegend.com/en-gb/products/totalseq-a0302-anti-mouse-hashtag-2-antibody-16104>
 TotalSeq™-A0303 anti-mouse Hashtag 3 Antibody <https://www.biolegend.com/en-gb/products/totalseq-a0303-anti-mouse-hashtag-3-antibody-16105>
 TotalSeq™-A0304 anti-mouse Hashtag 4 Antibody <https://www.biolegend.com/en-gb/products/totalseq-a0304-anti-mouse-hashtag-4-antibody-16106>

Human Total SeqC antibodies:

Hu.GPR15 <https://www.biolegend.com/en-gb/products/totalseq-a1298-anti-human-gpr15-antibody-25399>
 TotalSeq™-C Human Universal Cocktail V1.0 <https://www.biolegend.com/en-gb/products/totalseq-c-human-universal-cocktail-v1-0-19736>

Human Hashtag antibodies:

HTO-1 <https://www.biolegend.com/en-gb/products/totalseq-c0251-anti-human-hashtag-1-antibody-17162>
 HTO-2 <https://www.biolegend.com/en-gb/products/totalseq-c0252-anti-human-hashtag-2-antibody-17163>
 HTO-3 <https://www.biolegend.com/en-gb/products/totalseq-c0253-anti-human-hashtag-3-antibody-17164>
 HTO-4 <https://www.biolegend.com/en-gb/products/totalseq-c0254-anti-human-hashtag-4-antibody-17165>
 HTO-5 <https://www.biolegend.com/en-gb/products/totalseq-c0255-anti-human-hashtag-5-antibody-17166>
 HTO-6 <https://www.biolegend.com/en-gb/products/totalseq-c0256-anti-human-hashtag-6-antibody-18373>
 HTO-7 <https://www.biolegend.com/en-gb/products/totalseq-c0257-anti-human-hashtag-7-antibody-18374>
 HTO-8 <https://www.biolegend.com/en-gb/products/totalseq-c0258-anti-human-hashtag-8-antibody-18375>
 HTO-9 <https://www.biolegend.com/en-gb/products/totalseq-c0259-anti-human-hashtag-9-antibody-18376>

Eukaryotic cell lines

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

Cell line source(s)	HEK 293T cells (cat: CRL-3216), Jurkat T cells, clone E6-1 (cat: TIB-152), and HeLa 229 cells (cat: CCL-2.1) were purchased from the American Type Culture Collection (ATCC). Lenti-X 293T cells (cat: 632180) were purchased from Takara Bio. Platinum-E (Plat-E) cells (cat: RV-101) were purchased from Cell Biolabs. CHO-K1 cells stably transfected with Gα16 G protein were a gift from Eugene C. Butcher.
Authentication	Cell lines were not independently authenticated after purchase or receipt from the providing laboratories. Authentication was based on information provided by the vendors or source laboratories.
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell line used.

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	Gpr15 ^{-/-} mice were a kind gift from Eugene C. Butcher and were generated by backcrossing B6;129P2-Gpr15tm1.1Litt/J (The Jackson Laboratory, Strain #: 008769) to C57BL/6 wild-type mice over 12 generations. Rag2 ^{-/-} Il2rg ^{-/-} mice (Line #: 4111), CD45.1 C57BL/6 mice (Line #: 4007), and CD45.2 C57BL/6 mice (Line #: B6) were purchased from Taconic. Gpr15fl/fl (Strain #: 028324), CD8-cre (Strain #: 008766), and Foxp3-cre (Strain #: 016959) mice were purchased from The Jackson Laboratory. Mice were housed in a specific pathogen-free facility under a 12-h light:dark cycle at 22±2°C with 70% humidity. After weaning, mice were separated by sex and co-housed by genotype. Sex-matched male and female littermates aged 8-12 weeks were used for all experiments.
Wild animals	This study did not involve wild animals.
Reporting on sex	We used both sexes in animal studies.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All animal protocols (ASP#: LISB-7E, EIB-104, EIB-105) were approved by the Animal Care and Use Committee of the National Institute of Allergy and Infectious Diseases (NIAID) or the National Cancer Institute (NCI) in accordance with NIH and Department of Health and Human Services (HHS) guidelines.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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

Human peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque PLUS (Cytiva, cat: GE17-1440-02) by density-gradient centrifugation and washed twice with PBS.

For isolation of human intestinal lymphocytes, frozen biopsies were thawed at 37 °C, washed in cRPMI, and minced into small pieces. Intraepithelial lymphocytes (IELs) were isolated by rotating tissues in HBSS containing 10% FCS, 5 mM EDTA, and 5 mM dithiothreitol (Sigma, cat: 10197777001) at 37 °C for 40 min, then filtered through 100-µm cell strainers. Remaining tissue was digested in RPMI containing 1 mg/mL Collagenase IV (Sigma, C5138) and 10 U/mL DNase I (Roche, cat: 04716728001) at 37 °C for 1 h to isolate lamina propria (LP) cells. Cells were filtered and centrifuged on a 40% Percoll gradient at 1,800 rpm, 4 °C for 10 min.

For mouse lymphocytes preparation, spleen, mesenteric lymph nodes (MLN), thymus, small intestine, and large intestine were collected from euthanized mice. Spleen, MLN, and thymus were mechanically dissociated and filtered through 70-µm cell strainers in RPMI to generate single-cell suspensions. For intestinal cell isolation, visible fat and luminal contents were removed, intestines were opened longitudinally, and Peyer's patches were removed from the small intestine. Tissues were cut into 0.5–1 cm pieces and incubated at 37 °C for 30 min in HBSS containing 5 mM EDTA and 1 mM dithiothreitol to isolate intestinal epithelial cells (IECs) and IELs. Cell suspensions were filtered through 100-µm mesh filters, washed with RPMI containing 3% FBS, and centrifuged at 800 × g for 10 min. IECs and IELs were separated by centrifugation in 40% Percoll (GE Healthcare, cat: GE17-0891-01) at 1,800 rpm for 10 min. Remaining intestinal tissue was minced and digested at 37 °C for 40 min in RPMI containing 0.1 mg/mL Liberase (Roche, cat: 05401020001) and 10 U/mL DNase I. Digested tissue was filtered through a 70-µm cell strainer and centrifuged at 500 × g for 5 min. Intestinal LP lymphocytes were isolated by centrifugation in 40% Percoll at 1,800 rpm for 10 min.

For flow cytometry analysis of CD8+ T cell migration to mouse intestinal organoids, 2 days after co-culture, medium containing free CD8+ T cells was collected. Organoids were released from Matrigel using Cell Recovery Solution on ice for 30 min, and supernatants containing free CD8+ T cells were combined with the collected medium. Free CD8+ cells were pelleted by centrifugation at 500 × g for 5 min. To isolate organoid-incorporated CD8+ T cells, organoid pellets were incubated in 5 mM EDTA/PBS on ice for 10 min, mechanically dissociated by vigorous pipetting, and centrifuged at 500 × g for 5 min. Free and organoid-incorporated CD8+ T cells were subsequently analyzed by flow cytometry.

Instrument

Flow cytometry data were acquired on FACSsymphony A3 or LSRFortessa (BD Biosciences)

Software

We used the software FACSDiva (v9.1, v9.0, BD Biosciences) to collect the data and the software FlowJo (Treestar Inc., v10.9.0) to analyze the data.

Cell population abundance

Cell numbers for flow cytometry were titrated, based on past experience.

Gating strategy

For all experiments, we first selected lymphocytes based on FSCxSSC; then, we selected the singlets based on FSC-H x FSC-W, then SSC-H x SSC-W; then we selected viable cells based on FSC x Live-Dead gating.

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