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Last updated by author(s): May 21, 2022

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	Images for HE and IHC were acquired on Nikon DS-Ri2 with NIS-Elements F 4.60 software. Images for Immunofluorescence were acquired on Zeiss LSM 800 confocal microscope with ZEN 2012 software and were exported as the .tif files. Flow cytometry data were acquired with Attune NxT flow cytometer with Acoustic Focusing Cytometer software (Thermo fisher), and were exported as the .fcs files. Quantitative real-time PCR data was obtained using ABI Stepone plus system. Western Blot data was obtained using LICOR oddysey or Tanon5200 with TanonImage software. The scRNA-Seq libraries were generated using the 10X Genomics Chromium Controller Instrument and Chromium Single Cell 3′ V3 Reagent Kits (10X Genomics, Pleasanton, CA).All libraries were sequenced by illumina sequencer (Illumina, San Diego, CA) on a 150 bp paired-end run.
Data analysis	Flow cytometry analysis was performed using Flow Jo 10.0.7 software. To analyze imaging data, Fiji (version 1.0) was used. Software used to analyze the scRNA-Seq data include R statistical environment (v4.0.3) and Cellranger (v3.1.0), R packages: Seurat (v3.1.4); pySCENIC (0.9.5); QuSAGE (2.16.1); Monocle2. Website: CellphoneDB (v1.1.0). STRING (v11.5). Statistical tests were performed using Graph Pad Prism 9.0 software and are presented as the means ± SEM.

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

Single cell RNA-Seq Raw data files and have been uploaded to Gene Expression Omnibus public database (GSE198550). The bulk RNA sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) database under the NCBI Bioproject PRJNA795031. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository with the dataset identifier PXD033272. For Metabolomics data, metabolite abundance between samples can be found in Supplementary Table S2.

We have created the"Reviewer access"to the raw sequencing and proteomics data for the reviewers. The data can be got at the following URL:
1. For Proteomics data (180 days):
URL: https://www.iprox.cn/page/PSV023.html;?url=16530981980091bBP
Password: FAz6

2. For Single cell RNA-Seq Raw data (no time limited):
URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE193360
Password: ufqfyeumjjajtsp

3. For bulk RNA sequencing data (no time limited):
URL: https://dataview.ncbi.nlm.nih.gov/object/PRJNA795031?reviewer=j6bbr19is3f5n20r9vahufssas
No password required

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	The single-cell RNA-seq data reported in this study can be retrieved from Gene Expression Omnibus using accession code GSE193360.The RNA-seq data have been deposited in the NCBI Sequence Read Archive (SRA) database under the NCBI Bioproject PRJNA795031. Other data used in this study is provided within the article as Source Data Files or is available from the corresponding author upon reasonable request.
Data exclusions	No data were excluded.
Replication	All experiments were reliably reproduced. All reported data were from at least two independent experiments (each specified in figure legends) with exactly the same conditions established from pilot experiments. For organoid experiments independent experiments refer to fully independent cultures starting from different mice.
Randomization	For in vivo experiments, sex and age-matched mice were distributed randomly for each experiment according to their genotypes.
Blinding	For the clinical score in vivo and histological analysis, the assessment were conducted in a blinded fashion.

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

The following antibodies were used for immunofluorescence and immunohistochemistry:
Rabbit anti-ECSIT (Nuvus, NBP1-91858, 1:400), rabbit anti-YAP (CST, ,1:400), rabbit anti MUC2 (Abcam, ab76774, 1:100), rabbit anti ChgA (Nuvus, NB120-15160SS, 1:300), rabbit anti DCLK (Abcam, ab31704, 1:800), rabbit anti LYZ1 (Abcam, ab108508, 1:250), rabbit anti SCA1-Alexa Fluor 647 (Biolegend, 122517, 1:400), rabbit anti CCND2 (SAB, 27973-1, 1:200), rabbit anti EIF4A1 (Bioworld, BS7106, 1:100) ,rabbit anti eIF4G2 (CST, 5169, 1:400), mouse anti PCNA (Santa Cruz, sc-56, 1:300), rabbit anti OLFM4 (CST, 14369, 1:400), rabbit anti SOX9 (Sigma, AB5535, 1:300) and rabbit anti GFP tag (Proteintech, 50430-2-AP, 1:150). Secondary antibodies for immunofluorescence were anti-rabbit goat antibodies conjugated with Alexa A488 (Invitrogen, A11008, 1:600) and A555 (Invitrogen, A21429, 1:600). Secondary antibodies for immunohistochemistry were HRP conjugated goat anti-rabbit IgG (H+L) Secondary Antibody (Invitrogen, 31460, 1:500) and HRP conjugated goat anti-mouse IgG (H+L) Secondary Antibody (Invitrogen, 31430, 1:500)
The following antibodies were used for Immunoblotting:
rabbit anti-YAP (CST, 14074S, 1:1000), rabbit anti SCA1 (SAB, 47996, 1:1000), rabbit anti CCND2 (SAB, 27973-1, 1:1000), rabbit anti ATP5A (Bioworld, BS3814 , 1:1000), rabbit anti UQCRC2 (Bioworld, BS70602 , 1:1000), rabbit anti SDHB (Bioworld, BS8003, 1:1000), rabbit anti MTCO1 (Abcam, ab203912, 1:1000), mouse anti NDUFS8 (Santa Cruz, sc-515527, 1:1000), Goat anti Rabbit antiboy IRDye800CW (Licor, 925-32211, 1:5000), Goat anti mouse antiboy IRDye700CW (Licor, 92668070, 1:5000), anti-goat-HRP (Thermo Fish, 31402, 1:5000), and anti-mouse-HRP (Thermo Fish, 31340,1 :5000).
The following antibodies were used for immunoprecipitation:
rabbit anti EIF4A1 (Bioworld, BS7106, 1:50) ,rabbit anti eIF4G2 (CST, 5169, 1:50) and Anti-5-methylcytosine (5-mC)(Abcam,ab10805).
The following antibodies were used for flow cytometry:
anti-CD45-Alexa Flour 700 (eBioscience, 30-F11, 56-0451-82, 1:400), anti-CD4-APC-Cy7 (Biolegend, GK1.5, 100414, 1:400), anti-CD8a- PE (eBioscience, 53-6.7, 12-0081-83, 1:400), anti-NK1.1-PE-Cy7 (eBioscience, PK136, 25-5941-82, 1:400), anti-CD19-APC (eBioscience, 1D3, 17-0193-80, 1:400), anti-CD11b-FITC (Biolegend, 101206, M1/70, 1:400), anti-CD11c-PE (eBioscience, N418, 12-0114-82, 1:400) and FVD eFlour506 (eBioscience,1:1000)

Validation

All antibodies used were commercially available and have been validated by vendors and based on multiple publications. All antibodies used for flow cytometry have been titrated and used by our own lab in previous experiments.

Animals and other organisms

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

Laboratory animals

All mice were of the species Mus musculus (C57BL/6). Ecsit Floxed conditional knockout mice were generated by CRISPR/Cas9 based approach. Briefly, two sgRNAs were designed by CRISPR design tool (http://crispr.mit.edu) to target either a region upstream or downstream of the exon 4, and then were screened for on-target activity using a Universal CRISPR Activity Assay (UCATM, Biocytogen Inc, Beijing, China). To minimize random integrations, we employ a circular donor vector. The gene targeting vector containing 5′ homologous arm, target fragment (exon4), 3′ homologous arm was used as a template to repair the DSBs generated by Cas9/sgRNA. The two loxp sites were precisely inserted in both sides of target fragment of the Ecsit gene. T7 promoter sequence was added to the Cas9 or sgRNA template by PCR amplification in vitro. Cas9 mRNA, targeting vector and sgRNAs were co-injected into the cytoplasm of one-cell stage fertilized C57BL/6 eggs. The injected zygotes were transferred into oviducts of Kunming pseudopregnant females to generate F0 mice. F0 mice with expected genotype confirmed by tail genomic DNA PCR and sequencing were mated with C57BL/6 mice to establish germline-transmitted F1 heterozygous mice. F1 heterozygous mice were genotyped by tail genomic PCR, southern blot and DNA sequencing. The Ecsit knockin floxed mice were generated with CRISPR/Cas-mediated genome engineering by Cyagen Biosciences (Guangzhou, China) Inc. In brief, the gRNA to mouse ROSA26 gene, the donor vector containing "CAG promoter-loxP-3*SV40 pA-loxPKozak-Mouse Ecsit CDS-3xFLAG-rBG pA" cassette, and Cas9 mRNA were co-injected into fertilized mouse eggs to generate targeted conditional knockin offspring. F0 founder animals were identified by PCR followed by sequence analysis, which were bred to wildtype mice to test germline transmission and F1 animal generation. Ecsit -floxed mice were crossed with Villin-Cre mice (Vliin-Cre; the Jackson laboratory) to generate IEC-conditional Ecsit knockout mice (Ecsit fl/fl Villin-Cre). Ecsit -floxed mice were also crossed Villin-Cre ERT2 (Kindly provided by Dr. Sylvie Robine and Dr. Yeguang Chen) or Lgr5-Cre ERT2 (Kindly provided by Dr. Jun Qin), and then i.p injected with 3 mg tamoxifen (T5648; Sigma-Aldrich) dissolved in 300 l corn oil (C8267; Sigma-Aldrich) for five consecutive days to induce the expression of Cre recombinase to achieve conditional deletion of Ecsit in the intestinal epithelium (Ecsit fl/fl Villin-Cre ERT2, termed Ecsit cKO) or in Lgr5+ stem cell (Ecsit fl/fl Lgr5-Cre ERT2) respectively at a specific time (6-8week). The mice were euthanized 17 days after the first injection for phenotype analysis. The Ecsit knockin floxed mice were crossed with Ecsit fl/fl Villin-Cre ERT2 to rescue the expression of Ecsit in Ecsit deficient mice. For spontaneous bowel cancer model, we constructed Ecsit fl/fl Vil1in-Cre-ERT2 Apc min/+ mice (Apc min/+ mice was kindly provided by Dr. Jun Qin), and the Ecsit fl/fl Apc min/+ mice was regarded as control. Mice were gavaged with tamoxifen (4mg) every other day twice to conditionally knock down Ecsit in intestinal epithelium and euthanized the mice 2 months after the last administration. Mice were housed in conventional cages in an animal room at constant temperature (19–23 °C) and humidity (55 ± 10%) under a 12-h light–dark cycle and were allowed

	access to standard diet and water ad libitum. All animal experiments were conducted in accordance with the procedure approved by the Ethical Review Committee for Laboratory Animal Welfare of Nanjing Medical University.
Wild animals	N/A
Field-collected samples	N/A
Ethics oversight	All animal experiments were conducted in accordance with the procedure approved by the Ethical Review Committee for Laboratory Animal Welfare of Nanjing Medical University.

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	Small intestine and colon tissues were incubated at 37°C in PBS containing 2% FBS and 5 mM EDTA for 25 min. The remaining tissue was cut into small pieces and digested in PBS containing 2% FBS, Collagenase IV (0.5 mg/ml; Thermo) and DNase I (10 U/ml; Sigma-Aldrich) and then incubated at 37°C for 45min. Single cell suspensions were stained with anti-CD45-Alexa Flour 700 (eBioscience, 30-F11, 56-0451-82, 1:400), anti-CD4-APC-Cy7 (Biolegend, GK1.5, 100414, 1:400), anti-CD8a- PE (eBioscience, 53-6.7, 12-0081-83, 1:400), anti-NK1.1-PE-Cy7 (eBioscience, PK136, 25-5941-82, 1:400), anti-CD19-APC (eBioscience, 1D3, 17-0193-80, 1:400), anti-CD11b-FITC (Biolegend, 101206, M1/70, 1:400), anti-CD11c-PE (eBioscience, N418, 12-0114-82, 1:400) and FVD eFlour506 (eBioscience,1:1000) for FACS analysis (Thermo). All flow cytometry was performed on an Attune NxT Flow Cytometer (Thermo Fisher Scientific), and data were analyzed by FlowJo 10 software.
Instrument	Flow cytometry data was collected using Attune NxT flow cytometer (Thermo fisher).
Software	Flow cytometry data was analyzed using Flow Jo 10 software.
Cell population abundance	For flow cytometry analysis, 10^5 cells were stained in per sample.
Gating strategy	First, cell size gating and doublet removal were applied respectively on the SSC-A/FSC-A, and sequential FSC-A/FCS-H. From the selected singlets, dead cells were discriminated out by gating on live/dead dye negative population. Then, from live cells, the CD45 positive cells were gated, and CD4 T cells, CD8 T cells, B cells, NK cells, DCs and macrophages were gated from CD45 positive cells .

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