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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Leica LAS X Version 1.1, Bio-Rad CFX Manager Version 3.1.
Data analysis	ImageJ (Fiji, Version 1.51n), Microsoft Excel 2016, R Studio (1.1.453), Leica LAS X Version 1.1, FlowJo V10

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/reviewers upon request. 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

Bulk and single-cell RNA–seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE114988.
Source data for Fig. 1c, 1e, 1g, 2b, 2e, 4a, 5b, 5c, 6b and Supplementary Fig. 1a, 2a, 2b, 2d, 3b, 4b, 4c, 5a, 5b and 6 have been provided as Supplementary Table 2.

All other data supporting the findings of this study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-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. For the mouse experiments, based on our experience, 4 animals per condition are required to answer our scientific question. Results obtained were highly significant and consistent and did not require larger animal experimental groups.
Data exclusions	No experimental animals were excluded. For the single cell sequence analysis we performed some filtering steps which are mentioned in the paper: after quantifying transcript expression in all of the cells, we normalized by down sampling to a minimum number of 3000 transcripts and discarded all cells with less than 3000 transcripts. To reduce noise, wediscarded genes which were not expressed with at least 2 transcripts in one of the cells in the data set.
Replication	All attempts at replication were successful. The number of times each experiment has been repeated with similar results is stated in each figure legend. All methods are complete and techniques available and therefore experiments should be easily reproducible.
Randomization	Control and experimental male and female mice were randomly assigned into experimental groups.
Blinding	a) Single-cell and bulk mRNA-sequencing analysis was performed in an unbiased fashion with pooling all data. Experimental conditions were assigned following initial analysis. b) Histological analysis did not allow blinding, since phenotypes were too apparent (either morphologically or after performing stainings).

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Research animals
☐	☒ Human research participants

Antibodies

Antibodies used	Antibodies used A) goat anti-Chromogranin A from Santa Cruz (1:500). Catalogue number: sc-1488, polyclonal. No lot available anymore, similar results were obtained using rabbit anti-Chromogranin A from Labned (1:1000). Catalogue number: LN1401487 B) goat anti-Cholestocystokin from Santa Cruz (1:100). Catalogue number: sc-21617, polyclonal. Lot #B1816 C) rabbit anti-Neurotensin from Santa Cruz (1:100). Catalogue number sc-20806, polyclonal. Lot #A1910 D) goat anti-Secretin from Santa Cruz (1:100). Catalogue number sc-26630, polyclonal. Lot #H1915 E) goat anti-Somatostatin from Santa Cruz (1:100). Catalogue number sc-7819, polyclonal. Lot #E2912 F) goat anti-Serotonin from Abcam (1:1000). Catalogue number ab66047, polyclonal. Lot GR235902-22 G) rabbit anti-Gastric inhibitory protein from Abcam (1:500). Catalogue number ab22624-50, polyclonal. Lot GR325064-1 H) goat anti-Glp1 from Santa Cruz (1:100). Catalogue number sc-7782, polyclonal. Lot #K0915 I) rabbit anti-Peptide YY from Abcam (1:500). Catalogue number ab22663, polyclonal. Lot GR208949-20 J) guinea pig anti-Substance P from Abcam (1:200). Catalogue number ab10353, polyclonal. Lot GR3195542-1 K) rabbit anti-Glp1 from Abcam (1:200). Catalogue number ab22625, polyclonal. Lot GR3178933 L) donkey anti-rabbit Alexa 488 conjugated from Molecular probes (1:1000). Catalogue number A21206, lot 1927937 M) donkey anti-rabbit Alexa 568 conjugated from Molecular probes (1:1000). Catalogue number A10042, lot 1891789 N) donkey anti-rabbit Alexa 647 conjugated from Molecular probes (1:1000). Catalogue number A31573, lot 1874788 O) donkey anti-goat Alexa 488 conjugated from Molecular probes (1:1000). Catalogue number A11055, lot 1915848 P) donkey anti-goat Alexa 568 conjugated from Molecular probes (1:1000). Catalogue number A11057, lot 1711491 Q) donkey anti-goat Alexa 647 conjugated from Molecular probes (1:1000). Catalogue number A21477, lot 1739289

Validation	Antibodies A, B, C, D, E, F, G, H, L, M, N, O, P, Q are validated for the used purposes by the supplier and used in previous studies such as Basak, Beumer et al. 2017 (Ref 8). Antibody J is validated for the used purposes by the supplier and used in previous studies such as Grun et al. 2015 (Ref 7). Antibody I is validated for the purposes by the supplier and used in previous studies such as Brooks L et al. Fermentable carbohydrate stimulates FFAR2-dependent colonic PYY cell expansion to increase satiety. Mol Metab 6:48-60 (2017). Antibody K is validated for the used purposes by the supplier and used in previous studies such as Bohórquez DV et al. Characterization of basal pseudopod-like processes in ileal and colonic PYY cells. J Mol Histol 42:3-13 (2011). Additional references of all antibodies are found on the supplier websites. Antibodies H and J targeting Glp1 generated similar results, confirming specificity of the target.

Research animals

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

Animals/animal-derived materials	a) Species: Mus Musculus/Strain: Tac1-IRES2-Cre; Rosa26-CAG-loxP-stop-loxP-Ai14 mice/sex: male and female/age: 8-12 weeks b) Species: Mus Musculus/Strain: Gcg-Venus mice/sex male: age 12-16 weeks c) Species: Mus Musculus/Strain: Gip-Cre; Rosa26-CAG-loxP-stop-loxP-Ai14 mice/sex: male and female/age: 8-12 weeks d) Other mice used for experiments were wildtype C57BL/6, male/female and age between 8 and 12 weeks.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Human duodenal and ileal tissues were obtained from the UMC Utrecht with informed consent and the study was approved by the ethical committee of the UMC Utrecht (The Netherlands). Patients were diagnosed with a small or large intestinal cancer and from the resected intestinal segments, a sample was taken from normal mucosa for this study. Patients were a 75-year old male with a metastatic colorectal carcinoma, a 37-year old male with a ileal neuroendocrine tumor and a patient with a small intestinal cancer (age and gender not disclosed).

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ Magnetic resonance imaging

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	Single-cell preparations were prepared from intestinal organoids using TryplE and DNaseI. Single cells were stained with DAPI prior to flow cytometry to select live cells.
Instrument	Cells were sorted with a BD FACSAria II flow sorter.
Software	Data was collected using BD FACSDiva and analyzed using FlowJo (version v10)
Cell population abundance	Index sorting was performed for single cell sequencing. Single cell transcriptomes of sorted reporter positive cells confirmed high purity of each corresponding cell type. Moreover, transcriptomes always indicated presence of one cell type, suggesting no doublets were sorted. This confirms successful sorting of the respective reporters, as well as the viability of sorted cells.
Gating strategy	First gate: FSC-A vs DAPI (select for live, DAPI negative cells) Second gate: FSC-A vs SSC-A Third gate: SSC-W vs SSC-H Fourth gates: Venus (Gcg) or RFP (Tac1, GIP) vs FSC-A. Reporter positive cells generated clearly separated populations that could be easily gated for. Gating strategy is provided as Supplementary figure.

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