nature portfolio

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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	timsControl (Bruker) and FlexImaging (Bruker) were used to collect raw imaging mass spec data. Xcalibur 4.3 (ThermoFisher) was used to collect raw liquid chromatography mass spec data. BioTek Gens (Agilent) was used to collect bright-field and fluorescence images.
Data analysis	MET-MAP was used to infer the metabolic topography in liver and intestine and is available at https://github.com/raphael-group/MET-MAP. SCiLS Lab (Bruker, 2025a) was used for raw imaging mass spec data conversion . lsoScope (MATLAB 2024a) was used for raw imaging mass spec data analysis, including generating ion intensity matrices, k-mean clustering, and isotope labeling, and is available at (https://github.com/xxing9703/lsoscope). We wrote a new code for supervised portal-central axis construction, which is linked to a Code Ocean capsule (https://codeocean.com/capsule/5032416/tree/v1). El-Maven v0.6.1 (https://github.com/ElucidatalnC/EIMaven) was used to process LC-MS data. Isocorr (https://github.com/xxing9703/MIDview_isocorrCN) (MATLAB 2024a) was used for natural isotope correction in LC-MS data. Fiji (ImageJ 1.54g) was used to process MALDI images. R v3.6.6 (https://cran.r-project.org/) and OriginPro 2024b were used for all other data analysis and graphing data. Adobe Illustrator 2024 was used to make figures. Biorender.com (BioRender 2025) was used to make schematics in Figure 2 and Figure 4.

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

Liver and intestine spatial metabolomics data produced in this study can be browsed at https://metmap.princeton.edu/.
Data for liver spatial metabolomics available on Figshare: https://doi.org/10.6084/m9.figshare.29318279.v1.
Data for small-intestine epithelium spatial metabolomics available on Figshare: https://doi.org/10.6084/m9.figshare.29318342.v1.
Data for liver glutamine tracing: https://doi.org/10.6084/m9.figshare.29322569.v1
Data for liver lactate tracing: https://doi.org/10.6084/m9.figshare.29322635.v1
Data for liver fructose and glucose tracing: https://doi.org/10.6084/m9.figshare.29322665.v1
Data for small-intestine epithelium glutamine tracing: https://doi.org/10.6084/m9.figshare.29322713.v1
Data for small-intestine epithelium lactate tracing: https://doi.org/10.6084/m9.figshare.29322740.v1
Data for small-intestine epithelium fructose and glucose tracing – 90 seconds: https://doi.org/10.6084/m9.figshare.29322917.v1
Data for small-intestine epithelium fructose and glucose tracing – 10 minutes: https://doi.org/10.6084/m9.figshare.29322920.v1
Liver protein zonation data was obtained from "Supplementary Table 4" in the previously published source (Ben-Moshe et al, Nature metabolism, 2019): https://static-content.springer.com/esm/art%3A10.1038%2Fs42255-019-0109-9/MediaObjects/42255_2019_109_MOESM6_ESM.xlsx
Small intestine protein zonation was obtained from "Supplementary Table 3" in the previously published source (Harnik et al, Nature metabolism, 2021): https://static-content.springer.com/esm/art%3A10.1038%2Fs42255-021-00504-6/MediaObjects/42255_2021_504_MOESM2_ESM.xlsx
Metabolite HMDB accession numbers were obtained from HMDB (https://hmdb.ca/).
Lipids were assigned using LIPID MAPS (https://www.lipidmaps.org/).

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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 define a sample as a liver or intestine collected from one independent mouse. We used 10,000 < data points< 100,000 (imaging-mass spec pixels) per sample for analyzing spatial trends. We chose sample size that enabled confident conclusions about the observed spatial trends. Spatial metabolomics studies involved > 6 mice sampled from multiple cages which provided sufficient power to detect significant spatial gradients in > 90% of compounds.
Data exclusions	Data from mice where the ion count for isotopically labeled metabolites fell below the limit of detection were not used during analysis.
Replication	Spatial metabolomics and metabolic activity were measured across different mice (see figure legend for exact number of mice) on different days. Where feasible, we collected and analyzed serial tissue sections per mouse and these were included in the analysis to ensure technical reproducibility.

Randomization	We randomly assigned mice to different experimental groups (lactate-infused vs glutamine-infused and fructose-treated vs glucose-treated vs untreated mice).
Blinding	We did not use blinding in our data collection and analysis. Samples from different groups were prepared in the same way and analyzed using the same mass spec settings to avoid technical variation.

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	EpCAM/TROPl antibody (BLR 077G), Novus Biologicals, Catalog no. NBP3-14685, monoclonal, Lot 1. Secondary antibody: goat anti-rabbit cross-adsorbed, polyclonal, Alexa Fluor™ 568 (Catalog # A-11011), Invitrogen.
Validation	The manufacturer lists several antibody validation assays, including Western Blot, lmmunohistochemistry and lmmunoprecipitation (https://www.novusbio.com/products/epcam-trop 1-anti body-blr077 g_nbp3-14685 ?srsltid=Afm BOoquAHi6HXLly2-fW8ZU _j0V36e 12zRsorNyK-m2fH hQU9G6Y35R#datasheet).

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	Animal species: Mus musculus; strain: C57BL/6J; age: 12 -18 weeks
Wild animals	Wild animals were not used in the study
Reporting on sex	Findings apply to males. Sex was not considered in the study design as sex specific aims were not considered.
Field-collected samples	Study did not involve field-collected samples.
Ethics oversight	Mouse studies followed protocols approved by the Princeton University Animal Care and Use Committee.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*