

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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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

VisiumHD data was generated using user manual guide. Data was parsed using Space Ranger (v3.1.2, 10x Genomics). For smFISH and HCR imaging we used NIS elements software AR (v5.02.03).

Data analysis

Analysis was performed in Python 3.10 using HiVis v1.0.0. Image analysis was performed in QuPath 0.6.0. Processing code available at: <https://github.com/roynov01/HiVis>

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](#)

VisiumHD data of mouse livers generated in this study can be downloaded from Zenodo: <https://zenodo.org/records/17779250>

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

Sex wasn't considered in study design. The study only used previously published human datasets. No sex specific analysis was performed. Human colons are from 2 males and 3 females. Human liver sample is from a male.

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

Mouse liver analysis included 2 mice.
Human colon analysis included 5 samples.
Human liver and mouse intestine included 1 sample.
Mouse intestine Xenium included 1 sample.

Data exclusions

Some VisiumHD bins were filtered based on quality control procedures as described in the methods section of the manuscript.

Replication

Analysis of VisiumHD data was preformed for each sample interdependently, followed by a pooling procedure as described in the methods section of the manuscript.

Randomization

Not applicable, no experimental groups.

Blinding

Not applicable, no experimental groups.

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

smFISH membrane staining: FITC-anti-E-cadherin (BD, cat: 612131; lot: 9210587).

Validation

FITC-anti-E-cadherin antibody was validated by the manufacturer for their intended purpose.

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

Two-months-old C57BL/6 male mice were housed under regular 12h light-dark cycle with an ambient temperature around 21–23 °C, humidity of 40–60% and fed ad libitum were used in the experiments.

Wild animals

The study did not involve wild animals.

Reporting on sex

Only male mice were used in the experiment. No sex-based analysis was performed.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

Mouse experiments were approved by the Institutional Animal Care and Use Committee of the Weizmann Institute of Science (approval number 05220824–2) and performed in accordance with institutional guidelines.

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

Plants

Seed stocks

na

Novel plant genotypes

na

Authentication

na