nature research

Corresponding author(s): D.Hendriks, B.Artegiani, H. Clevers

Last updated by author(s): 07-08-2020

Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Leica software
Data analysis	IMARIS, ImageJ (Fiji)

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. 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

Not applicable.

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	No sample-size calculation was performed.
Data exclusions	No data has been excluded.
Replication	All the experiments described have been successfully replicated at least twice as independent experiments.
Randomization	Organoids to be analyzed were chosen randomly.
Blinding	The researchers were not blinded. Image analysis and processing was done by 2 individual researchers.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	A: anti-ZO-1 rabbit polyclonal 1:200 (Life Technology #402200) B: anti-MRP2 mouse monoclonal 1:100 (Abcam #ab3373) C: anti-beta catenin rabbit polyclonal 1:500 (Santa Cruz #sc-7199) D: anti-A1AT rabbit polyclonal 1:500 (Abcam, #ab9373) E: anti-AFP rabbit polyclonal 1:250 (Thermo Fisher Scientific, #PAS-16658) F: anti-Albumin goat polyclonal 1:500 (Bethyl, #A80-229A) G: anti-KRT7 mouse monoclonal 1:200 (Millipore, #MAB3226)
Validation	Antibodies are validated for the used purposes by the supplier and used in previous studies, such as in Artegiani et al. Cell Stem Cell 2019 (Antibody A) and Hu et al. Cell 2018 (Antibodies B, C, E, F) and Huch et al. Cell 2015 (Antibodies D and G).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Wild-type organoid lines from fetal and adult human liver tissues were generated in this study or established in previous studies from our group. All images of presented knock-in and knock-out lines were generated in a previous study from our groups (Artegiani et al. Cell Stem Cell 2019, Artegiani, Hendriks et al. Nature Cell Biology 2020)
Authentication	Not applicable.
Mycoplasma contamination	Regular mycoplasma contamination testing was performed and always gave negative results.
Commonly misidentified lines (See ICLAC register)	Not applicable.