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Last updated by author(s): Jan 27, 2019

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	Zen black, microscope software for Zeiss dual platform confocal/multiphoton 880 and light sheet Z.1. Imaris software (Bitplane) version 8.2.1 was used for 3D and 3D stereo visualization and segmentation of confocal and multiphoton data. Surfaces module was used for threshold based segmentation. For each segmented cell measurements of channel intensity were extracted.
Data analysis	GraphPad Prism 6 was used for segmented cell analysis and plotting.

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

All data generated or analysed during this study are included in this published article (and its supplementary information files)

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	Depending on the organoid size and origin and due to potential loss during the sample preparation, we ideally start the experiment with 50-200 organoids for confocal or multi-photon imaging and 100-200 organoids for light sheet imaging to obtain enough organoids for imaging experiment (usually 10-20 organoids were imaged per experiments and the remaining frozen down).
Data exclusions	No data exclusion.
Replication	10-20 organoids were analyzed per experiment (replicated at least 2 times). All replications were successful.
Randomization	Randomization is not relevant to our study since we describe a protocol of sample preparation for 3D imaging of all types of organoid.
Blinding	Blinding is not relevant to our study since we describe a protocol of sample preparation for 3D imaging of all type of organoid.

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	Table 1
Validation	All antibodies used are commercialized and have been previously tested. Information is included in table 1

Animals and other organisms

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

Laboratory animals	organoids from FVB/N mouse Female 6-10-week-old
Wild animals	*Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	All animal experiments conform to regulatory standards and were approved by the Walter and Eliza Hall Institute (WEHI) Animal Ethics Comittee.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Human airways, kidney, liver, breast cancer and CF (Cystic fibrosis) organoids.
Recruitment	All human organoids samples were obtained from biobanks through Hubrecht Organoid Technology (HUB, www.hub4organoids.nl).
Ethics oversight	Authorizations were obtained by the ethical committee UMC Utrecht (METC UMCU) at request of the HUB in order to ensure compliance with the Dutch medical research involving human subjects' act and informed consent was obtained from donors when appropriate.

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