nature research

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Last updated by author(s): Apr 10, 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	No software was used to collect data.
Data analysis	For single-cell analysis we used 10x Genomics CellRanger and Seurat v2 and v3 and RStudio. For statistical analysis we used GraphPad Prism 8. For image analysis we used ImageJ (FIJI), Leica LAS X Software (v3), and Imaris 8.

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

Single-cell RNA sequencing data have been uploaded to the Gene Expression Omnibus (Accession code GSE147206): https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE147206. In the supplementary material, we have provided HTML files that generate an online interface with which to explore our scRNA-seq analysis pipeline and evaluate additional cell cluster markers for the integrated and non-integrated datasets (Supplementary Data 1-7). In addition, the integrated datasets shown in Figure 2 can be queried via a data exploration portal hosted on our laboratory website: https://www.koehler-lab.org/resources. Source data behind Figures 1-4 are available within the manuscript 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	No statistical methods were used to determine sample size. Generally, we sought to technically replicate experiments >6 times over >3 separate experiments. These numbers were chosen based on previous studies in the organoid literature.
Data exclusions	No data were excluded.
Replication	The number of replications is noted throughout the manuscript. Also, see specific sections on reproducibility in the METHODS. For histology, we analyzed multiple organoids from each cell line at different timepoints to demonstrate the robustness of the technique. All replication attempts were successful unless noted, as in our Supplementary Note on methodology development.
Randomization	In general, the experiments were not randomized; however, organoid specimens used for histology and single-cell RNA-sequencing analysis were selected at random and not screened for quality or specific morphological characteristics.
Blinding	The investigators were not blinded during experiments and outcome assessments. The same investigators performed experiments and data analyses, which made blinding difficult. Our use of single-cell RNA-sequencing and unbiased clustering analysis helped to mitigate any potential bias from a lack of blinding.

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

Antibody Name (Species, Company, Cat. No., Dilution)
Aggrecan (Rabbit, Invitrogen, PA1-1745, 1:100)
alpha-smooth muscle actin (Mouse, Abcam, ab7871, 1:50)
βIII-Tubulin (TUJ1) (Mouse BioLegend 801202 1:100)
CD49f (Rat Invitrogen 12-0495-81 1:50)
Collagen 2A1 (COL2A1) (Mouse, Invitrogen, MA1-37493, 1:10)
Cytokeratin 5 (KRT5) (Rabbit, Invitrogen, MA5-14473, 1:50)
Cytokeratin 5 (KRT5) (Mouse, Invitrogen, MA5-12596, 1:50)
Cytokeratin 10 (KRT10) (Mouse, Santa Cruz, sc-23877, 1:50)
Cytokeratin 15 (KRT15) (Mouse, Santa Cruz, sc-47697, 1:50)
Cytokeratin 17 (KRT17) (Mouse, Santa Cruz, sc-393091, 1:50)
Cytokeratin 20 (KRT 20) (Rabbit, Cell Signaling Technology, 13063S, 1:100)
Cytokeratin 71 (KRT 71) (Rabbit, Invitrogen, PA5-83965, 1:500)
Cytokeratin 74 (KRT 74) (Rabbit, Sigma-Aldrich, HPA048596, 1:1000)
Cytokeratin 75 (KRT 75) (Rabbit, Invitrogen, PA553987, 1:50)
Cytokeratin 82 (KRT 82) (Rabbit, Sigma-Aldrich, HPA067230, 1:100)
E-cadherin (ECAD) (Mouse, BD Biosciences, 610181, 1:50)
EDAR (Goat, R&D Systems, AF745-SP, 1:50)
GATA3 (Mouse, Invitrogen, PIMA1028, 1:100)
Human nuclear antigen (Mouse, Abcam, ab191181, 1:100)

Integrin-alpha-8 (ITGA8) (Mouse, Santa Cruz, sc-365798, 1:50)
Islet1 (ISL1) (Mouse, DSHB, 39.4D5, 1:5)
Ki67 (Mouse, BD Biosciences, 550609, 1:100)
LHX2 (Rabbit, Millipore, ABE1402, 1:750)
Loricrin (Rabbit, Abcam, ab85679, 1:50)
Nephronectin (NPNT) (Mouse, R&D Systems, MAB8025, 1:50)
Neurofilament-heavy chain (NEFH) (Mouse, Cell Signaling Technology, 2836S, 1:100)
NFATC1 (Mouse, Invitrogen, MA3-024, 1:50)
P-cadherin (PCAD) (Mouse, Invitrogen, 32-4000, 1:50)
p75NTR (P75) (Rabbit, Cell Signaling Technology, 8238, 1:50)
pan-Cytokeratin (AE13) (Mouse, Santa Cruz, sc-57012, 1:50)
Parvalbumin (Mouse, Sigma-Aldrich, P3088, 1:1000)
PDGFRα (D13C6) (Rabbit, Cell Signaling Technology, 5241, 1:50)
Peripherin (Rabbit, Abcam, ab4666, 1:2500)
PMEL (Mouse, Novus, NBP2-29407, 1:100)
S100β (Rabbit, Abcam, ab52642, 1:75)
SCD1 (Rabbit, Sigma-Aldrich, HPA012107, 1:50)
SOX2 (Mouse, BD Biosciences, 561469, 1:100)
SOX10 (Mouse, Invitrogen, 14-5925-80, 1:50)
TFAP2A (Mouse, DSHB, 3B5, 1:5)

Validation

Previously published or manufacturer-validated antibodies were used for this study. For each antibody, the company website contains validation data. All antibodies were tested, in house, on PFA-fixed and cryosectioned human/mouse primary skin tissue samples.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	hESC lines: WA25 and WA09 (H9) - obtained from WiCell; hiPSC lines: mND2-0 (obtain from WiCell) and DSP-GFP (also known as AICS-0017, obtain from Coriell Institute)
Authentication	The cell lines were obtained under a materials transfer aggreement with the WiCell or Coriell Institute. Each line was determined to have a normal karyotype and the ability to generate all three germ layers at the time of derivation.
Mycoplasma contamination	The cell lines are negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No ICLAC lines were used.

Animals and other organisms

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

Laboratory animals	For xenograft experiments, 5-6 week-old female immunodeficient nude mice (NU/J) were purchased from The Jackson Laboratory.
Wild animals	No wild animals were used for this study.
Field-collected samples	No field-collected samples were used for this study.
Ethics oversight	All mouse experiments were approved by the Indiana University School of Medicine Animal Care and Use Committee (IACUC), protocol number 11077.

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 foetal and adult skin tissue samples were collected without any identifying information including sex, race, or gender.
Recruitment	No human participants were used in this study. Foetal tissue was obtain from misscarried specimens with the patients consent. Foetal tissue was procured by the University of Washington Birth Defects Research Laboratory. Adult human skin samples were procured by the Human Skin Disease Resource Center at Brigham and Women's Hospital-Harvard Medical School.
Ethics oversight	This work falls under NIH Exemption 4, thus it was designated as "Non-Human Subjects Research" by the Indiana University School of Medicine IRB.

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