nature research

Corresponding author(s): Joseph V. Bonventre
Kyle W. McCracken

Last updated by author(s): 06/14/2022

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 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	Nikon NIS Elements LabChart 7 (ADInstruments).
Data analysis	GraphPad Prism 9 Raw scRNA-seq data was processed using Seven Bridges Genomics' pipeline for BD Rhapsody. Data were then analyzed in RStudio (v1.4.1717; R version 4.1.1) using Seurat (v4.0.4) and Monocle2 (v2.20.0) packages. Azimuth web application (https://azimuth.hubmapconsortium.org)

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 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

scRNA-seq data are available at the Gene Expression Omnibus, under the accession number GSE206153.
RNA-seq data from mouse dissected tubules were downloaded from the Mouse Renal Epithelial Cell Atlas (esbl.nhlbi.nih.gov/MRECA/Nephron/)

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	Sample sizes were not predetermined using statistical calculation, but rather were empirically estimated based on previous studies (Morizane et al, Nat Biotech 2015).
Data exclusions	Data were not excluded from analyses.
Replication	All differentiation experiments were replicated using at least two cell lines (including one hESC and one hiPSC) and independently repeated a minimum of three times, and the differentiation strategies were also successfully replicated by at least 2-3 independent users. For each molecular assay, including IF staining and qPCR, a minimum of three wells were analyzed in entirety, with each containing a minimum of 20 organoids. The electrophysiologic studies in transwell platform (Fig. 4) have been repeated more than five times with respect to demonstrating amiloride-sensitive current. The aldosterone sensitivity experiment has only repeated four times. For the Foxi1-mediated induction of intercalated cells (Fig. 5), results were repeated in both hESC and hiPSC lines. The scRNA-sequencing experiment was only performed on one experiment, using two different time points. All attempts of molecular assays using either qPCR or IF staining with validated antibodies were successful.
Randomization	Allocation of individual samples into control and experimental groups was done randomly.
Blinding	Blinding was not relevant to this study, since most of the analyses were performed on different time points of stem cell differentiated tissues, and there were no comparisons between genotypes or therapeutic treatment 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
☒	☐	Human research participants
☒	☐	Clinical data
☒	☐	Dual use research of concern

Methods

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

Antibodies

Antibodies used

anti-ALDH1A3 Rabbit Novus NBP2-15339 1:200
anti-AQP2 Mouse Santa Cruz sc-515770 1:200
anti-AQP2 Rabbit Millipore 178612 1:200
anti-ATP6V1B1 Rabbit Sigma HPA031847 1:200
anti-CDH1 Mouse BD Biosciences 610181 1:500
anti-EMX2 Rabbit Sigma HPA065294 1:200
anti-FOXI1 Goat Abcam ab20454 1:200
anti-SCNN1B Rabbit Gift from L. Palmer 1:100
anti-GATA3 Rabbit Cell Signaling D13C9 1:300
anti-HNF1B Rabbit Sigma HPA002083 1:200
anti-human nuclear antigen Mouse Novus NBP2-34342 1:500
anti-GRHL2 Rabbit Sigma HPA004820 1:200
anti-HOXB7 Mouse R&D Systems MAB8040 1:200
anti-KRT8 Mouse Santa Cruz sc-8020 1:100
anti-LHX1 Rabbit Abcam ab229474 1:200
anti-PAX2 Rabbit BioLegend PRB-276P 1:200

anti-PAX2 Goat R&D Systems AF3364 1:200
anti-PAX8 Rabbit Abcam ab189249 1:400
anti-RET Goat R&D Systems AF-1485 1:200
anti-SCNN1B Rabbit obtained from Dr. Palmer (Columbia University) 1:200
anti-SOX9 Rabbit Novusbio NBP1-85551 1:200
anti-SLC26A4 Rabbit Sigma HPA042860 1:200
anti-SLC4A1 Rabbit Sigma HPA-015584 1:200
anti-TBXT Goat R&D Systems AF2085 1:200

Validation

anti-AQP2 (mouse) has been cited in 24 publications according to manufacturer's website, and collecting duct specific localization was demonstrated in Hreha et al, Physiologic Reports 2020.
anti-AQP2 (rabbit) has been cited in 4 publications according to manufacturer's website, and we confirmed collecting duct-specific in situ localization in archival human kidney samples.
anti-ALDH1A3 has been cited in 21 publications according to manufacturer's website, and it was recently shown to stain the leader cells of the nephric duct that also express the gene at the transcriptional level (Sanchez-Ferras Nature Commun. 2021).
anti-ATP6V1B1 has been cited in 2 publications according to manufacturer's website, and expected pattern of localization was demonstrated in the Human Protein Atlas.
anti-CDH1 has been cited in 5 publications according to manufacturer's website, and staining of the human protein was demonstrated in Hildebrand et al, Scientific Reports 2017.
anti-EMX2 was validated via a specific nuclear localization as shown in the Human Protein Atlas.
anti-FOXI1 has been cited in 9 publications according to manufacturer's website, and specific detection of the human protein was demonstrated in this study.
anti-SCNN1B was provided by Lawrence Palmer, and we confirmed the collecting duct-specific expression via staining of human kidney cortex.
anti-GATA3 has been cited in 38 publications according to manufacturer's website, and detection of human protein was demonstrated by Okae et al, Cell Stem Cell 2018.
anti-HNF1B has been cited in 15 publications according to manufacturer's website, and expected pattern of localization was demonstrated in the Human Protein Atlas.
anti-human nuclear antibody has been cited in 10 publications according to manufacturer's website, and the antibody clone is also distributed by several additional vendors. In control mouse explants with no human cells, we never observed positive staining.
anti-GRHL2 has been cited in 27 publications according to manufacturer's website, and expected pattern of localization was demonstrated in the Human Protein Atlas.
anti-HOXB7 was demonstrated to react with human antigen by Benedetti et al, EBioMedicine 2018.
anti-KRT8 has been cited in 45 publications according to manufacturer's website.
anti-LHX1 was demonstrated to give specific nuclear signal on both IF and IHC as shown on manufacturer's website.
anti-PAX2 (Rabbit) has been cited in 26 publications according to manufacturer's website, and human-specific kidney IF staining was validated in Lindstrom et al, JASN 2018.
anti-PAX2 (goat) has been cited in 4 publications according to manufacturer's website, and human-specific kidney expression was demonstrated in Tran et al, Developmental Cell 2019.
anti-PAX8 has been cited in 7 publications according to manufacturer's website, and expression in developing kidney cells validated by Ramalingham et al, Developmental Biology 2018.
anti-RET has been cited in 2 publications according to manufacturer's website, and human fetal kidney expression was validated by Menon et al, Development 2018.
anti-SOX9 has been cited in 15 publications according to manufacturer's website, and detection of human protein was validated in Kaczmarek-Hajek et al, ELife 2018.
anti-SLC26A4 and anti-SLC4A1 were able to detect protein in ICs in the human kidney as previously published in the Human Protein Atlas.
anti-TBXT has been cited in 71 publications according to manufacturer's website, and detection of human protein was validated in Foo et al, Molecular Therapy 2018.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

H9 (hESC) cell line was originally obtained from WiCell.
BJFF6 (hiPSC) cell line was provided by S. Jain via the ReBuilding a Kidney (RBK) Consortium. The line was originally generated by reprogramming of human foreskin fibroblasts from a healthy male donor. The line was produced at Washington University, and is maintained in the Kidney Translational Research Center.
For generation of the GATA3 reporter cell line, mScarlet construct was introduced into H9 cells as described in methods.
HEK293T/17 cells were obtained from ATCC.

Authentication

Pluripotency of hPSC lines was routinely validated using in vitro trilineage differentiation potential assays.

Mycoplasma contamination

Cell lines were periodically subjected to PCR-based mycoplasma screening for routine surveillance.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

Animals and other organisms

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

Laboratory animals

Pregnant female mice (CD1) from Charles River were sacrificed to collect E12.5 embryos.

Wild animals	Wild animals were not used in this study.
Field-collected samples	There were no field-collected samples in this work.
Ethics oversight	Institutional Animal Care and Use Committee of Brigham and Women's Hospital; Protocol 2016N000162.

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