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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.

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| 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 |
| \boxtimes | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| \boxtimes | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| \boxtimes | 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. |
| X | A description of all covariates tested |
| \boxtimes | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| \boxtimes | 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) |
| \boxtimes | For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i> |
| \times | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| \times | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| \boxtimes | Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |
| | Our web collection on <u>statistics for biologists</u> contains articles on many of the points above. |
| | |

Software and code

Policy information about <u>availability of computer code</u>

Data collection commercially available widefield and confocal microscopy are used for collecting every data except the 1-mm thick kidney tissue which was imaged by a custom light-sheet microscope (https://doi.org/10.1038/s41467-019-10534-0).

Data analysis the free software package ImageJ is sufficient to analyze most data sets. However the large mouse kidney data set (Fig. 6) was volumetrically rendered using Imaris, and the custom Wolfram Mathematica scripts was used for stitching tiling images (Fig. 4a and 4h).

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

Fig. 1c, Fig. 3a-d, and Fig. 5a and 5c are reproduced from Mao et al. (DOI: 10.1126/sciadv.aba4542). The data in Fig. 6 and supplementary movie 1 were previously shown in Mao et al. (DOI: 10.1126/sciadv.aba4542), but are now presented in an alternative format. The data shown in Fig. 3e-h, Fig. 4, Fig. 5b and 5d, Fig. 10, Fig. 12 has not been published previously. Additional examples of data are available from the corresponding author upon request.

| Field-sne | cific reporting | | | | | |
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| <u> </u> | · | | | | | |
| Life sciences | be below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection. Behavioural & social sciences Ecological, evolutionary & environmental sciences | | | | | |
| | ne document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf | | | | | |
| Tor a reference copy or a | ne declarient with an sections, see <u>natareteen / declarients/ in reporting sammal y natipal</u> | | | | | |
| Life sciences study design | | | | | | |
| All studies must dis | close on these points even when the disclosure is negative. | | | | | |
| Sample size | Sample size calculation was not performed. | | | | | |
| Data exclusions | Data were not excluded. | | | | | |
| Replication | All reported data have been reproduced at least three times. | | | | | |
| Randomization | Randomization was not performed as it was unnecessary for our experiments. | | | | | |
| Blinding | Blinding was not performed and was unnecessary for our experiments. | | | | | |
| 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 Methods | | | | | | |
| Antibodies Antibodies used | anti-podocalyxin (R&D Systems Inc., AF1156), aquaporin-1 (Abcam, ab15080), and cytokeratin 8+18 (Abcam, ab194130) | | | | | |
| Validation | Antibodies were validated by the vendors indicated above; the vendors list multiple references for each antibody. We performed no | | | | | |
| validation | additional validation steps. | | | | | |
| Animals and other organisms | | | | | | |
| Policy information a | about <u>studies involving animals</u> ; <u>ARRIVE guidelines</u> recommended for reporting animal research | | | | | |
| Laboratory anima | two-month-old C57BL/6 male mice | | | | | |
| 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.

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, Field-collected samples photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight University of Washington

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