nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio 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	We used version 4.2 of FV1000 software (Olympus, 2017), version 3.4 of ZEN software (Carl Zeiss, 2021), and BZ-X710 (Keyence, 2013) for image data collection.
Data analysis	We used version 7.8 of MetaMorph software (Universal Imaging, 2015), version 7.6.0 of Imaris software (Oxford Instruments, 2012), version 01.03.00.05 of BZ-X Viewer (Keyence, 2013), and version 1.54f of ImageJ software (National Institutes of Health, 2023) for analysis of image data. We used version 17.0.0 of JMP Pro software (SAS Institute, 2022) for statistical analysis.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The datasets analysed during the current study are available from the corresponding author on request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

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	4 mice per group were used for animal experiments including two photon microscopy based on the preliminary experiments.
Data exclusions	One sample was excluded from data of urinary albumin to creatinine ratio (ACR) because operative failure of ischemia reperfusion injury was confirmed by histology analysis.
Replication	We repeated ATP imaging experiments at least four times each and in vitro experiments three times each, in which all attempts at replication were successful.
Randomization	Mice were allocated into experimental groups randomly after excluding visibly sick or overweight/underweight individuals. For in vitro experiments, dishes with adequate confluency were randomly allocated to the experimental groups.
Blinding	Blinding was not possible because the procedures of ischemia reperfusion injury and two photon microscopy include the information of allocation such as ischemia duration.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

polyclonal anti-nephrin antibody (R & D Systems, AF3159, 1:500), monoclonal anti-Wilms Tumor Protein (WT-1) antibody (Abcam, ab89901, CAN-R9(IHC)-56-2, 1:200), anti-podocin affinity isolated antibody (Sigma-Aldrich, P0372, 1:200), anti-nestin affinity purified antibody (Immuno-Biological Laboratories, #18741, 1:200), polyclonal anti-GFP antibody (Abcam, ab13970. 1:200), monoclonal anti-CD31 antibody (BD Biosciences, 553370, MEC13.3, 1:200), monoclonal anti-synaptopodin antibody (PROGEN, 61094, G1D4, 1:200), Alexa Fluor-647 conjugated Phalliodin (ThermoFisher Scientific, A22287, 1:500), polyclonal anti-megalin antibody (Santa Cruz, sc-16478, 1:200), monoclonal anti-CD140b (PDGFRB) antibody (eBioscience, 14-1402-82, ABP5, 1:200), monoclonal anti-DRP1 antibody (abcam, ab184247, EPR19274, 1:200 for IHC and 1:1000 for WB) , polyclonal anti-beta-actin antibody (Cell Signaling Technology, 4967, 1:1000)

Validation

anti-nephrin antibody (R & D Systems, AF3159); The data sheet validates the application to IF of murine samples. anti-WT-1 antibody (Abcam, ab89901, Lot: GR3270281-3); The data sheet validates the application to IF of murine samples. anti-podocin antibody (Sigma-Aldrich, P0372, Lot: 048M4781V); Application to IF of murine samples has been previously published (Wehbi, B. et al. (2019) J Am Soc nephrol 30: 1238). anti-nestin antibody (Immuno-Biological Laboratories, #18741, Lot: OD-519); The data sheet validates application to IF and cross-reactivity to murine antigens. anti-GFP antibody (Abcam, ab13970, Lot: 1018753-2); The data sheet validates the application to IF of murine samples. anti-CD31 antibody (BD Biosciences, 553370, Lot: 9338730); The data sheet validates the application to IF of murine samples. anti-synaptopodin antibody (PROGEN, 65194); The data sheet validates the application to ICC-IF of murine samples. Alexa Fluor-647 conjugated Phalloidin (ThermoFisher Scientific, A22287, Lot: 2575967); Application to ICC-IF of murine samples has been previously published (Kay, JG. et al. (2006) J Biol Chem281: 11949). anti-megalin antibody (Santa Cruz, as-16478, Lot: L2104); Application to IF of murine samples has been previously published (Mori, P. K. et al. (2017) J Am Soc Nephrol 28: 278). anti-CD140b antibody (eBioscience, 14-1402-82, Lot: 2202687); The data sheet validates the application to IF of murine samples. anti-DRP1 antibody (abcam, ab184247, Lot: GR3369203-20); The data sheet validates the application to WB of murine samples. anti-beta-actin antibody (Cell Signaling Technology, Lot: 9); The data sheet validates the application to WB of murine samples.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The murine podocyte cell line used in this study was derived from transgenic H-2Kb-tsA58 mice (Mundel, P. et al. (1997) Exp Cell Res 236: 248). Primary cultured podocytes were isolated from 8-12-week old female wild type mice as described previously (Mundel, P. et al. (1997) J Am Soc Nephrol 8: 697. Mundel, P. et al. (1997) Exp Cell Res 236: 248). Murine embryonic fibroblasts (MEFs) were freshly isolated from GO-ATeam2 mouse embryos at E13.5.

Authentication

None of the cell lines we used were authenticated.

Mycoplasma contamination

None of the cell lines we used were tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in the current study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

BL6/J mice, GO-ATeam2 mice, Nphs1-Cre mice, Tie2-Cre mice, and GO-ATeam2 flox/+ mice were used. Female mice between 9 and 13 weeks of age, which were housed in a specific pathogen-free facility at 50 ± 10 % humidity and 24 ± 2 C under 14 hours/10 hours light and dark cycle with access to water and regular diet ad libitum and received a routine diet, were used for the experiments.

Wild animals

This study did not involve wild animals.

Reporting on sex

We analyzed only female mice because male mice were not applicable to glomerular observation with two photon microscopy due to their thicker surface layers.

Field-collected samples

This study did not involve any samples collected from the field.

Ethics oversight	All animal studies were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*