

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <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> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	LC-MS/MS analysis was carried out using a nano-LC 1200 system coupled to Orbitrap Lumos ETD mass spectrometer (Thermo Fisher). Respiration analysis was performed on a Seahorse XFe96 analyzer. Flow cytometry was performed using FACS Aria (Becton Dickinson, San Jose, CA). Odyssey Fc Imaging System was used to obtain western blot images. Image Studio (Li-COR, v5.2.5) was used for densitometry analysis for western blot. StepOnePlus Real-Time PCR System was used to collect RT-qPCR data. Immunofluorescence images except for STED/STORM were captured by FV1200 MPE confocal microscope (Olympus). N-STORM (Nikon Instruments) was used for STORM imaging, and STED imaging was performed on an STEDYCON (Abberior) and Eclipse Ti2 inverted imaging system (Nikon Instruments) with a Plan Aplanachromat 100X (NA 1.49) oil immersion objective (Nikon Instruments). Cryo-ET data collection was performed using a Titan Krios G3 300 keV FEG transmission electron cryo-microscope (Thermo Fisher). Molecular graphics and visualization in Cryo-ET were performed with UCSF Chimera. Human STOML2 structure was predicted by Contact-guided Iterative Threading ASSEmbly Refinement web tool (C-I-TASSER). Human NDUFS4 within the structure of the respirasome was obtained from the PDB database (5XTB).
Data analysis	NDUFS4 intensity and NADH diaphorase staining in glomeruli, and mitochondrial morphology were analyzed in Image J (Fiji, v2.0.0). The MS raw data was searched using Proteome Discoverer 2.1 software (Thermo Fisher) with Mascot algorithm against mouse NCBI refseq database updated 2020_0324. In STED analysis, JACoP plugin of mage J was used, and for STORM imaging analysis, ThunderSTORM plugin of image J was used. Maximum intensity projection (MIP) images were reconstructed from Nikon Nd2 files using Huygens Essential (Scientific Volume Imaging). SerialEM software was used for imaging of Cryo-ET. All statistical analyses were performed with GraphPad Prism version 9.3.1 (Graphpad Software). The ClusPro 2.0 web server ²⁵ was used to perform protein-protein docking simulation. The resulting docking structures were analyzed using PyMol Molecular Graphics Systems, version 2.0, Schrodinger, LLC.

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](#)

All data supporting the findings of this study are available in the main text or supplementary materials. The mass spectrometry proteomic data for mitochondrial proteome in murine podocytes, NDUFS4-APEX proteome, and murine podocyte complexome have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers, PXD041202, PXD041378, and PXD041203, respectively. Glomerular transcriptomic data (Nephroseq database version 5) were analyzed using Ju CKD Glom dataset (GSE104948).

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	Male and Female.
Reporting on race, ethnicity, or other socially relevant groupings	Race: Asian Ethnicity: Japanese
Population characteristics	1. Patients with biopsy-proven diabetic kidney disease 2. Healthy kidney donors as controls
Recruitment	1. Renal biopsy cohort 1 (Protocol biopsy group) Patients who agreed to join the NExT-DN Study (UMIN000024530) from 2/21/2017 to 12/1/2021 2. Renal biopsy cohort 2 (Clinical biopsy group) I. Patients who underwent renal biopsy from 12/1/2010 to 2/21/2017 and were diagnosed with kidney disease. II. Patients who were enrolled into previous clinical studies at Okayama University Hospital, Japan (No: 1009 and No: 1509-005[2063]). 3. Healthy kidney control who meet all of the following criteria I. Donors of kidney transplantation at Okayama University Hospital, Japan from 7/1/2017 to 12/1/2021 II. Donors without urine abnormalities such as hematuria and proteinuria. III. Donors who are not diagnosed with any kidney disease in the results of 0 hour-renal biopsy. IV. Donors who agree to keep their blood and urine sample in OKADAI BIOBANK (Biobank of Okayama university hospital).
Ethics oversight	The protocol was approved by the Institutional Review Board of the Ethics Committee of Okayama University Hospital and registered with the University Hospital Medical Information Network (UMIN) (identification number: UMIN000046398). Written informed consent was obtained from all patients prior to their inclusion in the clinical study.

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](https://www.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 predetermine the sample sizes. Sample sizes were indicated in the legend of each Figure and Supplementary Figure. For assays both in vitro and in vivo, there are generally at least 3-4 experimental/biological replicates for every assay and condition.
Data exclusions	No data were excluded from the analyses.
Replication	All attempts at replication were successful. Data reported in this manuscript were reproduced with at least 3 biologically independent replicates for the in vitro experiments and at least 3-4 independent mice per group.

Randomization

No randomization was performed for in vivo experiments, and experimental animals were analyzed based on age and sex-matched (male) controls. For cell cultured experiments, all samples in one experiment were treated at the same time and with the same condition.

Blinding

Blinding was not performed in this study except for human IHC analyses since the investigators needed to know the genotype of animals and sample information for downstream analyses. For histological analyses for human samples, clinical information was blinded for investigator/renal pathologists.

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

Primary antibodies

Anti-GST Alexa Fluor 680 (Santa Cruz, sc-138 AF680)
 Goat anti-Podocalyxin, biotinylated (R&D Systems, BAF1556)
 Guinea pig anti-Synaptopodin (Progen, GP94-N)
 Mouse anti-Actin (Cell Signaling, 4967)
 Mouse anti-FLAG M2 monoclonal (Sigma-Aldrich, F3165)
 Mouse anti-STOML2 (Proteintech, 60052-1-Ig)
 Mouse anti-VDAC (Abcam, ab14734)
 Mouse OXPHOS cocktail (Thermo Fisher, 45-8099)
 Rabbit anti-ATAD3A/B (Proteintech, 16610-1-AP)
 Rabbit anti-HA-Tag (Cell Signaling, 3724)
 Rabbit anti-Mitofilin (Proteintech, 10179-1-AP)
 Rabbit anti-Ndufs4 (Nobus, NBP1-31465)
 Rabbit anti-Ndufs4 (Abcam, ab137064)
 Rabbit anti-OPA1 (BD Biosciences, 612606)
 Rabbit anti-PAX8 (Proteintech, 10336-1-AP)
 Rabbit anti-Podocin (Sigma-Aldrich, P0372)
 Rabbit anti-STOML2 (Proteintech, 10348-1-AP)
 Rabbit anti-VDAC (Cell Signaling, 4661)
 Rabbit anti-Wilms Tumor Protein (Abcam, ab89901)
 Sheep anti-KIRREL3, biotinylated (R&D Systems, BAF4910)

Secondary antibodies

Donkey anti-mouse Alexa Fluor 488 (Thermo Fisher, A21202)
 Donkey anti-mouse Alexa Fluor 594 (Thermo Fisher, A21203)
 Donkey anti-rabbit Alexa Fluor 488 (Thermo Fisher, A21206)
 Donkey anti-rabbit Alexa Fluor 594 (Thermo Fisher, A21207)
 Donkey anti-rabbit Alexa Fluor 647 (Thermo Fisher, A21244)
 Goat anti-guinea pig Alexa Fluor 594 (Thermo Fisher, A11076)
 Goat anti-mouse DyLight 680 (Thermo Fisher, 35519)
 Goat anti-mouse DyLight 800 (Thermo Fisher, SA510172)
 Goat anti-mouse ATTO 488 (Rockland, 610-152-121)
 Goat anti-rabbit DyLight 680 (Thermo Fisher, 35519)
 Goat anti-rabbit DyLight 800 (Thermo Fisher, SA535571)
 Goat anti-rabbit IgG HRP Polymer (Vector Laboratories, MP-7451)

Validation

Primary antibodies

Anti-GST Alexa Fluor 680 (Santa Cruz, sc-138 AF680): <https://www.scbt.com/p/gst-antibody-b-14>
 Goat anti-Podocalyxin, biotinylated (R&D Systems, BAF1556): https://www.rndsystems.com/products/mouse-podocalyxin-biotinylated-antibody_baf1556
 Guinea pig anti-Synaptopodin (Progen, GP94-N): <https://us.progen.com//anti-Synaptopodin-SYNPO-N-terminus-guinea-pig->

polyclonal-serum/GP94-N

Mouse anti-Actin (Cell Signaling, 4967): <https://www.cellsignal.com/products/primary-antibodies/b-actin-antibody/4967>

Mouse anti-FLAG M2 monoclonal (Sigma-Aldrich, F3165): <https://www.sigmaaldrich.com/US/en/product/sigma/f3165>

Mouse anti-STOML2 (Proteintech, 60052-1-Ig): <https://www.ptglab.com/products/STOML2-Antibody-60052-1-Ig.htm>

Mouse anti-VDAC (Abcam, ab14734): <https://www.abcam.com/products/primary-antibodies/vdac1porin--vdac3-antibody-20b12af2-ab14734.html>

Mouse OXPHOS cocktail (Thermo Fisher, 45-8099): <https://www.thermofisher.com/antibody/product/OxPhos-Rodent-WB-Antibody-clone-Cocktail-Cocktail/45-8099>

Rabbit anti-ATAD3A/B (Proteintech, 16610-1-AP): <https://www.ptglab.com/products/ATAD3B-Antibody-16610-1-AP.htm>

Rabbit anti-HA-Tag (Cell Signaling, 3724): <https://www.cellsignal.com/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724>

Rabbit anti-Mitofilin (Proteintech, 10179-1-AP): <https://www.ptglab.com/products/IMMT-Antibody-10179-1-AP.htm>

Rabbit anti-Ndufs4 (Nobus, NBP1-31465): https://www.novusbio.com/products/ndufs4-antibody_nbp1-31465

Rabbit anti-Ndufs4 (Abcam, ab137064): <https://www.abcam.com/products/primary-antibodies/ndufs4-antibody-ep7832-ab137064.html>

Rabbit anti-OPA1 (BD Biosciences, 612606): <https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-opa1.612606>

Rabbit anti-PAX8 (Proteintech, 10336-1-AP): <https://www.ptglab.com/products/PAX8-Antibody-10336-1-AP.htm>

Rabbit anti-Podocin (Sigma-Aldrich, P0372): <https://www.sigmaaldrich.com/US/en/product/sigma/p0372>

Rabbit anti-STOML2 (Proteintech, 10348-1-AP): <https://www.ptglab.com/products/STOML2-Antibody-10348-1-AP.htm>

Rabbit anti-VDAC (Cell Signaling, 4661): <https://www.cellsignal.com/products/primary-antibodies/vdac-d73d12-rabbit-mab/4661>

Rabbit anti-Wilms Tumor Protein (Abcam, ab89901): <https://www.abcam.com/products/primary-antibodies/wilms-tumor-protein-antibody-can-r9ihc-56-2-ab89901.html>

Sheep anti-KIRREL3, biotinylated (R&D Systems, BAF4910): https://www.rndsystems.com/products/human-mouse-rat-kirrel3-neph2-biotinylated-antibody_baf4910

Secondary antibodies

Donkey anti-mouse Alexa Fluor 488 (Thermo Fisher, A21202): <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21202>

Donkey anti-mouse Alexa Fluor 594 (Thermo Fisher, A21203): <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21203>

Donkey anti-rabbit Alexa Fluor 488 (Thermo Fisher, A21206): <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206>

Donkey anti-rabbit Alexa Fluor 594 (Thermo Fisher, A21207): <https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21207>

Goat anti-rabbit Alexa Fluor 647 (Thermo Fisher, A21244): <https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21244>

Goat anti-guinea pig Alexa Fluor 594 (Thermo Fisher, A11076): <https://www.thermofisher.com/antibody/product/Goat-anti-Guinea-Pig-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11076>

Goat anti-mouse DyLight 680 (Thermo Fisher, 35519): <https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/35519>

Goat anti-mouse DyLight 800 (Thermo Fisher, SA510172): <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/SA5-10172>

Goat anti-mouse ATTO 488 (Rockland, 610-152-121): <https://www.rockland.com/categories/secondary-antibodies/mouse-igg-hl-antibody-atto-488-conjugated-pre-adsorbed-610-152-121/>

Goat anti-rabbit DyLight 680 (Thermo Fisher, 35568): <https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Secondary-Antibody-Polyclonal/35568>

Goat anti-rabbit DyLight 800 (Thermo Fisher, SA535571): <https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Secondary-Antibody-Polyclonal/SA5-35571>

Goat anti-rabbit IgG HRP Polymer (Vector Laboratories, MP-7451): <https://vectorlabs.com/products/enzyme-polymer/impress-hrp-goat-anti-rabbit-igg-kit>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Conditionally immortalized mouse podocytes were a kind gift from Jochen Reiser (Rush University, Chicago, IL). HEK293T cells was purchased from ACTT.
Authentication	Immortalized mouse podocytes are regularly checked for podocyte like morphology and podocyte marker protein expression such as synaptopodin and podocin by Western blot. Knockdown or overexpression of the target gene was analyzed by qPCR and Western blot analysis. HEK293T cells were authenticated by manufacturer (ATCC) and confirmed by microscopy looking at morphology.
Mycoplasma contamination	All cell lines tested negative for Mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No misidentified cell lines were used in this 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	All mice were maintained in a temperature-controlled environment (22oC) under a 12-hrs light/dark cycle with free access to chow
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Laboratory animals	and water. All mice were maintained with ad Librium access to food and water under the ambient temperature of 23°C. Type 1 and type 2 diabetic mice (Ins2Akita/+ on C57BL/6J background and Leprdb/db on C57BLKS/J background) were obtained from Jackson Laboratories (Stock Nos. 003548 and 000642).
Wild animals	No wild animals were used in this study.
Reporting on sex	Only male mice were used in this study.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas at MD Anderson Cancer Center and conducted according to the institutional and the US National Institutes of Health guidelines.

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	UMIN000046398 (UMIN: University Hospital Medical Information Network)
Study protocol	Study type: Retrospective cohort study (using previous data from ongoing prospective cohort study and retrospective cohort study) Narrative objectives To investigate novel targets and biomarkers to determinate pathophysiology and prognosis of diabetic kidney disease (DKD) using human renal tissue, blood, and urine. Basic objectives To explore the new therapeutic target of DKD Eligibility Age: not applicable Gender: male and female Key inclusion criteria 1. Renal biopsy cohort 1 (Protocol biopsy group) Patients who agreed to join the NExT-DN Study (UMIN000024530) from 2/21/2017 to 12/1/2021 2. Renal biopsy cohort 2 (Clinical biopsy group) I. Patients who underwent renal biopsy from 12/1/2010 to 2/21/2017 and were diagnosed with kidney disease. II. Patients who were enrolled into previous clinical studies at Okayama University Hospital, Japan (No: 1009 and No: 1509-005[2063]). 3. Healthy kidney control who meet all of the following criteria I. Donors of kidney transplantation at Okayama University Hospital, Japan from 7/1/2017 to 12/1/2021 II. Donors without urine abnormalities such as hematuria and proteinuria. III. Donors who are not diagnosed with any kidney disease in the results of 0 hour-renal biopsy. IV. Donors who agree to keep their blood and urine sample in OKADAI BIOBANK (Biobank of Okayama university hospital). Key exclusion criteria 1. Renal biopsy cohort 1 People who decline the entry of NExT-DN Study (UMIN000024530). 2. Renal biopsy cohort 2 Patients who withdraw the agreement of previous clinical studies of our department (No: 1009 and No: 1509-005[2063]). 3. Healthy kidney control People who decline the entry of this study.
Data collection	Clinical parameters at the time of renal biopsy and during follow-up periods after biopsy. Histopathological parameters that is assessed by at least two nephrologists and renal pathologist.
Outcomes	Primary outcome Correlation of novel biomarkers and pathophysiological targets in renal tissue, urine, and blood with pathological finding of diabetic kidney disease (DKD) Secondary outcome 1. Investigation of clinicopathologic indicators of renal progression in DKD I. eGFR 30-40% decline from baseline II. eGFR decline > 3-5 ml/min/1.73m²/year III. Commencement of dialysis or renal transplantation because of end stage renal disease IV. Development of albuminuric stage 2. Investigation of other major complications of diabetes I. Development of diabetic retinopathy II. CVD event III. Death

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Podocytes were incubated at 37°C with fresh media without serum containing 5µM MitoSOX Red mitochondrial superoxide indicator (Thermo Fisher). The cells were analyzed by flow cytometry with help from UT-MDACC FCCICF Core.
Instrument	Beckman Coulter Gallios Flow Cytometer (Beckman Coulter)
Software	Kaluza Analysis Software (Beckman Coulter)
Cell population abundance	In total, 10,000 events were recorded for each sample and analyzed using Kaluza Analysis software.
Gating strategy	Live cells were defined based on forward and side scatter, single cells were defined on the basis of forward scatter area vs forward scatter height. Mitochondrial superoxide levels were assessed by measuring MitoSox Red MFI (median fluorescence intensity)

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.