Supplementary Information

A flexible, multi-layered protein scaffold maintains the slit in between glomerular podocytes


Supplementary Figure legends

Supplementary Figure 1: Expression profile of NEPHRIN and NEPH1.
(A,B) Expression of Nphs1 in the cerebellum anlage is dependent on time and vanishes by E15,5. (C,D) Kidney and pancreas are the only other sites of embryonic (E13,5) expression of Nphs1. (E-H) Neph1 in contrast shows a widespread expression in the CNS, lung, kidney and gut. In newborn mice NEPHRIN on the protein level can only be detected in kidney and pancreas, but not in brain. (I) By contrast, NEPH1 can be readily detected in kidney, brain, heart, lung, liver, pancreas, gut and skeletal muscle. (J,K) Proof of constitutive Nphs1 knock-out by the absence of NEPHRIN protein. (L,M) Proof of constitutive Neph1 knock-out by the absence of NEPH1 protein.

Supplementary Figure 2: Expression profile of Neph2 and Neph3.
(A + insert) At E13.5 Neph2 expression is widespread in central and peripheral nervous tissue, but absent in kidney. (B + insert) At the same stage of embryonic
development Neph3 expression is restricted to forebrain, cerebellum anlage, pancreas and is lacking in kidney as well. (C,D) Immuno precipitation of brain lysates was used to confirm knock-out of NEPH2 and NEPH3 using the targeting strategies out-lined in Fig. 1 G,H. (E) RT-PCR of isolated mouse podocytes and mouse brain of Nphs1, Neph1, Neph2 and Neph3. Nphs1, Neph1 and Neph3 can be detected in isolated mouse podocytes of 8 week old animals while Neph2 mRNA is absent. (F) On a protein level also NEPH3 is absent in isolated mouse glomeruli, while it can be detected faintly in mouse brain.

Supplementary Figure 3: Residual slit diaphragms in mouse and human Nphs1 deletion show altered slit width and distance to the GBM. (A,D) Murine Nphs1 deletion leads to narrower and more apically located remnant SDs (17% open SDs, width: n=32 from 3 Nphs1 +/- animals vs n=312 from 3 wild-type animals; distance to GBM: n=13 from 3 Nphs1 +/- animals vs. n=94 from 3 wild-type animals; Student’s t-test). (B, E) In mice with Neph1 deletion, slit width was only very slightly reduced compared with wild-type animals while distance to the GBM was significantly increased (30% open SDs, width: n=188 from 3 Neph1 +/- animals vs n=243 from 3 wild-type animals; distance to GBM: n=120 from 3 Neph1 +/- animals vs. n=98 from 3 wild-type animals; Student’s t-test). (C,F) Similarly, in human patients with Fin major mutations remnant SDs were narrow and located in apical distance to the GBM (width: n=77 from 3 Fin major patients; distance to GBM n=46 from 3 Fin major patients).

Supplementary Figure 4: Residual slit diaphragms in human pathology specimen with congenital nephrotic syndrome. (A-D) In approximately 5-10% of SDs, residual slit diaphragms are still apparent in both fetal and infant tissue from
patients carrying *Fin major* mutations in *NPHS1*. Example of a kidney specimen of a *Fin major/major* fetus (*NPHS1* -/- without residual function) showing residual SD like cell-cell contacts (yellow arrows in overview A, C and details B, D). (E, F) In a nephrectomy specimen (E) of another *Fin major/major* patient at the age of 2 years we were able to indeed demonstrate NEPH1 immunoreactivity (F yellow arrows) using Immuno–EM.

**Supplementary Figure 5:** *Neph2*<sup>-/-</sup> and *Neph3*<sup>-/-</sup> mice do not exhibit any overt phenotype. (A-F) Compared with control (A, D) animals *Neph2*<sup>-/-</sup> (B, E) and *Neph3*<sup>-/-</sup> (C, F) animals show normal light microscopic and TEM appearance. (G, H) Neither *Neph2*<sup>-/-</sup> (G) nor *Neph3*<sup>-/-</sup> (H) 6 month old adult constitutive animals (black columns) do display any proteinuria compared with wild type animals (white columns; at least 6 - *Neph2*<sup>-/-</sup> or 8 – *Neph3*<sup>-/-</sup> animals per group were tested).

**Supplementary Figure 6:** NEPHRIN is absent from chicken glomerula. (A-F) NEPHRIN cannot be detected by immunofluorescence in chicken glomerula (A – ZO1 green ,B – NPHS1 red, C - MERGE, blue Hoe 33342) whereas NEPH1 can be easily and specifically detected in chicken podocytes (D – ZO1 green, E – NEPH1 red, F – MERGE, blue Hoe 33342). (G-I) The same holds true for PODOCIN and ZO-1 which can both be detected in podocytes within the chicken glomerulum (G – ZO1 green, H – PODOCIN red, I - MERGE, blue Hoe 33342). (J-L) WT-1, one of the main transcription factors active in mammalian podocytes, is also expressed in chicken (J - ZO-1 green, K - WT-1 red, I - MERGE, blue Hoe 33342).
Methods

Animals

All animal experiments were conducted according to the NIH Guide for the care and use of Laboratory animals as well as in accordance with the German law for the welfare of animals, and were approved by local authorities (Regierungspräsidium Freiburg G-09/23, G-10/100, X12/06J and X13/04J). Mice were housed in a SPF facility with free access to chow and water and a 12 h day/night cycle. Breeding and genotyping were done according to standard procedures. Urinary albumin and creatinine were measured using a fluorimetric albumin test kit (Progen, PR2005, Heidelberg, Germany) or enzymatic colorimetric creatinine kit (LT-SYS, Lehmann, Berlin, Germany) following the manufacturers’ instructions.

We generated a Nphs1 floxed mouse with loxP sites flanking exons 1B-5 using commercial support by Ozgene (Bentley, WA, Australia). The targeting vector included three loxP sites, one in the 5’-UTR of exon 1B, one in the 5’-UTR of Exon 1A and the third flanking the PGK-neo selection cassette downstream of exon 5. The complete targeting vector was then screened by sequencing and restriction enzyme digestions. Neph1 animals were generated by Genoway (Lyon, France) targeting Exons 12-15 of mouse Neph1 in a 9.7 kB mouse genomic DNA fragment in a C57Bl/6 BAC clone, which was recombinated into 129Sv/Pas ES cells. Recombination was confirmed using PCR and Southern blot. Male floxed mice for Nphs1 and Neph1 were crossed with female Sox2Cre deleter mice to obtain heterozygous constitutive knockout mice(1). Null mice were obtained by heterozygous breeding and genotyping was performed using standard procedures. We generated conditional knockout mice of the Neph2 and Neph3 loci by
homologous recombination in 129 Sv/J (129S7/SvEvBrd) embryonic stem cells.

Briefly, exon 2 of the endogenous Neph2 locus was replaced by a cDNA consisting of exon 2-16 flanked by loxP sites that was followed by an internal ribosomal entry site (IRES) to enable expression of beta-galactosidase from the bacterial lacZ gene. The Neph3 locus was targeted by inserting loxP sites into exon 1 upstream of the start codon and into the intronic sequence between exon 2 and exon 3. Cre-mediated recombination excises parts of the 5′-UTR and the coding sequence, including the regions encoding the start codon and the signal peptide, of the Neph3-transcript.

Gt(Rosa)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J mice were purchased from JaxLab (Bar Harbour, Massachusetts, USA) (2), hNPHS2Cre mice were a generous gift of MJ Möller (University of Aachen, Department of Nephrology, Aachen, Germany).

**Human archive material**

The use of human kidney samples was accepted by the local ethical committee (Hospital for Children and Adolescents, Helsinki, Finland). One sample was derived from an aborted fetus and the other was a nephrectomy specimen obtained from a 2 year old boy. Both patients carried a two base pair deletion after nucleotide 121 (nt121delCT), resulting in a stop codon shortly after the signal peptide of NPHS1 leading to a *Fin major* phenotype.

**Morphological analysis**

Kidneys were perfusion fixed in 4% phosphate buffered paraformaldehyde, embedded in paraffin and further processed for PAS (Periodic Acid–Schiff) staining. For ultrastructural analysis kidneys were also fixed in 4% phosphate buffered paraformaldehyde. Samples were postfixed in 1% osmium tetroxide in the same buffer for 1 h and stained *en bloc* in 1% uranyl acetate in 10% ethanol for 1 h,
dehydration in ethanol, and embedded in LX112 (Fisher-Scientific, Schwerte, Germany). Semithin sections were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined in a Jeol JEM 1200EX electron microscope (JEOL, Eching, Germany). For SEM mouse and chicken kidneys were perfused through the renal artery with phosphate-buffered saline (PBS, 0.9% NaCl in 10mM phosphate buffer, pH7.4) followed by paraformaldehyde (4%) lysine (75mM) periodate (10mM) fixative in 0.15M sucrose, 37.5mM sodium phosphate (modified PLP) and post-fixed overnight at 4°C in the same fixative. 200μm thick vibratome sections were cut and dehydrated in a series of graded ethanol solutions. Ethanol and baskets containing vibratome sections were placed in a critical point drying apparatus (Baltec, Wetlar, Germany), the samples were purged with cold liquid CO2 at elevated pressure, and then brought to supercritical pressure and temperature for incubation and equilibration. Then the pressure was slowly reduced, while maintaining supercritical temperatures. After the bleeding process was completed, dried samples were mounted onto placeholders with sticky pads, sputter-coated with gold and examined using a scanning electron microscope (FEI, Hillsboro, OR, USA).

Western blot and immunofluorescence
Tissues were glass-glass-homogenized in lysis buffer (containing either 20mM CHAPS and 1% Triton X-100 [WB shown in Suppl. Figure 1] or RIPA Buffer containing: 50mM Tris/HCl pH 7,5, 1 mM EGTA, 1 mM EDTA, 1% (w/v) Triton X-100, 0,1 % (w/v) SDS, 50 mM NaF, 150 mM NaCl, 0,5% (w/v) Na-Deoxycholate, 0,1 % (v/v) 2-mercaptoethanol, 1mM Na-Orthovanadate, Roche Ultra complete proteinase inhibitor cocktail and Roche Phospho-STOPP as indicated by the manufacturer (Roche, Mannheim, Germany) and ddH2O ad final volume; 15μl lysis
buffer per 1 mg of tissue were used [WB shown in Suppl. Figure 2]). After centrifugation (1000xg, 5min, 4 °C), the supernatant was recovered and the protein concentration was determined by DC Protein-Assay (Bio-Rad, Munich, Germany). Samples were heated after addition of 2x Laemmli buffer (including 100 mM DTT) at 42°C for 30 min. Equal amounts of protein (80μg per lane) were separated on SDS page. HRP coupled 2nd antibodies and ECL in combination with a conventional x-ray system (films: Fuji, Tokio, Japan; developer: AGFA, Mortsel, Belgium) were used to detect western blot bands. For immunofluorescence kidneys were frozen in OCT compound and sectioned at 5μm (Leica Kryostat, Wetzlar, Germany). The sections were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS), blocked in PBS containing 5% BSA + 5% Normal Donkey Serum (Jackson Immuno Research, Suffolk, UK) and incubated for 45 min with primary antibodies as indicated. After several PBS rinses, fluorophore-conjugated secondary antibodies (Life Technologies, Darmstadt, Germany) were applied for 30 min. Images were taken using a Zeiss fluorescence microscope equipped with a 20x and 63x water immersion objective (Zeiss, Oberkochen, Germany).

**Antibodies**

We generated a NEPH1 peptide-antibody against mNEPH1 aa 767-788 by immunizing a rabbit with the corresponding peptide coupled to Keyhole Limpet Hemocyanin using Freud’s complete adjuvant on d1 and Freund’s incomplete adjuvant on d20, d30 and d40. From d61 a boost was given every 15 days. The final bleed was performed on d130 after a positive immunoreactive testbleed on d120, and the serum was affinity purified against the immunogenic peptide used (Pineda Antikörper, Berlin, Germany). The following other antibodies were used: guinea pig anti-NEPHRIN (gp-NP2; Progen, Heidelberg, Germany), mouse anti-ZO1 (33-9100;
In situ hybridization

Mouse kidney postnatal day 1 RNA served to clone fragments of coding and 3'-UTR of mouse \textit{Neph1} using One-Step PCR Kit (Qiagen, Heidenheim, Germany). PCR fragments were inserted into \textit{pBluescript (SK+)} vector (Invitrogen, Carlsbad, CA) using \textit{SpeI} and \textit{XhoI} restriction sites. For mouse \textit{Nephrin}, \textit{Neph2} and \textit{Neph3} mouse kidney postnatal day 1 RNA served to clone PCR fragments using One-Step PCR Kit (Qiagen). PCR fragments were inserted into \textit{pBluescript (KS-)} vector (Invitrogen, Carlsbad, CA) using \textit{NotI} and \textit{MluI} restriction sites. \textit{pBluescript} Vector was linearized and digoxigenin-(DIG)-labeled antisense riboprobes were generated using T7-RNA-polymerase (Roche, Mannheim, Germany); for Neph1, T3 was used (Roche). For paraffin section ISH, slides were progressively rehydrated and permeabilized with proteinase K for 5 min. After prehybridization (20 min), hybridization with DIG-UTP probes took place overnight in standard saline citrate (SSC; pH 4.5; containing 50% formamide) at 68 °C. Specimens were then incubated with alkaline phosphatase-conjugated anti-DIG Fab fragments (Roche, Mannheim, Germany) at a dilution of 1:3000 for 2 h at room temperature. Alkaline phosphatase was detected using chromogenic conversion of BM Purple (Roche). Slides were then progressively dehydrated in xylol, and mounted. The following primers were used:
Isolation of Mouse Glomeruli and Podocytes

We essentially used the same method as described previously (5). Briefly, kidneys were dissected together with the abdominal aorta and transferred into dishes filled with 37°C prewarmed Hank’s buffered salt solution (HBSS). Each kidney was perfused slowly through the renal artery with 4 ml 37°C warm bead solution and 1 ml bead solution plus enzymatic digestion buffer [containing: collagenase 300 U/ml (Worthington, Collagenase Type II, USA), 1 mg/ml pronase E (Sigma P6911, Germany) DNase I 50 U/ml (Applichem A3778, Germany)]. Kidneys were minced into 1 mm³ pieces using a scalpel. After addition of 3 ml digestion buffer they were incubated at 37°C for 15 min on a rotator (100rpm). The solution was pipetted up and down with a cut 1000µl pipette tip every 5 min. After incubation all steps were performed at 4°C or on ice. The digested kidneys were gently pressed twice through
a 100 μm cellstrainer and the flow through was washed extensively with HBSS. After spinning down, the supernatant was discarded and the pellet resuspended in 2 ml HBSS. These tubes were inserted into a magnetic particle concentrator and the separated glomeruli were washed twice. Glomeruli were resuspended in 2 ml digestion buffer and incubated for 40 min at 37°C on a thermomixer shaking at 1400/min. During this incubation period the glomeruli were sheared with a 27G needle at 15 min, and mixed by pipetting twice at 5, 10, 15, 20 and 25 min using a glass pipette. Podocytes were loosened at 10, 20, 30 min by vortexing once. After 40 min the solution was vortexed three times and the digestion result controlled by fluorescence microscopy. Samples were put on a magnetic particle concentrator again to eliminate beads and glomerular structures void of podocytes. The supernatant was pooled and the magnetic particles discarded. The cell suspension (2 ml) was sieved through a 40 μm pore size filter on top of a 50 ml Falcon tube, rinsed with 10 ml of HBSS. Cells were collected by centrifugation at 1500 rpm for 5 min at 4°C, resuspended in 0.5 ml of HBSS supplemented with 0.1% BSA plus DAPI (1μg/ml). To separate GFP-expressing (GFP+) and GFP-negative (GFP-) cells, glomerular cells were sorted with a Mo-Flo cell sorter (Beckman Coulter) with a Laser excitation at 488nm (Power 200 mW) and a sheath pressure of 60 PSI. Cells were kept at 4°C before entering the FACS machine and thereafter, while temperature during the sorting procedure (approx. 3 min) was 22°C. Only viable (DAPI negative) cells were sorted (laser excitation 380nm, power 80 mW).

RT PCR of **Neph1, Neph2, Neph3** and **Nphs1**

Under RNase-free conditions RNA was extracted from mouse brain and isolated mouse podocytes with the chloroform/phenol method and DNase digested at the end of the preparation process. The RT reaction was performed using dNTPs (Promega,
Mannheim, Germany), random primers (Invitrogen, Karlsruhe, Germany), MMLV Reverse Transcriptase (Promega, Mannheim, Germany) and RNase out (Invitrogen, Karlsruhe, Germany) following the instructions of the manufacturers. 40ng of RNA was used for each reaction. RT-PCR was done using the Taq DNA Polymerase Kit from Invitrogen (Karlsruhe, Germany). The following primers were used:

\[
\begin{align*}
\text{mNeph1s} & \quad \text{CTGCCACCATCATTTGGTTC} \\
\text{mNeph1as} & \quad \text{GTGCTGACATTGGTGCTCCC} \\
\text{mNeph2s} & \quad \text{GATGCTGTCTTCCAGCTGTGCGT} \\
\text{mNeph2as} & \quad \text{CCCAGCATCCTCTTGGCGGAC} \\
\text{mNeph3s} & \quad \text{CCGCAACCGGCTTAGGAAGGGA} \\
\text{mNeph3as} & \quad \text{GCTGCACCAGCCACAATCCG} \\
\text{mNphs1s} & \quad \text{GGACTGGTTCGTCTTGTCGT} \\
\text{mNphs1as} & \quad \text{TCAAAGCCAGGTTTCCACTC}
\end{align*}
\]

Resulting products were visualized using a 2% Agarose Gel and sequenced to prove specificity.

**Structural modeling of NEPHRIN and NEPH1**

For mouse NEPHRIN databases (uniprot Q9QZS7, NCBI: NP_062332.2) predict 8 N-terminal immunoglobulin type C2 domains and one C-terminal fibronectin type III domain. A tenth Ig domain (between Ig6 and Ig7, aa650-753) was predicted here with sequence alignments of all mouse NEPHRIN Ig domains and with the use of PHYRE2 (6).
For all 10 separate domains of mouse NEPHRIN and 3 domains of mouse NEPH1 (uniprot Q80W68, ncbi: NM_019459.2) PHYRE2 was used to obtain optimal templates. The following PDB structures were used to generate a model from the N-to C-terminus of mouse NEPHRIN: 2yuv, 1vca, 2eo9, 1mfb, 2wwm, 1lc1, 2wwm, 2v5m, 1ie5, 2ed8 and Neph1: 3b43 (Ig3-4), 2cry (Ig5). For NEPH1 the Ig domains 1 and 2 of the mouse crystal structure were used (pdb: 4ofd).

Using the Swiss pdb-viewer missing loops were closed, single domains were connected and aligned in a linear order to obtain the longest possible protein structure. The arrangement of the Ig domains in the crystal structure of NEPH1 was not changed but two missing loops were generated. Also the c-terminal unfolded region was maximally spread and linked with a transmembran a-helix generated by the Swiss pdb-viewer. Both intracellular domains were omitted. The structures were visualized using PyMOL.

Electron tomography

For electron tomography (ET), kidney tissue was prepared as described above. Thick sections (300nm) were cut from Epon blocks using an EM UC7 ultramicrotome (Leica AG, Wetzlar, Germany) and transferred onto formvar-coated copper slot grids. 10nm colloidal gold particles (CMC, Utrecht, The Netherlands) were applied to the sections as fiducial markers. Samples were analyzed using a Tecnai F30 transmission electron microscope (FEI, Eindhoven, The Netherlands) operated at 300 kV. Tilt series were acquired between -60° and +60° using an increment of 1° and a magnification of 9400x on a US 4000 CCD camera (Gatan Inc., Pleasanton, CA, USA). Afterwards tilt series were aligned and reconstructed using IMOD software (7). For visualization of the filtration barrier, Amira software (FEI, Eindhoven, The Netherlands) was used.
Cryosectioning and cryo electron tomography

Isolated kidneys of mT/mG*hNphs2Cre mice (8) were first perfused with 1ml of 20% Dextran in PBS followed by a second perfusion with 1ml 25% Dextran containing 10nm Protein-A-Gold (CMC, Utrecht, The Netherlands). The tissue was cut into small pieces and placed in gold-plated copper carriers type 662 with 0.2μm recess (Wohlwend, Sennwald, Switzerland) filled with 20% Dextran. Carriers were closed with flat gold-plated type 663 carriers (Wohlwend) coated with Lecithin prior to high-pressure freezing with HPM-010 (Abra, Abrafluid AG, Widnau, Switzerland). Carriers were observed in a Linkam Cryostage on a Zeiss LSM 700 Confocal Microscope (Zeiss, Oberkochen, Germany) to identify fluorescent glomeruli. Distances of glomeruli to tissue edges and/or carrier edges were measured. Based on those measurements glomeruli were target trimmed and cryosectioned in an Ultracut FC6 (Leica, Wetzlar, Germany). Sections were transferred to C-flat CF 2/1 Grids and attached with a Haug Charging System (Haug, Leinfelden-Echterdingen, Germany). Grids were mounted in FEI autogrids and analyzed in a Titan Krios TEM (FEI company, Eindhoven, The Netherlands) operated at 300kV. Tomography tilt series between (-60° to +60°) were acquired using a nominal defocus of -5μm and a total dose < 60 e-/Å² on a US 4000 CCD camera (Gatan Inc, Pleasanton, CA, USA).

Statistics

Data are expressed as mean ± SEM. Statistical comparisons were performed using the GraphPad Prism Software Package 6.02 (GraphPad Software, La Jolla, CA. USA) with two-tailed Student’s t-test, Wilcoxon test or ANOVA including respective corrections where indicated. Differences with p values below 0.05 were considered significant.
### Supplementary Information References


Supplemental Table I

<table>
<thead>
<tr>
<th>Domain</th>
<th>Protein</th>
<th>PDB-code</th>
<th>sequence identity (%)</th>
<th>sequence similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEPHRIN</td>
<td>Ig1</td>
<td>2nd Immunoglobulin Domain of Slow Type Myosin-Binding Protein C</td>
<td>2yuv</td>
<td>25,3</td>
</tr>
<tr>
<td></td>
<td>Ig2</td>
<td>VCAM-1</td>
<td>1vca</td>
<td>20,4</td>
</tr>
<tr>
<td></td>
<td>Ig3</td>
<td>fifth ig-like domain from human Roundabout homo1</td>
<td>2eo9</td>
<td>26,2</td>
</tr>
<tr>
<td></td>
<td>Ig4</td>
<td>IGG1-LAMBDA SE155-4 FAB (LIGHT CHAIN)</td>
<td>1mfb</td>
<td>22,8</td>
</tr>
<tr>
<td></td>
<td>Ig5</td>
<td>Obscurin like Protein</td>
<td>2wwm</td>
<td>18,8</td>
</tr>
<tr>
<td></td>
<td>Ig6</td>
<td>Icam-1</td>
<td>1lc1</td>
<td>21,6</td>
</tr>
<tr>
<td></td>
<td>inter Ig6-7</td>
<td>Obscurin like Protein</td>
<td>2wwm</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Ig7</td>
<td>Dscam</td>
<td>2v5m</td>
<td>23,6</td>
</tr>
<tr>
<td></td>
<td>Ig8</td>
<td>NCAM Ig3</td>
<td>1ie5</td>
<td>30,6</td>
</tr>
<tr>
<td></td>
<td>Fibronectin</td>
<td>Netrin Rezeptor</td>
<td>2ed8</td>
<td>29,5</td>
</tr>
<tr>
<td>NEPH1</td>
<td>Ig1</td>
<td>Neph1</td>
<td>4ofd</td>
<td>95,5</td>
</tr>
<tr>
<td></td>
<td>Ig2</td>
<td>Neph1</td>
<td>4ofd</td>
<td>95,5</td>
</tr>
<tr>
<td></td>
<td>Ig3</td>
<td>Titin</td>
<td>3b43</td>
<td>21,4</td>
</tr>
<tr>
<td></td>
<td>Ig4</td>
<td>Titin</td>
<td>3b43</td>
<td>21,4</td>
</tr>
<tr>
<td></td>
<td>Ig5</td>
<td>Kirrel3</td>
<td>2cry</td>
<td>63,8</td>
</tr>
</tbody>
</table>

Summary of the respective templates used for modelling each individual Ig fold in NEPHRIN and NEPH1, respectively.
Supplemental Figure 2

A. Neph2 E13.5

B. Neph3 E13.5

C. +/+ flox/flox del/+ del/del

D. +/+ flox/flox del/+ del/del

E. Nephrin (416bp) Neph1(448bp) GAPDH(334bp)

F. NEPH1 NEPH2 NEPH3 TUBULIN

E. podocyte pod. (-) RT brain neg. ctrl. podocyte pod. (-) RT brain neg.ctrl. podocyte pod. (-) RT brain neg. ctrl.
Supplemental Figure 4

A

B

C

D

E

F

anti-NEPH1
Supplemental Figure 5

A - C: Images of kidneys from wild type, Neph2 const. KO, and Neph3 const. KO mice. Each panel shows a section of a kidney with labeled measurements of 200 nm.

D - F: Electron microscopy images of the same regions as in A - C, showing a higher magnification of the tubules and glomeruli.

G - H: Graphs showing the albumin/creatinine ratio for wild type (wt) and KO mice for Neph2 and Neph3. The data points are represented by circles, and the bars indicate the mean and standard deviation.