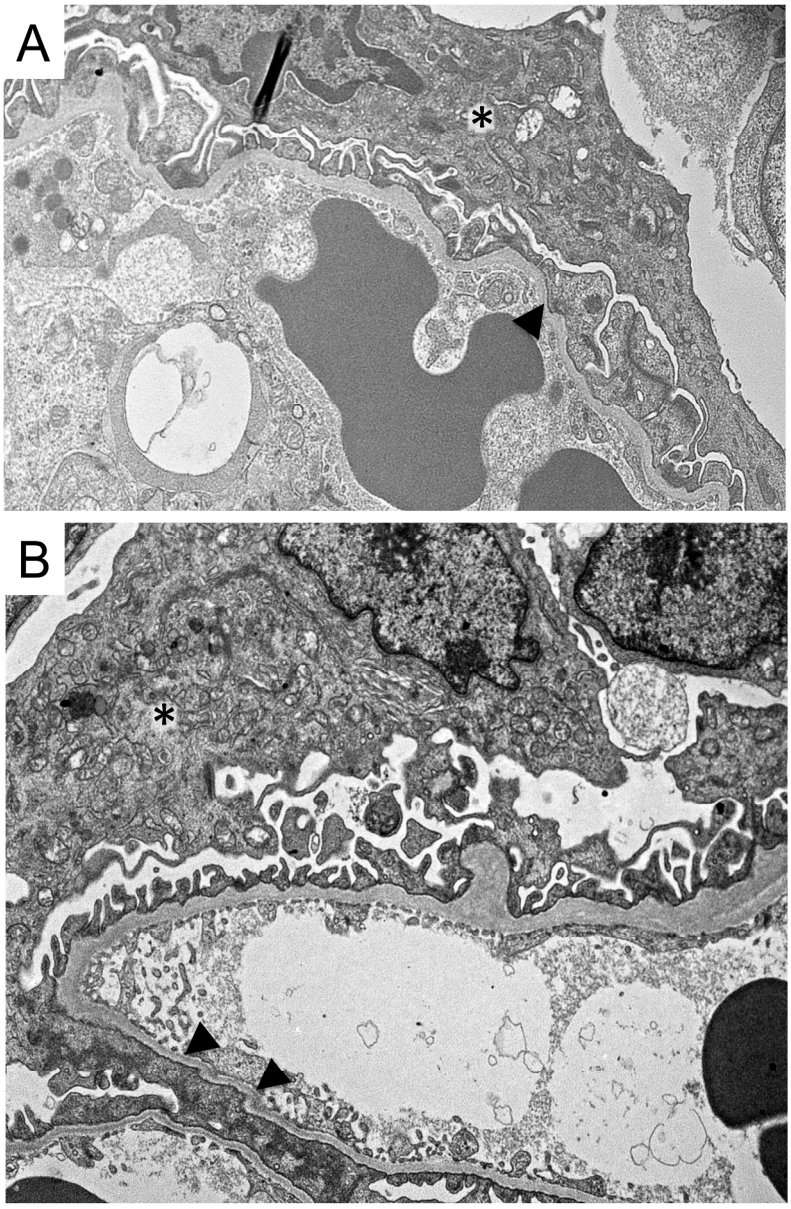

Supplementary Figure 1. Representative transmission EMs of injured podocytes in UNX/DOCA-salt treated floxed control (A) and podoRac1-/- (B) mice. Each displays segmental foot process widening and effacement (arrowheads) and accumulation of lysosomal and/or autophagic elements (asterisks). For comparison, a sham treated wild-type mouse is shown (C).
180x275mm (300 x 300 DPI)

Supplementary methods:

Isolation of glomeruli

Glomeruli were isolated for molecular analysis using magnetic beads as previously described.[1] Briefly, anesthetized mice were perfused via left ventricular access with 40 ml of warm (38C) phosphate buffered saline (PBS) containing approximately $8x10^7$ Dynabeads M-450 Epoxy (Invitrogen, Carlsbad, CA) at 70-mmHg pressure. Kidneys were removed, minced, and digested in Collagenase A (Roche, Nutley, NJ) at 37C for 30 minutes. After digestion, placing on ice, and sieving through a 100μM strainer, glomeruli were captured by means of a strong magnetic field. Glomeruli were washed several times with ice-cold PBS. Glomeruli for RNA isolation were picked manually by hand under the microscope and stored in RNAlater (Applied Biosystems/Ambion, Austin, TX) at -20C until RNA isolation. For protein preparation, glomeruli were briefly centrifuged and PBS was removed. Protein samples were stored at -80C until glomerular proteins were extracted in RIPA buffer (phosphate-buffered 0.9% NaCl containing 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate Complete (Roche, Nutley, NJ) protease inhibitors for Western Blot analysis.

Western Blot

Protein content of the samples was determined using a DC Protein Assay (Bio-Rad, Hercules, CA). Equal amounts of protein were diluted in loading buffer, boiled and separated by SDS-PAGE. Proteins were blotted onto a PVDF-membrane (Millipore, Billerica, MA) and membranes were blocked in 3% skim milk in TBS-Tween-20 (TBS-T) for 45 minutes at room temperature. Membranes were incubated with primary antibodies for 1 hour at room temperature or over night at 4°C. After three washing steps with TBS-T the membranes were incubated with HRP-conjugated secondary antibody. Immune complexes were visualized using Western Lightning ECL (Perkin Elmer, Waltham, MA). The following primary antibodies were used: anti-Cdc42(Cell Signaling Technology, Boston MA), anti-Rac1(Cytoskeleton Inc., Denver CO), anti-cofilin (Cell Signaling Technology, Boston MA), anti-phospho-cofilin (Cell Signaling Technology, Boston MA), and anti-actin (Sigma Aldrich, St. Louis, MO).

Urine protein analysis by SDS-PAGE and albumin to creatinine ratio by ELISA

Mouse serum albumin (2 μg) and 4 μl of urine of wild-type and podocyte specific Rac1 and Cdc42 knockout mice were heated to 95C for 5 minutes and separated by 12% SDS-PAGE followed by Coomassie blue staining. Urine albumin and creatinine was determined with the Albuwell M Test and Creatinine Companion kit (Exocel, Philadelphia, PA) according to manufacturer's instructions.

Histology and morphometry

At time of sacrifice, mice were perfused with 4% paraformaldehyde in PBS. Kidneys were removed and either placed in 10% buffered Formalin solution (Fisher Scientific, Pittsburgh, PA) or 4% glutaraldehyde. After 24 hours, samples were respectively stored in 70% ethanol or Sorensen's buffer prior to further processing. For light microscopic analysis, formalin fixed tissue was paraffin embedded using routine methods and 4 μm sections were cut and stained with periodic acid-schiff (PAS). For ultrastructural examination, glutaraldehyde fixed tissue was post-fixed in 1% osmium tetroxide and routinely embedded in Epon. Ultrathin sections (70-80 nm) were stained with uranyl citrate and lead citrate and examined with a transmission electron microscope (Phillips CM-100). Samples were also analyzed with an AMRAY 1910 Field Emission Scanning Electron Microscope.

Filtration slit frequency was determined on glomerular images at x7900 magnification by counting the number of podocyte filtration slits (slit diaphragms) across peripheral capillary loops and dividing by the length of the underlying glomerular basement membrane. On average, approximately 1300 filtration slits (range 768-2325) were counted per animal.

Real-time PCR analysis

RNA was isolated from mouse glomeruli using the RNeasy Kit with DNase digestion (Qiagen Valencia, CA). After reverse transcription using hexanucleotide primers (Roche, Nutley, NJ) and Superscript RT (Invitrogen, Carlsbad, CA), Real-time PCR was performed on each sample using TaqMan ABI 7900 Sequence

Detection System (Applied Biosystems, Foster City, CA). mRNA expression levels of target and housekeeping genes were quantified using the ΔΔCt technique.[2] Water was used for negative control. The following Applied Biosystems gene expression assays were used: nephrin (Mm01176615_g1), podocin (Mm01292252_m1), synaptopodin (Mm03413333_m1), and cyclophilin A (Mm00510343_m1).

Immunoflourescence staining

Paraffin sections of 4 μm thickness were used for immunofluorescence studies. Sections were de-waxed in Xylene and subsequently rehydrated. Tissue was permeabilized for 15 minutes in 0.3% Triton X-100 in PBS. Antigen retrieval was performed by microwaving the samples 10 minutes in Antigen Unmasking Solution (Vector Laboratories, Burlingame, CA). Sections were blocked with 10% goat serum in PBS with 0.15% Triton X-100. Primary antibody incubations were carried out over night at 4C. Three washing steps with PBS were performed and samples were incubated with fluorescence labeled secondary antibodies and DAPI as counterstain. Sections were washed and mounted in Mowiol. Primary rabbit polyclonal Podocin and Nephrin antibodies were a kind gift from Dr. L. Holzman (University of Pennsylvania, Philadelphia, PA). Monoclonal Synaptopodin antibody was purchased from GenWay (San Diego, CA). Alexa-Fluor 488- and 568-conjugated goat anti-rabbit and anti-mouse antibodies were used as secondary antibodies (Invitrogen, Carlsbad, CA). Negative controls were performed by substituting buffer for the primary buffer. Immunofluorescence images were obtained with a Leica DMIRB inverted microscope and an RT slider digital camera (model 2.3.1; Diagnostic Instruments) and collected with SPOT software (version 4.5; Diagnostic Instruments).

Protamine sulfate induced injury model

Perfusion of mouse kidney was performed as described previously.[3] Briefly, adult podoRac1-/- and floxed control Rac1-fl/fl mice, separate from those described above, were anesthetized with pentobarbital and maintained at 37°C using a heating pad. Perfusion was carried at a constant pressure of 70-mmHg through the vena cava with HBSS for 2 minutes followed by 2 mg/ml protamine sulfate (Sigma-Aldrich, St.Louis, MO) dissolved in HBSS for 15 minutes or HBSS alone. Kidneys were fixed by perfusion with 3% paraformaldehyde

in HBSS before further processing for transmission electron microscopy as described above.

References

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3. Verma R, Kovari I, Soofi A*, et al.* Nephrin ectodomain engagement results in Src kinase activation, nephrin phosphorylation, Nck recruitment, and actin polymerization. *J Clin Invest* 2006; **116:** 1346-1359.

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