Supplemental Material

Supplemental Figure Legends.

Figure S1.

A, B. Termini on proteins mutated in podocytopathies. Protein domain models were generated by EMBL-SMART algorithm including PFAM annotations. Termini were mapped against the respective uniprot ID. S1 A demonstrates termini on slit diaphragm proteins. TM, transmembrane domain.S1 B demonstrates termini on cytoskeletal proteins. C. Podocin was immunopurified from human glomeruli using the C-terminal antibody (Sigma), and both lysates and immunoprecipitates were separated by gel electrophoresis. One half of the gel was stained with Coomassie and the band running at approximately 34 kDa was subjected to LC-MS/MS analysis (Red box). The other half of the gel was subjected to immunoblotting followed by immunostaining for podocin and detection with a horseradish coupled antibody. The IgG bands run at 55 and 25 kDa. The lower diagram demonstrates the sequence coverage of human podocin (383 amino acids). Green represents high-confident peptides (Peptide FDR <0.01), yellow medium confident peptides (Peptide FDR <0.02). Peptides predominantly cover the C-terminus, but not the very distal N-terminus, consistent with a cleaved podocinproteoform running at 34 kDa.

Figure S2.

A. Analysis of PAN injury in podocytes by immunofluorescence. Podocytes were stained with anti-Vimentin and Phalloidin recognizing F-ACTIN. This experiment was unrelated to the TAILS study. A rearrangement of actin cytoskeleton (stress fibers) to the cell periphery was observed. The picture shows a representative result of rarification of intracellular stress fibers (arrows) with PAN, 24h 50 µg/mL.B. XTT uptake assay of PAN podocytes incubated with PAN. Podocytes were treated with PAN (c= 50 µg/mL) and with cytochalasin D (100nM) for 24h. XTT uptake was measured after 2h incubation. * = significance with $p<0.05$ in a one-way anova with Tukey's post test. C. Workflow of quantitative TAILS by stable isotope dimethyllabeling[1] in cultured podocytes to investigate protease perturbation in glomeruli. Both treated and control podocytes are lysed, and the proteins are digested with trypsin. Then, dimethyl labeling with either a heavy (stable-isotope labeled) or a light peptide modification was performed, and the protein lysates were pooled before measurement by nLC-MS/MS. This procedure allows quantification of both treatment groups in a single sample with minimal variability. Please see methods for more details.

Figure S3.

A. Histogram of cleavage sites by known proteases in human podocytes. Cathepsins were the most frequent known proteases involved in the observed cleavage sites. Cleavage sites of MAP12 were statistically overrepresented in the dataset. (Bonferroni-corrected Fisher's exact test, $p<0.05$) as compared to the until now discovered "terminome". B. Mapping of N-termini from cultured human podocytes to N-termini from mouse glomeruli. The distance between the observed human and the next mouse homologous mouse N terminus is depicted. Distances were determined using the CPHOS program [2]. Most cleavage sites mapped on common proteins were conserved and occurred at homologous (identical) positions within the protein.

Figure S4.

Cumulative histograms of cytoskeletal, mitochondrial and adhesion proteins. Distributions of cytoskeletal (A, uniprot keyword: cytoskeleton), of microtubule (B, keyword microtubule), of adhesion proteins (C, keyword adhesion) or of mitochondrial proteins (D, uniprot keyword: mitochondrion) were compared with the other proteins, respectively. Distributions were tested for differences by using a two-sided Kolmogorov-Smirnoff-Test. All distributions were significantly different ($p<0.001$).

Figure S5.

Overview of Neo-termini (cleavage sites, A) and immunoblots (B)for MYH9 protein in the presence and absence of PAN.

Figure S6.

Immunoblot analysis of glomeruli from WT1 heterozygous knockout mice (WT1KO/WT) and control (wildtype) littermates using an N-terminal Vimentin antibody.

Supplemental Tables

S1: Analysis of TAILS in Glomeruli.

The data contains an overview of N-termini discovered from mouse glomerular proteins. The table contains multiple tabs. Tab1 is a userfriendly overview of identification data. Tab 2 contains mass spectrometry features of termini. Tab 3 contains termini annotation using TopFinder. For further technical and mass-

spectrometry related details, and raw files, please consult the deposited .RAW files and primary search outputs (please see methods for repository access).

S2: Analysis of TAILS in cultured podocytes.

The table contains an overview of N-termini discovered from human cultured podocyte proteins and the quantification of PAN effect. The table contains multiple tabs. Tab1 is a userfriendly overview of quantification data. Tab2 contains mass spectrometry featuresof termini. Tab 3 contains termini annotation using TopFinder. For further technical and mass-spectrometry related details, and raw files, please consult the deposited .RAW files and primary search outputs (please see methods for repository access).

S3: Quantitative TAILS analysis in Glomeruli.

The table contains an overview of N-termini discovered from rat glomeruli proteins and the quantification of PAN effect. For further technical and mass-spectrometry related details, and raw files, please consult the deposited .RAW files and primary search outputs (please see methods for repository access).

References

1. Boersema PJ, Raijmakers R, Lemeer S, Mohammed S, Heck AJR: Multiplex peptide stable isotope dimethyl labeling for quantitative proteomics. *Nat. Protoc.* 4: 484–494, 2009

2. Zhao B, Pisitkun T, Hoffert JD, Knepper MA, Saeed F: CPhos: a program to calculate and visualize evolutionarily conserved functional phosphorylation sites. *Proteomics* 12: 3299–3303, 2012