**Supplemental material**

**Identification of an altered matrix signature in kidney ageing and disease**

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**Figure S1: Glomerular localization type IV collagen isoforms.** Wildtype mouse cryosections were stained for the different type IV collagen alpha chains (1-6) and the podocyte marker podocin. Col4a1 and Col4a2 localise to the glomerulus, specifically to the glomerular basement membrane and Bowman’s capsule basement membrane. Col4a3 and Col4a4 were detected in the GBM. Col4a5 was detected in the GBM and Bowman’s capsule. Col4a6 localized to Bowman’s capsule. Scale bar is 40 μm.

**Figure S2: Characterisation of *Col4a5-/-* mice.** (a) Cryosections of *Col4a5-/-* and *WT* mice were immunolabeled for Col4a5 and the podocyte marker podocin. Scale bar is 40 μm. (b) Albumin and creatinine concentration was measured in urine from *Col4a5-/-* and *WT* mice and albumin-creatinine ratios were calculated. N=5 mice per group. (c) Paraffin sections from *Col4a5-/-*and wild type mice were prepared and H&E stained or (d) Picro Sirius Red staining was performed. Right panel shows Picro Sirius stains exposed to polarised light. White arrowhead highlights organised collagen fibers in Bowman's capsule. Scale bar is 40 μm. (e) Percentage glomerulosclerosis and interstitial fibrosis and tubular atrophy (IFTA) was scored by a kidney pathologist. Between 62 and 118 glomeruli were examined per mouse. Pooled data from n=3 wild typemice and n=4 *Col4a5-/-* mice are shown. (f) Kidney samples from *Col4a5-/-* and wild type mice were fixed and transmission electron microscopy (TEM) performed. Yellow arrowhead highlights an area of thickened basement membrane. (g) Quantification of (f). Number of foot processes per length of GBM was calculated. GBM thickness was measured from TEM images. Pooled data from n= 3 mice per group.

**Figure S3: Overview of glomerular proteomic data.** (a) Gene Ontology enrichment map analysis of all proteins detected by mass spectrometry with altered abundance (>1.4 fold; *p*<0.1) in *Col4a1+/svc*, *Col4a3-/-*, *Col4a5-/-* mice at all analysed ages compared with aged, matched control mice. Overrepresented biological process terms are presented as nodes. (b) Comparison of wild type mice at 6-8 weeks and 16-18 weeks of age. Mass spectrometry data from matrix enriched protein fractions were filtered for known matrisome proteins. These proteins were mapped onto a merged human, mouse and rat interactome and a protein-protein interaction network of altered mouse matrisome proteins is shown. Nodes represent proteins and edges represent reported protein-protein interactions. Colour represents fold enrichment to datasets, with increased abundance in 16-18 week wild type mice in red and increased abundance in 6-8 week old wild type miceillustrated in blue. Proteins are grouped by function.

**Figure S4: Altered matrisome proteins. (**a) Selected profiles of matrix proteins detected with significantly altered protein abundance in *Col4a1+/svc* and young *Col4a3-/-* and *Col4a5-/-* mouse glomeruli by mass spectrometry. Data are presented as log10 fold change dot plots with the mean and standard deviation shown. (d) Immunofluorescence staining for Col4a1, Col4a2, Col4a3 and Col4a4 protein in both *Col4a5-/-* and *WT* mice. Scale bar is 70 μm.

**Figure S5: Altered matrisome proteins in *Col4a3-/-* mice.** (a,b) Mass spectrometry data from matrix enriched protein fractions were filtered for known matrisome proteins. These proteins were mapped onto a merged human, mouse and rat interactome. Nodes represent proteins and edges represent reported protein-protein interactions. Colour represents fold enrichment to datasets, with increased abundance in *Col4a3-/-* relative to control illustrated in red and decreased abundance in *Col4a3-/-* relative to control illustrated in blue. Proteins are grouped functionally. (a) Early time point, (b) late time point.

**Figure S6: Altered matrisome proteins in *Col4a5-/-* mice.** (a,b) Mass spectrometry data from matrix enriched protein fractions were filtered for known matrisome proteins. These proteins were mapped onto a merged human, mouse and rat interactome. Nodes represent proteins and edges represent reported protein-protein interactions. Colour represents fold enrichment to datasets, with increased abundance in *Col4a5-/-* relative to control illustrated in red and decreased abundance in *Col4a5-/-* relative to control illustrated in blue. Proteins are grouped functionally. (a) Early time point, (b) late time point.

**Figure S7: Human kidney data enrichment analysis.** (a) Gene Ontology enrichment map analysis of all proteins detected by mass spectrometry with altered abundance (>1.4 fold; p<0.1) in aged human glomerular samples compared with young human glomerular samples. Overrepresented biological process terms are presented as nodes, colour represents Bonferroni-corrected p value, the lower the p value the more intense the node colour. Orange represents terms increased with age and blue represents terms decreased with age. Edge weight represents overlap between the proteins in the connected nodes. (b) Gene Ontology enrichment map analysis of all proteins detected by mass spectrometry with altered abundance (>1.4 fold; p<0.1) in aged tubulointerstitial samples compared with young tubulointerstitial samples. Overrepresented biological process terms are presented as nodes. Orange represents terms increased with age and blue represents terms decreased with age.

**Figure S8: Defining the tubulointerstitial matrisome.** Tubulointerstitial matrix was isolated from human kidney samples and subjected analyzed by mass spectrometry. (a) Mass spectrometry data from matrix enriched protein fractions were filtered for known matrisome proteins. These proteins were mapped onto a merged human, mouse and rat interactome. Protein-protein interaction network of human tubulointerstitial matrix proteins. (b) Principal component analysis of tubulointerstitial matrix mass spectrometry data. (c) Interaction network demonstrating enrichment of matrix proteins to either glomerular (green) or tubulointerstitial (orange) compartments. Dashed lines represent interactions, nodes represent proteins. Node size relates to connectivity within the network.

**Figure S9: Identification of new tubulointerstitial matrix proteins.** (a) Immunofluorescence of human kidney sections for Col4a1-6 (green). DAPI stain was used to highlight nuclei. Scale bar is 50 μm. (b) Percentage of TI matrix proteins identified in the Human Protein Atlas database and their localization to different kidney compartments. (c) Venn diagram classifying the identified TI matrix proteins into different groups according to their localisation to the tubulointerstitium, tubular basement membrane (TBM), glomerular matrix (mesangium, glomerular basement membrane and Bowman’s capsule basement membrane) and blood vessel matrix in the Human Protein Atlas database.

**Figure S10: Defining the human kidney extracellular matrix.** Mass spectrometry data from matrix enriched protein fractions were filtered for known matrisome proteins. These proteins were mapped onto a merged human, mouse and rat interactome. Protein-protein interaction network shows combined tubulointerstitial and glomerular matrix fractions colour coded and grouped based on category of matrisome protein.

**Figure S11: One-hop interactors of the upregulated matrix signature proteins**. Gene ontology enrichment analysis was performed on the proteins that directly interact with the identified up regulated matrix signature proteins. Three clusters were identified and the enriched terms presented as bar charts with Benjamini significance presented on the x axis.

**Supplemental Videos**

**Video 1: SV1-Col4a1-16weeks.** Serial block face-scanning electron microscopy was performed on a *Col4a1+/SVC* mouse (16 weeks) kidney sample and 3 dimensional ultrastructure of the glomerular filtration barrier was reconstructed using imod software. Glomerular BM is shown in yellow, podocyte cell body in light blue, nuclei of parietal epithelial cells are shown in pink and bowman’s capsule basement membrane is shown in green.

**Video 2: SV2-Col4a3-16weeks.** Serial block face-scanning electron microscopy was performed on a *Col4a3-/-* mouse (16 weeks) kidney sample and 3 dimensional ultrastructure of the glomerular filtration barrier was reconstructed using imod software. Glomerular BM is shown in yellow, podocyte cell body in light blue, bowman’s capsule basement membrane is shown in green, thickness of Bowman’s capsule is highlighted through dark blue lines.

**Video 3: SV3-Col4a3-28weeks.** Serial block face-scanning electron microscopy was performed on a *Col4a3-/-* mouse (28 weeks) kidney sample and 3 dimensional ultrastructure of the glomerular filtration barrier was reconstructed using imod software. Glomerular BM is shown in yellow, podocyte cell body in light blue, bowman’s capsule basement membrane is shown in green and dark blue lines highlight thickened Bowman’s capsule.

**Video 4: SV4-Col4a5-16weeks.** Serial block face-scanning electron microscopy was performed on a *Col4a5-/-* mouse (16 weeks) kidney sample and 3 dimensional ultrastructure of the glomerular filtration barrier was reconstructed using imod software. Glomerular BM is shown in yellow, podocyte cell body in light blue, bowman’s capsule basement membrane is shown in green and the thickness of Bowman’s capsule is highlighted through dark blue lines.

**Supplemental Tables**

**Table S1.** Abundance of matrix proteins identified and quantified using Progenesis were categorised as either basement membrane, other structural or ECM associated. A: *Col4a1+/svc,* B: *Col4a3-/-* (young and adult), C: *Col4a5-/-* (young and adult). Progenesis was used to quantify proteins with the Hi-N setting, the three most abundant peptides were used for protein quantification. Anova (p) was extracted from Progenesis.

**Table S2.** Abundance of matrix proteins identified and quantified using Progenesis were categorised as either basement membrane, other structural or ECM associated. A: human glomerular matrix (G) and B: human tubulointerstitial matrix (T) at a range of ages (15, 29, 37, 61, 67 and 69 years).

**Table S3.** Signature matrix proteins were searched for their fold change gene expression levels of indicated disease datasets over control tissue samples. Data from the indicated datasets were extracted at a fold change of +/- 1.5 and p<0.05.

**Table S4.** Nephroseq v5 database was used for analysis of transcript levels of identified down regulated signature proteins.

**Table S5:** Normalized abundance for all primary datasets.