Supplementary Material

Complete Methods

UNx

Mice were anesthetized with ketamine/xylazine and body temperature was maintained with a warmed operation table (Föhr Medical Instruments, Germany), infrared light during recovery and warmed solutions. After deep anaesthesia a left sided flank incision was made. The vasculature of the left kidney was ligated with Mersilene 3-0 (Ethicon, Germany) and the kidney removed. The wound was closed with Vicryl 5-0 (Ethicon, Germany). After regaining consciousness adequate pain relief was given. The explanted kidney was perfused with magnetic beads and used for podocyte isolation.

Bone marrow transplantation

Recipient *mT/mG* wild type mice were lethally irradiated with 900 cGy in two doses given 5 h apart. Bone marrow cells were isolated from femura and tibiae of donor *mG* mice and injected intravenously ($5x10^6$ cells in 100 µl sterile PBS). After irradiation, mice were maintained on antibiotic water containing Enrofloxacin (10 mg/l, Bayer, Germany) for 4 weeks. For the determination of chimerism, peripheral blood cells were stained with Alexa Fluor 647 anti-mouse CD45 (clone 30-F11, BioLegend) and the ratio of mT/mG positive cells was determined via flow cytometry (CyAn ADP, Beckman Coulter).

Genotyping

Tail biopsies were incubated at 95°C in an alkaline lysis reagent (25 mM NaOH, 0,2 mM EDTA), neutralized with 40 mM TrisHCl and subsequently used for PCR with the following primers:

NPHS2.rtTA forward CGC ACT TCA GTT ACT TCA GGT CC TC and reverse GCT TAT GCC TGA TGT TGA TGA TGC; *pPEC.rtTA* forward AAT CGA GAT GCT GGA CAG GCA TCA TAC CCA and reverse GGC ATA GAA TCG GTG GTA GGT GTC TCT CTT; *TetOCre*

forward GCA TAA CCA GTG AAA CAG CAT TGC TG and reverse GGA CAT GTT CAG GGA TCG CCA GGC G; *mT/mG* forward (common) CTC TGC TGC CTC CTG GCT TCT and reverse (mutant) TCA ATG GGC GGG GGT CGT T and (wild type) CGA GGC GGA TCA CAA GCA ATA; *iDTR* forward (common) AAA GTC GCT CTG AGT TGT TAT and reverse (mutant) CAT CAA GGA AAC CCT GGA CTA CTG and (wild type) GGA GCG GGA GAA ATG GAT ATG.

Urinary and serum measurements

Urinary albumin and creatinine were measured with a Microflural Microalbumin Test kit (Progen) and an enzymatic Creatinine PAP kit (Labortechnik) following the manufacturer´s instructions. Albumin-to-creatinine ratio is given as mg albumin/mg creatinine.

Immunofluorescence staining of kidney sections

mT/mG mouse kidneys were perfused and incubated with 4 % PFA in PB at 4 °C for 3 hours. Kidneys were immersed in 15 % sucrose in PBS for 2 h and 30% sucrose overnight, then frozen in OCT compound and sectioned at 6 µm (Leica Kryostat). The sections were counterstained with Hoechst 33342 (Invitrogen). For paraffin embedding, the kidneys were incubated in 4 % PFA for 24 hours and then dehydrated in ethanol. PAS staining was done with 3 µm sections. Antigen retrieval was performed on 6 µm sections with citrate buffer (pH 6.0) for 30 min. Images were taken with a Zeiss fluorescence microscope with Apotome mode or a Zeiss laser scan microscope equipped with a 63x water immersion objective. To determine podocyte cell number WT1-positive cells were counted in >30 glomerular sections per mouse and the mean value of each animal used for statistical analysis.

Immunohistochemical staining of kidney sections

Kidneys were fixed in 4% paraformaldehyde in PBS, embedded in paraffin and sectioned at 4 µm (Leica Microtome). After deparaffinization, rehydration and antigen retrieval in 10mM sodium citrate buffer, sections were blocked with 2% BSA in PBS and incubated overnight with primary antibodies as indicated, followed by peroxidase blocking in 3% H_2O_2 for 10

minutes and incubation with HRP conjugated secondary antibodies (Dako) for 1 hour. DAB (Dako) was applied for 6 minutes. Sections were counterstained with hematoxylin.

Antibodies

The following antibodies were used: rabbit anti-active caspase 3 (AF835, R&D), mouse anti-PCNA (M08979, Dako), guinea pig anti-nephrin (GP-N2, Progen), rabbit anti-WT1 (ab15249, Abcam), mouse anti-desmin (M0760, Dako), rabbit anti-podocin (P0372, Sigma), rabbit anti-GFP (MBL-598, Biozol), rabbit anti-nitrotyrosine (AB5411, Millipore) and guinea pig anti-p62 (GP62-C, Progen). Fluorophore-conjugated secondary antibodies and nuclear staining reagent (To-Pro-3 T3605, Hoechst 33342) were obtained from LifeTechnologies.

Isolation of podocytes

Glomeruli were isolated by Dynabead perfusion as recently described in detail (19, 26). After subsequent digestion to single cells, the cells were fixed in 4% PFA, permeabilized with 0.5% Triton and stained with anti-podocin antibody (P0372, Sigma) labeled with Alexa Fluor 647 (Zenon labeling kit, Invitrogen).

Flow cytometry

Isolated and stained glomerular cells were resuspended in FACS buffer (0.5% BSA, 5 mM EDTA in PBS) and measured with FACS Calibur (BD).

Histological analyses

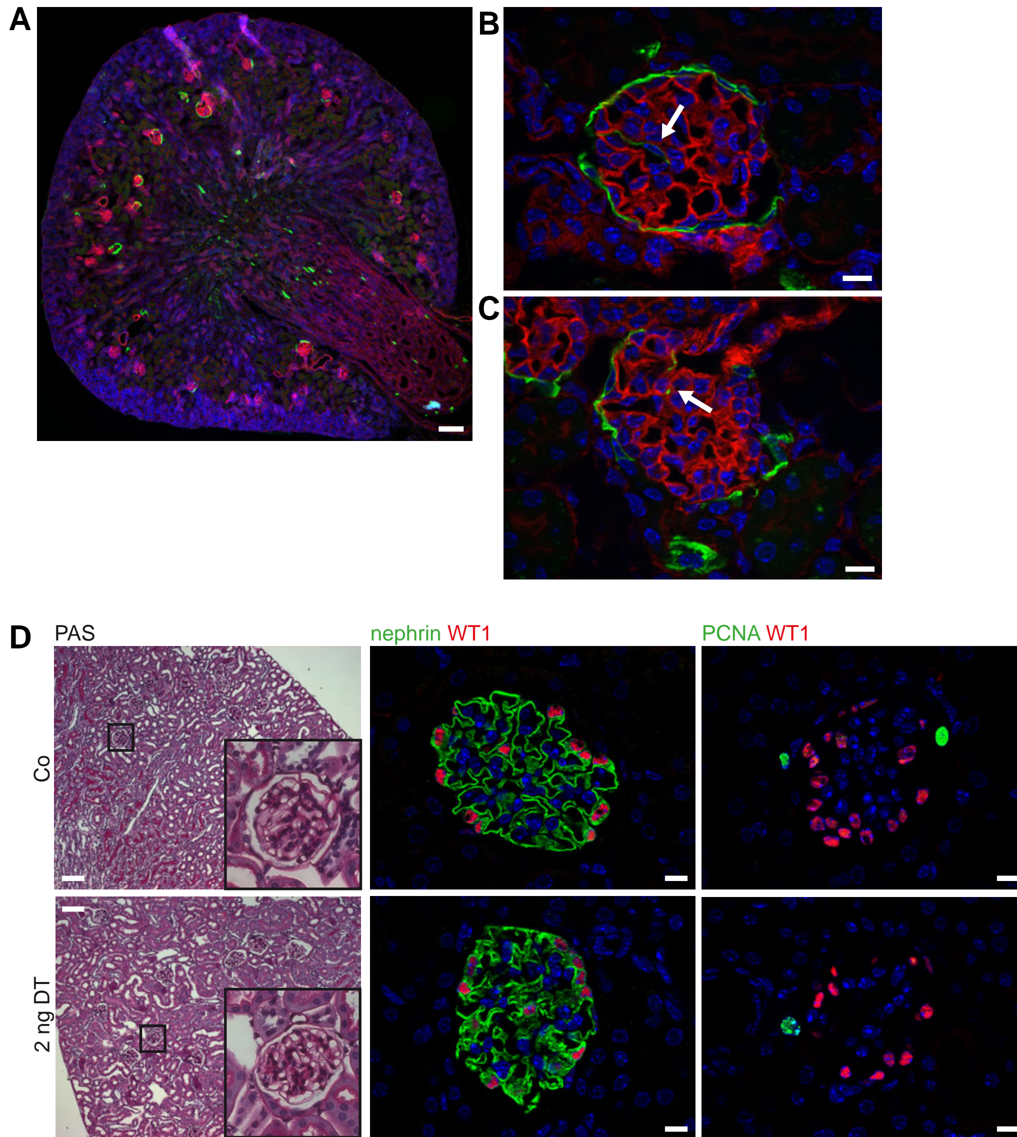
Quantitative stereological analyses of kidney sections were performed as described previously (1). Briefly, the mean glomerular volume (mean $v(Glom)$) was determined from the mean glomerular profile area (mean $A(Glom)$) of ~ 100 systematically sampled glomerular profiles per animal (2),. The physical disector principle was applied for counting podocytes (Q^-) as described, using semithin sections (3, 4). The numerical density of podocytes in glomeruli ($N_V(P/Glom)$) was calculated as the quotient of the sum of Q^- divided by the disector volume. The number of podocytes per glomerulus ($N(P,Glom)$) was calculated

multiplying *NV*(*P/Glom*) and mean *v*(*Glom*). Six glomerular profiles were evaluated (corresponding to 352–500 reference points). All results were corrected for embedding shrinkage (1).

References

1. Herbach, N., Schairer, I., Blutke, A., Kautz, S., Siebert, A., Goke, B., Wolf, E., and Wanke, R. 2009. Diabetic kidney lesions of GIPRdn transgenic mice: podocyte hypertrophy and thickening of the GBM precede glomerular hypertrophy and glomerulosclerosis. *Am J Physiol Renal Physiol* 296:F819-829.
2. Weibel, E.R., and Gomez, D.M. 1962. A principle for counting tissue structures on random sections. *J Appl Physiol* 17:343-348.
3. Hoeflich, A., Weber, M.M., Fisch, T., Nedbal, S., Fottner, C., Elmlinger, M.W., Wanke, R., and Wolf, E. 2002. Insulin-like growth factor binding protein 2 (IGFBP-2) separates hypertrophic and hyperplastic effects of growth hormone (GH)/IGF-I excess on adrenocortical cells in vivo. *Faseb J* 16:1721-1731.
4. Sterio, D.C. 1984. The unbiased estimation of number and sizes of arbitrary particles using the disector. *J Microsc* 134:127-136.

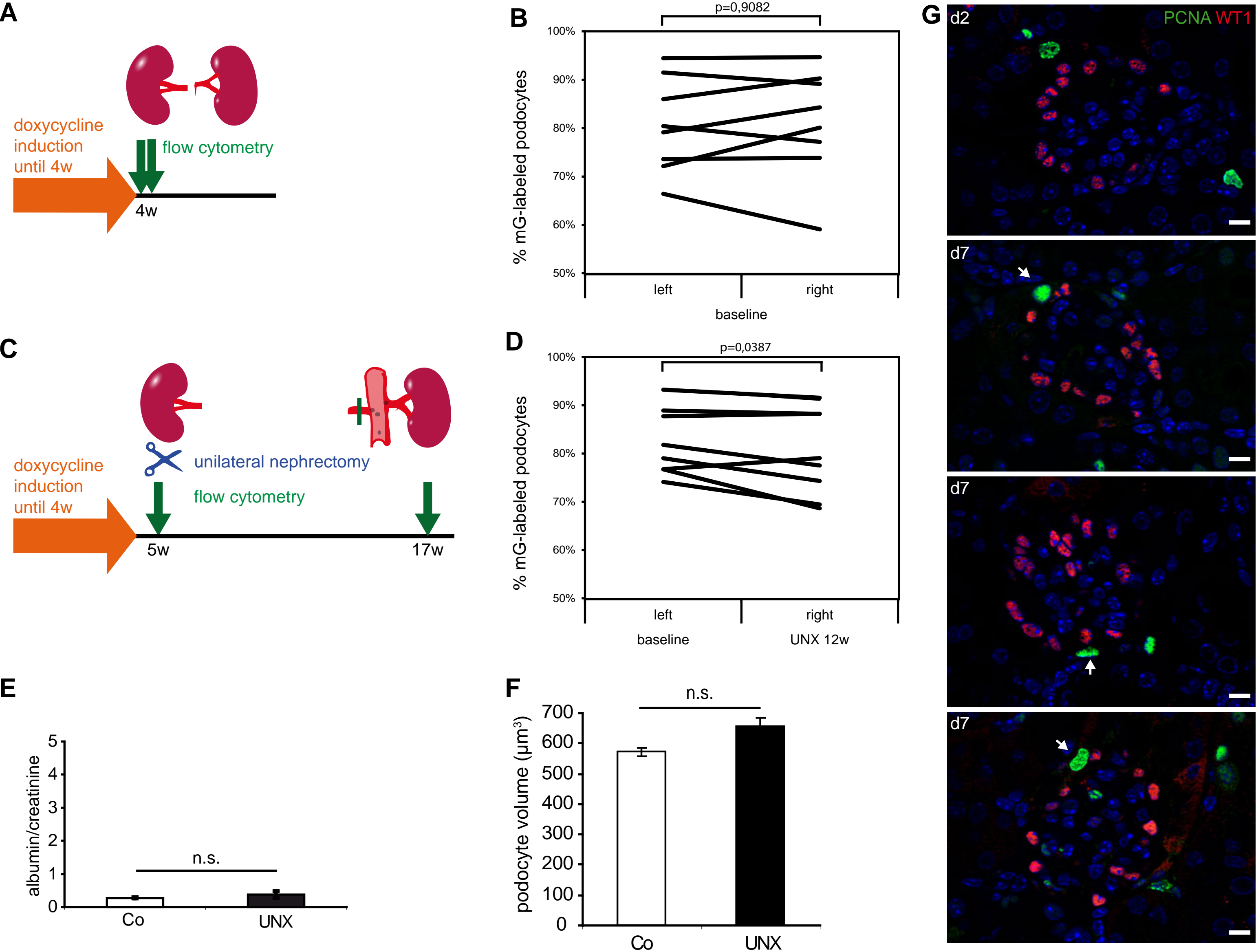
Supplementary Figure 1:



Supplementary Figure 1. PEC induction and diphtheria toxin mediated targeted podocyte ablation

(A) Embryonically induced *hPODXL.rtTA;tetO.Cre;mT/mG* kidney at day 1 after birth (P1) Scale bar, 100 µm. (B and C) 63x magnification of P1 kidney shows expression of mG in parietal epithelial cells around mature glomeruli (arrow: mG-positive podocyte). Scale bar, 10 µm. (D) Four weeks after 2 ng diphtheria toxin injection PAS, nephrin and PCNA staining is indistinguishable from control litter mates, whereas WT1 staining shows reduced numbers of podocytes. Scale bar, 100 µm PAS, 10 µm IF.

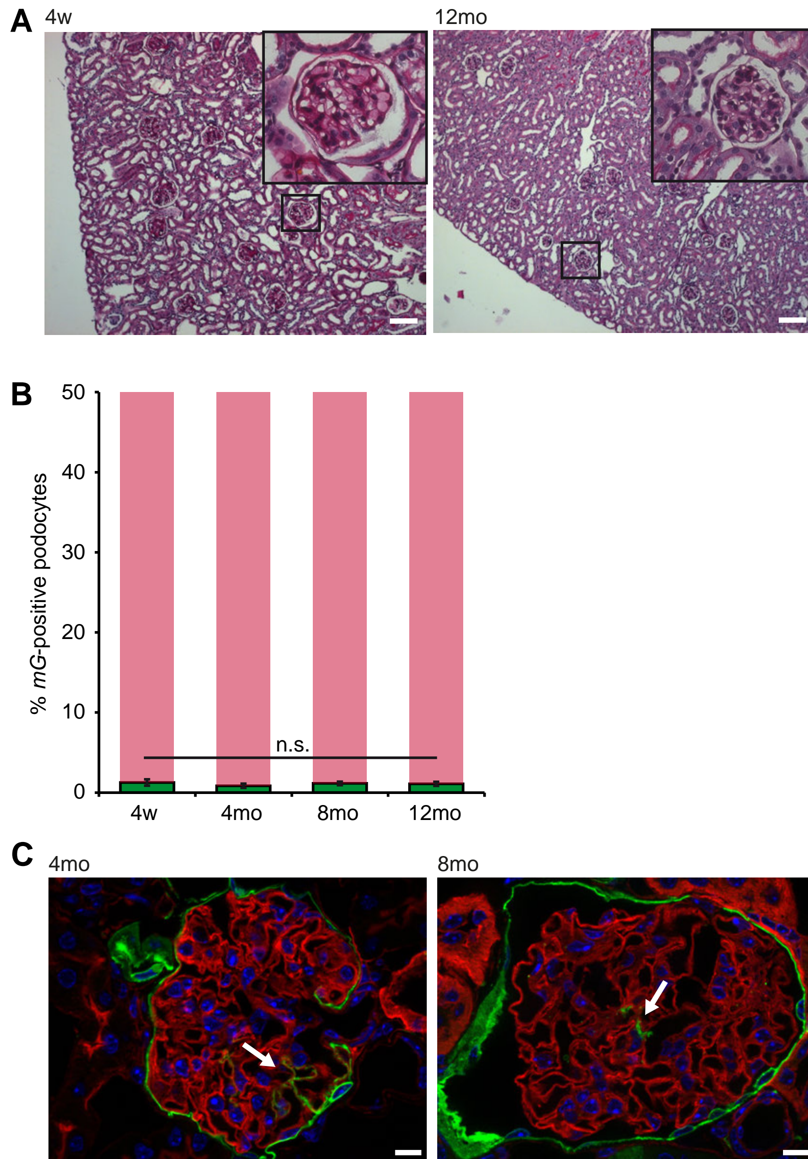
Supplementary Figure 2:



S2. Unilateral nephrectomy

(A) Schematic of comparison of induction levels of the two kidneys of the same mouse. (B) Comparison of induction levels between right and left kidney at the same time point shows no significant directional differences (p=0.9082). (C) Schematic of UNx in *hNPHS2.rtTA;tetO.Cre;mT/mG* mice. (D) Flow cytometry analysis of mG-labeled podocytes 12 weeks after nephrectomy compared to baseline values (p=0.0387). (E) The animals did not develop proteinuria 3 months after nephrectomy (p=0.3207). Error bars, SEM. n.s., not significant. (F) mean podocyte volume. n.s., not significant, p=0.057. (G) PCNA/WT1 staining of kidney sections 2 days or 7 days after unilateral nephrectomy shows activation of PCNA in parietal cells after 7 days (arrows), but no colocalization with WT1 at either time point. Scale bar, 10 µm.

Supplementary Figure 3:

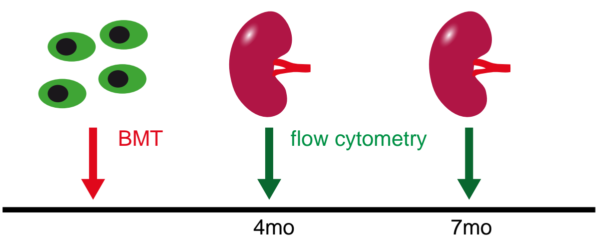


S3. Physiological podocyte turnover during aging: podocytes and PECs

(A) Representative PAS stainings of the renal cortex of *hNPHS2.rtTA;tetO.Cre;mT/mG* mice at 4 weeks and 12 months. Scale bar, 100 µm (B) Flow cytometry analysis of *hPODXL.rtTA;tetO.Cre;mT/mG* mice showed no increase of mG-positive podocytes at 4 months, 8 months and 12 months compared to 4 weeks. Error bars, SEM. n.s., not significant (4w vs. 12mo p= 0,6804). n≥6 per group. (C) mT/mG fluorescent images of 4 months and 8 months points showed labeled parietal cells and rare mG podocytes (arrows). Scale bar, 10 µm.

Supplementary Figure 4:

A



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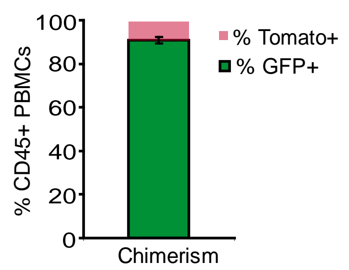

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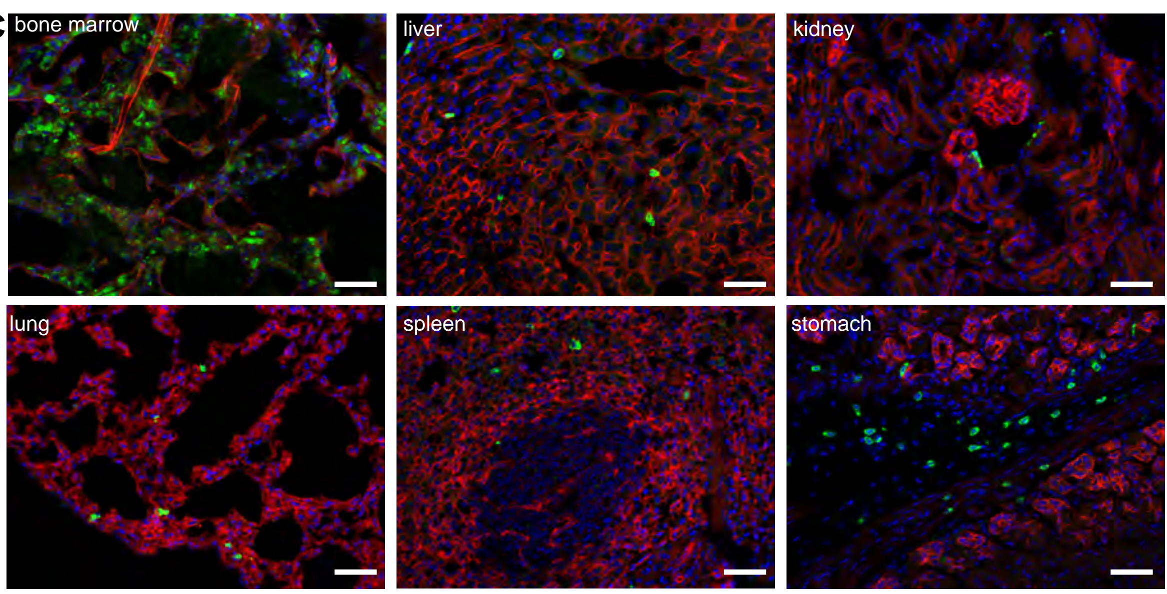

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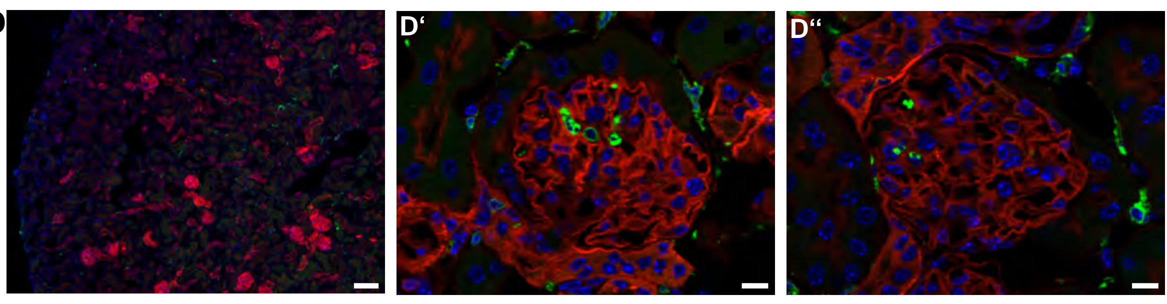

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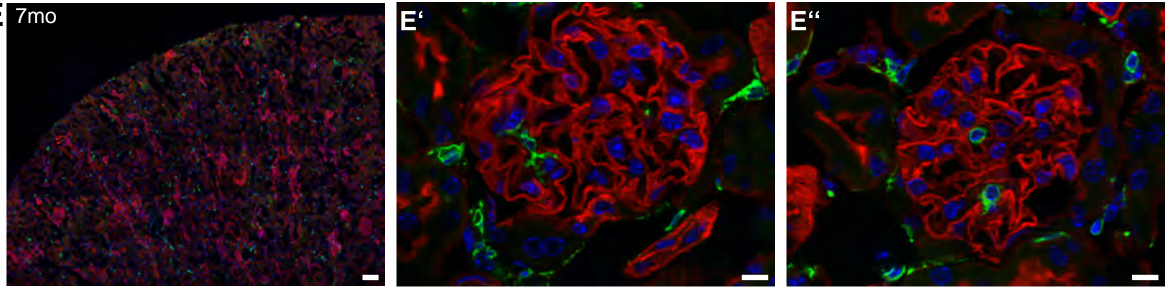

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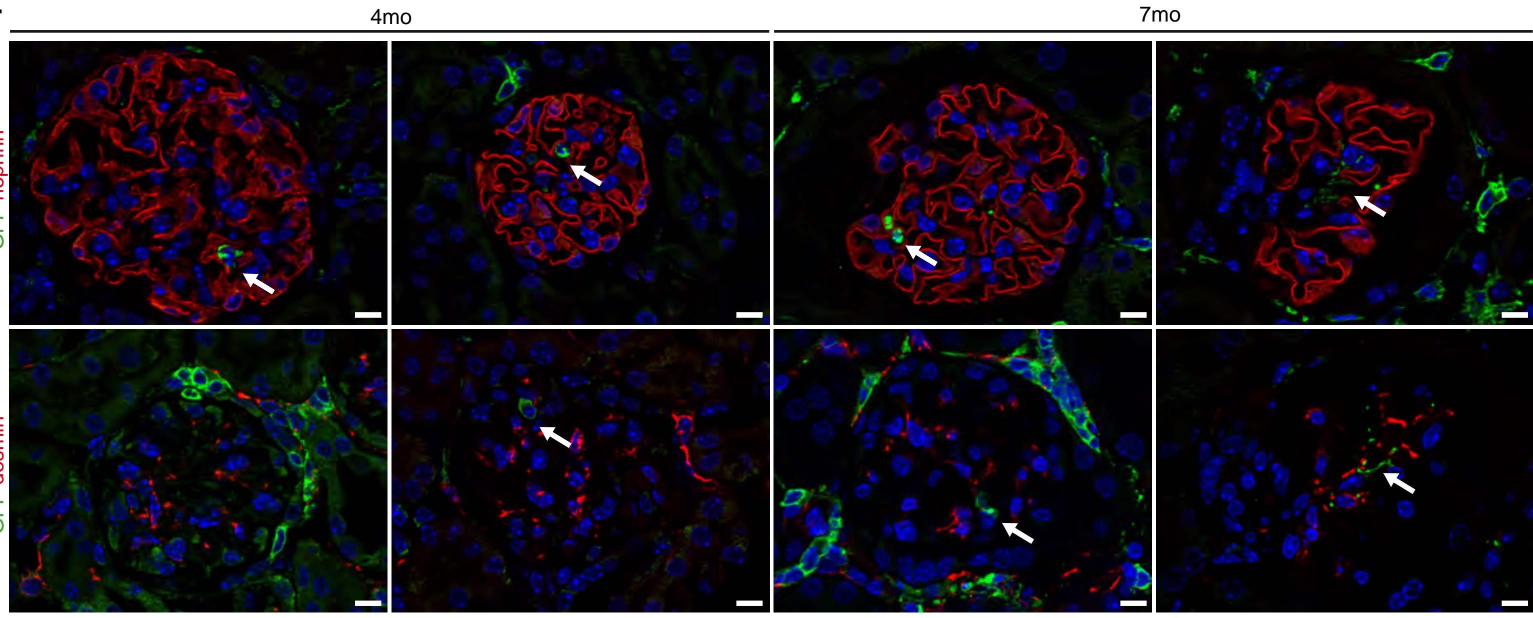
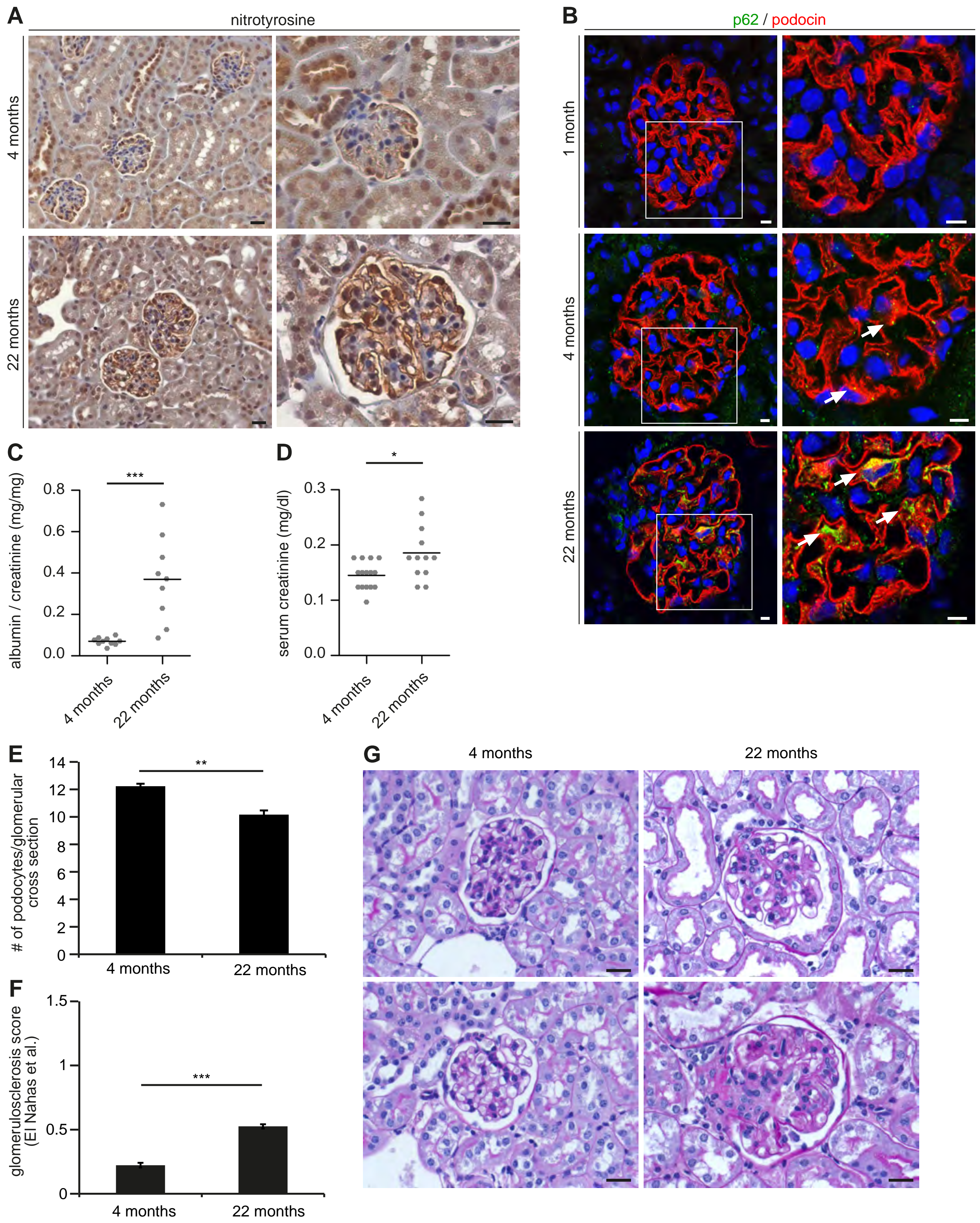


S4. Physiological podocyte turnover during aging: bone marrow cells

(A) Irradiated *mT* wild type mice received mG-labeled donor bone marrow. The kidneys were analyzed 4 months and 7 month after irradiation. (B) After 4 weeks > 89 % of CD45-positive peripheral blood mononuclear cells were mG-labeled. Error bar, SEM. (C) Tissue sections 11 days after bone marrow transplantation (BMT) showed mG-positive bone marrow and single cells in the liver, kidney, lung, spleen and stomach lining. Scale bar, 50 µm. After (D) 4 and (E) 7 months mG-positive cells have increased in the kidney (scale bar 100 µm), but not in the glomeruli (D', D'', E' and E'', scale bar 10 µm). (F) Staining of mG-positive cells in the kidney 4 months or 7 months after bone marrow transplantation did not reveal colocalization with podocyte marker nephrin or mesangial marker desmin. Scale bar, 10 µm. Arrows, mG-labeled glomerular cells.

Supplementary Figure 5:



S5. Accumulation of oxidized proteins and deposition of p62 aggregates in podocytes are hallmarks of kidney aging.

(A) Accumulation of the oxidative stress marker nitrotyrosine in glomeruli of aging mice. Scale bars, 20 µm. (B) Immunofluorescence staining of mouse kidney sections for p62 and the podocyte marker protein podocin reveals accumulation of p62 positive protein aggregates (arrows) in podocytes of aging mice. Scale bars, 5 µm. (C) Significantly increased albuminuria in 22 month old C57BL/6 wild type mice compared to 4 month old C57BL/6 wild type mice ($n>9$, *** $p=0.0003$). (D) Significantly increased serum creatinine in 22 month old C57BL/6 wild type mice compared to 4 month old C57BL/6 wild type mice ($n>12$, * $p=0.020$). (E) The number of podocytes per glomerular cross section at 4 and 22 months (** $p=0.0052$). Error bars, SEM. $n\geq3$, respectively. (F) Glomerulosclerosis score at 4 and 22 months (*** $p<0.0001$). Error bars, SEM. $n\geq3$, respectively. (G) PAS stainings at 4 and 22 months. Scale bars, 20 µm.