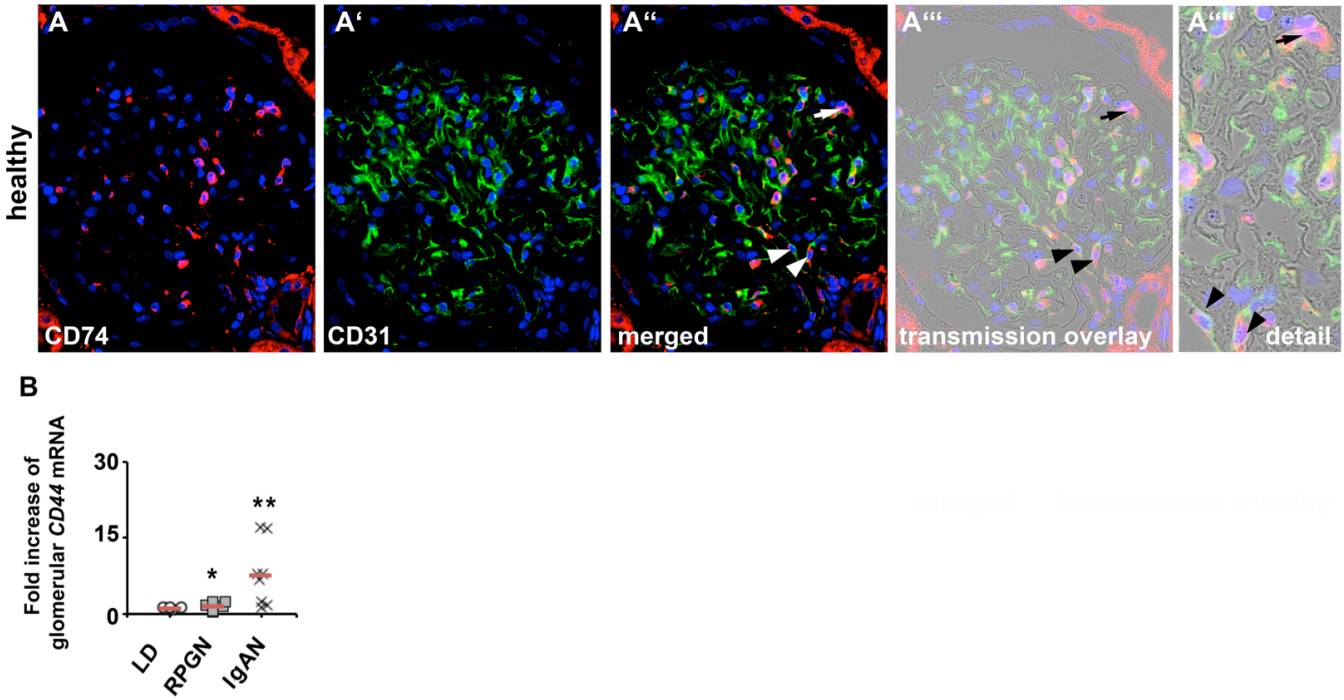
Suppl. Figure 1

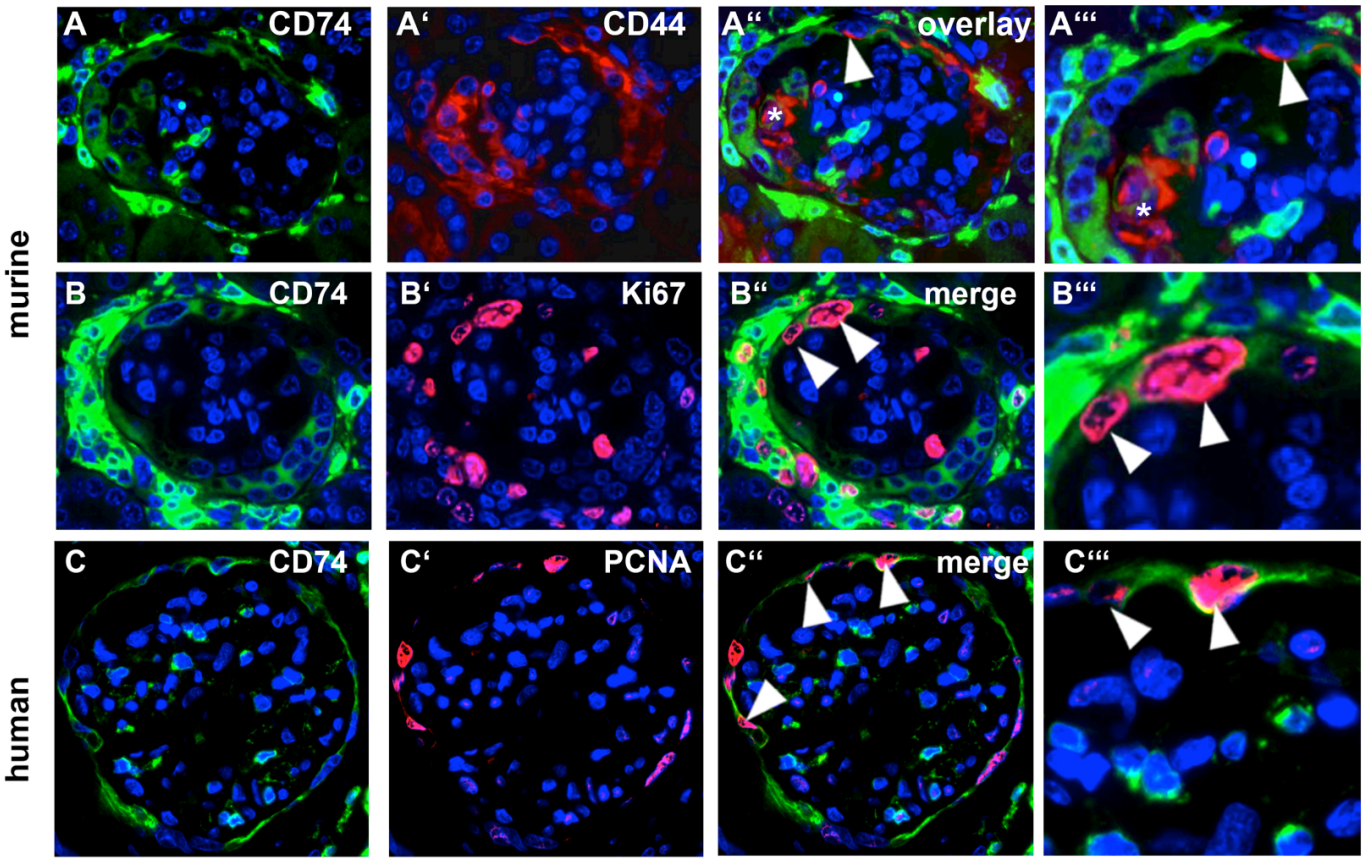


Suppl. Figure 1

Immunofluorescence of CD74 (A) and CD31 (A’) in healthy human kidney suggested a partial co-localization (A”, arrowhead). Overlay with light microscopy (A”’) confirmed the expression of CD74 by podocytes (arrows) and endothelial cells (arrowhead) showed in more detail in A””.

qRT-PCR results (B) obtained from microdissected glomeruli of patients with rapidly progressive glomerulonephritis (RPGN, n=10), IgA nephropathy (IgAN, n=8) and controls (pre-transplant allograft biopsies from living donors (LD, n=6). Compared to controls, a significant up-regulation of *CD44* was found in glomeruli from patients with glomerulonephritides including RPGN and IgAN. mRNA expression levels for each gene are shown as ratios calculated against GAPDH. Each dot represents one patient and red bars represents means. *p<0.05 vs. LD, **p<0.01 vs. LD, ***p<0.001 vs. LD.

Suppl. Figure 2

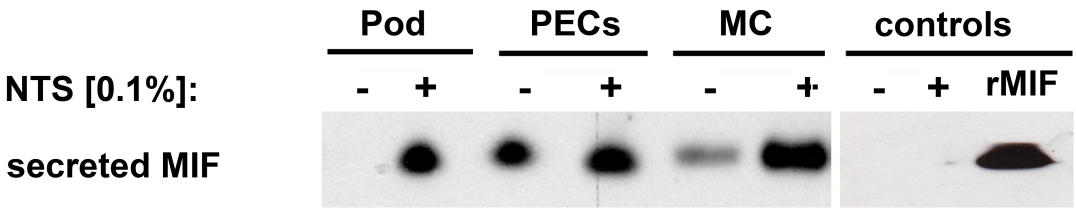


Suppl. Figure 2

Staining of CD74 (A) and CD44 (A’) in murine NTN in consecutive slides supported the co-expression of both receptors on PECs (arrowheads in A” and A”’) and mesangial cells (asterisk), in more detail shown in overlay (A”) and digital enlargement (A”’).

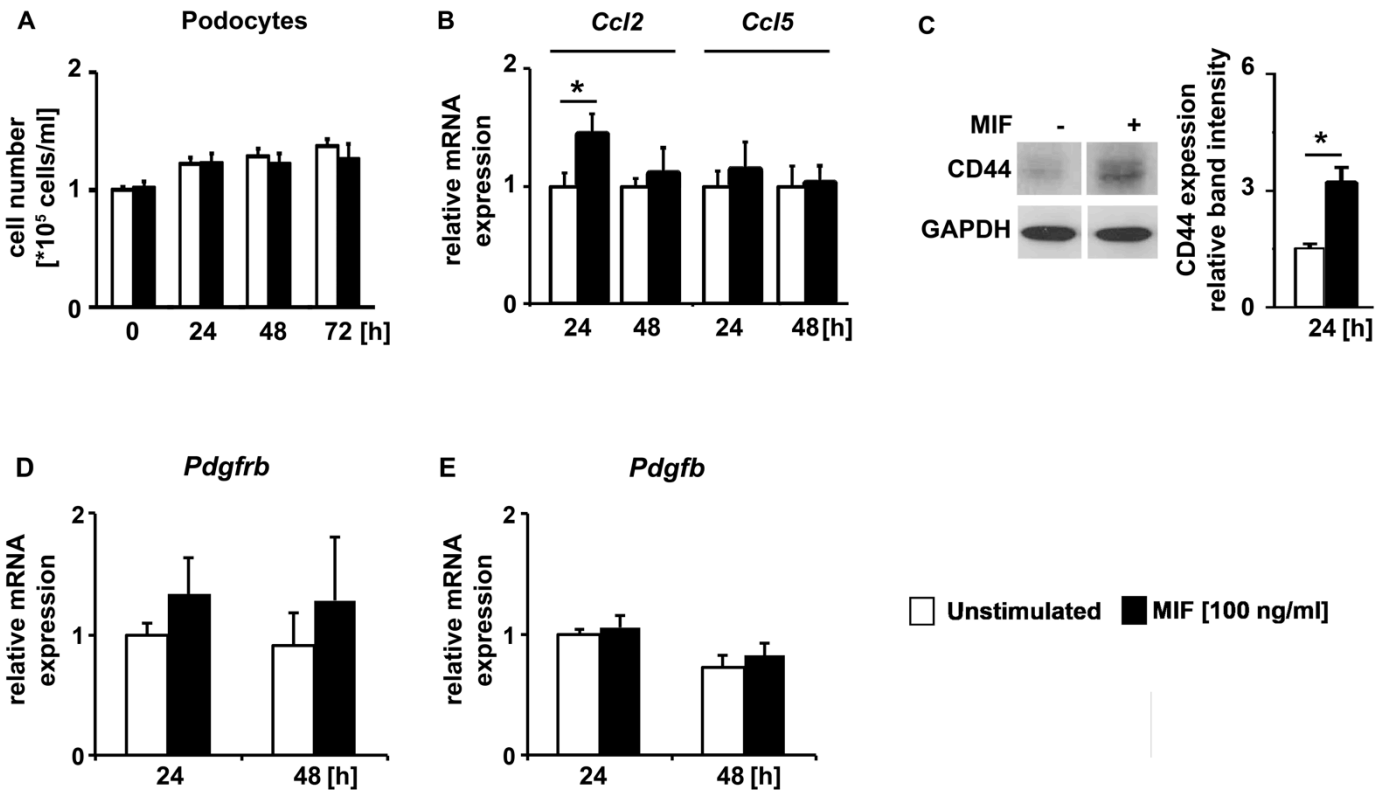
Double immunofluorescence staining for CD74 (green/Alexa488, B) and proliferation marker Ki67 (red/Alexa555, B’) in murine NTN revealed expression of CD74 in proliferating PECs (arrowhead in B” and B”’). In human kidney, CD74 (green/Alexa488, C) and proliferation marker PCNA (red/Alexa555, C’) confirmed expression of CD74 in proliferating PECs (arrowhead in C” and C”’). The last picture is a digital enlargement (B”’ and C”’).

Suppl. Figure 3



Suppl. Figure 3
MIF is secreted by glomerular cells after treatment with nephrotoxic serum.
Primary murine podocytes (Pod), parietal epithelial cells (PECs) and mesangial cells (MCs) were incubated for 24 h with 0.1% nephrotoxic serum (NTS) and cell-free supernatant was taken for TCA precipitation. Podocytes exhibited a strong secretion of MIF upon NTS stimulation. PECs and mesangial cells secreted MIF already in the steady state and even more after NTS stimulation.

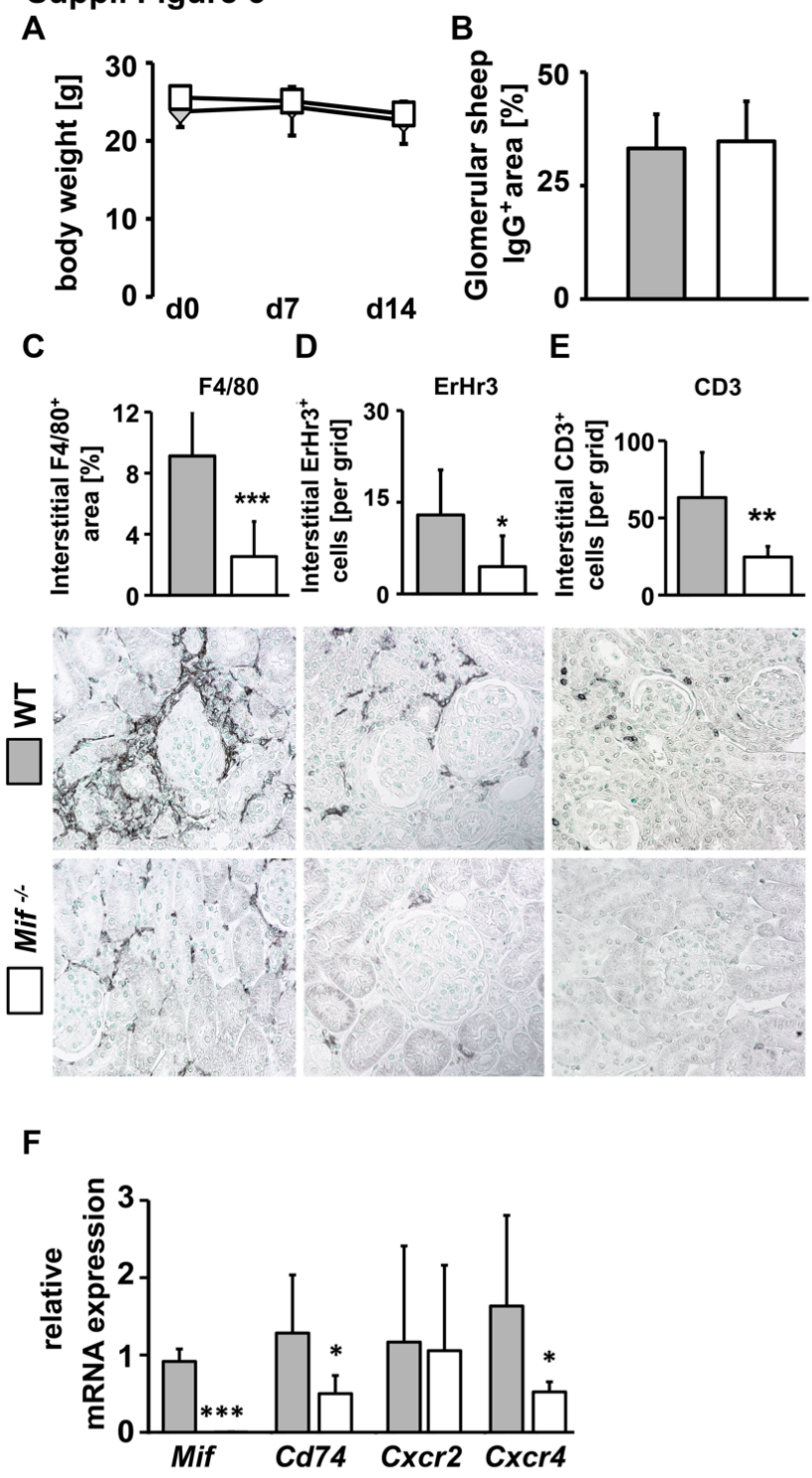
Suppl. Figure 4



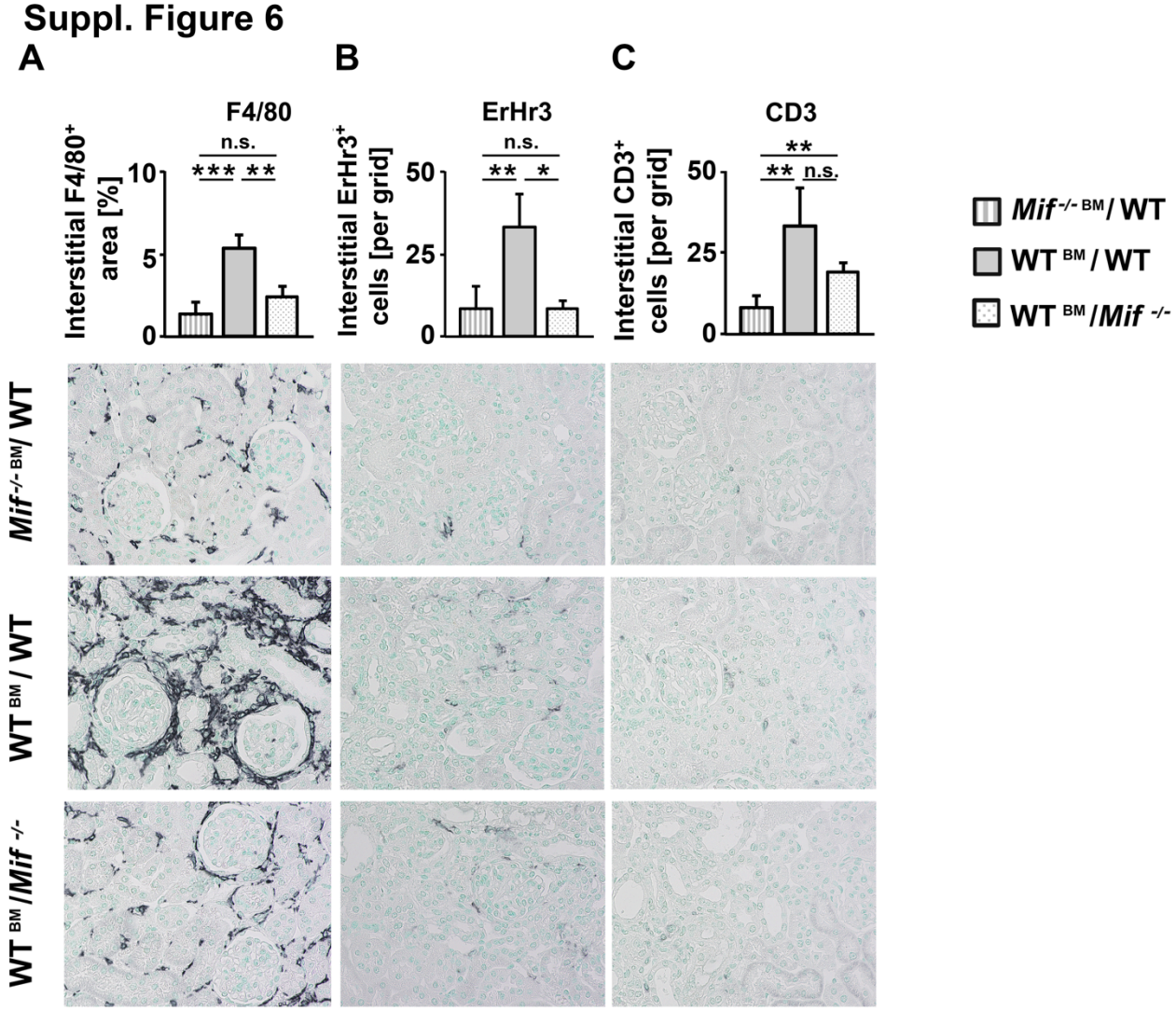
Suppl. Figure 4
MIF has no effect on podocyte proliferation, but influence chemokine and receptor expression in PECs.

Stimulation with 100 ng/ml rmMIF (black bars) had no effect on podocyte proliferation compared to unstimulated controls (white bars) (A).

Besides proliferative effects, MIF stimulation (black bars) of PECs significantly induced chemokine gene expression of *Ccl2* and *Ccl5* gene expression by trend compared to unstimulated controls (white bars) (B). Expression of CD74 co-receptor CD44 was also directly influenced by rmMIF (C). In contrast *Pdgfrb* (D) and *Pdgfb* (E) gene expression is not altered by MIF.



Suppl. Figure 5
MIF deficiency reduces interstitial immune cell infiltration
The decrease of body weight (A) during NTN was similar in *Mif*$^{-/-}$ mice (white squares, n=13) and WT (grey triangles, n=6) as was the binding of sheep IgG to the glomerular basement membrane (B), suggesting that *Mif* deficiency had no effect on disease induction. Infiltration of F4/80 (C), ErHr3 (D) and CD3 positive cells (E) was strongly reduced in *Mif*$^{-/-}$ compared to WT mice. Gene expression of MIF receptors *Cd74* and *Cxcr4* was also significantly decreased in *Mif*$^{-/-}$, whereas Cxcr2 was unchanged (F). Quantification was performed by assessing the positively stained cortical area (%) or the number of positively stained cells per grid. Data represent means ± SD. *p<0.05 vs. WT **p<0.01 vs. WT ***p<0.001 vs. WT

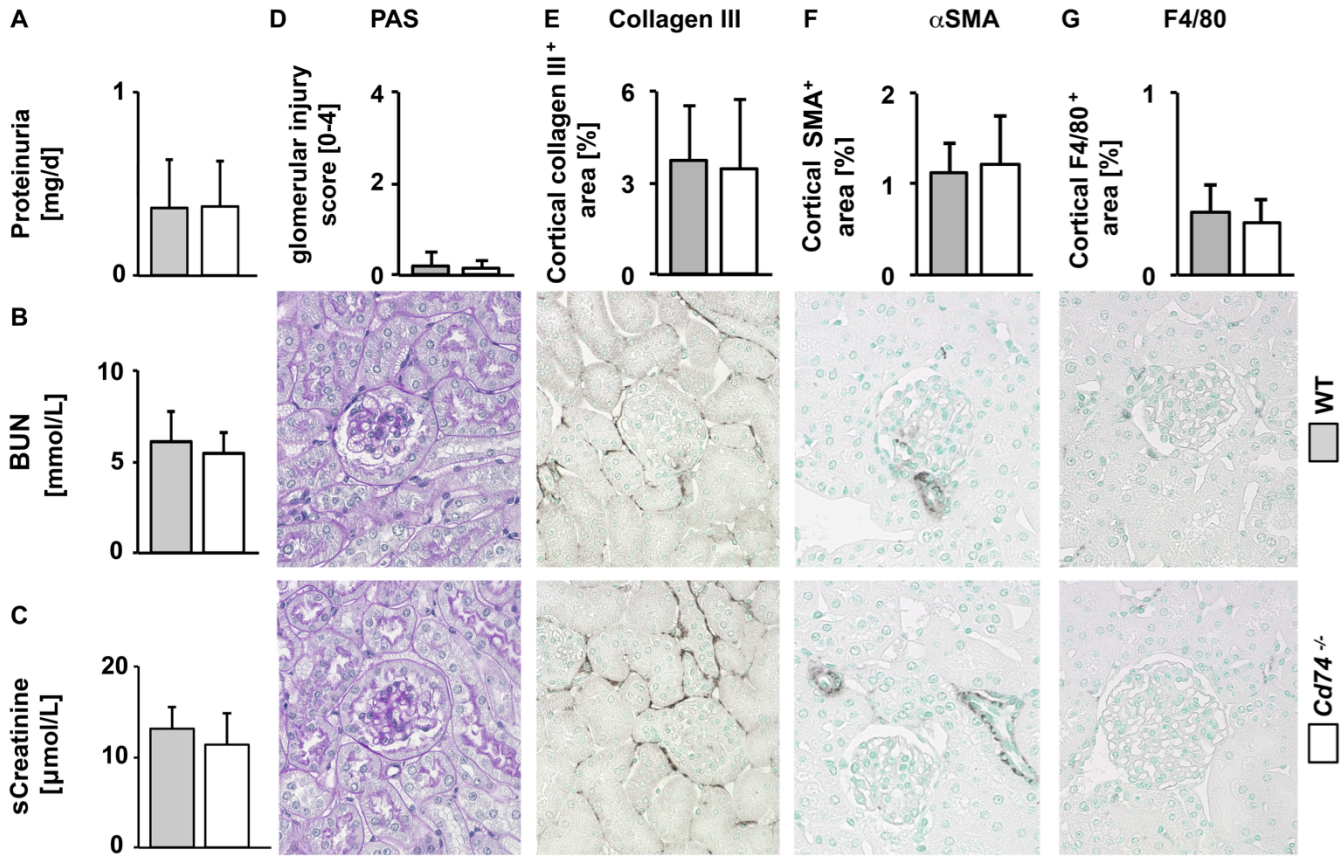


Suppl. Figure 6

Both local and bone marrow *Mif* deficiency reduces interstitial immune cell infiltration.

Infiltration of F4/80 (A), ErHr3 (B) and CD3 positive cells (C) was significantly reduced in WT recipients of *Mif*-deficient bone marrow ($Mif^{-/-BM}$/WT) as well as in *Mif*-deficient recipients with WT bone marrow (WT^{BM}/$Mif^{-/-}$) compared to WT mice with WT bone-marrow (WT^{BM}/WT). Quantification was performed by assessing the positively stained cortical area (%) or the number of positively stained cells per grid. Data represent means ± SD. *p<0.05 **p<0.01 ***p<0.001

Suppl. Figure 7



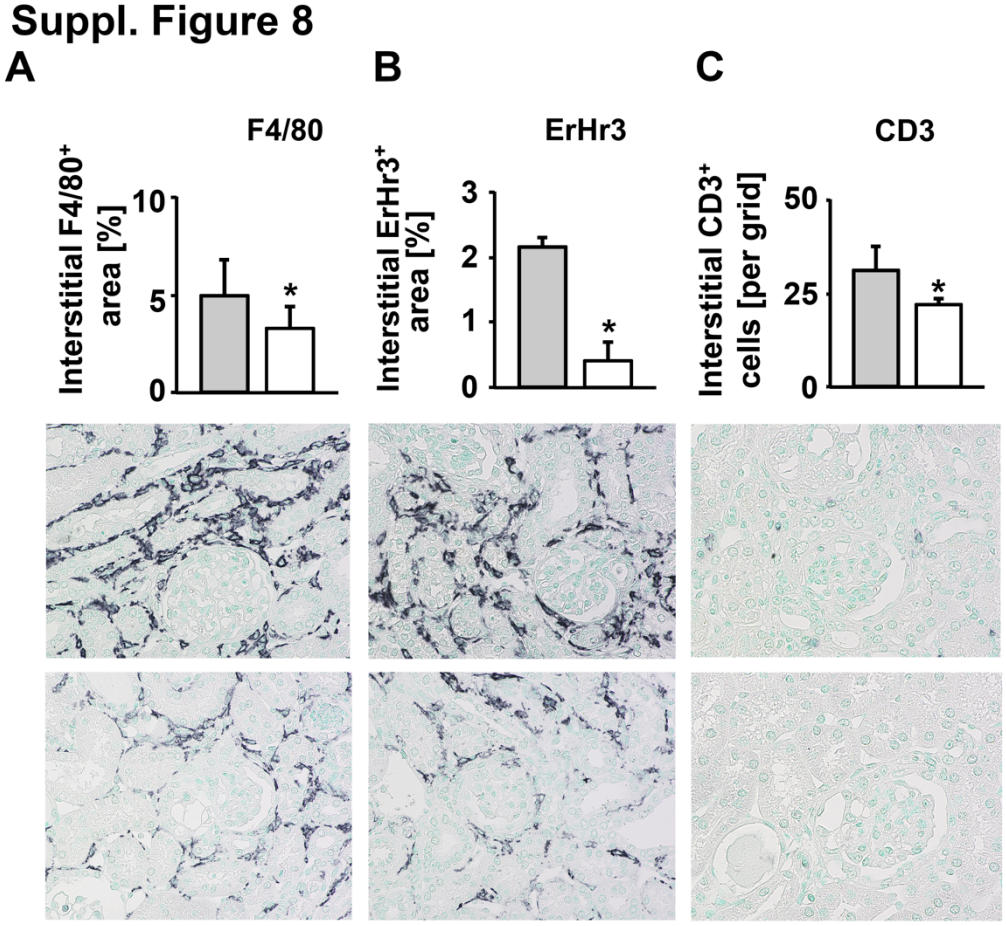
H

	CK [U/L]	LDH [U/L]	Calcium [mmol/L]	Potassium [mmol/L]	Sodium [mmol/L]	Creatinine [µmol/L]	Glucose [mmol/L]	Uric acid [µmol/L]	Urea [mmol/L]	Albumin [g/L]
WT (n=5)	246±208	417±106	2±0.15	5±0.5	152±0.6	20±1.5	8±3	191±55	8±0.9	21±1
Cd74$^{-/-}$ (n=5)	350±205	745±229	2±0.05	6±0.4	149±1*	20±5.6	6±1	146±21	10±0.4	22±2

Suppl. Figure 7

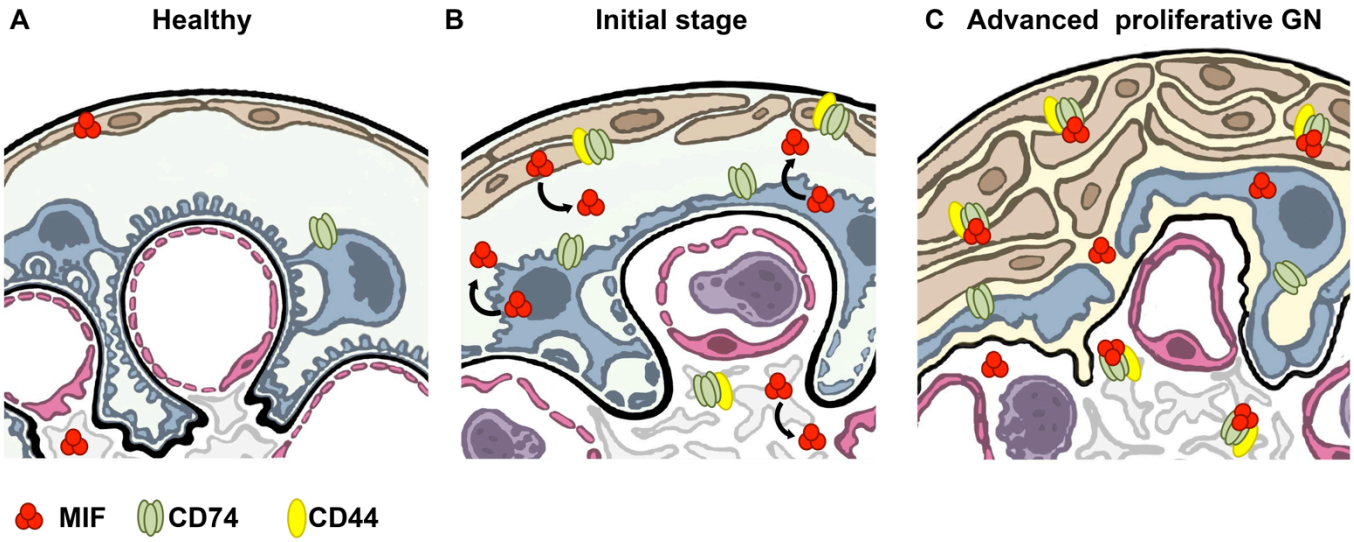
***Cd74*$^{-/-}$ mice showed no obvious spontaneous renal phenotype.**

Cd74$^{-/-}$ mice (white bars, n=5) showed no pathological changes in renal function assessed by proteinuria (A), BUN (B) or serum creatinine (C) and the values were similar to those found in WT mice (grey bars, n=5). No pathological changes were observed on renal histology, supported by quantification of glomerular injury score (D), collagen III deposition (E), mesangial/fibroblast activation (F) or immune cell infiltration (G). Analysis of blood biochemistry also showed no pathological changes (H). Quantification of immunohistochemical stainings was performed by assessing the positively stained cortical area (%) or the number of positively stained cells per grid. Data represent means ± SD. *p<0.05 vs. WT **p<0.01 vs. WT ***p<0.001 vs. WT.



Suppl. Figure 8
CD74 deficiency reduced interstitial immune cell infiltration
Infiltration of F4/80 (A), ErHr3 (B) and CD3 positive cells (C) was strongly reduced in *Cd74*$^{-/-}$ compared to WT mice. Quantification was performed by assessing the positively stained cortical area (%) or the number of positively stained cells per grid. Data represent means ± SD. *p<0.05 vs. WT **p<0.01 vs. WT ***p<0.001 vs. WT

Suppl. Figure 9



Suppl. Figure 9
Scheme of MIF signaling *via* CD44/CD74 in proliferative glomerular diseases.
In the healthy kidney (A), MIF is predominately expressed by PECs and mesangial cells whereas CD44 is not and CD74 only minimally expressed by podocytes and endothelial cells. After induction of podocytic stress (B), MIF is up-regulated and secreted (arrows) by mesangial cells, PECs and podocyte and binds to the *de novo* expressed CD74/CD44 receptor complex on PECs and mesangial cells. Binding of MIF to the CD74/CD44 receptor complex induces proliferation of mesangial cells and PECs (C). PECs – brown, podocytes – blue, mesangial cells- grey, monocytes/macrophages – purple, endothelium – pink, MIF – red, CD74 – green, CD44 – yellow