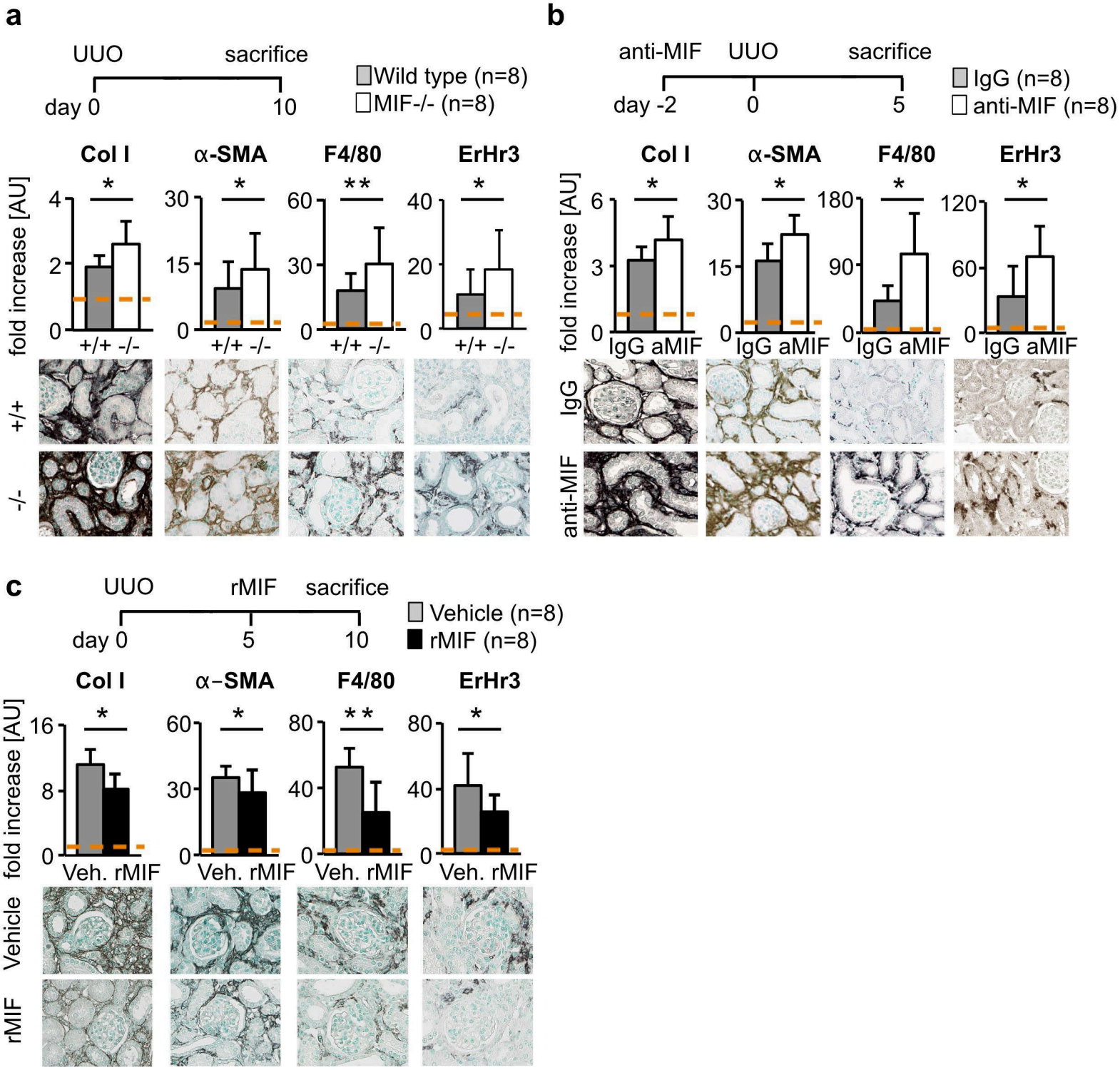
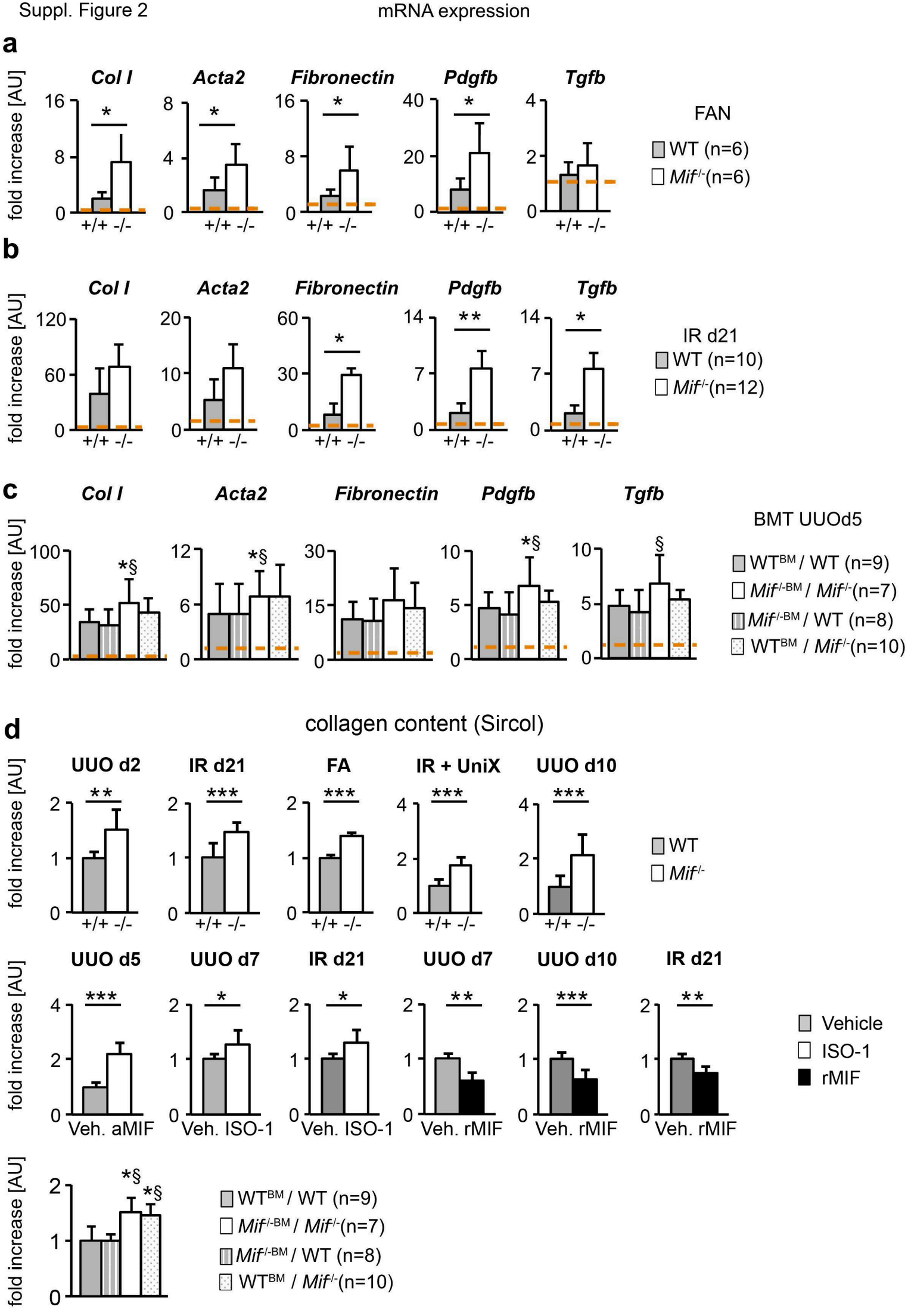
Suppl. Figure 1



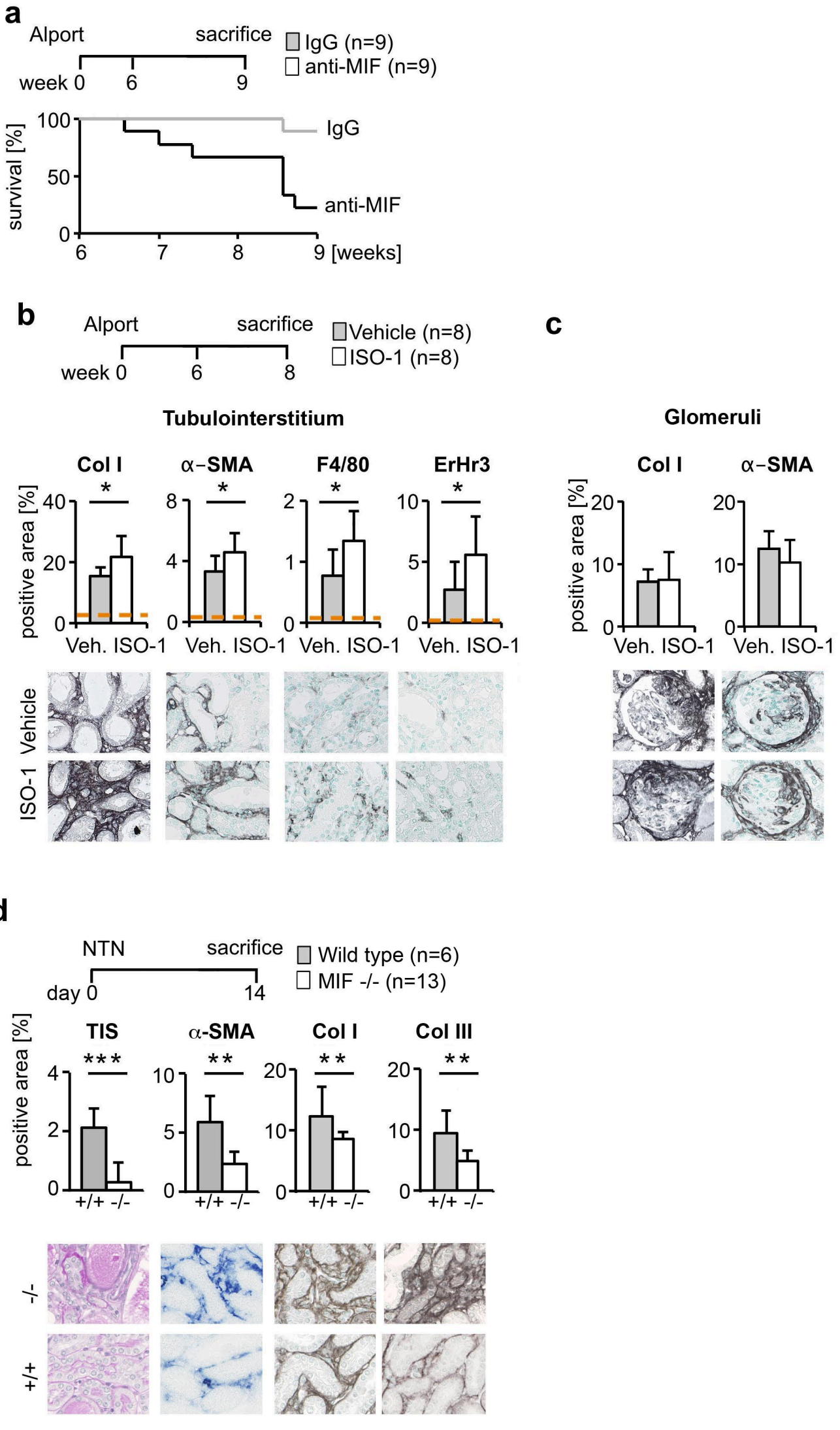
Suppl. Figure 1. MIF manipulation in obstructive nephropathy confirmed its anti-fibrotic effects
(a) In later stage of unilateral ureteral obstruction (UUO), i.e. day 10 after disease induction, compared to wild type littermates (+/+, n=8), all parameters of renal fibrosis and inflammation were increased in Mif-/- mice (-/-, n=8), including collagen type I (Col I) α–smooth muscle cell actin (α-SMA), number of infiltrating monocytes and macrophages (ErHr3 and F4/80). **(b)** Similarly, increased renal fibrosis and immune cell infiltration was found when MIF was inhibited via neutralizing anti-MIF antibody (aMIF) compared to isotype-matched control IgG in UUO (both 5 mg/kg BW; n=8 each group). **(c)** Daily treatment (i.p.) with recombinant murine MIF (rMIF; 40µg per animal and day), even when started in fully established and advanced disease stage, i.e. on day 5 of UUO, resulted in significantly decreased fibrosis and inflammation compared to vehicle-treated mice (n=8 each group).
Data are means ± SD. Values of healthy contralateral kidneys of WT or control mice were set as 1, represented by the orange, dashed line. *P<0.05; **P<0.01. AU=arbitrary units. BW=body weight

Suppl. Figure 2



Suppl. Figure 2. MIF deficiency increased the expression of pro-fibrotic genes
In **(a)** folic acid nephropathy (FAN) and **(b)** ischemia reperfusion injury (I/R), the mRNA analyses showed increased expression of the pro-fibrotic genes collagen 1 (*Col I*), α–smooth muscle cell actin (*Acta2*), fibronectin, platelet-derived growth factor B (*Pdgfb*) and transforming growth factor β1 (*Tgfb*) in *Mif* deficient mice (-/-) compared to wild type (WT) littermates (+/+). **(c)** $Mif^{-/-}$ mice with $Mif^{-/-}$ bone marrow ($Mif^{-/-BM}/Mif^{-/-}$) had also increased expression of pro-fibrotic genes compared to WT mice with WT bone marrow (WT^{BM}/WT). Bone marrow derived MIF is not involved in mediating the anti-fibrotic effects, since WT mice with $Mif^{-/-}$ bone marrow ($Mif^{-/-BM}$/WT) had similar expression as WT mice with WT bone marrow (WTBM/WT), whereas $Mif^{-/-}$ mice with WT bone marrow ($WT^{BM}/Mif^{-/-}$) had similar expression as $Mif^{-/-}$ mice with $Mif^{-/-}$ bone marrow ($Mif^{-/-BM}$/-$Mif^{-/-}$). **(d)** Changes in pro-fibrotic gene expression resulted in different collagen content measured by biochemical sircol assay. Data are means ± SD. Values of healthy or contralateral kidneys of WT were set as 1, represented by the orange, dashed line. *P<0.05 *vs.* WT or WTBM/WT; § P<0.05 *vs.* Mif-/-BM/WT; **P<0.01. AU=arbitrary units.

Suppl Figure 3

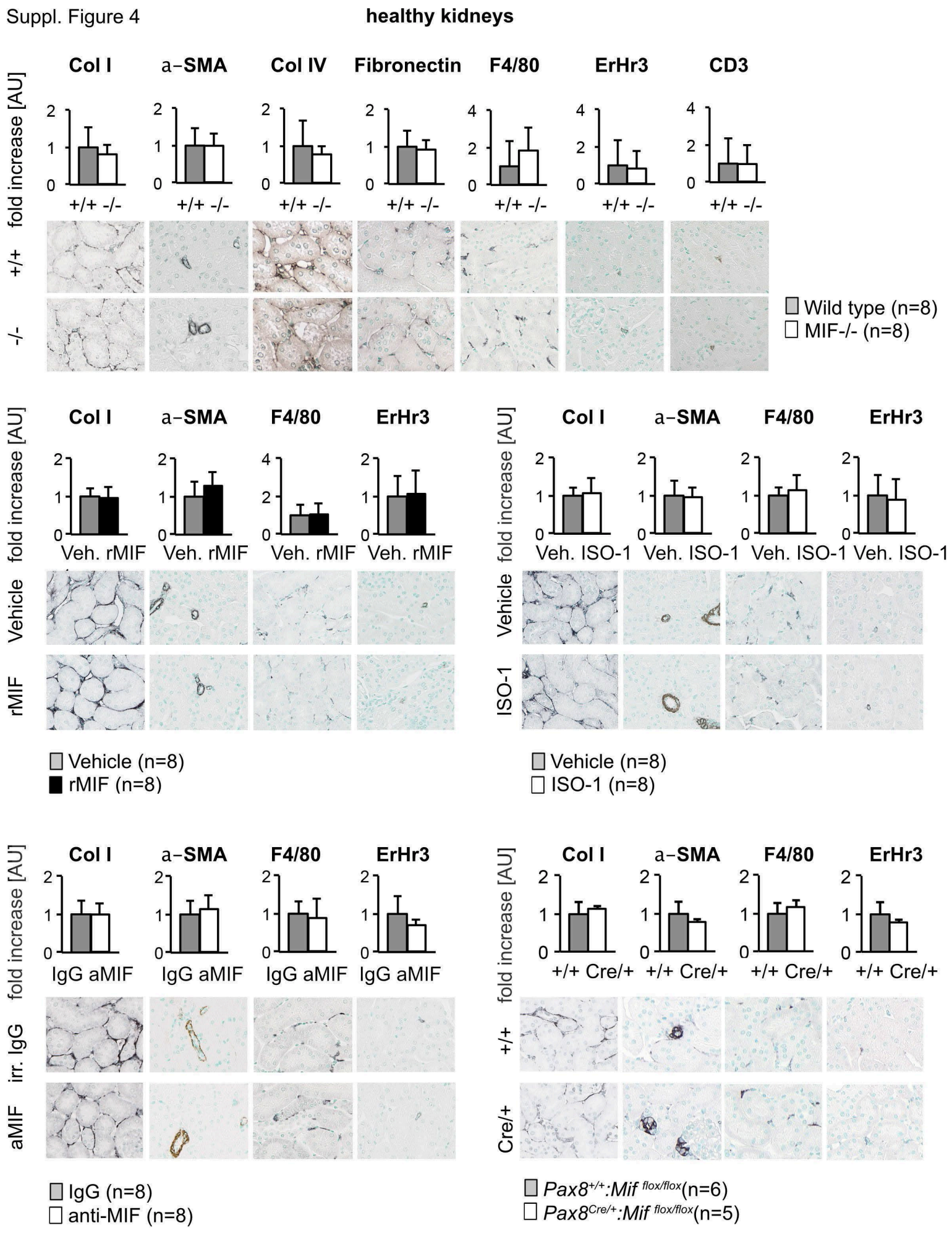


Suppl. Figure 3. MIF reduced secondary fibrosis to progressive glomerular injury

(a) *Col4a3*$^{-/-}$ (Alport) mice represent a murine model of non-inflammatory progressive glomerular disease leading to secondary tubulointerstitial fibrosis and death due to renal failure. Compared to daily treatment of *Col4a3*$^{-/-}$ mice with isotype-matched control IgG, treatment with MIF neutralizing antibody (anti-MIF; both 5 mg/kg BW i.p.) for three weeks, starting at the age of 6 weeks (n=9 each group), when the mice already have manifest fibrosis and decreased renal function, led to increased mortality. **(b)** Compared to vehicle treated *Col4a3-/-* mice, specific small-molecule inhibitor of MIF, ISO-1 (40 mg/kg BW daily i.p.), treated *Col4a3*$^{-/-}$ mice treated for two weeks starting at the age of 6 weeks, showed significantly more fibrosis and inflammation (n=8 each group). **(c)** Glomerular analysis of collagen type I (Col I) and α–smooth muscle cell actin (α-SMA) revealed no significant differences between ISO-1 and vehicle treated Col4a3$^{-/-}$ mice. **(d)** Analysis of tubulointersitial damage (TIS), α–smooth muscle cell actin (α-SMA), collagen type I (Col I) and collagen type III (Col II) in immune-mediated renal disease (nephrotoxic serum nephritis) revealed less renal fibrosis in *Mif*$^{-/-}$ (-/-; n=12) compared to wild type littermates (+/+; n=6).

Data are means ± SD. Values of healthy kidneys of WT mice were set as 1, represented by the orange, dashed line. *P<0.05.

Suppl. Figure 4

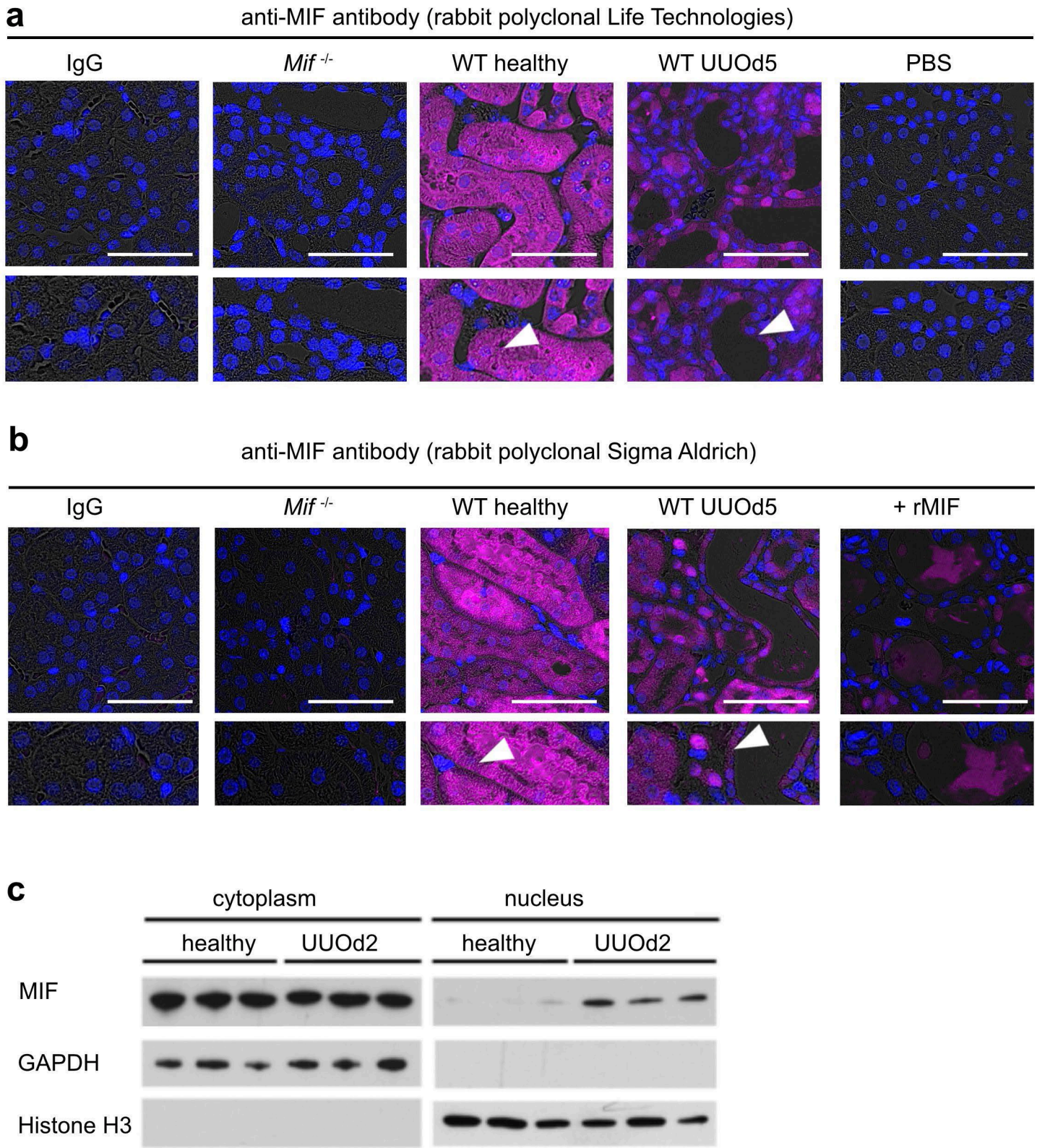


Suppl. Figure 4. MIF or its neutralization had no effect on healthy kidneys

Neither Mif deficiency (-/-), recombinant MIF (rMIF), anti-MIF antibody nor ISO-1 showed any effects on the expression of pro-fibrotic or inflammatory proteins in healthy, or contralateral non-fibrotic kidneys.

Data are means ± SD. Values of healthy kidneys of WT or control treated mice were set as 1. AU=arbitrary units

Suppl. Figure 5

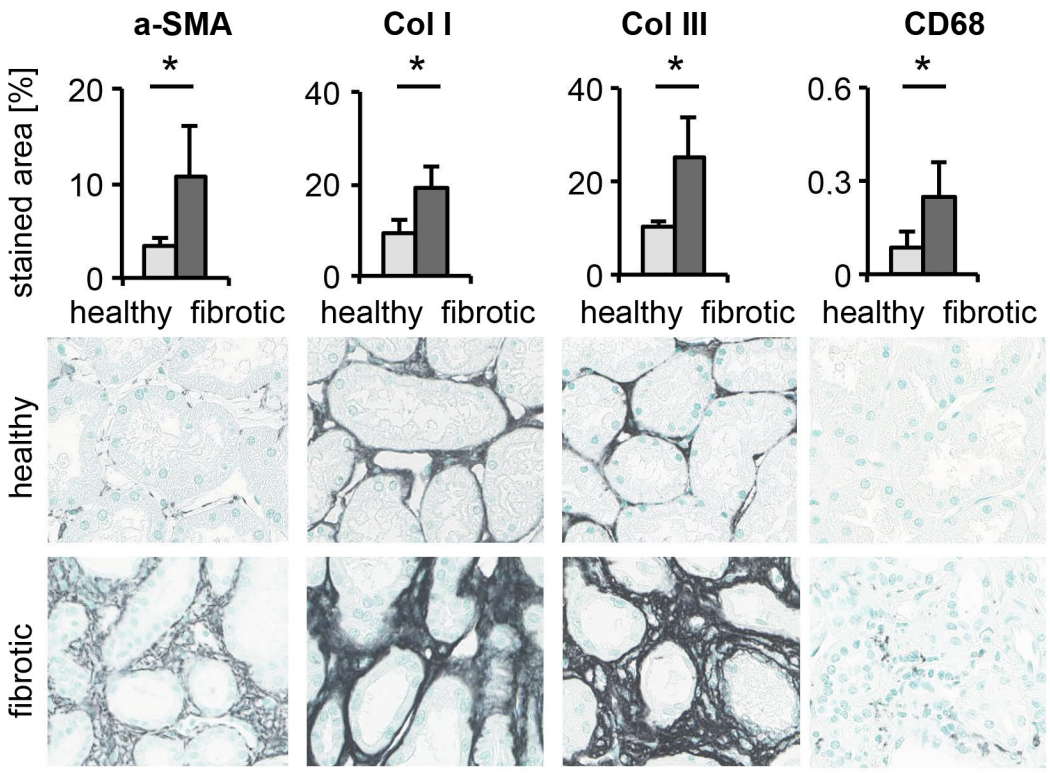


Suppl. Figure 5. Specificity of anti-MIF Antibodies

Anti-MIF antibodies from two different companies, **(a)** Life Technologies and **(b)** Sigma Aldrich showed similar staining pattern in both wild type healthy and diseased kidneys (day 5 of UUO). Negative controls using PBS or isotype-matched control IgG instead of primary antibody were negative. Similarly, no staining was found when Mif deficient mice were stained by both anti-MIF antibodies. Specificity of staining was further proven by pre-incubation of anti-MIF antibody with recombinant MIF (rMIF), which also resulted virtually absent staining. **(c)** Unexpected nuclear staining of MIF at day 2 of UUO was confirmed by western blot of cytoplasmic and nuclear fractions of healthy and diseased kidneys (n=3 for each group). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and histone H3 served as controls for cytoplasmic and nuclear fractions, respectively.

Suppl. Figure 6

a



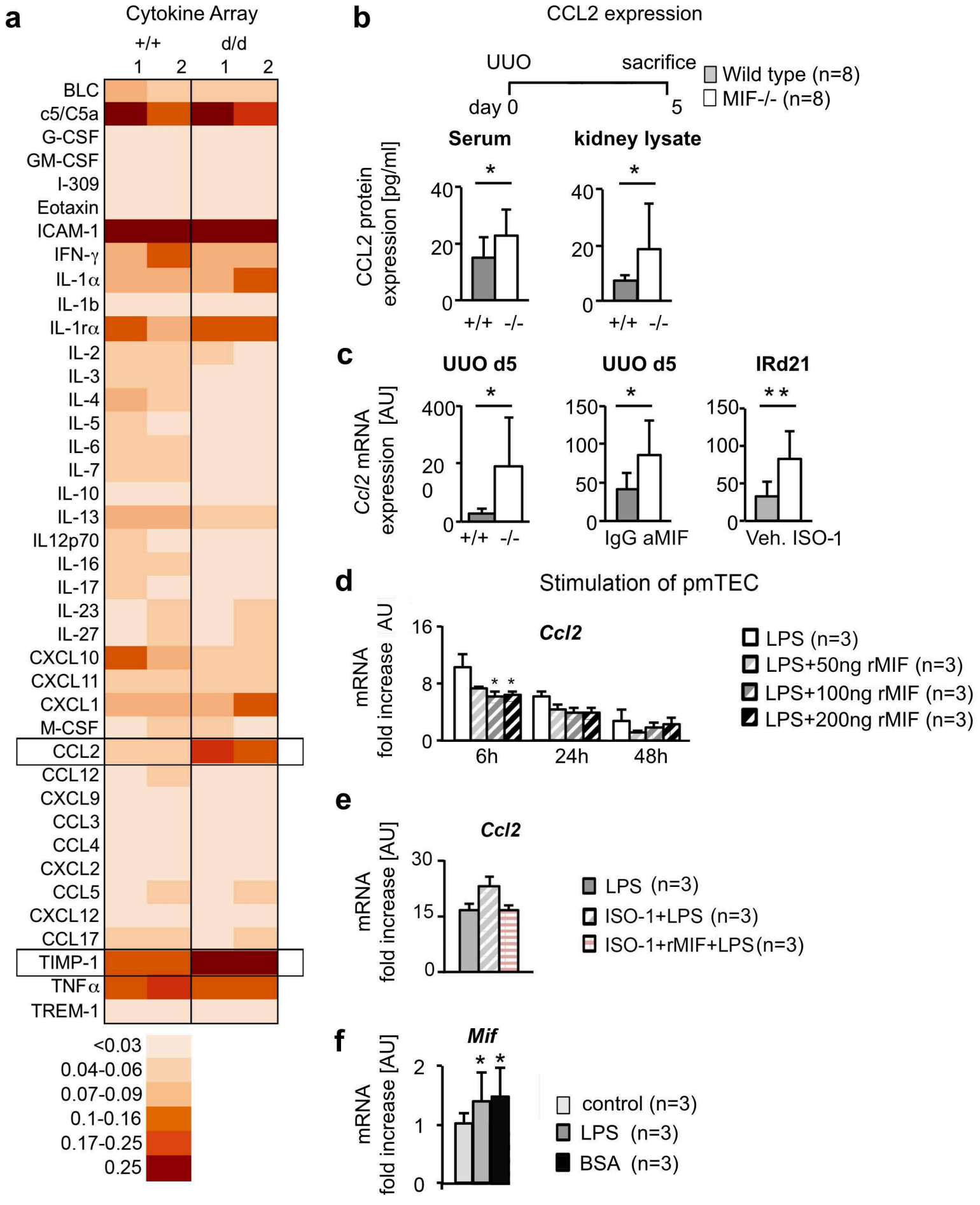
b

	healthy	fibrotic
Patients	6	6
Sex (male/female)	1/5	3/3
Age (yr)	58±9	65±9
sCrea (mg/dL)	0.6±0.2	2.25±2.4

Suppl. Figure 6. Characteristics of patients

(a) Immunohistochemical analyses of human tissue samples for α-SMA, collagen I, collagen III and CD68 were used to define “healthy” and “fibrotic” patients. **(b)** Characteristics of patients with and without fibrosis analyzed for MIF expression.

Suppl. Figure 7

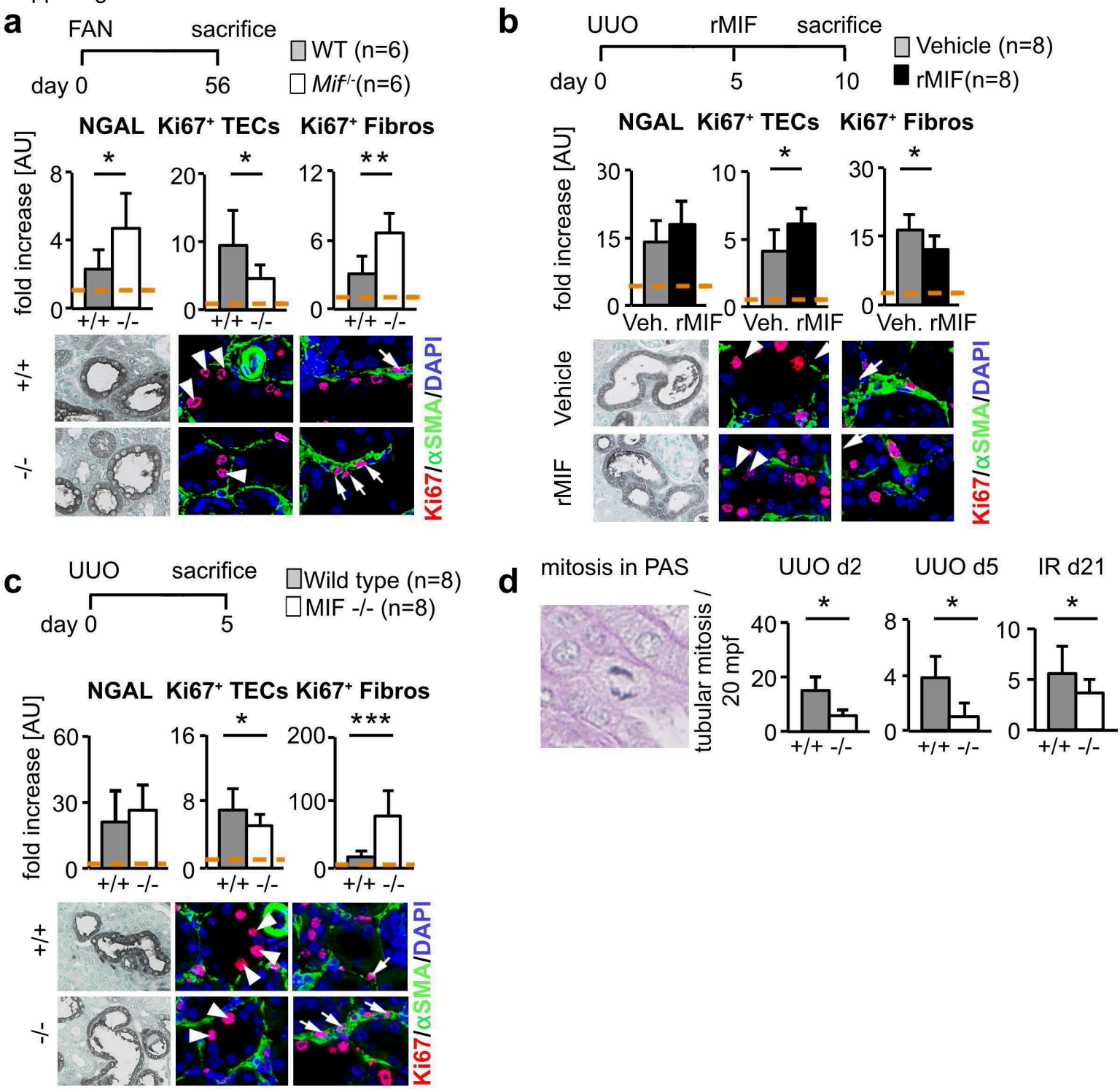


Suppl. Figure 7. MIF effects on CCL2 expression

(a) Heat-map of cytokine array demonstrated differences in CCL2 and TIMP-1 expression in *Mif*$^{-/-}$ mice compared to wild type littermates, whereas no differences in other cytokines where observed (n=2 per group). **(b)** *Mif* deficiency (-/-) increased CCL2 chemokine expression in obstructed kidney and circulating levels of CCL2 in the serum compared to wild type littermates (+/+), as analyzed by ELISA (n=8 per group). **(c)** Analyses of *Ccl2* mRNA expression in different experimental settings, e.g. unilateral ureteral obstruction (UUO) and ischemia reperfusion (I/R) in *Mif* deficient mice and in mice treated with neutralizing MIF antibody (aMIF) or a specific small-molecule inhibitor of MIF, ISO-1, confirmed the effect of MIF on chemokine expression. **(d)** In vitro, activation of primary murine tubular epithelial cells (pmTEC) with lipopolysacharide LPS (200 ng/ml) resulted in a 10-fold increase of *Ccl2* expression after 6h, which declined at 24h and 48h. Dose-response experiments showed that 100ng/ml of rMIF was most potent in reducing *Ccl2* expression, whereas 200 ng/ml had no additive effect. **(e)** Pre-incubation of ISO-1 with rMIF abrogated the effects on Ccl2 expression, supporting the specificity of ISO-1. **(f)** Both, stimulation with LPS and BSA resulted in increase of Mif mRNA expression already 6h after stimulation.

Data are means ± SD. Values of healthy contralateral kidneys of WT or control mice were set as 1, represented by the orange, dashed line. *P<0.05. AU=arbitrary units.

Suppl. Figure 8

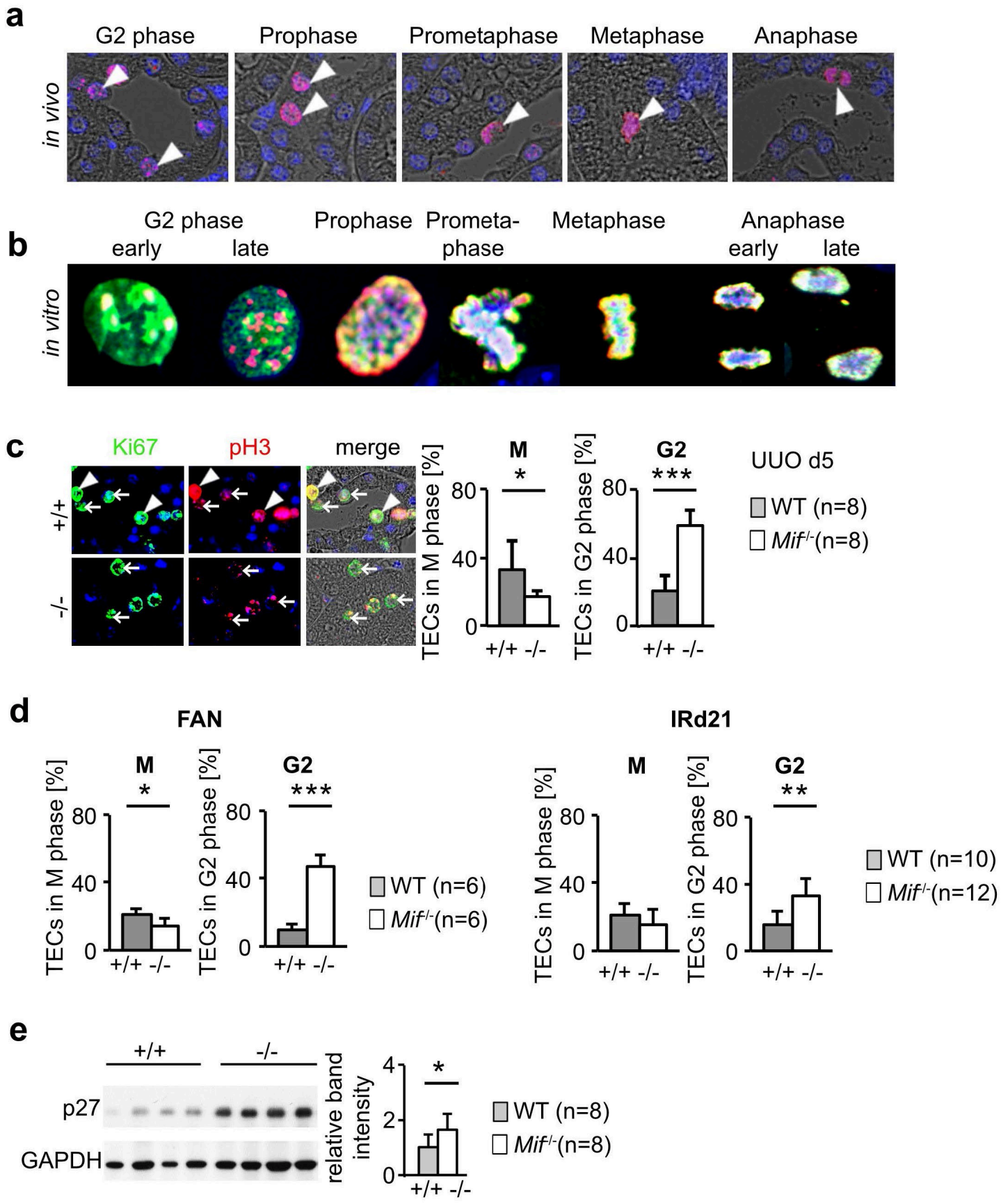


Suppl. Figure 8. MIF effects on cell cycle entry and proliferation

(a) In folic acid nephropathy (FAN) and **(c)** unilateral ureteral obstruction (UUO) day 5, compared to wild type littermates (+/+), *Mif* deficient mice (-/-) showed decreased proliferation of tubular cells (arrowhead pointing to a tubular cell with nucleus positive for proliferation marker Ki67 and counterstained with DAPI), while myofibroblast (Fibros) proliferation was increased (arrows pointing to interstitial cells stained for myofibroblast marker α-SMA with nuclei positive for Ki67 and counterstained with DAPI). **(b)** *Vice versa*, treatment with recombinant MIF (rMIF), starting at day 5 after ureteral obstruction (UUO) for 5 days, increased tubular cell (TEC) proliferation and inhibited proliferation of fibroblasts. **(d)** Decreased proliferation of tubular cells was also found in UUO day 2 and 5 and unilateral ischemia/reperfusion injury (I/R) day 21, as shown by number of tubular mitotic figures counted in Periodic Acid Schiff's staining (PAS).

All in vitro experiments were performed at least in triplicates. Data are means ± SD. Values of healthy contralateral kidneys of WT or control mice were set as 1, represented by the orange, dashed line. *P<0.05; **P<0.01; ***P<0.001. AU=arbitrary units, Dox=Doxycyclin.

Suppl. Figure 9

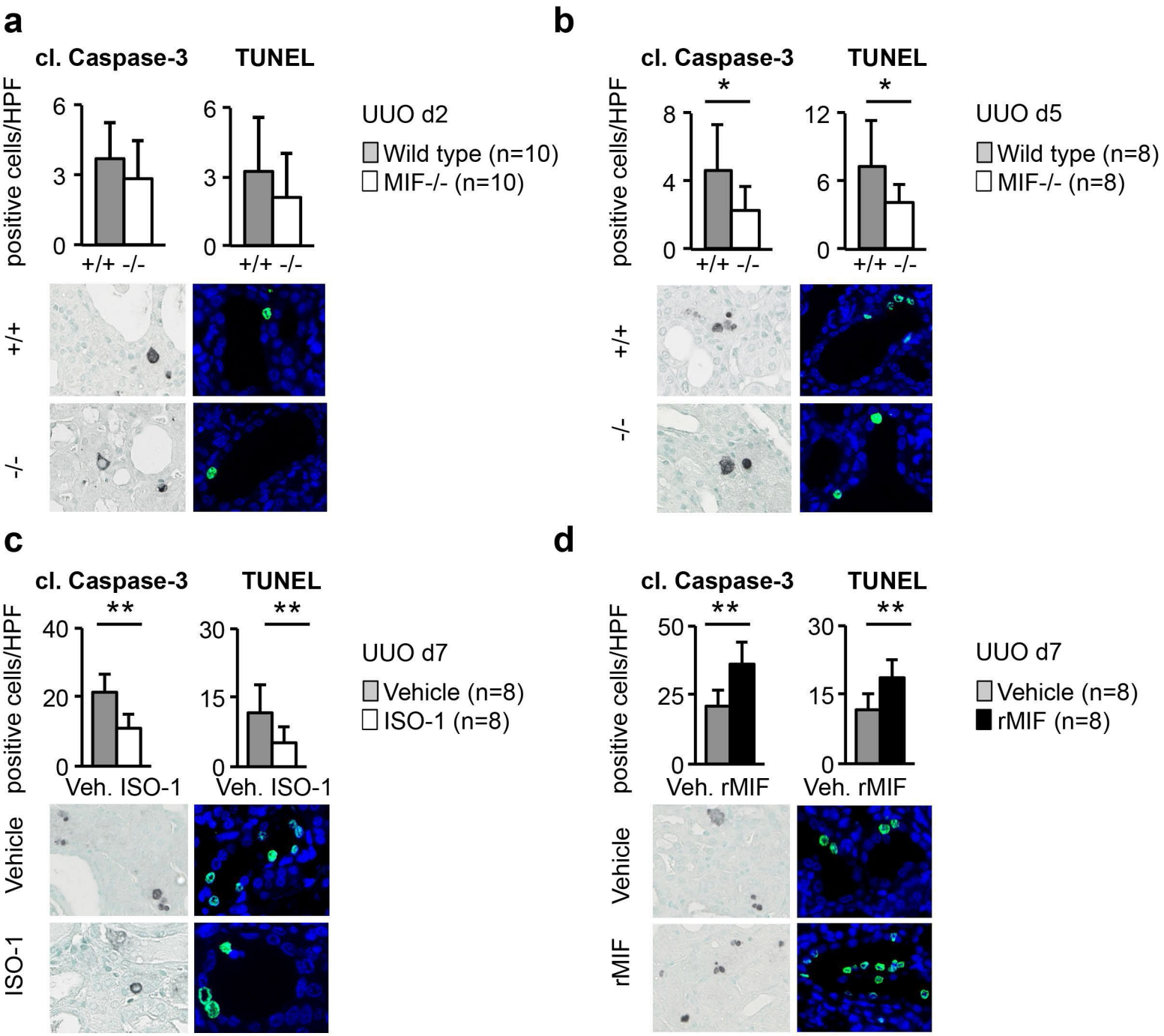


Suppl. Figure 9. MIF effects on cell cycle arrest

Chromatin pattern of tubular cells shown with immunostaining of antibodies to phosphorylated serine 10 of histone H3 and Ki67 allowed the specific counting of number of cells in various phases of cell cycle **(a)** *in vivo* and **(b)** in vitro. **(c)** Compared to wild type littermates (+/+), *Mif*$^{-/-}$ mice (-/-) showed an increased proportion of cells in G2 cycle arrest (arrow) and decreased number of mitosis (arrowhead) shown by Ki67/pH3 immunofluorescence staining (n=8 per group). The same was also observed in **(d)** late stages of fibrosis in other models, i.e. folic acid (FAN) and ischemia/reperfusion (I/R) day 21. **(e)** Arrest in G2 phase in *Mif*$^{-/-}$ mice was mediated by increased expression of cell cycle inhibitor p27Kip1 at day 5 of UUO, analyzed by Western-blot (n=8 per group, a representative blot of n=4 is shown).

Data are means ± SD. *P<0.05; **P<0.01; ***P<0.001.

Suppl. Figure.10



Suppl.Figure 10. MIF induced apoptosis of tubular cells

(a) In early stages of unilateral ureteral obstruction (UUO), i.e. day 2 after disease induction, only few cells underwent apoptosis as analyzed by cleaved caspase-3 (early apoptosis) and TUNEL (late apoptosis). *Mif*$^{-/-}$ mice exhibited a trend for decreased apoptosis compared to wild type littermates (+/+, n=8). In later disease stages with increasing numbers of apoptotic cells, *Mif* deficiency **(b)** and MIF inhibition by ISO-1 **(c)** decreased, whereas treatment with rMIF **(d)** increased apoptosis. Data are means ± SD. *P<0.05; **P<0.01; ***P<0.001.