Thrombospondin-1 plays a profibrotic and pro-inflammatory role during ureteric obstruction

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Thrombospondin-1 (TSP-1) is an endogenous activator of transforming growth factor-β (TGF-β), and an anti-angiogenic factor, which may prevent kidney repair. Here we investigated whether TSP-1 is involved in the development of chronic kidney disease using rats with unilateral ureteral obstruction, a well-known model to study renal fibrosis. Obstruction of 10 days duration induced inflammation, tubular cell atrophy, dilation, apoptosis, and proliferation, leading to interstitial fibrosis. TSP-1 expression was increased in parallel to that of collagen III and TGF-β. Relief of the obstruction at day 10 produced a gradual improvement in renal structure and function, the reappearance of peritubular capillaries, and restoration of renal VEGF content over a 7- to 15-day post-relief period. TSP-1 expression decreased in parallel with that of TGF-β1 and collagen III. Mice in which the *TSP-1* gene was knocked out displayed less inflammation and had better preservation of renal tissue and the peritubular capillary network compared to wild-type mice. Additional studies showed that the inflammatory effect of TSP-1 was mediated, at least in part, by monocyte chemoattractant protein-1 and activation of the Th17 pathway. Thus, TSP-1 is an important profibrotic and inflammatory mediator of renal disease. Blockade of its action may be a treatment against the development of chronic kidney disease.

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Chronic kidney diseases (CKDs) are a growing health problem. Renal fibrosis, especially interstitial fibrosis, is a final common pathway that leads to progressive loss of renal function in various CKDs. Our group and other investigators as well have investigated over the past years the mechanisms involved in the development of renal fibrosis in order to identify targets for therapy.[1–5] We were among the first few groups to report that regression of renal fibrosis was achieved following angiotensin II receptor antagonist at least in experimental models of hypertensive nephropathies.[6–8] These results were independently confirmed and extended to additional experimental models of nephropathies by other investigators.[9–11] As angiotensin II blockade is currently used and renal function still declines in most CKD patients, we believe that additional therapeutic approaches are required to improve renal function in CKD patients.

In addition to etiologic and nephroprotective treatments, facilitating renal repair represents a promising approach. Stem cell therapy has raised important hope to replace damaged tissues and promote renal repair.[12] Despite a better characterization of stem cells, their use remains too early for clinical application. On the other hand, the kidney possesses a remarkable capacity to regenerate. Most of the time, following acute kidney injury, the kidney heals. The model of ischemia/reperfusion has been used to identify molecular mechanisms central to repair.[13] Unilateral ureteral obstruction (UUO) generates progressive renal fibrosis.[14] It is considered as a model of renal interstitial fibrosis, although reversal of the obstruction permits the study of recovery. To date, most studies have investigated renal recovery after a few days of obstruction-generating tubular injury and dysfunction.[15,16] Renal repair after a longer time of UUO has been successfully demonstrated, but cellular and molecular mechanisms of renal repair after UUO release are largely unknown.[17,18]

Our goals were to show the endogenous capacity of the kidney to regenerate after severe injury induced by 10 days of UUO, and to investigate cellular and molecular mechanisms involved during the phase of repair. We focused our study on thrombospondin-1 (TSP-1), because it is an endogenous activator of transforming growth factor-β (TGF-β1), and an anti-angiogenic factor, which may prevent kidney repair.[19,20] TSP-1 is a 450-kDa homotrimeric extracellular matrix protein

with multiple domains that interact with different receptors, proteins, and proteoglycans.[21] Three regions of TSP-1 have been implicated in its anti-angiogenic property: the procollagen domain, the three type 1 repeats (TSRs), and the C-terminal domain. We used TSP-1 knockout mice (TSP-1 KO) to emphasize its major role in fibrosis development and repair.

Our findings show that renal repair involved an early decrease in TSP-1 and TGF-β1 expression followed by reappearance of peritubular capillaries, and regeneration of the proximal tubule. As TSP-1 inhibition reduced TGF-β renal content, prevented peritubular capillary rarefaction, and attenuated inflammation and tubular injury, targeting TSP-1 could be a new treatment against the development of CKDs.

RESULTS

Development of renal profibrotic lesions

UUO for 10 days induced tubular dilatation, extracellular matrix accumulation, interstitial infiltration, and cortical atrophy (Figure 1a and b, Supplementary Figure S1a and b online). In most dilated tubules, the epithelium was flattened or tubular membrane was denuded. Proximal epithelial cell brush border easily visible by Masson and periodic acid–Schiff staining in SHAM rats significantly decreased after UUO (Figure 1a and b, Supplementary Figure S1 online). Cellular interstitial infiltration increased after UUO ($P<0.05$, Figure 2a and b). The number of peritubular capillaries was reduced in the UUO group compared with the control group ($P<0.001$, Figure 3a and b).

Reversal of UUO

UUO relief was followed by progressive renal repair with epithelial cell replacement and extracellular matrix accumulation remodeling. As early as 7 days after reversible unilateral ureteral obstruction (RUUO), epithelial replacement of some tubular segments in the outer medulla was evident, as well as reappearance of brush border in the proximal tubules (Figures 1aC, D and 2c, d). This regeneration process continued with time and became evident at 15 days. In the RUUO 15 group, tubular dilatation and interstitial extracellular matrix accumulation disappeared (Figures 1aC, D and 2c, d). Tubules were next to each other, tightened as normal renal tissue. In the RUUO 15 group, morphology of the kidney appeared normal with very rare scattered area of scarred tissue. Ten days after UUO, cortical height was significantly lower compared with SHAM rats (2061 ± 79 µm and 3292 ± 255 µm, $P<0.005$). Fifteen days after UUO release, cortical thickness significantly improved compared with UUO (2427 ± 48 µm $P<0.01$), but was still lower than that in the SHAM group, $P<0.05$. Glomeruli displayed normal architecture in all groups. Peritubular capillary regeneration was delayed and was visible only in the RUUO 15 group (Figure 3c–e).

Functional improvement after UUO relief

To confirm that renal repair and matrix remodeling have functional consequences, we assessed cell viability in all groups and renal function in the RUUO + NX subgroup (the group that underwent right nephrectomy 15 days after reversal). Tissue viability index significantly decreased after UUO, $P<0.001$ (Supplementary Figure S1c online). Release of ureteral obstruction induced progressive functional improvement. RUUO 7 animals had intermediate level of cell viability, whereas viability index normalized in RUUO 15 rats. Serum creatinine was 32 ± 0.9 µmol/l in SHAM + NX rats, and 59.3 ± 10.5 µmol/l in RUUO + NX right nephrectomized rats, $P=0.076$, showing an important recovery of renal function in the left obstructed kidney, after UUO release. Urine albumin excretion was not different between these two groups: 222 ± 19 and 260 ± 44 mg/mmol creatinine, respectively ($P=0.47$).

Mechanisms of renal repair and TSP-1 expression

To elucidate cellular mechanisms of renal repair and remodeling, we assessed cellular proliferation and apoptosis. Both markers increased markedly after 10 days of UUO, $P<0.001$ (Supplementary Figure S1 online). As expected, the largest part of proliferative cells was observed within the interstitium in UUO animals (arrow), whereas apoptotic cells were mostly tubular cells. Interestingly, cellular proliferation increased further in RUUO 7 rats ($P<0.05$ vs. UUO group). At this time, proliferative cells were epithelial tubular cells (Supplementary Figure S1a online, box). Both apoptosis and proliferation decreased in the late phase of renal repair, but were maintained at a higher level than in the SHAM group.

To investigate the mechanisms involved in renal repair, we tested whether vascular endothelial growth factor (VEGF) could contribute to peritubular capillary regeneration after UUO release. Renal VEGF content strongly decreased after 10 days of UUO compared with the SHAM group ($P<0.001$) and remained low in the RUUO 7 group (Figure 3c). Renal VEGF content normalized in the RUUO 15 group.

As TGF-β1 is a major growth factor involved in renal fibrosis and repair, we assessed TGF-β1 renal content and type III collagen mRNA expression as a TGF-β-regulated gene (Figure 2c and d). Total renal TGF-β1 content increased after UUO (26.7 ± 2.3 pg/mg vs. 1.9 ± 0.3 pg/mg, for UUO and SHAM groups, respectively ($P<0.001$)). After 7 days of release, TGF-β1 content decreased and equaled the SHAM group levels (5.3 ± 1.8 pg/mg); it was maintained in these normal levels up to 15 days after release (3.4 ± 1.1 pg/mg). The elevation of TGF-β1 renal content was associated with six-fold increase of collagen type III mRNA expression in the UUO group compared with the SHAM group ($P<0.001$; Figure 2c and d). Collagen expression significantly decreased 15 days after release, $P<0.01$.

Among endogenous TGF-β activators, we assessed TSP-1 expression during renal fibrosis and repair. TSP-1 expression was faint and was limited to Bowman's capsule in SHAM rats (Figure 4a and c). In the UUO group, TSP-1 mRNA and protein expression increased, and protein was localized in dilated tubules on tubular membranes (Figure 4a–e). After ureteral obstruction release, mRNA and protein expression of TSP-1 were reduced. A faint expression persisted in the most dilated tubules. Importantly, the protein expression of TSP-1

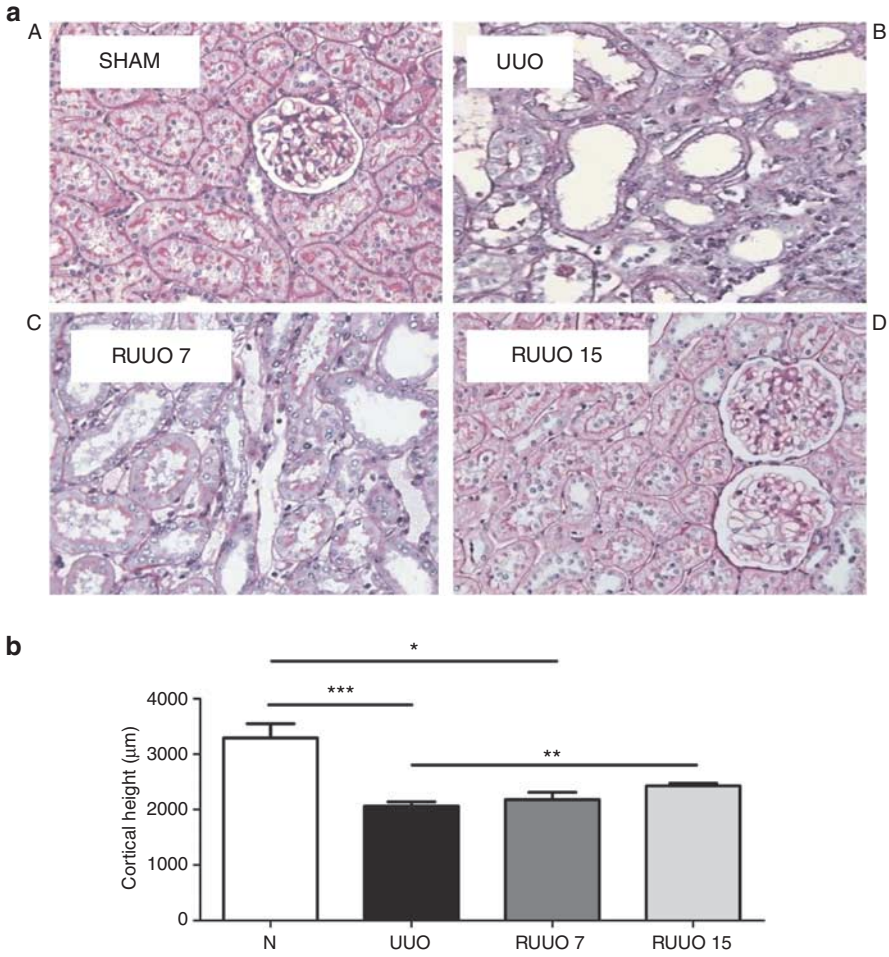

Figure 1 | Representative example of periodic acid–Schiff staining of SHAM, unilateral ureteral obstruction (UUO), and reversible UUO7, 15 (RUUO 7, 15). (**a**A) Normal renal architecture seen in the SHAM group. (B) After 10 days of UUO, tubular dilatation, flattening of the epithelium, loss of proximal epithelial cell brush border, and interstitial infiltration were observed. (C) Seven days after RUUO, partial epithelial replacement of tubular segments was evident. (D) Complete renal regeneration was observed 15 days after RUUO. (**b**) Fifteen days after relief of UUO, cortical height had increased, but cortical atrophy persisted compared with SHAM. Values are mean ± s.e.m., *$P<0.05$, **$P<0.01$, ***$P<0.005$. N, normal.

was closely related to TGF-β1 content ($r=0.749$, $P<0.0001$) and to collagen III expression ($r=0.634$, $P<0.001$) during fibrosis development and repair.

Genetic deficiency of TSP-1 preserves renal parenchyma from UUO-induced injury

To confirm the angiogenic effect of TSP-1 inhibition, we submitted TSP-1 KO and their control wild-type (WT) mice to UUO for 10 days. UUO induced cortical atrophy, capillary loss, and tubular lesions in both genotypes. However, these lesions were less severe in TSP-1 KO mice. UUO induced a reduction of peritubular capillaries in both groups, but to a lesser extent in TSP-1 KO mice (Figure 5). VEGF-A and VEGF-R2 mRNA expressions were similar in the two groups in normal kidneys and significantly decreased after UUO in both genotypes, $P<0.001$. However, TSP-1 KO animals exhibited a significantly higher expression of VEGF-A and VEGF-R2 mRNA expression after UUO than WT mice, $P<0.05$ (Figure 5). In the same way, renal VEGF content was similar in normal kidneys in both genotypes and decreased after UUO in WT mice ($P<0.001$), but not in TSP-1 KO mice ($P=0.18$), and thus it was significantly higher in TSP-1 KO UUO than in WT UUO mice ($P<0.05$, Figure 5e). To further assess renal tissue preservation, we measured cortex thickness and megalin expression as a marker of normal tubular phenotype. At baseline, cortical thickness was equal in the two genotypes (1059 ± 31 vs. $1058\pm30\,\mu m$). After UUO, cortical thickness was significantly reduced by 37.5% in WT mice ($P<0.005$), whereas the reduction observed in KO animals (14%) was not significant. Moreover, cortical thickness after UUO was significantly higher in TSP-1 KO mice than in WT mice, i.e., $935\pm96\,\mu m$ and $661\pm64\,\mu m$, respectively, $P<0.05$ (Figure 6a). Megalin is normally expressed by proximal tubular epithelial cells and is lost after tubular injury. Megalin expression was equivalent in the two genotypes of mice at baseline, i.e., $41.13\pm2.51\%$ and

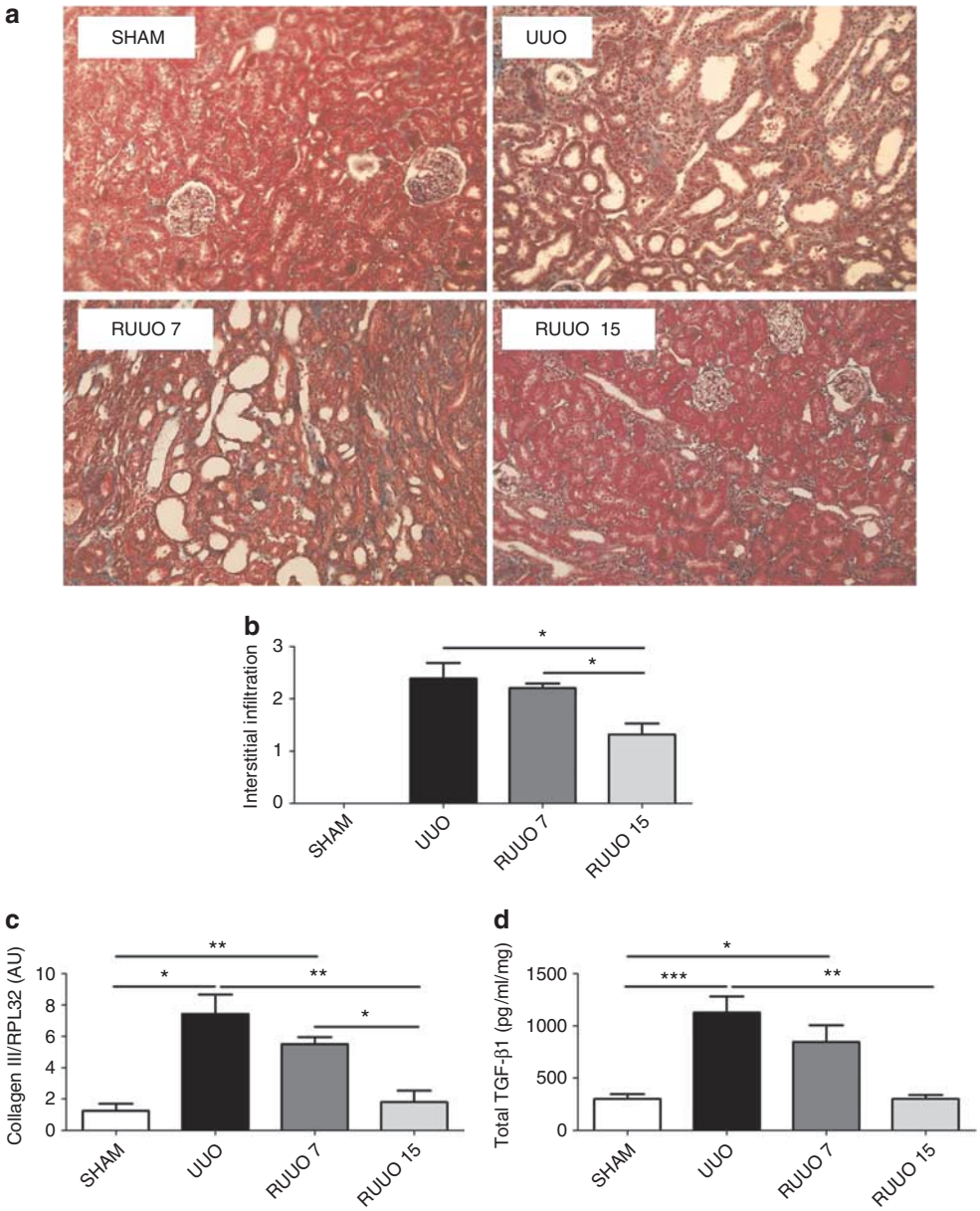

Figure 2 | Fibrosis and inflammation assessment after UUO and RUUO 7, 15. (**a**) Representative example of Masson's trichrome staining with cellular interstitial infiltration score. (**b**) Very rare interstitial cells were observed in the SHAM group. Ten days after unilateral ureteral obstruction (UUO), cellular interstitial infiltration was markedly increased. Seven days after reversible UUO (RUUO), cellular infiltration was still important. Fifteen days after RUUO, cellular infiltration significantly decreased compared with UUO rats. Quantitative real-time PCR of type III collagen. (**c**) UUO increased type III mRNA expression, which decreased at RUUO 15. (**d**) Total transforming growth factor-β (TGF-β) content in the renal cortex in the SHAM group, UUO, RUUO 7, and RUUO 15. Note the significant increase of TGF-β content in UUO. In RUUO 15, TGF-β level decreased to a level similar to that of SHAM. Values are mean ± s.e.m., $*P<0.05$, $**P<0.01$, $***P<0.005$.

$43.76 \pm 1.31\%$ of cortical area for WT and TSP-1 KO mice, respectively (Figure 6b). UUO decreased megalin expression in both groups, $P<0.001$. Interestingly, megalin expression was better preserved in TSP-1 KO mice compared with WT mice, i.e., 17.23 ± 1.67 vs. $12.82 \pm 0.67\%$ of cortical area in WT, $P<0.05$ (Figure 6b). Altogether, these results show that TSP-1 KO mice are protected against tubular injury with a better-preserved renal tissue and capillary network.

Genetic deficiency of *TSP-1* reduces inflammation from UUO-induced injury

At baseline, interstitial inflammatory cells were rare and mainly set around arteries. After UUO, important cortical interstitial cellular infiltration was observed in both genotypes. Semiquantitative analysis revealed that mean cellular infiltration score was significantly lower in TSP-1 KO mice, i.e., 1.9 ± 0.9 compared with 2.8 ± 0.2 in WT mice, $P<0.05$ (Figure 7a and b). Moreover, tubular cellular casts were only

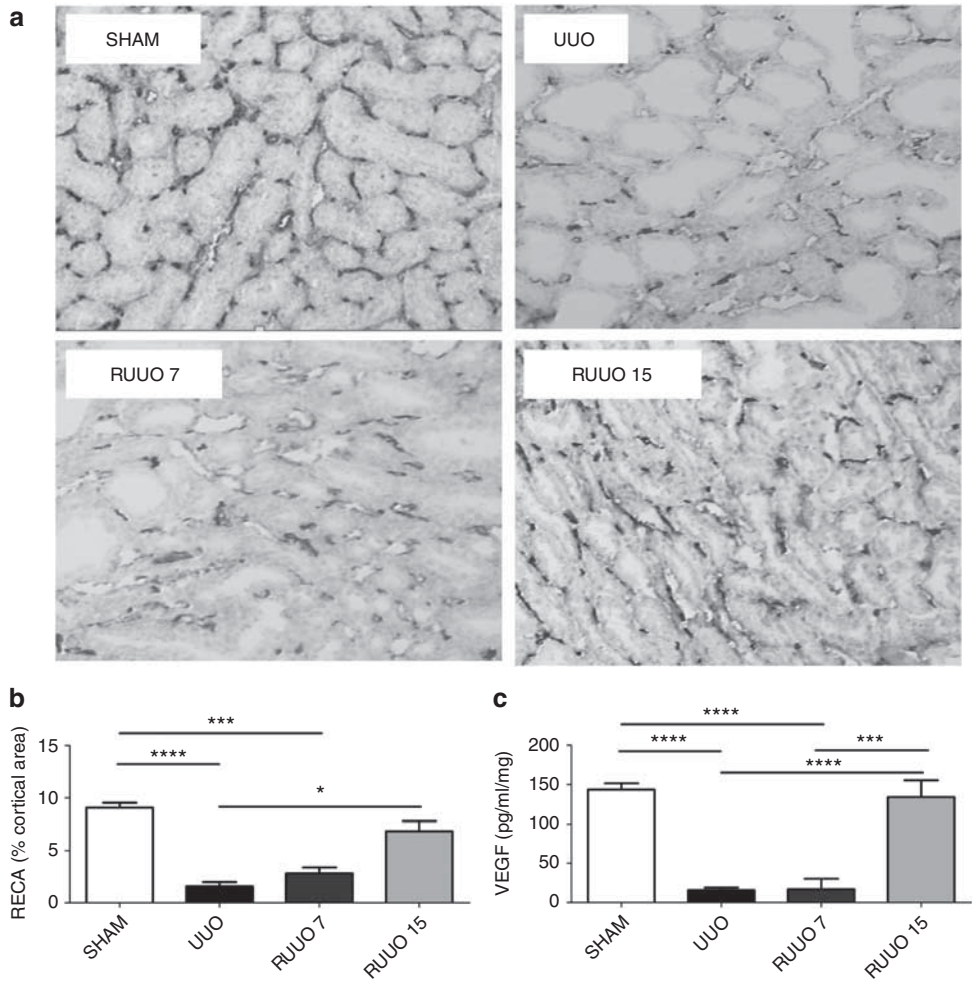

Figure 3 | Evaluation of microvascular network after UUO and RUUO 7, 15. (**a**) Representative example of peritubular capillary network revealed by anti-RECA antibody. RECA-positive cells highlight peritubular capillaries (PTC) in SHAM control. The surface area of RECA-positive cell significantly decreased in unilateral ureteral obstruction (UUO), indicating considerable areas of renal tissue devoid of PTC. Seven days after reversible UUO (RUUO), the rarefaction of PTC was sustained. After 15 days of RUUO, we observed a significant regeneration of PTC compared with UUO, but the value was still lower than that in the SHAM control group. (**b**) Quantification of RECA-positive cells by morphometric analysis. (**c**) Vascular endothelial growth factor (VEGF) content in renal cortex of SHAM, UUO, RUUO 7, and RUUO 15. Renal VEGF content significantly decreased after 10 days of UUO. This decrease was sustained in RUUO 7, but after 15 days of RUUO renal VEGF content returned to SHAM level. Values are mean ± s.e.m., $*P<0.05$, $***P<0.001$, $****P<0.001$.

observed in WT mice. In the non-obstructed kidney, MCP-1 mRNA expression was similar between the two genotypes. After UUO, MCP-1 mRNA expression increased in both groups. However, this increase was blunted in KO mice, $P<0.01$ (Figure 7c).

Previous studies have demonstrated that TGF-β1 is necessary for the differentiation of Th17 and T regulatory cells. These two pathways have opposite effects: Th17 has a pro-inflammatory role, whereas T regulatory pathway rather limits inflammation. As renal TGF-β1 level, inflammation, and, in particular, T lymphocyte (CD3 lymphocytes) infiltration were lower in UUO TSP-1 KO mice than in UUO WT mice (Figures 7d and 8a, b), we compared the activation of these two pathways. Therefore, we studied FOXP3, RORγT, and IL-17 mRNA expression. FOXP3 and RORγT are transcription factors, respectively, expressed by T regulatory and Th17 cells. IL-17 is a pro-inflammatory cytokine produced by Th17 cells.

In the normal kidney, IL-17 and RORγT were undetectable. FOXP3 was detectable in 6 out of 10 WT animals and 7 out of 11 TSP-1 KO mice, and its level was similar in the two genotypes. Although the expression of these markers was several-fold increased following UUO, overexpression of FOXP3 mRNA was similar between WT and TSP-1 KO UUO mice (Figure 8f). In contrast, overexpression of RORγT and IL-17 mRNA was blunted in TSP-1 KO compared with WT mice after UUO (Figure 8d–f). The ratio of RORγT to FOXP3 mRNA, used as a marker of the inflammation balance, was significantly lower in TSP-1 KO compared with WT mice ($P<0.05$; Figure 8g). These results suggest that TSP-1 invalidation reduces UUO-induced inflammation through inhibition of the Th17 pathway.

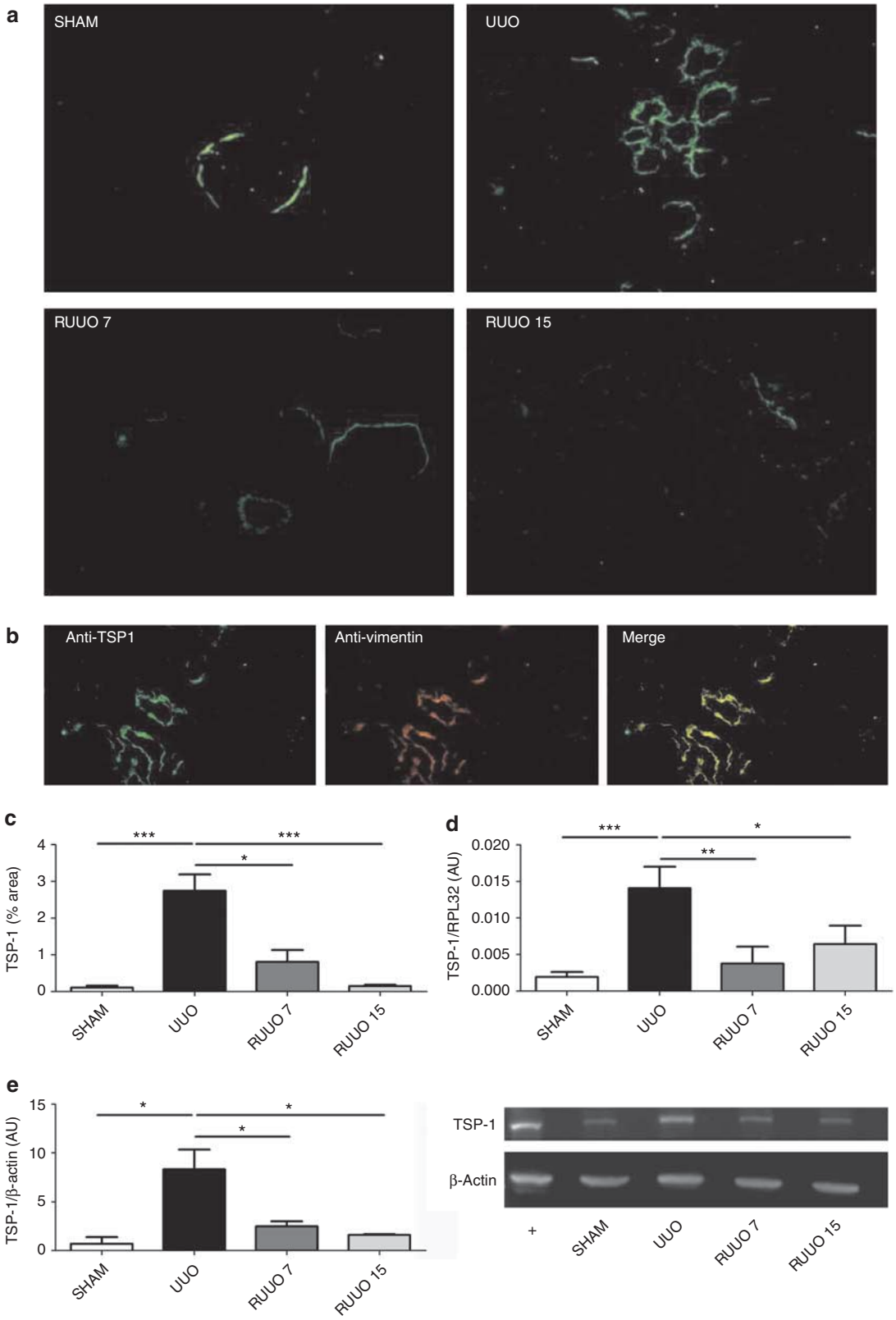

Figure 4 | Protein and mRNA expression of thrombospondin-1 (TSP-1). (**a**–**c**) Unilateral ureteral obstruction (UUO) induced an increased expression of TSP-1 confirmed by immunofluorescence, (**d**) real-time PCR, and (**e**) western blot analysis. (**b**) In the UUO group, TSP-1 was localized around the most dilated tubules and in dedifferentiated tubular epithelial cells that coexpressed vimentin filament. (**c**–**e**) TSP-1 expression decreased after relief of UUO compared with the UUO group. Values are mean ± s.e.m., $*P<0.05$, $**P<0.01$, $***P<0.005$.

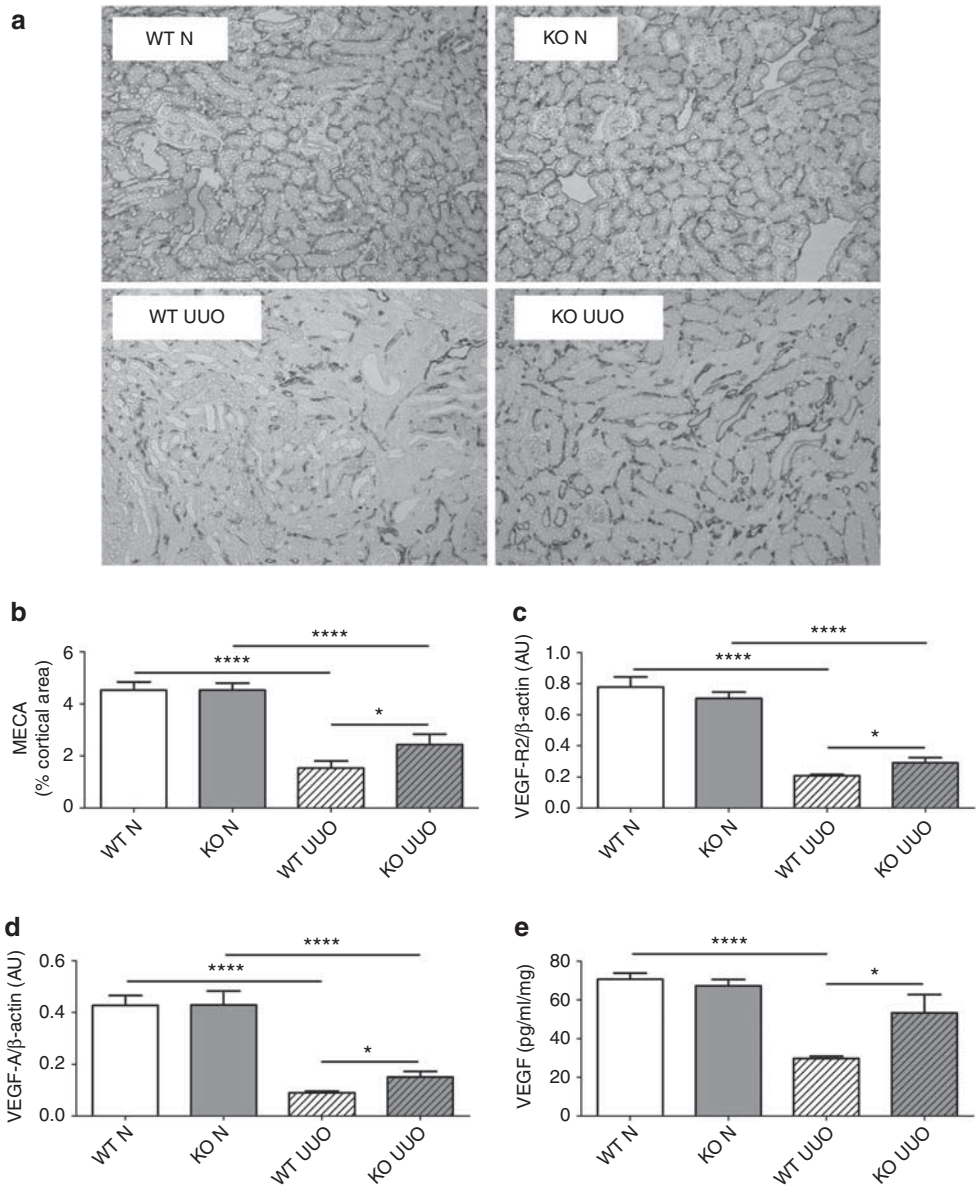

Figure 5 | Evaluation of microvascular network in WT and KO animals at baseline and after UUO. (**a**) Peritubular capillary network revealed by anti-MECA antibody in normal (N) and obstructed (unilateral ureteral obstruction, UUO) kidneys of wild-type (WT) and thrombospondin-1 (TSP-1) knockout (KO) mice. mRNA expression of (**c**) vascular endothelial growth factor (VEGF)-R2 and (**d**) VEGF-A by real-time quantitative PCR. (**e**) Renal VEGF-A content was assessed by enzyme-linked immunosorbent assay (ELISA). (**b**) Capillary rarefaction was alleviated in TSP-1 KO mice as the reduction of expression of (**d**, **e**) VEGF-A and (**c**) its receptor VEGF-R2. Values are mean ± s.e.m., $^{*}P<0.05$, $^{****}P<0.001$.

DISCUSSION

In the present study, we investigated cellular and molecular mechanisms of renal repair in rats with profibrotic, inflammatory lesions using the model of ligature relief after UUO. The kidneys of these animals showed tubular epithelium replacement, regeneration of peritubular capillaries, remodeling of extracellular matrix accumulation matrix, increased cell viability, and functional recovery. Repair was progressive, and renal parenchyma appeared almost normal 15 days after UUO release. Structural improvement was preceded by cellular proliferation and the reduction of TSP-1 and TGF-β1 expression. The involvement of TSP-1 in renal profibrotic, inflammatory lesions was demonstrated using TSP-1 KO mice. In these mice, the development of UUO lesions was blunted as evidenced by a lower renal interstitial inflammation and a better-preserved renal cortex. In addition, we showed that inflammation was mediated at least in part by MCP-1 and the activation of Th17 cell pathway.

UUO is widely utilized as a model of tubulointerstitial fibrosis.[14] This model has provided important insights into the molecular and cellular mechanisms of interstitial fibrosis and CKD progression.[22] Less is known about the mechanisms that promote tissue remodeling and regeneration. RUUO offers the advantages for studying mechanisms of renal

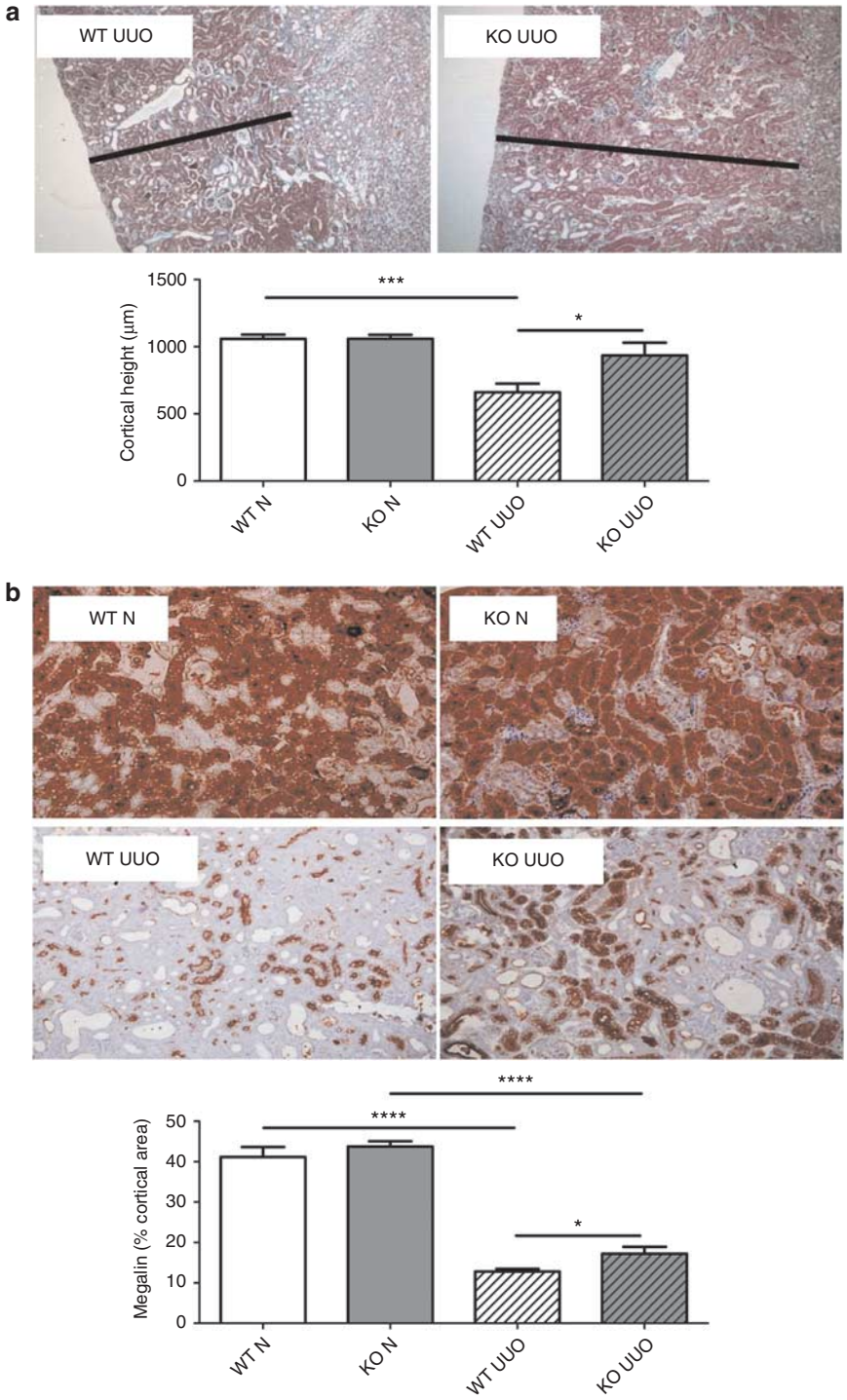

Figure 6 | Preservation of renal cortex in WT and KO animals at baseline and after UUO. (**a**) Representative example of Masson's trichrome staining of unilateral ureteral obstruction (UUO) kidneys of wild-type (WT) and thrombospondin-1 (TSP-1) knockout (KO) mice. (**b**) Renal megalin expression revealed by anti-megalin antibody in normal (N) and obstructed (UUO) kidneys of WT and TSP-1 KO (KO) mice. (**a**) TSP-1 KO mice displayed a better-preserved renal cortex and (**b**) megalin expression than WT mice after UUO. Values are mean ± s.e.m., $^{*}P<0.05$, $^{***}P<0.001$, $^{****}P<0.001$.

repair. Different approaches have been used to allow reversal of obstruction. In rats, surgical procedures included reimplantation of the ureter into the bladder or removal of the ureter previously placed between two silicone tubes with different diameters and ligated.[16,17,23] Post-obstructed kidney function was assessed after contralateral nephrectomy. More recently, in mice, Cochrane *et al.*[18] used microvascular clips to facilitate reversal of obstruction followed by bilateral

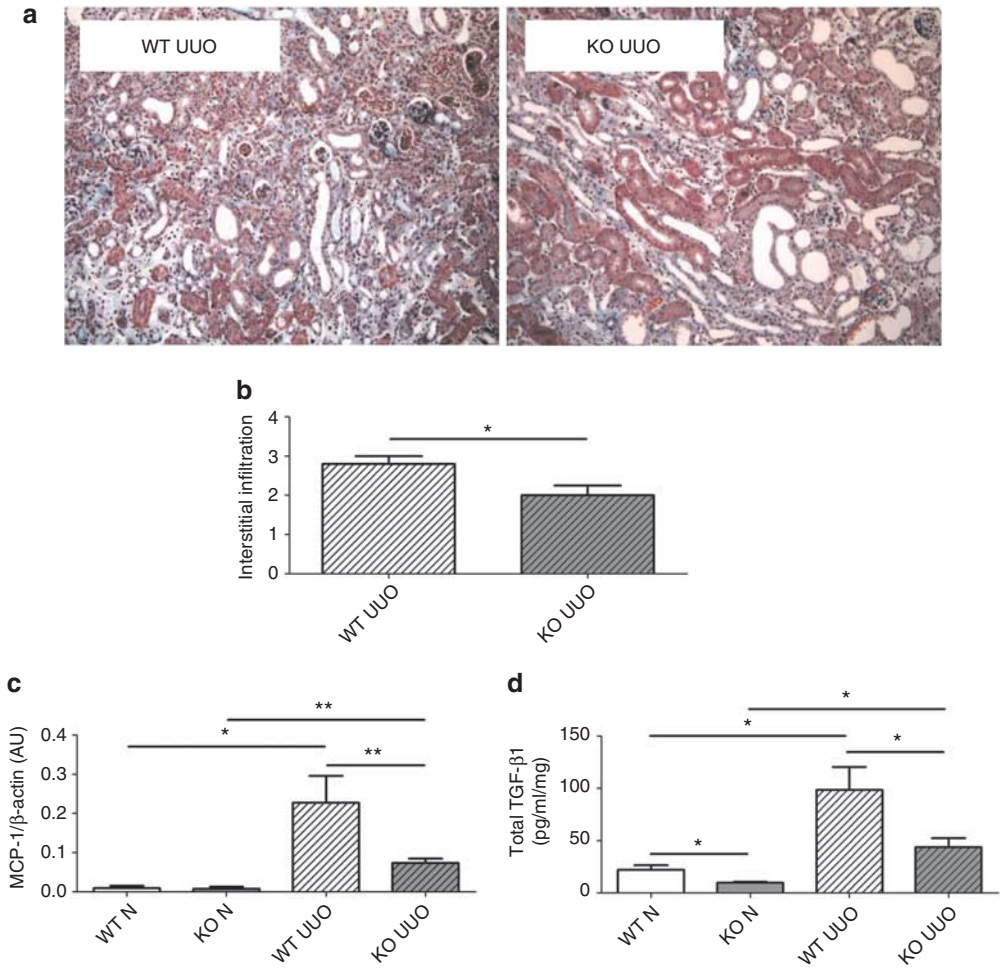

Figure 7 | Evaluation of fibrosis and inflammation in WT and KO animals after UUO. (**a**) Representative example of Masson's trichrome staining of unilateral ureteral obstruction (UUO) kidneys of wild-type (WT) and thrombospondin-1 (TSP-1) knockout (KO) mice and (**b**) inflammation score. (**c**) mRNA expression of MCP-1 by real-time PCR and (**d**) total transforming growth factor-β (TGF-β) content in the renal cortex in normal (N) and obstructed (UUO) kidneys of WT and TSP-1 KO (KO) mice. Note the significant decrease of MCP-1 mRNA and total TGF-β1 content in TSP-1 KO mice. Values are mean ± s.e.m., $*P<0.05$, $**P<0.01$.

cannulation of the ureters for functional assessment. To simplify the microsurgical procedure, we used the vascular clip and performed a contralateral nephrectomy, which allowed to measure the renal function of the left kidney and its recovery.

RUUO resulted in a remarkable restoration of kidney parenchyma as previously reported.[18] Our study brings new insights into cellular and molecular mechanisms involved in renal repair. This restoration was characterized by progressive disappearance of dilated tubules, regeneration of cortical thickness, and proximal tubular epithelium with reappearance of brush borders, improvement of peritubular capillary network, and removal of interstitial cell infiltration. Interestingly, the early phase of renal repair (7th day post removal) was characterized by increased cell proliferation. In a similar experiment in C57BL/6 strain mice, other investigators found a higher inflammation score and macrophage infiltration 7 days after release of obstruction than in UUO mice after 6 days of obstruction. In BALB/c mice, only a modest inflammation was observed, which resolved and normalized rapidly.[24] Interestingly, these results indicate that renal repair is preceded by cell proliferation and a possible cross talk between tubular epithelial and macrophage cells. Consistent with this hypothesis, it has been recently observed that CSF-1, a hematopoietic growth factor predominantly generated by tubular epithelial cells, promotes proliferation and activation of macrophages, and mediates renal repair after ischemia/reperfusion and UUO.[13] Although macrophages have classically been recognized as active players in progression of interstitial fibrosis,[25] several studies have suggested a beneficial antifibrotic role of infiltrating macrophages.[26] This apparent opposite role could depend on functional heterogeneity and different phenotypes of macrophages.[27,28]

To determine whether structural improvement has functional consequence, we assessed cell viability and renal function by measuring plasma creatinine and urinary albumin excretion. We showed an improvement of cell viability with important renal function recovery of the previously

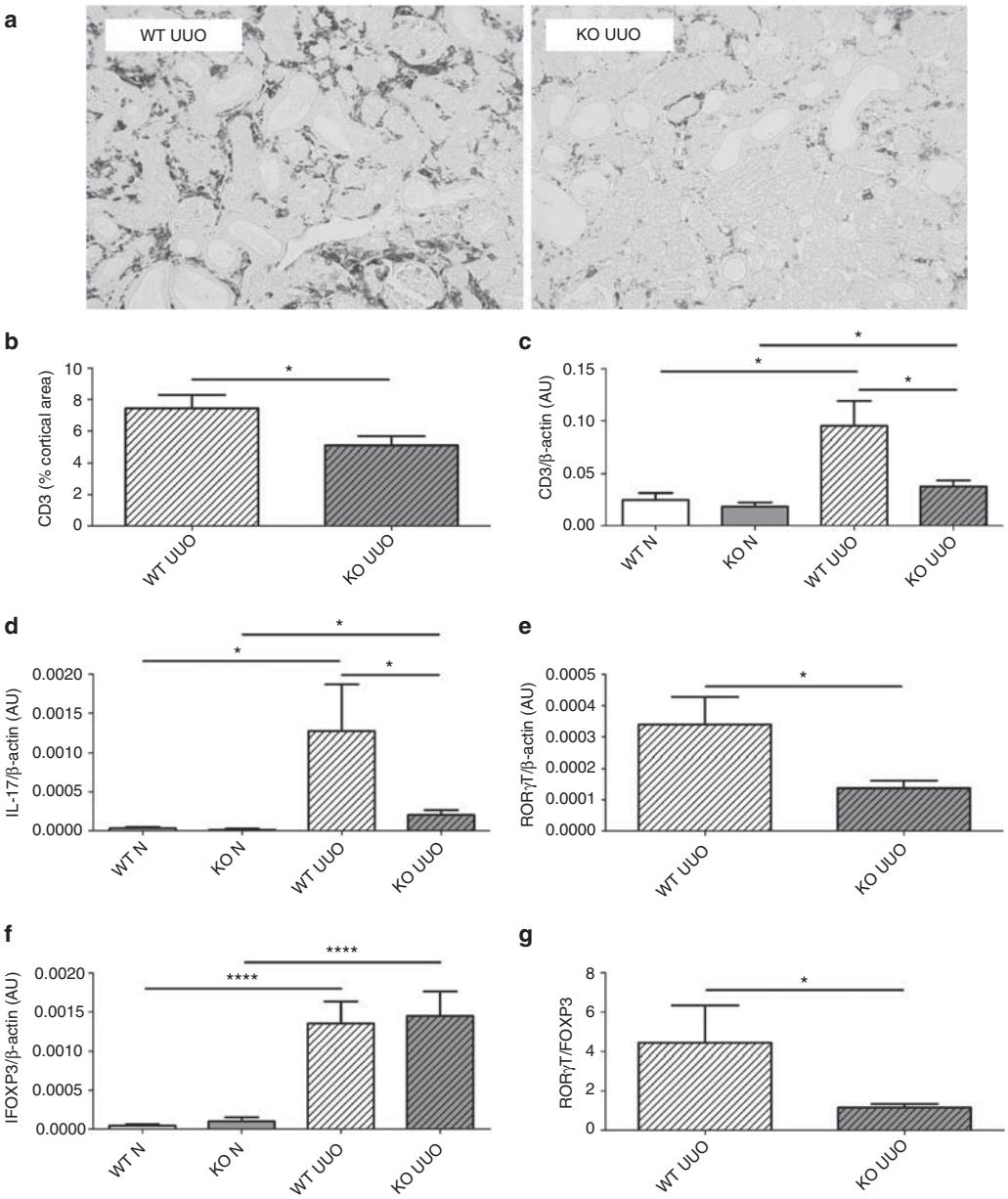

Figure 8 | Analysis of lymphocyte infiltration in WT and KO animals after UUO. (**a**, **b**) Interstitial infiltration by T lymphocytes revealed by anti-CD3 antibody and (**c**–**g**) mRNA expression of CD3, FOXP3, RORγT, IL-17, and the ratio of RORγT to FOXP3 in the renal cortex in normal (N) and obstructed (unilateral ureteral obstruction, UUO) kidneys of wild-type (WT) and thrombospondin-1 (TSP-1) knockout (KO) mice. (**a**, **b**) After UUO, interstitial CD3 lymphocyte infiltration was alleviated in TSP-1 KO (KO) mice compared with WT. (**c**) This was corroborated with a reduction of CD3 mRNA. The pro-inflammatory Th17 pathway was reduced in TSP-1 KO mice compared with WT after UUO. Values are mean ± s.e.m., $*P<0.05$, $****P<0.001$.

obstructed, severely injured kidney. In mice, glomerular filtration rate of RUUO kidneys was restored from 43 to 88% after 10 days of UUO.[18] Other authors have reported renal function recovery, but after a shorter time of obstruction. Glomerular filtration rate and renal blood flow returned to baseline 14 days after relief of 3 days of UUO.[15] These data clearly show that the restoration of renal structure is accompanied by functional recovery.

To date, the role of TGF-β1 during the repair process is unclear. TGF-β1 is a major factor involved in the progression of renal fibrosis.[29] On the other hand, TGF-β1, similar to FGF2 and VEGF, contributes to vasculogenesis and angiogenesis, which are required for repair.[30] We assessed the active form of TGF-β1 and the expression of its endogenous activator TSP-1.[19,31] We have previously demonstrated the involvement of TG2, another endogenous activator of TGF-β1 during UUO, and showed that mice with genetic deficiency of TG2 were partly protected from renal fibrosis because of reduced activation of TGF-β1.[3] The increased expression of TSP-1 has been observed during experimental and clinical nephropathies.[32–35] Besides the activation of TGF-β1, TSP-1 could contribute to renal fibrosis through its

anti-angiogenic effects.[36] We found that RUUO was followed by an early decrease in active TGF-β1 and TSP-1 at day 7, followed by a reduction in collagen III at day 15. In addition, the rise in renal VEGF content and peritubular capillaries was delayed, reaching the highest level at day 15. Different results of TGF-β during renal repair have been reported by other groups. In adult rats, after 3-day UUO followed by relief, total TGF-β1 renal content increased progressively with time.[15] Similarly, the release of UUO did not blunt the increased mRNA level of TGF-β1 in the post-obstructed kidney up to 14 days, but it significantly decreased 28 days after the release of obstruction.[37] The reasons for the discrepancies are unclear. It is possible that the duration of UUO (3 vs. 10 days) could be a determining factor in the activation of TGF-β1. In agreement to the hypothesis that TSP-1 is involved in TGF-β1 activation,[34] we found a close correlation not only between TSP-1 expression and TGF-β1 content, but also with mRNA collagen III expression and myofibroblasts. These observations further support a leading role of TSP-1 in the progression of renal fibrosis.

We further investigated the role of TSP-1 using TSP-1 KO mice[38] and showed an angiogenic effect when TSP-1 was genetically deleted. Although the increase in TSP-1 expression and the loss of VEGF expression were correlated with capillary loss and the development of fibrosis in aging rats and in the remnant kidney model,[33,39] the pro-angiogenic effect of TSP-1 inhibition had never been reported in CKDs before.[40–42] In addition to a better capillary network, TSP-1 KO mice displayed a better-preserved renal cortex and megalin expression than WT mice after UUO, suggesting a deleterious effect of TSP-1 in tubular epithelial cells. Accordingly, it has been shown that addition of TSP-1 peptide in rat proximal tubular cell culture favored cell apoptosis through caspace-3 activation.[43] Moreover, TSP-1-null mice exposed to renal ischemia/reperfusion injury demonstrated significant preservation of tubular structures.[43]

We investigated the mechanisms of TSP-1-induced renal inflammation because we observed less renal interstitial inflammation in TSP-1 KO mice compared with WT mice. We found that at least two mechanisms might contribute to decreased inflammation in TSP-1 KO mice: reduced activation of MCP-1 and decreased rates of CD3 T-cell infiltration through inhibition of the TGF-β1-induced Th17 pathway.[44,45] In support to our hypothesis, TSP-1 KO mice also exhibited lower levels of MCP-1 than WT animals in a wound healing model.[46] Other authors have shown that, *in vitro*, TSP-1 induced MCP-1 release by mononuclear cells in a dose-dependent manner.[47] Th17-associated transcription factors RORγT and interleukin 17 mRNA were not detectable in unobstructed kidneys, but were significantly increased after UUO. However, this increase was blunted in TSP-1 KO mice. This result could be explained by the blunted levels of TGF-β1 in KO animals. Indeed, two recent studies revealed that TSP-1 participates in the development of experimental autoimmune encephalomyelitis by enhancing T-cell infiltration and the differentiation of Th17 cells in a TGF-β1-dependent manner.[48,49] The role of Th17 cells in renal injury was previously studied in experimental glomerulonephritis in which IL-23- and IL-17-deficient mice developed less severe nephritis compared with WT animals.[47] In our model, TSP-1 KO mice showed reduced expression of IL-17 and RORγT associated with less severe tubular lesions. Altogether, these results suggest that Th17 cells might participate in tubular and glomerular injury.

In conclusion, our data provide new clues to the understanding of the mechanisms of renal repair after UUO relief. Structural regeneration was associated with functional recovery. Among the early and driving events of renal repair in RUUO are cell proliferation and decrease in TSP-1 and TGF-β1 levels. TSP-1 appears to be an important mediator, because its deletion is associated with protection against UUO induced renal inflammation and fibrosis. Thus, targeting TSP-1 could limit fibrosis development and favor renal repair in CKD patients.

MATERIALS AND METHODS

Animals and experimental design

UUO surgery was performed on male Sprague-Dawley rats (Charles River Laboratories, Arbresle, France) weighing 150–200 g. Surgery was performed under general anesthesia after intraperitoneal injection of pentobarbital sodium 65 mg/kg. After laparotomy, the left ureter was clamped with a non-traumatic microvascular clip (S&T Fine Science Tools, North Vancouver, CA). In control rats (SHAM), the ureter was manipulated but not ligated.

A first protocol was performed to study the mechanisms of renal repair after UUO release (RUUO). Ten days after UUO, the vascular clip was removed and rats were killed 7 or 15 days after UUO release (RUUO 7 and 15, respectively). They were compared with SHAM control, and rats were killed after 10 days of UUO ($n = 6$ per group). Reversal of obstruction was confirmed by resolution of hydronephrosis. The RUUO surgery was successful in more than 75% of the animals. Failure was due to excessive adhesions around the ligation, and those animals were excluded on gross examination.

To assess the renal function of the previously obstructed kidney at RUUO 15, a second protocol was performed. Two weeks after RUUO, the contralateral kidney was removed, leaving the previously obstructed kidney as a life-sustaining organ (RUUO + NX). Plasma creatinine and urinary protein excretion were measured 15 days later, to allow hemodynamic and renal function recovery after surgery. Values were compared with the values of SHAM group of rats that were killed 15 days after uninephrectomy (SHAM + NX) without UUO ($n = 5$ per group).

To determine the role of TSP-1, TSP-1 knockout (TSP-1 KO) mice generated on a C57/Bl6J background[38] and age-matched WT mice underwent UUO (8- to 10-week-old, $n = 13$ and 12, respectively) by double ligation of the left ureter under general anesthesia after intraperitoneal injection of xylazin and ketamin. Animals were killed 10 days after UUO. The right unobstructed kidney was used as normal control for each mouse.

Detailed methodology is described in the Supplementary Methods online.

DISCLOSURE

All the authors declared no competing interests.

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Author contributions

NS and NB carried out experiments and analyzed the data. SB, SV, and CJ participated in some experiments. J-CD, PR, and CC analyzed the data and were involved in writing the article. J-JB conceived, analyzed, and was involved in writing the article. All authors had final approval of the submitted version.

SUPPLEMENTARY MATERIAL

Figure S1. Representative example of cell proliferation revealed by PCNA antibody (A), and cellular apoptosis revealed by TUNEL assay (B).

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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