

Research Article

WNT1-inducible signaling pathway protein 1 regulates kidney inflammation through the NF- κ B pathway

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Inflammation is a pathological feature of kidney injury and its progression correlates with the development of kidney fibrosis which can lead to kidney function impairment. This project investigated the regulatory function of WNT1-inducible signaling pathway protein 1 (WISP1) in kidney inflammation. Administration of recombinant WISP1 protein to healthy mice induced kidney inflammation (macrophage accrual and production of tumor necrosis factor α (TNF- α), CCL2 and IL-6), which could be prevented by inhibition of nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B). Furthermore, inhibition of WISP1, by gene knockdown or neutralising antibody, could inhibit cultured macrophages producing inflammatory cytokines following stimulation with lipopolysaccharides (LPSs) and kidney fibroblasts proliferating in response to TNF α , which both involved NF- κ B signaling. Kidney expression of WISP1 was found to be increased in mouse models of progressive kidney inflammation-unilateral ureter obstruction (UJO) and streptozotocin (STZ)-induced diabetic nephropathy (DN). Treatment of UJO mice with WISP1 antibody reduced the kidney inflammation in these mice. Therefore, pharmacological blockade of WISP1 exhibits potential as a novel therapy for inhibiting inflammation in kidney disease.

Introduction

The growing prevalence of chronic kidney disease (CKD) is a major health concern and can be caused by a variety of factors including obesity, diabetes and hypertension. Current therapies have a limited ability to prevent chronic kidney inflammation and fibrosis and halt the progression of CKD. Consequently, many CKD patients will develop end-stage kidney disease (ESKD) and require dialysis or kidney transplantation to survive. Therefore, novel strategies are required to specifically target chronic inflammation, and its ability to promote fibrosis, in patients with CKD.

Chronic inflammation causes injury and fibrosis in kidney glomeruli and the tubulointerstitium, leading to progressive CKD and renal function impairment. Complex mechanisms underlie the development of kidney inflammation, which include kidney accumulation of infiltrating leukocytes and increased levels of inflammatory cytokines [1]. Common kidney inflammatory responses include the induction of proinflammatory cytokines, such as tumor necrosis factor α (TNF- α) and interleukin (IL)-6 (IL-6), and chemokines, such as monocyte chemoattractant protein-1 (MCP-1), and IL-8. Notably, TNF- α has the ability to increase induction of other potent cytokines and amplify the inflammatory response through promoting activation of the nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) and MAPK signaling pathways [2]. NF- κ B-p65 is a subunit of the NF- κ B transcription complex which is activated by

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inflammatory stimuli and translocates into the nuclei of inflammatory cells, where it plays a regulatory role in the inflammatory response [3]. NF- κ B also acts as a transcriptional regulator of survival genes or anti-apoptotic genes to regulate cell death, cell proliferation and transformation [4].

Activation of the Wnt/ β -catenin signaling pathway has been shown to correlate with progression of renal injury [5], with targeted inhibition of the Wnt pathway reducing the development of fibrosis in animal models of kidney disease [6,7]. In addition, a downstream factor of the Wnt signaling pathway, known as WNT1-inducible signaling pathway protein 1 (WISP1) [11], has been shown to be increased in mouse models of progressive CKD, in association with development of renal fibrosis [8]. However, it is unclear whether the association of WISP1 with kidney fibrosis is due to an effect of WISP1 on the development of kidney inflammation which precedes fibrosis and can drive a fibrotic response. Therefore, the current study focuses on the regulatory role of WISP1 in inflammatory responses associated with kidney injury and investigates the functional role of WISP1 in the progression of inflammation in a mouse model of CKD, with a view to exploring the potential benefits of targeting WISP1 in CKD with a pharmacological inhibitor.

Materials and methods

In vitro studies: cell culture

The RAW macrophage cells and rat kidney fibroblast cells were obtained from the American Tissue Culture Collection (Rockville, MD) and maintained in Dulbecco's modified Eagle's medium containing 10% serum and 5 mmol/l glucose.

Gene expression in RAW macrophages was assessed after 12 h of stimulation with lipopolysaccharide (LPS; 1 ng/ml) or high glucose (22.5 mM D-glucose) by quantitative real-time PCR.

Drugs and antibodies

Recombinant human WISP1 protein, IgG control and mouse WISP1 antibody were purchased commercially (R&D Systems, Minneapolis, MN) and used at specified concentrations. Typically, 24 h after cells were seeded, culture medium was replaced and cells were incubated for a further 1–5 days with WISP1 or TNF- α , depending on experiments. For Western blotting, primary antibodies were used to detect I κ B α (1:2000; Cell Signaling, Danvers, MA), WISP1 and secondary HRP-conjugated antibodies were either anti-mouse IgG or anti-rabbit IgG (1:2000; Cell Signaling).

RNA extraction and real-time PCR

RNA was isolated from cells and tissues using the Zymo Quick RNA (Zymo Research, Irvine, CA 92614). mRNA was reverse transcribed into cDNA using Agilent AffinityScript (Agilent, Santa Clara, CA 95051). Gene expression was analyzed by real-time (RT)-PCR, using the TaqMan system based on real-time detection of accumulated fluorescence (ABI Prism 7500; PerkinElmer, Foster City, CA). Fluorescence for each cycle was quantitatively analyzed by an ABI Prism 7500 Sequence Detection System (PerkinElmer). To control variation in the amount of DNA that was available for PCR in the different samples, gene expression of the target sequence was normalized in relation to the expression of an endogenous control, 18S rRNA (18S rRNA TaqMan Control Reagent kit, ABI Prism 7500; PerkinElmer). Details of primers and TaqMan probes for these genes have been previously reported [9]. Each experiment was conducted in four to six replicates. Results were expressed relative to control (untreated) cells/tissue, which were arbitrarily assigned a value of 1.

Transfection of shRNA of WISP1

Cells were seeded at 3×10^4 cells per well in 12-well plates. The following day, medium was replaced with OptiMEM (Invitrogen) and cells were transfected with lentivirus expressing Wisp1 shRNA using Lipofectamine 2000 (Invitrogen).

In each case, lentiviral controls were used at the same concentration. Cells were harvested 3–5 days post-transfection.

ELISA

The expression levels of TNF- α were determined in medium of RAW 264.7 cells using ELISA kits (R&D Systems, Inc., Minneapolis, MN, U.S.A.), according to the manufacturer's protocols.

Western blot analysis

Whole-cell lysates that contained 10–50 μg of protein were subjected to 10–12% SDS/PAGE and transferred on to polyvinylidene fluoride membranes by semidry transfer. Membranes were blocked in 5% skim milk/Tris-buffered saline with 0.1% Tween 20 for 1 h at room temperature. All primary and secondary antibody incubations were for 1 h at room temperature. Detection was by chemiluminescence and images captured on the UVITEC Imaging system (Cambridge, U.K.). ImageJ was used to analyze protein density.

Animal models

Two well-established animal models were used to explore the relationship between WISP1 and the development of kidney inflammation. Unilateral ureter obstruction (UO) was performed on C57BL/6 mice aged 7–8 weeks (20–25 g, $n=7-8/\text{group}$) [10], whereby the left ureter was visualized via a flank incision and ligated using double tracks with 5.0 surgical silk. This procedure results in rapidly progressive tubulointerstitial fibrosis, which resembles lesions seen in C.KD after 7 days.

Secondly, we induced type 1 diabetic nephropathy (DN) in C57BL/6 mice aged 7–8 weeks (20–25 g, $n=8/\text{group}$) using streptozotocin (STZ). Each mouse was administered with five daily intraperitoneal injections of STZ (55 mg/kg/day) to induce overt diabetes [11] and were assessed after 18 weeks for development of chronic kidney fibrosis.

Antibody treatment of UO mice

Male C57BL/6 mice (20–25 g) were randomly allocated into the treatment groups of IgG control or WISP1 antibody ($n=7/\text{group}$). UO surgery was performed on mice using 2% isoflurane as the inhalation anesthesia (Abbott Australasia, Kurnell, NSW, Australia) immediately following UO surgery, osmotic minipumps (ALZET Models 1007D Alzet, Cupertino, CA) administering control IgG or WISP1 antibody (5 $\mu\text{g}/\text{g}$ body weight/day for 7 days) were placed in the abdominal cavity prior to suturing and recovery. Seven days after UO surgery, kidney fibrosis was assessed.

Administration of recombinant human WISP1 protein and NF- κ B inhibitor

Male mice were delivered parthenolide (NF- κ B inhibitor, PTL) 3.5 $\mu\text{g}/\text{g}$ body weight or its vehicle (0.05% DMSO) 1 day prior to 2.5 $\mu\text{g}/\text{g}$ body weight recombinant WISP1 or saline by intraperitoneal injection [12]. The mice were killed after 48 h of WISP1 injection.

Histopathology analysis of kidney samples

Formalin-fixed paraffin-embedded kidneys were sectioned at 4 μm and stained with Masson's Trichrome to assess changes in kidney structure. Picosirius Red (PSR) staining was performed on kidney sections to visualize the distribution of interstitial collagen (Col) under bright-field and polarized microscopy as described previously [9].

Immunohistochemistry

For immunostaining, sections were treated with 5% rabbit or goat serum for 30 min and then incubated with primary antibody in 3% BSA overnight at 4°C. Sections were then washed three times with PBS for 10 min. Bound primary antibodies were detected using a fluorescently labeled secondary antibody.

Statistical analysis

Values are shown as means \pm SEM, unless otherwise specified. GraphPad Prism (GraphPad Software) was used to analyze data by unpaired Student's *t* test or by one-way analysis of variance followed by a Tukey's multiple comparison test. *P*-values <0.05 were considered significant.

Results

Administration of recombinant WISP1 into mice induces kidney inflammation

To examine the acute inflammatory effects of WISP1 in the kidney, we administered human recombinant WISP1 into C57BL/6 mice by intraperitoneal injection and studied the kidney response 48 h later. Compared with vehicle controls, mice which received WISP1 had increased accumulation of macrophages (F4/80⁺ cells) in their kidneys (Figure 1A), verified by counting the percentage of F4/80⁺ cells and increased kidney gene expression of proinflam-

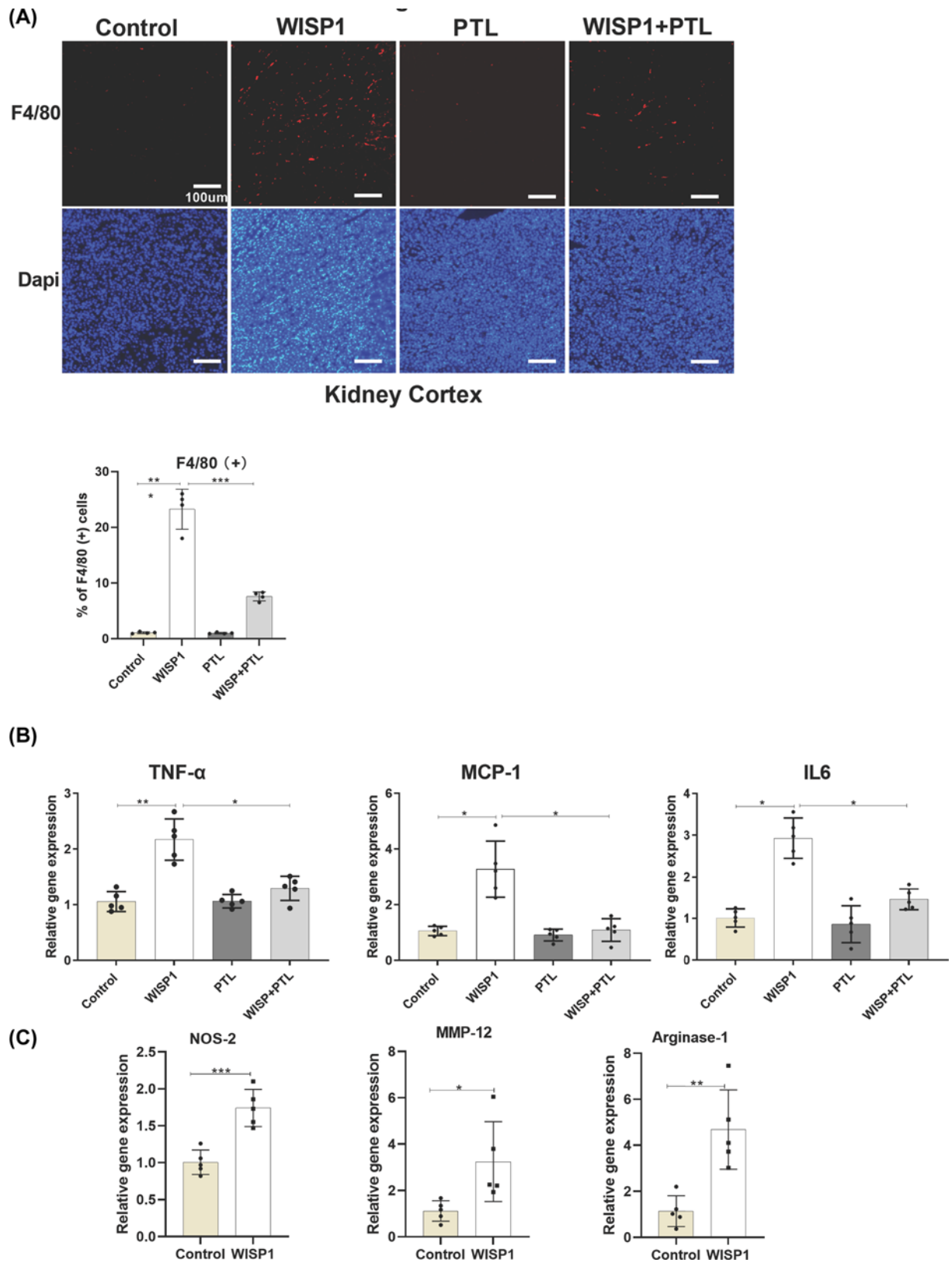


Figure 1. WISP1 induces inflammation in healthy mouse kidneys

(A) F4/80 positive cells were accumulated in mice kidney (kidney cortex) with WISP1 administration and reduced by PTL, which was verified by counting the percentage of F4/80 positive cells. (B) The expression of inflammation markers was up-regulated by WISP1 and alleviated by PTL treatment. (C) M1 and M2 marker expression. Results are expressed as the mean \pm SEM relative gene expression, *** P <0.001, ** P <0.01, * P <0.05. n =3–5.

matory molecules (TNF- α , MCP-1, IL-6) (Figure 1B). Pre-treatment of mice with the NF- κ B inhibitor parthenolide (PTL), but not vehicle alone (0.05% DMSO), prevented both the accrual of kidney macrophages and the increase in kidney pro-inflammatory gene expression seen in response to WISP1 administration. This indicates that the pro-inflammatory effects of WISP1 in the kidney are mediated by activation of NF- κ B. The pro-inflammation function of WISP1 was also demonstrated by the up-regulated M1 markers (Nos-2, MMP-12) and M2 markers (Arginase-1) (Figure 1C).

In comparison, acute exposure of mouse kidneys to recombinant WISP1 had no impact on the kidney morphology (Figure 2A) and kidney collagen accumulation (Figure 2B). Furthermore, quantitative PCR analysis of whole kidney found that acute exposure to WISP1 induced no changes in the gene expression of collagen 1 and fibronectin (Figure 2C), but did induce gene expression of α -smooth muscle actin (α -SMA) (Figure 2D). Our data to study other inflammatory cells that are involved in kidney injury showed no significant changes in CD3e marker of T cells in WISP1-treated normal kidneys at 48 h, indicating that WISP-1 does not promote T-cell infiltration (Supplementary Data S1).

WISP1 promotes inflammation by facilitating activation of the NF- κ B pathway

To verify that WISP1 regulates NF- κ B activity in macrophages, we treated cultured macrophages (RAW264 cells) with the recombinant WISP1 for 48 h in the presence or absence of NF- κ B inhibitor (PTL). Immunofluorescence staining demonstrated that WISP1 induces translocation of phosphorylated-p65 (P-p65) into nuclei, which can be inhibited by PTL (Figure 3A). The elevated gene expression of TNF- α was inhibited by PTL (Figure 3B). This translocation of P-p65 could also be induced by stimulating macrophages with the classical pro-inflammatory stimulant LPS (Figure 3C), which coincided with elevated gene expression of TNF- α , MCP-1 and IL-6, and was inhibited by PTL (Figure 3D). Interestingly, LPS-induced nuclear translocation of P-p65 in macrophages, and the corresponding increase in pro-inflammatory gene expression, were also inhibited by WISP1 knockdown with shRNA (expressed via lentiviral transfection) (Figure 4A–C) or WISP1 blockade with neutralizing antibody (Figure 5A,B). In addition, these strategies for inhibiting WISP1 activity reduced macrophage secretion of TNF- α induced by LPS (Figure 5C). These results suggest that WISP1 facilitates NF- κ B-p65 signaling in macrophages during inflammatory responses, which could help drive kidney injury.

WISP1 promotes kidney fibroblast proliferation

In addition to inducing kidney inflammation, administration of WISP1 to normal mice also increased kidney expression of α -smooth muscle actin at 48 h, suggesting that it may directly or indirectly regulate myofibroblast activity. It is known that α -SMA expression by fibroblasts is an indicator of fibroblast activation [13]. However, no increase in gene expression of collagen I or fibronectin was seen in WISP1-treated mice (Figure 2). To identify how WISP1 may increase myofibroblasts, we treated cultured kidney fibroblasts (NRK49F cells) with recombinant WISP1. This showed that WISP1 could induce kidney fibroblast proliferation, and was preventable by the addition of the NF- κ B inhibitor PTL (Figure 6A). Since TNF- α can also stimulate fibroblast proliferation [14] through NF- κ B pathway [15], we explored whether this mitogenic effect was dependent on WISP1. We found that kidney fibroblast proliferation in response to TNF- α could be reduced by either WISP1 knockdown using shRNA (Figure 6B) or treatment with a neutralizing anti-WISP1 antibody (Figure 6C). These studies indicate a role for WISP1 in promoting kidney fibroblast activity, particularly during an inflammatory response.

Kidney expression of WISP1 is increased in mouse models of progressive inflammatory kidney disease

Two common models of inflammatory kidney disease were examined to analyze the relationship of WISP1 to kidney inflammation. DN is an inflammatory kidney disease affecting both glomerular and tubulointerstitial compartments, which progresses in approximately one-third of patients [16]. It is also the most common cause of end-stage renal failure worldwide. Therefore, we examined kidney expression of WISP1, and its association with inflammation, in a mouse model of DN. After 16 weeks of STZ-induced diabetes, mice displayed elevated kidney gene expression of CD68, TNF- α and MCP-1 and α -SMA, which was accompanied by an increase in WISP1 gene expression ($P < 0.01$) (Figure 7A). In addition, immunofluorescence staining indicated the elevated expression of WISP1 in diabetic kidneys (Figure 7A). Infiltrating macrophages co-localized with WISP1 in DN model (white arrows). The profiles of non-diabetic/control and DN mice are in Supplementary Data S2.

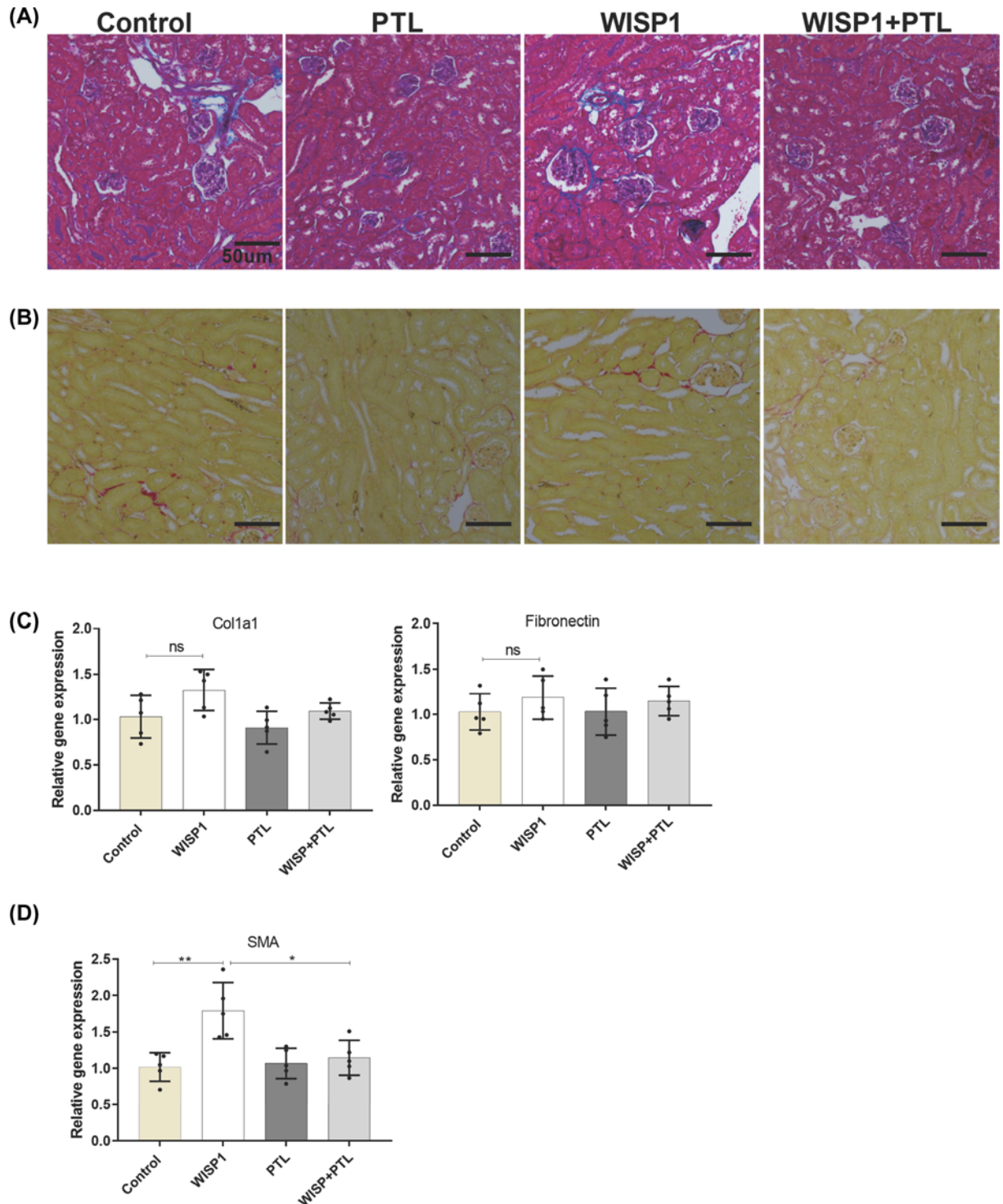


Figure 2. WISP1 induces no immediate changes to kidney structure

At 48 h after administration of WISP1 and/or PTL to healthy mice, there were: **(A)** no morphology changes (Masson staining) observed based on in the kidney at 48 h after administration of WISP1 and/or PTL in healthy mice. **(B)** No collagen (PSR staining) alteration was found in the WISP1-treated mice. **(C)** Q-PCR data showed no significant changes in collagen1a1 and fibronectin. **(D)** α -SMA expression were up-regulated with WISP1 treatment, reduced by PTL. Results are expressed as the mean \pm SEM relative gene expression, ** $P < 0.01$, * $P < 0.05$. $n = 3-5$.

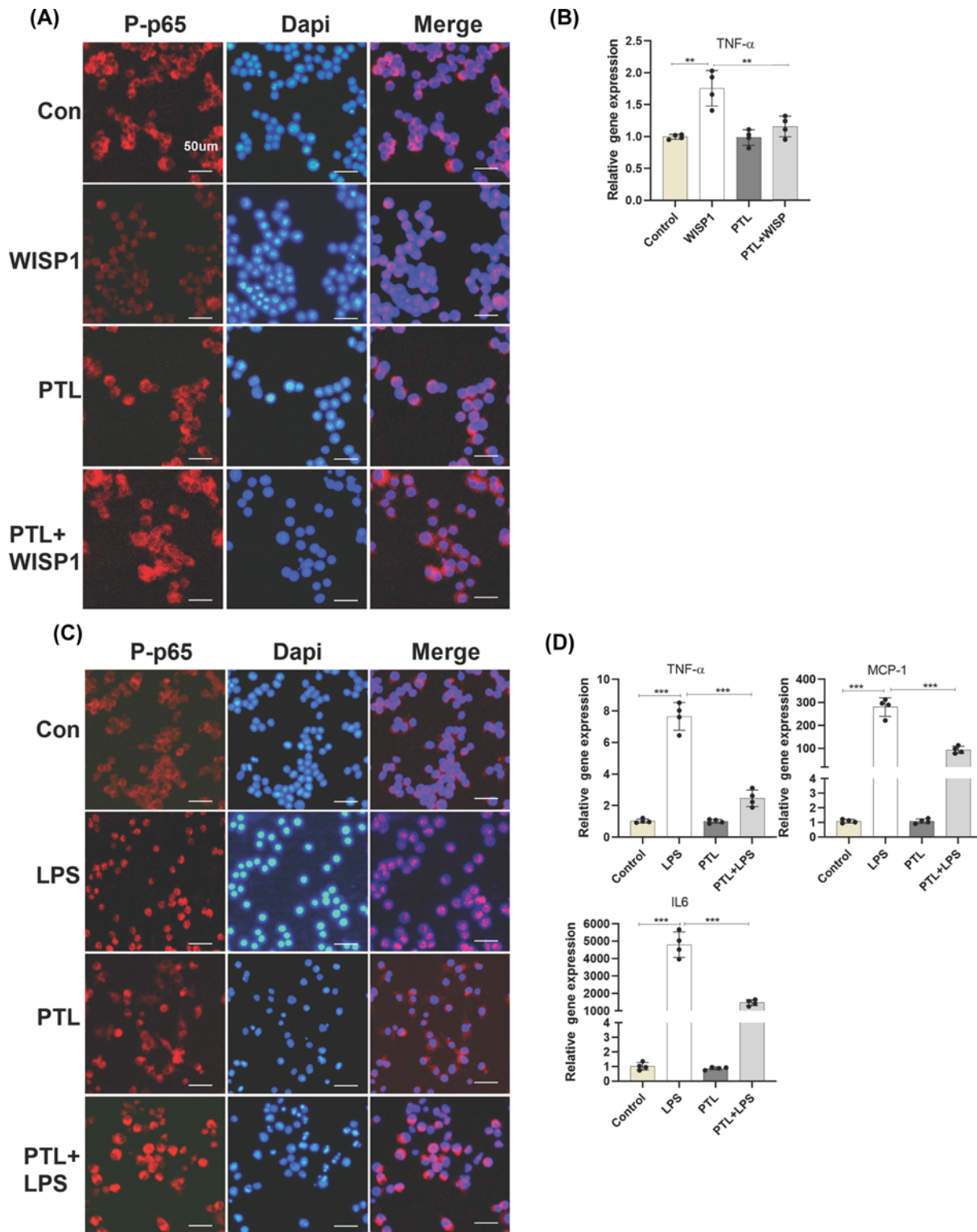


Figure 3. WISP1 regulates inflammation via the NF-κB signaling pathway in RAW macrophages

(A) The translocation of P-p65 into nucleus induced by WISP1 was inhibited by treatment of NFκB inhibitor PTL, with the inhibition on the up-regulated expression of TNF-α. (B,C) PTL inhibited the LPS-induced nucleus translocation of P-p65 and the up-regulated expression of inflammation markers. (D) Results are expressed as the mean ± SEM relative gene expression, *** $P < 0.001$, ** $P < 0.01$. $n = 3-5$.

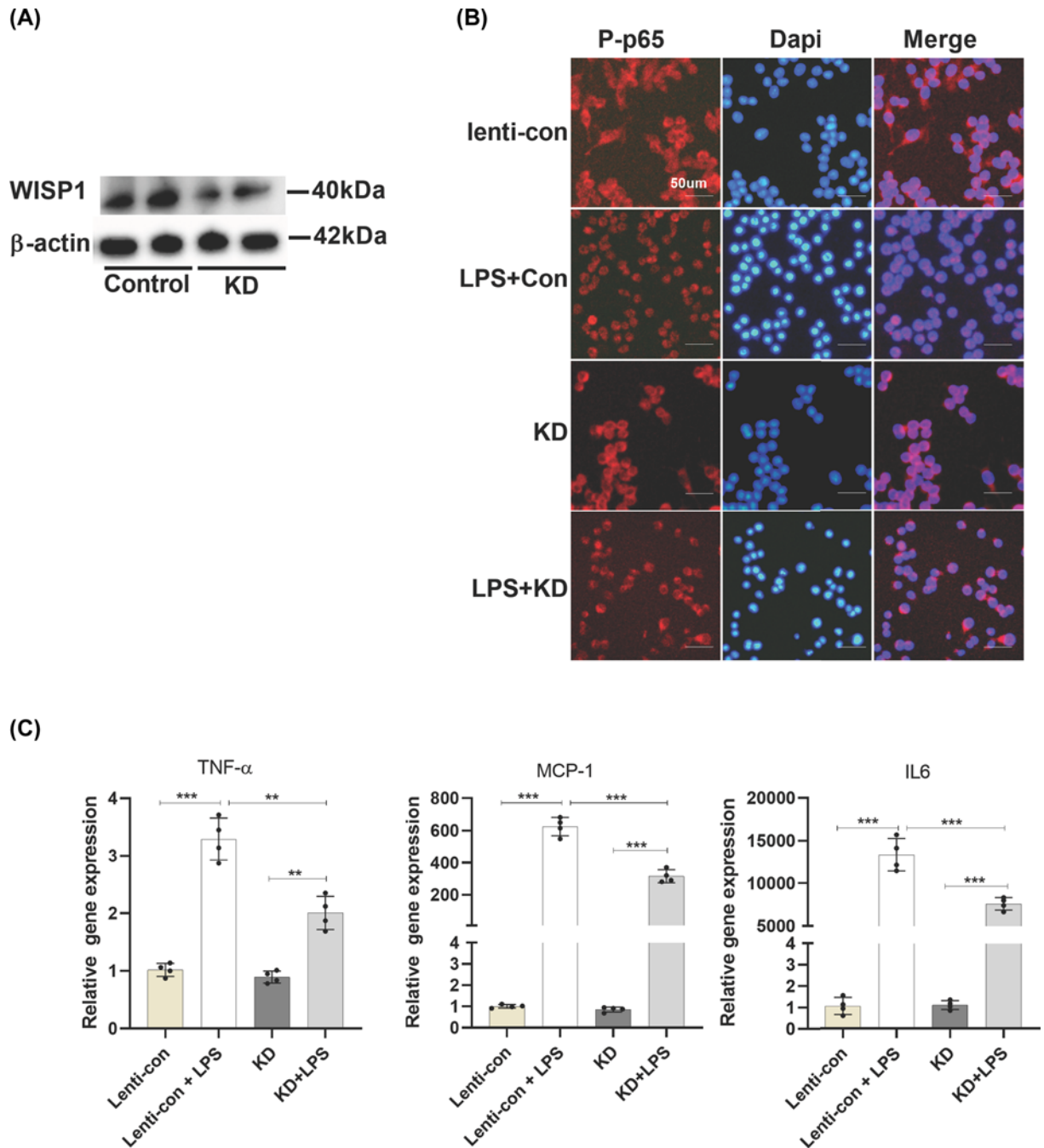


Figure 4. Knockdown of WISP1 inhibits LPS-induced inflammation in RAW macrophages

(A) Knockdown (KD) of WISP1 by shRNA. (B) Knockdown of WISP1 in LPS-stimulated RAW macrophages inhibited (B) the translocation of P-p65 into nucleus, and up-regulated (C) expression of inflammation markers. *** $P < 0.001$, ** $P < 0.01$. $n = 3-5$.

Similarly, quantitative PCR analysis revealed that treatment of RAW macrophages with high glucose (22.5 mM = 4.5 g/l) induced an increase in the gene expression of WISP1, TNF- α and MCP-1, compared with cells cultured with low glucose (5 mM = 1 g/l, Figure 7B).

We also examined WISP1 expression in a mouse model of rapidly progressive tubulointerstitial inflammation and fibrosis caused by UUU. Similar to the DN model, we found a significant increase in the gene expression of WISP1 at day 7 of UUU, which correlated with increases in inflammatory genes (TNF- α , MCP-1 and IL-6) (Figure 7C).

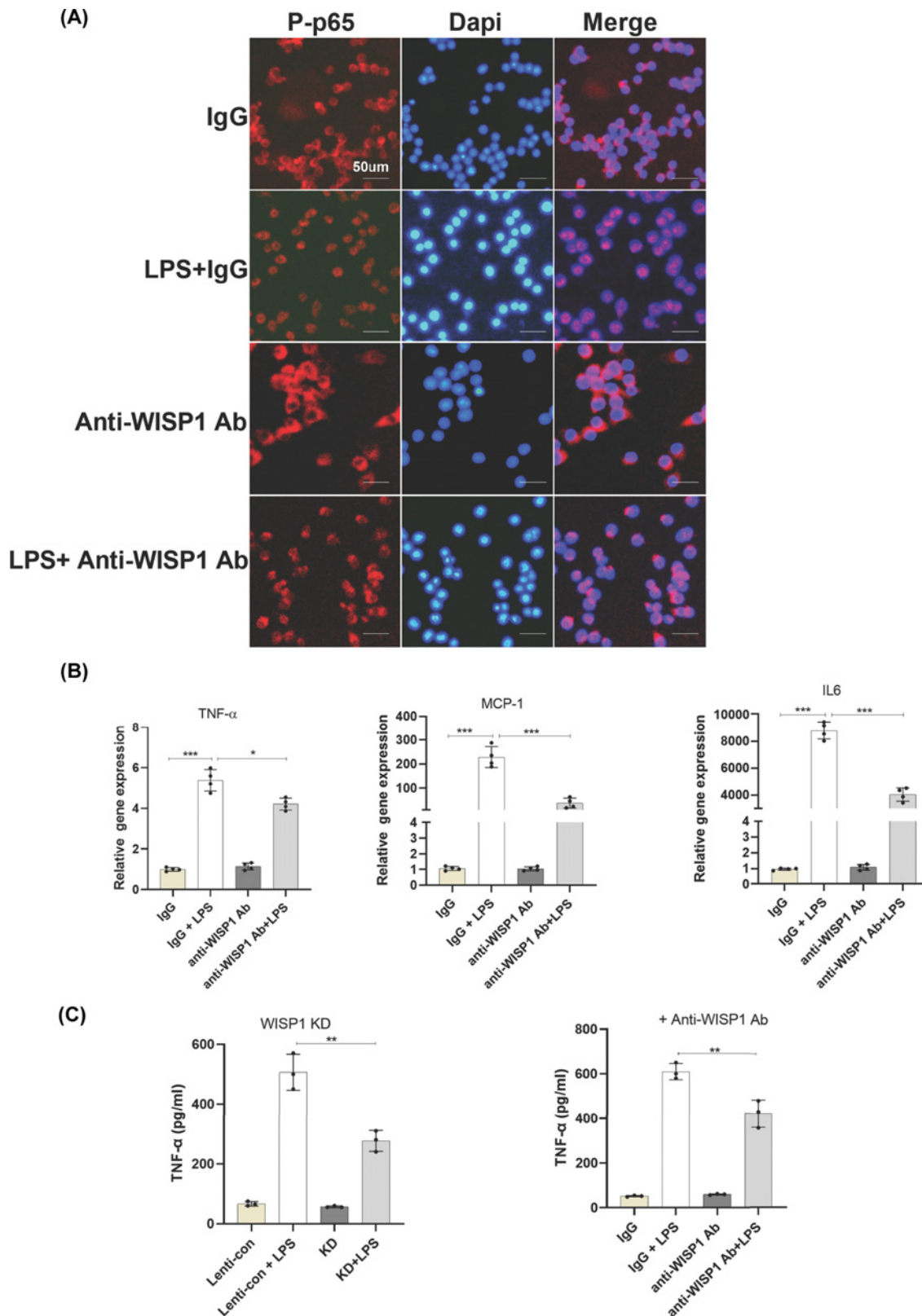
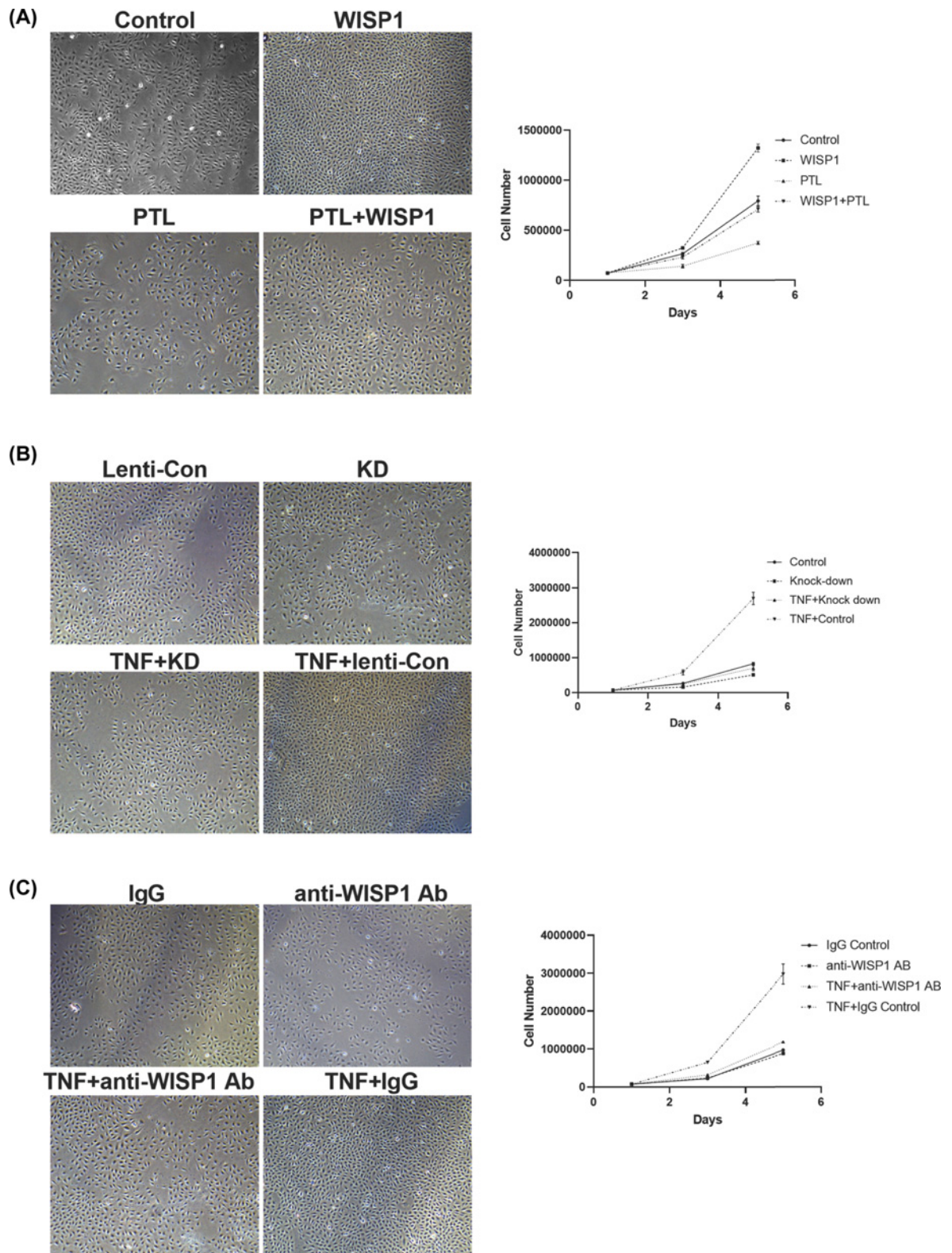


Figure 5. Blockade of WISP1 reduced inflammation in RAW macrophages

(A) The addition of WISP1 antibody (Ab) inhibited the translocation of P-p65 induced by LPS and the expression of inflammation markers (B,C). ELISA showed that the up-regulation TNF- α was reduced by WISP1 knockdown and the addition of antibody. Results are expressed as the mean \pm SEM relative gene expression, *** P <0.001, ** P <0.01, * P <0.05. n =3–5.



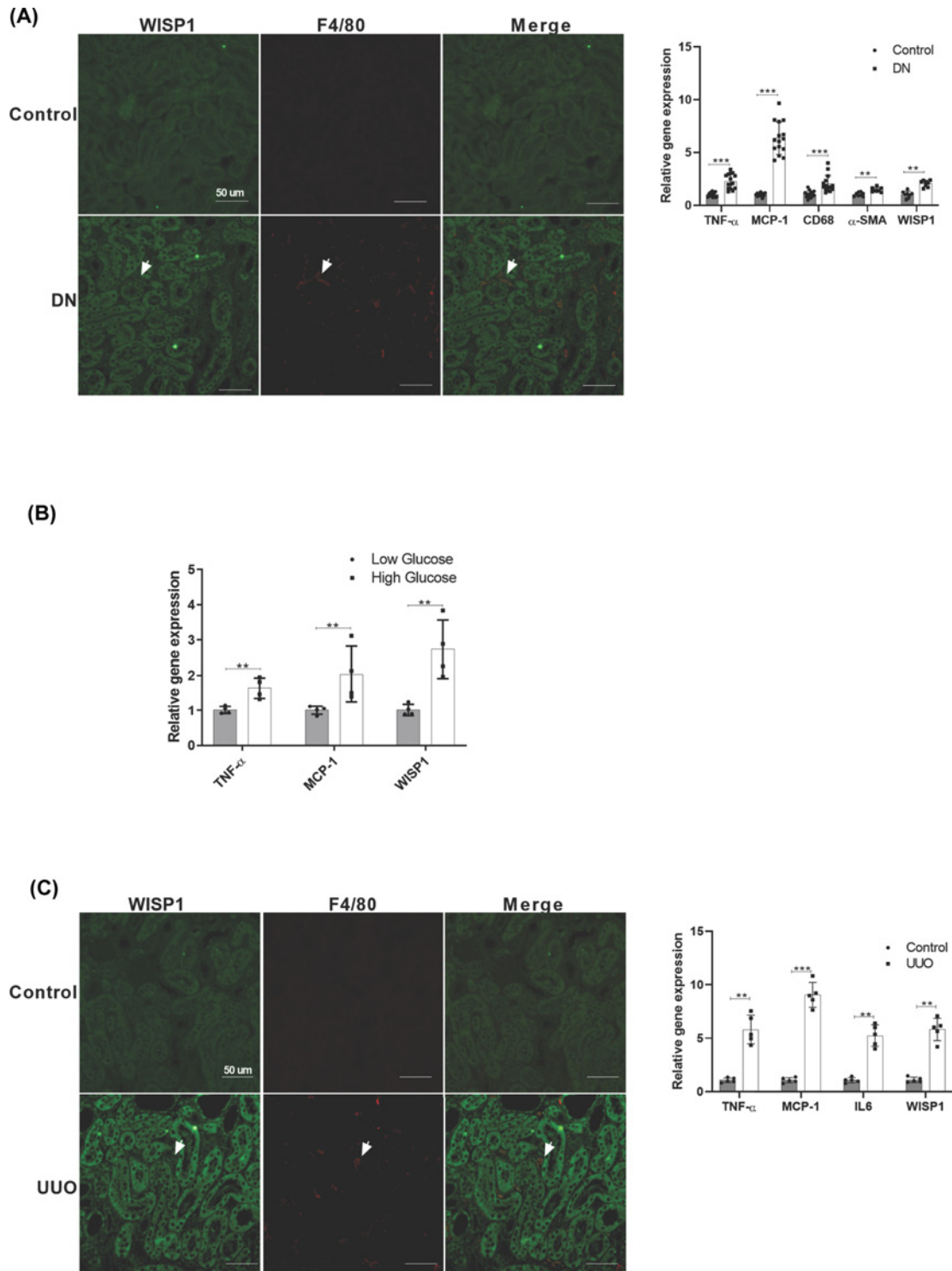


Figure 7. WISP1 expression in two different experimental models of kidney inflammation

(A) STZ-induced DN mice at 18 weeks demonstrated the increased expression of TNF- α , MCP-1, CD68, α -SMA and WISP1 gene by qPCR, compared with control mice ($n=12-15$ mice/group). Immunofluorescence staining indicated the elevated expression of WISP1 in DN model. Macrophage co-localized with WISP1 staining (white arrow). (B) High glucose-treated RAW cells expressed more WISP1, TNF- α and MCP-1 than low glucose-treated cells. (C) The kidneys of mice with 7 days post-UUO surgery showed an increased expression of inflammation genes, TNF- α , MCP-1 and IL6 and an up-regulation of WISP1, compared with control kidneys ($n=5$ mice/group). Immunofluorescence staining indicated the elevated expression of WISP1 in UUO model. Macrophage co-localized with WISP1 staining (white arrow). *** $P < 0.001$, ** $P < 0.01$.

Immunofluorescence staining indicated the elevated WISP1 in kidney with UO injury (Figure 7C). Infiltrating macrophages co-localized with WISP1 in UO model (white arrows). These data suggested WISP1 expression in UO kidneys correlates with increased macrophage infiltration in these kidneys.

Data from these models indicate that an up-regulation of WISP1 is a common feature of kidney injury which is associated with kidney inflammation. Kidney tubules also strongly express WISP1 in kidney disease, particularly in UO, which may also influence the inflammatory response.

Blockade of WISP1 reduces inflammation in mouse UO kidneys

We examined the functional role of WISP1 in inflammation in mouse UO kidneys by administering neutralizing WISP1 antibody to mice from the commencement of injury.

Compared with sham controls, UO kidneys from untreated mice displayed a marked increase in the gene expression of TNF- α , MCP-1 and IL-6 (Figure 8A), which was similar to UO mice that received control IgG. In contrast, UO mice which received WISP1 antibody had reduced kidney expression of these inflammation genes, indicating the suppression of kidney inflammation (Figure 8A). In addition, immunostaining showed a marked increase in F4/80⁺ macrophages in the kidneys of UO mice, which was significantly reduced by treatment of WISP1 antibody (Figure 8B), indicating that kidney macrophage accumulation in UO mice is dependent on WISP1. To ascertain whether WISP1-mediated inflammation in UO kidneys is dependent on NF- κ B signaling, we examined whether WISP1 antibody treatment could protect UO kidneys from the degradation of I κ B proteins which facilitates NF- κ B activation [17]. Western blotting analysis showed that I κ B- α was reduced in UO kidneys, which was inhibited by treatment with WISP1 antibody (Figure 8C). This demonstrates that WISP1-mediated inflammation in UO kidneys is dependent on NF- κ B signaling.

Discussion

The present study has identified a role for WISP1 in the induction of kidney inflammation. It has also demonstrated that kidney production of WISP1 correlates kidney inflammation during diabetes or ureter obstruction, and that pharmacological blockade of WISP1 reduces kidney inflammation. In addition, it has established that macrophages and NF- κ B-p65 signaling play a key role in WISP1-mediated inflammation. These findings demonstrate that WISP1 has the capacity to regulate inflammation in kidney diseases.

Macrophages appear to be the major effector cells of WISP1-mediated inflammation in the kidney. In support of this concept, we demonstrated that administering WISP1 to normal mice induces kidney macrophage accumulation. This correlated with increased kidney gene expression of pro-inflammatory cytokines (TNF- α , IL-6, MCP-1) which are readily produced by macrophages. *In vitro* studies performed by other researchers have shown that WISP1 can induce cultured mouse or human macrophages to produce and secrete these proinflammatory cytokines [18,19]. In comparison, peritoneal macrophages require activation of TLR4 by LPS before they will produce proinflammatory cytokines in response to WISP1 [19,20]. This latter finding also relates to our *in vitro* studies which demonstrated that LPS induction of pro-inflammatory cytokine gene expression in mouse RAW264 macrophages was dependent on WISP1 production by these cells. Therefore, activation of TLR4 may help facilitate WISP1-mediated inflammation by stimulating production of both WISP1 and its receptors on macrophages. Hence, *in vitro* and *in vivo* studies support the concept that WISP1 can promote kidney inflammation by stimulating the recruitment and activation of kidney macrophages. What remains to be determined is whether proximal tubular epithelial cells are also be important in the WISP1-mediated inflammatory response, since they are the most abundant cell type in the kidney and are capable of producing cytokines which can recruit and activate macrophages.

Activation of NF- κ B-p65 signaling appears to be the principal mechanism by which WISP1 facilitates kidney inflammation. Our *in vivo* studies found that pharmacological blockade of NF- κ B could prevent WISP1 from inducing inflammation in healthy kidneys. They also showed that treatment with WISP1 antibody reduced inflammation in diseased (UO) kidneys in association with reduced degradation of I κ B- α , which is an inhibitor of NF- κ B activation. Similarly, our *in vitro* studies demonstrated that blockade of either NF- κ B or WISP1 was able to significantly reduce induction of proinflammatory gene expression in macrophages by LPS. Induction of NF- κ B activation by WISP1 was also identified in immunofluorescence staining showing that both LPS and WISP1 induce nuclear translocation of NF- κ B in macrophages, which were inhibited by either gene knockdown or antibody blockade of WISP1. These findings indicate that WISP1-induced inflammation in kidney, particularly in macrophages, is mediated by NF- κ B signaling.

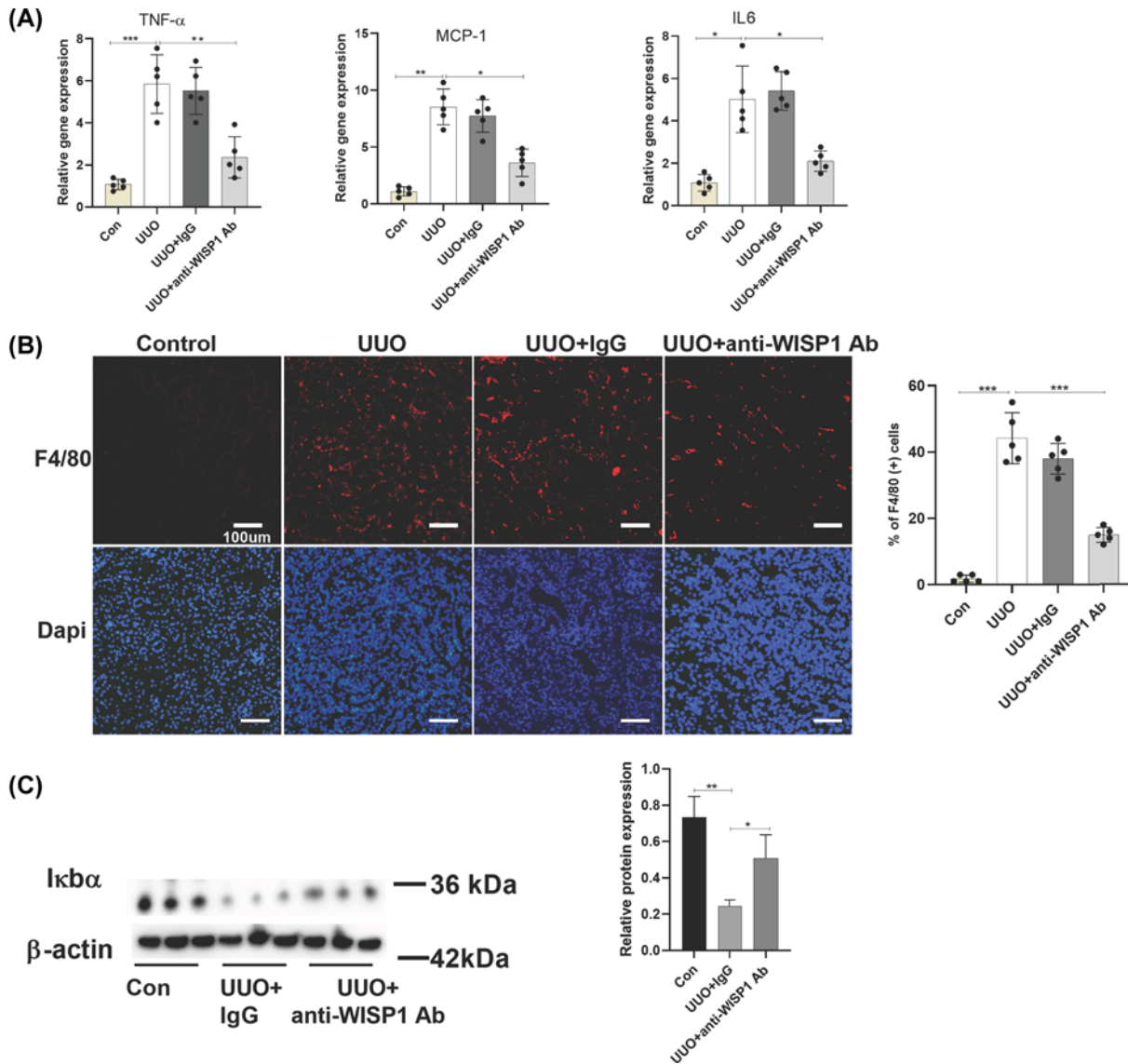


Figure 8. Treatment with WISP1 antibody alleviated the development of inflammation in kidney of UUO mice with 7 days post-injury

(A) Q-PCR analysis demonstrated that WISP1 antibody reduced inflammation gene expression in kidneys from UUO mice. (B) UUO kidneys without treatment or receiving IgG show an up-regulated F4/80 positive cells accumulation at 7 days. Administration of WISP1 antibody in UUO mice resulted in a reduction, verified by the percentage of F4/80 positive cells. (C) WISP1 antibody treatment rescued the degradation of $\text{I}\kappa\text{B}\alpha$ expression in UUO kidneys, by Western blotting. Results are expressed as the mean \pm SEM relative gene expression, *** P <0.001, ** P <0.01, * P <0.05. n =3–7.

WISP1 is associated with inflammation in patients with obesity, diabetes and diabetic kidney disease. Clinical studies have shown that WISP1 increases in visceral adipose tissue (VAT) during obesity and type 2 diabetes in association with adipose inflammation, accumulation of adipose tissue macrophages and reduced insulin sensitivity [18,21]. Furthermore, our examination of mouse DN has shown that gene expression of WISP1 increases with the expression levels of CD68 (a macrophage marker) and inflammatory cytokines (IL-6, TNF- α , MCP-1) in diabetic kidneys. Therefore, WISP1 regulation of inflammation may play a role in the development of type 2 diabetes and its kidney complications. This concept is supported by our finding that high levels of glucose induce WISP1 gene expression in macrophages and that macrophages are known to play a critical role in adipose inflammation, insulin resistance and diabetic kidney disease [22].

Our experiments validated WISP1 as a therapeutic target for progressive kidney disease. In our UUO model, kidney injury is characterized by an early inflammatory response involving macrophage infiltration and inflammatory cytokine production (TNF- α , IL-6, MCP-1). We showed that this correlated with increased kidney gene expression of WISP1. Furthermore, we established that treatment of UUO mice with a neutralising antibody targeting WISP1 reduced this inflammatory response, thereby demonstrating a functional role for WISP1 in kidney inflammation. What remains to be determined is whether therapeutic blockade of WISP1 would be effective at suppressing the progression of kidney inflammation in patients with DN, especially since this disease is the major cause of end-stage renal failure.

The study also identified a link between WISP1-mediated inflammation and kidney fibrosis. Given that kidney inflammation can often drive subsequent fibrotic responses, we explored whether WISP1 regulation of inflammation could promote renal fibrosis. Administration of WISP1 to healthy mice produced an inflammatory response and an increase in gene expression of α -SMA, suggesting a possible role in kidney myofibroblast accumulation. Both the inflammatory response and the increase in α -SMA were reduced by NF- κ B, suggesting that these events may be linked. We showed that an inflammatory stimulus (LPS) can induce WISP1, which can subsequently induce TNF- α production. Interestingly, TNF- α has been shown to promote fibroblast proliferation via the NF- κ B pathway [21]. Therefore, we examined whether WISP1 could induce proliferation of kidney fibroblasts. We found that WISP1 could directly stimulate kidney fibroblast proliferation via activation of NF- κ B, which is supported by similar findings in adult human dermal fibroblasts [23]. Furthermore, we showed that WISP1 was a mediator of TNF- α -induced proliferation of kidney fibroblasts. These findings suggest that WISP1 can have a role in promoting both inflammation and fibrosis in kidney diseases.

In conclusion, our study has established a functional role for WISP1 in mediating kidney inflammation via NF- κ B signaling. It has also demonstrated that therapeutic targeting of WISP1 can reduce inflammation in kidney disease. Given that macrophages appear to be the major effector cells of WISP1-mediated inflammation, it is likely that therapies targeting WISP1 will be most effective in macrophage-mediated kidney diseases, such as DN, which warrants further investigation.

Clinical perspectives

- CKD is growing worldwide with renal failure being a common end-point. It is characterized by the loss of kidney function and tubular fibrosis/inflammation. There is a clinical urgency to explore novel therapy for kidney injury due to the lack of effective treatments.
- In this project a new pro-inflammation factor, called WISP1, will be explored to understand the underlying mechanism in mediating kidney injury. We demonstrated *in vitro* and *in vivo* data to show that WISP1 has the potential to induce kidney inflammation. The transfection of shRNA of WISP1 into RAW macrophage cells alleviated the progress of inflammation progress. In addition, we report that WISP1 antibody provides significant protection from inflammation in a kidney injury model.
- Recently, the monoclonal antibody-based anti-fibrosis therapy has become a breakthrough in kidney disease treatment. In general, our work demonstrated WISP1 as a potential therapeutic target for kidney injury and the generation of WISP1 monoclonal antibody will provide a new idea for CKD treatment.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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CRedit Author Contribution

Bo Wang: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing. **Chenguang Ding:** Conceptualization, Supervision, Investigation, Methodology, Writing—original draft. **Xiaoming Ding:** Conceptualization, Supervision, Investigation, Writing—original draft. **Greg Tesch:** Data curation, Formal analysis, Writing—review & editing. **Jin Zheng:** Resources, Methodology. **PuYun Tian:** Formal analysis, Supervision. **Yang Li:** Resources. **Sharon Ricardo:** Data curation, Supervision, Methodology. **Hsin-Hui Shen:** Resources, Supervision. **Wujun Xue:** Conceptualization, Resources, Supervision, Funding acquisition.

Ethics Approval

Animal studies were performed at the Monash University/Monash Medical Centre Animal Facility (Clayton, Australia), approved by the Monash University/Monash Medical Centre Animal Ethics Committee, in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th edition (2004).

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Abbreviations

α -SMA, α -smooth muscle actin; CCL2, chemokine (C-C motif) ligand 2, also referred to as monocyte chemoattractant protein 1 (MCP-1); CKD, chronic kidney disease; Col, collagen; DN, diabetic nephropathy; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; P-p65, phosphorylated-p65; PTL, parthenolide; STZ, streptozotocin; TNF- α , tumor necrosis factor α ; UUO, unilateral ureter obstruction; WISP1, WNT1-inducible signaling pathway protein 1; WNT1, Wingless/INT family member 1.

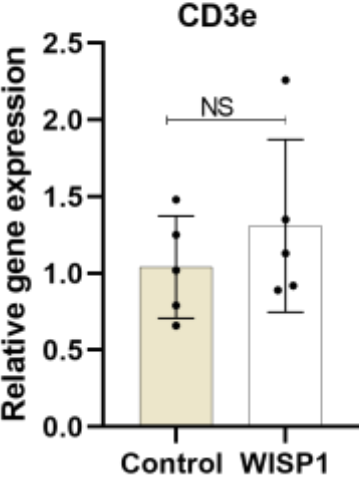
References

- 1 Cobo, G., Lindholm, B. and Stenvinkel, P. (2018) Chronic inflammation in end-stage renal disease and dialysis. *Nephrol. Dial. Transplant.* **33**, iii35–iii40, <https://doi.org/10.1093/ndt/gfy175>
- 2 Peschon, J.J., Torrance, D.S., Stocking, K.L., Glaccum, M.B., Otten, C., Willis, C.R. et al. (1998) TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. *J. Immunol.* **160**, 943–952
- 3 Bagaev, A.V., Garaeva, A.Y., Lebedeva, E.S., Pichugin, A.V., Ataulakhanov, R.I. and Ataulakhanov, F.I. (2019) Elevated pre-activation basal level of nuclear NF- κ B in native macrophages accelerates LPS-induced translocation of cytosolic NF- κ B into the cell nucleus. *Sci. Rep.* **9**, 4563, <https://doi.org/10.1038/s41598-018-36052-5>
- 4 Chen, F., Castranova, V. and Shi, X. (2001) New insights into the role of nuclear factor- κ B in cell growth regulation. *Am. J. Pathol.* **159**, 387–397, [https://doi.org/10.1016/S0002-9440\(10\)61708-7](https://doi.org/10.1016/S0002-9440(10)61708-7)
- 5 Edeling, M., Ragi, G., Huang, S., Pavenstadt, H. and Susztak, K. (2016) Developmental signalling pathways in renal fibrosis: the roles of Notch, Wnt and Hedgehog. *Nat. Rev. Nephrol.* **12**, 426–439, <https://doi.org/10.1038/nrneph.2016.54>
- 6 Tan, R.J., Zhou, D., Zhou, L. and Liu, Y. (2014) Wnt/ β -catenin signaling and kidney fibrosis. *Kidney Int. Suppl.* **4**, 84–90, <https://doi.org/10.1038/kisup.2014.16>
- 7 He, W., Dai, C., Li, Y., Zeng, G., Monga, S.P. and Liu, Y. (2009) Wnt/ β -catenin signaling promotes renal interstitial fibrosis. *J. Am. Soc. Nephrol.* **20**, 765–776, <https://doi.org/10.1681/ASN.2008060566>
- 8 Wang, B., Ding, X., Ding, C., Tesch, G., Zheng, J., Tian, P. et al. (2020) WNT1-inducible-signaling pathway protein 1 regulates the development of kidney fibrosis through the TGF- β 1 pathway. *FASEB J.* **34** (11), 14507–14520, <https://doi.org/10.1096/fj.202000953R>
- 9 Wang, B., Yao, K., Huuskes, B.M., Shen, H.H., Zhuang, J., Godson, C. et al. (2016) Mesenchymal stem cells deliver exogenous microRNA-let7c via exosomes to attenuate renal fibrosis. *Mol. Ther.* **24**, 1290–1301, <https://doi.org/10.1038/mt.2016.90>

- 10 Huuskos, B.M., Wise, A.F., Cox, A.J., Lim, E.X., Payne, N.L., Kelly, D.J. et al. (2015) Combination therapy of mesenchymal stem cells and serelaxin effectively attenuates renal fibrosis in obstructive nephropathy. *FASEB J.* **29**, 540–553, <https://doi.org/10.1096/fj.14-254789>
- 11 Tesch, G.H. and Allen, T.J. (2007) Rodent models of streptozotocin-induced diabetic nephropathy. *Nephrology (Carlton)* **12**, 261–266, <https://doi.org/10.1111/j.1440-1797.2007.00796.x>
- 12 Sanchez-Lopez, E., Rayego, S., Rodriguez-Diez, R., Rodriguez, J.S., Rodriguez-Diez, R., Rodriguez-Vita, J. et al. (2009) CTGF promotes inflammatory cell infiltration of the renal interstitium by activating NF-kappaB. *J. Am. Soc. Nephrol.* **20**, 1513–1526, <https://doi.org/10.1681/ASN.2008090999>
- 13 Yuan, Q., Tan, R.J. and Liu, Y. (2019) Myofibroblast in kidney fibrosis: origin, activation, and regulation. *Adv. Exp. Med. Biol.* **1165**, 253–283, https://doi.org/10.1007/978-981-13-8871-2_12
- 14 Battegay, E.J., Raines, E.W., Colbert, T. and Ross, R. (1995) TNF-alpha stimulation of fibroblast proliferation. Dependence on platelet-derived growth factor (PDGF) secretion and alteration of PDGF receptor expression. *J. Immunol.* **154**, 6040–6047
- 15 Hayden, M.S. and Ghosh, S. (2014) Regulation of NF-kappaB by TNF family cytokines. *Semin. Immunol.* **26**, 253–266, <https://doi.org/10.1016/j.smim.2014.05.004>
- 16 Duran-Salgado, M.B. and Rubio-Guerra, A.F. (2014) Diabetic nephropathy and inflammation. *World J. Diabetes* **5**, 393–398, <https://doi.org/10.4239/wjd.v5.i3.393>
- 17 Sanz, A.B., Sanchez-Nino, M.D., Ramos, A.M., Moreno, J.A., Santamaria, B., Ruiz-Ortega, M. et al. (2010) NF-kappaB in renal inflammation. *J. Am. Soc. Nephrol.* **21**, 1254–1262, <https://doi.org/10.1681/ASN.2010020218>
- 18 Murahovschi, V., Pivovarova, O., Ilkavets, I., Dmitrieva, R.M., Docke, S., Keyhani-Nejad, F. et al. (2015) WISP1 is a novel adipokine linked to inflammation in obesity. *Diabetes* **64**, 856–866, <https://doi.org/10.2337/db14-0444>
- 19 Chen, Z., Ding, X., Jin, S., Pitt, B., Zhang, L., Billiar, T. et al. (2016) WISP1-alpha5beta3 integrin signaling positively regulates TLR-triggered inflammation response in sepsis induced lung injury. *Sci. Rep.* **6**, 28841, <https://doi.org/10.1038/srep28841>
- 20 Ding, X., Tong, Y., Jin, S., Chen, Z., Li, T., Billiar, T.R. et al. (2018) Mechanical ventilation enhances extrapulmonary sepsis-induced lung injury: role of WISP1-alpha5beta5 integrin pathway in TLR4-mediated inflammation and injury. *Crit. Care* **22**, 302, <https://doi.org/10.1186/s13054-018-2237-0>
- 21 Barchetta, I., Cimini, F.A., De Gioannis, R., Ciccarelli, G., Bertocchini, L., Lenzi, A. et al. (2018) Procollagen-III peptide identifies adipose tissue-associated inflammation in type 2 diabetes with or without nonalcoholic liver disease. *Diabetes Metab. Res. Rev.* **34**, e2998, <https://doi.org/10.1002/dmrr.2998>
- 22 Tesch, G.H. (2017) Diabetic nephropathy - is this an immune disorder? *Clin. Sci. (Lond.)* **131**, 2183–2199, <https://doi.org/10.1042/CS20160636>
- 23 Ono, M., Masaki, A., Maeda, A., Kilts, T.M., Hara, E.S., Komori, T. et al. (2018) CCN4/WISP1 controls cutaneous wound healing by modulating proliferation, migration and ECM expression in dermal fibroblasts via alpha5beta1 and TNFalpha. *Matrix Biol.* **68–69**, 533–546

Figure Legend for Supplementary data

Supplementary data 1



Supplemental data 1. T-cell marker expression in WISP1 treated mouse kidney.

Supplementary data 2

Non-Diabetic mouse

	GRP 1	Wk 0		Wk 2		Wk 4		Wk 6		Wk 8		Wk 10		Wk 12		Wk 14		Wk 15		Kill Mon	
	BL6/ND	Sep4		Sep-18		Oct2		Oct16		Oct30		Nov13		Nov27		Dec11		Dec18		Dec-21	
	ET/ID	FBW	FBG	FBW	FBG	FBW	FBG	FBW	FBG	FBW	FBG	FBW	FBG	FBW	FBG	FBW	FBG	FBW	FBG	SBP	HbA1c
	mouse	(g)	(mM)	(g)	(mM)	(g)	(mM)	(g)	(mM)	(g)	(mM)	(g)	(mM)	(g)	(mM)	(g)	(mM)	(g)	(mM)		(%)
1	CP-19	23.4	7.8	25.7	9.3	24.8	5.4	26.2	7.4	27.4	7.8	28.5	7.8	29.4	7.4	31.1	9.2	29.9	7.1	106	4.3
2	CP-21	23.8	7.8	25.7	7	27	6.8	27.9	5.7	27.2	5.8	27.4	7.4	27.6	7.1	28.3	7.1	28	6.9	103	3.7
3	CP-22	26.2	7.1	28.7	5.6	29.2	5.9	29.9	7.3	30.2	5.9	29.8	5.7	30.3	5.4	31.8	5.6	31.2	5.9	103	3.7
4	CP-23	21.1	8.4	22.4	9.5	23.4	8.5	24.7	8	25.6	7.4	26	6.5	26.8	7.8	27.5	7	27.3	7.2	107	4.5
5	CP-25	22.9	6.9	24.5	8.2	25.3	7.7	26.6	8.4	27.9	8	31.5	7.3	30	8.5	30	8	30.1	7.8	108	4.2
6	CP-26	26.1	7	28.4	6.3	29.7	7.7	30.6	6.4	31.8	6.3	32.6	4.9	32.5	5.4	33.9	7.5	33.2	6.8	100	4.1
7	CP-27	25.4	7.5	27.5	7.9	28.5	7.5	29.3	7.7	30.2	7.2	30.7	7.7	31	8	32	7.9	31.5	7.6	97	4.4
8	CP-28	23.2	7.8	25.2	7.6	26.4	7.5	27.4	6.8	29	7.2	30.3	7.6	31	7.2	32.4	8.3	32	7.5	105	4.3
9	CP-29	26.9	8.5	29.1	7.4	29.7	6.9	31.5	7.8	32.3	8.8	32.5	7.3	32.1	7	33.6	7	33.1	7.3	106	4.3
10	CP-30	25.5	6.7	27.4	7.3	27.8	7.5	29.4	7.4	30.2	7.8	30.1	7.9	30.2	7.4	30.7	7	30.6	7.4	99	4.5
11	CP-31	25.2	9.7	27.1	9.4	26.4	7.2	29.3	8.3	30.3	7	30	7.9	31	7.8	33.1	8.4	32.9	7.7	101	4.5
12	CP-32	25.6	8.1	27.6	8.5	27.6	7.4	29.7	7	30.7	8	30.7	8	31	6.5	33	8.4	31.8	8.2	101	4.6
13	CP-33	25.5	7.7	27.1	8.3	26.8	8.1	29.5	8.3	30.4	6.7	31.4	7.5	33.5	7.9	32.8	8.5	32.3	8.3	100	4.3
14	CP-34	26.1	8.2	27.9	7.9	28.4	7.6	28.8	8.4	30.1	8.1	30.6	7.4	30.3	6.9	32.1	7.8	31.9	7.9	106	4.3
15	CP-35	26.6	8.1	28.7	7.2	30.3	7.3	30.3	6.4	32.4	6.7	34.2	5.9	34.1	7.5	35	8.1	34.3	8.1	103	4.1
	AV	24.9	7.8	26.9	7.8	27.4	7.3	28.7	7.4	29.7	7.2	30.4	7.1	30.7	7.2	31.8	7.7	31.3	7.4	103	4.3
	SD	1.6	0.8	1.8	1.1	2.0	0.8	1.8	0.8	2.0	0.9	2.0	0.9	1.9	0.9	2.0	0.9	1.9	0.6	3	0.3

LKW	RKW	BW	2KW/BW	Renal Fn		Glom Area
				Plasma Cystatin-C	UAER ACR	
(g)	(g)	(g)	(%)	(ng/mL)	(g/mol)	(%)
0.145	0.153	31.1	0.96	352	0.9	15.7
0.147	0.153	28.3	1.06	255	1.4	15.5
0.212	0.2	31.3	1.32	346	0.7	17.1
0.15	0.152	27.6	1.09	266	1.9	14.4
0.169	0.169	30.2	1.12	326	0.8	16.2
0.207	0.195	33.5	1.20	245	1.1	16.9
0.169	0.181	31.7	1.10	320	1.2	17.3
0.157	0.175	31.8	1.04	335	0.9	17.5
0.19	0.187	33.5	1.13	298	0.9	16.6
0.173	0.17	30.2	1.14	354	4.7	14.7
0.184	0.186	33.3	1.11	310	0.8	16.7
0.171	0.152	31.4	1.03	346	0.7	18.7
0.173	0.191	31.5	1.16	369	3.2	17.3
0.163	0.184	32.1	1.08	394	1.6	18.7
0.186	0.205	34.9	1.12	393	1.2	19.7
0.173	0.177	31.5	1.11	327.27	1.5	16.87
0.020	0.018	1.9	0.08	45.95	1.1	1.47

Diabetic Mouse

	GRP 2	PreSTZ			Wk 2		Wk 4		Wk 6		Wk 8		Wk 10		Wk 12		Wk 14		Wk 15	
	BL6/STZ				Aug-7		Aug-21		Sep-4		Sep-18		Oct-2		Oct-16		Oct-30		Nov-6	
	ET/ID	FBW	FBG	STZ	FBW	FBG	FBW	FBG	FBW	FBG	FBW	FBG	HbA1c	FBW	FBG	FBW	FBG	FBW	FBG	FBW
	mouse	(g)	(mM)	uL	(g)	(mM)	(g)	(mM)	(g)	(mM)	(g)	(mM)	(%)	(g)	(mM)	(g)	(mM)	(g)	(mM)	(g)
1	CP-2	21.1	6.6	166	19.8	18.7	19.7	19.3	19.8	21.4	19.7	21.6	10.3	19.7	21.8	19.9	20.9	21.6	25.9	21.4
2	CP-3	24.3	8.6	191	22.4	20.3	23.0	25.5	22.0	23.6	22.3	23.7	9.9	22.2	21.2	22.6	27.9	24.1	32.8	24.2
3	CP-4	25.0	6.9	197	21.2	24.6	21.1	23.9	20.7	20.9	21.9	24.4	7.7	21.3	20.9	21.2	21.5	20.9	22.6	19.8
4	CP-5	22.3	6.7	175	20.7	20.5	21.2	21.1	20.9	21.8	22.2	21.6	9.6	21.7	21.9	19.6	18.9	18.1	25.9	19.1
5	CP-6	25.1	6.9	197	23.7	23.4	23.8	23	23	21.9	23.2	29.4	10.4	23.6	21.8	23.4	24.1	23.7	27.6	23.4
6	CP-7	23.7	6.8	186	22.6	22.2	22	27.8	21.8	24.1	22.7	28.3	10	22.2	25.8	21.1	20	20.5	23.7	21.9
7	CP-8	24.0	6.2	189	22.1	21.9	20.7	29.8	20.4	23.5	20.8	28.1	9.8	21.4	22.6	22.8	23.6	22.9	25.4	24
8	CP-9	23.4	7.9	184	22.4	25	23.5	26	23.1	24.6	23.4	24.3	12.9	23.4	26.4	23.9	23.5	24.6	28.2	25
9	CP-11	25.3	7.5	199	23.6	19.4	23.7	24.3	22.5	25.2	23.7	26	12	23.1	25.4	23.2	27.2	23.1	28.1	23
10	CP-12	21.8	7.1	171	20.6	20.4	19.9	25.2	18.7	23.2	19.1	23.8	10.1	19.1	24.1	20	29.1	21.4	27.5	20.3
11	CP-13	22.3	7.4	175	20.7	21.1	19.9	20.9	19.5	18	20.4	22.5	13.2	19.7	19.8	19.7	26.8	20.9	24.3	19.8
12	CP-16	26.5	8.3	208	24.6	21.3	25.3	23.1	24.7	28.4	25.3	26.5	10.8	25.6	25.6	25.7	25.5	26.0	29	25.3
13	CP-17	23.9	7.9	188	22.3	19.4	22.4	25.5	22.6	27.4	23.1	23.6	11.7	24.3	26	24.3	30.4	23.8	23.3	23.9
14	CP-18	25.3	8	199	25.4	23	24.8	17.7	25.2	23.8	26.0	25.8	10.9	25.9	23	26.1	24.3	28.0	25	27.6
15	CP-20	25	7.2	197	25.2	26	24.7	16.2	24.9	21.4	25.3	25.5	9.9	25.7	23.6	25.9	19	26.1	26	26.2
	AV	23.9	7.3	188	22.5	21.8	22.4	23.3	22.0	23.3	22.6	25.0	10.6	22.6	23.3	22.6	24.2	23.0	26.4	23.0
	SD	1.5	0.7	12	1.7	2.2	1.9	3.7	2.0	2.6	2.0	2.4	1.4	2.2	2.1	2.3	3.6	2.6	2.6	2.5

Kill			Renal Hypertrophy			Renal Fn Plasma Cystatin-C	UAER	Glom Area
FBG	SBP	HbA1c	LKW	RKW	BW	2KW/BW	ACR	Coll 4
(mM)		(%)	(g)	(g)	(g)	(%)	(ng/mL)	(%)
29	99	10.6	0.168	0.18	21.3	1.63	514	20.3
29.9	102	12.7	0.188	0.199	24.4	1.59	484	24
18.6	107	8.7	0.228	0.242	21.4	2.20	342	26.5
23.1	108	9.5	0.187	0.198	17.3	2.23	344	27.9
32.7	106	12.4	0.204	0.214	24.5	1.71	394	21.3
25.9	106	8.9	0.165	0.206	21.9	1.69	567	22.7
28.1	102	11.3	0.187	0.191	24	1.58	466	21.7
27.5	97	12.9	0.209	0.209	25.3	1.65	373	20.2
31.5	102	13.6	0.169	0.175	22.1	1.56	460	22.4
31.7	105	12.2	0.16	0.173	21	1.59	392	23.1
28	105	12.9	0.182	0.207	20.7	1.88	359	22.9
26	107	11.4	0.217	0.207	27.2	1.56	315	17.3
27.5	100	11	0.222	0.218	24.6	1.79	324	18.6
29.1	101	10.3	0.204	0.228	28.2	1.53	301	21
24.8	100	11	0.184	0.198	26.8	1.43	368	16.7
27.6	103	11.3	0.192	0.203	23.4	1.71	400.20	21.77
3.6	3.4	1.5	0.022	0.019	2.9	0.23	79.61	3.05

Abbreviation in Supplementary data 2

Wk: Week

STZ: streptozotocin

FBW: Fasting Body Weight

FBG: Fasting Blood Glucose

SBP: systolic blood pressure

BW: Body Weight

LKW: Left kidney weight

RKW: Right kidney weight

Glom Area: Glomerular Area

AV: Average

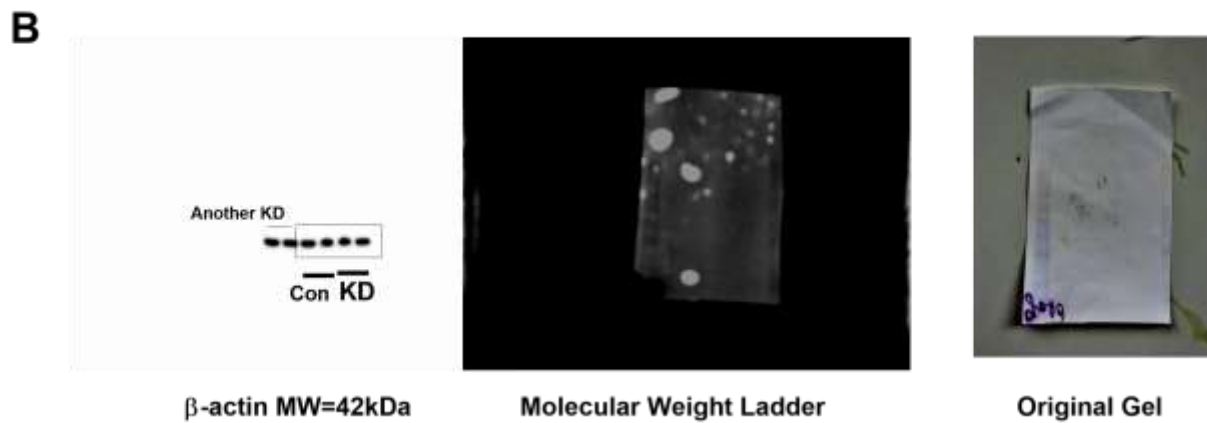
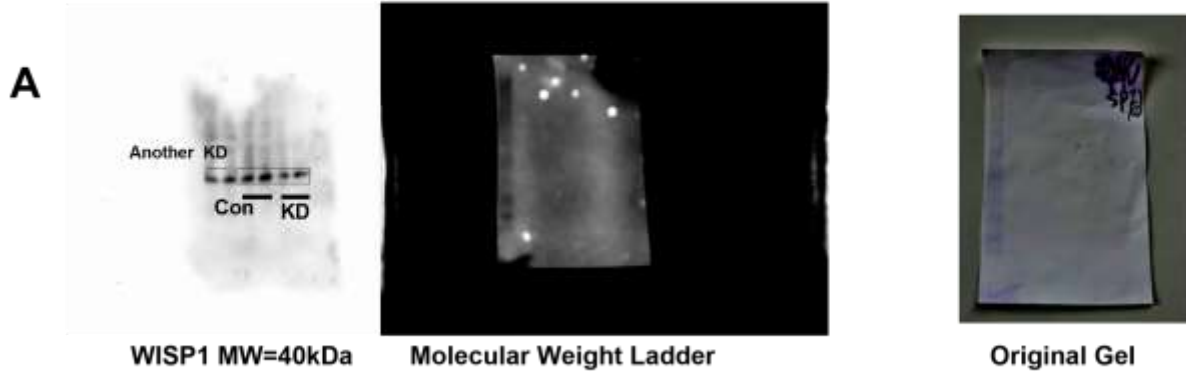
SD: standard deviations

UAER: urinary albumin excretion rate

ACR: Urine albumin to creatinine ratio

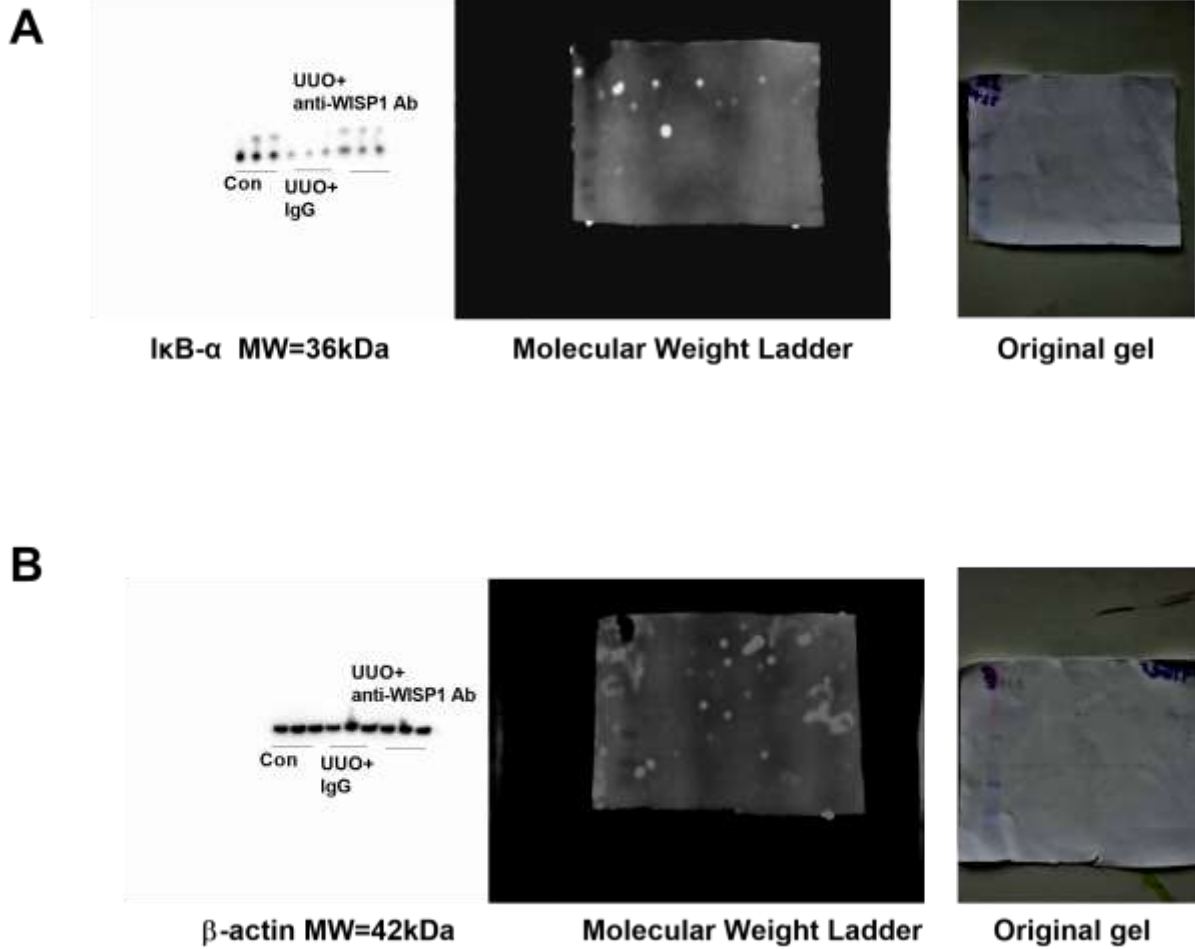
Supplemental data 2. The data for non-diabetic and STZ-induced diabetic nephropathy mouse model with abbreviation table. (Refer to Fig 7A).

Supplementary Data 3. Original Western blot for Figure 4



Supplemental data 3. The original western blotting images to show the knock-down of WISP1 in RAW macrophages. (Refer to Fig 4A). Images for “protein of interest (A for WISP1 and B for beta-actin)”, “molecular weight ladder” and “original gel”.

Supplementary data 4. Original western blot for Figure 8



Supplemental data 4. The original western blotting images to show the IκB- α expression in mouse kidney. (Refer to Fig 8C). Images for “protein of interest (A for IκB- α and B for beta-actin)”, “molecular weight ladder” and “original gel”.