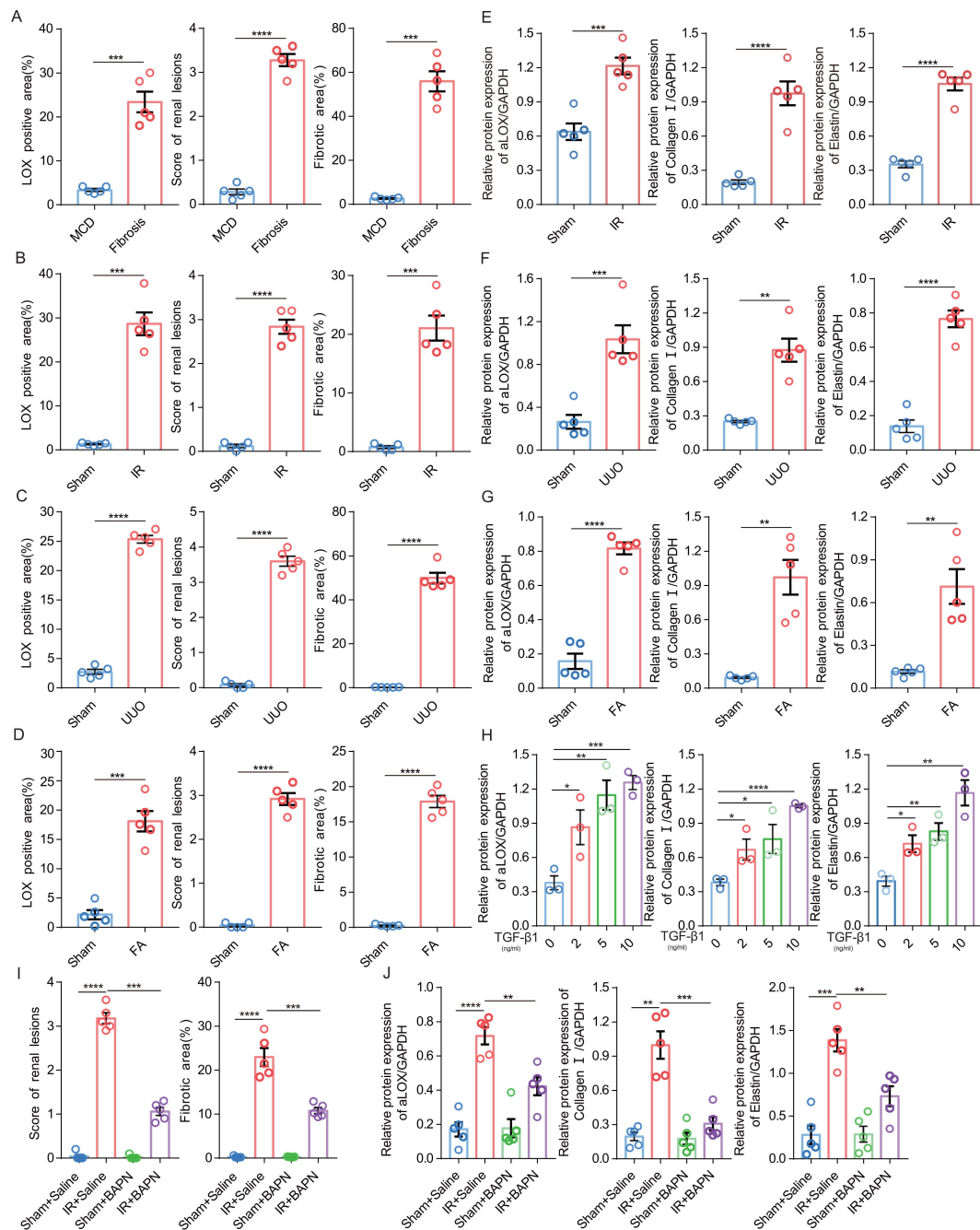


## SUPPLEMENTAL FIGURE LEGENDS



**Supplemental Figure 1. The quantitative analysis data of Figure 1.**

(A) Quantitative analysis of immunohistochemical staining with LOX antibody, HE and Masson's staining in kidney sections from patients with and without renal fibrosis ( $n = 5$ ). Original magnification,  $\times 200$ . Bar = 100  $\mu\text{m}$ .

(B-D) Quantitative analysis of immunohistochemical staining of LOX, HE and Masson's staining in kidney sections from renal fibrosis models induced by IR, UUO, FA ( $n = 5$ ). Original magnification,  $\times 200$ . Bar = 100  $\mu\text{m}$ .

magnification,  $\times 200$ . Bar = 100  $\mu\text{m}$ .

**(E-G)** Quantitative analysis of aLOX (activated aLOX), Collagen I and Elastin levels detected by Western immunoblots in kidneys collected from IR-, UUO- and FA- mice models ( $n = 5$ ).

**(H)** Quantitative analysis of the expression of aLOX, Collagen I and Elastin levels by Western immunoblots in NRK-52E cells treated with TGF- $\beta$ 1 ( $n = 3$ ).

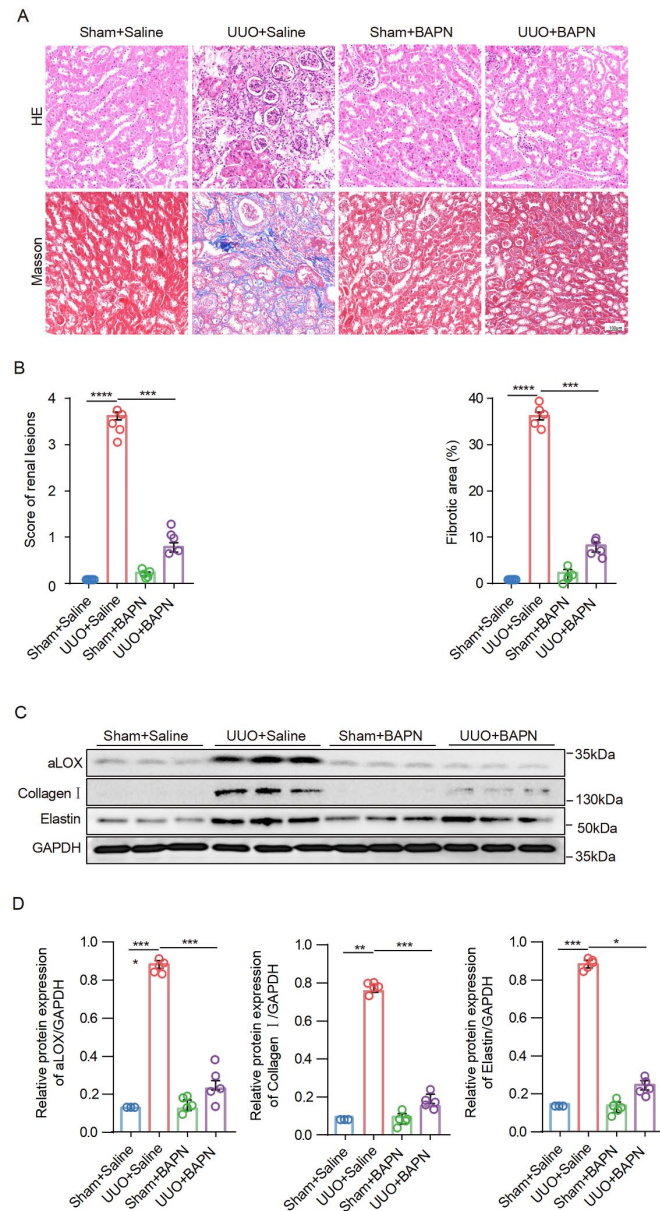
For BAPN treatment, four groups ( $n = 5$  / each group) of C57BL/6 mice were prepared: Sham + Saline; Sham + BAPN (100 mg/kg/day); IR + Saline; and IR + BAPN (I-J).

**(I)** Quantitative analysis of HE and Masson's staining of kidney sections. Original magnification,  $\times 200$ . Bar = 100  $\mu\text{m}$ .

**(J)** Quantitative analysis of aLOX, Collagen I and Elastin levels in mouse kidneys detected by Western immunoblots.

Each bar represents the mean  $\pm$  SEM, statistics used included a 2-tailed t test (2 groups, in A-G) or 1-way ANOVA (multiple groups, in H-J). \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , \*\*\*\*,  $P < 0.0001$ .

IR, ischemia-reperfusion injury; FA, folic acid-treated; UUO, unilateral urethral obstruction.

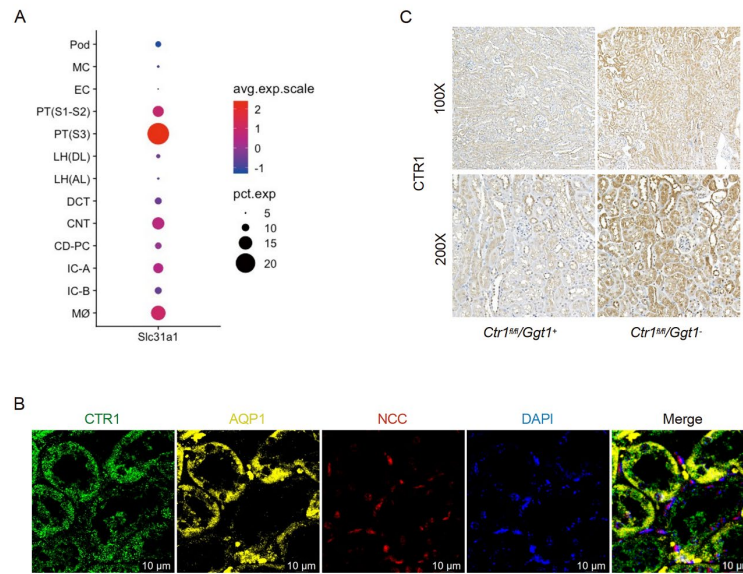


**Supplemental Figure 2. Inhibition of LOX activity attenuates renal fibrosis in UUO mice.**

**(A-B)** Representative histological images and quantitative analysis of the kidney. H&E staining and Masson's trichrome staining of kidney sections from UUO- mice models ( $n = 5$ ). Original magnification,  $\times 200$ . Bar = 100  $\mu\text{m}$ .

**(C-D)** Western blot analysis and quantitative analysis of aLOX, Collagen I, and Elastin protein expression in kidney tissues from Sham and UUO mice treated with Saline or BAPN ( $n = 5$ ).

Each bar represents the mean  $\pm$  SEM, statistics used included 1-way ANOVA. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , \*\*\*\*,  $P < 0.0001$ .

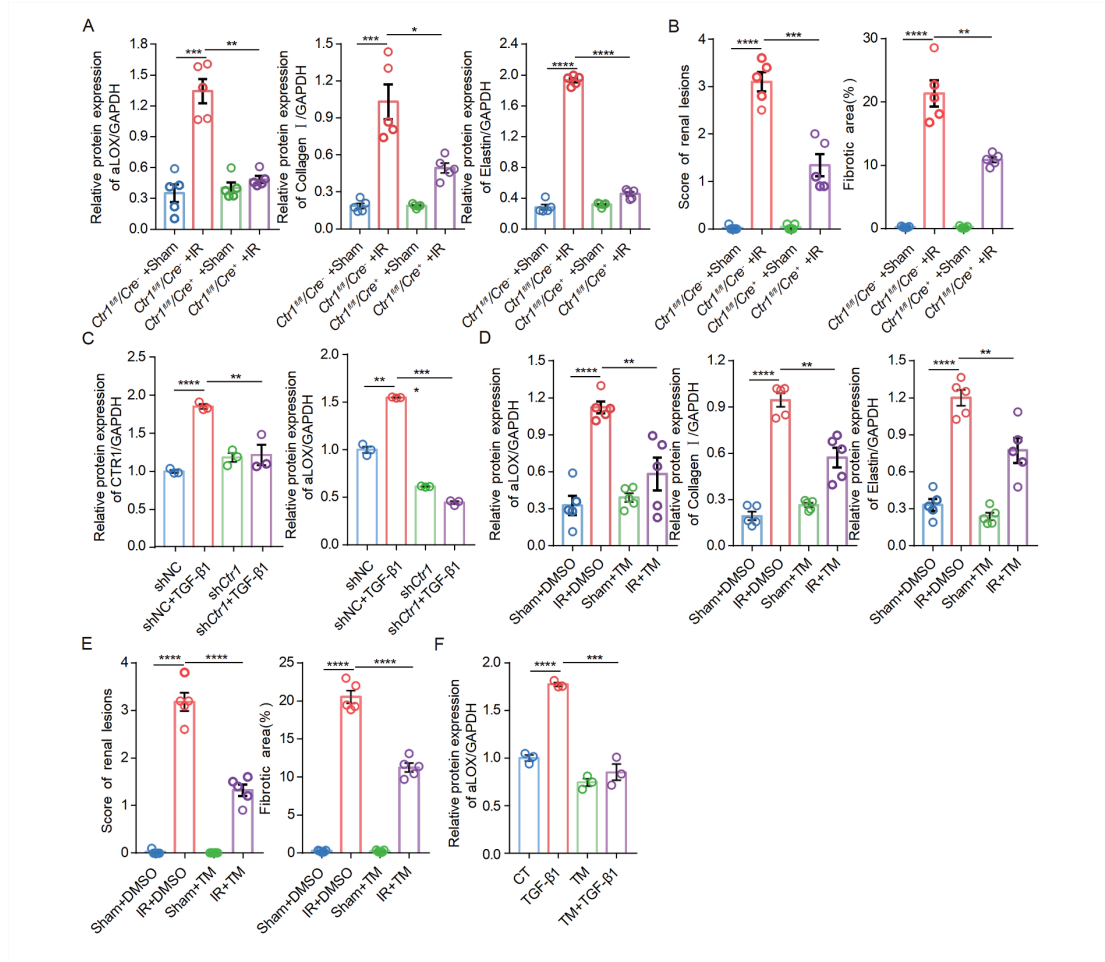


**Supplemental Figure 3. The expression of CTR1 in normal mouse kidney and the identification of *Ctrl<sup>fl/fl</sup>/Cre<sup>-/-</sup>* and *Ctrl<sup>fl/fl</sup>/Cre<sup>+/+</sup>* mice.**

(A) The expression profile of CTR1 across various cell types in the single-cell sequencing data of normal mouse kidneys. The data was obtained from the ‘Healthy Mouse Dataset: Wu et al, JASN 2019; and RBK RID: 14-4KBC’ in <https://humphreyslab.com/SingleCell/>.

(B) The co-localization of CTR1 (green) with AQP1 (yellow) and NCC (red) was analyzed by immunofluorescent co-staining in renal tissues ( $n=3$ ). AQP1, Aquaporin-1. NCC, Na-Cl cotransporter. Original magnification,  $\times 400$ . Bar = 10  $\mu$ m.

(C) Representative images of immunohistochemical staining with CTR1 in kidney sections from *Ctrl<sup>fl/fl</sup>/Cre<sup>-</sup>* and *Ctrl<sup>fl/fl</sup>/Cre<sup>+</sup>* mice ( $n=5$ ).



**Supplemental Figure 4. The quantitative analysis data of Figure 2 and Figure 3.**

For the analyses of *Ctrl* manipulated mice, *Ctrl*<sup>fl/fl</sup>/*Cre*<sup>-</sup> and *Ctrl*<sup>fl/fl</sup>/*Cre*<sup>+</sup> mice were randomly divided into the following four groups (*n* = 5/each group): *Ctrl*<sup>fl/fl</sup>/*Cre*<sup>-</sup> mice + sham; *Ctrl*<sup>fl/fl</sup>/*Cre*<sup>-</sup> mice + IR; *Ctrl*<sup>fl/fl</sup>/*Cre*<sup>+</sup> mice + sham; and *Ctrl*<sup>fl/fl</sup>/*Cre*<sup>+</sup> mice + IR (A-B).

(A) Quantitative analysis of the expression of aLOX, Collagen I and Elastin levels detected by Western immunoblots in mouse kidneys.

(B) Quantitative analysis of HE and Masson's staining of kidney sections. Original magnification, ×200. Bar = 100 μm.

(C) Quantitative analysis of aLOX and CTR1 detected by Western immunoblots in NRK-52E cells treated with TGF-β1 after downregulation of *Ctrl* (*n* = 3).

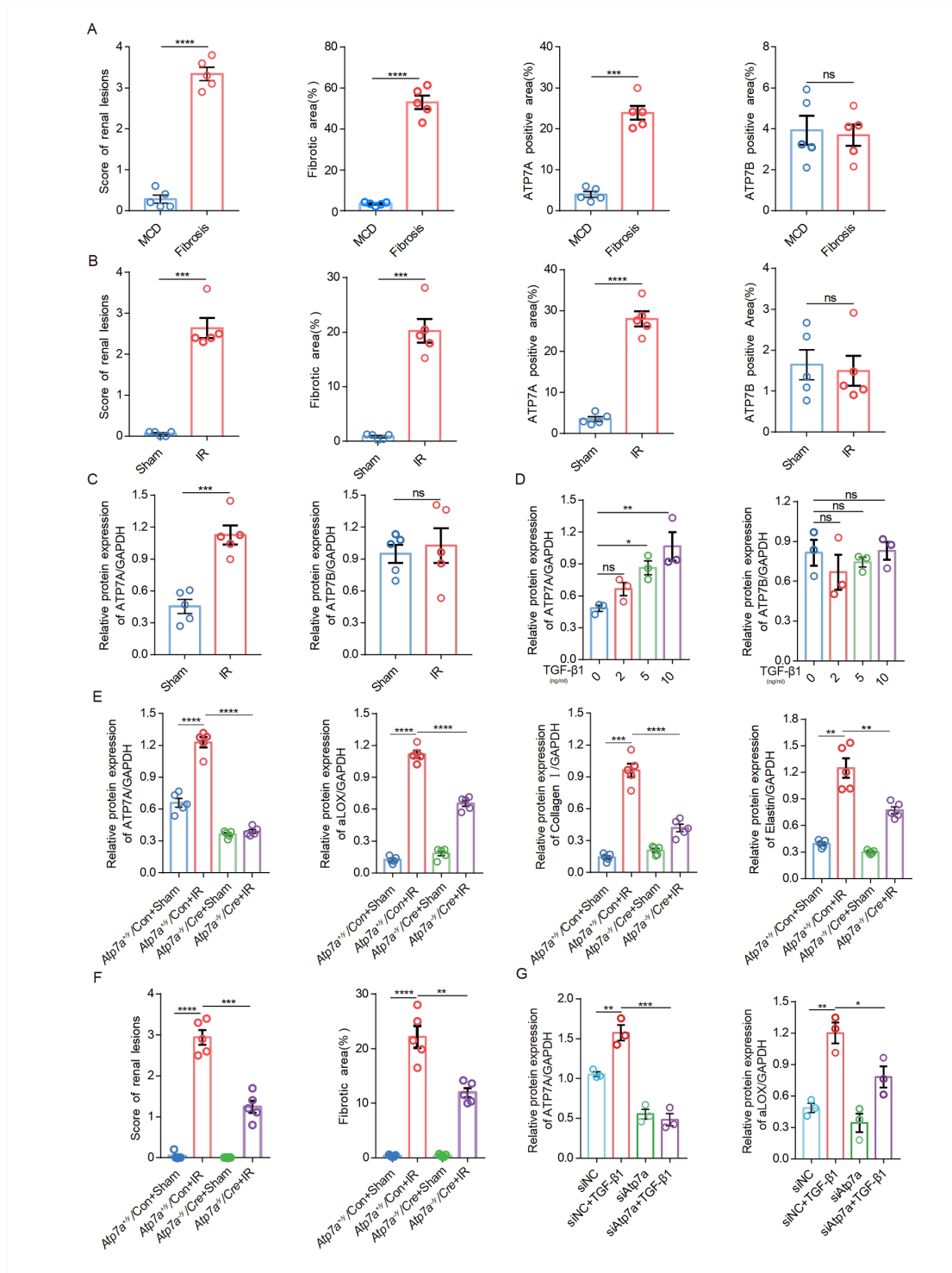
For TM treatment, four groups of C57BL/6 mice were prepared (*n* = 5/each group): Sham + DMSO; Sham + TM (30 mg/kg); IR + DMSO; and IR + TM (D-E).

(D) Quantitative analysis of aLOX, Collagen I and Elastin levels detected by Western immunoblots in mouse kidneys.

(E) Quantitative analysis of HE and Masson's staining of kidney sections. Original magnification,  $\times 200$ . Bar = 100  $\mu\text{m}$ .

(F) Quantitative analysis of aLOX detected by Western immunoblots in NRK-52E cells treated with or without TM after stimulated with TGF- $\beta 1$  ( $n = 3$ ).

Each bar represents the mean  $\pm$  SEM, statistics used included 1-way ANOVA. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , \*\*\*\*,  $P < 0.0001$ .



**Supplemental Figure 5. The quantitative analysis data of Figure 4 and Figure 5.**

(A) Quantitative analysis of immunohistochemical staining with ATP7A and ATP7B, HE and Masson's staining in kidney sections from patients with and without renal fibrosis ( $n = 5$ ). Original magnification,  $\times 200$ . Bar = 100  $\mu$ m.

(B) Quantitative analysis of immunohistochemical staining of ATP7A and ATP7B, HE and Masson's staining in kidney sections from renal fibrosis models induced by IR ( $n = 5$ ). Original magnification,

×200. Bar = 100 μm.

(C) Quantitative analysis of ATP7A and ATP7B expression in mouse kidneys detected by Western immunoblots ( $n = 5$ ).

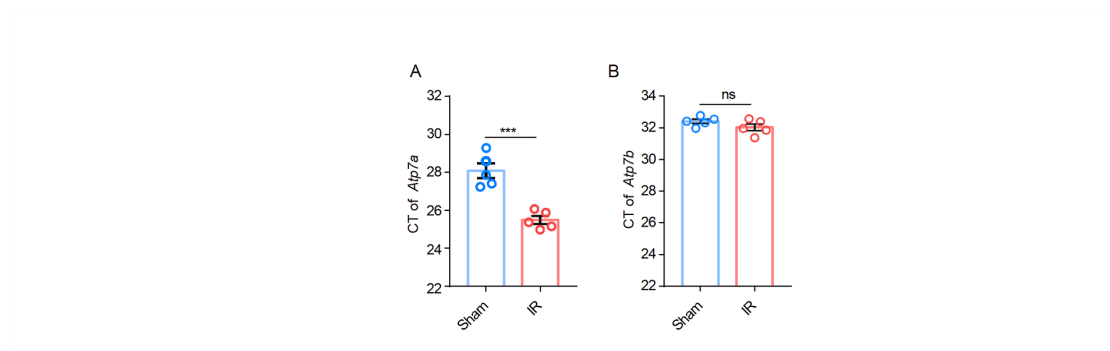
(D) Quantitative analysis of ATP7A and ATP7B expression detected by Western immunoblots in NRK-52E cells treated with TGF-β1 ( $n = 3$ ).

(E) Quantitative analysis ATP7A, aLOX, Collagen I and Elastin expression detected by Western immunoblots in mouse kidneys among different groups ( $n = 5$ ).

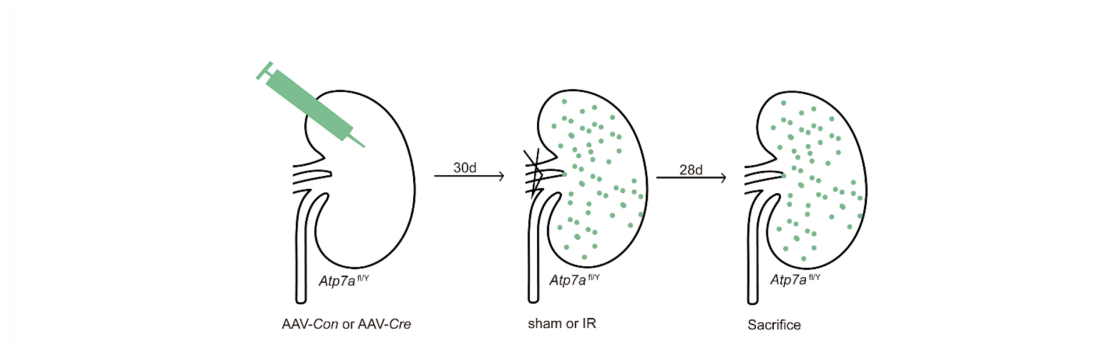
(F) Quantitative analysis of HE and Masson's staining of kidney sections ( $n = 5$ ). Original magnification, ×200. Bar = 100 μm.

(G) Quantitative immunoblots of ATP7A and aLOX detected by Western immunoblots in NRK-52E cells among different groups ( $n = 3$ ).

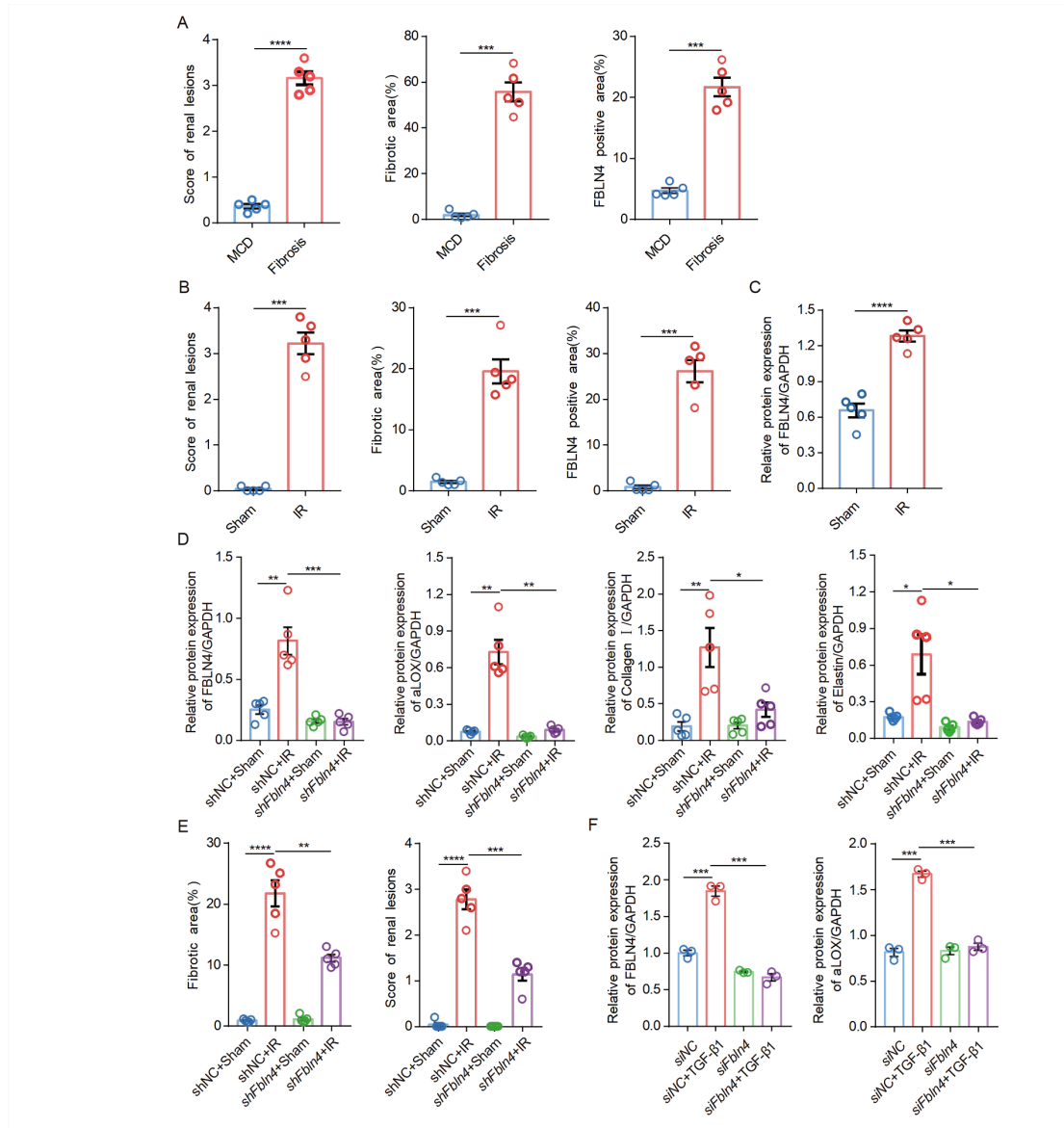
Each bar represents the mean ± SEM, statistics used included a 2-tailed t test (2 groups, in A-C) or 1-way ANOVA (multiple groups, in D-G). \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , \*\*\*\*,  $P < 0.0001$ .



**Supplemental Figure 6. The RT-qPCR analysis of *Atp7a* (A) and *Atp7b* (B) mRNA in kidney subjected by IR injury.** CT: cycle threshold. ( $n = 5$ ), Each bar represents the mean ± SEM, statistics used included a 2-tailed t test. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ .



**Supplemental Figure 7. The time points for AAV injection and IR or Sham surgery.**



**Supplemental Figure 8. The quantitative analysis data of Figure 8.**

(A) Quantitative analysis of immunohistochemical staining with FBLN4, HE and Masson's staining in kidney sections from patients with and without renal fibrosis ( $n = 5$ ). Original magnification,  $\times 200$ . Bar = 100  $\mu\text{m}$ .

(B) Quantitative analysis of immunohistochemical staining with FBLN4, HE and Masson's staining in kidney subjected by IR injury ( $n = 5$ ). Original magnification,  $\times 200$ . Bar = 100  $\mu\text{m}$ .

(C) Quantitative analysis of FBLN4 detected by Western immunoblots in kidney tissues among different groups ( $n = 5$ ).

All mice were randomly divided into four groups ( $n = 5$  / each group): sham-operated mice treated with AAV9-Con (shNC + Sham), sham-operated mice treated with AAV9-sh*Fbln4* (sh *Fbln4* +

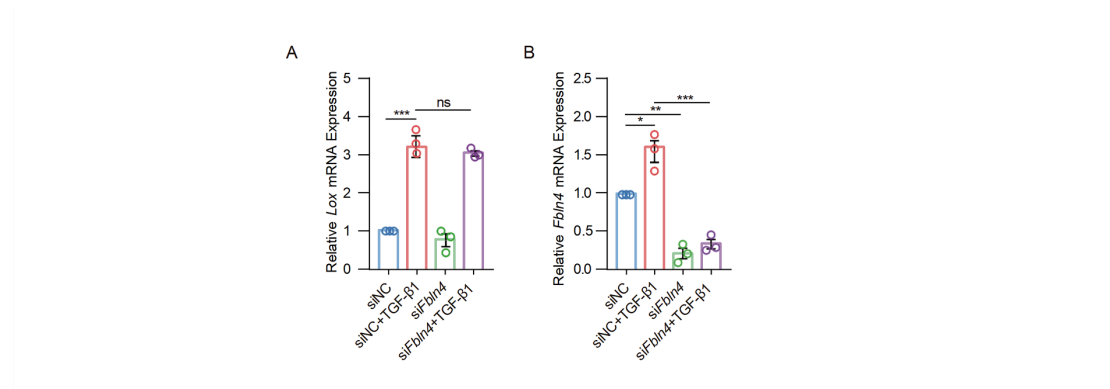
Sham), IR mice treated with AAV9-Con (shNC + IR) and IR mice treated with AAV9- sh *Fbln4* (sh *Fbln4* + IR) (**D-E**).

(**D**) Quantitative analysis of FBLN4, aLOX, Collagen I and Elastin expression detected by Western immunoblots in mouse kidneys among different groups.

(**E**) Quantitative analysis of HE and Masson's staining of kidney sections. Original magnification,  $\times 200$ . Bar = 100  $\mu\text{m}$ .

(**F**) Quantitative analysis of FBLN4 and aLOX detected by Western immunoblots in NRK-52E cells among different groups ( $n = 3$ ).

Each bar represents the mean  $\pm$  SEM, statistics used included a 2-tailed t test (2 groups, in A-C) or 1-way ANOVA (multiple groups, in D-F). \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , \*\*\*\*,  $P < 0.0001$ .



**Supplemental Figure 9. The RT-qPCR analysis of *Lox* and *Fbln4* mRNA expression.**

(**A-B**) RT-qPCR analysis of *Lox* and *Fbln4* mRNA expression in NRK-52E cells transfected with siNC or si*Fbln4* and treated with or without TGF- $\beta$ 1 ( $n = 3$ ).

Each bar represents the mean  $\pm$  SEM, statistics used included 1-way ANOVA. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , \*\*\*\*,  $P < 0.0001$ .

| Name                    | Forward Primer                  | Reverse Primer                 |
|-------------------------|---------------------------------|--------------------------------|
| <i>Gapdh</i> -<br>mouse | 5' AAGGTCGGTGTGAACGGATT 3'      | 5'CGCTCCTGGAAGATGGTGAT 3'      |
| <i>Atp7a</i> -<br>mouse | 5' TGGGAAAGTGAATGGTGTCCA 3'     | 5'ACGGTATTGGTTAAGACAGGGA3'     |
| <i>Atp7b</i> -<br>mouse | 5' GGGGACGATGCCTGAACAG 3'       | 5' GCCGGGCAAAGCAAGTTTAG 3'     |
| <i>Gapdh</i> -rat       | 5' GGCAAGTTCAACGGCACA 3'        | 5' CCATTTGATGTTAGCGGGAT 3'     |
| <i>Fbln4</i> -rat       | 5' AGACATTGACGAGTGCGAAACAG 3'   | 5' AGGACAGAGGCAGCGGTTG 3'      |
| <i>Lox</i> -rat         | 5' GTTCAGCATATAGGGCGGATGTCAG 3' | 5' GGCGGCTTGGTAAGAAGTCAG AC 3' |

Table 1 Sequences of the primer pairs used for real-time PCR.