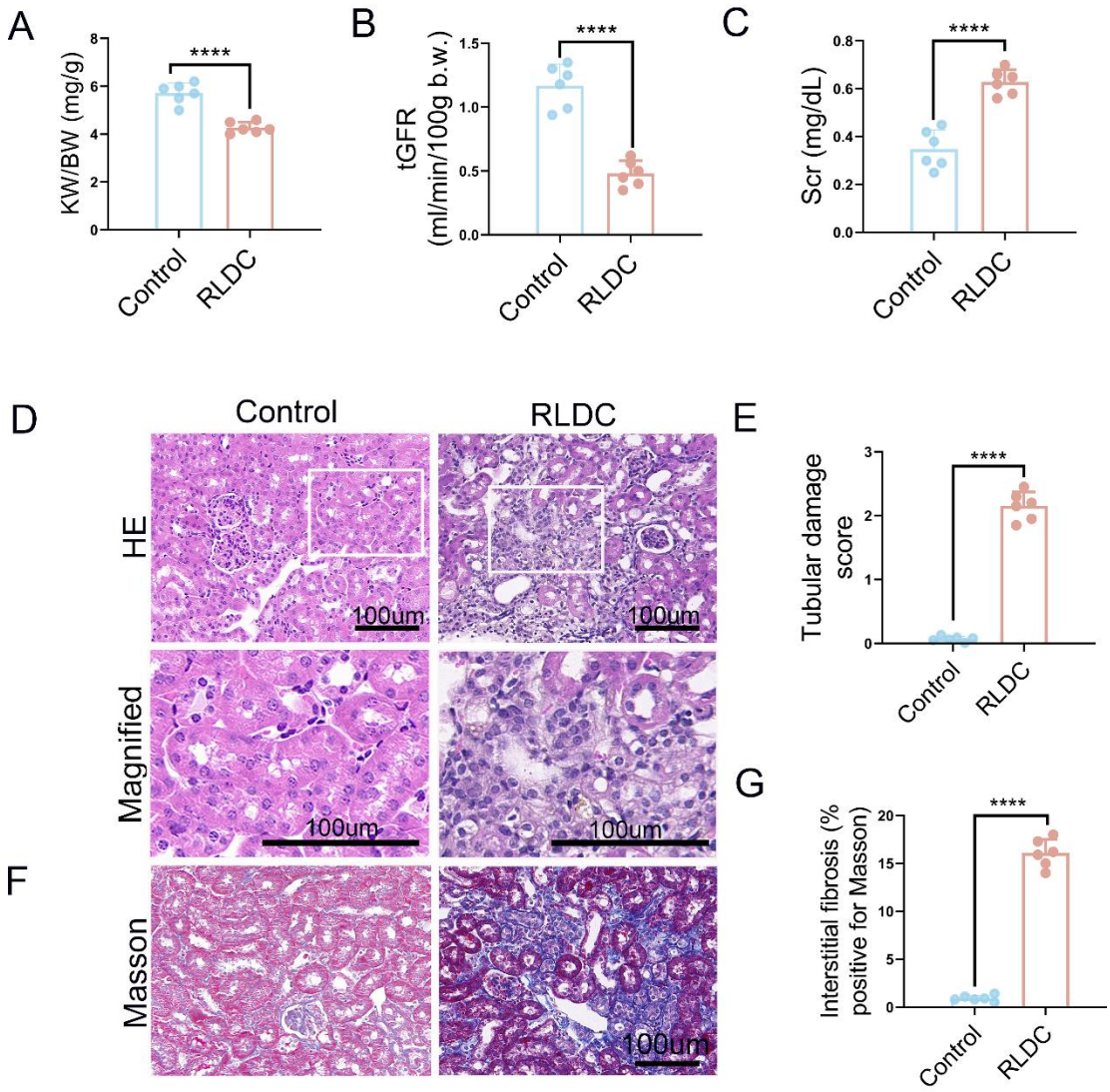
Supplementary Data

The Foxo4/Tcf12/TGF-β signaling pathway promotes senescence and fibrosis after cisplatin nephrotoxicity

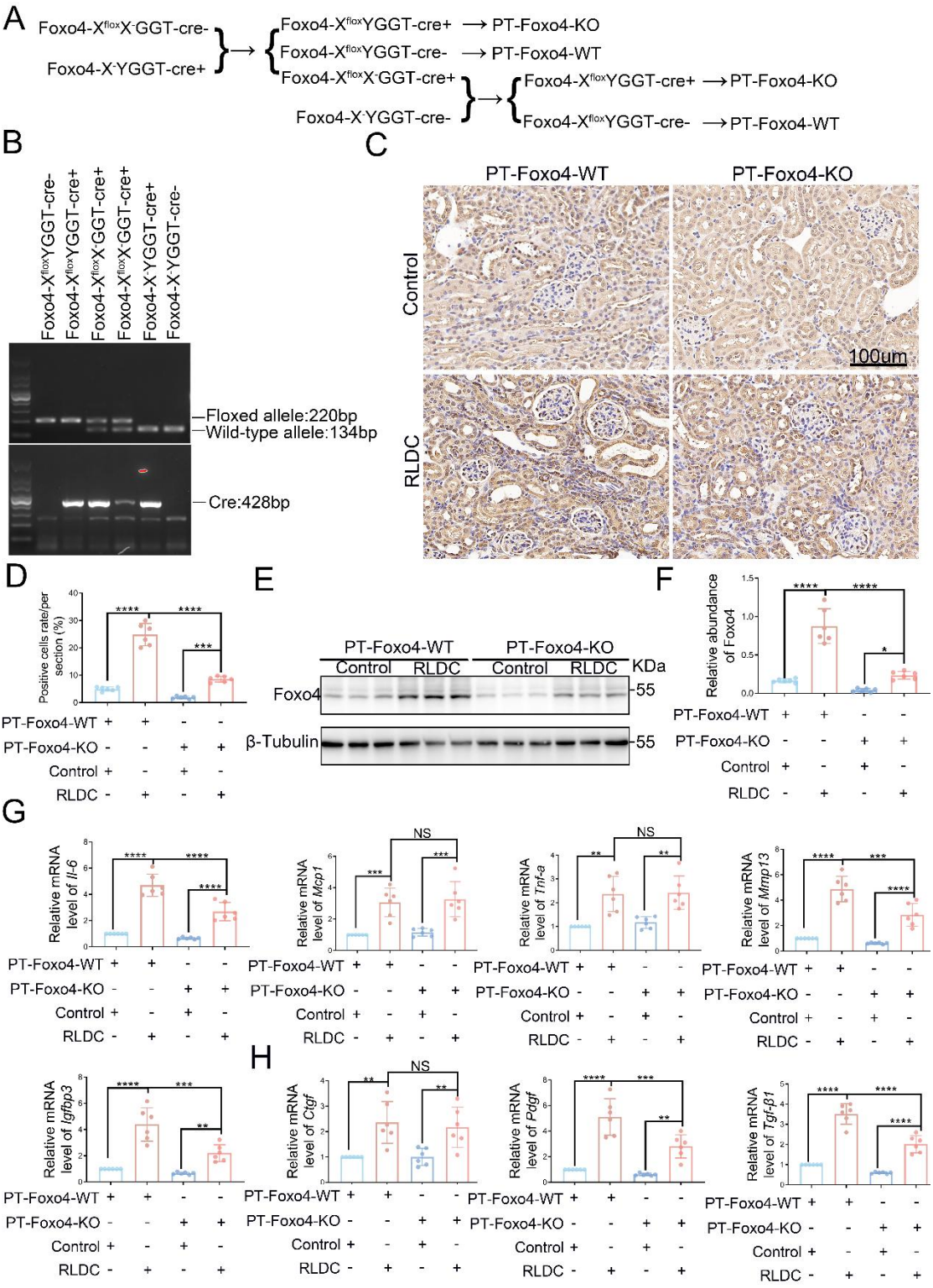
Fang Xu[1], Honglin Li[1], Yu Xiang[1], Yuqing Zeng[1], Yun Han[1], Wenwen Wu[1], Ying Fu[1], Juan Cai[1], Fanghua Li[2], Dongshan Zhang[3], Yu Liu[1*], Zheng Dong [1,4*]



Supplementary Figure 1: Chronic kidney problems after RLDC treatment in mice.

C57BL/6 mice were intraperitoneally injected with 8 mg/kg cisplatin or saline (Control) once a week for 4 weeks (in vivo RLDC treatment). 4 weeks after RLDC, kidneys were harvested for analysis, and the body weights and tGFR levels of mice were measured. **(A)** Kidney weight to body weight ratio. (**B**) tGFR. (**C**) Scr. (**D**) H&E staining of renal histology. Original magnification x 400. Lower panels are enlarged images of the boxed areas in the upper panels. Scale Bar:100μM. (**E**) Tubular damage score. (**F**) Masson staining of renal fibrosis. Original magnification x 400. Scale

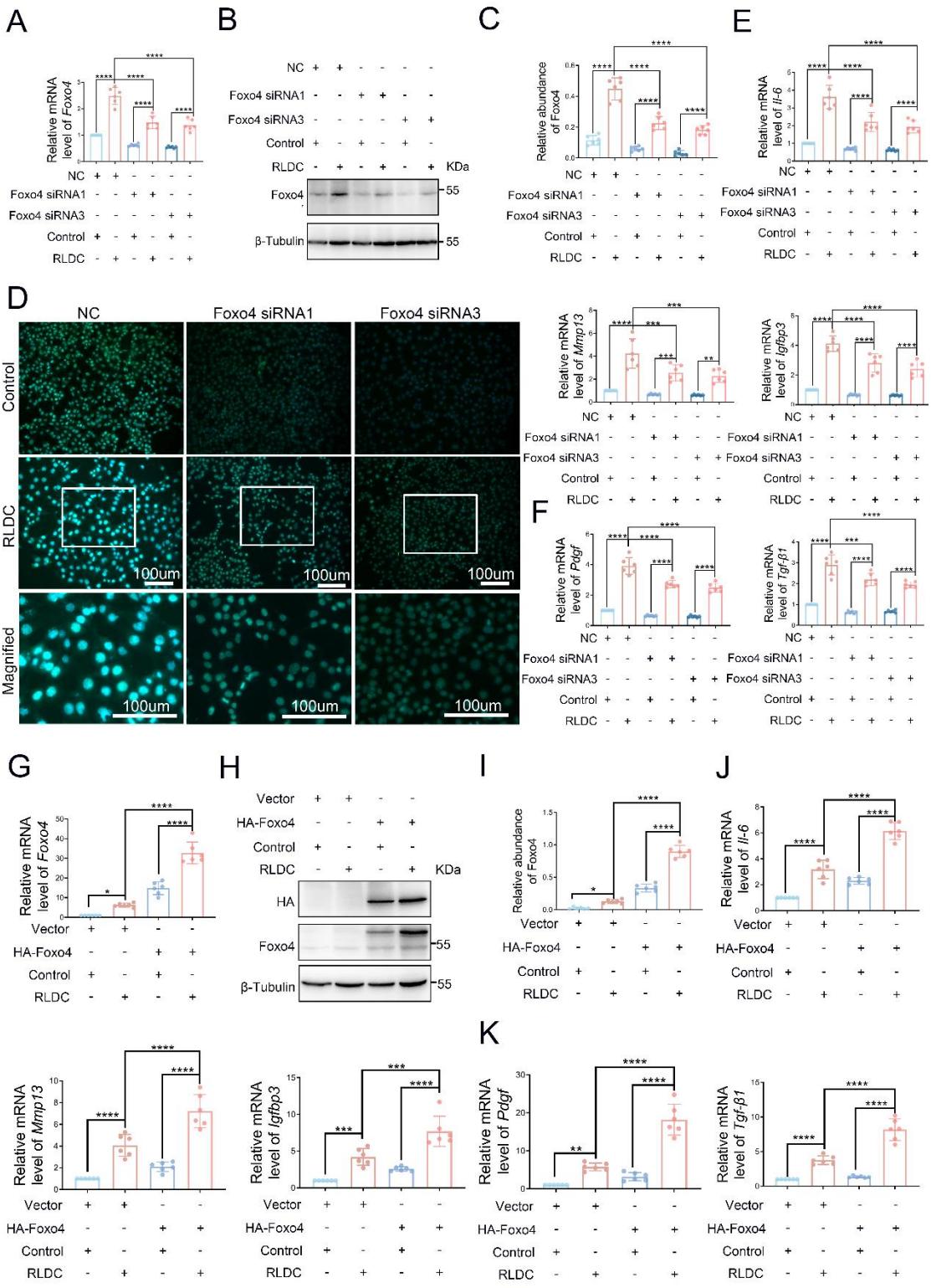
Bar:100μM. (**G**) Quantification of tubulointerstitial fibrosis in the kidney cortex. Data are expressed as mean ± SD (n = 6). P values <0.05 were considered significant, with P values presented as follows: NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.



Supplementary Figure 2: Creation and characterization of PT-Foxo4 -KO mouse model.

(A) Breeding protocol for the creation of PT-Foxo4 -KO mice. **(B)** PCR detection of

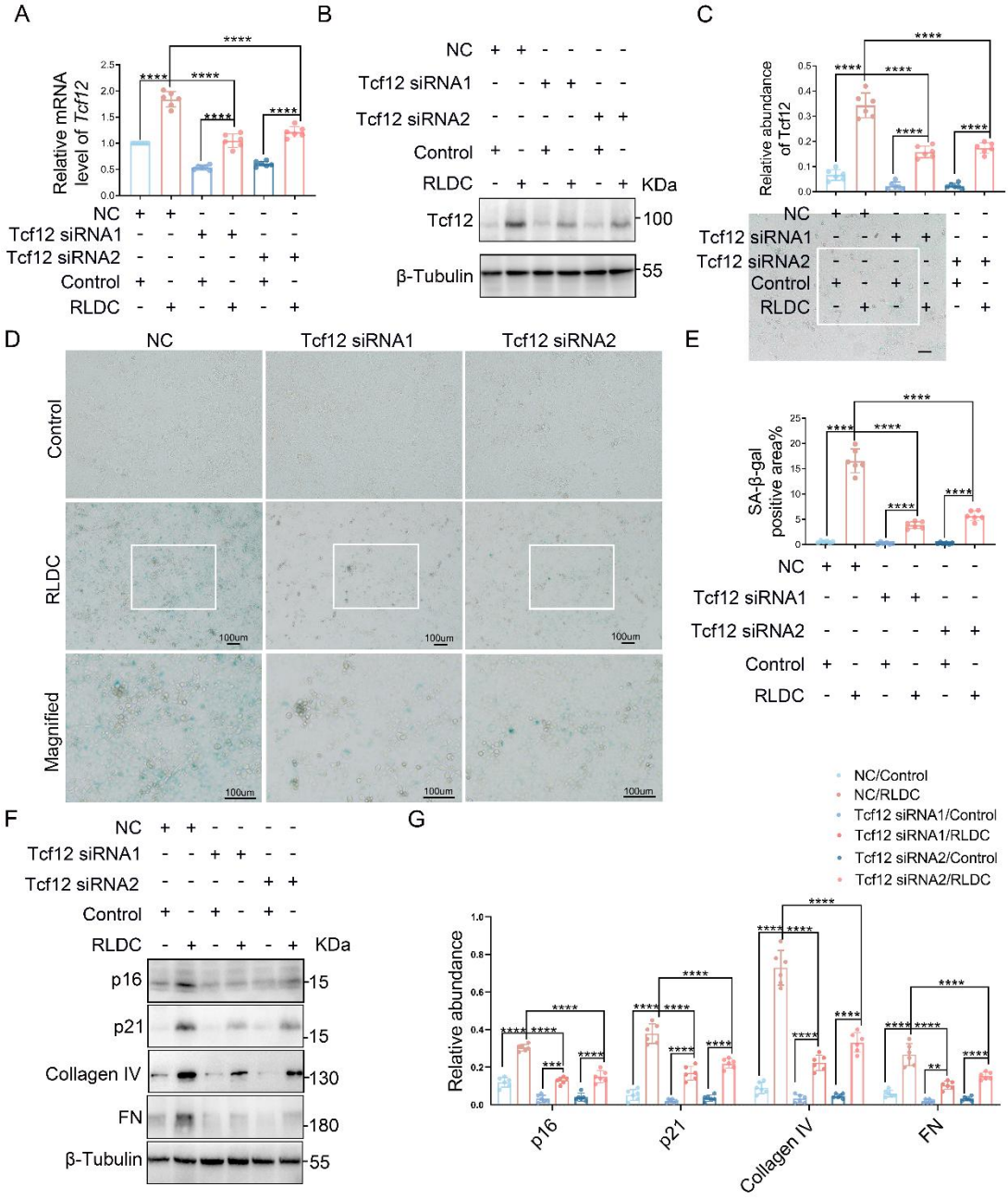
wild-type and floxed alleles of Foxo4 and GGT-Cre allele. **(C-F)** PT-Foxo4-WT and PT-Foxo4-KO mice were subjected to RLDC treatment. 4 weeks later, kidneys were harvested for analysis. **(C)** Immunohistochemical staining of Foxo4. Original magnification x 400. Scale Bar:100μM. **(D)** Semi-quantification of immunohistochemistry staining. **(E)** Immunoblot analysis of Foxo4. **(F)** Densitometry analysis of immunoblot bands. (**G**) RT-qPCR analysis of *Il-6*, *Mcp1*, *Tnf-a*, *Mmp13*, and *Igfbp3* mRNA expression. (**H**) RT-qPCR analysis of connective tissue growth factor (*Ctgf*), *Pdgf*, and *Tgf-β1* mRNA expression. Data are expressed as mean ± SD (n = 6). P values <0.05 were considered significant, with P values presented as follows: NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001



Supplementary Figure 3: Knockdown of Foxo4 and overexpression of Foxo4 in RLDC treatment of kidney tubular cells.

(A-F) BUMPT cells were transfected with Foxo4 siRNA or negative control (NC)

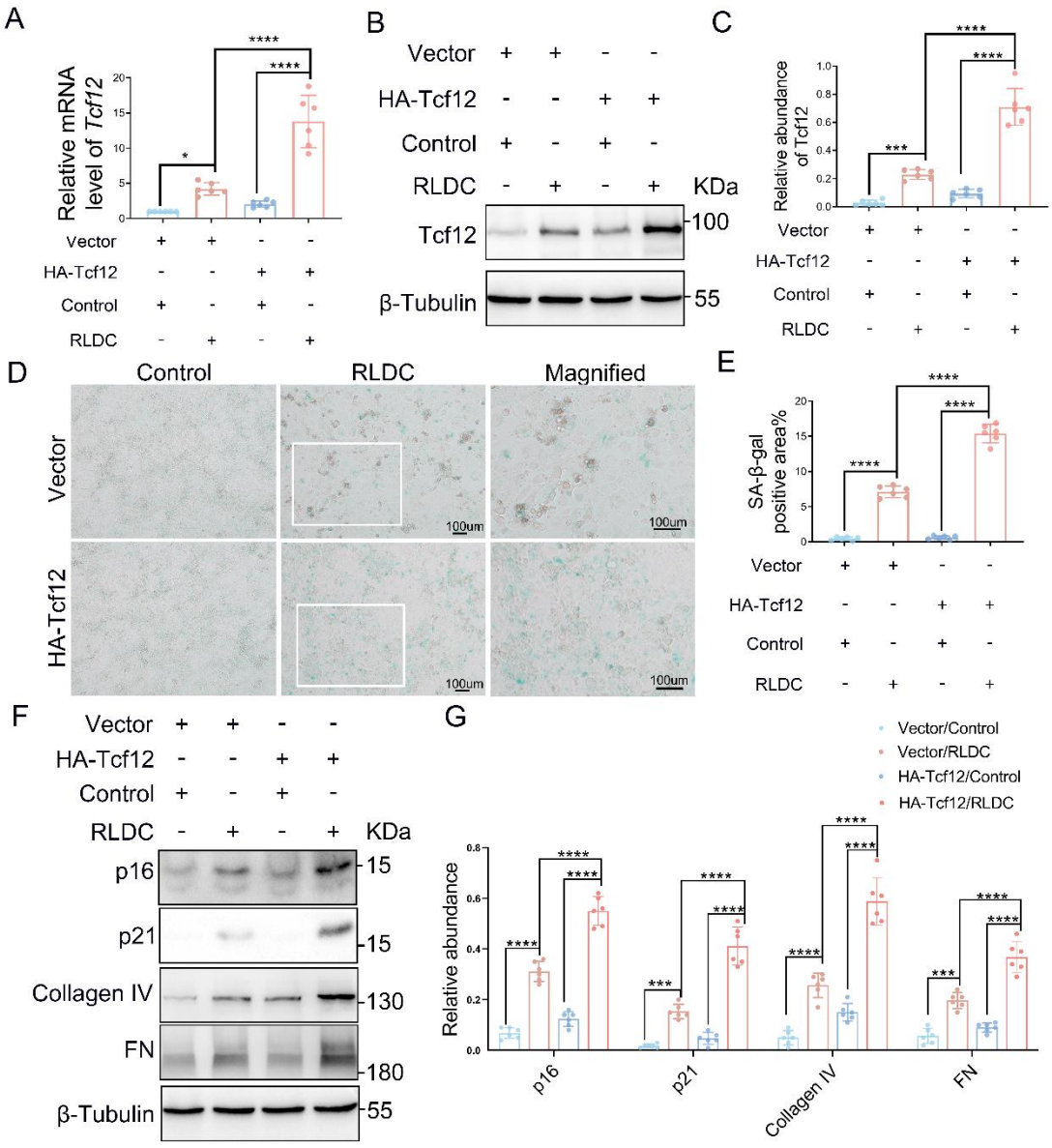
siRNA, and then subjected to in vitro RLDC treatment or left untreated to collect cell lysate for analysis. (**A**) RT-qPCR analysis of *Foxo4* mRNA. (**B**) Immunoblot analysis of Foxo4 with densitometry analysis (**C**). (**D**) Immunofluorescence of Foxo4 in BUMPT cells. Scale bar:100μm. (**E**) RT-qPCR analysis of *Il-6*, *Mmp13*, and *Igfbp3* mRNA expression. (**F**)RT-qPCR analysis of *Pdgf*, and *Tgf-β1* mRNA expression. (G-K) BUMPT cells stably transfected with HA-Foxo4-GFP or empty vector were subjected to in vitro RLDC treatment or left untreated to collect cell samples for analysis. (**G**)RT-qPCR analysis of *Foxo4* mRNA. (**H**) Immunoblot analysis of HA and Foxo4 (**I**) Densitometry analysis of immunoblot bands. (**J**) RT-qPCR analysis of *Il-6*, *Mmp13*, and *Igfbp3* mRNA expression. (**K**)RT-qPCR analysis of *Pdgf*, and *Tgf-β1* mRNA expression. Data are expressed as mean ± SD (n = 6). P values <0.05 were considered significant, with P values presented as follows: NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.



Supplementary Figure 4: Knockdown of Tcf12 ameliorates senescence and fibrotic response during RLDC treatment of kidney tubular cells.

BUMPT cells were transfected with Tcf12 siRNA or negative control (NC) siRNA, and then subjected to in vitro RLDC treatment or untreated to collect cell samples for analysis. **(A)** RT-qPCR analysis of *Tcf12* mRNA. **(B)** Immunoblot analysis of Tcf12 with densitometry analysis in **(C)**. **(D)** Representative images of SA-β-gal staining.

Scale bar:100μm. **(E)** Quantification of SA-β-gal staining. **(F)** Immunoblot analysis of p16, p21, Collagen IV and FN with densitometry analysis in **(G)**. Data are expressed as mean ± SD (n = 6). P values <0.05 were considered significant, with P values presented as follows: NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.

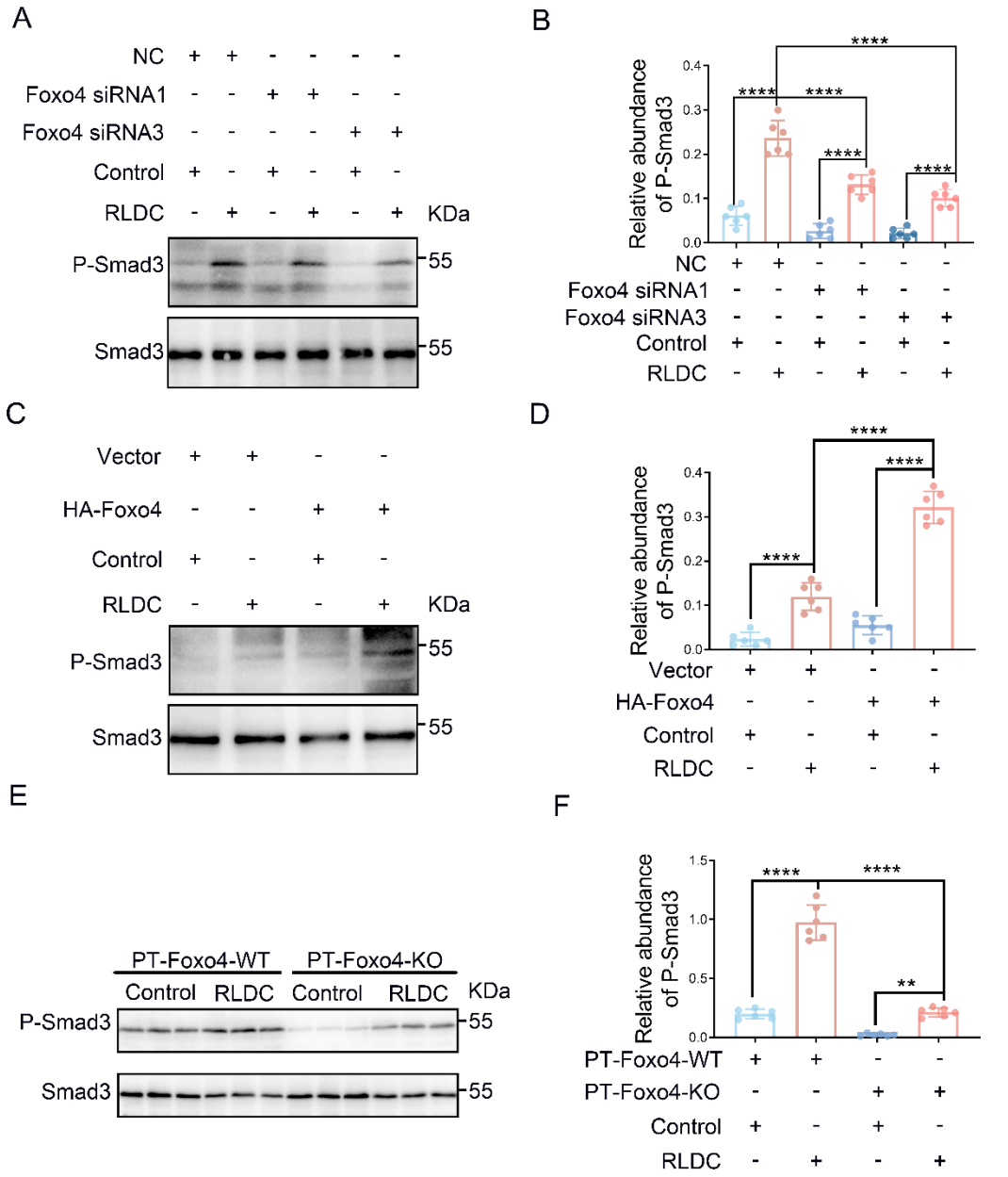


Supplementary Figure 5: Overexpression of Tcf12 enhances senescence and fibrotic response during RLDC treatment of kidney tubular cells.

BUMPT cells stably transfected with HA- Tcf12-GFP or empty vector were subjected to RLDC treatment or left untreated to collect cell samples for analysis. (**A**) RT-qPCR analysis of *Tcf12* mRNA. (**B**) Immunoblot analysis of Tcf12 with densitometry analysis in (**C**). (**D**) Representative images of SA-β-gal staining. Scale bar:100μm. (**E**) Quantification of SA-β-gal staining. (**F**)Immunoblot analysis of p16, p21, Collagen IV and FN with densitometry analysis in (**G**). Data are expressed as mean ± SD (n =

6). P values <0.05 were considered significant, with P values presented as follows:

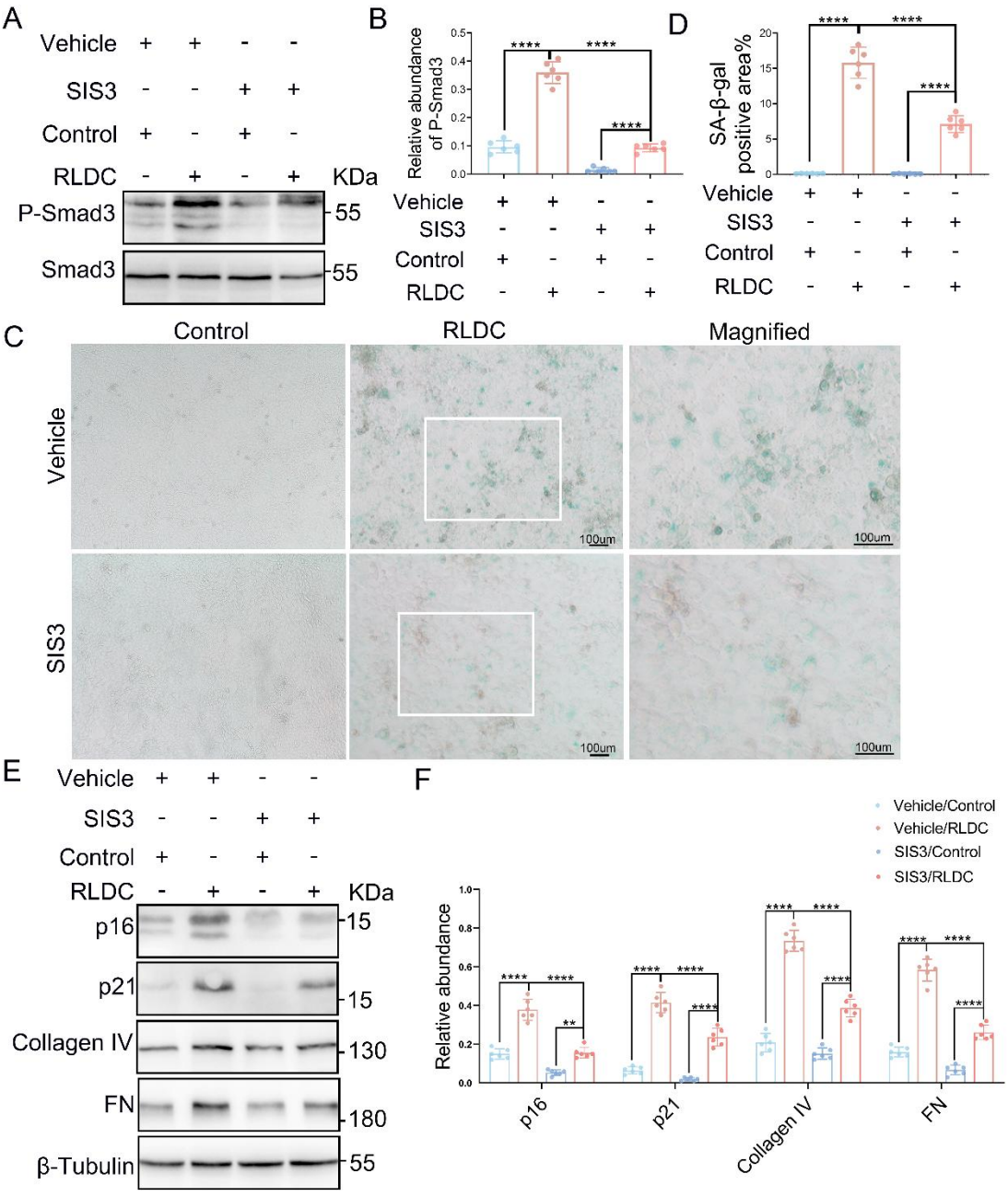
NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.



Supplementary Figure 6: Foxo4 and Tcf12 enhance TGF-β signaling during RLDC treatment of kidney tubular cells and mice.

(**A, B**) BUMPT cells were transfected with Foxo4 siRNA or negative control (NC) siRNA, and then subjected to RLDC treatment or left untreated to collect cell samples for analysis. (**A**) Immunoblot analysis of P-Smad3 with densitometry analysis in (**B**). (**C, D**) BUMPT cells stably transfected with HA-Foxo4-GFP or empty vector were

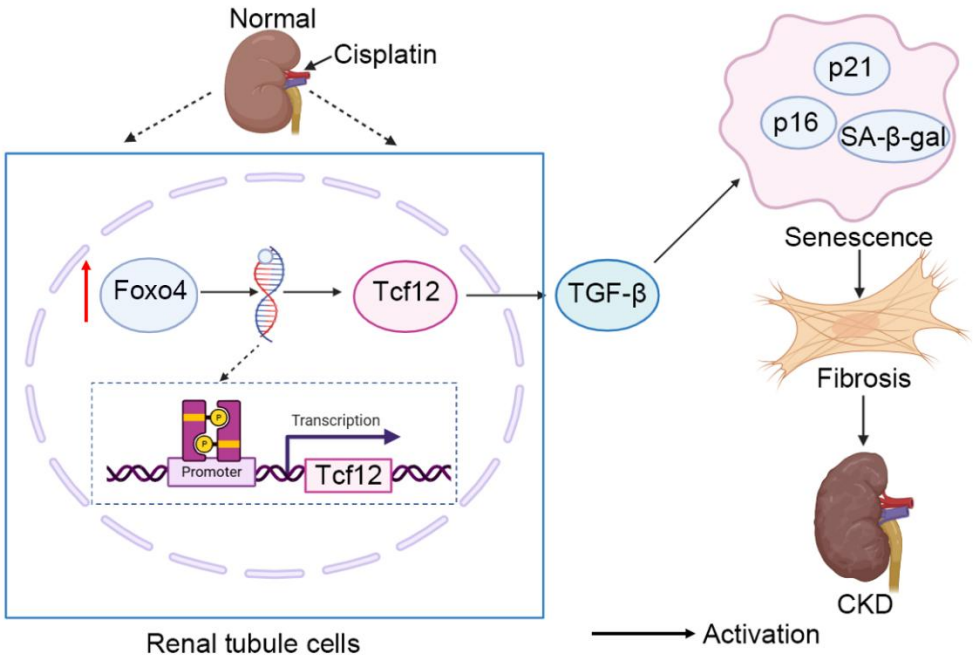
subjected to RLDC treatment or left untreated to collect cell samples for analysis. (**C**) Immunoblot analysis of P-Smad3 with densitometry analysis in (**D**). (**E, F**) PT-Foxo4-WT and PT-Foxo4-KO mice were subjected to RLDC treatment to collect samples 4 weeks later for renal function and histology analysis. (**E**) Immunoblot analysis of P-Smad3 with densitometry analysis in (**F**). Data are expressed as mean ± SD (n = 6). P values <0.05 were considered significant, with P values presented as follows: NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.



Supplementary Figure 7: SIS3 ameliorates senescence and fibrotic response during RLDC treatment of kidney tubular cells.

BUMPT cells were treated with 5 μM SIS3 (a selective inhibitor of Smad3) or DMSO for 1h, and then subjected to RLDC treatment or left untreated to collect cell samples for analysis. (**A**) Immunoblot analysis of P-Smad3 with densitometry analysis in (**B**). (**C**) Representative images of SA-β-gal staining. Scale bar:100μm. (**D**) Quantification

of SA-β-gal staining. (**E**) Immunoblot analysis of p16, p21, Collagen IV and FN with densitometry analysis in (**F**). Data are expressed as mean ± SD (n = 6). P values <0.05 were considered significant, with P values presented as follows: NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.



Supplementary Figure 8: The Foxo4/Tcf12/ TGF-β signaling pathway in tubular cell senescence and renal interstitial fibrosis after cisplatin nephrotoxicity

RLDC treatment induces Foxo4 in kidney tubule cells, which activates Tcf12 transcription and expression. Tcf12 then promotes the activation of the TGF-β signaling pathway, leading to tubular cell senescence and interstitial fibrosis for maladaptive kidney repair and CKD progression. Figure created with BioRender.com.

Supplementary Table 1. The basic clinical information of PC and CKD patients

Variable	PC (6)	CKD (6)
Age(years) (mean)[SD]	**62.7±5.7**	**55.4±5. 5**
Gender (Male:Female)	**3：3**	**4 ： 2**
eGFR ml/min/1.73 m^2(mean) [SD]	**110.8±3.2**	**42.0±10.9$^{\#}$**
Urine Protein		**+： 1**
	-	**++： 2**
		+++： 3

Note:— indicate the lack of proteinuria.

Data are expressed as mean ± SD (n = 6). #p < 0.05, CKD groups versus PC groups.

Supplementary Table 2. Primers used for mRNA quantitation by real-time PCR

Gene	Forward primers	Reverse primers
Foxo4	5′- GATGGGTCTTTGTCAGCAGGAGAAG -3′	5′- AGCAGGAGGTGGTGGTGTATCAG -3′
Tcf12	5′- CTCTCAGGCTGTCAGTCTAGTCTC -3′	5′- CTTGTGGCAGAGAATGAGTAGTATGG -3′
Tgf-β1	5′- GCGGACTACTATGCTAAAGAGG-3′	5′- CCGAATGTCTGACGTATTGAAGA-3′
Il-6	5′-TCTATACCACTTCACAAGTCGGA-3′	5′-GAATTGCCATTGCACAACTCTTT-3′
Tnf-a	5′-CAGGCGGTGCCTATGTCTC-3′	5′-CGATCACCCCGAAGTTCAGTAG-3′
Mcp1	5′- TTTTTGTCACCAAGCTCAAGAG-3′	5′-TTCTGATCTCATTTGGTTCCGA -3′
Mmp13	5′-ACAGGCTCCGAGAAATGCAA-3′	5′-CCACATCAGGCACTCCACAT -3′
Igfbp3	5′-CACCGAGTGACCGATTCCAA-3′	5′-GTCAACTTTGTAGCGCTGGC-3′
Ctgf	5′-GACCCAACTATGATGCGAGCC-3′	5′-TCCCACAGGTCTTAGAACAGG-3′
Pdgf	5′-TCTCTGCTGCTACCTGCGTCTG-3′	5′-CGTCTTGCACTCGGCGATTACAG
β-actin	5′-CAAGTGCTTCTAGGCGGACTGTTACTG-3′	5′-AAACAAAGCCATGCCAATGTTGTCTCTTAT-3′