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Supplemental information

MicroRNA-10a/b inhibit TGF-β/Smad-induced renal fibrosis by targeting TGF-β receptor 1 in diabetic kidney disease

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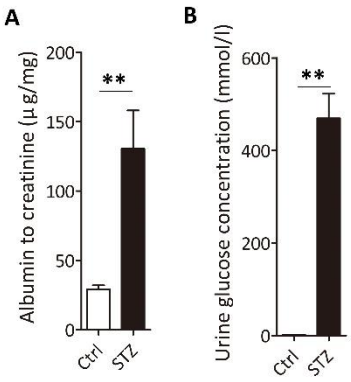

Figure S1. Albumin and glucose increased in urine from DKD mice.

(**A**, **B**) Albumin to creatinine ratio (**A**) and glucose concentration (**B**) in urine from STZ-treated mice. Data were expressed as means ± SEM, n=6 for each group. Student's t test was used for the comparison of two groups. *$p < 0.05$, **$p < 0.01$.

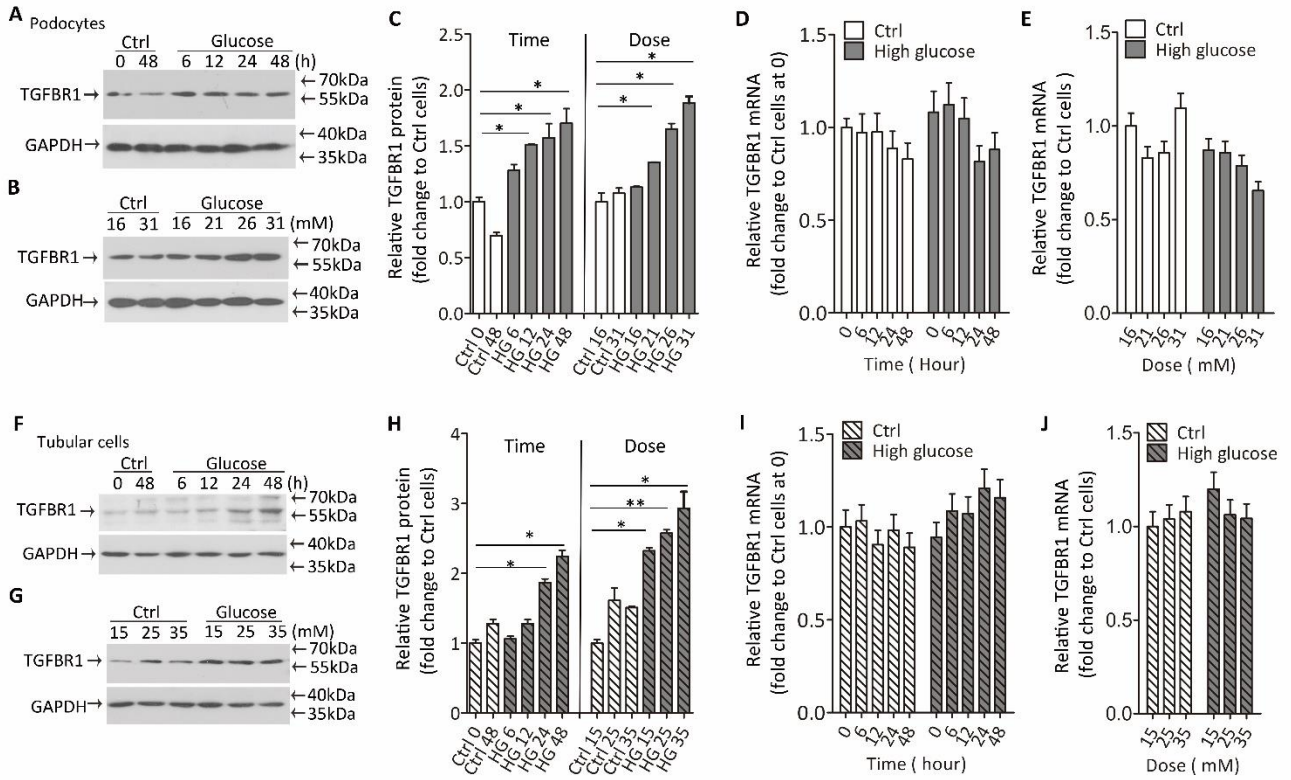

Figure S2. High glucose induces TGFBR1 protein in a time- and dose-dependent manner *in vitro*.

(**A-C**) Representative images of western blot for TGFBR1 in podocytes treated with glucose for various hours at 26mM (**A**) or at various concentrations for 24 hours (**B**), and quantification data (**C**). Equivalent mannitol was used as osmotic control. (**D**, **E**) TGFBR1 mRNA was not altered in podocytes treated with glucose for indicated time (**D**) or concentration (**E**). (**F-H**) Representative images of western blot for TGFBR1 in tubular cells treated with glucose for various hours at 25mM (**F**) or at various concentrations for 24 hours (**G**), and quantification data (**H**). Equivalent mannitol was used as osmotic control. (**I**, **J**) TGFBR1 mRNA was not altered in tubular cells treated with glucose for indicated time (**I**) or concentration (**J**). Data were expressed as means ± SEM. Student's t test was used for the comparison of two groups. ANOVA were used for comparison among multiple groups. *$p < 0.05$, **$p < 0.01$.

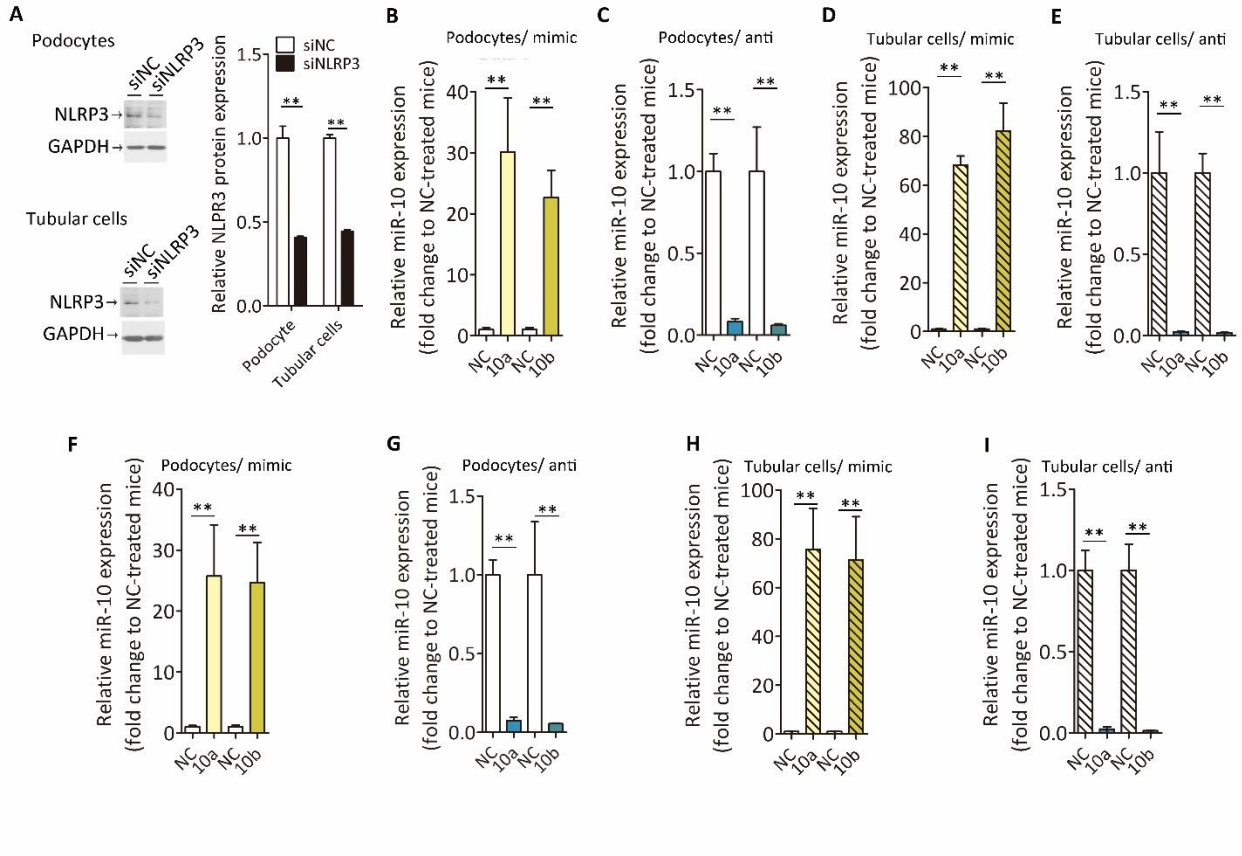

Figure S3. Efficiency of siRNA and miRNA transfection in podocytes and tubular cells.

(**A**) NLRP3 expression in podocytes and tubular cells treated with siRNA targeting NLRP3 (siNLRP3) or negative control (siNC). (**B-E**) Expression of miR-10a or 10b in podocytes (**B**, **C**) or tubular cells (**D**, **E**) co-transfected with siNLRP3 and miR-10a or 10b mimic or antisense. (**F-I**) Expression of miR-10a or 10b in podocytes (**F**, **G**) or tubular cells (**H**, **I**) transfected with miR-10a or 10b mimic or antisense. Data were expressed as means ± SEM. Student's t test was used for the comparison of two groups. *$p < 0.05$, **$p < 0.01$.

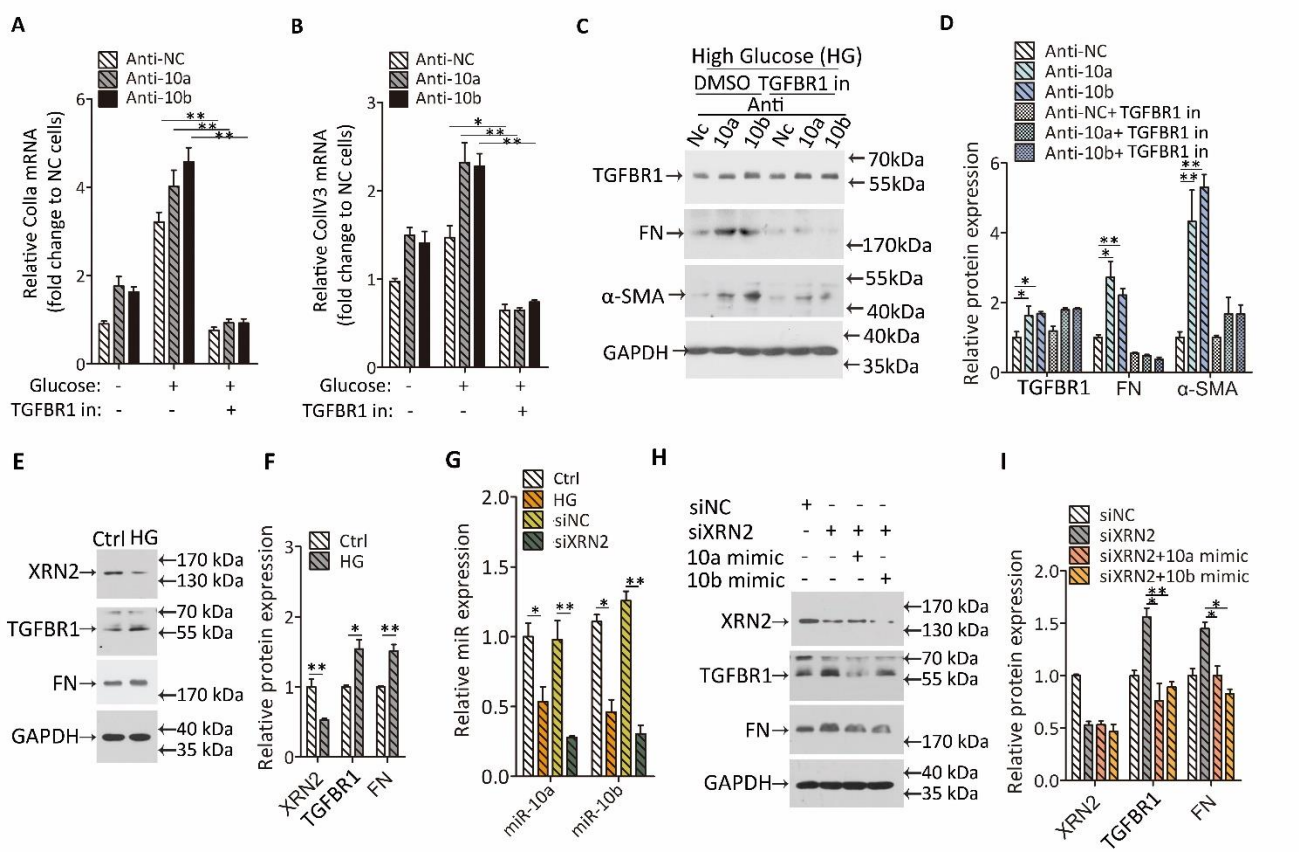

Figure S4. miR-10a/b suppress fibrosis dependent on TGFBR1, and are modulated by XRN2 in tubular cells.

(**A**, **B**) Relative ColIa (**A**) and ColIV3 (**B**) mRNA expression in tubular cells transfected with miR-10a or 10b antisense, 25mM glucose incubation was used after TGFBR1 inhibitor treatment (TGFBR1 in). (**C**, **D**) Western blot for TGFBR1, FN and α-SMA protein in tubular cells transfected with miR-10a or 10b antisense in the presence of TGFBR1 inhibitor (**C**), and quantification data (**D**), DMSO was used as dissolvant control. (**E**, **F**) Western blot for XRN2, TGFBR1, and FN protein expression in tubular cells treated with high glucose for 24 hours (**E**), and quantification data (**F**). (**G**) Relative miR-10a/b expression in tubular cells treated with high glucose (HG), siRNA targeting XRN2 (siXRN2) was introduced to evaluates its function in miR-10a or 10b expression, and RNA sequence without binding site was used as control (siNC). (**H**, **I**) Western blot for XRN2, TGFBR1, and FN protein expression in tubular cells treated with siRNA targeting XRN2 (siXRN2), 24 hours later, miR-10a or miR-10b

mimic was transfected into tubular cells. Data were expressed as means ± SEM. Student's t test was used for the comparison of two groups. ANOVA were used for comparison among multiple groups. *p < 0.05, **p < 0.01.

Table S1.

Clinical data in patients with biopsy proven diabetic kidney disease (n=19).

Variables	
Male, n (%)	10 (52.6%)
Age at biopsy (year)	51.74 ±2.02
Systolic blood pressure (mmHg)	146.4 ±4.70
Diastolic blood pressure (mmHg)	84.79 ±3.13
Serum creatinine (μmol/L)	136.8 ±15.96
Serum Urea (mmol/L)	8.94 ±0.75
Serum albumin (g/L)	29.49 ±1.74
Fasting blood glucose (mmol/L)	7.96 ±0.68
Estimated glomerular filtration rate ml/ min/ 1.73m^2	45.02 ±4.24
Urinary protein excretion (g/24h)	3.73 ±0.57