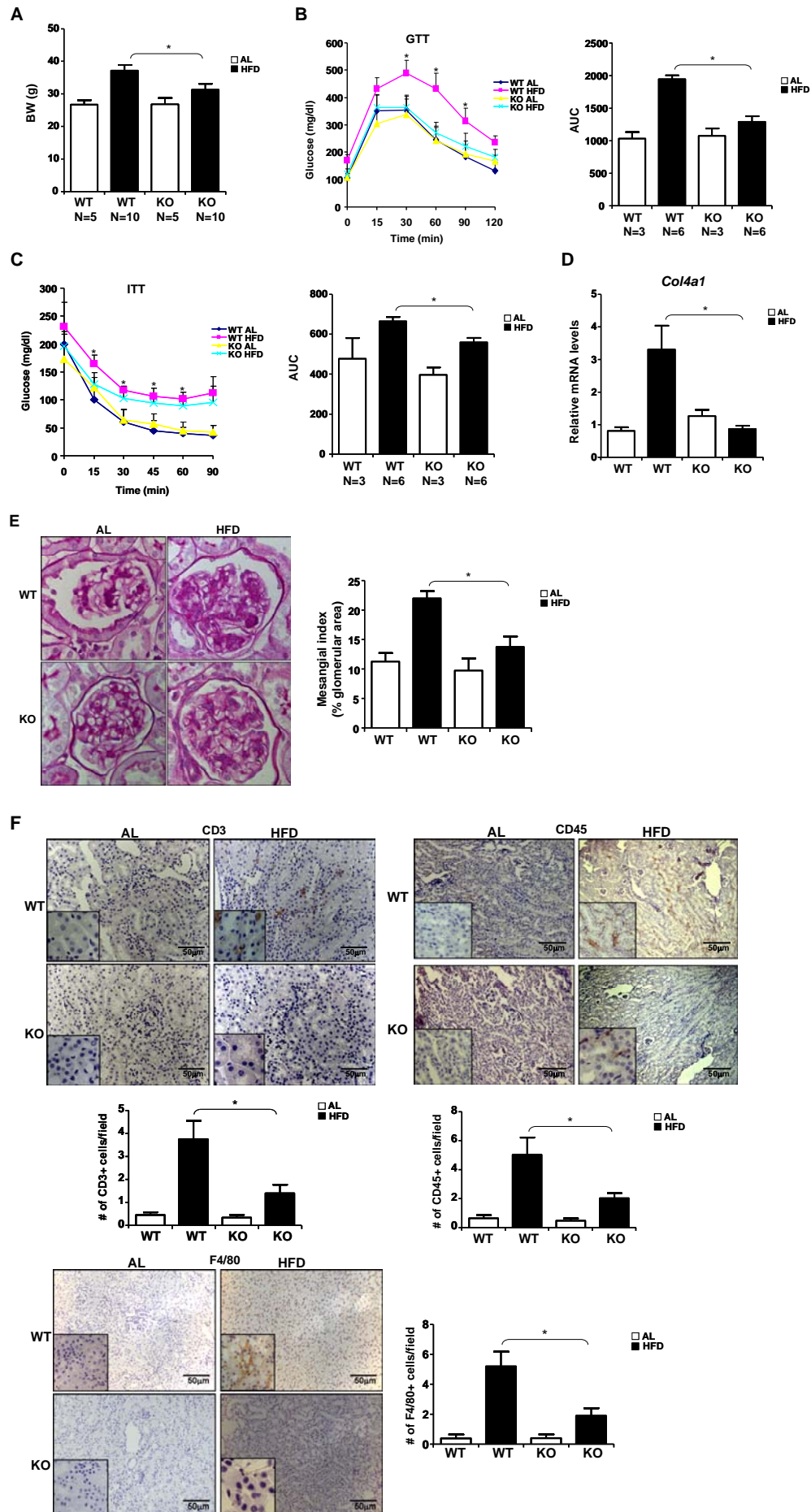**Xu HH *et al*: MRTF-A epigenetically regulates renal fibrosis in diabetic nephropathy**
Supplemental material
Supplemental figures: 15
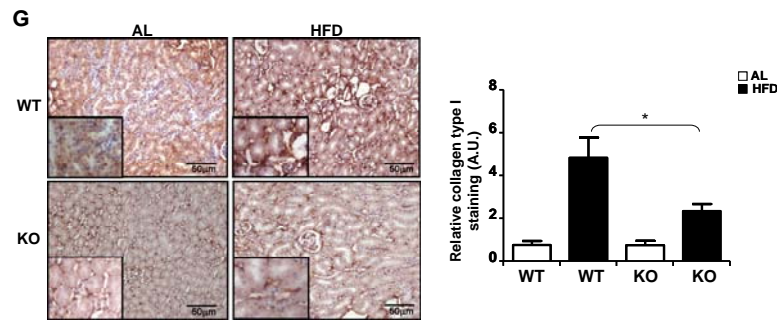**Supplemental table: 1**

A

BW (g)

WT N=5 | WT N=10 | KO N=5 | KO N=10

□ AL
■ HFD

B

GTT

Glucose (mg/dl) vs Time (min)

WT AL
WT HFD
KO AL
KO HFD

AUC

WT N=3 | WT N=6 | KO N=3 | KO N=6

□ AL
■ HFD

C

ITT

Glucose (mg/dl) vs Time (min)

WT AL
WT HFD
KO AL
KO HFD

AUC

WT N=3 | WT N=6 | KO N=3 | KO N=6

□ AL
■ HFD

D

Col4a1

Relative mRNA levels

WT | WT | KO | KO

□ AL
■ HFD

E

AL   HFD

WT

KO

Mesangial index (% glomerular area)

WT | WT | KO | KO

□ AL
■ HFD

F

CD3
AL   HFD

WT

KO

50µm

CD45
AL   HFD

WT

KO

50µm

# of CD3+ cells/field

WT | WT | KO | KO

□ AL
■ HFD

# of CD45+ cells/field

WT | WT | KO | KO

□ AL
■ HFD

F4/80
AL   HFD

WT

KO

50µm

# of F4/80+ cells/field

WT | WT | KO | KO

□ AL
■ HFD

Fig.S1: Wild type (WT) or MRTF-A deficient (KO) mice were fed with a high-fat diet (HFD) or a control diet (AL) for 16 weeks. (**A**) Body weight was measured before the mice were sacrificed for histology. N=5-10 mice for each group. (**B, C**) Glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed as described under *Methods*. N=3-6 mice for each group (**D**) Expression of Col4a1 was assessed by qPCR. N=4 mice for each group. (**E**) Paraffin-embedded kidney sections were stained with periodic acid-Schiff (PAS). Mesangial index was calculated by dividing PAS-positive area by total glomerular area. N=4 mice for each group (**F**) Immunohistochemistry was performed with anti-CD3, anti-F4/80, or anti-CD45. N=4 mice for each group. (**G**) Immunohistochemistry was performed with anti-collagen type I. N=4 mice for each group.

A ITT

B

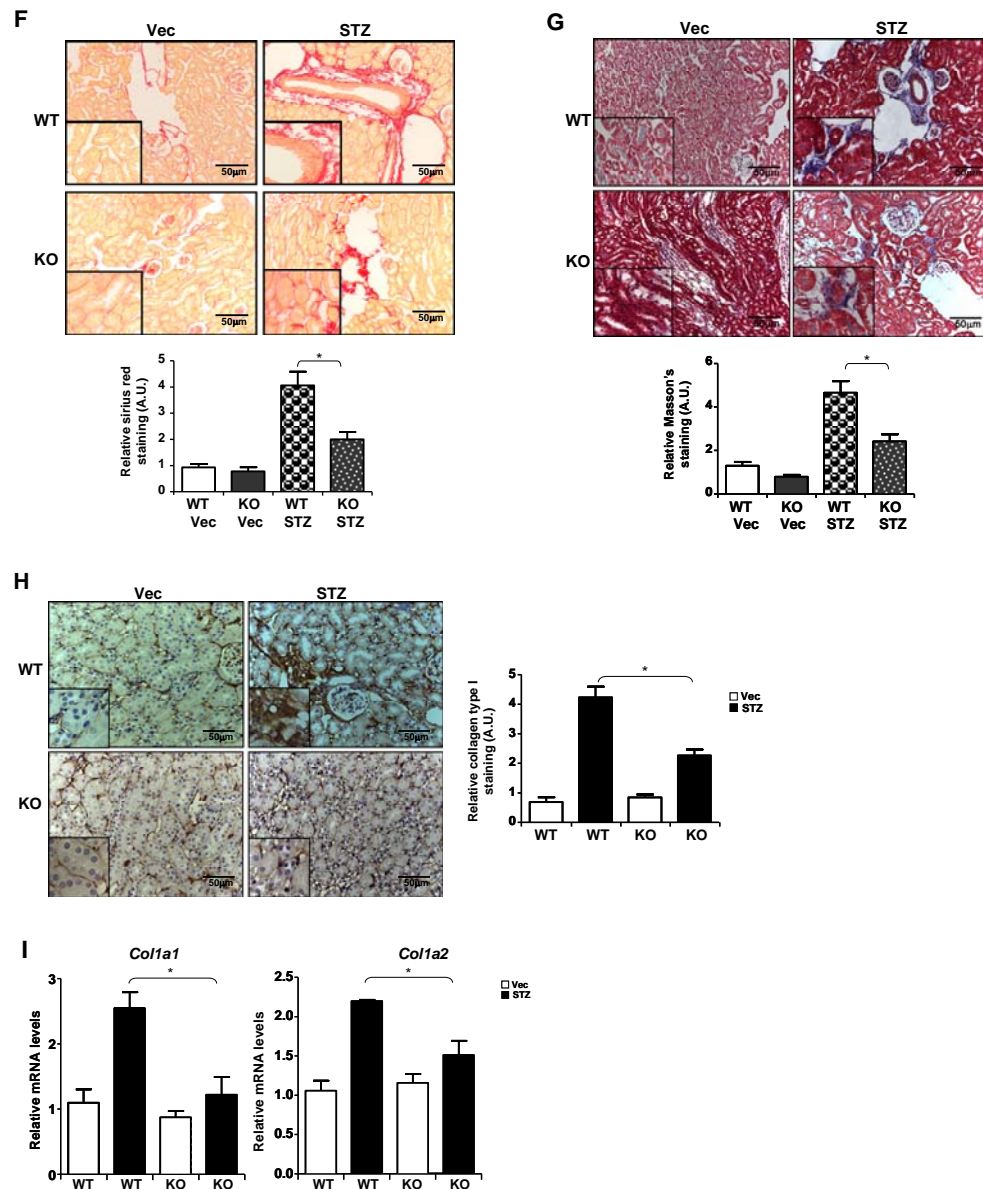**C** *Col4a1*

D

E CD3   CD45   F4/80
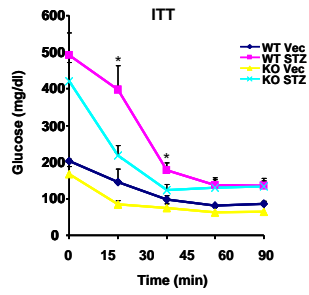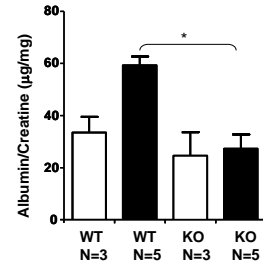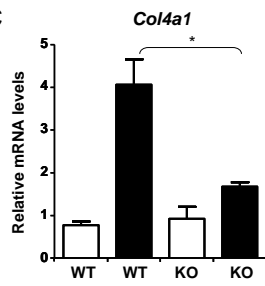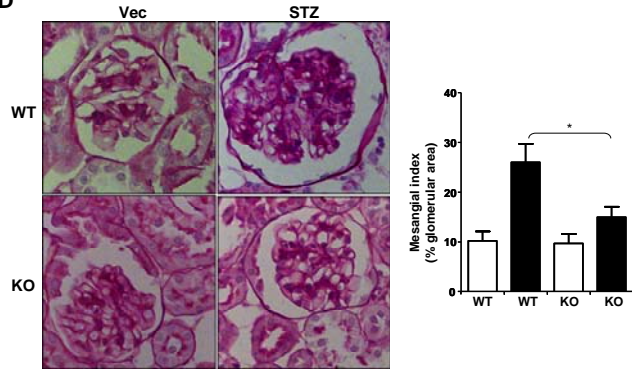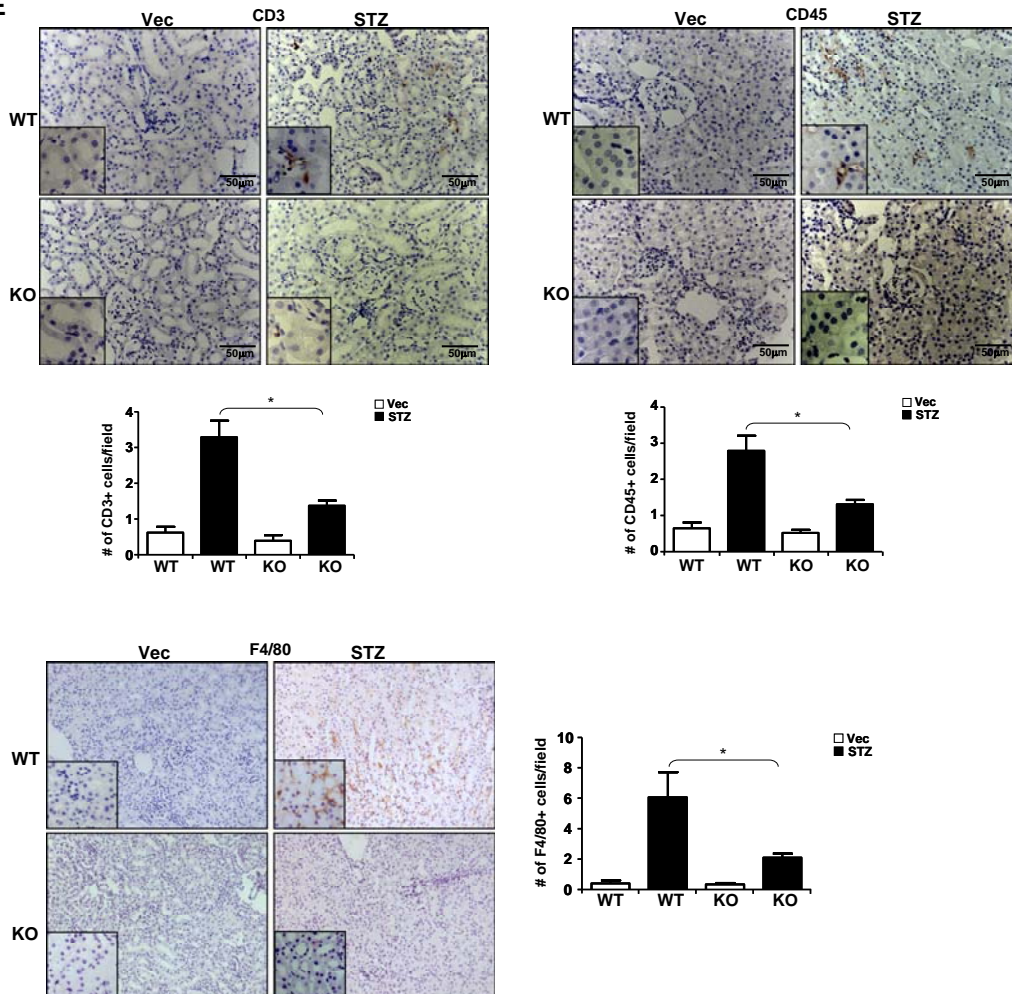
Fig.S2: Wild type (WT) or MRTF-A deficient (KO) mice were injected with STZ or vehicle (Vec) and sacrificed after 16 weeks. (**A**) ITT was performed as described under *Methods*. N=3-5 mice for each group (**B**) Urinary albumin excretion was measured as described under *Methods*. N=3-5 mice for each group. (**C**) Expression of Col4a1 was assessed by qPCR. N=4 mice for each group. (**D**) Paraffin-embedded kidney sections were stained with periodic acid-Schiff (PAS). (**E**) Immunohistochemistry was performed with anti-CD3, anti-F4/80, or anti-CD45. N=3-6 mice for each group. (**F, G**) Renal fibrosis was evaluated by picrosirius red and Masson's trichrome stainings and quantified by Image Pro. N=3 mice for each group. (**H**) Immunohistochemistry was performed with anti-collagen type I. N=3 mice for each group. (**I**) Expression of type I collagen in the kidneys was examined by qPCR. N=5 mice for each group.
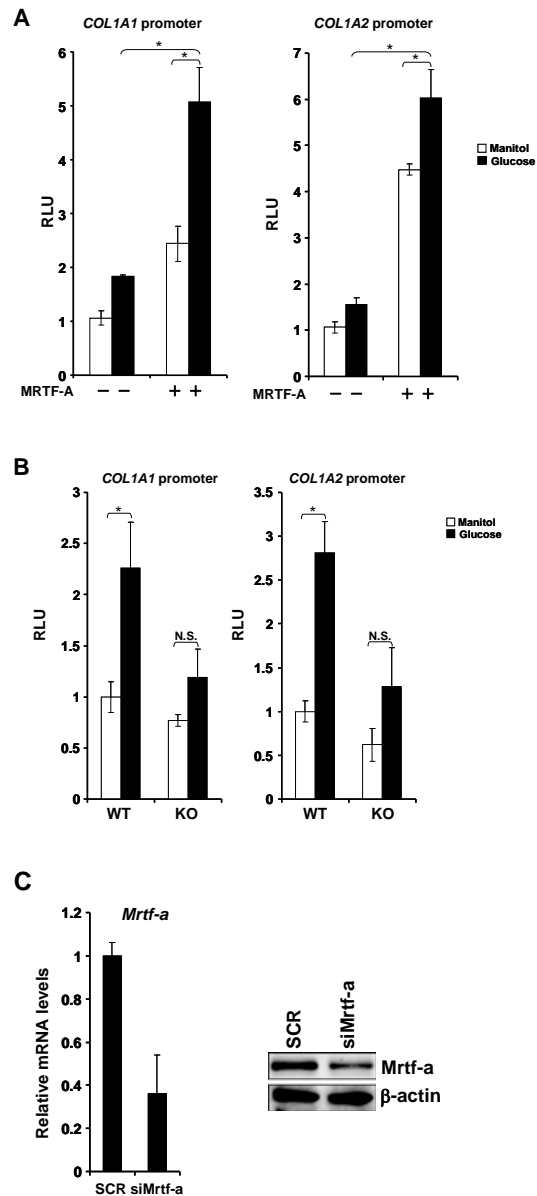
Fig.S3: (**A**) Collagen promoter luciferase constructs were transfected into HK-2 cells with or without MRTF-A followed by treatment with glucose. Data are expressed as relative luciferase unit (RLU). (**B**) Collagen promoter luciferase constructs were transfected into wild type (WT) or MRTF-A deficient (KO) MEF cells followed by treatment with glucose. Data are expressed as relative luciferase unit (RLU). N.S., no statistical significance (**C**) NRK-52E was transfected with MRTF-A siRNA or scrambled siRNA. MRTF-A expression was measured by qPCR and Western.

| | | Fold Change (comparing to control group) | |
| | | Group 1 | |
Position	Symbol	Fold Change	Comments
A01	Acta2	0.2864	OKAY
A02	Agt	3.8593	B
A03	Akt1	1.5762	OKAY
A04	Bcl2	0.9816	OKAY
A05	Bmp7	1.2669	OKAY
A06	Cav1	0.8633	OKAY
A07	Ccl11	2.6656	B
A08	Ccl12	1.8431	OKAY
A09	Ccl3	1.4774	OKAY
A10	Ccr2	6.6572	A
A11	Cebpb	3.0664	OKAY
A12	Col1a2	0.4331	OKAY
B01	Col3a1	0.2618	OKAY
B02	Ctgf	0.8564	OKAY
B03	Cxcr4	0.8786	OKAY
B04	Dcn	0.6535	OKAY
B05	Edn1	0.6886	OKAY
B06	Egf	1.6027	A
B07	Eng	0.4585	OKAY
B08	Fasl	0.9634	C
B09	Grem1	1.2652	OKAY
B10	Hgf	2.5321	OKAY
B11	Ifng	0.2199	B
B12	Il10	0.1087	B
C01	Il13	5.326	B
C02	Il13ra2	0.4202	B
C03	Il1a	1.4942	OKAY
C04	Il1b	3.2264	OKAY
C05	Il4	0.3217	B
C06	Il5	0.8752	B
C07	Ilk	0.8369	OKAY
C08	Inhbe	0.3868	B
C09	Itga1	1.0664	OKAY
C10	Itga2	0.8484	OKAY
C11	Itga3	1.3354	OKAY
C12	Itgav	1.1209	OKAY
D01	Itgb1	1.1793	OKAY
D02	Itgb3	0.8077	OKAY
D03	Itgb5	1.4687	OKAY
D04	Itgb6	1.1381	OKAY
D05	Itgb8	1.4247	OKAY
D06	Jun	1.3419	OKAY
D07	Lox	0.5164	OKAY
D08	Ltbp1	0.7853	OKAY
D09	Mmp13	1.5288	OKAY
D10	Mmp14	1.2718	OKAY
D11	Mmp1a	2.5493	OKAY
D12	Mmp2	1.6445	OKAY
E01	Mmp3	0.4699	A
E02	Mmp8	0.3823	OKAY
E03	Mmp9	0.5309	OKAY
E04	Myc	0.9717	OKAY
E05	Nfkb1	1.2248	OKAY
E06	Pdgfa	0.7345	OKAY
E07	Pdgfb	1.1305	OKAY
E08	Plat	1.376	OKAY
E09	Plau	1.6146	OKAY
E10	Plg	0.9634	C
E11	Serpina1a	0.9634	C
E12	Serpine1	1.0722	OKAY
F01	Serpinh1	1.2828	OKAY
F02	Smad2	1.2608	OKAY
F03	Smad3	1.6185	OKAY
F04	Smad4	1.1698	OKAY
F05	Smad6	1.883	OKAY
F06	Smad7	1.5073	OKAY
F07	Snai1	1.6818	B
F08	Sp1	1.4902	OKAY
F09	Stat1	1.7463	OKAY
F10	Stat6	1.3604	OKAY
F11	Tgfb1	1.7116	OKAY
F12	Tgfb2	1.1421	OKAY
G01	Tgfb3	1.1346	OKAY
G02	Tgfbr1	1.502	OKAY
G03	Tgfbr2	1.5594	OKAY
G04	Tgif1	0.9524	OKAY
G05	Thbs1	1.2435	OKAY
G06	Thbs2	1.13	OKAY
G07	Timp1	0.3729	OKAY
G08	Timp2	0.641	OKAY
G09	Timp3	0.9255	OKAY
G10	Timp4	0.9634	C
G11	Tnf	1.9686	OKAY
G12	Vegfa	0.9916	OKAY
H01	Actb	1.0929	OKAY
H02	B2m	0.893	OKAY
H03	Gapdh	1.4623	OKAY
H04	Gusb	1.0247	OKAY
H05	Hsp90ab1	1.1973	OKAY
H06	MGDC	0.9634	C
H07	RTC	1.0292	OKAY
H08	RTC	1.0745	OKAY
H09	RTC	1.1236	OKAY
H10	PPC	0.8813	OKAY
H11	PPC	0.874	OKAY
H12	PPC	0.9256	OKAY

Comments:

A: This gene's average threshold cycle is relatively high (> 30) in either the control or the test sample, and is reasonably low in the other sample (< 30).
These data mean that the gene's expression is relatively low in one sample and reasonably detected in the other sample suggesting that the actual fold-change value is at least as large as the calculated and reported fold-change result.
This fold-change result may also have greater variations if p value > 0.05; therefore, it is important to have a sufficient number of biological replicates to validate the result for this gene.

B: This gene's average threshold cycle is relatively high (> 30), meaning that its relative expression level is low, in both control and test samples, and the p-value for the fold-change is either unavailable or relatively high (p > 0.05).
This fold-change result may also have greater variations; therefore, it is important to have a sufficient number of biological replicates to validate the result for this gene.

C: This gene's average threshold cycle is either not determined or greater than the defined cut-off value (default 35), in both samples meaning that its expression was undetected, making this fold-change result erroneous and un-interpretable.

Fig.S4: Identification of novel MRTF-A target genes by PCR array. Mouse fibrosis PCR array was performed as described under *Methods* with RNA prepared from MRTF-A deficient renal tubular epithelial cells treated with high glucose (Group 1) or wild type RTEs treated with high glucose (control group).
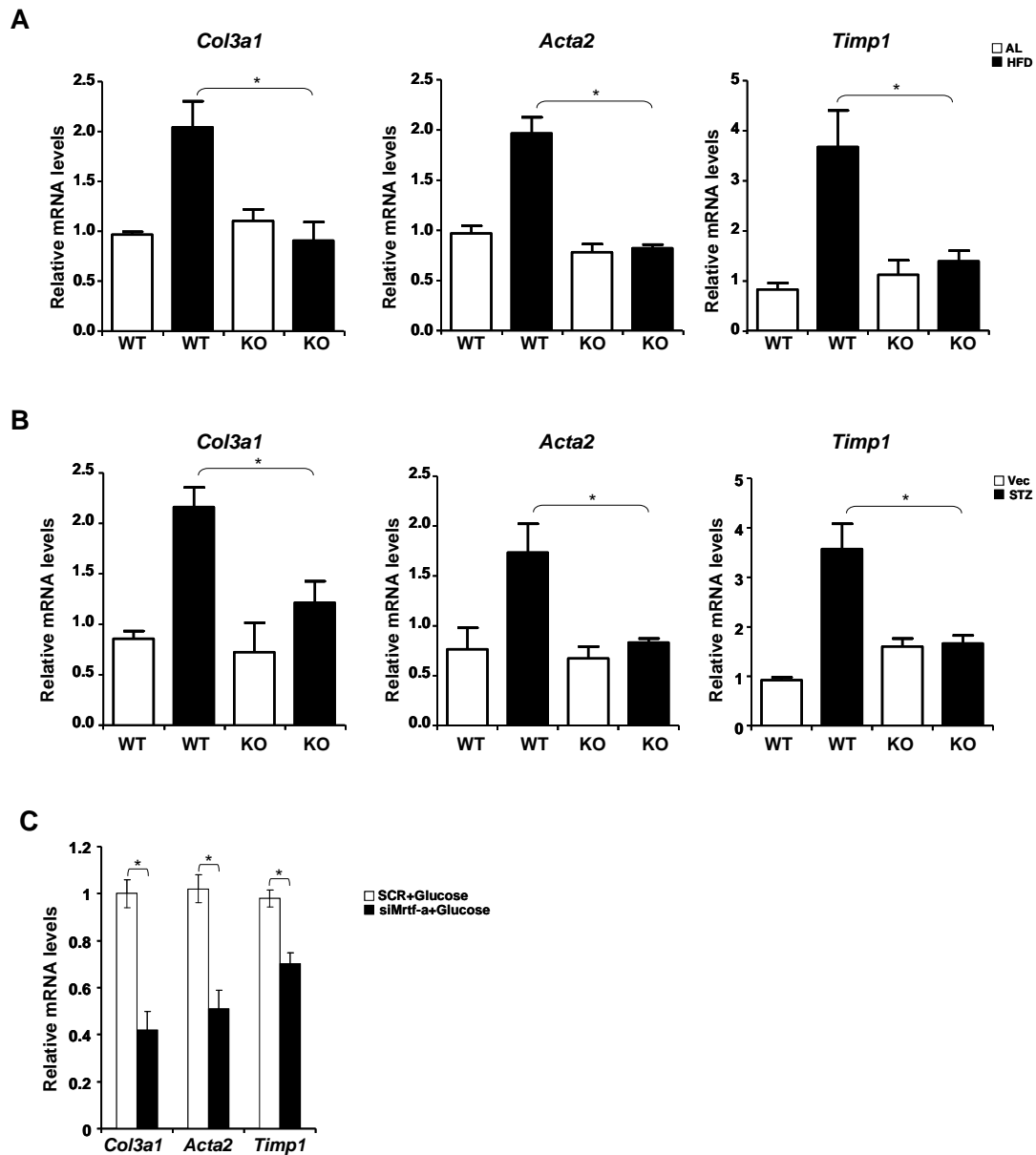
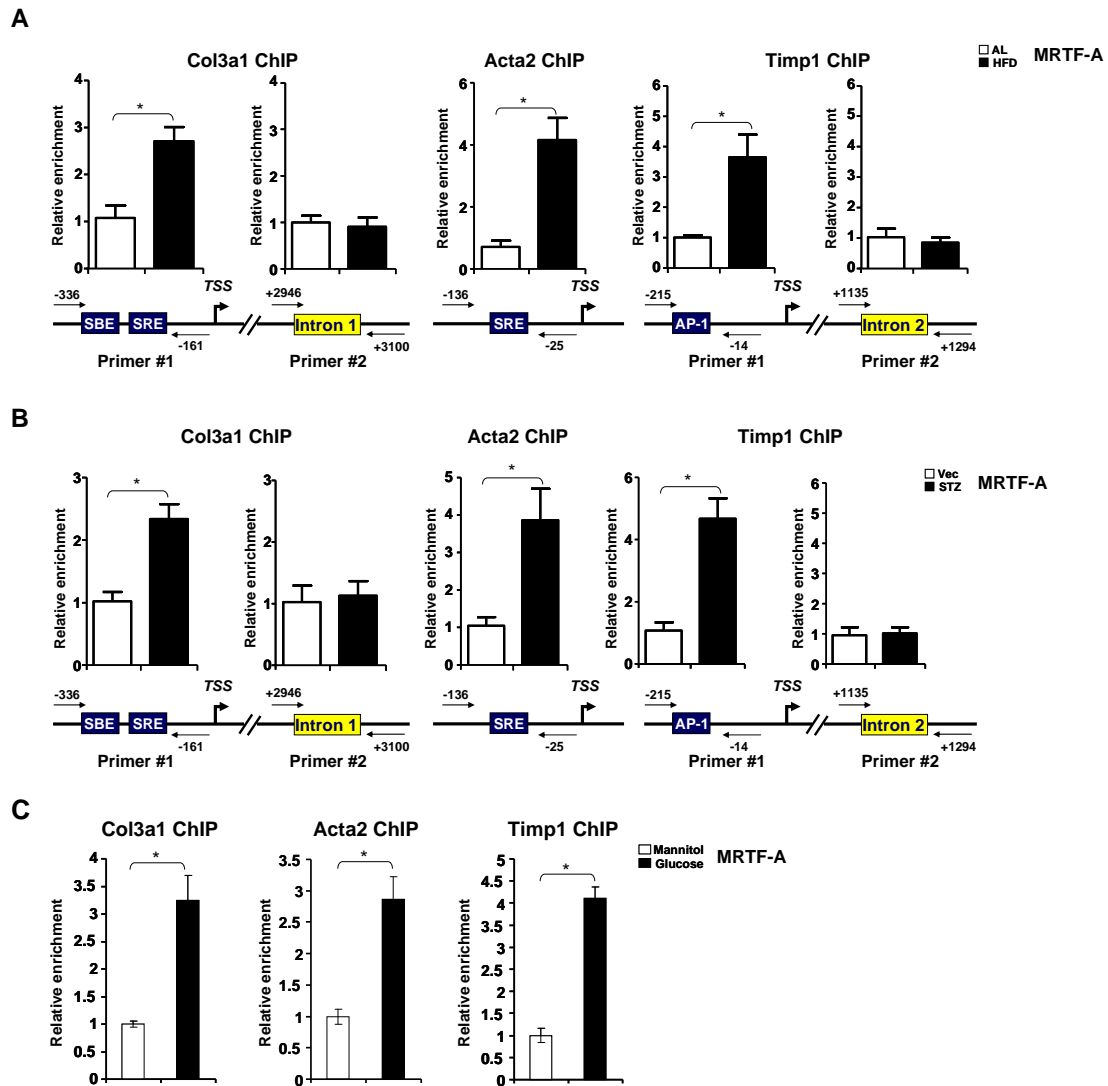**Fig.S5:** Validation of MRTF-A target genes *in vivo* and *in vitro*. (**A**) Wild type (WT) or MRTF-A deficient (KO) mice were induced to develop diabetic nephropathy by high-fat diet (HFD). Expression of *Col1a3*, *Acta2*, and *Timp1* in the kidneys was examined by qPCR. N=5 mice for each group. (**B**) Wild type (WT) or MRTF-A deficient (KO) mice were induced to develop diabetic nephropathy by STZ injection. Expression of *Col1a3*, *Acta2*, and *Timp1* in the kidneys was examined by qPCR. N=5 mice for each group. (**C**) NRK-52E cells were transfected with indicated siRNAs followed by treatment with glucose. Expression of *Col1a3*, *Acta2*, and *Timp1* in the kidneys was examined by qPCR.

Fig.S6: Direct binding of MRTF-A to new target genes *in vivo* and *in vitro*. (**A**) Wild type (WT) or MRTF-A deficient (KO) mice were induced to develop diabetic nephropathy by high-fat diet (HFD). ChIP assays were performed using kidney lysates with anti-MRTF-A. SBE, Smad-binding element; SRE, serum response element/CArG box. N=3 mice for each group. (**B**) Wild type (WT) or MRTF-A deficient (KO) mice were induced to develop diabetic nephropathy by STZ injection. ChIP assays were performed using kidney lysates with anti-MRTF-A. N=3 mice for each group. (**C**) NRK-52E cells were treated with glucose or mannitol for 24 hours. ChIP assays were performed using kidney lysates with anti-MRTF-A.
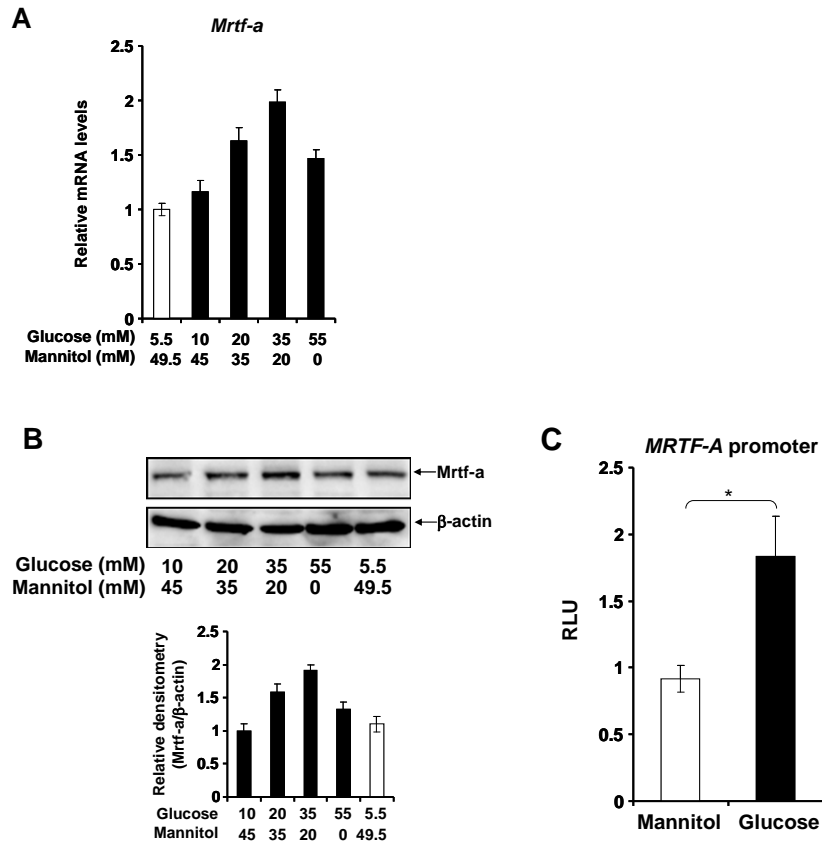
Fig.S7: (**A, B**) NRK-52E cells were treated with glucose of indicated concentrations for 24 hours. Expression of MRTF-A was measured by qPCR (A) and Western (B). (**C**) A MRTF-A promoter-luciferase fusion construct was transfected into NRK-52E cells followed by treatment with glucose or mannitol.
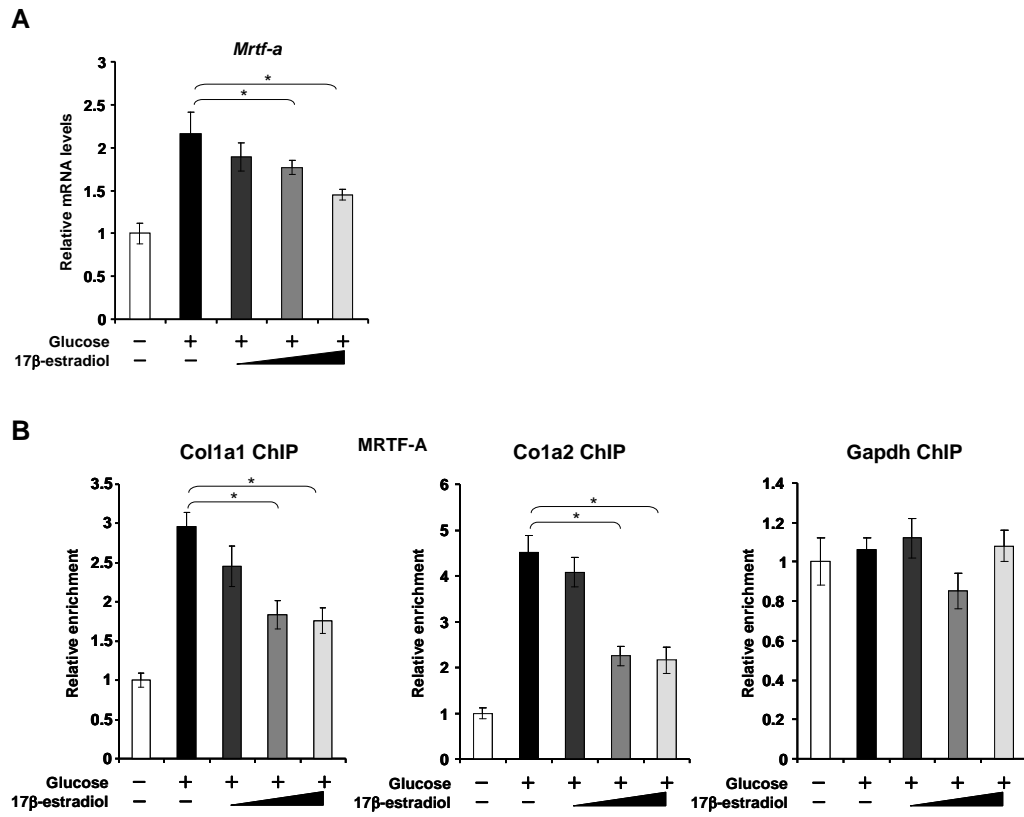
Fig.S8: (**A**) NRK-52E cells were treated with glucose (35mM) in the presence or absence of 17β-estradiol (10^{-7}-10^{-9}M) for 24 hours. Expression of MRTF-A was measured by qPCR. (**B**) NRK-52E cells were treated with glucose (35mM) in the presence or absence of 17β-estradiol (10^{-7}-10^{-9}M) for 24 hours. ChIP assays were performed with anti-MRTF-A.
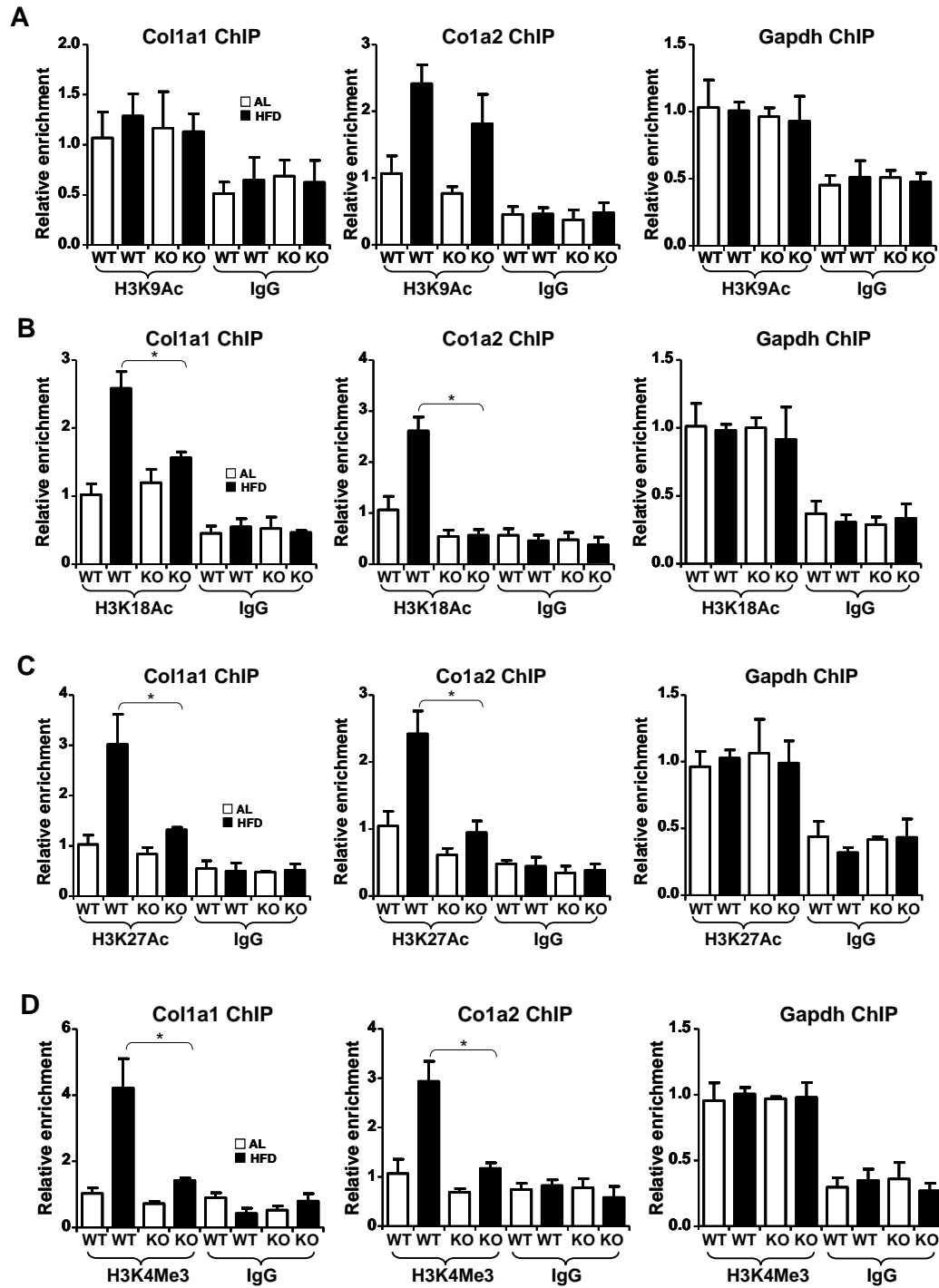
Fig.S9: (**A-D**) WT or KO mice were induced to develop diabetic nephropathy by HFD. ChIP assays were performed using kidney lysates with anti-H3K9 (A), anti-H3K18 (B), anti-H3K27 (C), and anti-H3K4Me3 (D). N=3 mice for each group
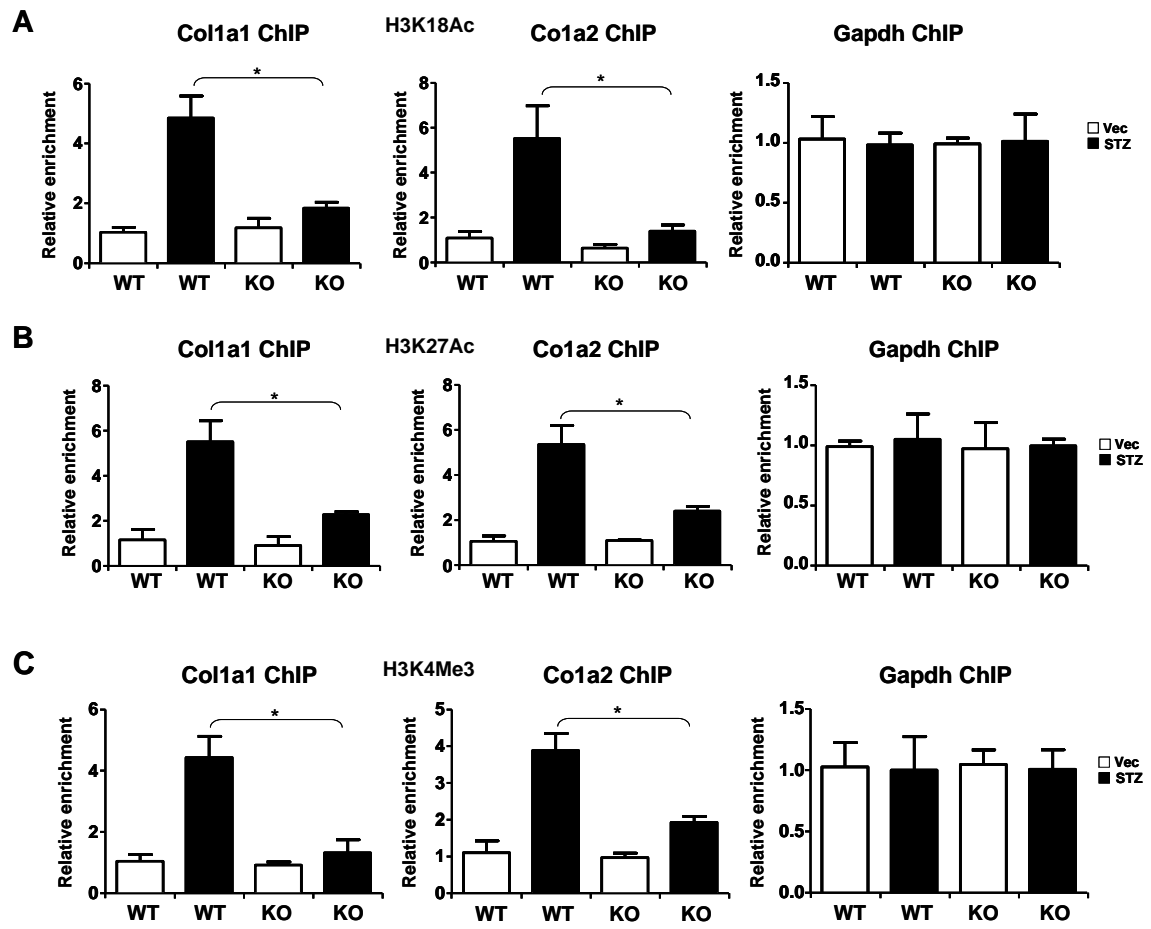
Fig.S10: (**A-C**) WT or KO mice were induced to develop diabetic nephropathy by STZ injection. ChIP assays were performed using kidney lysates with anti-H3K18 (A), anti-H3K27 (B), and anti-H3K4Me3 (C). N=3 mice for each group
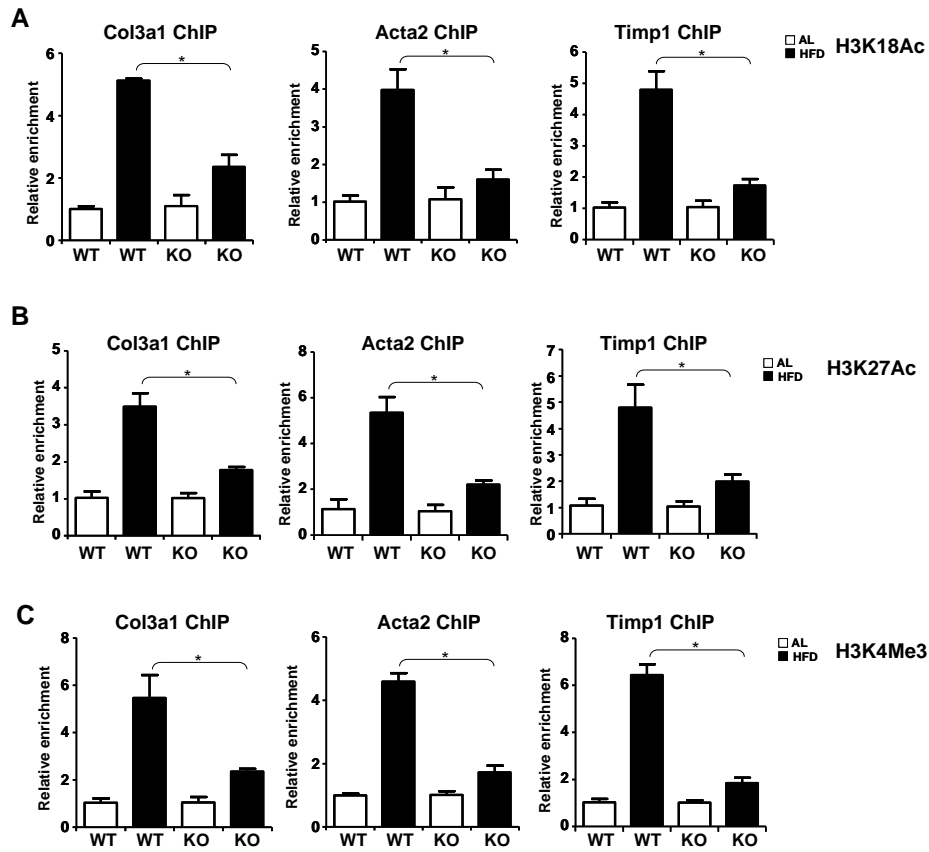
Fig.S11: (**A-C**) WT or KO mice were induced to develop diabetic nephropathy by HFD. ChIP assays were performed using kidney lysates with anti-H3K18 (A), anti-H3K27 (B), and anti-H3K4Me3 (C). N=3 mice for each group
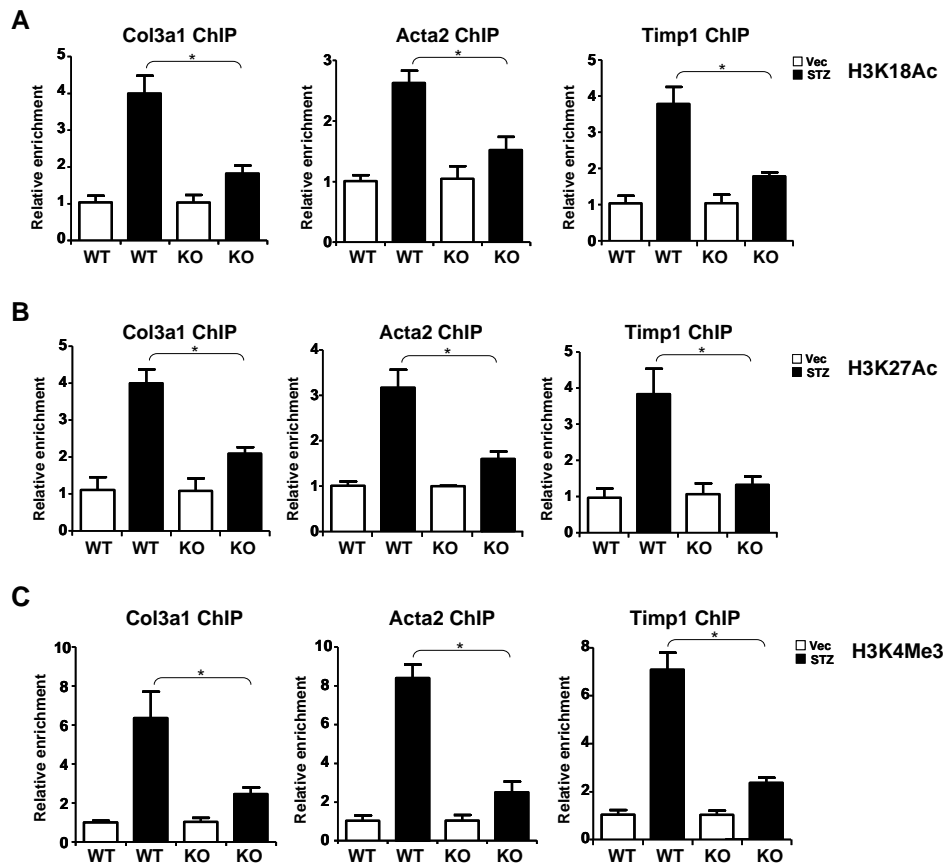
Fig.S12: (**A-C**) WT or KO mice were induced to develop diabetic nephropathy by STZ injection. ChIP assays were performed using kidney lysates with anti-H3K18 (A), anti-H3K27 (B), and anti-H3K4Me3 (C). N=3 mice for each group
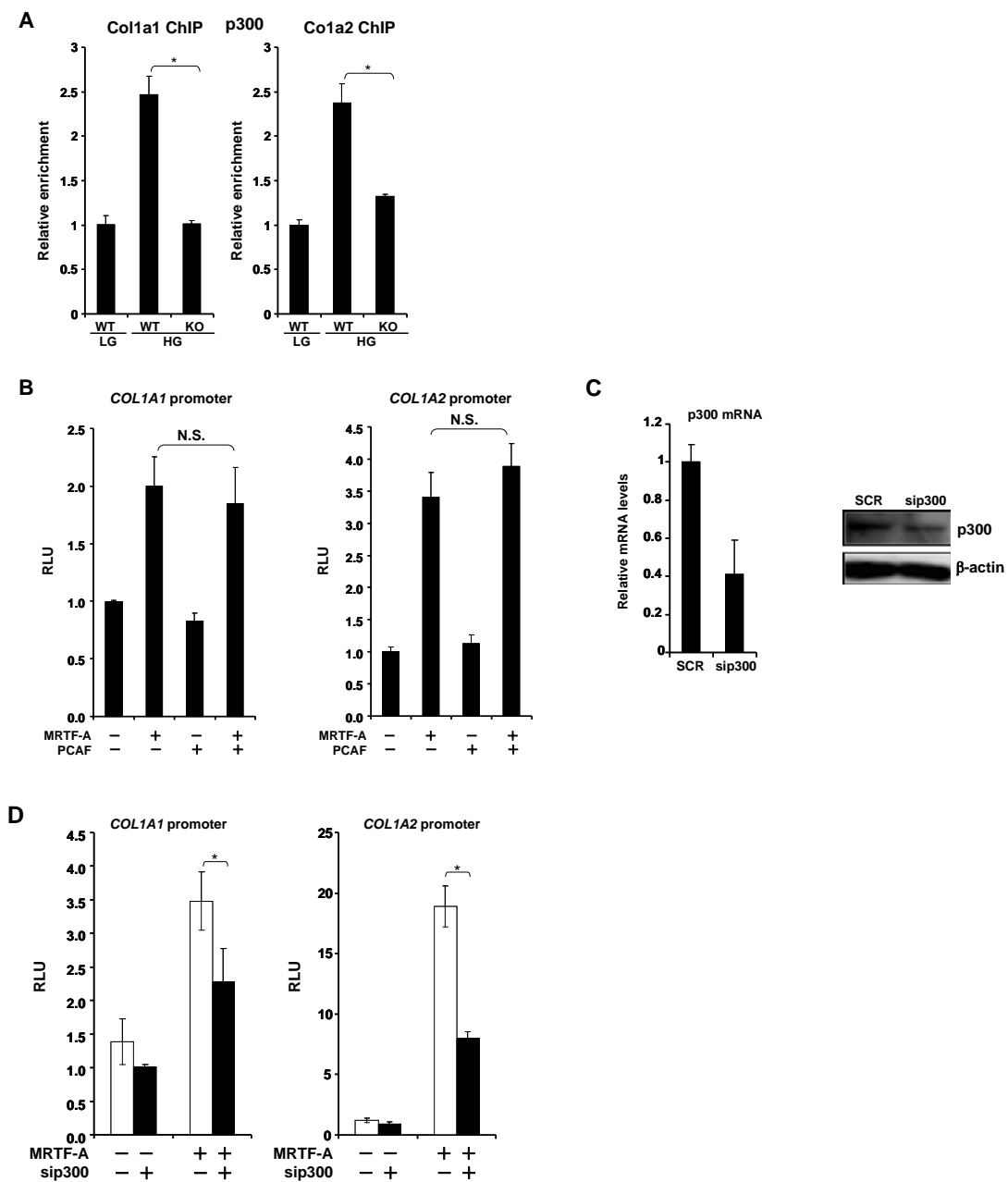
Fig.S13: (**A**) Primary renal tubular epithelial cells were isolated from WT or KO mice and treated with glucose. ChIP assays were performed with anti-p300. (**B**) Collagen promoter luciferase constructs were transfected into NRK-52E cells with indicated expression constructs. (**C**) NRK-52E was transfected with p300 siRNA or scrambled siRNA. p300 expression was measured by qPCR and Western. (**D**) Collagen promoter luciferase constructs were transfected into NRK-52E cells with indicated expression constructs and siRNAs.
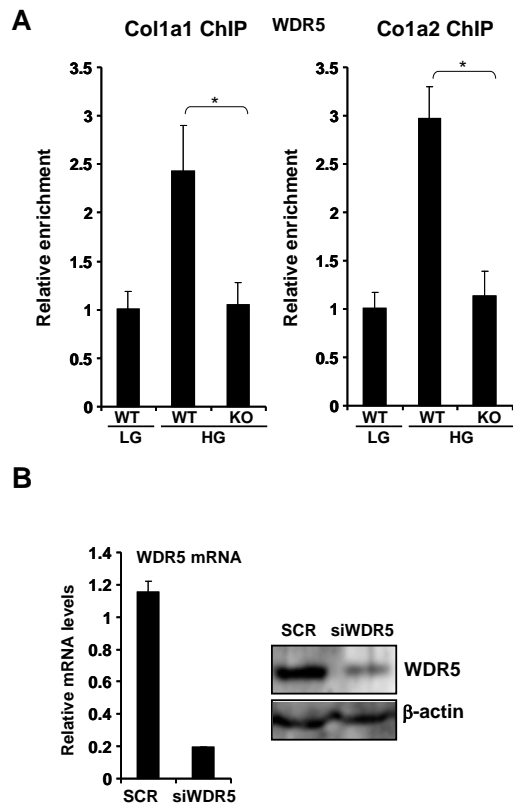
Fig.S14: (**A**) Primary renal tubular epithelial cells were isolated from WT or KO mice and treated with glucose. ChIP assays were performed with anti-p300. (**B**) NRK-52E was transfected with WDR5 siRNA or scrambled siRNA. WDR5 expression was measured by qPCR and Western.
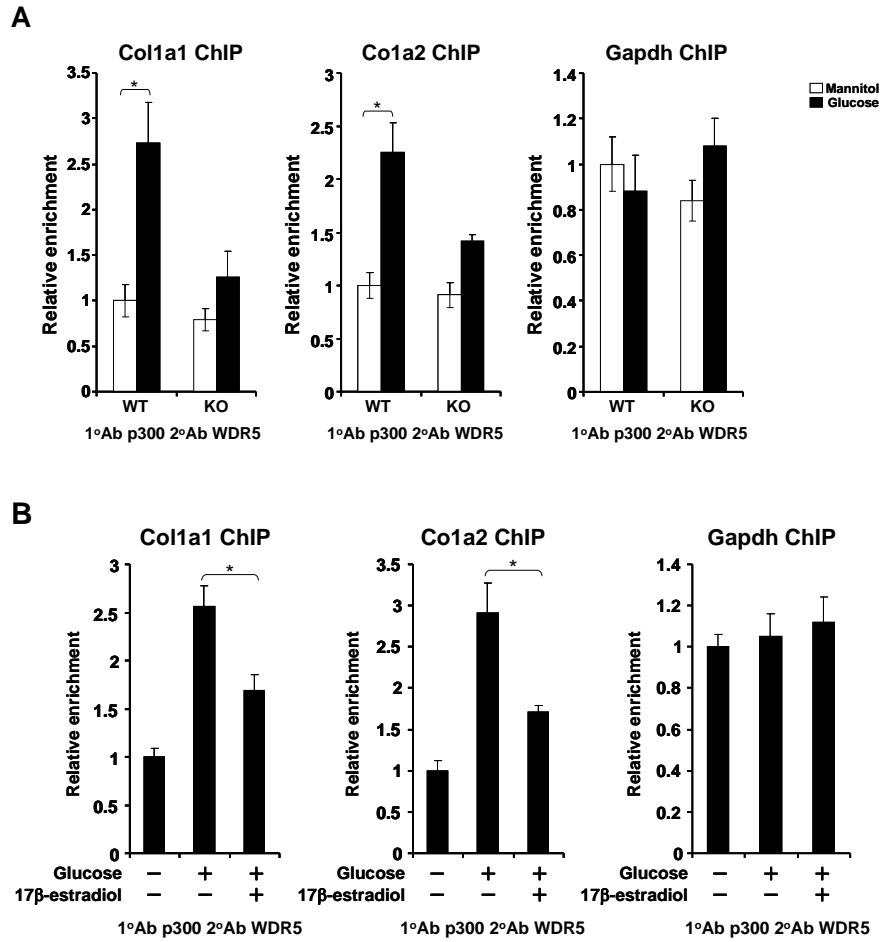
Fig.S15: (**A**) Primary renal tubular epithelial cells were isolated from WT or KO mice and treated with glucose or mannitol. Re-ChIP assays were performed with indicated antibodies. (**B**) NRK-52E cells were treated with glucose (35mM) in the presence or absence of 17β-estradiol (10^{-7}M) for 24 hours. Re-ChIP assays were performed with indicated antibodies.

Table I: ChIP Real-time qPCR primers

Gene name	Primer sequences
Mouse Col1a1	Forward: 5'- ATTTGAAGTCCCAGAAAG -3'
	Reverse: 5'- AGAAACTCCCGTCTGCTC -3'
Mouse Col1a2	Forward: 5'-CTTCGTGCATGACTTCAGCTTT-3'
	Reverse: 5'-CGTCCTTTAGCATGGCAAGAC-3'
Mouse Col1a3 #1	Forward: 5'- GACTCTGGCAAAACTCAAAGTATCA-3'
	Reverse: 5'- TAGGAATGTGCTTTGTGATAGCCT -3'
Mouse Col1a3 #2	Forward: 5'- AGACCTTCATTCCCAGCTACTTG-3'
	Reverse: 5'- CTCTCTACCACTGACCTGCATCTC -3'
Mouse Acta2	Forward: 5'-AGCAGAACAGAGGAATGCAGTGGA AGA GAC-3'
	Reverse: 5'-CCTCCCACTCGCCTC CCA AACAAGGAGC-3'
Mouse Timp1 #1	Forward: 5'- AGGACTGTGCATGACGTGGAG-3'
	Reverse: 5'- ACAGTGGAGAATAAATGTCCATGC -3'
Mouse Timp1 #2	Forward: 5'- TGTGGTCAAGCAAAGCATCTG-3'
	Reverse: 5'- TGGGTTTGTAGCTCAATTGTGC -3'
Rat Col1a1	Forward: 5'- ATCCTTCTGATTTGAGGTC -3'
	Reverse: 5'- AGGTGAAACTCCCGTCTG -3'
Rat Col1a2	Forward: 5'-GACATGCTCAAGTGCTGAGTCAC-3'
	Reverse: 5'-AGATTGCACAATGTGACGTCG-3'
Rat Col3a1	Forward: 5'- ATCCTTCTGATTTGAGGTC -3'
	Reverse: 5'- AGGTGAAACTCCCGTCTG -3'
Rat Timp1	Forward: 5'-CTCTGCCACCCCTCACCA-3'
	Reverse: 5'-GGACTGGATGGGCCTCGT-3'
Rat Acta2	Forward: 5'- CATGCACGTGGACTGTACCT -3'
	Reverse: 5'- AAAGATGCTTGGGTCACCTG -3'
Rat Gapdh	Forward: 5'- ATCACTGCCACCCAGAAGACTGTGGA -3'
	Reverse: 5'-C TCATACCAGGAAATGAGCTTGACAAA -3'