## TABLE S1: Characteristics of non-diabetic and STZ-induced diabetic C3H and Hcb-19 mice

| Characteristics | C3H wildtype(WT) | | Hcb-19 (TxNIP mutant) | | p-value |
|---|---|---|---|---|---|
| | Non-diabetic | Diabetic | Non-diabetic | Diabetic | |
| Glycemia (mmol/L) | 8.3±0.7 | 33.3±0*** | 5.4±1.1*** | 33.1±0.5$^{\#\#\#}$ | ***p<0.0001 vs WT non Diabetic,$^{\#\#\#}$p<0.0001 vs Hcb19 non-diabetic |
| Body weight (g) | 21±1.5 | 20±0.7 | 32±1.5*** | 21±2.1$^{\#\#\#}$ | ***p<0.0001 vs WT non Diabetic,$^{\#\#\#}$p<0.0001 vs Hcb19 non-diabetic |
| Left kidney weight (mg) | 191±28.3 | 234±23.4 | 303±20.6*** | 256±35.1$^{\#\#}$ | ***p<0.0001 vs WT non Diabetic,$^{\#\#}$p<0.001 vs Hcb19 non-diabetic |
| Left kidney weight/ body weight | 9.4±0.7 | 12.5±1.8* | 9.4±0.2 | 13.3±0.9$^{\#\#\#}$ | *p<0.01 vs WT non Diabetic,$^{\#\#\#}$p<0.0001 vs Hcb19 non-diabetic |
| Albumin/ Creatinine ratio (µg/mg) | 28.4±16.8 | 384.8±184.4*** | 212.9±83.4*** | 217.5±84.9*** | ***p<0.0001 vs WT non Diabetic |

Results are expressed as mean±SD

**FIGURE S1. Renal parameters of STZ-diabetic and non-diabetic TxNIP$^{+/+}$ (WT), TxNIP$^{-/-}$ (KO) and TxNIP$^{+/-}$ (HET).** Assays were performed as described in Methods after 24 weeks of diabetes. (A) 24h urine albumin excretion (µg), (B) urine albumin/creatinine ratio (µg/mg), (C) 24h proteinuria (µg) and (D) Serum Cystatin C (pg/mL). Results are expressed as mean± SE (n= 6-7 mice/condition). $^{*}p<0.05$ diabetic WT vs non-diabetic WT, diabetic KO and diabetic HET, $^{**}p<0.01$ diabetic WT vs non-diabetic WT and diabetic KO, and $^{***}p<0.001$ diabetic WT vs non-diabetic WT.

**FIGURE S2. TxNIP mRNA content of renal cortex.** TxNIP$^{+/+}$ (WT), TxNIP $^{-/-}$ (KO), and TxNIP $^{+/-}$ (HET) mice were rendered diabetic or not with STZ as in Methods. Renal cortex TxNIP mRNA was determined by real time RT-PCR and corrected for *actb* and normalized to nondiabetic WT. Results are mean± SE from 3-5 mice/condition. $^{**}p<0.01$ diabetic HET vs non-diabetic WT and diabetic KO, $^{***}p<0.001$ diabetic WT vs non-diabetic WT and diabetic KO, $^{\#\#}p<0.01$ diabetic HET vs non-diabetic HET.

**FIGURE S3. Mesangial matrix expansion, glomerular fibrosis, Nox4 expression and Electron Micrographs from nondiabetic and STZ-induced diabetic C3H and Hcb-19 mice.** (A) Representative images of Periodic Acid Schiff (PAS) stained glomeruli. (B) Quantitation of mesangial matrix expansion from PAS stained sections as described in Methods. (C) Collagen IV IHC. (D) Quantitation of collagen IV as percentage of glomerular area. (E) TGFβ1 staining. (F) Quantitation of TGFβ1. (G) Nox4 IHC in glomeruli. (H) Quantitation of Nox4. Results are expressed as mean ± SD, n=4-5mice/condition. $^{*}p<0.05$ diabetic C3H vs nondiabetic C3H and nondiabetic Hcb19 vs nondiabetic C3H. $\#p<0.01$ diabetic C3H vs diabetic Hcb-19. (I) Representative electron micrographs (magnification x 15,000) of glomeruli from non-diabetic and diabetic C3H and Hcb-19 mice where arrows= slit diaphragm, *FP= foot process and GBM=glomerular basement membrane.

**FIGURE S4. Histopathological markers of renal fibrosis in STZ-diabetic and non-diabetic TxNIP $^{+/+}$ (WT), TxNIP $^{-/-}$ (KO) and TxNIP $^{+/-}$ (HET) mice**. Representative images of markers of fibrosis in non-diabetic and STZ-diabetic HET mice assessed by collagen IV (*A*, *B*), TGFβ *(C, D)*, and renal interstitial fibrosis (Masson's Trichrome staining) (*E*,*F*). Staining of mesangial matrix was assessed with PAS (*G*, *H*). Quantitation of the staining is shown in the graphs in the bottom panels (*I-L*). HET (n=3 mice/condition), WT and KO (n=5 mice/condition). Results are means ± SE; $^{*}p<0.05$ diabetic WT vs non-diabetic WT and diabetic KO, $^{**}p<0.01$ diabetic WT vs non-diabetic WT and diabetic HET, and $^{***}p<0.001$ diabetic WT vs non-diabetic WT, diabetic KO and diabetic HET.

**FIGURE S5. Expression of nephrin in glomeruli.** *(A)* Representative images of nephrin staining (IHC) in glomeruli from nondiabetic and diabetic WT and TxNIP KO mice. Quantitation is shown in the graphs in the bottom panel (n=4 mice/condition). Results are means ± SE relative to WT designated as 1.0; $^{*}p<0.05$ diabetic WT vs diabetic KO and $^{***}p<0.001$ diabetic WT vs non-diabetic WT.

**FIGURE S6. Morphological EM changes in glomerular basement membrane and podocyte foot process.** (*A)* Representative electron micrographs (magnification approx. x15,000) of non-diabetic and diabetic TxNIP$^{+/-}$ (HET) mice where arrows= slit diaphragm, *FP= foot process and GBM=glomerular basement membrane. (*B*) Quantification of GBM thickness, and (*C*) podocyte foot process effacement

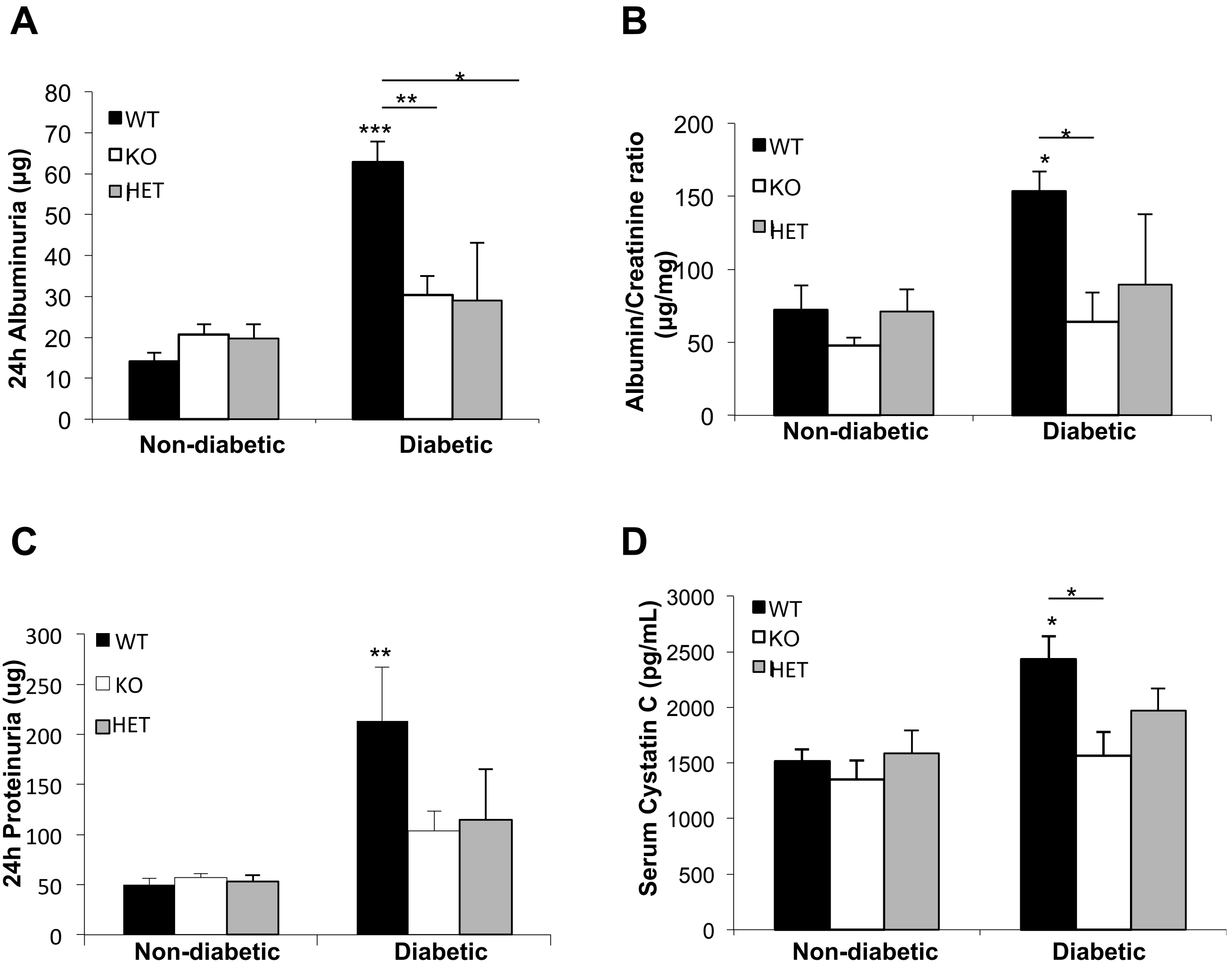
quantified as in Methods from 9 different fields from each of 3 mice/condition. Results are expressed as means± SE. *$p<0.01$ diabetic WT vs non-diabetic WT and diabetic KO, ***$p<0.001$ diabetic WT vs non-diabetic WT, diabetic KO and diabetic HET.

**FIGURE S7. Markers of oxidative and nitrosative stress and Nox4 expression.** Representative images of glomerular nitrosative stress in non-diabetic and diabetic TxNIP$^{+/-}$ HET mice, as indicated by glomerular nitrotyrosine staining (*A*,*B*), and its quantification *(E)* (n=3-5 mice/ condition). Representative images of glomerular Nox4 expression in HET mice (*C,D)* and its quantification (*F*) (n=3-5 mice/condition). Results are means± SE. **$p<0.001$ diabetic WT vs non-diabetic WT and diabetic KO, and ***$p<0.001$ diabetic WT vs non-diabetic WT and diabetic KO, $^{\#}p<0.01$ diabetic HET vs non-diabetic HET, and $^{\$\$}p<0.001$ diabetic HET vs diabetic KO. (*G)* ROS detected as 24h urinary 8-hydroxy-2-deoxy-guanosine levels (pg) from 3-7 mice/condition. *(H*) Expression of *Nox4* mRNA, corrected for the housekeeping gene, *actb,* and normalized to non-diabetic WT in renal cortex. Results are means± SE (n=3-5 mice/condition). *$p<0.05$ diabetic WT vs non-diabetic WT and diabetic KO, **$p<0.01$ diabetic KO vs non-diabetic KO and diabetic WT, ***$p<0.001$ diabetic WT vs non-diabetic WT and diabetic HET, and $^{\#}p<0.01$ diabetic HET vs non-diabetic HET.

**FIGURE S8. Oxidative and nitrosative stress in tubular cells.** *(A*) Representative images of tubular Nox4 and RNS as indicated by nitrotyrosine staining (n=3 mice/ condition). Quantitation of tubular *(B)* Nox4 and *(C)* Nitrotyrosine . Results are mean ± SE. *$p<0.05$ diabetic WT vs non-diabetic WT and diabetic TxNIP KO.

**FIGURE S9. Markers of glomerular inflammation.** (*A*) Representative images of glomerular inflammation indicated by F4/80 staining in glomeruli from non-diabetic and diabetic TxNIP$^{+/-}$ (HET) mice. Quantification of staining intensity is shown in the right panel. Expression of (*B*) *IL1β* and (*C*) *TNFα* mRNA corrected for the housekeeping gene *actb*, and normalized to non-diabetic WT in renal cortex. Results are mean± SE (n= 3-5 mice/ condition) *$p<0.05$ diabetic WT vs non-diabetic WT, diabetic KO and diabetic HET, **$p<0.001$ diabetic WT vs non-diabetic WT and diabetic KO, and $^{\$}p<0.05$ diabetic HET vs non-diabetic HET.

**Figure S1**


A
24h Albuminuria (µg)
0
10
20
30
40
50
60
70
80
WT
KO
HET
***
**
*
Non-diabetic
Diabetic
B
Albumin/Creatinine ratio (µg/mg)
0
50
100
150
200
WT
KO
HET
*
*
Non-diabetic
Diabetic
C
24h Proteinuria (ug)
0
50
100
150
200
250
300
WT
KO
HET
**
Non-diabetic
Diabetic
D
Serum Cystatin C (pg/mL)
0
500
1000
1500
2000
2500
3000
WT
KO
HET
*
*
Non-diabetic
Diabetic

# Figure S2

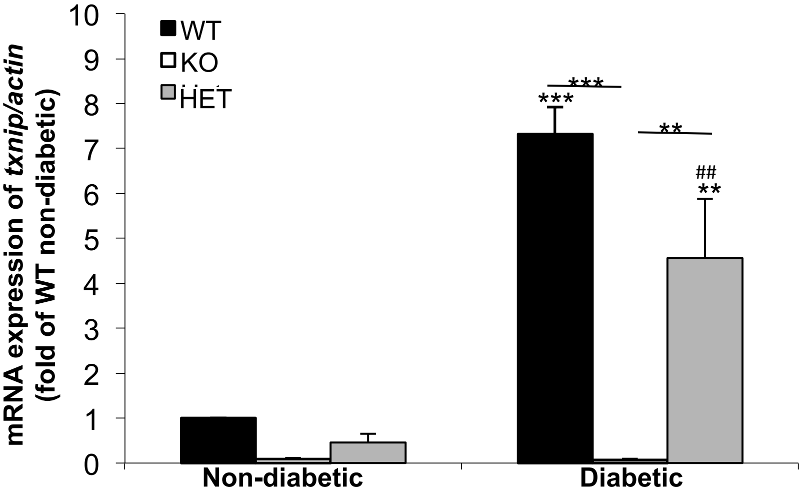
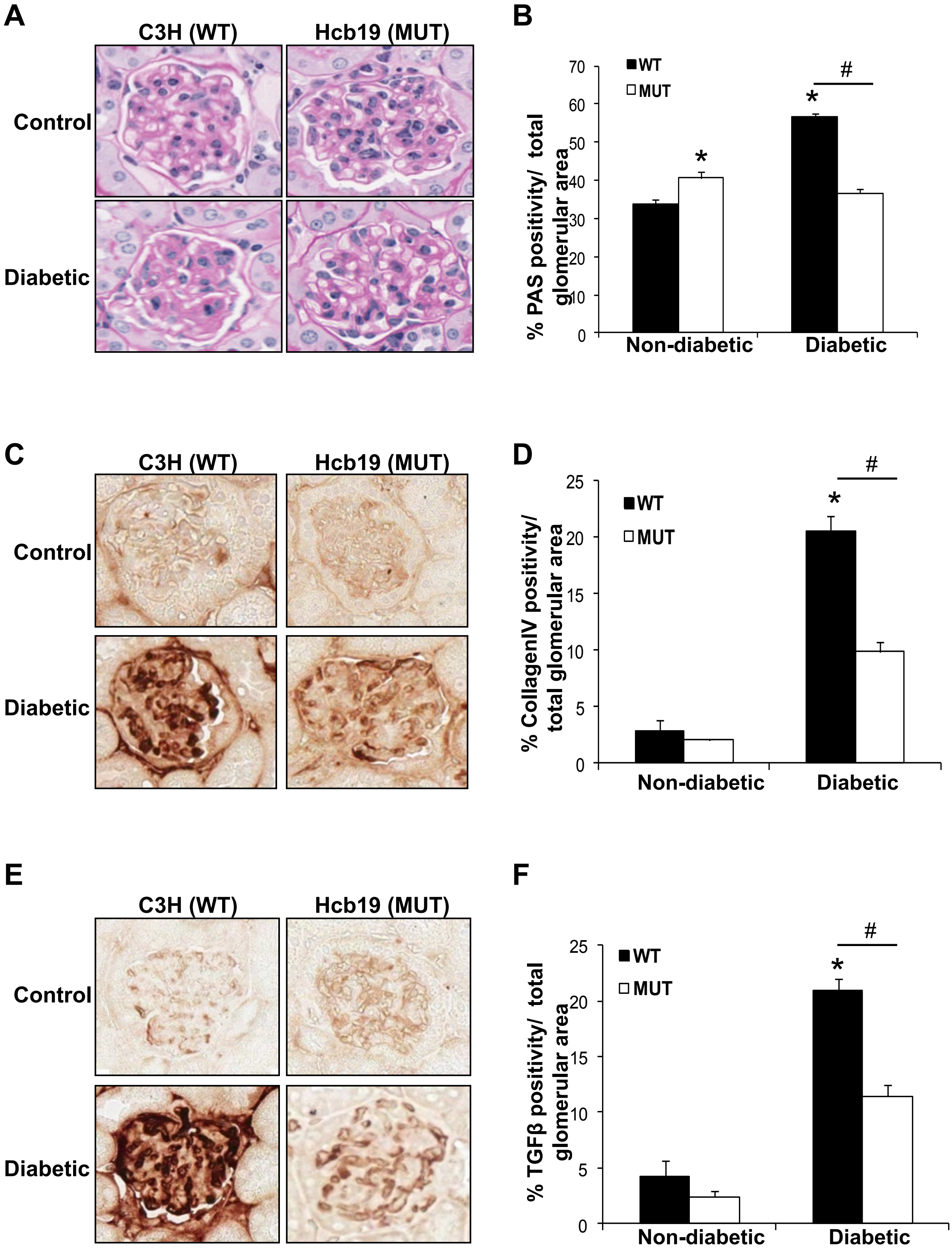
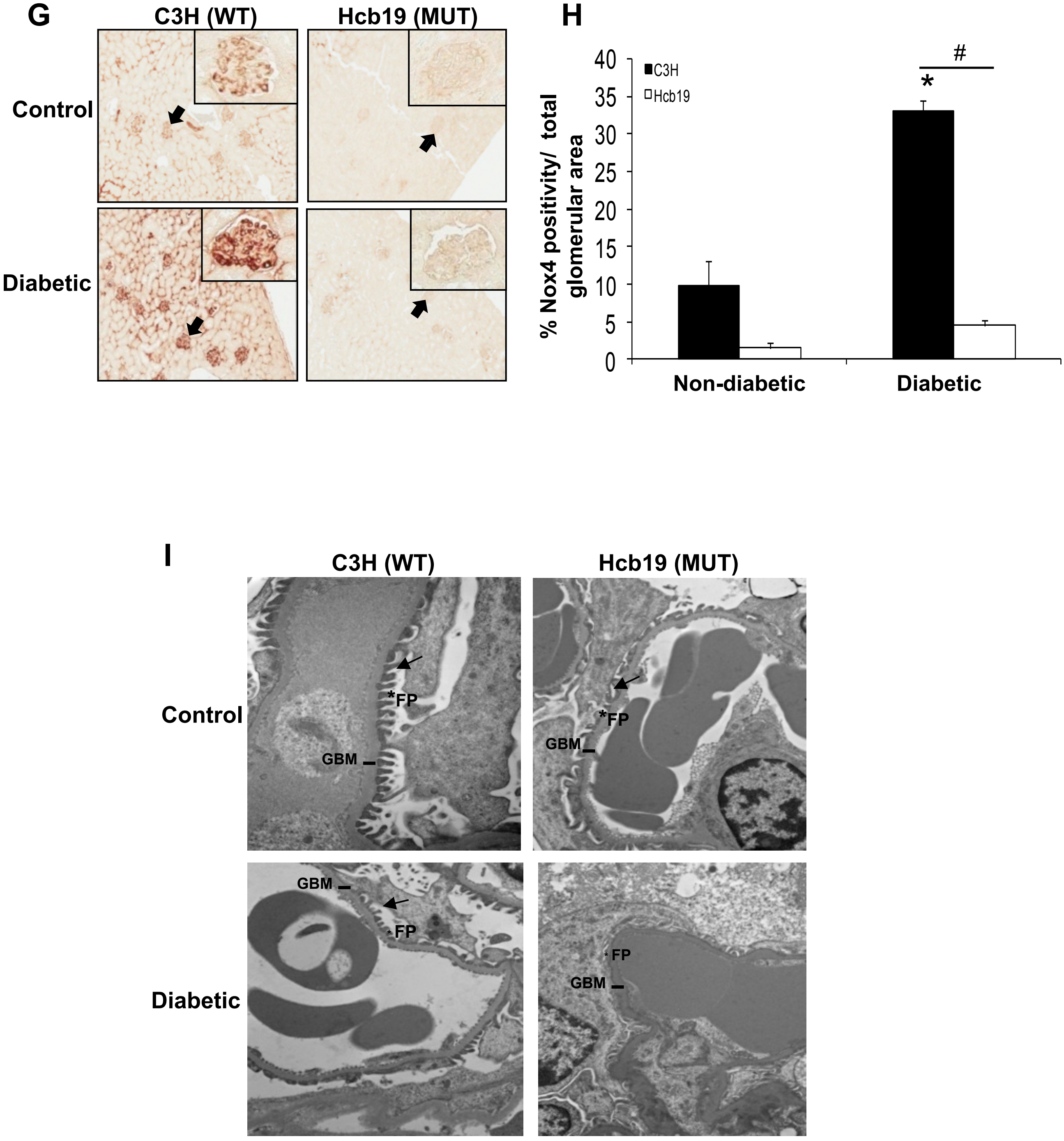

mRNA expression of *txnip/actin* (fold of WT non-diabetic)
10
9
8
7
6
5
4
3
2
1
0
WT
KO
HET
***
***
**
##
**
Non-diabetic
Diabetic

Figure S3

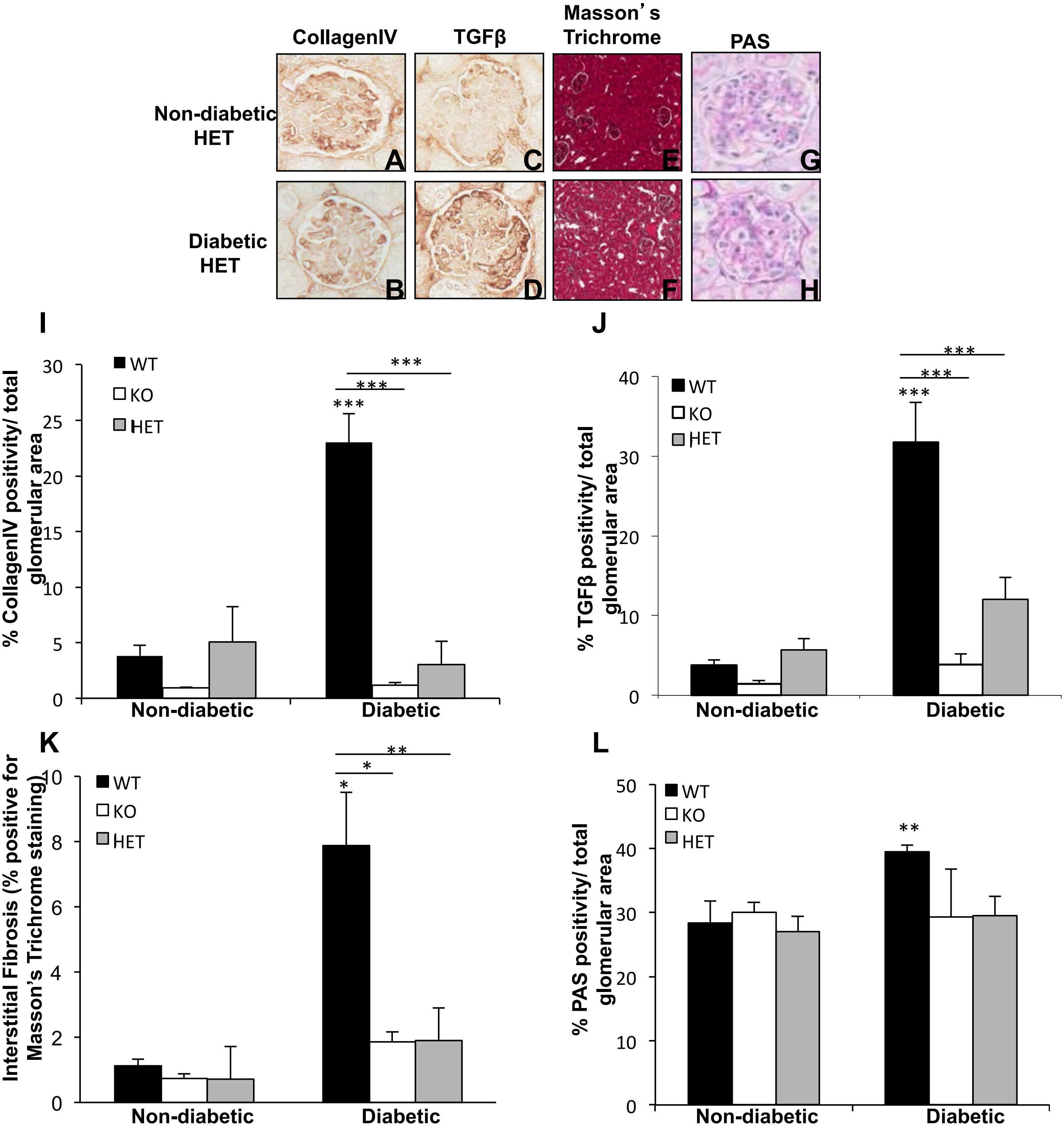

A
C3H (WT)
Hcb19 (MUT)
Control
Diabetic
B
WT
MUT
#
*
*
% PAS positivity/ total glomerular area
0
10
20
30
40
50
60
70
Non-diabetic
Diabetic
C
C3H (WT)
Hcb19 (MUT)
Control
Diabetic
D
WT
MUT
#
*
% CollagenIV positivity/ total glomerular area
0
5
10
15
20
25
Non-diabetic
Diabetic
E
C3H (WT)
Hcb19 (MUT)
Control
Diabetic
F
WT
MUT
#
*
% TGFβ positivity/ total glomerular area
0
5
10
15
20
25
Non-diabetic
Diabetic

# Figure S3

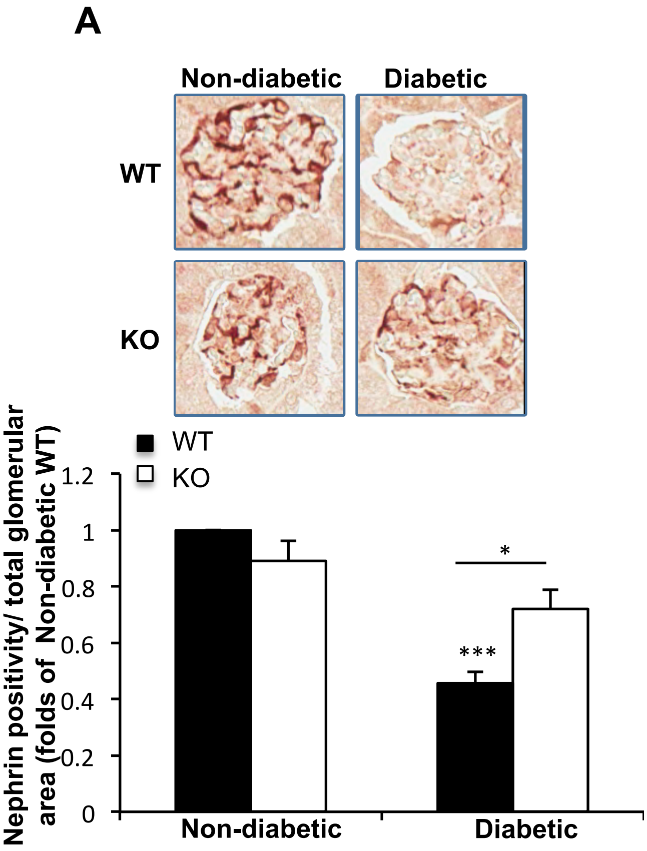

G
C3H (WT)
Hcb19 (MUT)
Control
Diabetic
H
% Nox4 positivity/ total glomerular area
40
35
30
25
20
15
10
5
0
■C3H
□Hcb19
#
*
Non-diabetic
Diabetic
I
C3H (WT)
Hcb19 (MUT)
Control
Diabetic
*FP
GBM

Representative images

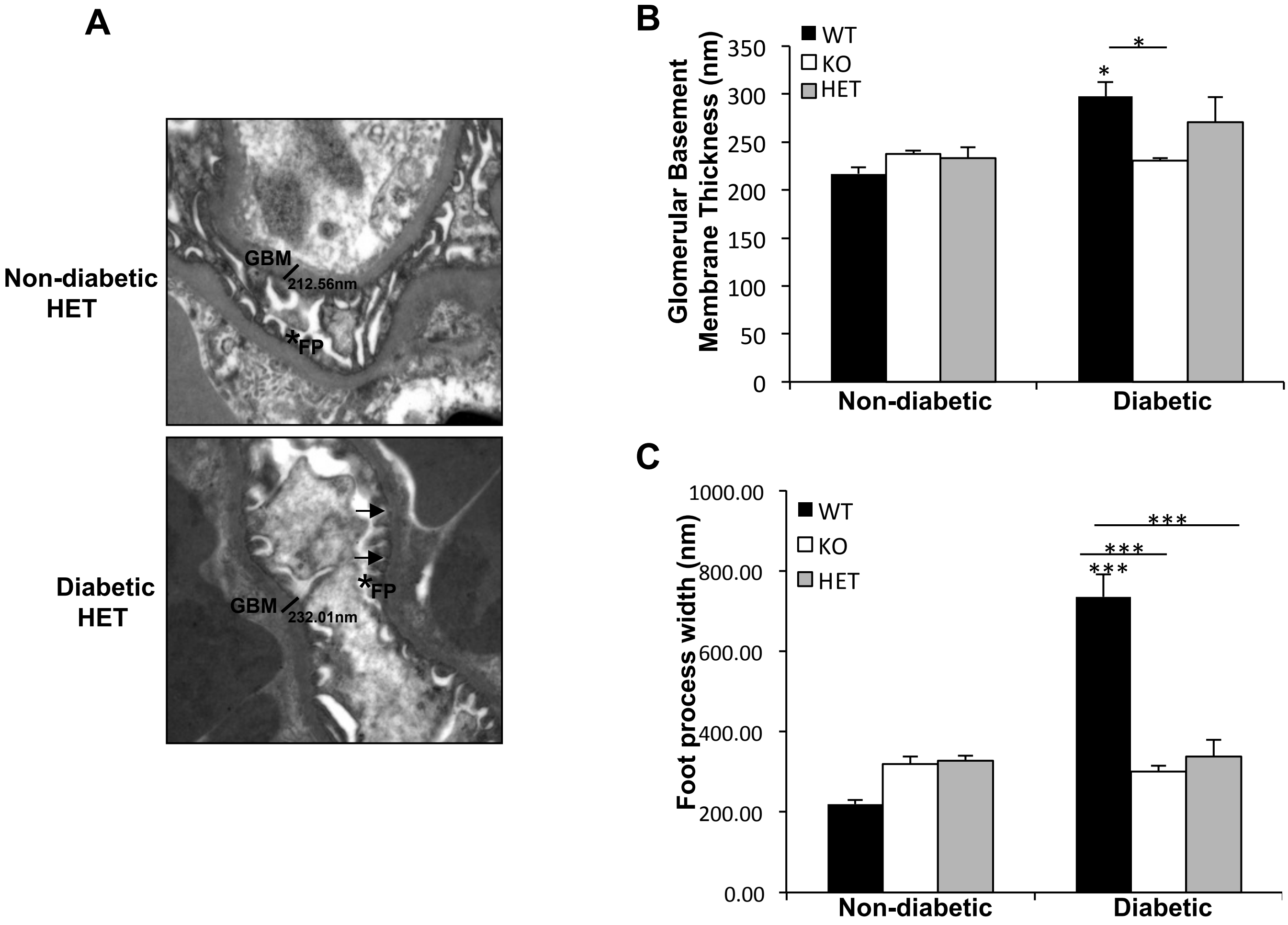
# Figure S4


CollagenIV
TGFβ
Masson's Trichrome
PAS
Non-diabetic HET
Diabetic HET
A
B
C
D
E
F
G
H
I
■ WT
☐ KO
▨ HET
% CollagenIV positivity/ total glomerular area
30
25
20
15
10
5
0
***
***
***
Non-diabetic
Diabetic
J
■ WT
☐ KO
▨ HET
% TGFβ positivity/ total glomerular area
40
30
20
10
0
***
***
***
Non-diabetic
Diabetic
K
■ WT
☐ KO
▨ HET
Interstitial Fibrosis (% positive for Masson's Trichrome staining)
10
8
6
4
2
0
**
*
*
Non-diabetic
Diabetic
L
■ WT
☐ KO
▨ HET
% PAS positivity/ total glomerular area
50
40
30
20
10
0
**
Non-diabetic
Diabetic

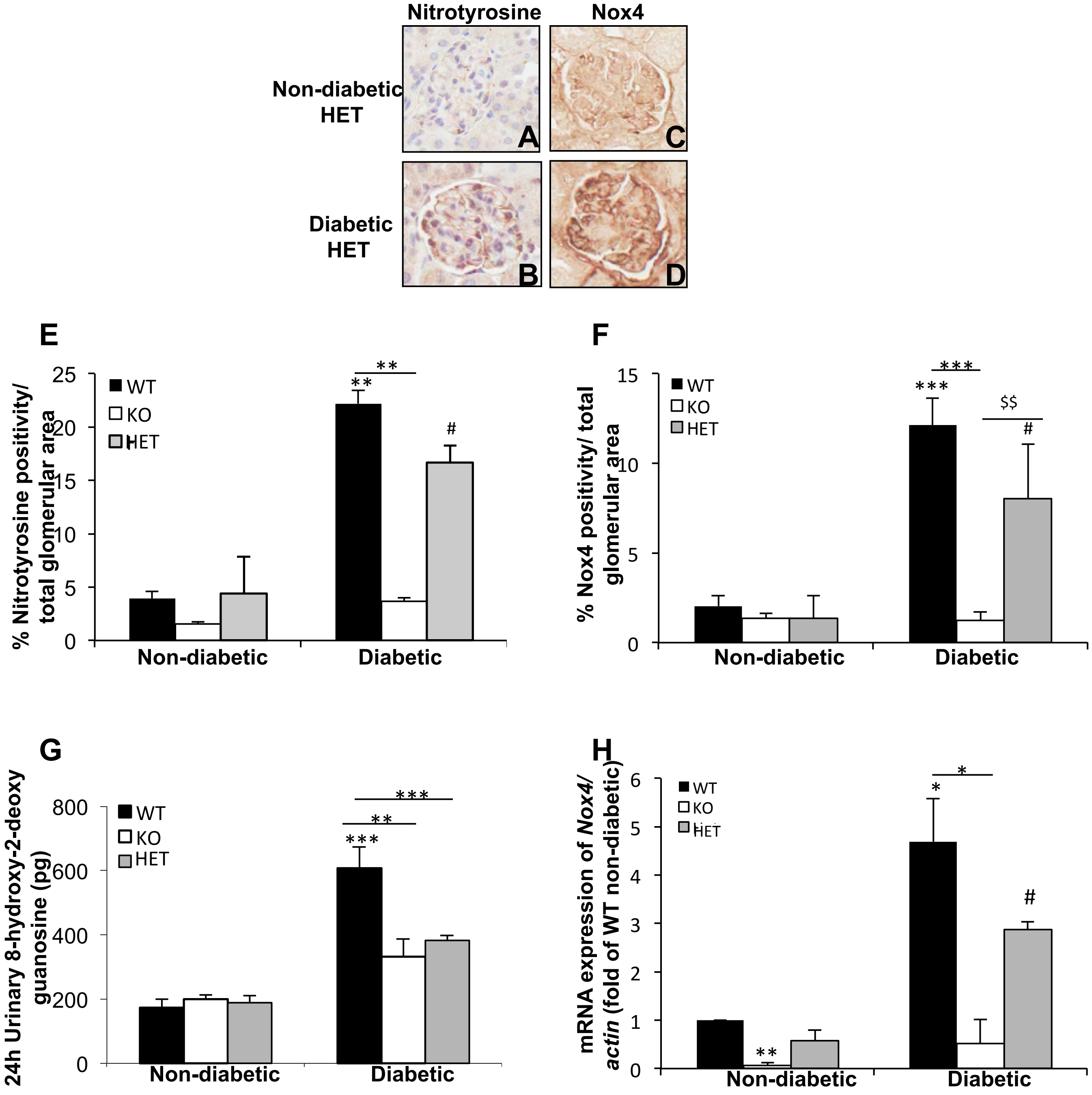
# Figure S5


A
Non-diabetic
Diabetic
WT
KO
■ WT
□ KO
Nephrin positivity/ total glomerular area (folds of Non-diabetic WT)
1.2
1
0.8
0.6
0.4
0.2
0
*
***
Non-diabetic
Diabetic

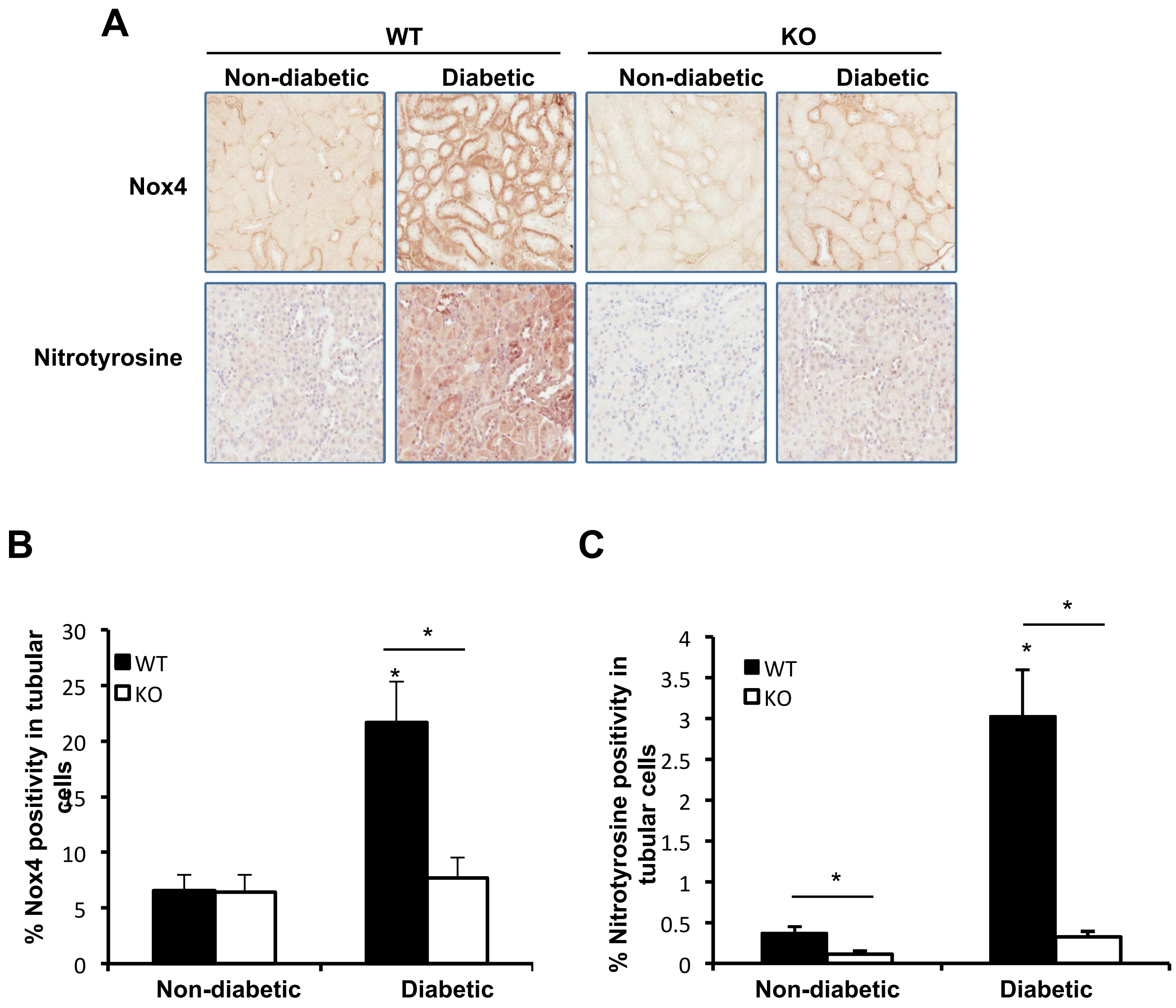
# Figure S6


A
Non-diabetic HET
GBM
212.56nm
*FP
Diabetic HET
GBM
232.01nm
*FP
B
Glomerular Basement Membrane Thickness (nm)
0
50
100
150
200
250
300
350
WT
KO
HET
*
*
Non-diabetic
Diabetic
C
Foot process width (nm)
0.00
200.00
400.00
600.00
800.00
1000.00
WT
KO
HET
***
***
***
Non-diabetic
Diabetic

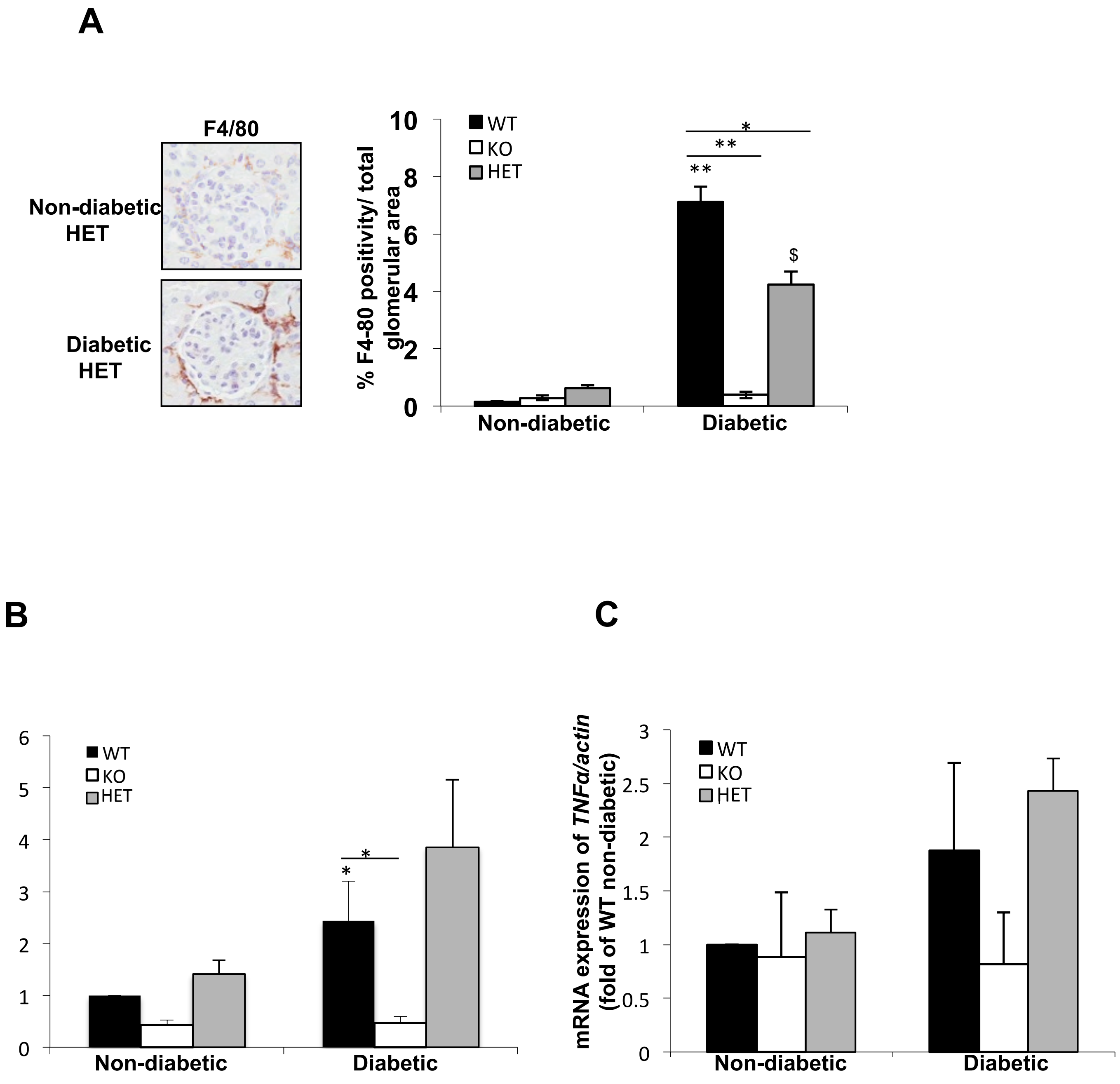
# Figure S7


Nitrotyrosine
Nox4
Non-diabetic HET
Diabetic HET
A
B
C
D
E
% Nitrotyrosine positivity/ total glomerular area
0
5
10
15
20
25
WT
KO
HET
**
**
#
Non-diabetic
Diabetic
F
% Nox4 positivity/ total glomerular area
0
5
10
15
WT
KO
HET
***
***
$$
#
Non-diabetic
Diabetic
G
24h Urinary 8-hydroxy-2-deoxy guanosine (pg)
0
200
400
600
800
WT
KO
HET
***
**
***
Non-diabetic
Diabetic
H
mRNA expression of Nox4/ actin (fold of WT non-diabetic)
0
1
2
3
4
5
6
WT
KO
HET
*
*
**
#
Non-diabetic
Diabetic

# Figure S8


A
WT
KO
Non-diabetic
Diabetic
Non-diabetic
Diabetic
Nox4
Nitrotyrosine
B
% Nox4 positivity in tubular cells
30
25
20
15
10
5
0
■ WT
□ KO
*
*
Non-diabetic
Diabetic
C
% Nitrotyrosine positivity in tubular cells
4
3.5
3
2.5
2
1.5
1
0.5
0
■ WT
□ KO
*
*
*
Non-diabetic
Diabetic

# Figure S9


A
F4/80
Non-diabetic HET
Diabetic HET
% F4-80 positivity/ total glomerular area
0
2
4
6
8
10
■ WT
☐ KO
▨ HET
**
**
*
$
Non-diabetic
Diabetic
B
mRNA expression of *IL1β/actin* (fold of WT non-diabetic)
0
1
2
3
4
5
6
■ WT
☐ KO
▨ HET
*
*
Non-diabetic
Diabetic
C
mRNA expression of *TNFα/actin* (fold of WT non-diabetic)
0
0.5
1
1.5
2
2.5
3
■ WT
☐ KO
▨ HET
Non-diabetic
Diabetic