Supplemental materials for

Independent of Renox, NOX5 promotes renal inflammation and fibrosis in diabetes by activating ROS-sensitive pathways

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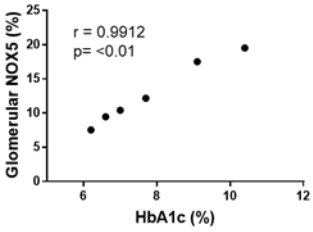
Fig. S1 **Table S1**



Case	Gender	Age (yrs)	Diabetes Status	Diabetes Duration (yrs)	BMI	eGFR	Urine Albumin (mg/24h)	BP (mmHg)	HbA1c (%)	Glomerular NOX5 % area
1	Male	66	Type 2	2	28	44	8.5	139/76	6.2	7.53
2	Female	72	Type 2	22	35	42	2.7	140/80	7.7	12.16
3	Female	70	Type 2	21	32	52	9	161/83	6.6	9.44
4	Male	74	Type 2	7	NA	48	138	130/70	9.1	17.50
5	Male	64	Type 2	31	28	30	1200.0	132/78	7	10.43
6	Male	59	Type 2	12	28	39	1750	200/90	10.4	19.51

Foot note: non-diabetic controls were kidney donors, clinical data are not available (NA)

Fig. S1 and table S1: Correlation between HbA1c and glomerular NOX5 expression (Fig. S1) as well as clinical data from human diabetic individuals (table S1). Pearson correlation analysis in diabetic individuals demonstrating a significant positive correlation between the glomerular NOX5 protein expression and the glycated haemoglobin ($r = 0.9912$, $p < 0.01$).

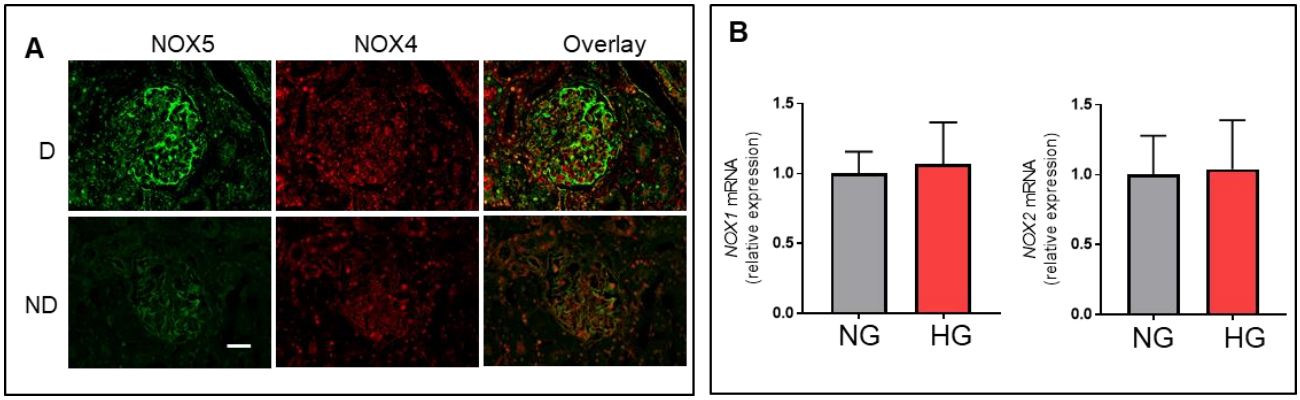


Fig. S2: **A**: Immunostaining of NOX5 and NOX4 in human kidney biopsies obtained from diabetic (D) and non-diabetic (ND) individuals. Scale bar, 50 µm in all photomicrographs. **B**: Gene expression of *NOX1* and *NOX2* in human mesangial cells exposed to normal glucose (NG, 5 mM) and high glucose (HG, 25 mM). Data are shown as mean ± SEM.

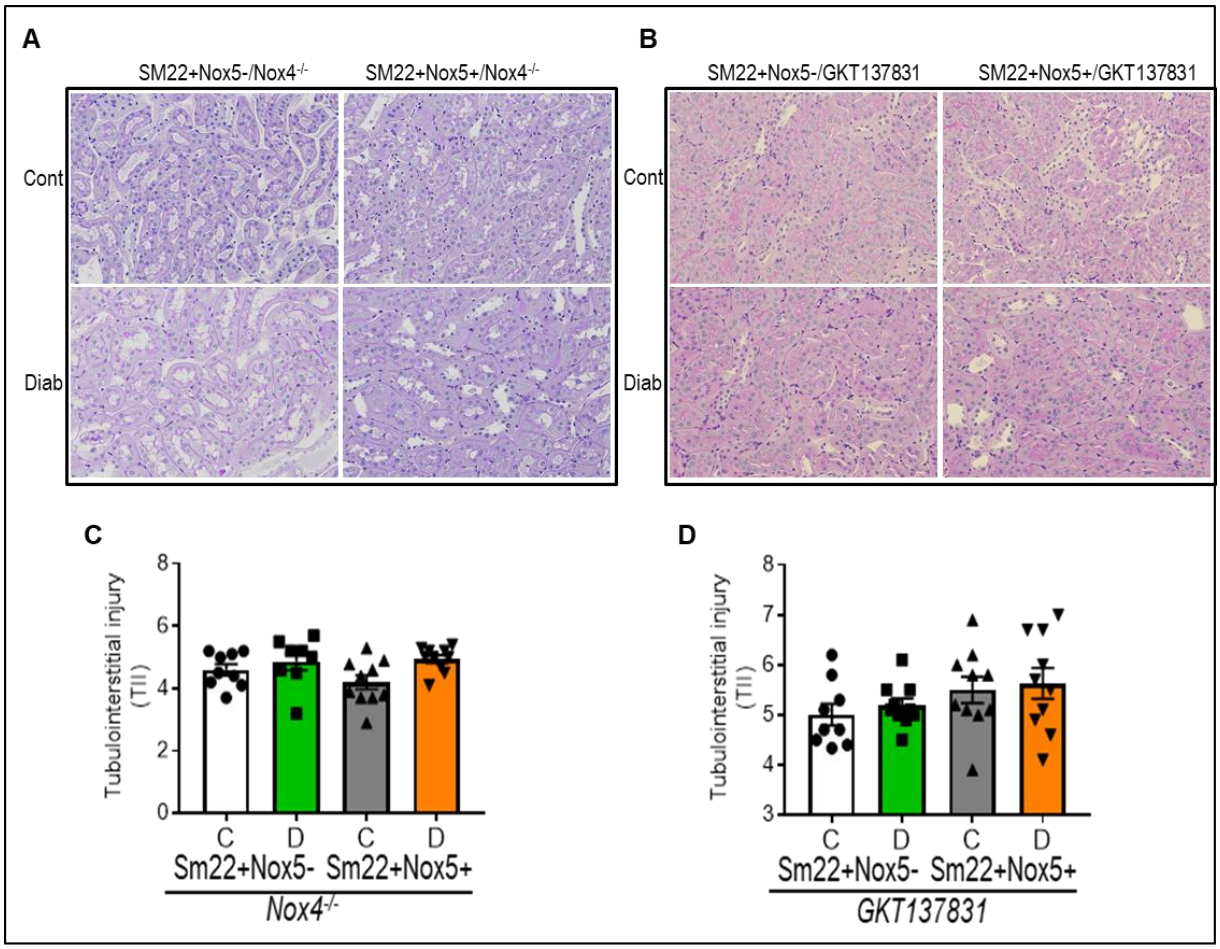


Fig. S3: *NOX5* transgenic mice showed no effects on TII in diabetes. Periodic Acid-Schiff staining (**A-B**) and the scoring of TII (**C-D**) in all groups of Nox4 knockout (*Nox4*$^{-/-}$) mice with and without *NOX5* expression as well as in GKT137831 treated mice with and without *NOX5* expression after 10 weeks of STZ- diabetes. Scale bar, 50 µm in all photomicrographs (n= 8-10/gp). Data are shown as mean ± SEM. *P* values were calculated using the two-tailed unpaired t-test and one-way ANOVA with Tukey's test for multiple comparison.

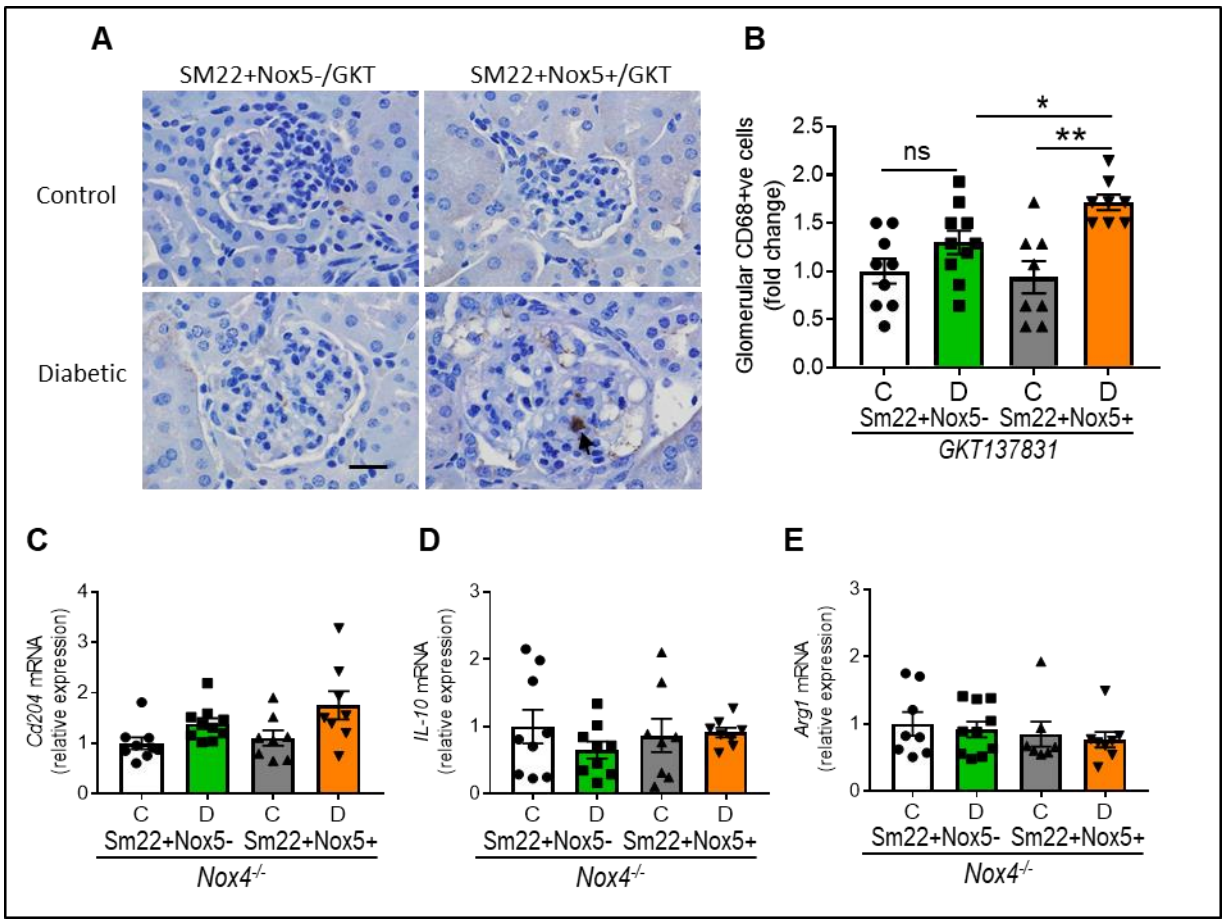


Fig. S4: **Increased number of glomerular CD68 positive cells in *NOX5* transgenic mice in diabetes**. A-B: Immunostaining of glomerular CD68 (A) and its quantitation (B), in GKT137831 treated mice with and without *NOX5* expression after 10 weeks of STZ- diabetes (n=8-10/gp). Scale bar, 20 µm in all photomicrographs. Renal cortical gene expression of *Cd204* (C), *IL-10* (D) and *Arg1* (E) in Nox4 knockout (*Nox4*$^{-/-}$) mice with and without *NOX5* expression after 10 weeks of STZ-diabetes (n=8-10/gp). Data are shown as mean ± SEM. Asterisks represent p-values for comparisons of the indicated groups: * < 0.05, ** < 0.01 and ns, not significant statistically. *P* values were calculated using the two-tailed unpaired t-test and one-way ANOVA with Tukey's test for multiple comparison.

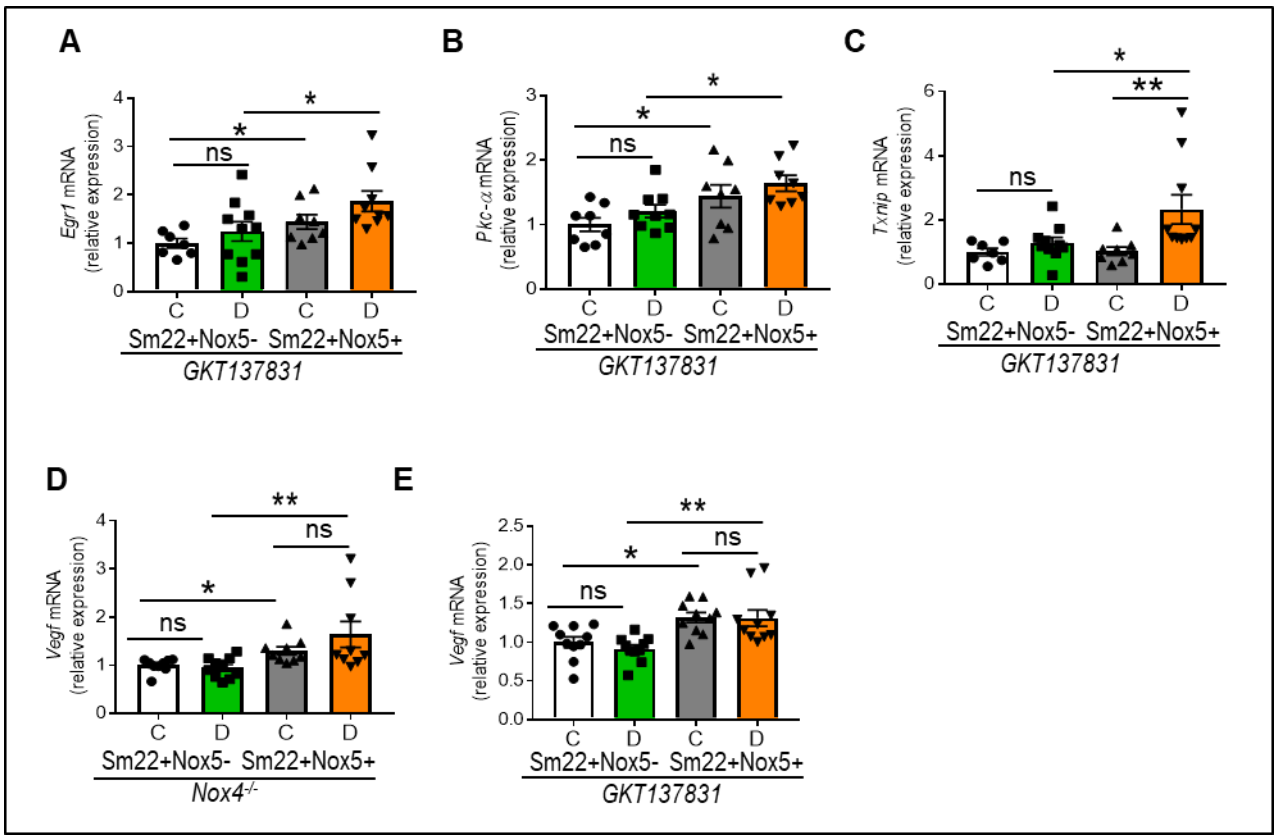


Fig. S5: Modulation of renal redox sensitive factors *Egr-1, Txnip, Pkc-α* and as well as growth factor *Vegf* by *NOX5 in DKD*. Renal cortical gene expression of ***Egr-1*** (A), ***Pkc-α*** (B) and ***Txnip*** (C) in GKT137831 treated mice and ***Vegf (D, E)*** in both Nox4 knockout (*Nox4*$^{-/-}$) or GKT137831 treated mice with and without *NOX5* expression after 10 weeks of STZ- diabetes (n=8-10/gp). Data are shown as mean ± SEM. Asterisks represent p-values for comparisons of the indicated groups: * < 0.05, ** < 0.01 and ns, not significant statistically. *P* values were calculated using the two-tailed unpaired t-test and one-way ANOVA with Tukey's test for multiple comparison.

Fig. S6: Schematic representing the pathogenic role of NOX5 in DKD

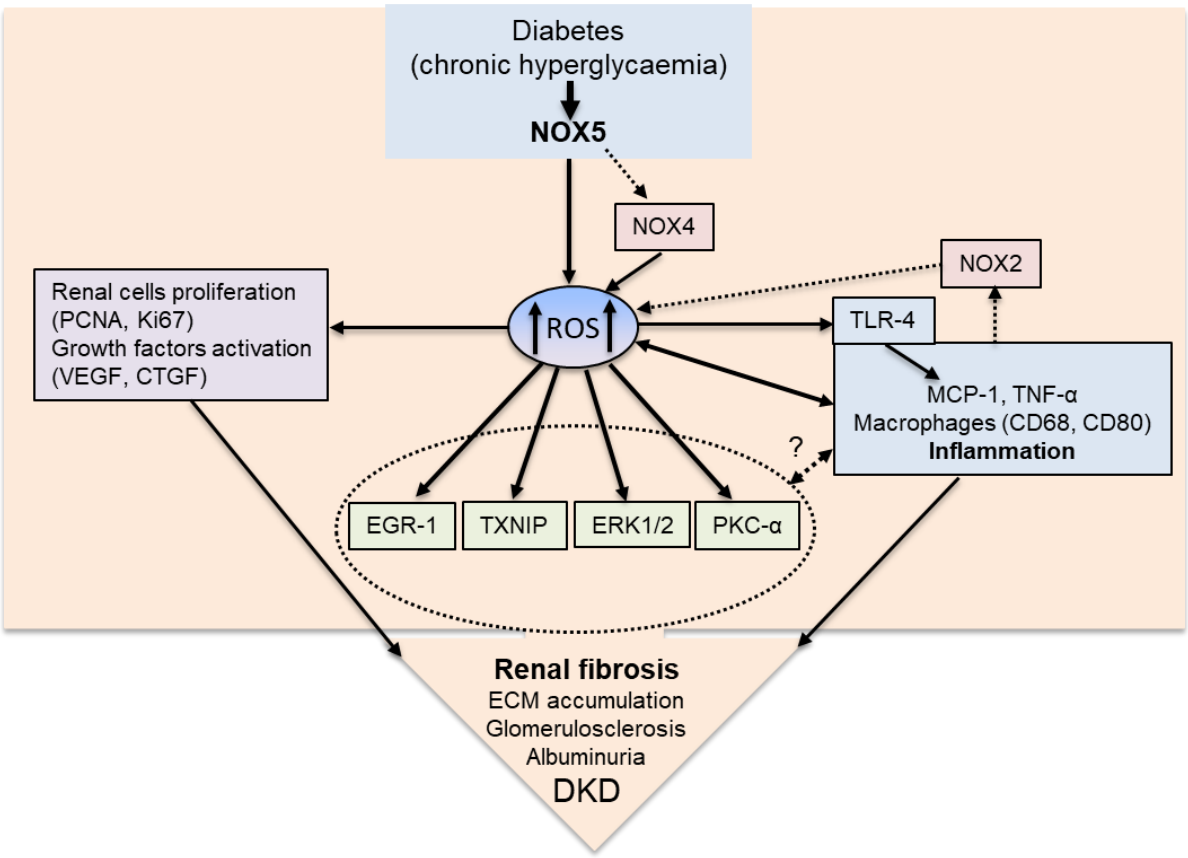


Table S2. Mouse probes and primers for RT-PCR

Genes	Probe Sequence (6-FAM 5'-3')	Forward Primer 5'-3'	Reverse Primer 5'-3'
Nox2	CAACTGGACAGGAACCT	AGTGCGTGTTGCTCGACAAG	CCAAGCTACCATCTTATGGAAAGT
Mcp-1	AATGGGTCCAGACATAC	GTCTGTGCTGACCCCAAGAAG	TGGTTCCGATCCAGGTTTTTA
Egr-1	SYBER	TCGGCTCCTTTCCTCACTCA	GCATCATCTCCTCCAGTTTGG
Txnip	SYBER	CTGAGTGCTGCGATCAAAGG	CGTTGGGAGAAAGCCTCAAA
Pkc-α	CGATCCCAGTCCCAG	AGACAAAGACCGGCGACTGT	TTAGCTCTGAGACACCAAAGGAAA
Ki-67	SYBER	CAAAAGGCGAAGTGGAGCTT	TGTTTCGCAACTTTCGTTTGTG
Pcna	CACAGCTGTACTCCTGTTC	TCAAGAGAAAGTTTCAGACTATGAAATGA	AAATTCACCAGATGGCATCTTTATT
α-SMA	TGCCAGATCTTTTCC	GACGCTGAAGTATCCGATAGAACA	GGCCACACGAAGCTCGTTAT
Ctgf	ACTGCCTGGTCCAGAC	GCTGCCTACCGACTGGAAGA	CTTAGAACAGGCGCTCCACTCT
Vegf	CTGTACCTCCACCATGC	GCACTGGACCCTGGCTTTACT	ATGGGACTTCTGCTCTCCTTCTG
Fn1	CCCCGTCAGGCTTA	ACATGGCTTTAGGCGGACAA	ACATTCGGCAGGTATGGTCTTG
Col1	CAGTGCCCTAACGGT	GGCGGTACACAGTCAGACCAT	GGAATAGCCGATCCACAGTGA
Erk1	TCAACCCAAACAAGCG	TGCTGGACCGGATGTTAACC	CCAGCGCTTCCTCTACTGTGA
Cd80	SYBER	TGGGAAAAACCCCCAGAAG	CCCCAAAGAGCACAAGTGTGT
Cd204	SYBER	GGAGGAGAGAATCGAAAGCATTT	TCTGGAAGCGTTCCGTGTCT
Arg1	SYBER	GAAAGTTCCCAGATGTACCAGGAT	CGATGTCTTTGGCAGATATGCA
IL-10	CATGGCCCAGAAAT	GATGCCCCAGGCAGAGAA	CACCCAGGGAATTCAAATGC
TNF-α	TCACCCACACCGTCAG	GGCTGCCCCGACTACGT	TTTCTCCTGGTATGAGATAGCAAATC

Table S3. Human probes and primers for RT-PCR

Genes	Probe Sequence (6-FAM 5'-3')	Forward Primer 5'-3'	Reverse Primer 5'-3'
NOX1	AAAGCAATTGGATCACAAC	TTGCAGCCGCACACTGA	GGCCACCAGCTTGTGGAA
NOX2	CCTCCTGCCATGACT	AGAGGGTTGGAGGTGGAGAATT	GCACAAGGAGCAGGACTAGATGA
NOX4	TCCATTTGCATCAATACT	GGCTGGAGGCATTGGAGTAA	CCAGTCATCCAACAGGGTGTT
NOX5	CCTGCTGACTAAACTG	CGGTCTTTCGAGTGGTTTGTG	CCTCGGCCTGGTCCATCT
MCP-1	CAGGAAACCAATATCCA	CAAAGCAGGGCTCGAGTTG	CCTGGGACTAGACTTGATGTCTCA
TLR-4	ATCCATGAAGGTTTCC	GGCCATTGCTGCCAACAT	CAACAATCACCTTTCGGCTTTT
EGR-1	SYBER	TCGGATCCTTTCCTCACTCG	GCATCATCTCCTCCAGCTTAGG
PKC-α	CGATCCCAGTCCCAG	TCAGACAAAGACCGACGACTGT	TCCGAAACTCCAAAGGAAAGG
CTGF	ACTGCCTGGTCCAGAC	GCGGCTTACCGACTGGAA	GGAACAGGCGCTCCACTCT
COL1	AAAGGTGCTGCTGGAGA	TTTCCTTGCACCTTTTCCAAA	TGTGTTGTGCACGTGGATTG
COL3	CCCATTGGACCACCAGG	ACCTCCTGGCAAAGATGGAA	GAGCCCTCAGATCCTCTTTCAC
TXNIP	SYBER	TGCCACCACCGA CTTATACTGA	CATGCTCACTGCACATTGTTGT