SUPPLEMENTARY DATA

Glomerular endothelial mitochondrial dysfunction is essential and characteristic of diabetic kidney disease susceptibility .

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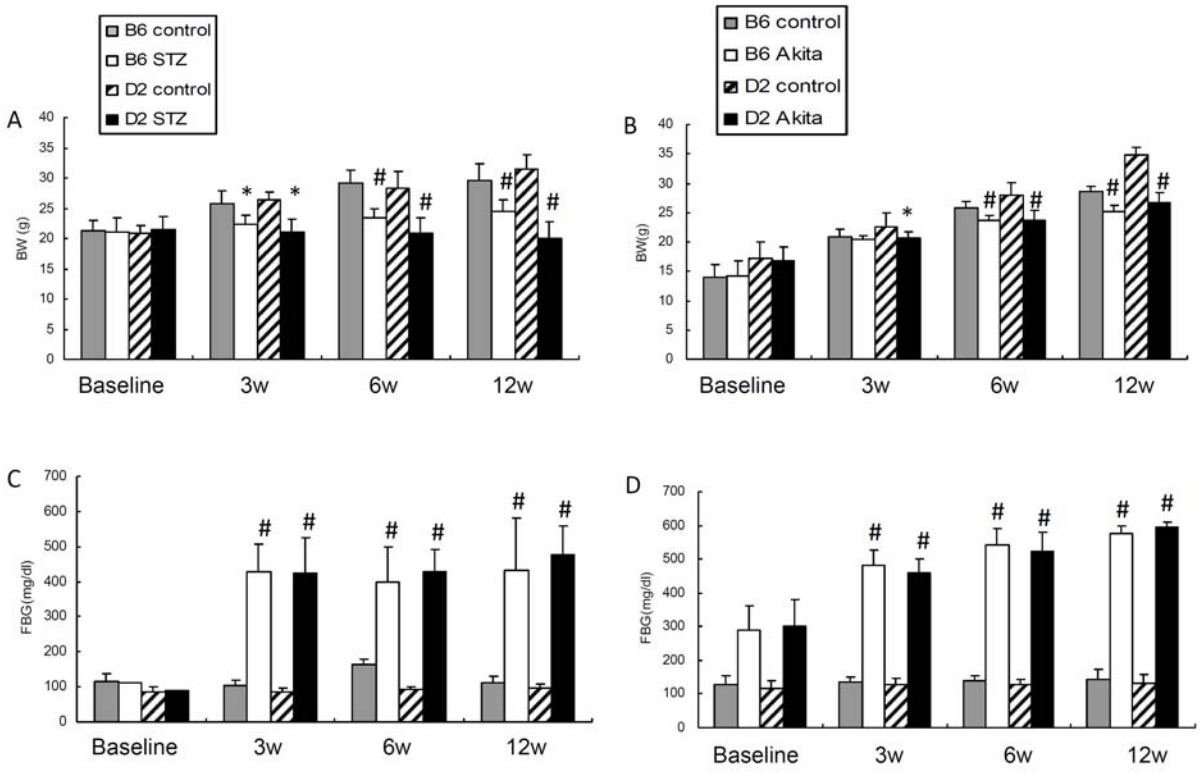
SUPPLEMENTARY DATA

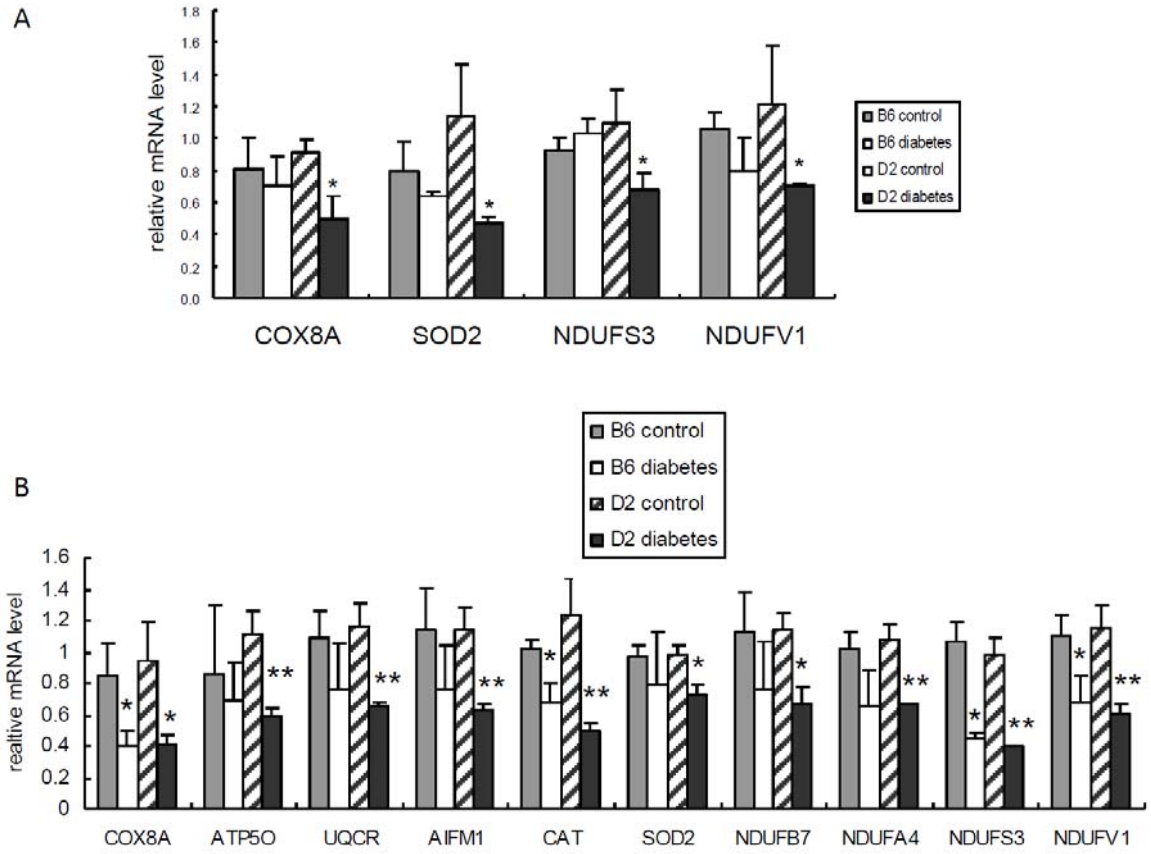
Supplementary Figure 1. Body weight (BW) and fasting blood glucose (FBG) in STZ treated and Akita mice.

Bar graph shows the mean +/-SD of BW in A) control or STZ treated B6 or D2 mice and B) Akita B6 or D2 mice at baseline, 3, 6, 12 weeks of diabetes as indicated. FBG levels +/-SD of C) control or STZ treated B6 or D2 mice, and of D) Akita B6 or D2 mice at baseline, 3, 6, 12 weeks of diabetes. *P < 0.05, **P < 0.01, or # P < 0.001.



SUPPLEMENTARY DATA

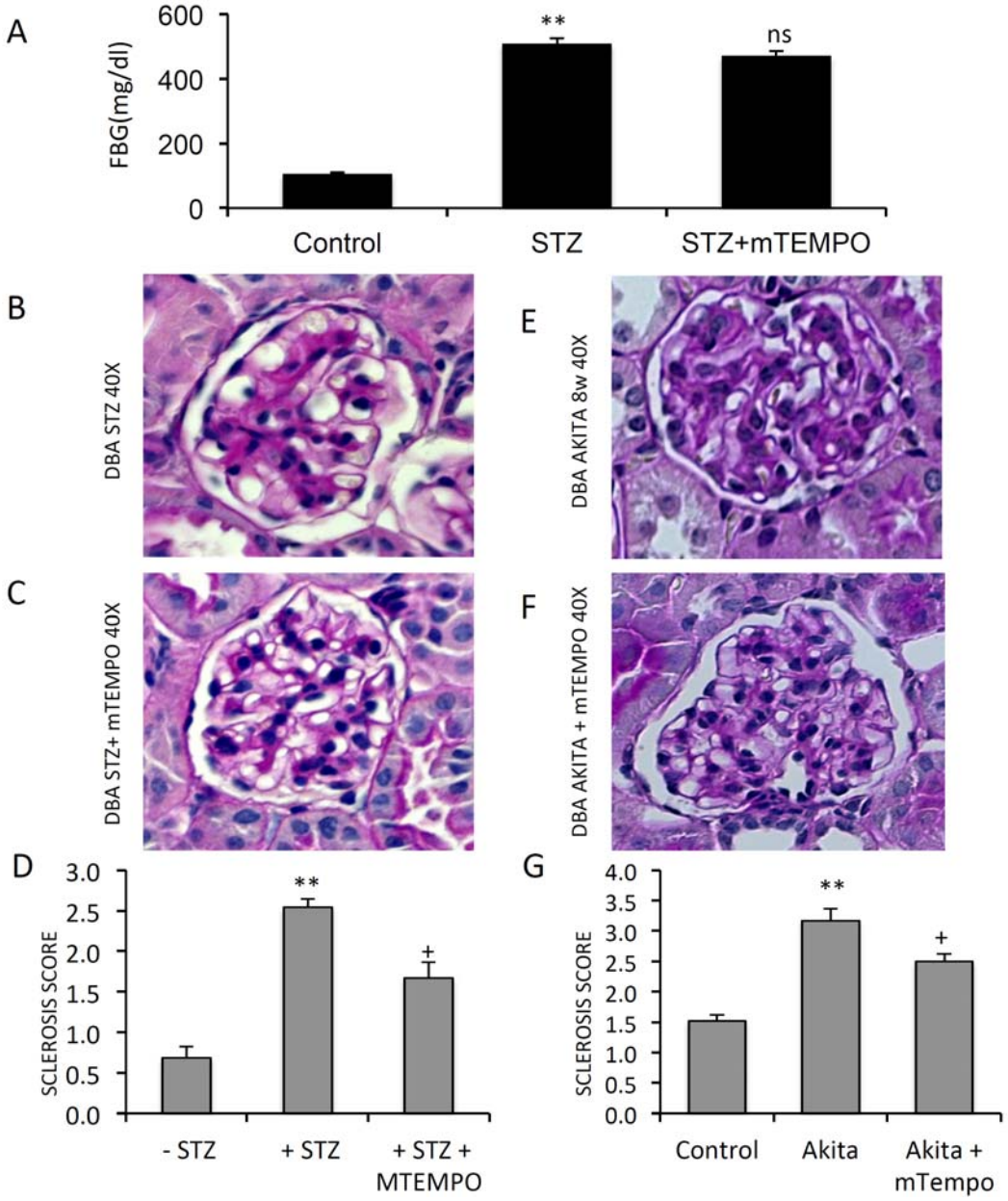
Supplementary Figure 2. Mitochondrial gene expression in diabetic B6 and D2 mice glomeruli
Total RNA was prepared from glomeruli and cell lysates (Qiagen, Valencia, CA) and reversely transcribed into single strand cDNA with SuperScript II reverse transcriptase(Life Technologies, Invitrogen). The cDNA was amplified using SYBR-Green PCR Master Mix (Applied Biosystems) and gene-specific exon–exon junction spanning primers (sequences available upon request), normalized the murine beta-actin gene or Gapdh. Relative mRNA expression levels of selected respiratory chain complex genes in STZ treated B6 and D2 mice with A) 3 weeks and B) 6 weeks of diabetes compared with respective non-diabetic controls. *P < 0.05, **P < 0.01.



SUPPLEMENTARY DATA

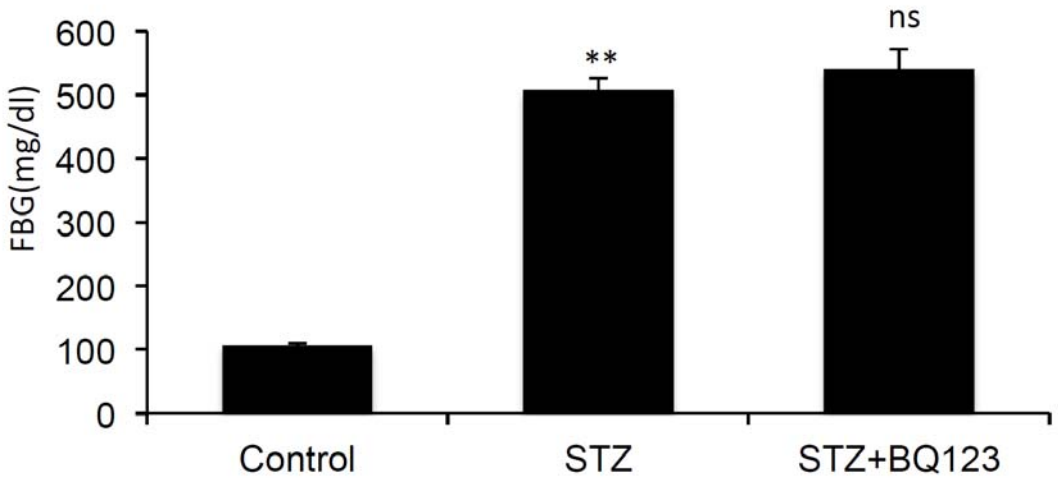
Supplementary Figure 3. MitoTEMPO ameliorated glomerulosclerosis of STZ-D2 and Akita-D2 diabetic mice

A) FBG levels of control, STZ-D2 and STZ-D2 co-treated with mitoTEMPO with 3 weeks of diabetes. PAS staining of D2 kidneys B) STZ-D2 mice showing severe sclerosis, C) STZ-D2 co-treated with mitoTEMPO (1mg/kg/day). D) Sclerosis score of STZ-D2 with/without mitoTEMPO. PAS staining of E) Akita-D2 mice, F) Akita-D2 co-treated with mitoTEMPO (1mg/kg/day) and G) Sclerosis index score of Akita-D2 with/without mitoTEMPO. The Sclerosis index score in D) and E) was performed as previously described (1), for each glomerulus and graded from 0 to 4+ as follows: 0 represents no lesion, 1+ represents sclerosis of <25% of the glomerulus, while 2+, 3+, and 4+ represents sclerosis of >25% to 50%, >50% to 75%, and >75% of the glomerulus. + P< 0.05, **P < 0.01, ns not significant.



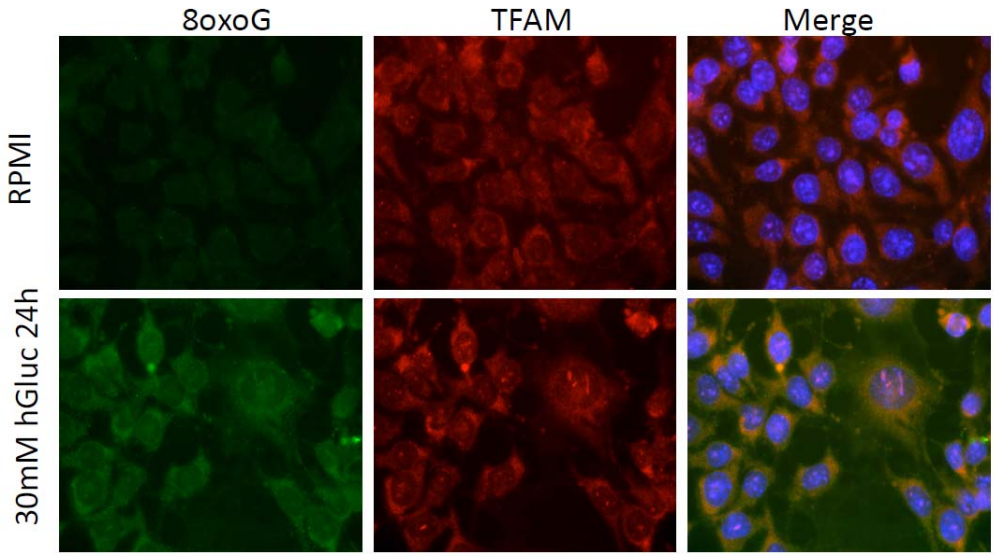
SUPPLEMENTARY DATA

Supplementary Figure 4. BQ-123 does not affect STZ-D2 FBG
A) FBG levels of control, STZ-D2 and STZ-D2 co-treated with BQ-123 with 3 weeks of diabetes. **P < 0.01, ns not significant.



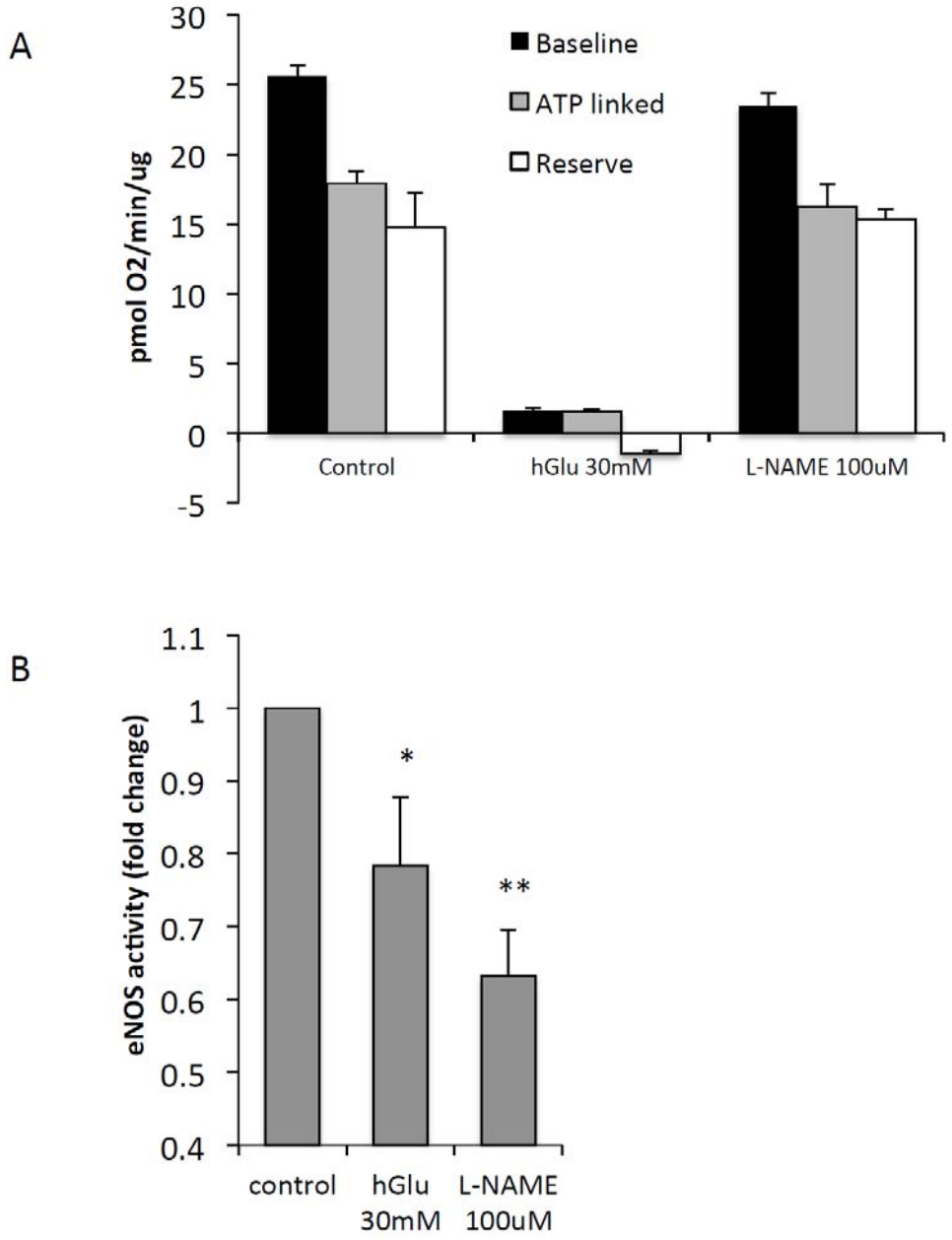
SUPPLEMENTARY DATA

Supplementary Figure 5. hGluc induced mStress in mGECs
8oxoG and TFAM staining of mGEC controls and mGECs in hGluc for 24h.



SUPPLEMENTARY DATA

Supplementary Figure 6. mitochondrial and endothelial function with hGluctreatment of mGECs
A) baseline OCR, ATP linked OCR calculated as the difference between basal and oligomycin-sensitive OCR, and reserve capacity OCR of mGEC controls (glucose (5mM) + D-mannitol (25mM)), or treated with hGluc, or NOS inhibitor L-NAME. B) eNOS activity of mGEC controls in RPMI, or treated with hGluc, or NOS inhibitor L-NAME.



SUPPLEMENTARY DATA

Supplementary Table 1.
Top ten differentially represented pathways in glomeruliaser 6 weeks of diabetes. # Down suggests reduced pathway activity, * Up suggests increased pathway activity

Ingenuity Canonical Pathways	Ratio (reg. genes/total pathway (%))	
	Down	Up
6 weeks of diabetes (DBA/2J STZ : DBA/2J control)		
1. Oxidative Phosphorylation	33/166 (20%) #	1/166 (1%)
2. Mitochondrial Dysfunction	29/172 (17%) #	3/172 (2%)
3. Nitrogen Metabolism	10/134 (7%) #	1/134 (1%)
4. LPS/IL-1 Mediated Inhibition of RXR Function	15/205 (7%) #	8/205 (4%)
5. Sulfur Metabolism	6/61 (10%) #	0/61 (0%)
6. Xenobiotic Metabolism Signaling	19/294 (6%) #	7/294 (2%)
7. Crosstalk between Dendritic Cells and Natural Killer Cells	2/98 (2%)	9/98 (9%) *
8. Activation of IRF by Cytosolic Pattern Recognition Receptors	1/73 (1%)	8/73 (11%) *
9. Antigen Presentation Pathway	0/39 (0%)	6/39 (15%) *
10. Dendritic Cell Maturation	3/173 (2%)	12/173 (7%) *

SUPPLEMENTARY DATA

Supplementary Table 2. Selected respiratory chain complex genes involved in mitochondrial function and oxidative phosphorylation (OXPHOS).

Symbol	Gene name	Pathways
AIFM1	apoptosis-inducing factor, mitochondrion-associated, 1	Mitochondrial dysfunction
ATP5O	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit	OXPHOS
CAT	catalase	Mitochondrial dysfunction
GSR	glutathione reductase	OXPHOS
COX6A1	cytochrome c oxidase subunit VIa polypeptide 1	Mitochondrial dysfunction, OXPHOS
COX6B1	cytochrome c oxidase subunit Vib polypeptide 1	Mitochondrial dysfunction, OXPHOS
COX7C	cytochrome c oxidase subunit VIIc	Mitochondrial dysfunction, OXPHOS
COX8A	cytochrome c oxidase subunit 8A (ubiquitous)	Mitochondrial dysfunction, OXPHOS
COX5B	cytochrome c oxidase subunit Vb	Mitochondrial dysfunction, OXPHOS
NDUFS8	NADH dehydrogenase (ubiquinone) Fe-S protein 8	Mitochondrial dysfunction, OXPHOS
NDUFV1	NADH dehydrogenase (ubiquinone) flavoprotein 1	Mitochondrial dysfunction, OXPHOS
NDUFS3	NADH dehydrogenase (ubiquinone) Fe-S protein 3	Mitochondrial dysfunction, OXPHOS
NDUFA4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4	Mitochondrial dysfunction, OXPHOS
NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7	Mitochondrial dysfunction, OXPHOS
NDUFA3	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 3	Mitochondrial dysfunction, OXPHOS
NDUFB9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 9	Mitochondrial dysfunction, OXPHOS
PRDX3	peroxiredoxin 3	Mitochondrial dysfunction
SOD2	superoxide dismutase 2, mitochondrial	Mitochondrial dysfunction
UQCR	ubiquinol-cytochrome c reductase,	OXPHOS

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Supplementary Table 3. Human pathology report

	n	DM type	mean BMI	% HTN	mean age	Female:Male
control	6	none	24.1 +/- 2.5	0%	37 +/- 18.5	1:5
mild to moderate	7	2	29.1 +/- 6.6	100%	63.2 +/- 15.7	0:7
mild to moderate	1	1	28.4	100%	28.0	0:1
Moderate to severe	5	2	N/A	100%	46.8 +/- 10.1	3:2
Advanced	1	2	N/A	100%	51	1:0

REFERENCES

1. Ma, L.J., and Fogo, A.B. 2003. Model of robust induction of glomerulosclerosis in mice: importance of genetic background. *Kidney Int.* 64:350-355.