Supplemental Data

Neutrophil extracellular traps induce glomerular endothelial cell dysfunction and pyroptosis in diabetic kidney disease

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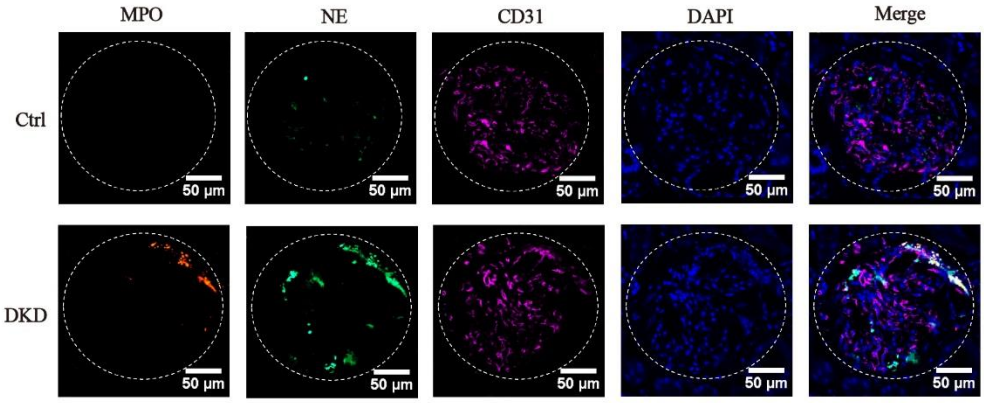
Supplementary Tables 1. Clinical characteristics of the study population.

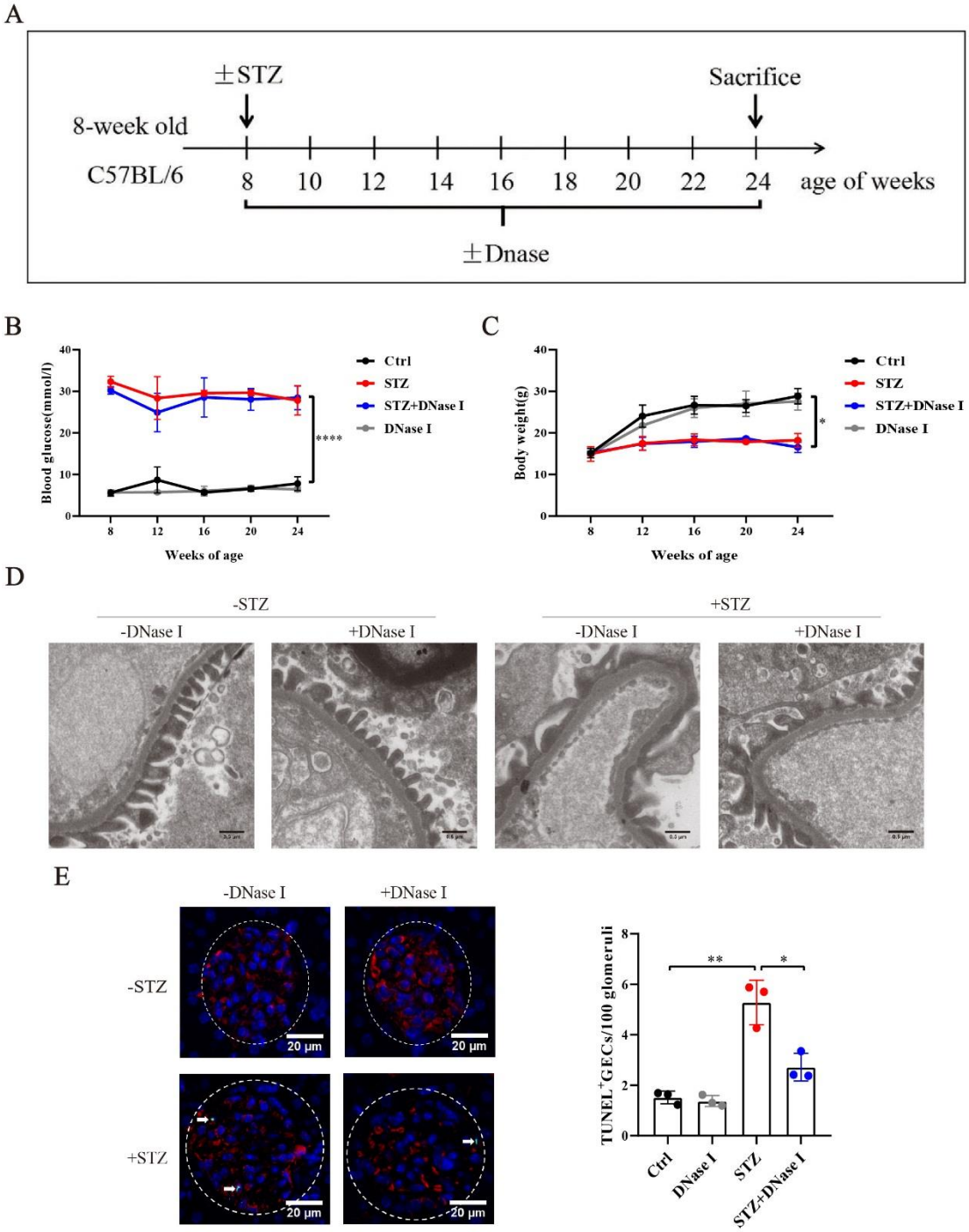
Characteristic	Non-DKD (n=70)	DKD(n=70)	P-value
Age (years)	60.17±9.28	60.00±9.16	0.9485
Men (%)	42 (60.00)	43 (61.43)	0.8630
BMI (kg/m^2)	23.63±2.61	24.97±5.64	0.0719
HbA1c (%)	8.51±1.20	9.13±2.01	0.0689
LDL cholesterol(mmol/l)	2.71±1.01	2.83±1.20	0.5400
Total cholesterol (mmol/l)	4.53±1.13	4.74±1.47	0.3482
MPO-DNA (OD405)	0.32±0.06	0.37±0.09	<0.001
Albuminuria (UACR, mg/g)	3.70 (2.15-7.53)	790.55 (504.85-1711.28)	<0.0001
eGFR (ml/min per 1.73 m^2)	93.68±27.96	69.93±37.57	<0.0001

Data were presented as n (%), mean±SD or median (interquartile range).

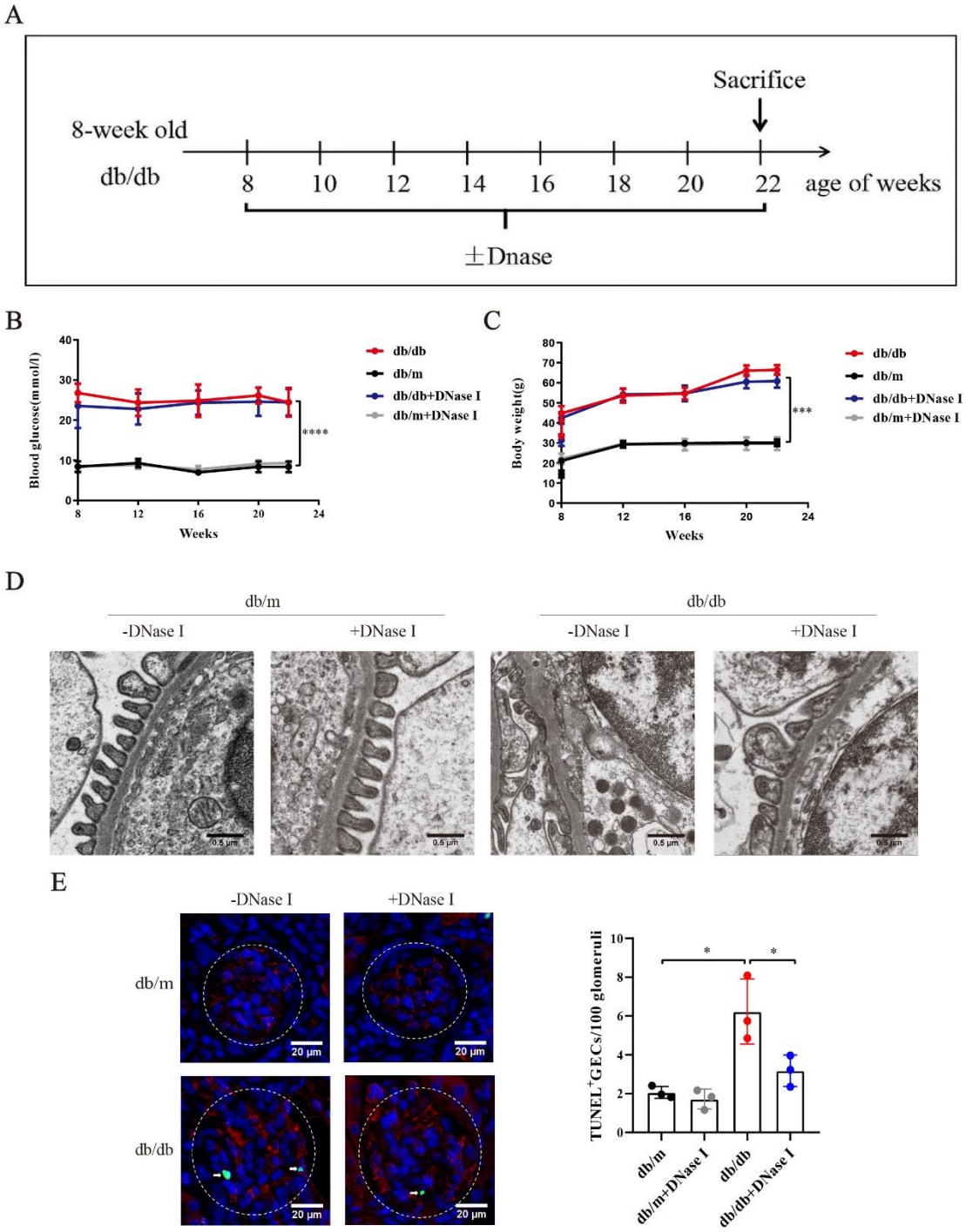


Supplementary Figure 1. Immunostaining for NETs in kidney sections.

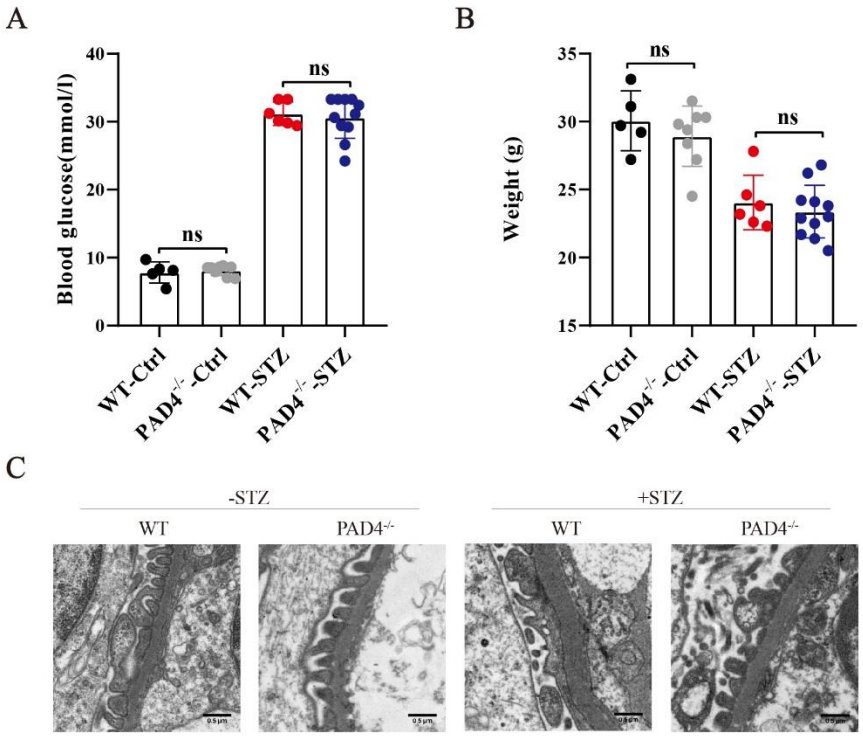
Immunofluorescence colocalization of NETs (MPO and NE) and CD31 in kidney tissues of patients with DKD and volunteers without diabetes from nephrectomy. Glomeruli are outlined with dotted lines.



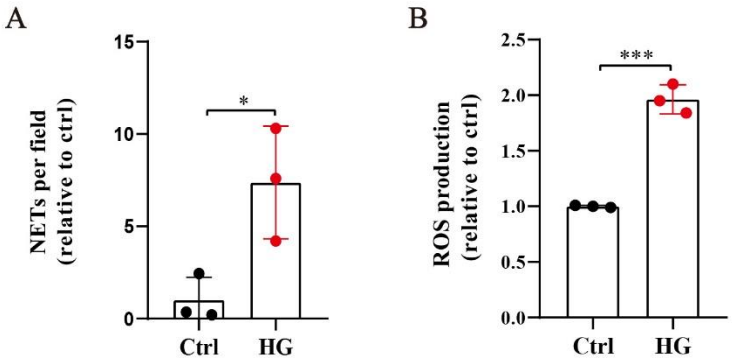
Supplementary Figure 2. Mouse model of STZ-induced diabetes. (A) Schematics of the experimental design. Type 1 diabetes was induced by STZ in 8-week-old C57BL/6 mice, and treatment with DNase I until euthanasia. **(B and C)** Monthly blood glucose and weight measurements of control and diabetic mice. **(D)** Transmission electron microscopy of mouse kidneys. **(E)** Co-localized staining and quantification of CD31 and TUNEL in mouse kidneys. **P*＜0.05; ***P*＜0.01, *****P*＜0.0001.



Supplementary Figure 3. Mouse model of db/db. (A) Schematics of the experimental design. db/db mice, a model of type 2 diabetes, also were treated with DNase I from 8-week-old to euthanasia. **(B and C)** Monthly blood glucose and weight measurements of control and diabetic mice. **(D)** Transmission electron microscopy of mouse kidneys. **(E)** Co-localized staining and quantification of CD31 and TUNEL in mouse kidneys. *$P<0.05$; ***$P<0.001$, ****$P<0.0001$.



Supplementary Figure 4. STZ-induced diabetes model in PAD4$^{-/-}$ mice. (A and B) Blood glucose and weight of WT and PAD4$^{-/-}$ mice with injection of STZ or citrate buffer vehicle. **(C)**Transmission electron microscopy of mouse kidneys. NS, not significant.



Supplementary Figure 5. High glucose induces NETs formation occurs in a ROS-dependent manner. (A) NETs release of human neutrophils incubated with high glucose. **(B)** DCF-DA fluorescence intensity indicating ROS production. *$P<0.05$; ***$P<0.001$.

Supplementary Tables 2. Primer sequences used for qRT-PCR.

Gene name			Sequence (5'->3')
ASC	Homo sapiens	F	CTCAAGAAGTTCAAGCTGAAGC
		R	TAGGTCTCCAGGTAGAAGCTG
NLRP3	Homo sapiens	F	GCTTGCCGACGATGCCTTCC
		R	GCTGTCATTGTCCTGGTGTCTTCC
IL-1β	Homo sapiens	F	CCTGAGCACCTTCTTTCCCTT
		R	GCGTGCAGTTCAGTGATCGTAC
IL-18	Homo sapiens	F	CTGCCAACTCTGGCTGCTAA
		R	TTTGTTGCGAGAGGAAGCGA
GAPDH	Homo sapiens	F	CACCCACTCCTCCACCTTTGA
		R	CCACCCTGTTGCTGTAGCCA

RESEARCH DESIGN AND METHODS

Electron microscopy

Kidney tissues used for transmission electron microscopy were prepared and detected by the electron microscope lab of Chongqing Medical University. The samples were harvested and fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer for 24 h at 4 °C. The samples were then sectioned and placed on copper mesh grids and then observed under transmission electron microscopy (JEOL, 100 CXII). Cells used for scanning electron microscopy were fixed with 2% glutaraldehyde, dehydrated with increasing concentrations of ethanol, dried in a CO2 critical point dryer, coated with conductive material, and then observed by scanning electron microscopy (Hitachi, SU8010).

Reactive oxygen species detection

Human blood neutrophils were incubated with 10 μM DCFH-DA (Beyotime, S0033S) in 1640 medium containing penicillin/streptomycin without serum for 20 mins. The cells were washed three times with serum-free cell culture medium and then detected by a fluorescence microplate reader (Synergy H1).

Cell viability assay

A Cell Counting Kit-8 (CCK-8) assay (Beyotime, C0038) was used to detect the viability of HRGECs. HRGECs were inoculated onto 96-well plates and incubated with different concentrations of NETs for 24 h. Ten microliters of CCK-8 was added

to the culture medium and incubated at 37 °C for 2 hours. The absorbance of each well was measured by a microplate reader (Thermo Scientific MULTISKAN GO) at 450 nm.

Quantitative real-time PCR (qRT–PCR)

Total RNA from cells was extracted using TRIzol reagent (Takara) according to the manufacturer's instructions. cDNA synthesis was performed using EvoScript Universal cDNA Master Mix (Roche, 07912455001) according to the manufacturer's instructions. FastStart Essential DNA Green Master Mix (Roche, 06924204001) was used to quantify the relative mRNA levels of ASC, NLRP3, interleukin-1β (IL-1β), and interleukin 18 (IL-18). qRT–PCR was performed with a CFX96TM Real-Time System (BIO-RAD), with GAPDH serving as an internal control. The primer sequences are listed in Supplemental Table 2.