Electronic Supplementary Material

Methods

Use of experimental animals-ethic statement

The animals were allowed to acclimatize to laboratory conditions for a period of at least one week before any experimental procedures were initiated. They were housed in individually ventilated cages lined with an absorbent bedding material with no more than 6 mice per cage. The room temperature and humidity was maintained at 19°C–23°C and 55%, respectively. All animals had free access to a standard diet and water *ad libitum*. The feeding boxes were cleaned and disinfected every 3 days, and the water was changed on a daily basis to prevent infectious diseases. Animals were inspected for signs of illness and/or unusual behavior by research staff at least once per day.

Human study population

T1DM was defined as onset of diabetes before the age of 25 and the need for continued insulin therapy within 3 months of diagnosis. Patients with diabetic nephropathy (DN+) had a documented positive history of early morning urine albumin creatinine ratio (ACR) ≥3.5 mg/mmol, arterial hypertension defined as treatment with on one or more anti-hypertensive agents and/or blood pressure (BP) ≥130/80mmHg, and diabetic retinopathy but no other kidney or urinary tract disease. Patients without diabetic nephropathy (MA-) had a history of normal ACR (<2.5mg/mmol in men and <3.5mg/mmol in women), normal BP (<130/80 mmHg) on no antihypertensive agents and duration of diabetes exceeding 20 years. All patients had preserved renal function (serum creatinine <150μmol/L), and a positive

diagnosis of diabetic retinopathy. Healthy donors are defined as having no diagnosis of diabetes and no history of hypertension BP <135/80mmHg. Other exclusion criteria include a history of cancer or current treatment for cancer, other auto-immune diseases and acute infection.

ELISA for ANXA1

A homemade sandwich ELISA was used to measure serum and tissue levels of ANXA1. Briefly, ELISA-treated plates (Nunc MaxiSorp, ThermoScientific, UK) were incubated overnight with capture antibody 20µg/ml (mouse monoclonal antibody, generated in house) in bicarbonate buffer (25 mM $NaHCO_3$, 25 mM $Na2CO_3$, pH 9.6). The plate was then washed 3 times with bicarbonate buffer and blocked in blocking buffer (0.1 % BSA, PBS) for 1 h at 37 °C. Then 100 µl of sample and standard in assay diluent (Tween-20 0.05 % (v/v), PBS) where loaded and incubated for 1 h at 37 °C, then washed 5 times with wash buffer (0.9% (w/v) NaCl, 0.05 % (v/v) Tween-20, dH_2O). Following this wells where incubated with 1µg/ml of detecting antibody (rabbit polyclonal anti-ANXA1; Invitrogen, UK) for 1 h at 37 °C. After 5 washes, immuno-complexes were detected by adding the goat-anti-rabbit IgG with conjugated alkaline phosphatase for 30 min. After 5 washes, the substrate, p-Nitrophenyl phosphate (Simga Aldrich, UK) was added and left for 30 min for full development of colour. The plate was then read absorbance at 405 nm and corrected at 540 nm as a reference wavelength, using a spectofluorimeter (Tecan Infinite M200 Pro, Tecan, Austria).

Histological analysis

Kidney samples were obtained at the end of the experiment and fixed in 10 % neutral-buffered formalin for 48 h and histology staining was performed. Briefly, kidney tissue was embedding in paraffin and processed to obtain 4 μm sections. After deparaffinisation and rehydrated through graded alcohols to distilled water. The sections were then incubated with saturated in Periodic Acid Schiffs (Sigma, UK) solution for 30 min. washed in distilled water. Counterstaining was performed with Harris hematoxylin. Or sections were treated with phosphomolybdic acid (Polysciences, Warrington, PA), rinsed in distilled water and stained with picrosirius red (Polysciences, Warrington, PA) for 60 min. Sections were then immediately immersed in hydrochloride acid for 2 min (Polysciences, Warrington, PA). In both cases sections were then dehydrated through graded alcohols and cleared before mounting with coverslips. Images were acquired using a NanoZoomer Digital Pathology Scanner (Hamamatsu Photonics K.K., Japan) and analysed using the NDP Viewer software. The diameter of 20 randomly selected glomeruli where measured in all kidneys at magnification (200×) in a double-blinded manner. Additionally 10 randomly selected fields of view from each kidney were used to assess structural alteration of the proximal convoluted tubules.

Western Blot Analysis

Semi-quantitative immunoblot analyses of phosphorylation of p38, JNK, ERK1/2 and Akt were carried out in tissue samples as described before (42),(43). Briefly, heart and kidney samples were homogenized in protein homogenization buffer and centrifuged at 1300g for 5 min at $4^{O}C$. To obtain the cytosolic protein fraction,

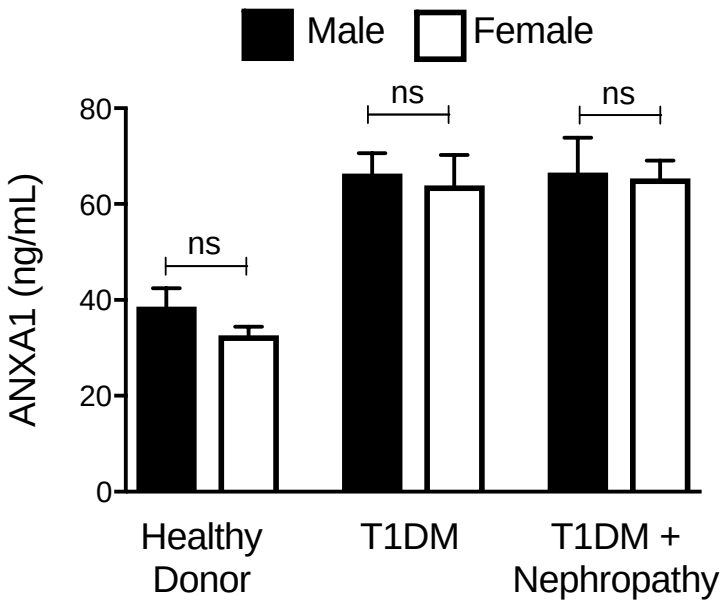
supernatants were centrifuged at 16000g at 4^{O}C for 40 min. Protein content was determined on cytosolic extracts using bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific Inc, Rockford, IL). Proteins were separated by 8% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinyldenediflouoride (PVDF) membrane, which were blocked with a solution of 5% dry milk in TBS-Tween for 2h. Membranes were incubated with a primary antibody [rabbit anti-total Akt (1:1000); mouse anti-pSer^{473} Akt (1:1000); rabbit anti-total p38 (1:1000); rabbit anti-pThr^{180}/Tyr^{182} p38 (1:1000); rabbit anti-total JNK (1:1000); rabbit anti-pThr^{183}/Tyr^{185} JNK(1:1000); rabbit anti-total ERK1/2 (1:1000); mouse anti-pTyr^{202}/Tyr^{204} ERK1/2 (1:2000)]. Membranes were incubated with a secondary antibody conjugated with horseradish peroxidase (1:2000) for 30 min at room temperature and developed with ECL detection system. The immunoreactive bands were visualised by autoradiography and the densitometry analysis was performed using Gel Pro Analyser 4.5, 2000 software (Media Cybernetics, Silver Spring, MD, USA). The membranes were stripped and incubated with alpha tubulin monoclonal antibody (1:5000) and subsequently with an anti-mouse antibody (1:2000) to assess gel-loading homogeneity. Densitometry analysis of the related bands is expressed as relative optical density, and normalised using the related WT + vehicle or sham band.

ESM Table 1

	Healthy donors	Diabetic (no nephropathy)	Diabetic (plus nephropathy)
Patients (n)	20	20	22
Sex (F %)	9(45)	13(65)	11(50)
Age (year)	48.3±1.9	42.2±2.2	54.5±2.3
Diabetic duration (year)	n/a	30.5±2.2	38.7±2.1 *
Creatinine (mmol/l)	n/a	64.9±3.2	93.7±1229*
BMI (kg/m^2)	n/a	26.8±0.9	26.3±0.8
Serum CRP (pg/m)	n/a	627.8±80.6	1469.2±225.2*

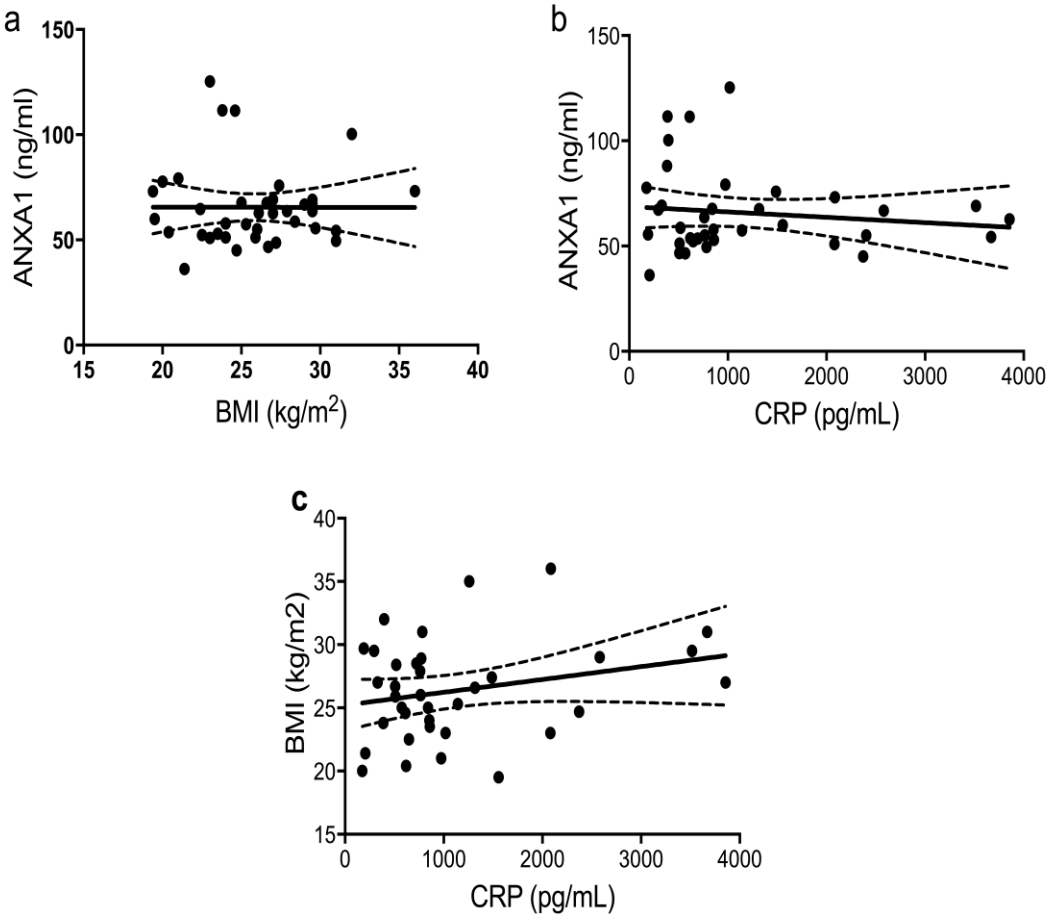
ESM Table 1. Detail of patients included in the study. Number of patients per group, sex, age, diabetic duration and serum creatinine level, BMI and plasma CRP. Experimental groups: Healthy donors, patients with type 1 diabetes without diabetic nephropathy and patients with type 1 diabetes with diabetic nephropathy. Data analyzed by Student's t-test and expressed as mean±SEM. *$p<0.05$ vs. Diabetic (no nephropathy).

ESM Figure 1



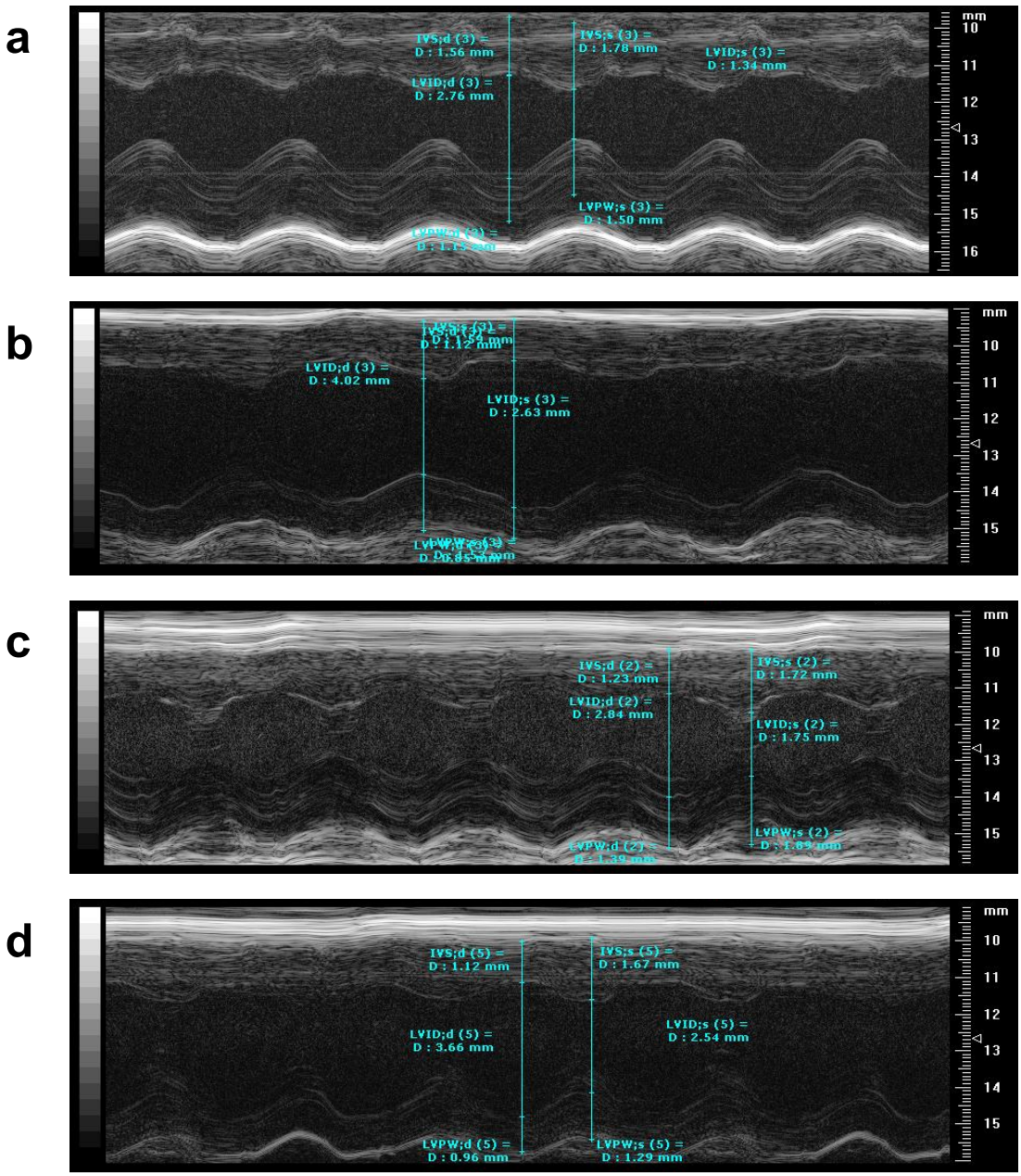
ESM Figure 1. Plasma ANXA1 concentration measured in healthy donors and patients with type 1 diabetes with/without nephropathy. Data analyzed by a one-way ANOVA followed by a Bonferroni *post-hoc* test, and expressed as mean ± SEM.

ESM Figure 2.



ESM figure 2. Correlation data show (A) plasma ANXA1 vs. plasma CRP and (B) plasma ANXA1 vs. BMI and (C) BMI vs. plasma CRP in patients with type 1 diabetes with/without nephropathy. Linear regressions were calculated by the least squares method and their significance estimated by a Fisher F test. $P<0.05$ was considered to be significant.

ESM Figure 3.

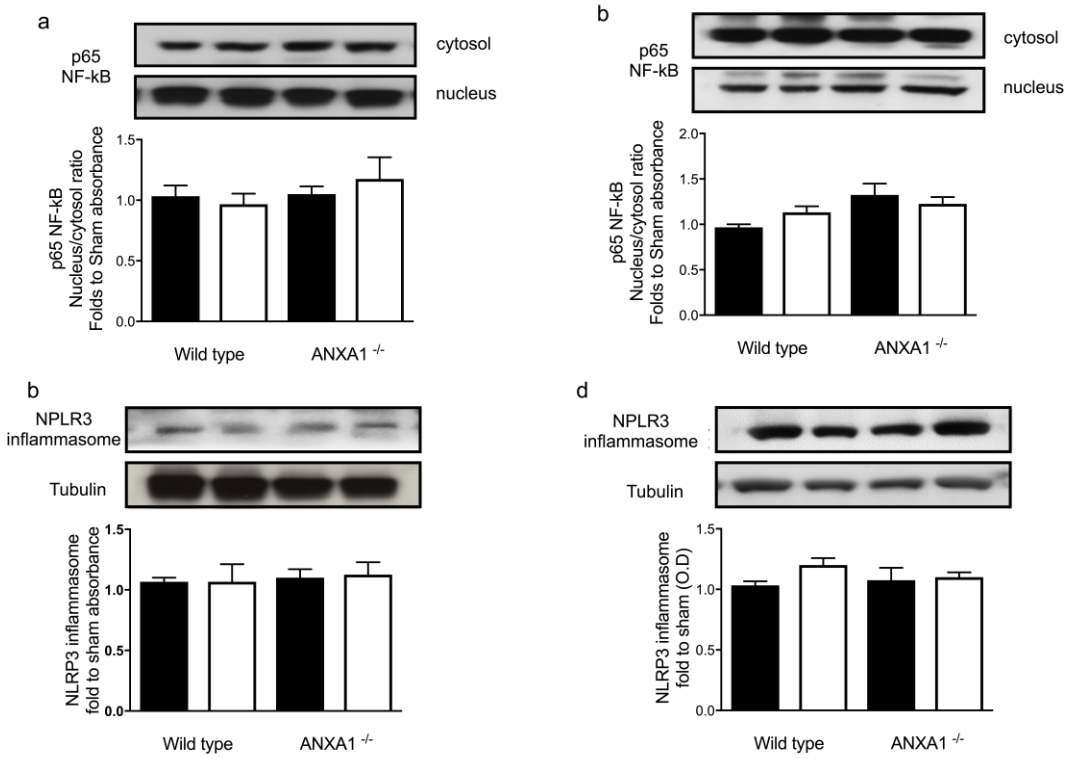


ESM Figure 3.

Representative M-mode images from echocardiography WT + Veh (a) WT + STZ (b) ANXA1$^{-/-}$ + Veh (c) and ANXA1$^{-/-}$ + STZ (d). Ejection fraction (EF) and fractional shortening (FS) are calculated using 3 measurements intraventricular septum (IVS), left ventricular internal dimension (LVID) and left ventricular posterior wall (LVPW).

Values were taken at the level of the papillary muscles in both systolic (s) and diastolic (d) phases.

ESM figure 4.



ESM figure 4. Western blot data showing p65 nuclear translocation from cytosol to nucleus (A and B) and inflammasome assembly (C and D) in the heart (A and C) and kidney (B and D).