Supplemental Material

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1. Supplementary Methods

Patients and Samples

Thirty patients with diabetes and biopsy-proven DN diagnosed at Peking University First Hospital, were enrolled in this study. Patients with other known kidney diseases were excluded. Routine clinical and pathological data of these patients were obtained. Kidney biopsy samples were collected to measure ANXA1 protein expression in kidneys. Urine and plasma samples were collected on the day of kidney biopsy for detecting urinary ANXA1 levels and plasma ANXA1 levels, accordingly. Control kidney samples (n=7) were obtained from the healthy kidney poles of individuals receiving tumor nephrectomies without diabetes or kidney diseases. We also collected urine and plasma samples from 18 DM patients without kidney injuries from the outpatient clinic of the Department of Endocrinology of our hospital. Urine and plasma samples of 10 age- and sex-matched healthy donors were collected. These healthy controls did not have a history of hypertension, diabetes, cardiovascular diseases, and kidney diseases.

Kidney biopsy samples from patients with DN (n=17) were obtained from the European Renal cDNA Bank (ERCB) for gene expression profiling.[1] The ERCB is a multicenter study established to collect kidney biopsy tissues for gene expression analysis at the time of a clinically indicated biopsy. Biopsies were obtained from patients after informed consent with approval of the local ethics committees. Tissue obtained from healthy living transplant donors (n=21) was used as the control group. Kidney tissues from ERCB were processed prior to transcriptional profiling as

previously described.[2] Collected kidney tissues were stored in RNAlater (ThermoFisher Scientific) and manually microdissected into glomerular and tubulointerstitial compartments. Tubulointerstitial transcriptional data were used to assess the reliability of microdissection, targeting 16-fold to 64-fold enrichment of tubulointerstitial-selective transcripts. RNA from each compartment was processed and analyzed using Affymetrix GeneChip Human Genome U133A and U133 Plus 2.0 platforms. Tubulointerstitial transcriptome data for patients from ERCB cohort have been deposited into GEO under the accession number, GSE104954.

Protocol biopsy specimens were collected from 69 American Indians with type 2 diabetes enrolled in Renoprotection in Early Diabetic Nephropathy in Pima Indians trial (Clinicaltrials.gov identifier NCT00340678).[3] The participants were followed annually for routine measurements of albuminuria and GFR. GFR was measured by the urinary clearance of iothalamate (iGFR).[4] Healthy tissues obtained from living transplant donors (n=18) were used as the control group. Owing to ethical considerations, privacy protection, and to avoid identifying individual study participants in this vulnerable population, the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases has stipulated that individual-level gene expression and genotype data from the Pima Indian study cannot be made publicly available.

Experimental Animals

The DNA sequencing results showed a 10-base deletion of *Anxa1* in heterozygous knockout (KO) mice (Supplementary Figure S1a). Total protein from

mice liver tissues was subjected to Western blot, and total RNAs from mice kidney tissues were subjected to quantitative PCR (qPCR) analysis. The results showed that ANXA1 expression was not detected in *Anxa1* KO mice (Supplementary Figure S1c and d).

To further test for a protective role of ANXA1, mice with transgenic overexpression of the *Anxa1* transgene (*Anxa1*-Tg) were generated. We performed agarose gel-electrophoresis on DNA isolated from wild type and transgenic animals. As demonstrated in Supplementary Figure S1b, *Anxa1* transgenic mice expressed a 461bp transgenic marker compared with WT mice. ANXA1 protein level was also overexpressed in *Anxa1*-Tg mice, demonstrated by Western blot and qPCR (Supplementary Figure S1c and e).

Animal Studies

WT mice, *Anxa1* knockout mice and *Anxa1* transgenic mice were fed with a high-fat diet (HFD) (60% fat, HfkBio, China) for 1 month, followed by intraperitoneal injection of 50 mg/kg streptozotocin (STZ) (S0130; Sigma-Aldrich Corp.) for 5 days. Mice were maintained for 20 weeks of HFD feeding. Mice fed with a standard fat diet (SFD: 10% fat) were used as non-diabetic controls. In another set of experiments, WT and Anxa1 KO mice were randomly assigned either SFD or HFD for 16 weeks.

For the treatment study in db/db mice, 10 weeks db/db mice were maintained on the Ac2-26 (0.5 mg/kg, 1 mg/kg, 2 mg/kg, in PBS) or vehicle (PBS) treatment for 10 weeks (every 48 hours, intraperitoneally). Age-matched db/m mice treated with

vehicle served as a non-diabetic control group. The Ac2-26 peptide was synthesized by GL Biochem (China) and the purity of Ac2-26 was 96.47%. For the treatment study in diabetic *Anxa1* KO mice, all four mice groups were induced DN with HFD/STZ as above described. After STZ injection, mice were maintained for 16 weeks of HFD feeding. At the same time, one group of diabetic *Anxa1* KO mice and one group of diabetic WT mice were maintained on the Ac2-26 treatment (2 mg/kg, in PBS) and the other 2 groups of mice were treated with vehicle (PBS) for 16 weeks (every 48 hours, intraperitoneally).

Blood and Urine Examination

The concentration of urinary and plasma ANXA1 was measured using Human ANXA1 ELISA kit according to the manufacturer's instructions (ab222868; Abcam). Urine albumin concentration was measured using a kit from Bethyl Laboratories, Inc. (E99-134). Urine creatinine level was measured using Creatinine Companion kit (#1012; Exocell). The level of urine albumin was normalized to urine creatinine (uACR). Serum creatinine (Scr) and blood urea nitrogen (BUN) were measured by the automated biochemical analyzer (Beckman Coulter Inc.). Blood glucose, blood cholesterol and triglyceride levels were determined using commercial kits (#240, #180, #220; Biosino).

Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT) Test

After 16 weeks of feeding, HFD mice fasted overnight were intraperitoneally injected with glucose (1 g/kg) or insulin (0.5 units/kg) for the glucose tolerance tests and insulin tolerance tests. Blood was collected from the tail vein at 0, 15, 30, 60, 90,

and 120 min after injection. The blood glucose level was measured using commercial kits (Biosino).

Histological Analysis

For kidney tissues of patients, the presence of sclerotic glomeruli was calculated as the percentage of the total number of glomeruli in the biopsy samples. Tubulointerstitial inflammation was scored semi-quantitatively, based on the percentage of the tubulointerstitial compartment affected (0: none, 1: < 25%, 2: 25–50%, 3: > 50%).[5]

Twenty glomeruli per kidney section were analyzed under 400 magnification for mice kidney tissues. Glomerular area was measured by tracing around the perimeter of the glomerular tuft. Mesangial matrix expansion area was defined as the PAS-positive and nuclei-free area in the mesangium and expressed as a ratio to a total glomerular area. The positive staining signals were quantified using Image-Pro Plus (Media Cybernetics, Bethesda, MD). The tubulointerstitial injury index was assessed by evaluating the amount and severity of tubule dilation, atrophy, and loss of tubular cells. Twenty views of a kidney section were photographed (magnification, ×200) and scored with 0 for no injury, 1 for < 25%, 2 for 25%-50%, 3 for 50%-75%, and 4 for > 75% of the proportion with tubulointerstitial injury.[6] Histological analysis was performed in a blinded manner. For the evaluation of fibrosis, kidney tissues were stained with Sirius Red (G1470; Solarbio) in accordance with the manufacturer's protocols.

Immunohistochemistry and Immunofluorescence

Paraffin-embedded kidney sections were stained with antibodies against α-smooth muscle actin (α-SMA) (ab32575; Abcam), and F4/80 (MCA497; Serotec). Immunofluorescence staining was performed using anti-ANXA1 (ab214486; Abcam), anti-podocalyxin (MAB1658; R&D Systems), anti-AQP-1 (sc-25287; Santa Cruz), anti-integrin α8 (sc-365798; Santa Cruz), anti-CD68 (ab955; Abcam), and anti-CD31 antibodies (sc-376764; Santa Cruz).

Transmission Electron Microscopy

Briefly, kidney tissues for electron microscopy were fixed in 3% glutaraldehyde and 1% osmium tetroxide (OsO4), followed by dehydration in graded ethanol series and washed with acetone, and finally embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate. Sections were then examined with a transmission electron microscope (JEM-1230; JEOL, Japan). Image-Pro Plus was used to measure the GBM thickness, foot process width and endothelial fenestrations.[7-9] All glomeruli were selected from each mouse and fifteen electron micrographs were taken in each glomerulus.

Real-time quantitative Polymerase Chain Reaction (qPCR) analysis

Total RNA was extracted from kidney tissues of HFD/STZ *Anxa1* KO mice and HFD/STZ WT mice. Mouse Inflammatory Cytokines and Receptors RT^2 Profiler PCR Array System were used (PAMM-011A; SABiosciences Corporation).

Total RNA was extracted from cultured cells or mouse kidney tissues. Real-time qPCR was performed using SYBR Green reagent (A25742; Applied Biosystems).

Relative gene expression was obtained after normalization with β-actin and followed by comparison with the control groups.

Flow Cytometry

We sacrificed the mice and harvested the right kidneys. After washing in PBS, the kidneys were cut into pieces and ground with homogenizer until there was no visible massive tissue. Washed the tissue with PBS three times and centrifuged at 1000 rpm for 3 minutes. Filtered the tissue to remove residue and collected the single cell suspension. Diluted 100μl single cell suspension to 1ml with PBS and incubated with FITC-CD86/PE-CD206 antibody (Biolegend) for 30min at room temperature. Flow cytometry was performed using BD FACS Calibur.

Cell Culture and Treatment

HK-2 cells were cultured in Dulbecco's modified Eagle medium/nutrient mixture F-12. One day before transfection, cells were plated and grown to ~50% confluence. HK-2 cells were transfected with siRNAs targeting human ANXA1 (HSS100503; Invitrogen) using X-tremeGENE siRNA Transfection Reagent (#4476093001; Roche Applied Science) according to the manufacturer's instructions. Scrambled siRNAs (#12935200; Invitrogen) were applied to parallel cultures as negative controls. When cell confluency reached to 80% confluence, cells were stimulated with high glucose and palmitic acid (HGPA; final concentration 30 mmol/l glucose and 300 μmol/l of the saturated free fatty acid palmitate [16:0]) for 24 h then harvested.[10] The blank group (containing only the medium) was served as the normal control group. The

control group was served as the isotonic solvent control group using mannitol and endotoxin-free BSA.

To investigate the pro-resolution function of Ac2-26 in HK-2 cells, cells were stimulated with HGPA and treated with Ac2-26 (15 μmol/l) for 36 h. In addition, HK-2 cells were pretreated with WRW4 (10 μmol/l; MeilunBio), known to antagonize ALX/FPR2,[11] or Boc-MLF (1 μmol/l; MeilunBio), known to selectively antagonize FPR1, or Boc-MLF (10 μmol/l), as pan-FPR antagonist,[12] prior to Ac2-26 treatment.

Western Blot

Total proteins were extracted from cultured HK-2 cells or mice kidney tissues using RIPA buffer (150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP40, 1 mM EDTA and 50 mM Tris pH 8.0). Equal amounts of protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Then samples were transferred to PVDF membrane and incubated with the primary antibody in 5% milk. After blocking with 5% milk, the membranes were incubated with antibodies against NF-κB p65 (ab32536; Abcam), NF-κB p65 (phospho S536) (ab76302; Abcam), IκBα (#9242; Cell Signaling Technology), phospho-IκBα (#2859; Cell Signaling Technology), ANXA1 (ab214486; Abcam), β-actin (ab6276; Abcam), and GAPDH (ab181602; Abcam) and subsequently incubated with peroxidase-conjugated secondary antibodies (Proteintech). Proteins were visualized using the chemiluminescence substrate kit (Millipore). Target proteins were quantified using Image J software.

Co-immunoprecipitation (Co-IP)

Co-IP was carried out using a Pierce Crosslink Magnetic Co-IP kit (#88805; ThermoFisher Scientific). Briefly, Cells or kidney tissues were lysed and the protein concentrations were measured. Then, 500 μg of protein in 500 μl of supernatant was incubated with 5 μg anti-p65, anti-ANXA1 or anti-IgG antibodies coated on beads on a rotator overnight at 4°C. The beads were washed to remove non-bound material and eluted in a low-pH elution buffer that could dissociate bound antigen from the antibody-crosslinked beads. The precipitate was separated by SDS-PAGE and detected by immunoblotting.

Electrophoretic Mobility Shift Assay (EMSA)

The p65 double-stranded oligonucleotide probe was synthesized (5'-C A T C G G A A A T T T C C G G A A A T T T C C G G A A A T T T C C G G C -3' / 5' -G C C G G A A A T T T C C G G A A A T T T C C G G A A A T T T C C G A T G -3') and the 3' and 5' ends of p65 were labeled with biotin (Takara Bio Inc.).[13] Nuclear extracts were extracted using NE-PER Nuclear and Cytoplasmic Extraction kit (ThermoFisher Scientific) according to the manufacturer's instructions. The nuclear extract proteins (5 μg) and biotin-labeled p65 probes (20 fmol) were incubated together for 20 min at room temperature, followed by loading onto a native 4% polyacrylamide gels using 0.5×Tris-borate-EDTA. The gel was pre-run and run with 0.5% Tris-Borate-EDTA and processed according to the manufacturer's instructions. Competition experiments were done by co-incubation of a 200-fold excess (4 pmol) of unlabeled double-stranded oligonucleotide in the DNA-protein binding reaction. For supershift

analysis, 4 μg of anti-p65 antibody (Abcam) were pre-incubated for 30 min at room temperature before the binding reaction.

Surface Plasmon Resonance (SPR) Analysis

The ligand, p65 (Lifespan Bio., Seattle WA, USA), was immobilized onto a CM5 sensor chip surface (GE Healthcare). The carboxymethyl dextran surface of the CM5 sensor chip was first activated by a 6-min injection of a 1:1 mixture of 0.4 M N-ethyl-N-(3-diethylaminopropyl) carbodiimide and 0.1 M N-hydroxysuccinimide (NHS) at a flow rate of 10 μl/min. Then, a solution of p65 (50 ng/ml; LS-G79230-20; LifeSpan) in immobilization buffer (10 mM sodium acetate, pH 5.0; GE Healthcare) was fluxed over the reactive matrix at a flow rate of 10 μl/min, with the NHS esters of the substrate spontaneously reacting with the protein amines to form covalent links. SPR measurements were performed by using multicycle kinetics which consists of injections of increasing concentrations of the analyte over a functionalized sensor chip surface. Solutions of ANXA1 (#3770-AN-050; R&D Systems) or Ac2-26 of 5 different concentration was made by PBS containing 0.05%(v/v) Tween 20 and ANXA1 protein or Ac2-26 peptide. The solutions were fluxed over the sensor chip surface for 120 s by using a flow rate of 30 μl/min.The binding assay also included three start-up cycles using buffer to equilibrate the surface, as well as a zero concentration cycle of analyte in order to have a blank response usable for double reference subtraction. The data was analyzed using the Bia Evaluation 4.1 software with a simple 1:1 langmuir binding model.

Statistical Analysis

The continuous data were expressed as mean ± s.d., or median and interquartile range (IQR). Categorical data were described by absolute frequencies and percentages. For clinical data, student's *t*-test or Mann-Whitney U test was used as appropriate to compare independent continuous variables. Differences of qualitative results were compared using chi-square test. Correlations between ANXA1 and clinical-pathological parameters were determined by Pearson or Spearman analysis. Cumulative kidney survival curves were generated using the Kaplan-Meier method and compared using the log-rank test. Murine *in vivo* experiments were performed with a group size of n = 5-6. A student's *t*-test was used for a single comparison. For multiple comparisons, data were analyzed by one- or two-way ANOVA as appropriate, with post hoc comparison using Tukey's test. For *in vitro* experiments, at least 3 independent experiments were performed, and statistical significance was determined using Student's *t*-test or two-way ANOVA followed by Turkey's test as appropriate. Analyses were performed with SPSS statistical software package (SPSS 24.0; IBM Corp., Armonk, NY).

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2. Supplementary Figures

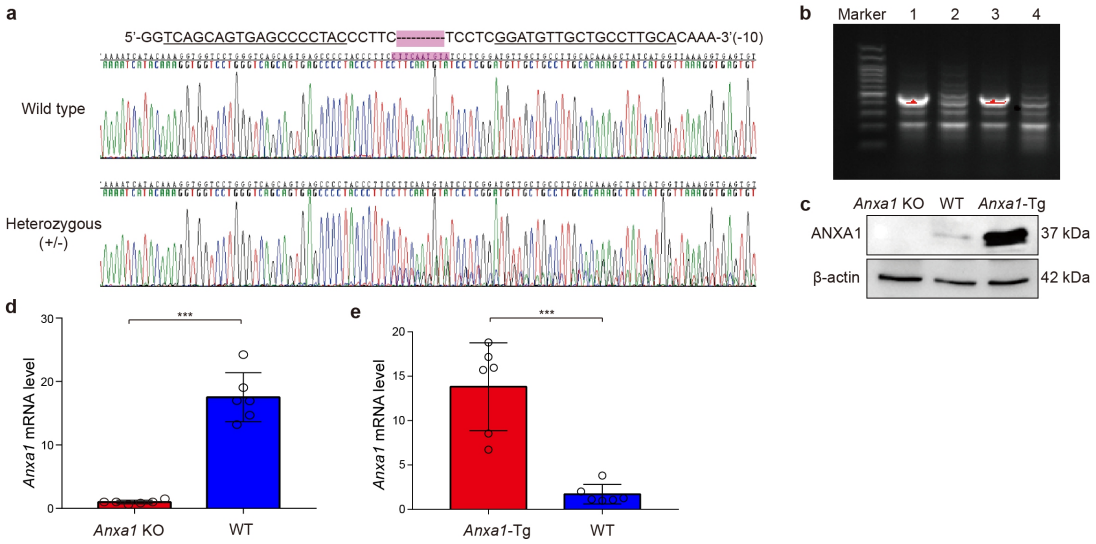

Figure S1. Identification of Annexin A1 (*Anxa1*) knockout mice and *Anxa1* transgenic mice.

(**a**) The DNA sequencing results of WT mice (up) and *Anxa1* knockout heterozygote mice (below). The double peak showed a 10 bases deletion (CTTCAATGTA) in one strand. (**b**) The agarose gel electrophoresis of *Anxa1* transgenic mice (the first and third lines) and WT mice (the second and fourth lines). The positive transgenic marker is 461 base pairs. (**c**) Western blot demonstrated ANXA1 expression was not detected in *Anxa1* KO mice, and overexpressed in *Anxa1* transgenic mice. (**d**) RT-qPCR analysis showing *Anxa1* mRNA levels in kidneys in SFD *Anxa1* KO and SFD WT mice. n = 6. (**e**) RT-qPCR analysis showing *Anxa1* mRNA levels in kidneys in SFD *Anxa1-Tg* mice and SFD WT mice. n = 6. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 determined by Student *t* test. *Anxa1*-Tg, *Anxa1* transgenic; KO, knockout; SFD, standard fat diet; WT, wild type.

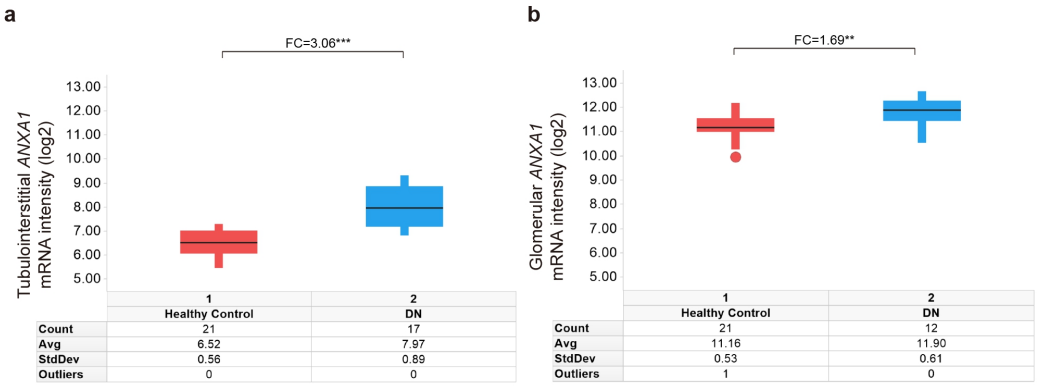

Figure S2. Annexin A1 (*ANXA1*) mRNA in kidney biopsies from patients with diabetic nephropathy (DN).

(**a**) *ANXA1* mRNA (log_2) levels in the tubulointerstitial compartment in advanced DN patient's samples from ERCB (n = 17) and healthy controls (n = 21). (**b**) *ANXA1* mRNA (log_2) levels in the glomerular compartment in advanced DN patient's samples from ERCB (n = 12, who had glomerular data among 17 DN patients) and healthy controls (n = 21). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. FC, fold change; HC, healthy controls.

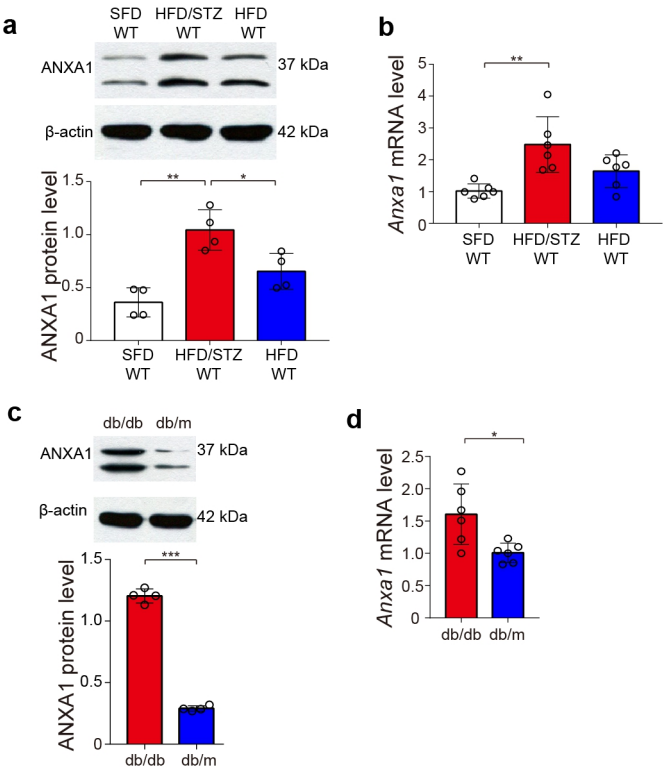

Figure S3. Western blot and RT-qPCR were used to detect the expression of ANXA1 in experimental animals.

(**a**) Western blot and (**b**) RT-qPCR analyzing the ANXA1 levels in kidneys of SFD WT mice (left), HFD/STZ WT mice (mid), and HFD WT mice (right). (**c**) Western blot and (**d**) RT-qPCR analyzing the ANXA1 levels in kidneys of db/db mice (left) and db/m mice (right). Data analyses were performed by Student *t* test for 2 groups and 1-way ANOVA followed by a Tukey test for 3 groups. Data expressed as mean ± SD.*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

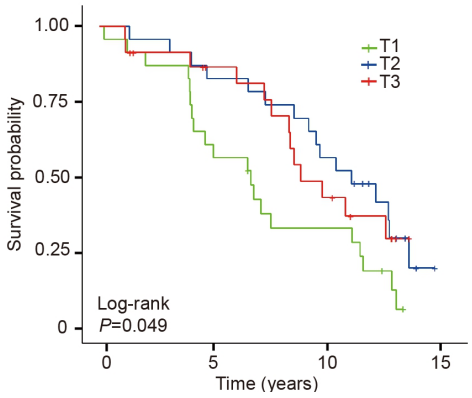

Figure S4. *ANXA1* mRNA levels associated with kidney outcomes.
According to tertiles, Kaplan-Meier survival curves depicting kidney survival in low (T1), medium (T2), and high (T3) *ANXA1* mRNA groups (log-rank test) in the Pima Indians cohort during a follow-up of 9.0 ± 4.0 years.

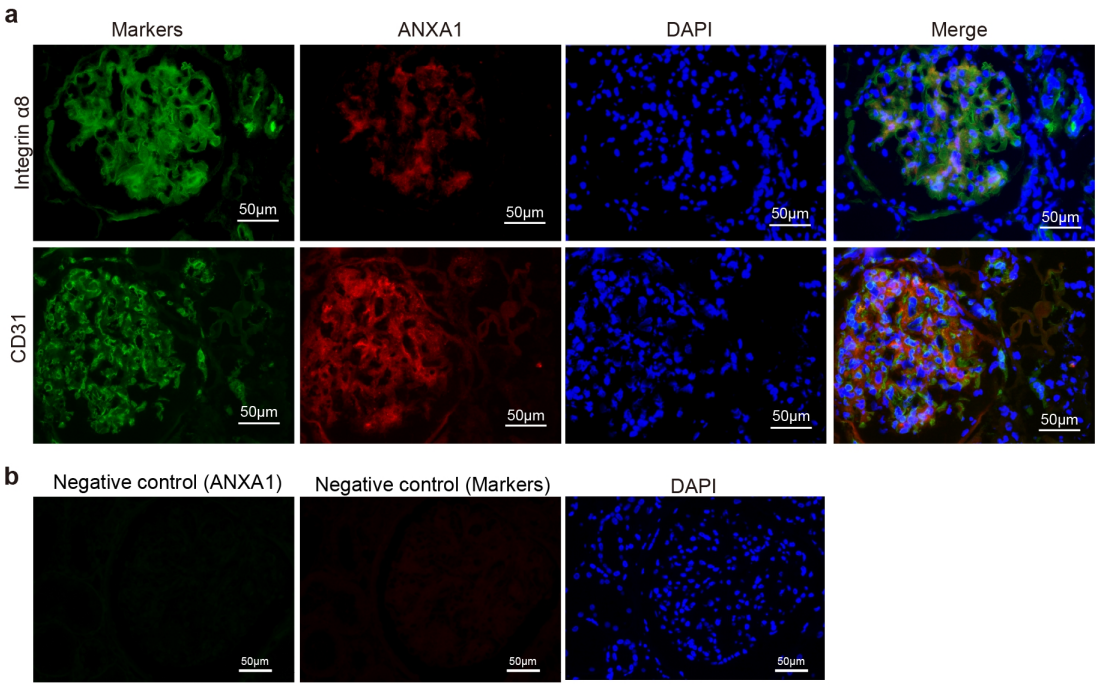

Figure S5. Immunofluorescence colocalization analyses.

(**a**) Representative fluorescent images showed the expression of ANXA1 in mesangial cells (integrin α8) and endothelial cells (CD31) in the kidneys from DN patients. (**b**) For colocalization experiments, the negative control without primary antibody was used and no background fluorescence was observed. Bar = 50 μm.

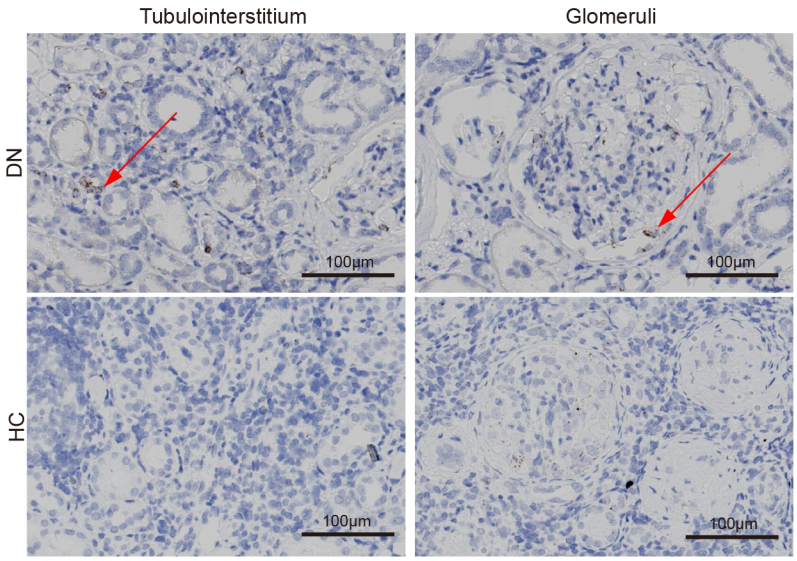

Figure S6. *In situ* hybridization (ISH) analyses were performed to detect the source of ANXA1.

(Top) ISH showed that ANXA1 was mainly expressed in parietal epithelial cells and tubular epithelial cells in kidneys from DN patients (red arrow). (Bottom) There was only a minor amount of ANXA1 expression in kidneys from healthy controls (HCs). Bar = 100 μm.

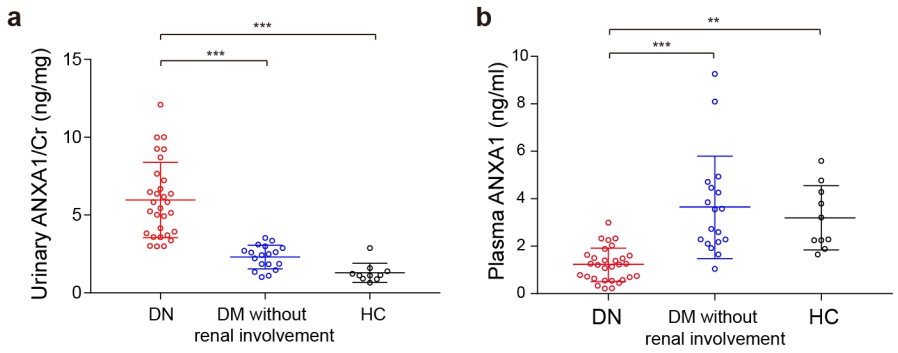

Figure S7. Urine and plasma levels of ANXA1 in patients with DN.

(**a**) The level of urinary ANXA1/Cr in DN patients was significantly higher than that in DM patients without kidney involvement and healthy controls. (**b**) The level of plasma ANXA1 in DN patients was significantly lower than that in DM patients without kidney involvement and healthy controls. Data analyses were performed by 1-way ANOVA followed by a Tukey test for 3 groups and expressed as mean ± SD. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

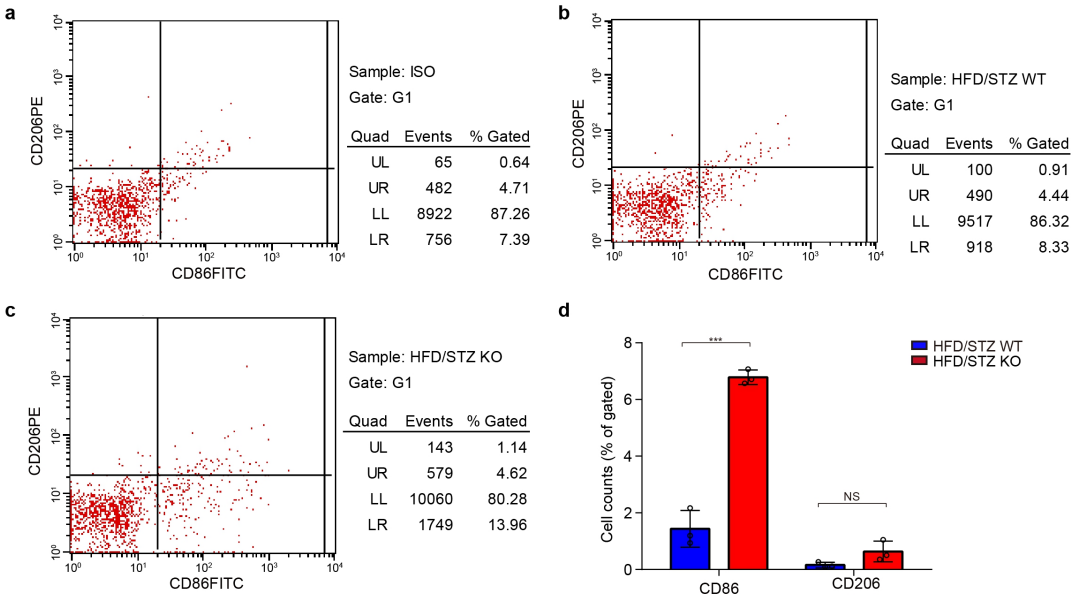

Figure S8. Flow cytometry testing M1 and M2 macrophages in HFD/STZ *Anxa1* KO mice and HFD/STZ WT mice.

Representative flow cytometry results of (**a**) isotype control (ISO), (**b**) HFD/STZ WT mice and (**c**) HFD/STZ *Anxa1* KO mice. (**d**) Statistical data for flow cytometry results. Data analyses were performed by Student *t* test. ***$P < 0.001$.

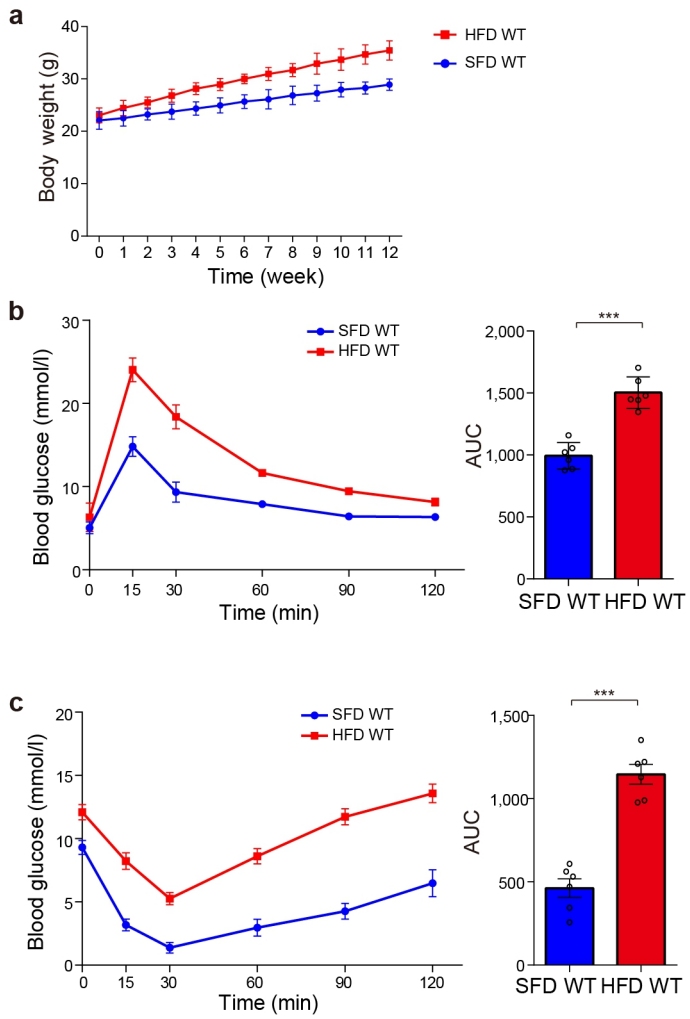

Figure S9. HFD-induced diabetic mice had impaired glucose tolerance and insulin tolerance.

(**a**) Body weight in SFD WT mice and HFD WT mice (n = 6). (**b**) Glucose tolerance in SFD WT mice and HFD WT mice (n = 6). (**c**) Insulin tolerance in WT mice and HFD mice (n = 6). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 determined by Student *t* test. HFD, high fat diet; SFD, standard fat diet.

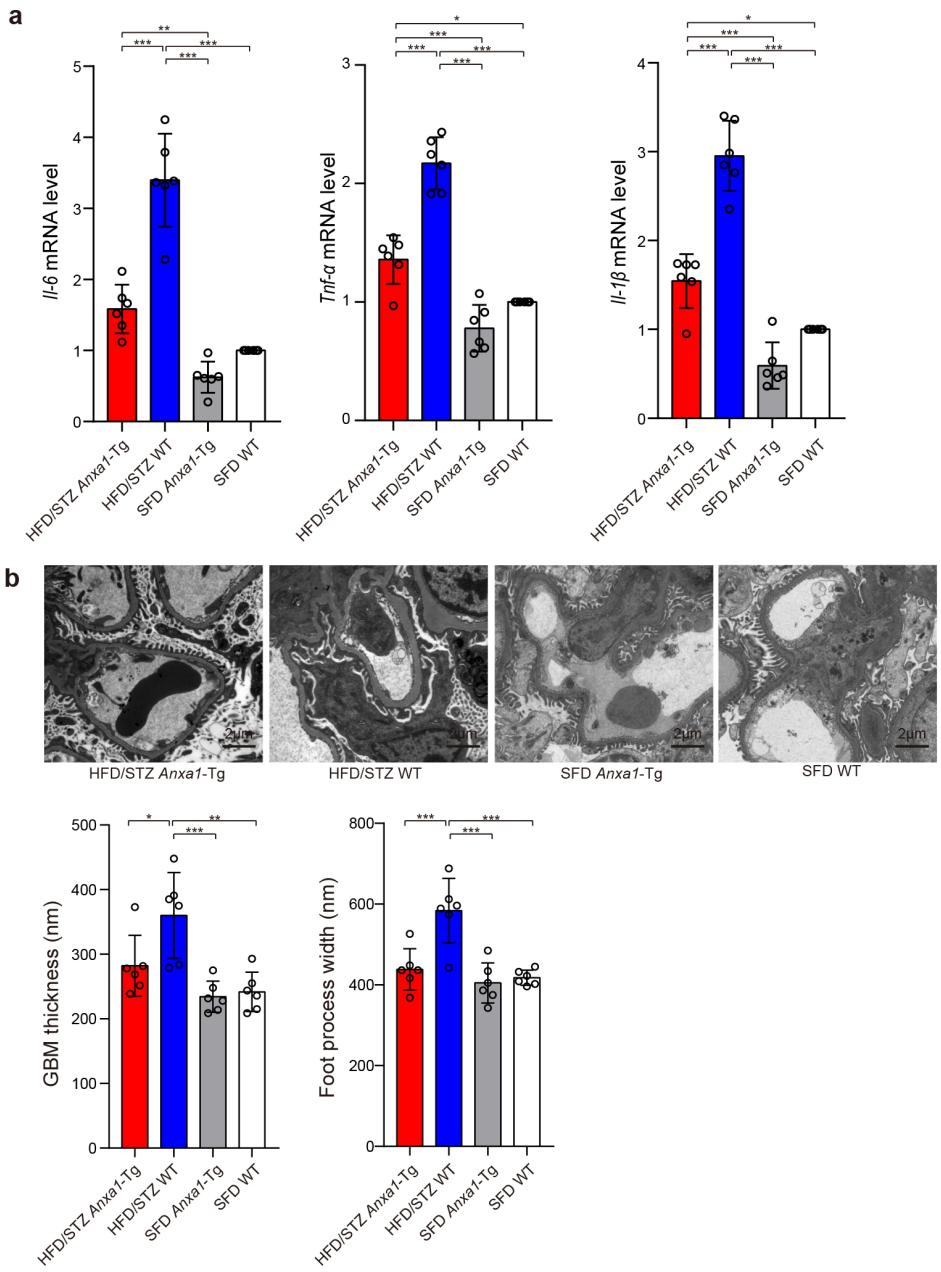

Figure S10. Overexpression of ANXA1 reduced the expression of inflammatory factors.

(**a**) RT-qPCR analyzed the mRNA expression level of interleukin-6 (*Il-6*), tumor necrosis factor-α (*Tnf-α*) and interleukin-1β (*Il-1β*) in *Anxa1*-Tg mice and WT mice (n = 6 per group) (**b**) Representative transmission electron microscopy images from WT and *Anxa1*-Tg mice. Bar = 2 μm. Quantification of mean foot process width and quantification of mean glomerular basement membrane (GBM) thickness. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 determined by 2-way ANOVA followed by Tukey test. *Anxa1*-Tg, *Anxa1* transgenic; SFD, standard fat diet.

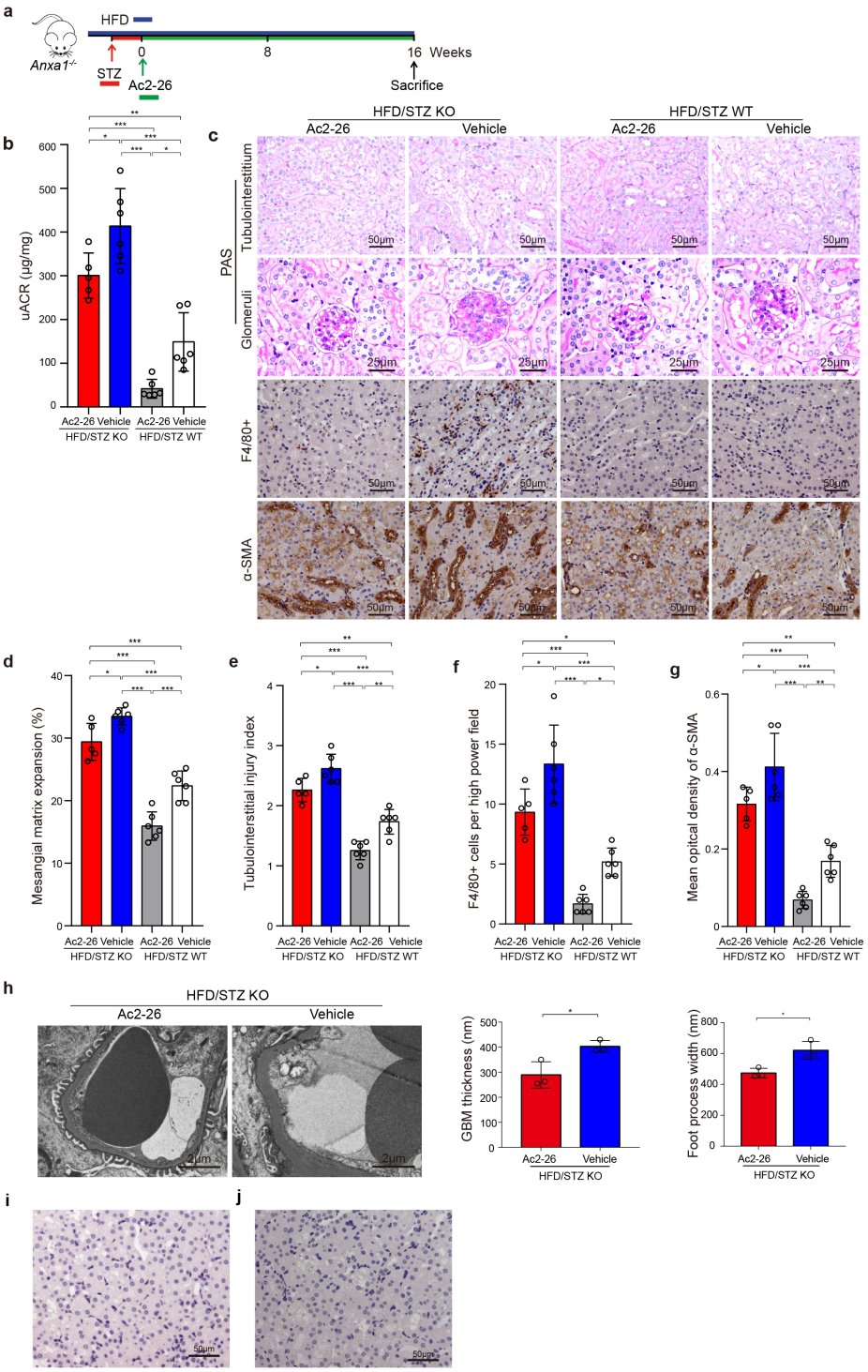

Figure S11. Ac2-26 administration ameliorated inflammation in diabetic *Anxa1* KO mice.

(**a**) Study design overview. Eight-week-old *Anxa1* KO mice were fed with a high-fat diet for 1 month, followed by i.p. injection of 50 mg/kg STZ for 5 days. After STZ injection, mice were maintained for 16 weeks of HFD feeding with Ac2-26 treatment or with vehicle (PBS) before sacrifice. n = 5 to 6 per group. (**b**) Four groups were evaluated for urine albumin-to-creatinine ratio (uACR). (**c**) Representative photomicrographs of periodic acid-Schiff (PAS) staining (bar = 50 μm for

tubulointerstitium and bar = 25 μm for glomeruli), F4/80 staining (bar = 50 μm), and α-SMA (bar = 50 μm). (**d**) Quantification of mesangial matrix expansion. (**e**) Quantification of tubulointerstitial injury index. (**f**) Quantitative analysis of F4/80 staining. (**g**) Quantitative analysis of α-SMA expression. (**h**) Representative transmission electron microscopy (TEM) images in kidney cortex tissues isolated from diabetic *Anxa1* KO mice with and without Ac2-26 treatment. Quantification of mean glomerular basement membrane (GBM) thickness, and mean foot process width in 2 groups (n = 3). Bar = 2 μm. (**i**) Immunohistochemical staining for F4/80 with the negative control in kidney tissues from HFD/STZ-induced diabetic mice. (**j**) Immunohistochemical staining for α-SMA with the negative control in kidney tissues from HFD/STZ-induced diabetic mice. Throughout, data analyses were performed by Student *t* test for 2 groups and 2-way ANOVA followed by a Tukey test for 4 groups. Data expressed as mean ± SD. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. KO, knockout; WT, wild type.

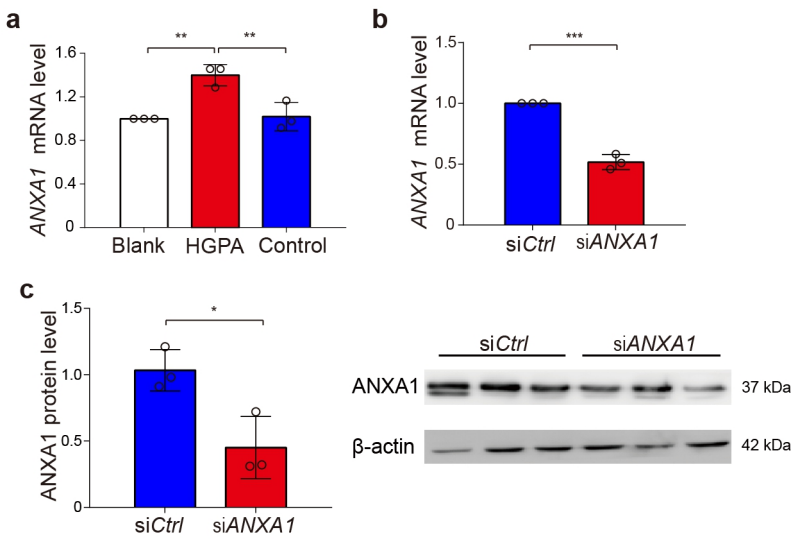

Figure S12. RT-qPCR and Western blot were used to detect the expression of ANXA1 in HK-2 cells.

(**a**) *ANXA1* mRNA levels were analyzed by RT-qPCR in HK-2 cells with HGPA treatment (n = 3). (**b**) RT-qPCR and (**c**) Western blot analyzing the expression level of ANXA1 in HK-2 cells (n = 3). The blank group was served as a normal control group. Mannitol and endotoxin-free BSA served as an isotonic solvent control group. Data analyses were performed by Student *t* test for 2 groups and 1-way ANOVA followed by a Tukey test for multiple groups. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. HGPA, high glucose plus palmitic acid; si*ANXA1*, siRNA against ANXA1; si*Ctrl*, negative control siRNA.

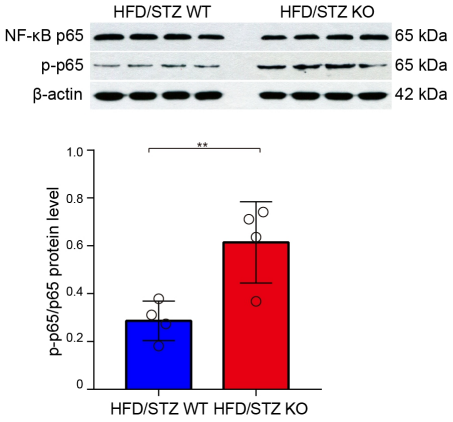

Figure S13. The phosphorylation levels of NF-κB p65.

Representative Western blot images and the analyses of phosphorylated p65 and p65 in HFD/STZ WT mice and HFD/STZ *Anxa1* KO mice. $^{**}P < 0.01$, determined by Student *t* test.

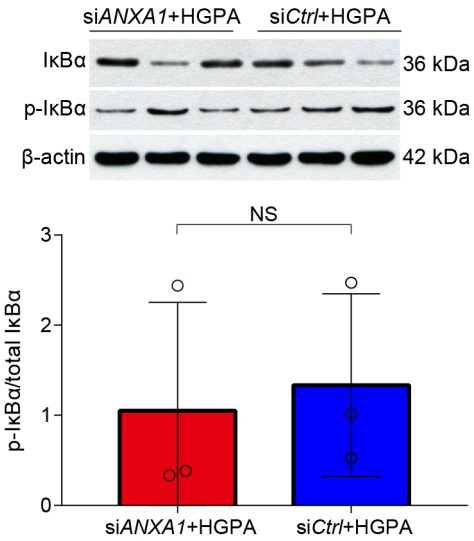

Figure S14. The degradation of IκBα in si*ANXA1* group treated with HGPA. Western blot showed that the phosphorylation levels of IκBα were not significantly altered in si*ANXA1* group treated with HGPA compared with si*Ctrl* group treated with HGPA (by Student *t* test). HGPA, high glucose plus palmitic acid; NS, no significance; si*ANXA1*, siRNA against ANXA1; si*Ctrl*, negative control siRNA.

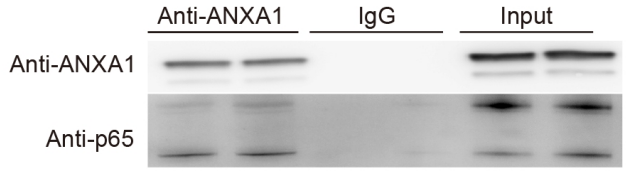

Figure S15. Coimmunoprecipitation of ANXA1 and p65 in kidney tissues.
Total protein lysates of kidney tissues from HFD/STZ WT mice were immunoprecipitated using an antibody against ANXA1 and immunoblotting against the p65.

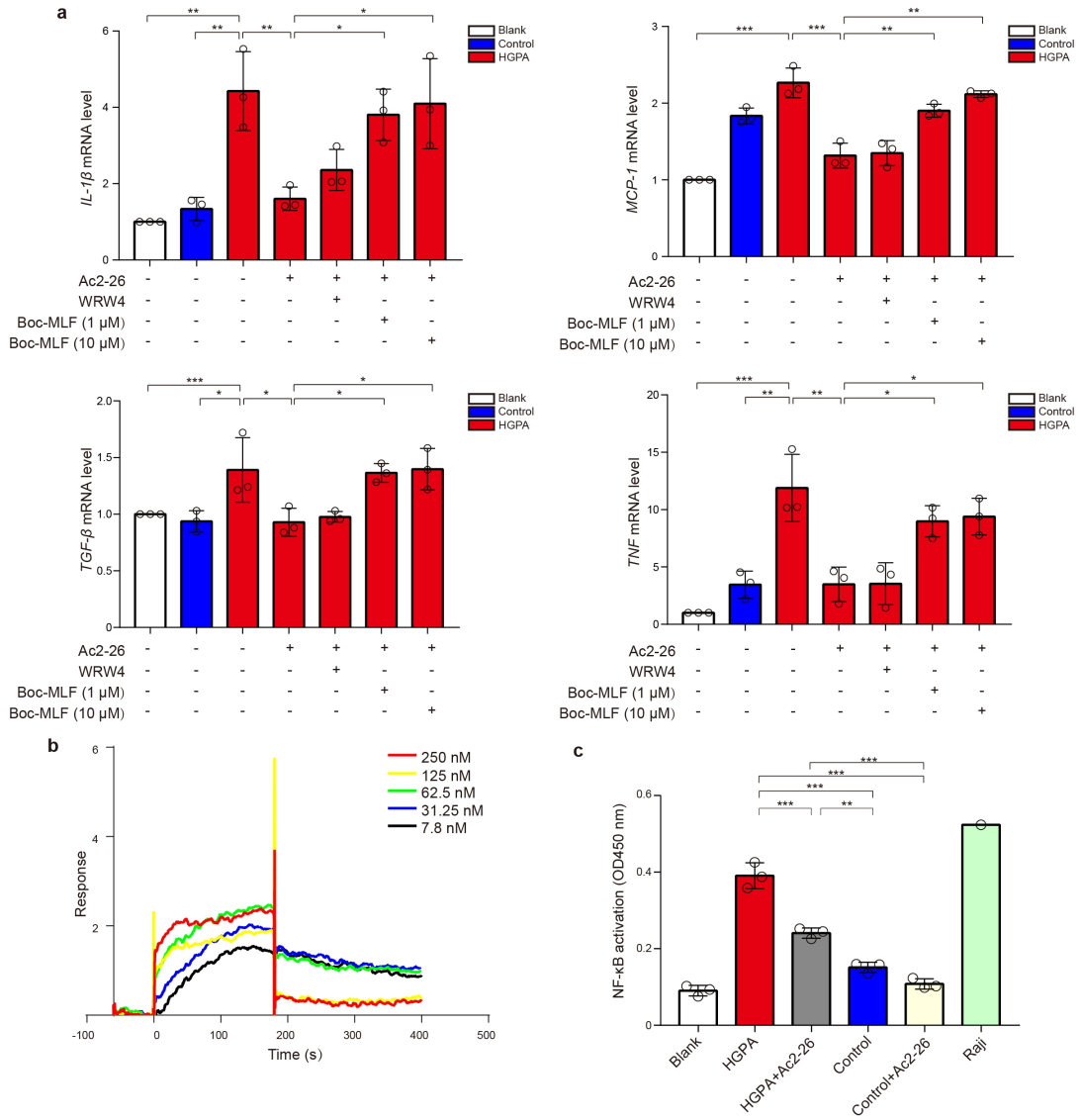

Figure S16. Ac2-26 played the pro-resolving role *via* FPR1 in HK-2 cells.
(**a**) The mRNA expression level of *IL-1β, MCP-1*, *TGF-β* and *TNF* in HK-2 cells treated with Ac2-26, WRW4, Boc-MLF(1 μmol/L) and Boc-MLF(10 μmol/L) by RT-qPCR. (**b**) The SPR response curves generated from Ac2-26 binding to p65. The results suggested that Ac2-26 did not interact with p65 directly. (**c**) The activation of NF-κB in HK-2 cells treated with different conditions. Data analyses were performed by 1 or 2-way ANOVA followed by a Tukey test and expressed as mean ± SD.*$P <$ 0.05, **$P < 0.01$, and ***$P < 0.001$.

3. Supplementary Tables

Table S1. Primer sequences used in real-time PCR analysis.

Gene	Primer Sequence 5’ to 3’	
	Forward	Reverse
Human_ ANXA1	5'-GCAGGAATATGTTCAAACTGTG	5'-CCTTATGCAAGGCAGCGA
Human_ IL-1β	5'-CCTGTCCTGCGTGTTGAAAGA	5'-GGGAACTGGGCAGACTCAAA
Human_ TGF-β	5'-GCGCATCCTAGACCCTTTCTC	5'-CAGAAGGTGGGTGGTCTTGAA
Human_ TNF	5'-CCCAGGCAGTCAGATCATCTTC	5'-GCTGCCCCTCAGCTTGAG
Human_ β-actin	5'-GAAGTGTGACGTGGACATCC	5'-CCGATCCACACGGAGTACTT
Hunan_ GAPDH	5'-GAAGGTGAAGGTCGGAGTC	5'-GAAGATGGTGATGGGATTTC
Mouse_ 18S	5'-GTAACCCGTTGAACCCATT	5'-CCATCCAATCGGTAGTAGCG
Mouse_ β-actin	5'-GTGACGTTGACATCCGTAAAGA	5'-GTAACAGTCCGCCTAGAAGCAC
Mouse_ Mcp-1	5'-AGAGCCAGACGGGAGGAAG	5'-CCAGCCTACTCATTGGGATC
Mouse_ Il-10	5'-TGAATTCCCTGGGTGAGAAG	5'-TCACTCTTCACCTGCTCCACT
Mouse_ Il-1α	5'-CGAAGACTACAGTTCTGCCATT	5'-GACGTTTCAGAGGTTCTCAGAG
Mouse_ Il-1β	5'-CGCAGCAGCACATCAACAAGAGC	5'-TGTCCTCATCCTGGAAGGTCCACG
Mouse_ Tnf-α	5'-TGTCTACTCCCAGGTTCTCT	5'-GGGGCAGGGGCTCTTGAC
Mouse_ Il-6	5'-TTCCATCCAGTTGCCTTCTT	5'-ATTTCCACGATTTCCCAGAG
Mouse_ Anxa1	5'-CCTCATCTTCGCAGAGTGTTTC	5'-ACGATGGTTGTGAGGCACTTCT

Table S2. General data of patients from China for measuring intrarenal ANXA1 expression.

Parameters	DN	HC
Number of patients	30	7
Age, years (mean±s.d.)	51.23±9.75	52.29±15.85
Sex, M/F	22/8	5/2
Diabetes history, years (median, IQR)	10 (8, 15)	-
Type of diabetes, type 1/2	(0/30)	-
Scr, μmol/l (median, IQR)	181.98 (121.78, 387.17)	-
BUN, mmol/l (median, IQR)	27.17 (16.67, 32.30)	-
eGFR, ml/min/1.73m^2 (mean±s.d.)	38.07±26.81	-
Urinary protein, g/24h (mean±s.d.)	5.57±3.72	-
Fasting glucose, mmol/l (median, IQR)	7.27 (6.34, 12.27)	-
HbA1c, % (median, IQR)	7.25 (5.78, 8.35)	-
Total cholesterol, mmol/l (mean±s.d.)	5.21±2.28	-
Triglycerides, mmol/l (median, IQR)	1.99 (1.33, 3.37)	-
HDL-C, mmol/l (mean±s.d.)	1.05±0.31	-
LDL-C, mmol/l (mean±s.d.)	3.07±1.33	-
Glomerulosclerosis, % (mean±s.d.)	32.19±24.78	-
Tubulointerstitial lesions		
Tubulointerstitial inflammation, -/+/++/+++	0/2/7/21	-
IFTA score, grade 0/1/2/3	0/3/7/20	-

[Abbreviations] BUN, blood urea nitrogen; DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HC, healthy controls; HDL-C, high-density lipoprotein cholesterol; IFTA, interstitial fibrosis and tubular atrophy; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Scr, serum creatinine; s.d. standard deviation.

Table S3. General data of patients from European Renal cDNA Bank with late DN for measuring intrarenal *ANXA1* mRNA expression.

Parameters	Living donors	DN	*P* value
Number of samples	21	17	-
Age, years (mean±s.d.)	47.45±11.74	58.30±10.71	<0.010
Sex, M/F	11/10	12/5	0.253
eGFR, ml/min/1.73 m^2 (mean±s.d.)	104.66±31.33	44.31±24.90	<0.001

[Abbreviations] DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; s.d. standard deviation; - not available.

Table S4. General data of American Indians with early DN for measuring intrarenal *ANXA1* mRNA expression.

Parameters	Living donors	DN	*P* value
Number of samples	18	69	-
Age, years (mean±s.d.)	47±13	45±10	0.632
Sex, M/F	9/9	18/51	0.050
HbA1c, % (median, IQR)	-	9 (8, 11)	-
uACR, mg/g (median, IQR)	-	31 (9, 157)	-
iGFR, ml/min (mean±s.d.)	108±32	146±52	0.005
Diabetes duration at biopsy, years (mean±s.d.)	-	16±6	-

[Abbreviations] iGFR, glomerular filtration rate was measured by the urinary clearance of iothalamate; IQR, interquartile range; uACR, urine albumin to creatinine ratio; s.d. standard deviation, - not available.

Table S5. General data of patients for measuring urinary and circulating ANXA1 levels.

Parameters	DN	DM without kidney involvement	HC
Number of patients	30	18	10
Age, years (mean±s.d.)	51.23±9.75	44.56±9.88	44.50±18.70
Sex, M/F	22/8	15/3	8/2
Diabetes history, years (median, IQR)	10 (8, 15)	-	-
Type of diabetes, type 1/2	(0/30)	(0/18)	-
Scr, μmol/l (median, IQR)	181.98 (121.78, 387.17)	57.60 (45.10, 64.25)	-
BUN, mmol/l (median, IQR)	27.17 (16.67, 32.30)	-	-
eGFR, ml/min/1.73m^2 (mean±s.d.)	38.07±26.81	182.72±48.31	-
Urinary protein, g/24h (mean±s.d.)	5.57±3.72	-	-
uACR mg/g (mean±s.d.)	-	5.31±1.55	-
Fasting glucose, mmol/l (median, IQR)	7.27 (6.34, 12.27)	9.90±4.13	-
HbA1c, % (median, IQR)	7.25 (5.78, 8.35)	6.65 (5.98, 9.35)	-
Total cholesterol, mmol/l (mean±s.d.)	5.21±2.28	5.46±1.34	-
Triglycerides, mmol/l (median, IQR)	1.99 (1.33, 3.37)	1.38 (0.87, 1.91)	-
HDL-C, mmol/l (mean±s.d.)	1.05±0.31	1.45±0.41	-
LDL-C, mmol/l (mean±s.d.)	3.07±1.33	3.04±1.17	-
Glomerulosclerosis, % (mean±s.d.)	32.19±24.78	-	-
Tubulointerstitial lesions			
Tubulointerstitial inflammation, -/+/++/+++	0/2/7/21	-	-
IFTA score, grade 0/1/2/3	0/3/7/20	-	-

[Abbreviations] BUN, blood urea nitrogen; DM, diabetes mellitus; DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HC, healthy controls; HDL-C, high-density lipoprotein cholesterol; IFTA, interstitial fibrosis and tubular atrophy; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Scr, serum creatinine; s.d. standard deviation; uACR, urine albumin to creatinine ratio.

Table S6. Physical and biochemical parameters of experimental animals.

Variable	*Anxa1* KO		WT	
	HFD/STZ	SFD	HFD/STZ	SFD
Weight, g	29.87±2.45[b)]	27.98±1.34	26.50±1.90	27.98±0.43
Kidney weight, g	0.26±0.03[a) c)]	0.18±0.02	0.24±0.02[a) c)]	0.19±0.01
Kidney/body weight	0.86±0.06[a) c)]	0.66±0.04	0.90±0.10[a) c)]	0.67±0.04
Blood glucose, mmol/l	25.20±1.83[a) c)]	8.04±1.87	24.52±1.89[a) c)]	7.49±1.69
Scr, mg/dl	0.089±0.03[a) c)]	0.026±0.02	0.053±0.02	0.028±0.03
BUN, mg/dl	30.83±5.31[a) c)]	21.83±4.07	25.50±2.59	21.83±3.67
Total cholesterol, mmol/l	7.26±1.55[a) c)]	1.10±0.22	7.99±0.55[a) c)]	1.15±0.40
Triglycerides, mmol/l	1.12±0.18	1.04±0.12	1.19±0.19[a)]	0.79±0.16
uACR, μg/mg	339.95±102.31[a) b) c)]	68.32±14.89	139.05±38.86[a)]	40.81±6.02

[Abbreviations] Data are shown as mean±s.d. n = 6 per group. BUN, blood urea nitrogen; HFD/STZ, high-fat diet plus streptozotocin; KO, knockout; Scr, serum creatinine; SFD, standard fat diet; uACR, urine albumin to creatinine ratio; WT, wild type. Data analyses were performed by two-way ANOVA followed by a Tukey's test for four groups.

[a)] ANOVA $P<0.05$ versus WT mice +SFD diet.

[b)] ANOVA $P<0.05$ versus WT mice +HFD/STZ.

[c)] ANOVA $P<0.05$ versus *Anxa1* KO mice +SFD diet.

Table S7. Quantification of mRNA by PCR array.

	HFD/STZ KO group			HFD/STZ WT group			SFD KO group			SFD WT group		
	1	2	3	1	2	3	1	2	3	1	2	3
Actb	15.23	15.61	15.61	16.76	16.78	16.43	16.51	16.54	16.39	16.48	15.96	16.38
Aimp1	21.46	21.23	21.54	21.79	21.36	21.15	21.67	22.02	21.61	21.87	21.51	21.60
Bmp2	25.54	26.01	25.80	26.24	27.34	26.27	26.99	27.74	27.27	26.90	26.78	26.95
Ccl1	30.35	30.22	30.15	30.57	32.26	30.86	31.50	31.98	31.70	31.54	32.03	31.31
Ccl11	25.55	25.82	26.19	25.69	29.54	26.31	26.61	26.61	26.60	26.89	26.74	26.50
Ccl12	20.69	21.66	21.50	25.85	25.84	24.96	22.93	24.17	23.78	26.15	25.34	25.98
Ccl17	24.54	25.75	25.16	27.21	29.20	26.80	28.77	28.13	27.49	28.13	27.95	28.32
Ccl19	20.78	21.60	21.64	22.58	23.24	22.24	21.78	22.87	23.40	22.69	22.67	23.26
Ccl2	20.69	21.40	21.26	25.44	25.91	23.30	24.38	23.87	22.99	25.41	26.30	25.93
Ccl20	23.61	24.27	24.53	27.81	30.54	26.79	29.26	29.50	27.58	29.87	28.96	30.73
Ccl22	25.75	26.81	26.37	28.46	29.78	27.62	28.88	28.62	27.90	28.93	28.89	29.43
Ccl24	30.62	29.66	30.42	29.68	34.11	30.55	30.43	30.32	29.53	30.31	30.32	31.82
Ccl3	25.29	25.55	25.68	28.49	28.90	27.78	27.74	27.58	27.34	28.80	28.92	28.68
Ccl4	23.71	24.67	24.68	25.60	27.38	26.00	25.36	25.68	25.60	26.44	26.28	26.60
Ccl5	21.68	22.67	21.25	25.15	25.80	23.24	24.13	25.27	24.14	24.49	25.44	25.45
Ccl6	20.41	21.13	20.75	23.50	23.92	22.53	23.36	23.76	22.77	23.58	23.85	23.47
Ccl7	21.94	23.42	22.94	26.60	28.45	26.22	26.71	26.24	25.48	28.46	29.40	28.53
Ccl8	20.38	21.48	20.85	23.81	26.36	24.60	27.01	25.13	24.84	26.61	25.01	24.62
Ccl9	25.69	26.77	26.35	29.54	30.73	28.01	29.90	29.69	28.56	29.49	30.38	29.67
Ccr1	24.38	24.77	25.22	27.21	26.52	26.51	27.35	26.92	27.50	27.47	27.63	27.57
Ccr10	27.14	27.02	27.16	27.54	27.76	27.30	27.79	27.24	27.64	27.31	27.28	27.37
Ccr2	21.18	22.23	21.76	24.79	24.74	23.27	24.18	23.57	22.83	23.95	24.19	23.79
Ccr3	21.55	22.40	22.25	25.79	25.74	24.52	24.63	24.52	23.88	25.37	25.16	25.41
Ccr4	27.59	28.40	28.48	29.49	29.52	28.70	29.44	29.14	28.66	29.59	29.66	29.43
Ccr5	21.64	22.63	22.33	26.54	26.13	24.92	24.82	24.69	24.42	26.54	26.23	26.28
Ccr6	26.32	26.91	27.41	27.39	29.50	27.53	28.34	28.48	27.77	28.39	27.52	28.47
Ccr8	28.33	30.32	29.74	32.28	32.69	29.57	32.12	35.19	30.32	32.60	30.53	30.68
Cd40lg	27.42	28.83	28.47	29.69	32.25	28.89	29.21	30.41	29.99	29.55	29.68	30.38
Csf1	22.50	23.40	23.28	25.27	25.41	24.60	25.31	24.80	24.99	25.64	25.30	25.77
Csf2	21.22	20.25	20.44	20.19	20.66	20.58	20.83	21.01	21.24	20.48	20.35	20.34
Csf3	29.30	28.96	29.20	29.84	30.88	30.61	29.82	30.44	30.43	30.32	30.07	30.14
Cx3cl1	22.26	21.62	21.45	22.36	22.26	21.75	22.57	22.70	23.28	22.74	22.30	22.57
Cxcl1	22.97	23.57	23.82	26.85	27.35	25.42	27.86	27.55	26.64	28.87	28.85	28.80
Cxcl10	21.29	21.19	21.56	24.29	24.80	22.28	22.13	22.77	22.43	24.21	24.35	24.76
Cxcl11	30.79	29.96	29.77	29.55	31.17	29.49	29.83	30.35	30.53	31.43	31.02	32.74
Cxcl12	20.76	20.51	20.77	20.78	20.72	20.60	20.58	20.55	21.74	21.34	20.65	21.36
Cxcl13	25.14	25.81	26.01	25.76	27.80	25.79	25.66	26.25	25.53	26.26	25.87	25.96
Cxcl15	31.71	29.93	31.48	30.40	30.96	30.45	30.82	30.75	30.70	31.28	30.59	30.70
Cxcl5	21.63	22.36	22.16	28.98	30.35	26.63	29.75	31.01	28.02	30.01	30.40	30.90
Cxcl9	26.66	27.73	27.57	29.88	30.42	28.80	28.00	29.96	29.61	30.39	29.72	30.27
Cxcr2	26.24	27.83	27.40	28.93	28.36	31.23	28.36	29.61	30.50	28.51	28.34	28.51
Cxcr3	24.34	24.97	25.16	26.89	27.88	25.59	26.26	26.68	25.95	27.19	26.71	26.84
Cxcr5	28.22	28.98	29.49	28.99	30.22	29.63	29.54	29.85	29.45	29.74	29.85	29.61
Fasl	26.51	26.61	27.18	28.95	28.79	27.34	27.54	28.69	27.84	28.76	29.87	29.67
Ifng	28.37	28.46	29.02	30.73	31.35	29.58	29.00	31.41	29.65	29.93	29.81	29.97
Il10ra	22.71	23.70	23.28	25.46	26.00	24.50	25.45	25.46	24.77	25.81	25.64	25.95
Il10rb	20.68	20.69	20.97	21.78	21.53	21.34	21.75	21.78	21.56	21.74	21.48	21.36
Il11	26.26	26.79	26.76	29.17	30.35	28.37	29.40	29.71	29.26	30.34	29.73	29.75
Il13	33.58	30.83	30.84	31.82	31.35	33.83	32.89	31.73	30.82	33.22	34.32	32.52
Il15	23.33	22.45	22.90	22.66	22.89	22.91	22.72	23.27	22.71	23.25	23.17	22.91
Il16	24.23	24.84	24.74	26.27	26.35	25.60	26.74	28.48	26.46	27.13	26.61	27.15

Il17a	31.57	29.99	30.38	29.63	33.46	30.70	30.86	31.30	30.57	30.87	30.99	30.83
Il17b	30.80	29.36	29.98	29.29	31.84	29.96	29.83	29.60	29.50	30.19	30.41	30.22
Il17f	24.90	23.84	24.75	23.73	25.58	24.19	23.83	24.02	23.68	24.71	24.56	24.38
Il1a	27.61	27.55	27.76	28.24	27.73	28.41	27.62	28.37	27.30	28.01	27.50	28.42
Il1b	23.38	23.83	23.81	26.62	26.64	26.46	25.75	26.63	25.99	26.17	26.17	26.13
Il1r1	23.28	23.70	23.69	24.83	24.96	24.44	25.32	24.95	25.32	24.88	24.70	24.87
Il1rn	23.64	24.44	24.40	28.41	29.49	27.72	29.75	28.18	26.77	29.64	30.21	29.28
Il21	31.40	34.27	33.02	33.84	34.69	37.01	35.90	36.98	33.81	36.27	36.45	34.42
Il27	28.26	28.65	29.32	30.22	30.12	29.94	30.39	31.21	30.74	30.61	32.25	33.59
Il2rb	24.92	25.58	25.63	27.23	27.83	25.87	27.16	27.32	27.32	28.21	27.41	27.89
Il2rg	22.41	23.60	23.43	25.70	26.20	24.51	25.24	25.19	24.38	25.78	25.42	25.61
Il3	32.46	32.00	32.35	33.81	33.58	33.16	33.13	36.17	33.38	33.19	33.27	33.28
Il33	21.58	22.37	22.30	24.24	24.26	23.44	24.48	24.44	24.44	24.48	24.36	24.64
Il4	27.99	27.32	28.00	27.59	28.25	27.39	27.37	27.59	27.30	27.72	27.65	27.59
Il5	28.76	27.41	27.97	27.43	28.40	27.87	27.70	27.48	27.25	27.85	28.09	27.91
Il5ra	24.78	24.90	25.19	26.59	25.70	24.48	25.39	26.18	25.49	24.79	24.72	24.39
Il6ra	24.16	24.82	24.82	25.85	26.37	25.40	26.51	26.02	26.19	26.50	26.28	26.48
Il6st	19.44	19.24	19.55	20.33	19.98	20.16	20.33	20.34	20.36	20.86	20.36	20.44
Il7	25.89	25.50	25.63	26.58	26.32	25.99	27.16	26.69	26.54	26.88	26.23	26.48
Lta	28.47	28.22	28.54	28.19	29.96	28.95	28.73	28.70	28.85	30.01	29.68	29.28
Ltb	23.95	24.54	24.27	26.31	28.28	25.72	26.55	27.46	26.44	27.52	26.72	27.16
Mif	17.39	16.79	17.43	17.45	17.57	16.70	17.28	17.22	17.28	17.32	17.47	17.50
Nampt	20.15	19.44	19.78	19.75	19.72	18.94	19.92	19.97	19.84	20.17	19.63	19.86
Osm	27.49	28.52	28.62	30.50	30.59	29.71	29.86	31.31	29.98	30.38	30.34	30.80
Pf4	24.46	25.39	25.33	26.45	27.45	27.20	27.21	26.97	26.25	27.96	27.30	27.40
Spp1	11.61	11.99	12.17	15.78	15.79	14.19	15.62	15.48	14.94	15.95	15.88	15.94
Tnf	25.27	26.28	25.89	28.94	29.51	28.60	28.27	28.16	27.94	29.42	29.50	29.68
Tnfrsf11b	26.48	26.65	26.84	28.17	28.48	27.78	27.88	27.97	27.83	28.37	27.95	27.27
Tnfsf10	21.90	20.78	21.45	22.13	21.50	21.65	21.86	22.00	21.57	22.62	22.16	22.39
Tnfsf11	26.71	25.78	26.38	25.40	27.59	26.15	26.18	26.45	26.24	26.80	26.87	26.66
Tnfsf13	24.73	24.93	25.21	26.67	26.35	26.33	27.21	26.48	25.98	26.84	26.35	26.25
Tnfsf13b	23.50	24.35	24.26	25.97	26.61	25.78	26.61	26.33	26.27	26.84	26.88	26.80
Tnfsf4	30.59	32.87	33.46	35.47	N/A	36.16	32.75	35.92	36.45	33.73	37.03	33.49
Vegfa	20.73	20.03	20.78	20.59	20.37	20.54	20.72	20.67	20.61	20.50	20.22	20.30