Supplementary Methods

Albumin, creatinine and unacylated ghrelin measurement

For assessing albuminuria in the STZ treated mice, albumin:creatinine ratio (ACR) was measured in the urine samples taken directly from the bladder at euthanisation. Albumin concentration was analysed using a modified version of the Albumin ELISA kit manufactured by Bethyl Laboratories Inc. (E90-134, Bethyl Laboratories Inc., Montgomery, USA). For details see [23]. Creatinine was measured using The Creatinine Companion assay (1012, Exocell Inc., Philadelphia, USA).

Plasma was collected at euthanisation and treated with the protease inhibitor aprotinine. Then the levels of Unacylated ghrelin were measured using an ELISA (KT-365, GENTAUR MOLECULAR PRODUCTS BVBA, Kampenhout, Belgium).

Gene expression analysis by Real-Time Quantitative PCR

After the kidneys were snap frozen in liquid nitrogen, they were stored in -80˚ C until further processing took place. For RNA extraction, the kidneys were transferred to TRIzol® (Life Technologies, Burlington, Canada) and homogenized on a Tissuelyzer II (Qiagen, Mississauga, Canada). RNA purification was performed using the RNeasy mini kit (Qiagen, Mississauga, ON, Canada) and cDNA synthesis using SuperScript™ VILO™ cDNA Synthesis Kit (Life Technologies, Burlington, Canada), both according to the manufacturer's instructions.

Gene expression was analysed using the QuantStudio 12K Flex Real-Time PCR System with Taqman fast universal PCR master mix and Taqman probes (Life Technologies, Burlington, Canada). The gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Ct values was found for each gene targets and then first normalized to the average of the housekeeping genes (Rps 13, Rpl27 and Actb) and then to the average of the vehicle group. The specific TaqMan probes used can be found in supplementary Table 1

Gene symbol	Gene name/alias	Taqman probe
Target genes		
CCL2	Chemokine (C-C motif) ligand 2/monocyte chemoattractant 1 (MCP-1)	Mm00441242_m1
CDKN1A	Cyclin-dependent kinase inhibitor 1A/ p21	Mm00432448_m1
HAVRC1	hepatitis A virus cellular receptor 1/ Kidney Injury Marker 1 (KIM-1)	Mm00506686_m1
LCN2	lipocalin 2/neutrophil gelatinase-associated lipocalin (NGAL)	Mm01324470_m1
Housekeeping genes		
RPL27	ribosomal protein L27	Mm01245874_g1
RPS13	ribosomal protein S13	Mm01731323_g1
ACTB	betaactin	Mm00607939_s1

Supplementary Table 1 Overview of the names and Taqman probes used both for target genes and housekeeping genes in the Real-Time Quantitative PCR

Immunohistochemical staining

After fixation in formalin and subsequently 70% ethanol, the kidneys were divided in half longitudinally and processed on a Leica Asp300S (Leica Microsystems, Ballerup, Denmark). Kidneys were hereafter embedded in paraffin blocks and 3 µm sections were cut. The Discovery ULTRA Staining Module (Ventana Medical Systems, Roche, Rotkreuz, Switzerland) was used for all immunohistochemical (IHC) staining. Slides were first baked for 60 min followed by 16 min pre-treatment with Cell Conditioning 1 (950-124, Roche Diagnostics, Rotkreuz, Switzerland). In both protocols the antibodies where incubated for 60 min. In Supplementary Table 2, antibodies as well as amplification and chromogenic detection method is listed.

Antibody		**Amplification**		**Chromogenic detection**	
Product	Company	Product	Company	Product	Company
Rb Ki67/ NB600-1252	Novus Biologicals, Abingdon, UK	Discovery Rabbit OmniMap HRP,/760-4647	Roche Diagnostics, Rotkreuz, Switzerland	Discovery Purple kit, 760-229	Roche Diagnostics
Gt KIM-1/ AF1812	R&D, Abingdon, UK	Discovery Goat OmniMap HRP/760-4311			

Supplementary Table 2 Overview of the antibody, amplification method and chromogenic detection used for IHC staining of Ki67 and KIM-1 on the kidneys.

After scanning with the Nanozoomer 2.0 and images were analysed using Visiopharm Integrator System software (Visiopharm, Hoersholm, Denmark). At first, the kidney was detected using an automated tissue detection protocol as previously published [40]. KIM-1 staining was quantified using the Contrast Red-Green (50-∞) channel in the whole kidney and KI-67 staining using the Contrast Red-Green (5-∞) channel and the Chromaticity Green (∞-0.3) channel in a region of interest containing only the cortex.