**Supplemental Material**

**Materials and Methods**

**Peripheral Blood Mononuclear Cells (PBMCs) isolation**

Peripheral Blood Mononuclear Cells (PBMCs) were isolated using Lymphosep (density 1.077 g/ml, Biosera) from freshly collected whole blood; blood was then diluted (1:1 dilution) with phosphate-buffered saline (PBS). The diluted whole blood was carefully layered over the separation medium (1/2 x the volume of the sample) and the two phases were kept separated before the centrifugation. Samples were then centrifuged at 400xg for 30 min at 20°C. PBMCs were collected after acquisition of the plasma and platelets layer; cells were then washed with PBS and processed for proteasome peptidase activity.1

**Measurement of proteasome peptidase activity**

For measuring proteasome peptidases activity, dissected kidneys or isolated PBMCs were lysed on ice in a 26S proteasomes isolation buffer as previously described.1 Protein content was adjusted with Lowry assay and supernatants were immediately used (after the addition of the fluorogenic substrate) to determine the CT-L proteasomal peptidase activity; all measurements were performed in duplicates. Emitted fluorescence was recorded at Spark® Tecan microplate reader (Tecan Group Ltd., Maennedorf, CH) a VersaFluor Fluorometer System (Bio-Rad laboratories, Hercules, CA, USA) at excitation and emission wave lengths of 380 nm and 460 350 and 440 nm, respectively, using a commercially available 20S proteasome activity kit (Item No. 10008041, Cayman Chemicals, Ann Arbor, MI · USA).

**Blood Pressure Recordings**

Systolic blood pressure (SBP) and heart rate (HR) were measured using the tail-cuff method in conscious trained mice (tail-cuff plethysmography, Kent Scientific CODA STD). The researcher performing the experiment was blinded to the study. Animals were acclimated to the retainers, prior to the conduction of the experiments. Ten acclimation and twenty measurement cycles were performed and after the exclusion of the false measurements, at least 15 cycles were averaged per mouse. All blood pressure measurements were performed at exactly the same time each day for all groups and HR was measured for the confirmation of the robustness of the SBP of the data.2

**Histology**

Murine kidney sections (3 mm) were fixed in 4% Formalin for 24h and subsequently sliced in 5 μm sections. Slides were deparaffinized in xylene and rehydrated in serial ethanol concentrations. Sections were Hematoxylin-Eosin stained for histology evaluation by a double blinded pathologist and glomerular volume, as a marker of renal disease and biomarkers of renal-derived hypertension,3 were calculated using an open-source software (Image J, NIH, USA). For the measurement of glomerular volume 3 slides per animal and n=4-6 animals per group, considering a globular shape of the glomeruli. Glomerular volume was subsequently averaged per animal before the statistical analysis.

**Biochemical Analysis**

Blood samples collected in heparin and were initially centrifuged at 5000 x rpm for 15 minutes. Serum Creatine was measured by the Jaffee kinetic method, whereas serum Urea-bound-nitrogen (BUN) was measured via a Glutamate dehydrogenase-dependent method in the human and murine samples according to the manufacturer’s instructions (#000216 and #001526 respectively, BIOSIS, Athens, Greece). Creatinine and BUN levels were measured photometrically with a microplate reader (Tecan® Infinite Pro, Tecan, Switzerland). Lactate Dehydrogenase (LDH) was measured using a photometric test, while C-Reactive Protein (CRP) was analyzed by tholosimetry. Measurements were obtained using the Dimension EXL with LM Integrated Chemistry System (Siemens Healthineers, Germany). Full Blood Count (FBC), for white blood cells (WBCs), lymphocytes, neutrophils and hematocrit were performed using the automated hematology analyzer pocH-100iV (Sysmex, Japan). Angiotensin Converting Enzyme (ACE) activity was measured using a commercially available kit and according to the manufacturer’s instructions (#MAK377, Sigma Aldrich, Germany). Angiotensin II (AngII) was measured using a commercially available kit (# RAB0010, Sigma Aldrich, Germany) provided for AngII measurement in plasma samples, which is a competitive ELISA kit based on immune-binding of AngII on a specific Anti-Angiotensin II antibody. In this assay, a biotinylated Angiotensin II peptide is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated Angiotensin II peptide competes with endogenous (unlabeled) AngII for binding to the anti-Angiotensin II antibody. This provides the kit with higher specificity for AngII compared to other angiotensin peptides. For avoidance of artefactual ex-vivo angiotensin generation or destruction plasma samples were diluted immediately to Assay Diluent C according to the manufacturer’s instructions. Urine protein was measured photometrically in the urine samples originating from the 24h metabolic cage experiments by the Lowry Method (DC Protein Assay, Bio-Rad Laboratories, Athens, Greece).

**Plasma and urine electrolyte Analysis**

Na+, K+ and plasma protein levels were measured in serum and urine samples to evaluate renal function and electrolyte imbalances. Electrolyte analysis in the urine was performed using a 24-hour urine collection via the metabolic cages. The analysis was based on an indirect potentiometric procedure performed in the Dimension EXL with LM Integrated Chemistry System from Siemens Healthineers. Na+ and K+ levels were expressed as mmol/L, whereas plasma protein levels were expressed as g/dL.

**Urine pH measurement**

Spot urine samples were collected from the animals to measure urinary pH. Samples were placed on AUTION sticks 10 PA, which were subsequently inserted in the automated system AUTION ELEVEN AE-4020. The urinalysis was based on a photometric method.

**Proteomic Analysis**

**Sample preparation for proteomic analysis**

The kidneys were homogenized in FASP lysis buffer (4% SDS, 0.1 M DTE, 0.1 M Tris–HCl pH 7.6) using the bullet blender homogenizer (Next Advance, Troy, NY). Protein concentration was determined by Bradford assay. Protease inhibitors (Roche, Basel, Switzerland) were added at a final concentration of 3.6%. Protein extracts (200 μg/sample) were processed using filter-aided sample preparation (FASP) as previously described 4,5. Briefly, the protein extracts were mixed with 200 μL of urea buffer (8M Urea in 0.1M Tris-HCl pH 8.5, UA buffer) in Amicon filter units (0.5ml, 30kDa MW cut-off) and were centrifuged at 16,000 rcf for 15 minutes. Alkylation of proteins was performed by addition of 100μL of 0.05 M iodoacetamide in UA buffer for 20 min in the dark, followed by a centrifugation at 16,000 rcf for 10 min at RT. Afterwards, 100 μL of ammonium bicarbonate buffer (50mM NH4HCO3 pH 8, in ultrapure water) were added to the filter unit and centrifuged at 16,000 rcf for 10 minutes twice. Tryptic digestion was performed overnight at room temperature in the dark, using a trypsin to protein ratio of 1:100. Peptides were eluted by centrifugation at 16,000 rcf for 10 minutes, lyophilized, and stored at −80°C until further use.

**LC-MS/MS Analysis**

All LC-MS/MS experiments were performed on the Dionex Ultimate 3000 UHPLC system coupled with the high-resolution nano-ESI QExactive mass spectrometer (Thermo Scientific). Each sample was reconstituted in 200 μL loading solution composed of 0.1 % v/v formic acid. A 5 μL volume was injected and loaded on the Acclaim PepMap 100, 100 μm × 2 cm C18, 5 μm, 100 Ȧ trapping column with the ulPickUp Injection mode with the loading pump operating at flow rate 5 μL/min. For the peptide separation the Acclaim PepMap RSLC, 75 μm × 50 cm, nanoViper, C18, 2 μm, 100 Ȧ column retrofitted to a PicoTip emitter was used for multi-step gradient elution. Mobile phase (A) was composed of 0.1 % formic acid and mobile phase (B) was composed of 100% acetonitrile, 0.1% formic acid. The peptides were eluted under a 240-minute gradient from 2% (B) to 33% (B). Flow rate was 300 nL/min and column temperature was set at 35 °C. Gaseous phase transition of the separated peptides was achieved with positive ion electrospray ionization applying a voltage of 2.5 kV. For every MS survey scan, the top 10 most abundant multiply charged precursor ions between m/z ratio 300 and 2200 and intensity threshold 500 counts were selected with FT mass resolution of 60,000 and subjected to HCD fragmentation. Tandem mass spectra were acquired with FT resolution of 15,000. Normalized collision energy was set to 33 and already targeted precursors were dynamically excluded for further isolation and activation for 45 sec with 5 ppm mass tolerance.4,5

**MS data processing and quantification**

Raw files were analysed with Proteome Discoverer 1.4 software package (Thermo Finnigan), using the Sequest search engine and the Uniprot mouse (*Mus musculus*) reviewed database, downloaded on November 22, 2017, including 16,935 entries. The search was performed using carbamidomethylation of cysteine as static and oxidation of methionine as dynamic modifications. Two missed cleavage sites, a precursor mass tolerance of 10 ppm and fragment mass tolerance of 0.05 Da were allowed. False discovery rate (FDR) validation was based on q value: target FDR (strict): 0.01. Label free quantification was performed by utilizing the precursor area values of each sample as defined by the Proteome Discoverer 1.4 software package. Samples were analyzed individually (not pooled) and assigned to distinct groups. For a small number of peptide sequences where no peptide precursor area could be retrieved (although the peptides were identified) the missing values were replaced by zero. In addition, if a peptide was not identified in a particular sample the missing values were replaced by zero. For the quantitation-statistical analysis, only the peptides which were present in at least 50% of the samples in at least one group were further considered. The precursor area values were subjected to the following normalization method within each sample prior to quantification analysis: normalized peak area = (peptide peak area/sum of peptides peak area) × 106. Protein expression values were calculated as the sum of all the normalized peptide areas that were assigned for a given protein. Principal Component Analysis (PCA) were herein engaged, as implementation via the SIMCA v14.1 software (Umetrics, Umea, Sweden). The nonparametric Mann–Whitney test was utilized for defining statistical significance between the two groups and P value ≤0.05 was considered statistically significant.

**Pathway Analysis**

Pathway analysis was performed using the ClueGO plug-in v2.5.4 in Cytoscape v3.8.2. The significant and up-(fold change ≥2) or down-regulated (fold change ≤0.5) in cases versus controls proteins were included in the analysis. Ontologies were retrieved from the REACTOME pathways database (organism: *Mus musculus*, 2,240 terms with 9292 available unique genes, updated on February 29, 2019) and only statistically significant pathways (Benjamini-Hochberg corrected p-value ≤0.05, two-sided hypergeometric test) were taken into account. The rest of the settings were used as default. For simplification, the leading term from each group is presented. The percentage of detected features over all associated genes per group is also displayed as “% associated genes” in the graph.

**Metabolic cages experiments**

For the monitoring of the food and water intake, urine volume and feces weight, mice were accommodated in metabolic cages (Metabolic Cage MMC100, Hatteras Instruments) for 24 hours. After 24 hours food, water and feces weight, urine volume and urine protein excretion were calculated.

**Metabolomic Analysis**

**Sample preparation for metabolomic analysis**

*Urine Samples*

500μL of urine were centrifuged using a NEYA 16R centrifugation apparatus (REMI, Mumbai, India) at 10000 rpm, 5 minutes, 4oC and diluted with 1000μL methanol-water (1:1 v/v).6 Then, 600μL were withdrawn, evaporated to dryness, using HyperVAC-LITE centrifugal vacuum concentration (Hanil Scientific Inc, Gimpo, Korea) and reconstituted using 150μL of internal standard solution (Internal standard (IS): 1 g/mL yohimbine in ACN-water 95-5 v/v).

*Plasma Samples*

200μL of plasma were diluted 3-fold with frozen methanol and centrifugated at 10000 rpm, 5 minutes, 4oC. An 350μL aliquot of the supernatant was evaporated to dryness and reconstituted with 150μL of IS.7

*Kidney Samples*

Kidney samples were homogenized employing a tissue homogenizing CKMix lysing kit (Bertin Corp.) and methanol-water (1:1v/v), using a CRYOLYS EVOLUTION tissue homogenizer (Bertin Instruments, France). The volume of solvent was adjusted to the weight of each sample using 1000 μL of solvent for 100 mg of tissue. Homogenization was completed in two rounds. At the first round, half of the solvent was used, and the “hard” mode of homogenizer was applied. Subsequently, after centrifugation and removal of the supernatant, the rest of solvent was added, and the “soft” applied. After centrifugation, the supernatant was pooled with the initially collected. Subsequently, 500μL of the total extract of each sample were evaporated to dryness and reconstituted with 150μL of IS 8. All solvents used in the study were of high purity (LC-MS grade).

**Chromatography**

All LC-MS experiments were performed with a Dionex UltiMate 3000 RSLC (Thermo Fischer Scientific, Dreieich, Germany) coupled to a Maxis Impact QTOF mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an Electrospray Ionization source performing both positive and negative ionization. 5μL volume of each sample were injected on an Acqutiy UPLC BEH Amide column, 2.1 x 100 mm, 1.7 μm, (Waters, Ireland), equipped with a pre-column of the same material. Column temperatures was set at 30oC. In the positive ionization the mobile phase was consisted of water (A) and ACN-water 95-5 (B), both amended with 5mM Ammonium Formate (Fischer Scientific) and acidified with 0.01% Formic Acid (Sigma-Aldrich, Steinheim, Germany). In the negative ionization the mobile phase was consisted of water (A) and ACN-water 95-5 (B) both amended with 10mM Ammonium Formate. In both ionization modes, the same gradient elution program was performed: 100% (B) for 2 minutes, decrease to 5% (B) in 10 minutes and remain for 5 minutes. Initial conditions were restored in 0.1 minute and the column re-equilibration lasted 8 minutes. The flow rate was 0.2mL/min and the injection volume were 5μL.

**Mass spectrometry**

Concerning ESI-QTOF system, nitrogen used as nebulizer gas set at 2 bar pressure and as drying gas with flow rate 10 L/min. The drying temperatures was set at 200oC. Capillary voltage was set at 3500V for positive mode and 2.5kV for negative mode. The end plate offset was 500V. Both MS and bbCID modes of data acquisition were performed, with the collision energy to elevate from 24V to 36V in both polarities of bbCID mode. For the external calibration, sodium formate was infused in the MS system for both polarities. HPC algorithm was performed for calibration and the limit of mass width was set at 1 mDa. The same solution was infused in the system before every injection, as the first segment of analysis, to permit the internal calibration of each sample during data treatment. During the analysis, samples were randomized, and QC samples were used to control the drift of analysis and provide the ability for signal correction, if it was required. Three types of QC samples were used, one for each type of biological specimen, composed as a pool sample of the extracts.

**MS data processing**

Raw files were analyzed employing TASQ Client 2.1 (Bruker Daltonics, Bremen, Germany). For metabolites detection, an in-house metabolites database created using the IROA-MSMLS metabolite standards library. The same conditions of the biological samples analysis were also applied for the database development and precursor ions, in-source fragments, fragments and adducts had been recorded. For the targeted metabolomic analysis the database was imported in TASQ as a list of potential metabolites, and the screening of the included metabolites was applied for all samples. Internal calibration of every sample was performed automatically via TASQ, adopting 5 mDa as mass error and 3000 abs. as minimum intensity. Gaussian smoothing was performed with the S/N ratio set at 10. For metabolites detection, extracted ion chromatogram width was set at 5 mDa and retention time tolerance at 0.2 min with the isotopic profile tolerance was being to 100 mSigma. Concerning integration parameters Area abs threshold was set at 2000 and intensity abs threshold at 1000. Yohimbine was used as the internal standard for signal and time correction.

The features from the analyzed samples that were annotated as detected metabolites were only those with the correct accurate mass that where closest to the standards reference retention time and additionally had at least one ion of qualification (fragment or adduct) recorded. Thus, ninety metabolites have been detected and submitted to statistical analysis.

Metabolite set enrichment analysis (Top 25 pathways) was performed using the online free access software MetaboAnalyst 5.0. Samples were submitted in rows (ID type: Compound Names), without sample normalization, data transformation and scaling and analysed with KEGG metabolite set library (metabolite sets containing at least 5 entries). Enrichment Ratio was computed by Hits/Expected, where hits = observed hits; expected = expected hits.9

**Statistical analysis**

Multivariate statistical analysis was performed using SIMCA 14.1 software (Umetrics, Sweden). PCΑ and Partial Least-Squares Discriminant Analysis (PLS-DA) models have been created for each type of biological specimen, to point out the discrimination between groups and the most differentiative metabolites.

PCA and PLS-DA models were created applying UV data scaling. PLS-DA models were submitted to permutation testing (59 random permutations) for evaluation of their efficacy and risk assessment of models to be spurious.

**Subcellular fractionation and protein localization**

For the separation of the nuclear and cytosolic cell fractions from the renal tissue, pulverized kidney tissue was initially lysed in hypotonic buffer (20mM Tris-HCl pH=7.4, 10mM NaCl, 3mM MgCl2) and incubated at 4oC for 15 min. Subsequently 5% NP4O detergent was added and homogenates were centrifuged for 10 min at 3000rpm at 4oC. The supernatant contained the cytoplasmic fraction. The pellet containing the nuclear fraction was resuspended in 50μl of Cell Extraction Buffer (10mM Tris pH=7.4, 100mM NaCl, 2mM Na3VPO4, 1%Triton-X-100, 1mM EDTA, 10% glycerol, 1mM EGTA, 0.1%SDS, 1MM NaF, 0.5% Na-deoxycholate, 20mM Na4P2O7) and incubated for 30 min at 4oC with vortexing at 10 min intervals. Subsequently homogenates were centrifuged at 14000 x g at 4oC for 30 min and the supernatant corresponds to the nuclear fraction. The cytoplasmic and nuclear fractions were used for western blot analysis.

**Western blot analysis**

Kidney tissue powder was extracted as previously described in the subcellular fractionation experiments or with lysis buffer (1% Triton X-100, 20 mM Tris pH 7.4-7.6, 150 mM NaCl, 50 mM NaF, 1 mM EDTA,1 mM EGTA, 1 mM Glycerolphosphatase, 1% SDS, 100 mM phenylmethylsulfonyl fluoride, and 0.1% protease phosphatase inhibitor cocktail) for the whole extract analysis. After centrifugation (11,000xg, 15 min, 4°C), supernatants were used for protein analysis, as previously described1. The following primary antibodies were used: pmTOR (mammalian target of rapamycin; Ser2448) (D9C2) (Rabbit mAb, #5536), mTOR (Rabbit mAb, #2983), p-Raptor (regulatory-associated protein of mTOR; Ser792) (Rabbit mAb, #2083), Raptor (Rabbit mAb, #2280), p-eNOS (endothelial nitric oxide synthase; Ser1177) (Rabbit mAb, #9570), eNOS (Rabbit mAb, #9572), iNOS (inducible nitric oxide synthase, Rabbit mAb #13120) p-AMPKα (AMP-activated kinase α; Thr172) (Rabbit mAb, #2535), AMPKα (Rabbit mAb #5831), p-Akt (protein kinase B; Ser473) (Rabbit mAb, #9271), Akt Antibody (Rabbit mAb, #9272), p-NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells, Rabbit mAb #3033), NF-κB (Rabbit mAb #8242) Cleaved caspase-1 (Rabbit mAb #89332), VEGFR2 (Rabbit mAb #9698), VEGF (Rabbit mAb #65373) β-tubulin (Rabbit mAb, #2146), α-actinin (Rabbit mAb #6487), LC3B (Microtubule-associated protein 1A/1B-light chain 3B, Rabbit mAb, #3868), GAPDH (Glyceraldehyde 3-phosphate dehydrogenase, Rabbit mAb, #5174) (Cell Signaling Technology). For the mineralocorticoid receptor signalling axis the following primary antibodies were used: MCR (Mineralocorticoid Receptor, Mouse mAb, sc-53000) β-ΕNaC (Epithelial Sodium Channel 1 Subunit Beta Mouse, mAb, sc-25354) (Santa Cruz, Texas, USA), p-SGK1 (Ser78) (Serum and glucocorticoid-inducible kinase, Rabbit mAb #5599), SGK1 (Rabbit mAb #12103) and Nedd4 (Neural precursor expressed, developmentally down-regulated protein 4, Rabbit mAb #2740). All primary Abs were used at a dilution of 1:1000. PVDF membranes were then incubated with secondary antibodies for 2 h at room temperature [1:2000 dilution, goat anti-mouse (#7076) and goat anti-rabbit HRP (#7074); Cell Signaling Technology, Beverly, MA, USA] and developed using the GE Healthcare ECL Western Blotting Detection Reagents (Thermo Scientific Technologies). Relative densitometry was determined using a computerized software package (Image J, NIH, USA), and relative ratios were used for statistical analysis.1

**Real-Time Polymerase Chain Reaction**

For RNA isolation, snap-frozen kidneys from the treated animals were pulverized and extracted by the standardized Trizol protocol. RT-PCR was performed with the CFX96 Real-Time PCR Detection System (Bio-Rad, Munich, Germany). Isolated RNA was reverse-transcribed to cDNA using high-capacity cDNA reverse transcription kit (FastGene Scriptase II cDNA, Nippon Genetics, Japan). The following primer pairs were designed (Primer-Blast, NCBI, NIH) and used in order to detect mRNA expression of (Eurofins Genomics AT, GmbH) were analyzed using the SYBR® Green method (Eva Green, Solis BioDyne, Estonia) according to the manufacturer’s instructions.10 Real-time PCR primers’ sequences are presented in **Table S4**.

**Measurement of ADAMTS-13 Activity with FRETS-VWF73 fluorescent substrate.**

For the measurement of ADAMTS-13 activity 4μl of citrated murine plasma was diluted in 100 μl FRETS Buffer (5mM Bis-Tris, 25 mM CaCl2, 0,005% Tween 20, pH=6) and was incubated with 100 μl of 4μM FRETS-VWF73 (#3224-s, PeptaNova, Germany) at 30oC for 1h and fluorescence was recorded every 5min excitation 340nm, emission 440nm). ADAMTS-13 activity was calculated as the slope of the linear regression of the fluorescence according to the previously published protocols.11,12

**Primary Murine Collecting Duct Tubular Epithelial Cell (PrCDTECs) isolation and *in vitro* experiments.**

Primary Murine Collecting Duct Tubular Epithelial Cell (PrCDTECs) were isolated from freshly harvested renal collecting ducts from adult C57BL6/J male mice.13 Briefly the intramedullary area of the kidneys was excised and minced. The tissue particles were digested in 2ml of digestion buffer [Collagenase IV 1mg/ml, DNAase I 0.1mg/ml, BSA 1mg/ml in Dulbecco’s Modified Eagle’s Medium (DMEM) high glucose] at 37oC under gentle shaking for 15min. Subsequently 4ml of DMEM was added and the digestion mixture was centrifuged at 200 x g for 2 min resulting in the isolation of renal tubules. Renal tubules were cultivated in DMEM high glucose (10% Fetal Bovine albumin, 1% Penicillin-Streptomycin) and 2-3 days after, the PrCDTECs, sprouting from the tubules, reached confluency. Confluent PrCDTECs were used in 96 well or 24 well plates containing round glass coverslips for 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) experiments, assessing cellular viability, or immunofluorescent microscopy respectively. For the cellular viability experiments MTT was used at a concentration of 0.5mg/ml.14 The half-maximal inhibitory concentration (IC50) for Carfilzomib and Sodium chloride (NaCl) were calculated and then prophylactic therapies against Cfz and NaCl-dependent cytotoxicity were performed. Eplerenone (Mineralocorticoid receptor blocker) and Metformin at a concentration range of 0-500 μΜ was used for the in vitro experiments and according to the concentrations used in in vitro experiments in the literature.15 The selection of Metformin and Eplerenone as promising prophylactic therapies was based on our *in vivo* proteomic and molecular data in which AMPKα and transport of organic ions and small molecules emerged as significantly regulated pathways by Carfilzomib, with the latter being mainly orchestrated by the mineralocorticoid receptor axis (**Figure 2**).16

**Immunohistochemistry**

Immunohistochemistry staining in human renal biopsies in the Control and Carfilzomib-nephrotoxicity samples was performed using VECTASTAIN® Universal Quick HRP Kit (# PK-7800, Vector Laboratories, Newark, USA) after deparaffinization and rehydration of the samples. For SGK-1 and MR staining SGK1 Rabbit monoclonal antibody (#12103, Cell Signaling Technology, Beverly, MA, USA) and MCR antibody (Mineralocorticoid Receptor; Mouse mAb, sc-53000) were used at concentrations 1:100 and 1:300 respectively. Histology was scored by a double-blinded pathologists on 6 different areas of each biopsy per slide.

**Immunofluorescence microscopy**

For the immunofluorescence (IF) microscopy experiments, PrCDTECs were seeded on round glass coverslips in 24 well plates until they reached confluency. Subsequently cells were treated accordingly and fixed in 4% paraformaldehyde (PFA). After permeabilization of the cells with 0.25% SDS, anti-SGK-1 (Rabbit mAb #12103, Cell Signaling), anti-Rabbit Alexa Fluor 488 secondary Ab (Goat Anti-Rabbit IgG H&L, ab150077, Abcam) and DAPI (Antifade medium with DAPI, Thermo Scientific) were used. IF was recorded on an upright microscope (Leica DMRA2 Microscope, Leica Microsystems, Wetzlar, Germany) and IF was quantified using a computerized software package (Image J, NIH, USA).

**Data Deposition**

All proteomic data are available in the cloud-based repository Mendeley data (<https://data.mendeley.com/datasets/mcxknx4s7v/draft?a=a126f1b5-e845-45b5-b724-71e97cb707be>) and will be publicly available upon publication of the manuscript, whereas all metabololomics data are available in MassIVE database (Study identifier: MSV000089596; <https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?accession=MSV000089596>).

**Supplemental Tables**

**Table S1**: Individual Patients Characteristics and features of renal biopsies

|  |
| --- |
| **Table S1** |
| **Ascension Number** | **Gender** | **MM Immunoglobulin Type** | **Age** | **Years since Diagnosis** | **FSGS%/Age-Corrected FGS% Value** | **Fibrosis** | **Multifocal Atrophy** | **ΤΜΑ** | **LCCD** |
| **1** | ♂ | IgGκ | 67 | 13 | 16.6%/23.5% | 15% | 5% | N/D | N/D |
| **2** | ♂ | IgGκ | 65 | 4 | 82.8%/23.5% | 5% | 75% | Yes | N/D |
| **3** | ♂ | IgGκ | 71 | 4 | 35%/23.5% | 5% | 5% | N/D | N/D |
| **4** | ♀ | IgGκ | 70 | 6 | 58.3%/ 25% | 35% | N/D | Yes | N/D |
| **5** | ♂ | IgGκ | 70 | 7 | 73%/25% | 35-40% | N/D | N/D | N/D |
| **6** | ♂ | IgGλ | 56 | 12 | 27.27%/18% | 35-40% | N/D | N/D | Λ |
| **7** | ♀ | IgGκ | 64 | 1 | 16.6% /22% | 20-25% | N/D  | N/D | N/D |

*FSGS: Focal Segmental Glomerulosclerosis, MM: Multiple Myeloma, N/D: Not Detected, LCCD: Light Chain Deposition Disease, TMA: Thrombotic Microangiopathy.*

**Table S2**: **Carfilzomib increases K+ sparing and Na+ retention in the urine in the four doses protocol.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Table S2** | **Control (N/S 0.9%) (n=6)** | **Cfz (8 mg/kg)****(n=6)** | **P Value** |
| **Plasma** |
| Na+ (mmol/L) | 165.0 ± 0.6 | 163.5 ± 1.1 | 0.06 |
| K+ (mmol/L) | 4.2 ± 0.1 | 4.7 ± 0.8 | 0.6 |
| Na+: K+ ratio | 42.4 ± 3.4 | 38.3 ± 4.6 | 0.5 |
| Protein (g/dL) | 4.5 ± 0.6 | 3.7 ± 0.1 | 0.2 |
| HCT % | 36.0 ± 1.8 | 37.5 ± 0.5 | 0.2 |
| **Urine** |
| Na+ (mmol/L) | **89.2 ± 4.2** | **67.8 ± 7.2\*** | **0.03** |
| K+ (mmol/L) | **180.3 ± 23.0** | **242.1 ± 17.0\*** | **0.05** |
| Na+: K+ ratio | **0.5 ± 0.1** | **0.3 ± 0.1\*\*** | **0.01** |
| pH | 5.8 ± 0.1 | 6.9 ± 0.5 | 0.07 |

*Plasma and Urine Electrolytes and Hematocrit in the 4 doses Protocol \*P<0.05 and \*\*P<0.01 vs Control, Two-Tailed, Unpaired Student’s T-Test. Na+: Sodium, K+: Potassium, HCT: Hematocrit*

**Table S3: Eplerenone prevents Carfilzomib-induced K+ sparing and Na+ retention in the urine in the four doses protocol.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table S3** | **Control (N/S 0.9%) (n=6)** | **Cfz (8 mg/kg)****(n=6)** | **Eplerenone (165 mg/kg)****(n=6)** | **Cfz + Eplerenone****(n=6)** |
| **Plasma** |
| Na+ (mmol/L) | 165.0 ± 0.5 | 162.2 ± 1.0 | **158.3 ± 0.5\*\*** | **155.3 ± 1.2\*\*\*\*,##** |
| K+ (mmol/L) | 3.9 ± 0.2 | 3.9 ± 0.3 | 4.5 ± 0.1 | 4.1 ±0.3 |
| Na+: K+ ratio | 42.0 ± 2.8 | 42.3 ± 2.8 | 35.75 ± 1.1\*\*, ## | 38.9 ± 3.0 |
| Protein (g/L) | 4.5 ± 0.6 | 3.6 ± 0.2 | 4.0 ± 0.2 | 4.3 ± 0.2 |
| HCT % | 34.5 ± 2.1 | 37.8 ± 0.4 | 37.5 ± 5.5 | 38.8 ± 3.5 |
| **Urine** |
| Na+ (mmol/L) | **89.1 ± 4.2** | **67.4 ± 7.4\*** | **86.2 ± 5.9#** | **82.3 ± 3.9#** |
| K+ (mmol/L) | **184.2 ± 18.2** | **249.08 ± 19.0\*\*\*** | **178.0 ± 24.56####** | **179.9 ± 14.9####** |
| Na+: K+ ratio | 0.6 ± 0.1 | **0.2** ± 0.1 | **0.6** ± 0.1 | **0.5** ± 0.1 |
| pH | 5.8 ± 0.1 | **6.8 ± 0.4** | **6.5 ± 0.4** | **6.3 ± 0.2** |

*Plasma and Urine Electrolytes and Hematocrit in the 4 doses Protocol with Eplerenone co-administration. \*\*P<0.01 and \*\*\*\*P<0.001 vs Control, ##P<0.01 vs Cfz, One Way ANOVA, Tukey's multiple comparisons test***.** *Na+: Sodium, K+: Potassium, HCT: Hematocrit*

**Table S4:** Primers’ Sequences, melting temperatures and product size used within the study**.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Table S4** | Forward Primer | Reverse Primer | Product Size |
| *Mus musculus* solute carrier family 9 (sodium/hydrogen exchanger), member 1 (Slc9a1; *Nhe1*) | GCCTCATGAAGATAGGTTTCCAT(Tm= 58.28) | GCAGCCCCACTACGATCAG(Tm= 60.23) | 84 |
| *Mus musculus* solute carrier family 5 (sodium/glucose cotransporter), member 1 (Slc5a1), (*Sglt-1*) | GGCTGACATCTCAGTCATCG(Tm= 58.15) | GTGGAAAACATGGCCCACAG(Tm= 59.68) | 72 |
| *Mus musculus* ATPase, Na+/K+ transporting, alpha 1 polypeptide (Atp1a1), (*Na+/K+ ATPase*) | CGGGGCCATTCTTTGTTTCC(Tm= 59.75) | CCCCGAGGTACAGATCATCG(Tm= 59.40) | 83 |
| *Mus musculus* sodium channel, non-voltage-gated 1 beta (Scnn1b), (β-*Enac*) | CACCCTGAGCAGGAAGGGTA(Tm= 60.91) | GGCTGGAGATTCCTCAATGGT (Tm= 59.79) | 82 |
| *Mus musculus* solute carrier family 12, member 1 (Slc12a1) (*Nkcc2*) | AAGCGGGAATTGGTCTTGGA(Tm= 59.60) | TGCAGAAGTTGACAACCCAGT(Tm= 60.06) | 80 |
| *Mus musculus* solute carrier family 14 (urea transporter), member 2 (Slc14a2) (*Uta-1*) | AAGGTGATCTCTGCAACAACAT(Tm=58.24) | AGCAAACAGGAAAGATCTCCCA(Tm= 59.62) | 80 |
| *Mus musculus* aquaporin 2 (*Aqp2*), | GCCACCTCCTTGGGATCTATT(Tm= 59.22) | AACTTGCCAGTGACAACTGC(Tm= 59.26) | 82 |
| *Mus musculus* glyceraldehyde-3-phosphate dehydrogenase (Gapdh) | CCCAGCTTAGGTTCATCAGGT(Tm= 59.44) | GCCAAATCCGTTCACACCG(Tm= 59.79) | 87 |

 **Supplemental Figures**



**Figure S1**



**Figure S2**



**Figure S3**

****

**F**

**Figure S4**

**Figure Legends**

**Figure S1: Graphical Workflows of the in vivo protocols. A.** Workflows of the two-, **B.** four-doses protocols and **C.** four-doses protocol with the co-administration of Eplerenone in the *in vivo* murine models.

**Figure S2: Two doses of Carfilzomib did not increase Serum Creatine and BUN, whereas elevated LDH and circulating neutrophils.** Graphs of **A.** Serum Creatinine (mg/dL), **B.** Serum Urea-Bound-Nitrogen (BUN) (n=5-8 per group) and **C.** Renal % LLVY Chemotrypsin-Like activity expressed as Fold change of Control (n=5-8 per group) **D.** Total blood cell count (x103 cells) (n=5-8 per group) and **E.** Graph of Lactate dehydrogenase (LDH, U/L) and C-reactive protein (CRP, mg/L) (n=5-8 per group) **F-G.** Representative hematoxylin-eosin histology images of kidney tissue and graph of percentage of positive samples presenting immune cell (IC) infiltration. (n=5-8 per group; 40x, bar corresponds to 15 μm). Blue scatter bars refer to the Control and red scatter bars refer to Cfz groups. Data are presented as mean±sem. *Student’s T-Test, unpaired, two-way. \*P<0.05.*

**Figure S3: Four doses of Carfilzomib did not induce Thrombotic microangiopathy (TMA) *in vivo*.** **A.** Graph of platelet (PLT) count (x105) and Platelet Width Distribution (PDW) (n=5 per group). **B.** Graph of ADAM-TS13 activity quantified as Fluorescence (AU)/min. **C-D.** Representative western blot images and relative densitometry analysis of cleaved Von Willebrand Factor (cl. vWF) normalized to total protein content (ponceau) (n=5 per group). Blue scatter bars refer to the Control and red scatter bars refer to Cfz groups. Data are presented as mean±sem. *Student’s T-Test, unpaired, two-way. \*P<0.05*

**Figure S4: Only four doses of Carfilzomib increase SBP *in vivo*, an effect prevented by eplerenone.** Graphsof Systolic Blood Pressure (SBP) and Heart Rate (HR) in the **A, B.** Two doses (n=5-8 per group) **C, D.** Four Doses (n=5-6 per group) and **E, F.** Carfilzomib and Eplerenone coadministration in the four doses protocol (n=6 per group). *Student’s T-Test, unpaired, two-way. \*P<0.05. One-way ANOVA, Tukey’s Post-hoc Test. \*\*\*P<0.005*

**References**

1. Efentakis P, Kremastiotis G, Varela A, et al. Molecular mechanisms of carfilzomib-induced cardiotoxicity in mice and the emerging cardioprotective role of metformin. *Blood*. Feb 14 2019;133(7):710-723. doi:10.1182/blood-2018-06-858415

2. Efentakis P, Psarakou G, Varela A, et al. Elucidating Carfilzomib's Induced Cardiotoxicity in an In Vivo Model of Aging: Prophylactic Potential of Metformin. *Int J Mol Sci*. Oct 11 2021;22(20)doi:10.3390/ijms222010956

3. Hoy WE, Bertram JF, Denton RD, Zimanyi M, Samuel T, Hughson MD. Nephron number, glomerular volume, renal disease and hypertension. *Curr Opin Nephrol Hypertens*. May 2008;17(3):258-65. doi:10.1097/MNH.0b013e3282f9b1a5

4. Latosinska A, Vougas K, Makridakis M, et al. Comparative Analysis of Label-Free and 8-Plex iTRAQ Approach for Quantitative Tissue Proteomic Analysis. *PLoS One*. 2015;10(9):e0137048. doi:10.1371/journal.pone.0137048

5. Stroggilos R, Mokou M, Latosinska A, et al. Proteome-based classification of Nonmuscle Invasive Bladder Cancer. *Int J Cancer*. Jan 1 2020;146(1):281-294. doi:10.1002/ijc.32556

6. Want EJ, Wilson ID, Gika H, et al. Global metabolic profiling procedures for urine using UPLC-MS. *Nat Protoc*. Jun 2010;5(6):1005-18. doi:10.1038/nprot.2010.50

7. Dunn WB, Broadhurst D, Begley P, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat Protoc*. Jun 30 2011;6(7):1060-83. doi:10.1038/nprot.2011.335

8. Katz SE, Fassbender CA. Improved sample preparation procedure for determining chlortetracycline in chicken tissue and related organs. *Bull Environ Contam Toxicol*. Jan-Feb 1971;6(1):11-6. doi:10.1007/BF01559065

9. Pang Z, Chong J, Zhou G, et al. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res*. Jul 2 2021;49(W1):W388-W396. doi:10.1093/nar/gkab382

10. Efentakis P, Varela A, Chavdoula E, et al. Levosimendan prevents doxorubicin-induced cardiotoxicity in time- and dose-dependent manner: implications for inotropy. *Cardiovasc Res*. Mar 1 2020;116(3):576-591. doi:10.1093/cvr/cvz163

11. Kokame K, Matsumoto M, Fujimura Y, Miyata T. VWF73, a region from D1596 to R1668 of von Willebrand factor, provides a minimal substrate for ADAMTS-13. *Blood*. Jan 15 2004;103(2):607-12. doi:10.1182/blood-2003-08-2861

12. Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol*. Apr 2005;129(1):93-100. doi:10.1111/j.1365-2141.2005.05420.x

13. Ding W, Yousefi K, Shehadeh LA. Isolation, Characterization, And High Throughput Extracellular Flux Analysis of Mouse Primary Renal Tubular Epithelial Cells. *J Vis Exp*. Jun 20 2018;(136)doi:10.3791/57718

14. Zhao Y, Zhao H, Zhang Y, et al. Isolation and epithelial co-culture of mouse renal peritubular endothelial cells. *BMC Cell Biol*. Nov 30 2014;15:40. doi:10.1186/s12860-014-0040-6

15. Xiao J, Shimada M, Liu W, Hu D, Matsumori A. Anti-inflammatory effects of eplerenone on viral myocarditis. *Eur J Heart Fail*. Apr 2009;11(4):349-53. doi:10.1093/eurjhf/hfp023

16. Fuller PJ, Young MJ. Mechanisms of mineralocorticoid action. *Hypertension*. Dec 2005;46(6):1227-35. doi:10.1161/01.HYP.0000193502.77417.17