Supplementary Methods

Breading environment for mice

The breeding environment was the same as previously described (1, 2). Mice were bred in our animal facility in Virginia Commonwealth University housed in temperature-controlled (20−22°C) polycarbonate cages with corn cob bedding. They were maintained in a 12:12 light/dark cycle and free access to food and water. All experiments have been approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

Cell culture

Human proximal tubular epithelial cells (HK-2) were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, MA) with 10% fetal bovine serum and streptomycin (100ug/ml) /penicillin (100 IU/ml, Gibco, MA) in a humidified incubator with 95% air and 5% CO_2 at 37 °C. HK-2 cells were grown to 80% confluence in normal medium and then starved with serum-free DMEM for 3 hours before experiments.

Primary culture of mouse kidney proximal tubular cells (3-5)

Renal cortices were dissected and washed in sterile ice-cold Krebs-Henseleit saline (KHS, pH 7.4, containing 119 mM NaCl, 4.7 mM KCl, 1.9 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, and 25 mM $NaHCO_3$).The sliced pieces were then digested in a solution of 10 mL of KHS containing 1mg/ml collagenase (270 U/mg, Worthington) and 1mg/ml soybean trypsin inhibitor (10000 U/mg, Sigma Aldrich) for 30 min at 37 °C. The supernatant was filtered through 200 μm and 80 μm mesh sieves Large proximal tubules were retained on the 80-μm sieve and then flushed in the reverse direction with KHS/1% BSA. Tubules were collected and cultured in hormonally defined serum-free medium supplemented with epidermal growth factor (EGF, 10 ng/ml) and triiodothyronine (T3, 5 $x10^{-12}$ M). The basal medium consists of DMEM/F12 (Gibco) with supplement of streptomycin (100ug/ml) /penicillin (100 IU/ml, Gibco), 50 nM selenium, 5 μg/ml transferrin, 5 μg/ml insulin, and 50 nM hydrocortisone. Identification of proximal tubular cells was

confirmed by immunostaining of megalin (1:200, Santa Cruz) (6). a marker for proximal tubules. A cortical collecting duct cell line (M-1 cell) was used as negative control.

Western blot

Cells or renal cortex were homogenized in Tris-lysis buffer containing protease and phosphatase inhibitors (Sigmafast™ Protease Inhibitor Cocktail Tablet, Sigma Aldrich). After sonication, homogenates were centrifuged at 14000 rpm at 4°C for 15 minutes. Supernatant was obtained for protein concentration measurement with Bio-Rad Protein Assay (BioRad Laboratories). Protein samples were prepared in 4X protein loading buffer boiled at 100°C for 10 minutes before SDS-PAGE. Total protein (15μg) was loaded into 10% or 12% polyacrylamide gel followed by electroblotting onto a PVDF membrane. Membranes were blocked with 5% TBST-blocking grade blocker (BioRad Laboratories) and incubated with primary antibodies against rabbit fatty acid amide hydrolase (FAAH, for human species, Proteintech, for mouse species, Cell Signaling Technology), α-smooth muscle actin (α-SMA, Abcam), collagen I/III (Millipore Corp), neutrophil gelatinase-associated lipocalin (NGAL, Cell Signaling Technology), E-cadherin (BD Biosciences), or primary antibodies against mouse IL-1β (Cell Signaling Technology) in a dilution of 1:1000 overnight at 4°C. GAPDH (1:5000, Proteintech) or β-actin (1:5000, Proteintech) were used as internal controls. Blot signals were amplified by HRP-conjugated secondary antibodies (Cell Signaling Technology) in a concentration of 1:5000 for one hour and incubated with SuperSignal™ West Pico PLUS Substrate (Thermo Fisher Scientific). Protein bands were detected with Odyssey@ Fc imaging system and intensity of bands was determined by ImageJ software. Band intensity ratios with loading controls were obtained from each single sample and normalized against mean value in control group.

Immunohistochemical (IHC) staining in kidney tissues

Paraffin-embedded kidney sections were used for immunohistochemistry staining. In brief, paraffin-embedded sections were deparaffinized and processed to subsequent antigen retrieval, endogenous peroxidase blocking with 3% H_2O_2, and nonspecific sites blocking in 10% serum. Primary antibodies against FAAH (1:1000 for mouse species, Cell

Signaling Technology), TGF-β1 (1:100, Abcam, Cambridge), CD68 (1:100, ABBiotech), CD43 (1:400, LSbio) were incubated overnight at 4 °C. Rabbit secondary antibody was then incubated for 1 hour at room temperature followed by streptavidin HRP incubation, DAB developing, and counterstained with hematoxylin. Staining area was calculated by using ImageJ (NIH, http://rsbweb.nih.gov/ij/).

Immunocytochemical staining in cultured cells

Cells were cultured on glass coverslips, fixed in 4% paraformaldehyde, incubated with antibody against with zonula occludens protein-1 (ZO-1) (Millipore Corp), α-SMA (1:300, Abcam), or megalin (1:200, Santa Cruz) at 4 °C overnight, and then incubated with fluorescence conjugated-secondary antibody for 1 hour at room temperature. Average positive areas were calculated by Image J (6).

Preparation for tissue sections

Mouse kidneys were cut longitudinally and fixed in 4% paraformaldehyde for at least 24 hours and then switched to 70% ethanol. The fixed tissues were embedded in paraffin and cut into 5μm sections.

Periodic-Acid Schiff staining

Periodic-Acid Schiff kit (Sigma Aldrich) was used for histological assessment. Kidney sections were immersed in periodic acid solution for 5 minutes and then fifteen minutes of Schiff staining. Tubular damage is defined as tubular atrophy, tubular dilation, damage of tubular epithelial cells, loss of brush border in proximal tubules, vacuolization, formation of tubular cast. The extent and scores of tubular injuries were determined on a scale of 0–4, as follows: 0 = no injury; 1 = 1–25% injured area; 2 = 26–50% injured area; 3 = 51–75% injured area; 4 > 75% injured area. Assessment of tubular injuries was semi-quantitatively scored by three blinded evaluators.

Picrosirius red staining

Picrosirius red staining (Abcam, Cambridge) was used to visualize collagen I/III staining in tubulointerstitial area. Kidney sections were stained in picrosirius red solution for 60 minutes with subsequent two changes of 0.5% acetic acid solution. The percentage area of collagen I/III staining was analyzed using ImageJ.

Masson's trichrome staining

Masson's trichrome staining (Sigma Aldrich) was used for fibrosis elvaluation. Briefly, the paraffin-embedded kidney sections were refixed in Bouin Solution for 1 hour at 60°C. Mixture of Weigert's Hematoxylin A and B solutions was applied to sections followed by incubation of Biebrich scarlet-acid fuschin solution. Later, phosphomolybdic-phosphotungstic acid was utilized as a decolorizer. Aniline blue along with 1% acetic acid was added to differentiate the tissue sections. The percentage area of blue collagen was calculated using ImageJ.

Liquid chromatography/tandem mass spectrometry (LC-MS/MS) for measurement of lipids.

Aix-point calibration curve was prepared for each analyte along with analyte free controls. Deuterated internal standards, 8 pmol of AEA-d_8 was added to each calibrator, control and sample. The analytes were extracted three time with chloroform/methanol (2:1, v/v) and a 0.73% sodium chloride mixture (Fisher Scientific). The organic phase was collected after centrifuged at 3000 rpm at 4°C for 5 minutes. The chloroform was collected and pooled. Dried residues were reconstituted in 0.1 ml of chloroform followed by 1 ml of cold acetone (Pharmco, Fisher Scientific, PA). After centrifugation for 5 min at 10000 rpm at 4°C, the upper layer was collected and dried under nitrogen gas. The extracts were reconstituted with 0.1 ml of methanol and placed in an auto-sampler vials for analysis. The ultra-performance liquid chromatography tandem mass spectrometer (LC-MS/MS) analysis was performed on a Sciex 6500 QTRAP system with an IonDrive Turbo V source for TurbolonSpray® (Sciex, Ontario, Canada) attached to a Shimadzu UPLC system (Kyoto, Japan) controlled by Analyst software (Sciex, Ontario, Canada). Chromatographic separation was performed on a Discovery® HS C18 Column 15cm x 2.1mm, 3µm (Supelco: Bellefonte, PA) kept at 40°C with a 10 µL injection volume. The mobile phase

consisted of A: acetonitrile and B: water with 1 g/L ammonium acetate and 0.1% formic acid. The following gradient was used: 0.0 to 2.4 minutes at 40% A, 2.5 to 6.0 minutes at 40% A, hold for 2.1 minutes at 40% A, then 8.1 to 9 min 100% A, hold at 100% A for 3.1 min and return to 40% A at 12.1 min. The flow rate was 1.0 mL/min and total run time was 14 minutes. The source temperature was set at 600°C, and curtain gas had a flow rate of 30 mL/min. The ionspray voltage was 5000 V, with the ion source gases 1 and 2 having flow rates of 60 mL/min. The acquisition mode used was Multiple Reaction Monitoring (MRM). The following ion transitions (*m/z*) with collision energies (eV) in parentheses were monitored in positive ion mode: AEA: 348 >62 (13) and 348>91 (60); AEA-d_8: 356 >63 (13).Calibration curves were constructed for AEA, 2-AG and arachidonic acid based on peak area ratios for the calibrators and internal standards.

siRNA transfection

FAAH or CB2 siRNA was transfected in HK-2 cells (Silencer Pre-designed siRNA, Ambion, Thermo Fisher Scientific) using Lipofectamine 3000 transfection reagent (Thermo Fisher Scientific) according to the manufacturer's guidance. For a six-well plate, each well was transfected with 75 pmol of siRNA in Opti-MEM medium (Gibco). Following 5 h incubation, cells were then maintained in normal medium overnight and subjected to TGF-β1 stimulation. A negative control siRNA (Silencer, Ambion, Thermo Fisher Scientific) was employed to reflect a baseline for target gene knockdown.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). The significance of differences in mean values within and between multiple groups was evaluated using one-way ANOVA by a Bonferroni's multiple comparisons test or two-way ANOVA followed by a Tukey's multiple comparisons test. Student's t-test was used to evaluate statistical significance of differences between two groups. $P < 0.05$ was considered statistically significant.

References

1. L. E. Wise, J. P. Harloe, A. H. Lichtman, Fatty acid amide hydrolase (FAAH) knockout mice exhibit enhanced acquisition of an aversive, but not of an appetitive, Barnes maze task. *Neurobiol Learn Mem* **92**, 597-601 (2009).
2. B. Lyu, W. Wang, X.-Y. Ji, J. K. Ritter, N. Li, Detrimental role of sphingosine kinase 1 in kidney damage in DOCA-salt hypertensive model: evidence from knockout mice. *BMC Nephrology* **21**, 173 (2020).
3. S. D. Chung, N. Alavi, D. Livingston, S. Hiller, M. Taub, Characterization of primary rabbit kidney cultures that express proximal tubule functions in a hormonally defined medium. *J Cell Biol* **95**, 118-126 (1982).
4. S. Terryn *et al.*, A primary culture of mouse proximal tubular cells, established on collagen-coated membranes. *Am J Physiol Renal Physiol* **293**, F476-485 (2007).
5. M. Kamiyama, M. K. Garner, K. M. Farragut, H. Kobori, The establishment of a primary culture system of proximal tubule segments using specific markers from normal mouse kidneys. *Int J Mol Sci* **13**, 5098-5111 (2012).
6. M. Hara-Chikuma, A. S. Verkman, Aquaporin-1 Facilitates Epithelial Cell Migration in Kidney Proximal Tubule. *Journal of the American Society of Nephrology* **17**, 39-45 (2006).