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Research Article

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FABP5 suppression impairs the adaptive response to acute kidney injury

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Abstract

Background. Fatty acid binding protein 5 (FABP5) is a FABP family intracellular fatty acid transporter. Under physiological conditions, FABPs play a crucial role in lipid metabolism, contributing to membrane biogenesis, lipid-mediated signaling, lipogenesis, and lipid degradation. Dysregulated expression of some FABPs has been associated with the development of acute kidney injury (AKI), and FABPs were proposed as biomarkers of AKI. However, the specific role of FABP5 has not been addressed.

Methods. Functional assays were performed to explore the role of FABP5 in cultured tubular cells and mice. In cultured cells, FABP5 activity was pharmacologically inhibited with SBFI-26. In vivo, folic acid-induced AKI (FA-AKI) was studied in FABP5-deficient mice and in WT mice treated with SBFI-26.

Results. Kidney FABP5 expression was reduced in FA-AKI. Inhibition of FABP5 activity in cultured tubular cells increased cellular stress and death. Mechanistically, FABP5 inhibition depleted lipid droplets, impaired fatty acid delivery to mitochondria, reduced ATP levels, and induced oxidative stress and mitochondrial loss. Consistently, both FABP5 deficiency and pharmacological inhibition of FABP5 increased the severity of AKI despite decreasing inflammatory cell infiltrates. Mechanistically, FABP5 deficiency decreased resilience to AKI by further impairing lipid metabolism, without overt compensatory responses in core glycolytic enzymes.

Conclusions. These results identify the loss of FABP5 as a maladaptive mechanism during AKI that contributes to increasing its severity. Preservation of FABP5 may confer

resilience to AKI. Clinical trials of FABP5 inhibitors for malignancy or pain should consider the potential risk of decreasing kidney resilience.

Keywords: FABP5, AKI, lipid metabolism, mitochondrial stress, inflammation, nephroprotection.

Plain English summary

Acute kidney injury (AKI) is a potentially lethal kidney condition that may require kidney replacement therapy and can accelerate the progression of chronic kidney disease. Currently, no effective pharmacological treatment exists for AKI. We now show that the levels of a protein termed FABP5 are lowered during AKI. Loss or inhibition of FABP5 causes stress and death of kidney cells in culture and increases AKI severity. This identifies FABP5 as a kidney-protective factor whose loss during AKI contributes to reduced kidney resilience. Mechanistically, loss of FABP5 lowers energy availability in kidney cells. Treatment aimed at preserving FABP5 activity may protect the kidneys from injury. By contrast, FABP5 inhibitors, which are being studied to treat malignancy or pain, may reduce kidney resilience.

Introduction

Acute kidney injury (AKI) is common and associated with an increased risk of death and of chronic kidney disease (CKD) progression [1]. Current therapeutic strategies remain largely supportive, reflecting an incomplete understanding of the cellular and molecular mechanisms that modulate tubular cell injury and recovery during acute stress. Increasing evidence indicates that metabolic dysfunction drives tubular cell injury, contributing to the initiation, severity, and persistence of kidney injury [2,3].

Kidney proximal tubular cells are among the most metabolically active cells in the body and rely predominantly on mitochondrial fatty acid oxidation (FAO) to sustain ATP production required for solute transport and cellular repair [4]. Mitochondrial dysfunction and impaired FAO are central features of AKI, leading to ATP depletion, oxidative stress, and cell death, which are interrelated processes [5-7]. Concurrent alterations in lipid metabolism and tubular repair promote lipotoxicity and a profibrotic shift [5,8,9]. During AKI, FAO and mitochondrial oxidative phosphorylation (OXPHOS) are suppressed, and proximal tubular cells shift toward glycolysis, a process that is ineffective under physiological conditions. Thus, short-term glycolytic shift can be protective, but persistent glycolysis promotes tubular atrophy, EMT, and fibrosis [10,11]. Restoring FAO activity is a major therapeutic focus to attenuate AKI [12-16]. However, although transcriptional suppression of FAO has been extensively studied, far less is known about the role of altered intracellular fatty acid trafficking in limiting mitochondrial oxidation and energy production during AKI [7-20]. Intracellular fatty acid handling requires tightly regulated transport between membranes, lipid droplets, cytosol, mitochondria, and peroxisomes. Fatty acid binding proteins (FABPs) function as cytosolic lipid chaperones that bind fatty acids of various chain lengths. Fatty acid binding protein 5 (FABP5) preferentially binds long-chain fatty acids, facilitating their targeted delivery to specific intracellular destinations, thereby coupling lipid availability to mitochondrial metabolism, redox balance, and gene regulation [21,22]. FABP5 deficiency has been linked to impaired mitochondrial respiration, oxidative stress, and pathological tissue remodeling in cardiovascular and fibrotic disease models [23]. Furthermore, Sodium-Glucose Cotransporter (SGLT2) inhibitors, which are kidney and cardioprotective drugs, increase circulating FABP5 levels [24]. Thus, FABP5 might naturally protect cells from damage. Beyond tubular metabolism, endothelial injury, pericyte detachment, and

capillary rarefaction exacerbate hypoxia and limit nutrient delivery, while maladaptive inflammatory responses further amplify tissue damage. However, the role of FABP5-mediated fatty acid trafficking in tubular and non-tubular compartments during AKI remains unknown.

From a translational perspective, metabolic interventions aimed at restoring mitochondrial function and FAO have shown promise in experimental AKI, yet clinical translation has been limited. One potential explanation is that targeting transcriptional regulators of FAO alone may be insufficient if intracellular lipid delivery to mitochondria is impaired. Identifying proximal metabolic bottlenecks that acutely limit energy production may therefore provide new therapeutic opportunities. In this context, FABP5 represents an attractive candidate, as it operates upstream mitochondrial oxidation by controlling fatty acid availability and facilitating lipid-sensitive transcription factors that regulate mitochondrial function, such as Peroxisome Proliferator-Activated Receptors (PPAR) family members [25].

We have now investigated the mechanistic role of FABP5 in renal tubular metabolism and AKI using integrated in vitro and in vivo approaches. We show that FABP5 expression is rapidly downregulated during nephrotoxic AKI and that pharmacological inhibition or genetic deficiency of FABP5 increases the severity of AKI. Mechanistically, targeting FABP5 disrupts fatty acid trafficking from lipid droplets to mitochondria. This defect results in impaired FAO, acute ATP depletion, mitochondrial dysfunction, and oxidative stress, despite activation of compensatory transcriptional programs. In vivo, FABP5 deficiency disrupted tubular integrity, mitochondrial homeostasis, inflammatory signaling, and microvascular stability. Finally, transcriptomic analyses revealed that FABP5 deficiency selectively impairs protective metabolic and reparative pathways while promoting stress and damage programs during AKI. Together,

these findings identify FABP5 as a key metabolic gatekeeper linking fatty acid trafficking, mitochondrial energy production, and tissue resilience in AKI, with potential implications for the development of metabolism-based therapeutic strategies.

Methods

Animal model

All animal procedures were conducted in accordance with the European Directive 2010/63/EU for the protection of animals used for scientific purposes and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the IIS-FJD Animal Research Ethics Committee and the Consejería de Medio Ambiente y Ordenación del Territorio, Comunidad de Madrid (PROEX 242.2/21).

Folic acid nephropathy is a classic model of acute kidney injury (AKI) characterized by acute renal failure, tubular cell death, interstitial leukocyte infiltration, and subsequent tubular regeneration, which has been reported in humans [26,28]. C57BL/6 wild-type mice (WT) were obtained from Charles River (Chatillon-Sur-Charlaronne, France). FABP5 knockout (FABP5^{-/-}) mice were obtained from Dr Gökhan S. Hotamışligil's laboratory (Harvard T. H. Chan School of Public Health, Boston, MA) under a material transfer agreement. Folic acid-induced AKI (FA-AKI) was induced in 12- to 14-week-old WT (n = 10) or FABP5^{-/-} male mice (n = 11) that received a single intraperitoneal injection of 250 mg/kg folic acid (FA) (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.3 mol/L sodium bicarbonate. WT (n = 8) and FABP5^{-/-} (n = 7) mice injected with the vehicle alone served as controls. Female mice were subjected to the same experimental conditions. In addition, to evaluate the effect of the FABP5 inhibitor SBFI-26 (SBFI) (H4-117506B, MedChem Express, Monmouth Junction, South Brunswick, NJ, USA), a group of WT mice was treated with SBFI alone (n=3), and another WT group was treated with SBFI-

26 before FA-AKI induction (n=4). Control untreated WT (n = 3) or FABP5^{-/-} (n = 3) mice injected with the FA vehicle were included as control groups. The inhibitor was resuspended in 10% DMSO and diluted with 90% corn oil (v/v), then administered intraperitoneally at a dose of 20 mg/kg. Mice in both groups received the inhibitor 4 hours before folic acid or vehicle injection, followed by a second dose 24 hours later. All animals were euthanized 48 hours after folic acid or vehicle dosing.

To assess the transition from acute to chronic kidney injury, male C57BL/6 WT (n = 5) and FABP5^{-/-} mice (n = 4) were administered a single intraperitoneal injection of 250 mg/kg folic acid and euthanized 15 days post-AKI induction. Control WT (n=3) or FABP5^{-/-} (n=3) groups were included for comparison.

Euthanasia was performed under deep anesthesia induced with ketamine (35 mg/kg; Ketolar, Pfizer, New York, NY, USA) and xylazine (5 mg/kg; Rompun, Bayer, Leverkusen, Germany). Blood for serum analytical assessment was collected from the saphenous vein into EDTA-coated tubes. Plasma was obtained by centrifugation at 2.500 rpm for 15 minutes. Kidneys were perfused *in situ* with cold saline before removal. One kidney was snap-frozen in liquid nitrogen for RNA and protein studies, and the other was fixed and paraffin-embedded for immunohistochemistry. Plasma urea and creatinine levels were measured using an autoanalyzer Roche/Hitachi cobas® c701/702 (Roche Diagnostics, Risch-Rotkreuz, Switzerland).

Cell cultures and treatments

Murine proximal tubular cells (MCT) are a well-characterized proximal tubular epithelial cell line originally derived from the renal cortex of SJL mice and suitable for studying the molecular mechanisms of kidney injury [29-30]. MCT were cultured in RPMI 1640 medium (Gibco, Waltham, MA, USA) supplemented with decomplemented fetal bovine

serum 10% (FBS) (Gibco, Waltham, MA, USA), 2 mM glutamine (Gibco, Waltham, MA, USA), 100 U/ml penicillin (Gibco, Waltham, MA, USA), and 100 µg/ml streptomycin (Gibco, Waltham, MA, USA). Cells were grown at 37 °C in a humidified atmosphere with 5% CO₂. HK-2 cells (ATCC, CRL-2190™, Manassas, VA, USA) are derived from a normal, human adult male kidney. Mouse C3H-derived fibroblast-like embryo cells (C3H/10T1/2, Clone 8; ATCC) are a well-characterized cell line typically used as an in vitro model of pericytes [31,32]. C3H/10T1/2 cells were cultured in Basal Medium Eagle (Gibco, Waltham, MA, USA) supplemented with 10% DFBS (Gibco, Waltham, MA, USA), 2 mM glutamine (Gibco, Waltham, MA, USA), 100 U/ml penicillin (Gibco, Waltham, MA, USA), and 100 µg/ml streptomycin (Gibco, Waltham, MA, USA). Cells were grown at 37 °C in a humidified atmosphere with 5% CO₂.

Primary proximal tubular epithelial cells (PTEC) were obtained from C57BL6j WT mice. The kidney cortex was decapsulated, dissected from the medulla and minced into small fragments (approximately 1 mm³). Then, the fragments were digested with 0.5 mg/ml type II collagenase (Gibco, Waltham, MA, USA) at 37 °C for 15 min. After digestion and centrifugation, the tissue fragments were gently filtered through a 100 µm cell strainer under pressure. The resulting pellets were suspended in erythrocyte lysis buffer for 10 minutes, centrifuged, and finally suspended in PBS and gently passed through a 40-µm cell strainer. The filtrate, which contains proximal tubular fragments, was centrifuged at 1500 rpm for 10 minutes at room temperature. The resulting pellet was resuspended in RPMI 1640 medium supplemented with 10% DFBS, 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 100 µg/ml insulin-transferrin-selenite (ITS). Medium and reagents were provided by Gibco (Waltham, MA, USA). The proximal tubular epithelial cells were maintained at 37 °C in a humidified atmosphere containing 5% CO₂ and used for experiments upon reaching confluence.

Unless otherwise indicated, experiments were performed in medium containing 10% FBS, as cells did not survive SBFI stimulation under serum-free conditions. Subconfluent cells were incubated overnight in serum-free medium and stimulated the following day in the presence of serum. Cells were treated with SBFI26 (MCE, Monmouth Junction, New Jersey, USA), added to the medium at various concentrations and for varying durations. Where indicated, inhibitors were added 30 minutes before SBFI stimulation: 25 μ M Z-Val-Ala-DL-Asp(OMe)-fluoromethylketone (z-VAD) (Bachem, Bubendorf, Switzerland), 30 μ M necrostatin 2 racemate (Nec-1s) (Selleck Chemicals, Houston, Texas, USA), 1 μ M ferrostatin-1 (Fer) (Sigma-Aldrich, Darmstadt, Germany), 100 μ M/mL Atglistatin (DEUP) (Sigma-Aldrich, Darmstadt, Germany), or Lys05 (MCE). Additionally, inhibitors were also tested as single treatments. Stock solutions of the stimuli and inhibitors were made following the specified manufacturers' instructions.

Transcriptomics analysis

Transcriptomic analyses were performed on kidney samples from the FA-AKI model, including WT and FABP5-deficient mice subjected to FA-induced AKI (AKI-WT, n = 4; AKI-FABP5^{-/-}, n = 4), along with their respective control groups (WT, n = 4; FABP5^{-/-}, n = 4). Before RNA extraction, kidney samples were homogenized and treated with DNase to remove genomic DNA contamination. Before RNA extraction, samples were homogenized and treated with DNase to eliminate any remaining genomic DNA. Total RNA was extracted using the MagMAX mirVana Total RNA Isolation kit according to the manufacturer's instructions (Applied Biosystems, ThermoFisher Scientific, Waltham, MA, USA). The quantification of the extracted RNA was performed using fluorimetry on a Quantus fluorometer (Promega Corporation, Madison, WI, USA). Dual-indexed mRNA libraries were prepared using the QuantSeq 3' mRNA-Seq V2 Library Prep Kit with UDI

(Lexogen GmbH, Vienna, Austria), according to the user guide. According to the manufacturer's recommendations, 250 ng of starting material was used. Library quality was evaluated using the QiaXcel Advanced System (Qiagen, Hilden, Germany). Sequencing was performed using a NovaSeq X System (Illumina, San Diego, CA, USA, using a paired-end 2×150 bp configuration based on Sequencing by Synthesis (SBS).

Bioinformatic Analysis

Raw sequencing data were processed through a bioinformatic pipeline including quality control, trimming, alignment, and gene expression quantification. Adapter sequences and low-quality bases, including poly(A) tails, were removed before analysis. Quality of the resulting FASTQ files was assessed using FastQC (v0.11.9). Trimmed reads were aligned to the mouse reference genome (GRCm39) using STAR (v2.7.10), allowing for spliced read mapping. Duplicate reads were removed based on unique molecular identifiers (UMIs) using UMI-tools. Gene-level reading counts were obtained using HTSeq-count (v2.0.2) with gene annotations from the NCBI GTF reference file. Gene expression analyses were performed in R using Bioconductor packages. Normalization and differential analysis between groups were performed in R using DESeq2 [33]. Statistical significance was determined by calculating p-values for each gene, followed by multiple testing correction using the Benjamini–Hochberg method to control the false discovery rate (FDR; q-value). Genes were considered to be differentially expressed if they exhibited an absolute $\log_2$ fold change of at least 1 ($|\log_2\text{FC}| \geq 1$) and an adjusted p-value (q-value) lower than 0.05. ($\text{q-value} \leq 0.05$). Data processing and visualization were performed using additional R packages, including ggplot2 [34] and pheatmap [35]. Gene functional annotation was performed using the Gene Set Enrichment Analysis (GSEA) software [36] with the GSEA Preranked approach, which is based on $\log_2$ fold-change–

ranked gene lists. Differentially expressed genes (DEGs) were further analyzed for functional enrichment using Metascape [37], based on Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and REACTOME pathways/reactions. Data visualization of DEGs and functional enrichment results, including volcano plots and heatmaps, was performed using GraphPad Prism (v8.0.2).

Gene expression assays

Total RNA was extracted using the TRI Reagent method (Invitrogen, Carlsbad, CA), and 1 µg RNA was reverse transcribed with the High-Capacity cDNA Archive Kit (Applied Biosystems™, Waltham, MA, USA). Quantitative PCR was performed in a 7500 Real Time PCR System with the Prism 7000 System SDS software using the commercial master mix Premix Ex Taq (Takara, Otsu, Japan) and predesigned TaqMan® Gene Expression Assays (Applied Biosystems™, Thermo Fisher Scientific, Waltham, MA, USA) for target and housekeeping genes. RNA expression of different genes was normalized to Glyceraldehyde-3-phosphate (GAPDH) expression and Peptidylprolyl isomerase A (PPIA) expression. The results were expressed as fold-change (n-fold) relative to control, and then the row Z-scores were calculated for each gene.

Protein expression assays

Western blot

Samples were homogenized in lysis buffer (50 mM Tris HCl, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 0.2% Triton X-100, 0.3% NP-40, and 0.1 mM PMSF) supplemented with a protease inhibitor cocktail (PIC) (Sigma-Aldrich, St. Louis, MO, USA) and a phosphatase inhibitor cocktail (PHIC) (Sigma-Aldrich), each at a 1:100 dilution. Proteins were quantified using a Pierce BCA protein assay kit (Thermo Fisher Scientific, Waltham,

MA, USA). Proteins were separated by 12% SDS-PAGE under reducing conditions. After electrophoresis, samples were transferred into nitrocellulose membranes (AmershamTM ProtranTM- Cytiva, Marlborough, MA, USA). Membranes were blocked with 5% skimmed milk in TBS with 0.1% Tween 20 (TBS-T) for 1 hour at room temperature, and then incubated overnight at 4°C with primary antibodies in the same blocking solution or 5% Bovine Serum Albumin (BSA) in TBS-T. The following primary antibodies were used to detect specific proteins of interest: anti-FABP5 (1:1000; D1A7T) and antiphospho-AMPK (1:1000; T172) were from Cell Signaling Technology (Danvers, MA, USA), anti-p62 (1:1000; E-AB-62289 Elabscience, San Diego, CA, USA), and anti-LC3B (1:1000; NB100-2220 Novus Biologicals, Centennial, CO, USA). Then, blots were washed with TBS-T and incubated with appropriate horseradish peroxidase–conjugated secondary antibody (1:5000; Cytiva, Marlborough, MA, USA). After this, the membrane was washed with TBS-T and subsequently incubated with the peroxidase substrate (Merck Millipore Corporation, Burlington, MA, USA) for chemiluminescent detection using the ImageQuant LAS 4000 imaging system and software (GE Healthcare Life Sciences- Cytiva, Marlborough, MA, USA). Anti- α -Tubulin (1:5000; MAB374 Sigma-Aldrich, St. Louis, MO, USA), anti-vinculin (1:1000; AB155120 Abcam, Cambridge, United Kingdom), and anti-GAPDH (1:5000; MAB374 Millipore Corporation, Burlington, MA, USA) were used as loading controls to assess protein loading homogeneity. The results were expressed as fold-change (n-fold) relative to the control.

Immunohistochemistry

Paraffin-embedded sections were stained using standard histology procedures. Immunostaining was performed on 3 μ m-thick tissue sections that were deparaffinized, rehydrated, and antigen retrieved using the PT Link system (Dako, Glostrup, Denmark)

with sodium citrate buffer (10 mM) adjusted to pH 6-9, depending on the marker. For colorimetric immunohistochemistry, endogenous peroxidase was blocked with 3% hydrogen peroxide (Millipore, Burlington, MA, USA), and protein blockade was performed using 10% species-specific serum diluted in 4% BSA in PBS (the same animal species as the animal source of primary antibodies) or, when indicated, using a casein-blocking solution (Vector Laboratories, Newark, CA, USA). Sections were then incubated overnight at 4°C with primary antibodies: anti-FABP5 (1:50; D1A7T), anti-F4/80 (1:300; D2S9R), and anti-VCAM-1 (1:200; # 32653) from Cell Signaling Technology, Danvers, MA, USA, and anti-PCNA (1:100; Ab29), anti-MPO (1:500; Ab139748), anti- α -Smooth Muscle Actin (α -SMA; 1:300; Ab7817), anti-CD3 (1:100; Ab16669), and anti-VEGF-1 (1:100; Ab52917) from Abcam, Cambridge, United Kingdom. Afterwards, sections were incubated with the appropriate biotinylated secondary antibodies (1:200; Merck Millipore Corporation, Burlington, MA, USA) and avidin-biotin complex (AB Complex) (Vector Labs, Newark, CA, USA). Finally, sections were washed, developed with 3,3'-diaminobenzidine (DAB) as chromogen (Dako (Agilent Technologies), Glostrup, Denmark), counterstained with Carazzi's hematoxylin (# 05-060 12/L, Bio Optica, Milan, Italy), dehydrated, and mounted in DPX medium (Sigma-Aldrich, St. Louis, MO, USA). Images were obtained by optical microscopy (BX53F2 model; Olympus Corporation, Tokyo, Japan) and quantified with Image Pro Plus software (MediaCybernetics, Bethesda, MD, USA). Results are expressed as the number of positive cells in 10 randomly selected low-power (20 $\times$) or high-power (40 $\times$) fields per kidney, and as the percentage of stained area, considering the total area as 100%. Negative controls included non-specific immunoglobulin and omission of the primary antibody.

Immunofluorescence

Immunofluorescence was performed on cultured cells and tissue samples. Cells were fixed with 4% paraformaldehyde, whereas tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval as described above. Both cultured cells and tissue samples were then permeabilized with 0.2% Triton X-100/PBS. Autofluorescence was quenched with 50 mM ammonium chloride (NH₄Cl), and nonspecific protein binding was blocked with 10% species-specific serum diluted in 4% BSA in PBS (from the same animal species as the source of the primary antibodies). Samples were incubated overnight at 4 °C with primary antibodies diluted in PBS containing 1% BSA and 10% species-specific serum. The following primary antibodies were used to detect specific proteins of interest: rabbit monoclonal anti-FABP5 (1:50; D1A7T, Cell Signaling Technology), rabbit monoclonal anti-PDGFR (1:100; Ab32570, Abcam), and mouse monoclonal anti- α -SMA (1:200; A2547, Sigma-Aldrich). Subsequently, samples were incubated for one hour at room temperature with the appropriate fluorophore-conjugated secondary antibodies diluted in 1% BSA in PBS. The following secondary antibodies were used: Alexa fluorTM 633 goat anti-rabbit IgG (H+L) (1:200, A21070), Alexa fluorTM 488 donkey anti-rabbit IgG (H+L) (1:200; A21206), and Alexa fluorTM 488 rabbit anti-mouse IgG (H+L) (1:200; A11059), all from ThermoFisher Scientific, Waltham, MA, USA. Colocalization studies were performed by either simultaneous or sequential incubation of primary antibodies, depending on their species specificity. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (1:10.000; D9564; Sigma-Aldrich). Finally, stained samples were mounted with ProLongTM Gold antifade reagent (Invitrogen, Waltham, MA, USA). Images were acquired using a fluorescence microscope (BX53F2 model, Olympus Corporation, Tokyo, Japan) or confocal microscopy (TCS SP5, Leica-Microsystems, Wetzlar, Germany) and analyzed using the imaging software "LAS AF" (LEICA) (Leica-microsystems, Wetzlar, Germany).

Fluorescence tissue staining

For tissue samples, 8 µm-thick cryosections embedded in OCT compound were fixed with 4% paraformaldehyde, washed with PBS, and incubated with 200 ng/mL BODIPY® 493/503 (Invitrogen™, Waltham, MA, USA) for 30 min. Then, samples were washed with PBS, and nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; 1:10,000; Sigma-Aldrich). Finally, stained samples were mounted with ProLong™ Gold antifade reagent (Invitrogen). Images were acquired using a confocal fluorescence microscope (SP5; Leica Microsistemas, L'Hospitalet de Llobregat, Spain).

Sirius Red staining

Paraffin-embedded 3 µm-thick tissue sections were deparaffinized, rehydrated, and incubated for five days in 70% ethanol at 4°C. Then, sections were incubated for 30 minutes with a working solution prepared 48 hours before use by mixing 10 ml of a 1% (w/v) Sirius Red solution with 90 ml of picric acid. A 1% (w/v) Sirius Red solution was prepared by dissolving 1 g of Direct Red80 (Sirius Red) (Sigma-Aldrich, St. Louis, MO, USA) in 100 ml of distilled water. After staining, the samples were washed, dehydrated, and mounted in DPX medium (Merck, Darmstadt, Germany). Finally, images were acquired using an optical microscope (BX53, Olympus Corporation, Tokyo, Japan) and quantified using ImageProPlus software (Media Cybernetics, Bethesda, MD, USA). Results are expressed as relative staining area, normalized to the corresponding control.

Cell viability assays

Cell cytotoxicity was evaluated using the Cell Counting Kit - 8 (CCK8) assay kit (reference CK04; Dojindo Laboratories, Tokyo, Japan) following the manufacturer's protocol. Absorbance was measured at 450 nm in a Tecan Infinite®200 PRO with Tecan

i-control 2.0.10.0 software (Tecan Group Ltd., Männedorf, Switzerland). Results are expressed as a percentage of cell viability normalized to the control (100%).

For the evaluation of cell death through plasma membrane alterations, we used the PE Annexin V apoptosis detection kit combined with 7-AAD staining (BD Pharmingen, BD Biosciences, Franklin Lakes, NJ, USA) developed according to the manufacturer's instructions, followed by flow cytometry (FC) to classify necrotic and apoptotic cell populations using a BD FACSCanto II flow cytometer (BD Biosciences).

In renal tissue, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was performed in 3-mm-thick sections of paraffin-embedded tissue with the In Situ Cell Death Detection Kit Fluorescein (Roche, Basel, Switzerland) according to the manufacturer's protocol. TUNEL-positive cells were counted in ten randomly chosen fields under a fluorescence microscope (BX53 model; Olympus Corporation, Tokyo, Japan). Results are expressed as TUNEL-positive cells, normalized to the corresponding control.

Fluorescent pulse-pulse chase assay

For fluorescent fatty acid pulse-chase assays, MCTs were pulsed with Red C12 overnight in complete medium (10% FBS) to allow its intracellular storage, then replaced with fresh, nutrient-deprived medium (0% FBS) to simulate nutrient stress, a condition associated with AKI that mobilizes fatty acids from intracellular deposits, including lipid droplets (LDs) [38]. Briefly, MCT cells were seeded on optical-bottom imaging dishes (Imaging Dish CG 1.0, 130-098-282; Miltenyi Biotec, Bergisch Gladbach, Germany). Cells growing in RPMI medium containing 10% FBS were incubated for 16 h with 1 μ M BODIPY™ 558/568 C12 (Red C12; D-3835; Invitrogen). Afterwards, the medium was renewed, and the cells were exposed to SBFI-26 at different times. Control cells were

allowed to grow in 10 % FBS RPMI or starved in 0 % FBS RPMI. Thirty minutes before the end of the experiment, 200 ng/mL BODIPY® 493/503 (D3922; Invitrogen) or 100 nM MitoTracker™ Green FM (M7514; Invitrogen) were added. Live-cell images were acquired using a confocal microscope (TCS SP5, Leica) with a 63x oil-immersion objective and an additional optical zoom of 3x. To analyze the degree of colocalization between Red C12/MitoTracker™ or Red C12/BODIPY®, images were processed using Fiji ImageJ (NIH, Bethesda, MD, USA). Colocalisation for RedC12 on MitoTracker™ or BODIPY® channels was analyzed by Mander's overlap coefficient (MOC) (Fiji-ImageJ, JACOP plug-in) and using a fluorescence intensity threshold (tM) to remove the background signal [39,40].

Oxidative stress detection

For the detection of mitochondrial oxidative stress, the fluorescent probe MitoSOX™ (reference M36008; Invitrogen, Waltham, Massachusetts, USA) was used. Cells were seeded in black-walled, clear-bottom 96-well plates (reference CLS3603; Corning, Corning, New York, USA) and treated according to the experimental design. Subsequently, cells were incubated with MitoSOX™ following the manufacturer's protocol, and fluorescence intensity was measured using a Tecan Infinite®200 PRO and Tecan i-control 2.0.10.0 software (Tecan Group Ltd.). The results were expressed as fold-change (n-fold) relative to the control. For the detection of general oxidative stress, the fluorescent probe CM-H2DCFDA (C6827; Invitrogen), which measures intracellular reactive oxygen species, was used. After treatment with the corresponding stimulus, cells were incubated with CM-H2DCFDA for 3 hours at 37 °C. After staining, cells were trypsinised, washed, and transferred to FACS tubes containing RPMI supplemented with

10% FBS for flow cytometric analysis on the BD FACSCanto II (BD Biosciences, Franklin Lakes, New Jersey, USA).

Measurement of mitochondrial membrane potential

For the detection of depolarized mitochondria, the Image-iT™ TMRM Reagent kit (I34361; Invitrogen) was used. Previously treated cells were collected and centrifuged for 5 min at 1500 rpm, then resuspended in serum-free medium containing 100 nM TMRM and incubated for 30 min at 37 °C. Subsequently, 400 µL of the same serum-free medium was added, and fluorescence was measured using a BD FACSCanto II flow cytometer (BD Biosciences, Franklin Lakes, New Jersey, USA). Results are expressed as TMRM-positive cells, normalized to the corresponding control.

Oil Red O cell staining

Cultured cells were fixed with 4% paraformaldehyde and washed with distilled water. Oil Red O (ORO) staining was performed by incubating the samples for 20 minutes with a working solution obtained by diluting a saturated Oil Red O solution in isopropanol with distilled water (1:1.5, v/v). After staining, the samples were washed with distilled water to remove excess dye and counterstained with Carazzi's hematoxylin (Bio Optica, Milan, Italy). Finally, plates were air-dried completely and visualized directly with optical microscopy (BX53 model; Olympus Corporation, Tokyo, Japan). Images were analyzed using the ImageProPlus software (Media Cybernetics) to measure the area positively stained with Oil Red O. Results are expressed as relative Oil Red O staining area, normalized to the corresponding control.

Tissue lipid peroxidation assay

Lipid peroxidation was analyzed using the Thiobarbituric Acid Reactive Substances (TBARS) Assay kit (Cayman Chemical, Ann Arbor, Michigan, USA). TBARS assay was performed on homogenized tissue according to the manufacturer's protocol. Absorbance was measured with the colorimetric reader TECAN infinite F200 and Tecan i-control 2.0.10.0 software (Tecan Group Ltd.). Results are expressed as MDA equivalents per μg of total protein.

Cell proliferation assay

Cell proliferation was analyzed using the Cell Proliferation ELISA, BrdU (colorimetric) kit (Reference 11 647 229 001; Roche, Basilea, Switzerland). The assay was performed on cultured cells that had previously been treated according to the experimental design, following the manufacturer's protocol. Absorbance was measured using the colorimetric reader TECAN infinite F200 and Tecan i-control 2.0.10.0 software (Tecan Group Ltd). Results are expressed as the percentage of absorbance normalized to the corresponding control, which was set as 100%.

Luminescent ATP detection assay

For the detection of luminescent ATP, the Luminescent ATP Detection Assay Kit (reference ab113849; Abcam, Cambridge, UK) was used. Cells were seeded in 96-well plates with opaque black walls and transparent bottoms (reference 10158721; Thermo Fisher Scientific, Waltham, Massachusetts, USA) and treated according to the experimental design. The luminescent ATP Detection Assay was then performed following the manufacturer's protocol, and luminescent intensity was measured using a Tecan Infinite®200 PRO and Tecan i-control 2.0.10.0 software (Tecan Group Ltd.). Results are expressed as relative concentrations normalized to the control.

Cellular bioenergetic analysis

The Cell Mitochondrial Stress Test Assay (Agilent Technologies, Santa Clara, CA, USA) was used to measure the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using a Seahorse XF Pro Analyzer (Agilent). These measurements provided information on ATP-Production Coupled Respiration (Last rate measurement before Oligomycin Injection-Minimum rate measurement after Oligomycin injection), Basal Respiration (Last rate measurement before first injection- Non-Mitochondrial Oxygen Consumption), and Maximal Respiration (Maximum rate measurement after FCCP injection-Non-Mitochondrial Oxygen Consumption) [41]. MCT cells were seeded in XF Pro M microplates (Agilent) at a density of 4×10^3 cells/well in 80 μ L of medium. Immediately after seeding, plates were kept at room temperature for 1 h before transferring to the incubator to minimize disturbances in plate-wide cell distribution [42]. The following day, cells were treated with SBFI (10 μ M) for 2 h. Culture medium was then carefully removed, and wells were washed with 200 μ L of XF RPMI (Agilent) supplemented with 1 mM sodium pyruvate, 2 mM glutamine, 10% FBS, and 10 mM D-glucose. Next, 180 μ L/well of the same medium was added, and cells were incubated for 1 h at 37 °C in a non-CO₂ incubator. After this period, and immediately before starting the assay readout, the plate medium was replaced with the same volume of fresh medium. For the bioenergetic assay, mitochondrial complex inhibitors were injected automatically. In the Real-Time ATP assay, oligomycin (oligo), p-trifluoromethoxyphenylhydrazine (FCCP), and rotenone/antimycin A (rot/AA) were added sequentially to final concentrations of 1.5 μ M, 10 μ M and 0.5 μ M, respectively. Measurements were performed with 3-6 technical replicates, and experiments were independently repeated at least three times. Experimental design and data analysis were carried out using Seahorse Wave Pro and XF Pro Controller software (Agilent Technologies, v10.0.1.72).

Culture medium conditioning experiments

Conditioned medium experiments were performed to evaluate fatty acid transfer from FABP5 inhibitor-treated MCT cells to untreated recipient cells. MCT cells were treated with the FABP5 inhibitor SBFI for 24 h, a time point at which LD depletion was observed. After treatment, the medium was replaced with serum-free RPMI (0% FBS) to eliminate inhibitor residues and ensure that free fatty acids originated exclusively from the cells. After an additional 24 h, the conditioned medium was collected and transferred to MCT cells previously serum-starved for 48 h to deplete LD-associated fatty acid stores. Following incubation with the conditioned medium, LD area in recipient cells was assessed by ORO (O0625, Sigma-Aldrich, Merck) staining, as a readout of fatty acid transfer from donor cells.

Statistics

Statistical analysis was performed using GraphPad Prism (Dotmatics, San Diego, CA). Data distribution was assessed using the Shapiro-Wilk test. Results are presented as mean $\pm$ standard error or the mean (SEM), and comparisons were performed using the unpaired *t*-test (two groups) or one-way ANOVA (multiple groups), followed by Tukey's post hoc test when appropriate. For non-normally distributed variables, comparisons were performed using the Mann–Whitney U test (two groups) or the Kruskal–Wallis test followed by Dunn's post hoc test (multiple groups). A two-tailed *p*-value < 0.05 was considered statistically significant.

Results

FABP5 expression in kidney cells and tissue

In-house transcriptomic data from murine folic acid-induced AKI (FA-AKI), deposited in GEO (GSE273063), revealed significant downregulation of FABP5 at 24 hours (fold change = 0.549; FDR = 0.005). Reduced kidney FABP5 expression in FA-AKI mice was validated by PCR (**Fig. 1A**) and Western blot (**Fig. 1B**). We further examined FABP5 immunoreactivity in kidney tissue by immunohistochemistry (**Fig. 1C**). In control kidneys from WT mice, FABP5 was diffusely expressed in kidney tubules and, more intensely, in endothelial cells, as previously reported [43], as well as in adjacent cells with a morphology consistent with pericytes. In kidneys from mice with AKI, there was an overall reduction in FABP5 expression and immunoreactivity, but cellular localization appeared to be preserved. FABP5 staining was absent in FABP5^{-/-} mice, confirming the specificity of tubular and interstitial FABP5 staining (**Fig. 1C**). Microvascular expression was further confirmed by double immunofluorescence staining of FABP5 and the pericyte marker Platelet-Derived Growth Factor Receptor (PDGFR) (**Fig. 1D**). In line with these findings, non-diseased human normal tissue sections show a similar FABP5 localization pattern, as reported in the Human Protein Atlas (**Fig. S1A**) [44].

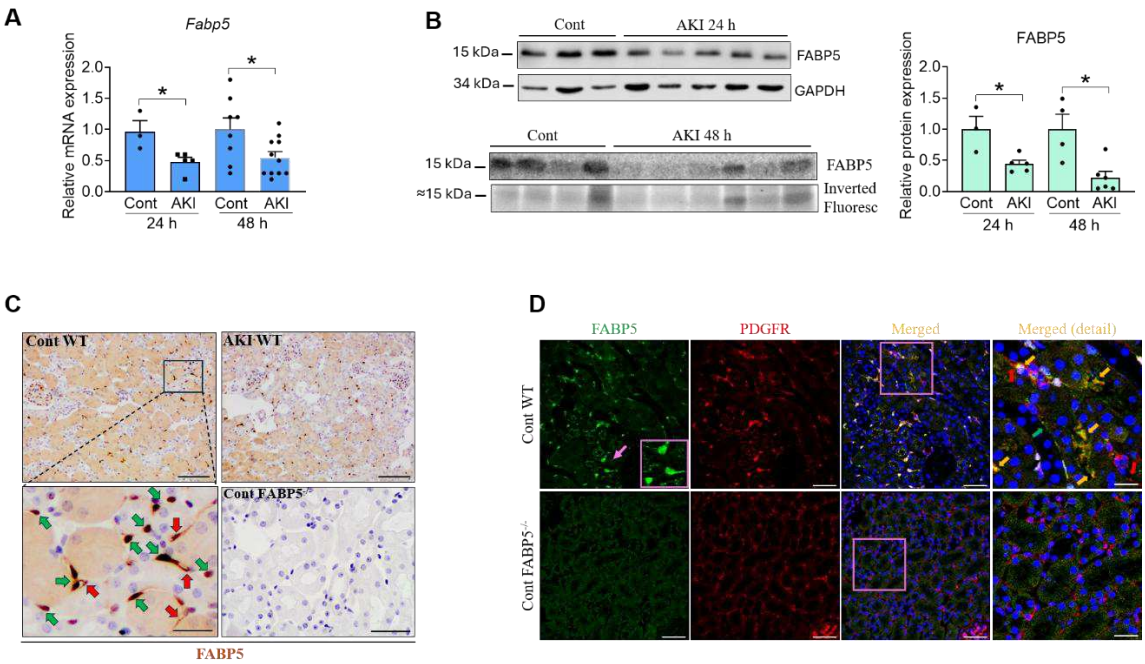


Fig. 1 FABP5 is expressed across several renal cell types and is reduced during AKI. A-B Kidney FABP5 expression was assessed at 24 h and 48 h after FA-AKI induction (AKI) by RT-qPCR (**A**) and Western blot (**B**), and normalized to time-matched wild-type (WT) control mice without AKI (Cont). Data are presented as mean $\pm$ SEM; * $p \leq 0.05$ vs Cont. **C** FABP5 expression and localization in kidney sections were analyzed by immunohistochemistry at 48 h post-AKI induction. The black box indicates the region shown at higher magnification below. Red arrows indicate FABP5 staining in cells morphologically compatible with pericytes, and green arrows with endothelial cells. Original magnification 200 $\times$; scale bars: 100 μ m. **D** Immunofluorescence analysis of kidney sections from WT and FABP5^{-/-} mice showing FABP5 (green fluorescence) colocalized with cells expressing PDGFR (red fluorescence). Representative images from mice of both genetic backgrounds are shown. Original magnification 200 $\times$, scale bars 50 μ m. A group of FABP5-expressing cells is indicated (pink arrow) and outlined (pink square). The boxed region is shown at higher magnification in the merged image on the right. Arrows indicate cells expressing FABP5 (green arrows), PDGFR (red arrows), or both proteins (orange arrows).

FABP5 inhibition sensitizes cells to nutritional stress and impairs lipid delivery to mitochondria

We studied the role of FABP5 in normal tubular metabolism. Murine proximal tubular cells (MCT) expressed FABP5. The FABP5 pharmacological inhibitor SBFI reduced FABP5 protein levels, suggesting that FABP5 expression may be regulated by its functional activity. FABP5 was also expressed in the human proximal tubular cell line HK2, and in murine PTEC (**Fig. S1B**). In MCT cells, serum depletion alone did not decrease cell viability. However, SBFI dose-dependently induced cell death within 8 hours in serum-starved MCT cells but not in cells cultured with 10% FBS (**Fig. S2A**). Cell death was mitigated by the pan-caspase inhibitor z-VAD-fmk. In contrast, necrostatin and ferrostatin had no protective effects, indicating that FABP5 inhibition in the absence of nutritional support preferentially induces apoptosis rather than regulated necrosis (**Fig. S2B, C**). Therefore, 10% SBF was used in subsequent experiments to study the role of FABP5.

Next, we performed fluorescence pulse-chase assays to track the intracellular trafficking of fluorescent fatty acid Red C12 in tubular cells stimulated with SBFI (**Fig.**

2). MCTs were pulsed with Red C12 overnight in complete medium (10% FBS) to allow its intracellular storage. The medium was then replaced with fresh, nutrient-deprived medium (0% FBS) to simulate nutrient stress, a condition associated with AKI that mobilizes fatty acids from intracellular deposits, including lipid droplets (LD) [38]. In the presence of 10% FBS, the Red C12 signal predominantly colocalized with the LD marker BODIPY 493/503 after 6 to 48 h, with only minor detection in mitochondria, consistent with its lower abundance relative to fatty acid content in FBS (**Fig. 2A and 2D; Fig. S3 A,D,E,H**). Under serum depletion (0% FBS), the Red C12 signal in LDs was higher than in 10% FBS cells, indicating that Red C12 was the sole external lipid source. Moreover, Red C12 was progressively detected in mitochondria from 6 to 48 h, while the BODIPY signal gradually decreased over time, indicating lipid mobilization from LDs to mitochondria to support energy production (**Fig. 2B and 2D; Fig. S3 B,D,F,H**). However, FABP5 inhibition resulted in a progressive loss of the Red C12 signal in LD that was uncoupled from mitochondrial transfer, as evidenced by the absence of Red C12 labelling in mitochondria (**Fig. 2C and 2D; Fig. S3. C,D,G,H**). Thus, in the absence of FABP5 activity, lipids can exit LDs but fail to reach mitochondria, even when the activities of other FABPs are preserved. This effect was further supported by Oil Red O (ORO) staining in murine MCT (**Fig. 2E**) and PTEC and in human tubular HK2 cells (**Fig. S3I**). ORO showed markedly reduced lipid storage after FABP5 inhibition, indicating increased LD degradation. In agreement with these results, FABP5 inhibition reduced mRNA expression of Perilipin 2 (*Plin2*), a key component of the LD membrane that correlates with LD abundance, and increased transcription of Patatin-like phospholipase domain-containing 2 (*Pnpla2*), which encodes Adipose Triglyceride Lipase (ATGL), the main lipolytic enzyme involved in lipid degradation during starvation [38] (**Fig. 2F**). Accordingly, pretreatment with the lipase activity inhibitor DEUP before FABP5

inhibition promoted LD accumulation in metabolically active, proliferating cells, as determined by ORO staining, and prevented the loss of LDs induced by FABP5 inhibition (**Fig. 2G**). Moreover, a similar effect was observed in cells pretreated with the autophagy inhibitor Lys05 [45] (**Fig. 2H**). These findings indicate that FABP5 inhibition blocks fatty acid delivery to mitochondria, resulting in LD depletion dependent on both lipase activity (e.g., ATGL) and autophagy-mediated lipolysis.

Finally, the fate of fatty acids not delivered to mitochondria upon FABP5 inhibition was examined. For this, LD-depleted MCT cells were incubated with conditioned medium from SBFI-stimulated cells under 0% FBS conditions (**Fig. S3J**). After 24 h of incubation in conditioned medium, ORO-positive lipid content increased (**Fig. S3K**). This suggests that conditioned medium from FABP5-inhibited cells contained fatty acids that could be taken up by receptor cells, that is, that FABP5 inhibition activated compensatory mechanisms promoting fatty acid extrusion to reduce cytosolic fatty acid levels and potential lipotoxicity.

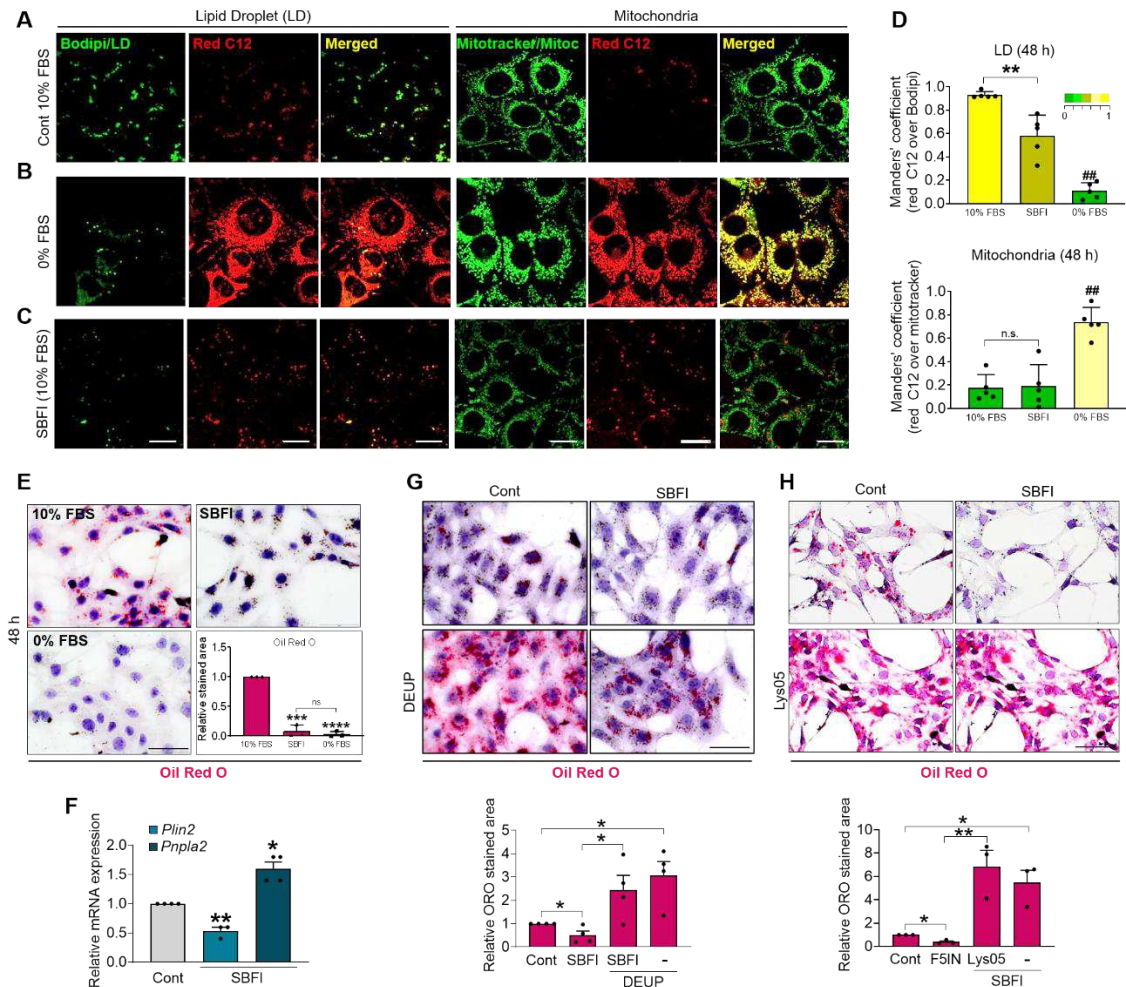


Fig. 2 FABP5 inhibition disrupts fatty acid delivery to mitochondria and lipid storage in renal tubular cells.

A-D Fatty acid fluorescence pulse-chase assay in MCT cells upon FABP5 inhibition. Representative confocal images show MCT cells stained with BODIPY493/503 (Bodipy) or MitoTracker (green signal) to trace the fatty acid Red C12 (red signal) after 48 h of treatment with the FABP5 inhibitor SBFI-26 (SBFI, 50 μ M) in medium 10% FBS (**C**). This condition was compared to Red C12 trafficking in control cells maintained in 10% FBS (10% FBS) (**A**) or in starved cells without serum (0% FBS) (**B**). Colocalization between Red C12 and Bodipy (upper bar graph) or MitoTracker (lower bar graph) was quantified using Manders' coefficient. The color scale shows the distribution of Red C12 signal across lipid droplets (LD) and mitochondria. Data are expressed as mean $\pm$ SEM. $^{**}p < 0.01$ and $^{##}p < 0.01$ vs 10% FBS (**D**). Images were taken using a 63 $\times$ objective with 3 $\times$ optical zoom. Scale bars: 25 μ m. **E** Lipid content was assessed by OIL Red O staining in MCT cells treated with SBFI under the same experimental conditions and quantified relative to serum-containing medium. Original magnification 600 $\times$, scale bars: 50 μ m. **F** *Plin2* and *Pnpla2* mRNA expression levels were measured by qRT-PCR following 24 h of SBFI treatment. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs Cont. **G-H** Lipid content was evaluated by OIL Red O staining in cells treated with 50 μ M SBFI alone or in combination with inhibitors of lipase (DEUP, 100 μ M) (**G**) or autophagy (Lys05, 10 μ M) (**H**) for 48 h. Red O-stained area was quantified relative to the area and normalized to the cell number per field. Images were captured at 600 $\times$ magnification; scale bars, 50 μ m. Data are presented as mean $\pm$ SEM from 3–5 experiments; $^{*}p < 0.05$; $^{**}p < 0.01$.

FABP5 inhibition impairs energy generation and induces mitochondrial stress and injury in tubular cells

We investigated whether disrupting fatty acid delivery to mitochondria impacts energy metabolism and mitochondrial function in renal tubular cells. Intracellular ATP content rapidly decreased by $\approx 30\%$ within 30-60 minutes following FABP5 inhibition, reaching a nadir of 70 % at 4 hours. By contrast, a similar decrease was reached in serum-starved cells only after 48 h, demonstrating unrestricted access to stored lipids when FABP5 activity is preserved (**Fig. 3A**). AMPK, which is activated in response to energy deficit via an increased AMP/ATP ratio, was phosphorylated as early as 1 hour of FABP5 inhibition (**Fig. 3B**). AMPK activation was accompanied by the upregulation of AMPK target genes (*Ppara*, *Ppargc1a*), which are known transcriptional regulators of FAO, as well as of PPAR α /PGC-1 α -regulated FAO genes (*Cpt1a*, *Acadm*, *Acox1*). Genes involved in glycolysis (*Pkm*, *Pfkb3*) remained unchanged (**Fig. 3C**).

To assess the impact of FABP5 on mitochondrial bioenergetics in MCT cells, we performed a Seahorse XF Cell Mito Stress Test. FABP5 inhibition significantly reduced basal respiration and ATP-production coupled respiration, suggesting reduced ATP-linked mitochondrial respiratory flux under basal conditions. Maximal respiration following carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) was only modestly reduced and did not differ significantly between groups. Following rotenone, oxygen consumption rate (OCR) fell to similarly low levels across conditions, supporting comparable non-mitochondrial respiration (**Fig. 3D–F**). Collectively, these data show that acute FABP5 inhibition preferentially attenuates ATP-linked mitochondrial respiration without evidence of overt early mitochondrial dysfunction at the studied time point. Bioenergetic limitations from reduced mitochondrial fatty acid delivery and ATP production may lead to mitochondrial dysfunction. FABP5 inhibition decreased

membrane potential ($\Delta\Psi_m$), indicating that FABP5 helps sustain the steady-state electrochemical gradient (**Fig. 3G**). It also increased mitochondrial (**Fig. 3H**) and cytoplasmic reactive oxygen species (ROS) (**Fig. 3I**). FABP5 inhibition upregulated key antioxidant genes (*Cat*, *Sod*, *Hmox*), suggesting a response to oxidative stress and activation of protective pathways such as *Nrf2*, along with the upstream endoplasmic reticulum stress-responsive gene *Atf6* (**Fig. 3J**). Moreover, FABP5 inhibition dampened gene programs related to mitochondrial biogenesis, as shown by reduced mitochondrial Rnr2 and Nd1 DNA copy number (**Fig. 3K**). FABP inhibition reduced cell proliferation within 24 hours (**Fig. 3L**). Even under FBS-growing conditions, FABP inhibition induced a slight but statistically significant increase in total, apoptotic, and necrotic cell death over 24–48 hours, as assessed by the 7-AAD/Annexin V assay and visualized by microscopy (**Fig. 3M**). Collectively, these findings suggest that FABP5, in addition to maintaining energy homeostasis, contributes to mitochondrial integrity, thereby supporting cell proliferation and viability, potentially through its role in lipid transport and metabolism.

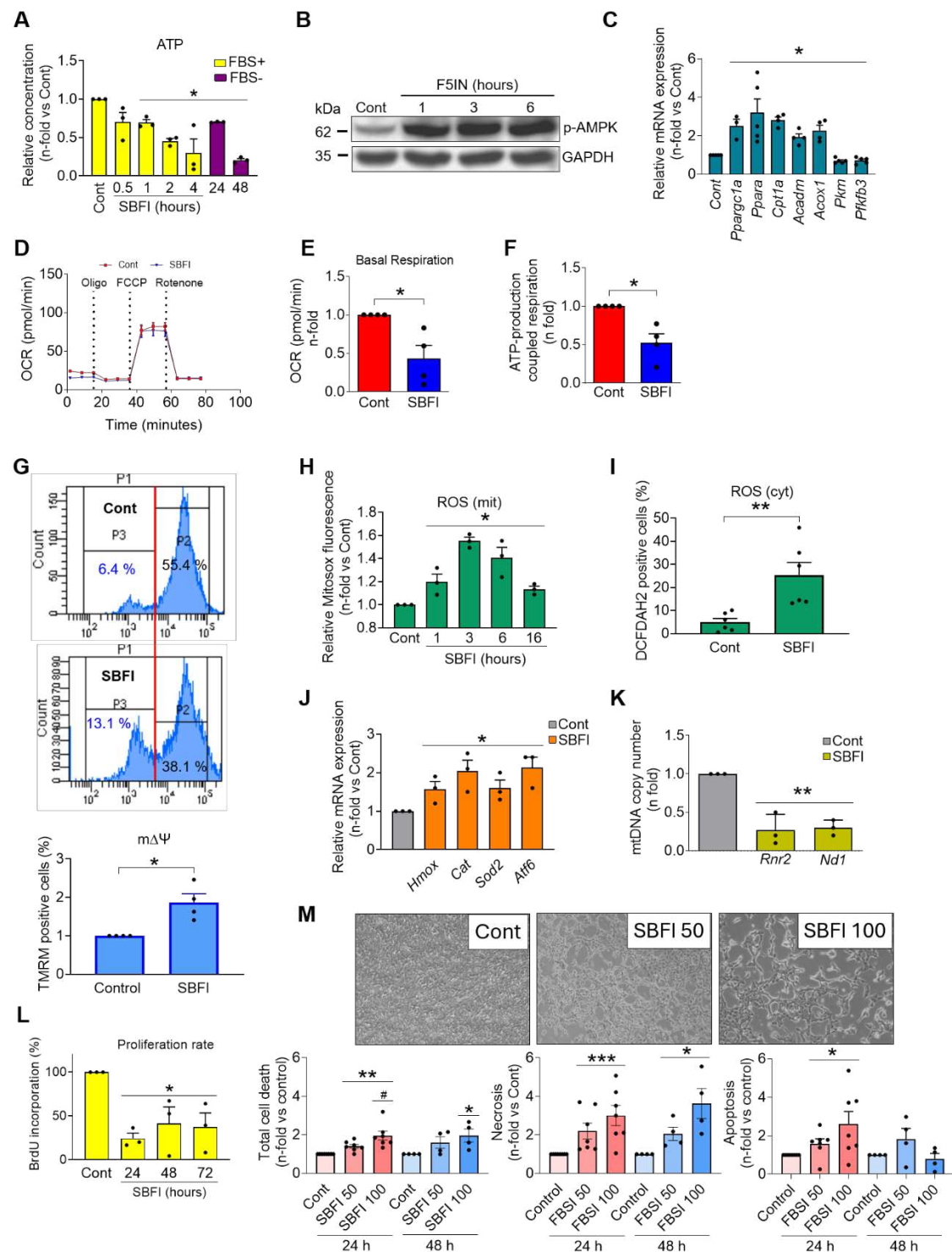


Fig. 3 Pharmacological FABP5 inhibition impairs energy production, mitochondrial integrity, and activates AMPK-dependent adaptation in tubular cells. MCT cells were treated with 50 μ M SBFI for variable times depending on the assay. Data represent the mean $\pm$ SEM from 3–6 independent experiments and normalized to untreated controls. **A** Relative ATP levels were assessed using a luminescent assay in MCT cells treated with SBFI in the presence or absence of FBS for the indicated times. *p < 0.05 vs control (Cont). **B** Time-course of p-AMPK expression after SBFI treatment assessed by Western blot. Representative image. **C** mRNA expression of *Ppargc1a*, *Ppara*, *Cpt1a*, *Acadm*, *Acox1*, *Pkm*, and *Pfkfb3* measured by RT-qPCR

after 6 h SBFI treatment. *p < 0.05 vs Cont. **D-F** Seahorse analysis of mitochondrial respiration. Cells cultured in 10% FBS-containing medium were incubated with SBFI before analysis. Oxygen consumption rate (OCR) profiles during the mitochondrial stress test after sequential injections of oligomycin, FCCP, and rotenone/antimycin A (**D**), basal respiration (**E**), and mitochondrial ATP production (**F**). *p < 0.05 vs Cont. **G** Mitochondrial membrane potential (mΔΨ) measured by TMRM staining and flow cytometry. Histograms show TMRM fluorescence distribution in control and SBFI-treated cells; bar graph shows percentage of TMRM-positive cells. *p < 0.05. **H-I** Oxidative stress induced by SBFI assessed by flow cytometry using MitoSOX™ for mitochondrial ROS (**H**) and DCFDAH2 for total ROS (**I**). *p < 0.05 and **p < 0.01 vs Cont. **J** RT-qPCR of *Hmox*, *Cat*, *Sod2*, and *Atf6*. *p < 0.05 vs Cont. **K** RT-qPCR of *Rnr2* and *Ndl*. **p < 0.01 vs Cont. **L** Cell proliferation assessed by BrdU incorporation. *p < 0.05 vs Cont. **M** Cell death assessed by 7-AAD/Annexin V staining and flow cytometry. *p < 0.05, **p < 0.01, ***p < 0.001 between indicated groups; #p < 0.05 vs Cont.

FABP5 deficiency increases the severity of nephrotoxic AKI

The overall impact of FABP5 deficiency was studied *in vivo* in FA-AKI induced in FABP5^{-/-} and WT male mice (**Fig. 4A**). FABP5 deficiency by itself did not modify baseline kidney function, as determined by plasma urea and creatinine levels, but increased the severity of AKI, as indicated by a greater elevation of these markers compared with wild-type (WT) mice (**Fig. 4B**). FABP5 deficiency increased the expression of the tubular stress/injury biomarker gene *Ngal* (**Fig. 4C**) and tubular cell death (**Fig. 4D**). However, kidney cell proliferation, assessed by nuclear PCNA immunodetection (**Fig. 4E**) and *Mki67* mRNA (**Fig. 4F**), failed to increase proportionally in FABP5^{-/-} mice despite their greater injury severity, remaining at levels comparable to those observed in WT mice. A gender-matched group of FABP5^{-/-} and WT female mice confirmed that FABP5 deficiency was associated with impaired renal function, as reflected by increased plasma urea and creatinine levels (**Fig. S4**).

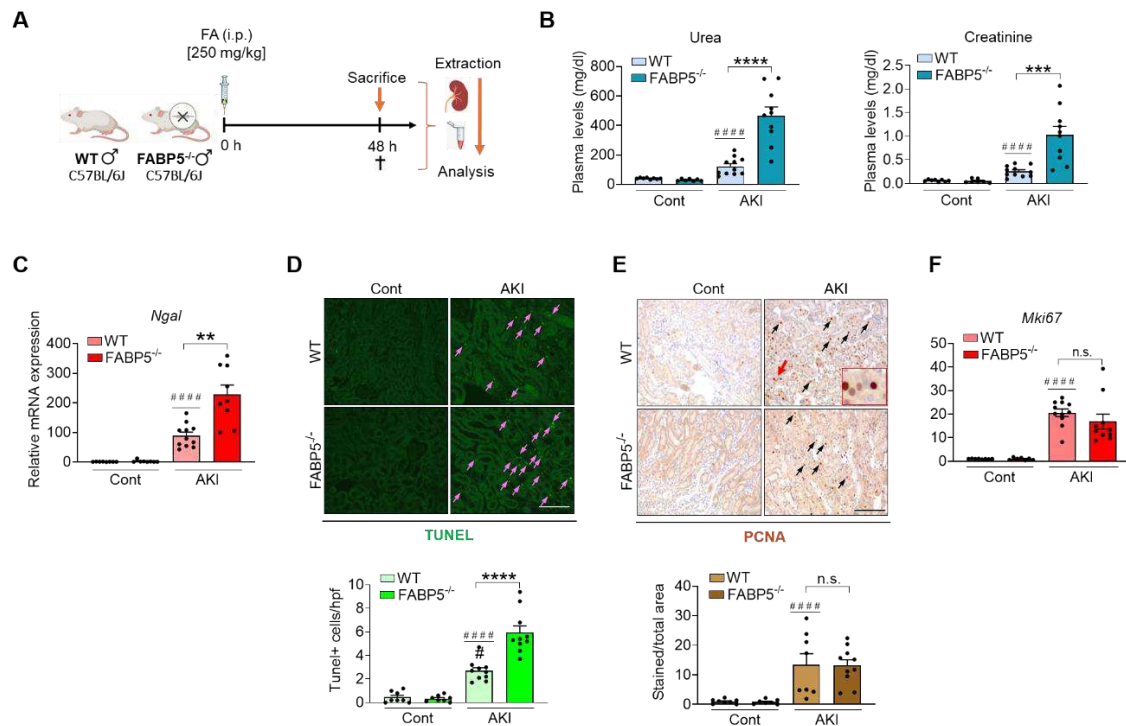


Fig. 4 FABP5 deficiency increases AKI severity without increasing proliferation. **A** Schematic representation of the experimental model. **B** Plasma levels of urea (left) and creatinine (right). **C** Kidney mRNA expression of *Ngai* measured by RT-qPCR. **** $p < 0.0001$ vs Cont WT; ** $p < 0.01$ vs AKI WT. **D** Cell death in kidney sections was detected by TUNEL staining and quantified as the number of positive cells/high-power field (hpf). The figure shows representative pictures for each experimental group taken at an original magnification of 400 $\times$; pink arrows indicate TUNEL-positive cells. Scale bars, 50 μ m. **** $p < 0.0001$ vs Cont WT; **** $p < 0.0001$ vs AKI WT. **E** Proliferating cells in kidney sections were detected by PCNA immunohistochemistry. Representative images from original samples taken at 400 $\times$; black arrows indicate PCNA-positive nuclei of proliferating cells; the red arrow points to a tubule with positive nuclei, which is shown at higher magnification. Scale bars, 50 μ m. PCNA staining was quantified as stained area/total area and normalized to control. **** $p < 0.0001$ vs Cont WT; n.s. non-significant. **F** Kidney mRNA expression of *Mki67* measured by RT-qPCR. **** $p < 0.0001$ vs Cont WT; n.s. non-significant.

Transcriptomic profiling reveals modulation of molecular pathways involved in the increased AKI severity in FABP5-deficient mice

Further functional analysis explored the kidney transcriptomic profiles of WT and FABP5-deficient mice with FA-AKI (**Fig. 5A; Table S1**). In control mice without AKI, 96 genes (19 upregulated and 77 downregulated) were differentially expressed ($|\log_2FC| \geq 1$, $q\text{-value} \leq 0.05$) between FABP5^{-/-} and WT mice. Compared with their respective

control mice, 586 differentially expressed genes (301 upregulated and 285 downregulated) were detected in WT mice with AKI, whereas 800 genes (429 upregulated and 371 downregulated) were identified in FABP5-deficient mice with AKI, indicating broader transcriptional alterations in FABP5-deficient mice following AKI. Furthermore, direct comparison between kidneys from AKI FABP5^{-/-} and AKI WT mice identified 165 differentially expressed genes (116 upregulated and 49 downregulated), further supporting genotype-dependent transcriptional differences.

Gene Set Enrichment Analysis (GSEA) revealed no major differences in enriched biological pathways between control FABP5^{-/-} and WT mice (**Table S1**). However, GSEA identified 30 gene sets positively enriched and 2 negatively enriched in kidneys from AKI WT mice compared to control WT mice, and 30 positively enriched and 3 negatively enriched gene sets in kidneys from FABP5^{-/-} mice (**Table S1**). Among the most significantly enriched gene sets in both AKI phenotypes compared with controls, 29 (74.4%) were shared between WT and FABP5^{-/-} mice with AKI, although some showed different significance relative to their controls, as detailed below. Of these, 27 (93.1%) were positively enriched in AKI in both phenotypes, while 2 (6.9%) were negatively enriched. Six (15.4%) gene sets were exclusively enriched in AKI WT, and 4 (10.3%) in AKI FABP5^{-/-}, indicating greater dependence on FABP5 status (**Fig. 5B; Table S1**).

Among the major pathways enriched in both mouse AKI genotypes and previously implicated in the disease pathogenesis (**Table S1; Fig. 5C**), some were less enriched in FABP5^{-/-} mice. These include Allograft Rejection [46], which is focused on T cell-driven inflammation, Inflammatory Response [47], and Glycolysis [11], whereas TNF α Signaling via NF κ B [48], MYC Targets V1/V2, TGF β 1 Signaling, and the Reactive Oxygen Species pathways were more enriched in FABP5^{-/-} mice [49-51]. The Unfolded Protein Response was the most enriched pathway upregulated uniquely in AKI FABP5^{-/-}

mice, whereas Fatty Acid Metabolism, which is fundamental for tubular cell function, was the only downregulated pathway [52]. Interferon Gamma and Alpha responses and DNA Repair were upregulated only in AKI WT mice, suggesting inactivation of some proinflammatory and reparative pathways in AKI FABP5^{-/-} mice [53-55]. Most of these pathways were also identified among the 9 hallmarks positively enriched and the 13 hallmarks negatively enriched in direct comparisons between AKI-FABP5^{-/-} and AKI-WT mice (**Fig. 5D**) (**Table S1**).

When integrated into broader functional networks using Metascape (**Fig. 5E**) (**Table S1**), genes downregulated in AKI FABP5^{-/-} in comparison with WT AKI mice were mainly enriched in clusters related to lipid and oxidative metabolism, cellular transport, and tubular homeostasis. Conversely, upregulated genes were enriched in clusters associated with ribosome biogenesis and protein translation, endocrine and metabolic regulation, and structural cellular reorganization. Overall, these results indicate metabolic dysfunction, energetic failure, reduced epithelial function (transport, detoxification, and xenobiotic metabolism), and impaired protein processing as likely contributors to the more severe AKI in FABP5^{-/-} mice.

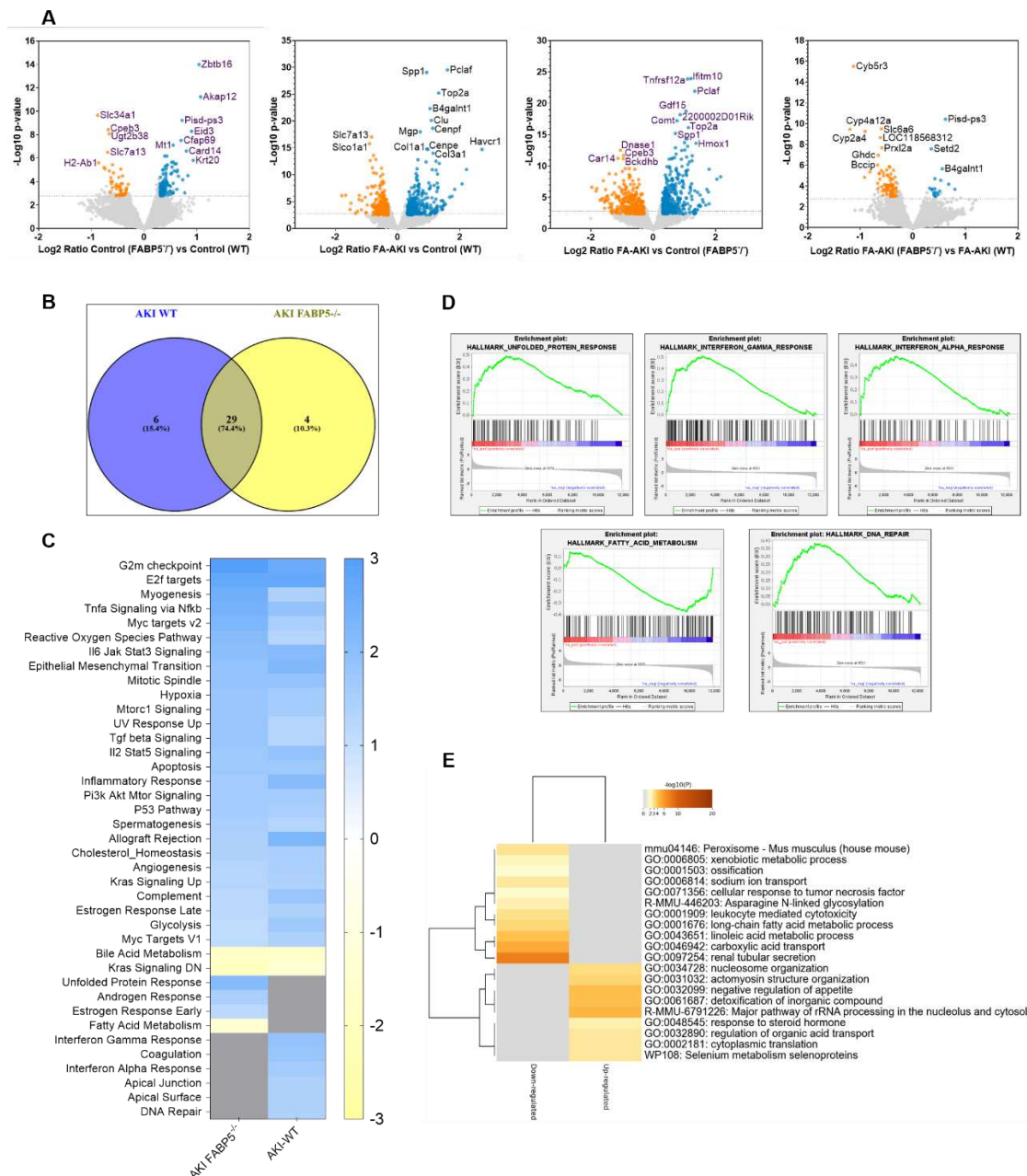


Fig. 5 Transcriptomic reprogramming in FABP5^{-/-} kidneys during AKI. **A** Volcano plots showing differentially expressed genes in control or AKI kidneys from wild-type (WT) and FABP5^{-/-} mice. Differentially Expressed Genes (DEG) are highlighted in blue (upregulated) and orange (downregulated), while non-significant genes are shown in grey. **B-E** Gene Set Enrichment Analysis (GSEA) identified biological pathways differentially enriched in kidneys following AKI induction in FABP5-deficient (AKI FABP5^{-/-}) and wild-type (AKI WT) mice. **B** Venn diagram showing the overlap of differentially enriched hallmark pathways in kidneys from WT-AKI and FABP5^{-/-} AKI mice that were compared against their corresponding control counterparts. A substantial number of differentially expressed pathways is shared between genotypes (n=29), while distinct gene subsets are uniquely regulated in FABP5-deficient kidneys (n=4) or WT mice (n=6), indicating FABP5-independent and FABP5-dependent transcriptional programs. **C** Heatmap showing normalized enrichment scores (NES) for Hallmark gene sets shared between FABP5^{-/-}

^{-/-} AKI and WT AKI kidneys, as well as pathways uniquely enriched in each phenotype. **D** GSEA enrichment plots for selected Hallmark gene sets derived from the comparison between FABP5^{-/-} AKI and WT AKI kidneys. Enrichment plots of representative positively and negatively enriched pathways in FABP5^{-/-} AKI kidneys, potentially relevant to FA-AKI. **E** Metascape functional enrichment analysis from DEGs in kidneys from FABP5^{-/-} AKI mice compared to WT AKI mice. Enriched Gene Ontology (GO) terms and pathways clustered into functional categories supporting a role for FABP5 in regulating metabolic and stress-adaptive programs during FA-AKI.

FABP5 deficiency exacerbates critical pathological events during AKI

Next, we explored individual components of molecular pathways found to be regulated by FABP5. FABP5-deficient mice with AKI exhibited significantly altered expressions of genes involved in inflammation, oxidative stress, lipid metabolism, and mitochondrial function (**Figure S5**).

AKI in WT mice was associated with increased mRNA expression of proinflammatory chemokines (**Fig. S5**) (**Fig. 6A**) and enhanced immune cell infiltration, including MPO⁺ neutrophils, F4/80⁺ mononuclear phagocytes, and CD3⁺ lymphocytes (**Fig. 6B**). Unexpectedly, AKI in FABP5^{-/-} mice induced less chemokines (**Fig. 6A**) (**Fig. S5**) and recruited fewer immune cells than in WT AKI mice (**Fig. 6B**). In this regard, FABP5 inhibition attenuated the proinflammatory cytokine response to TWEAK stimulation in cultured MCT cells (**Fig. S6A**).

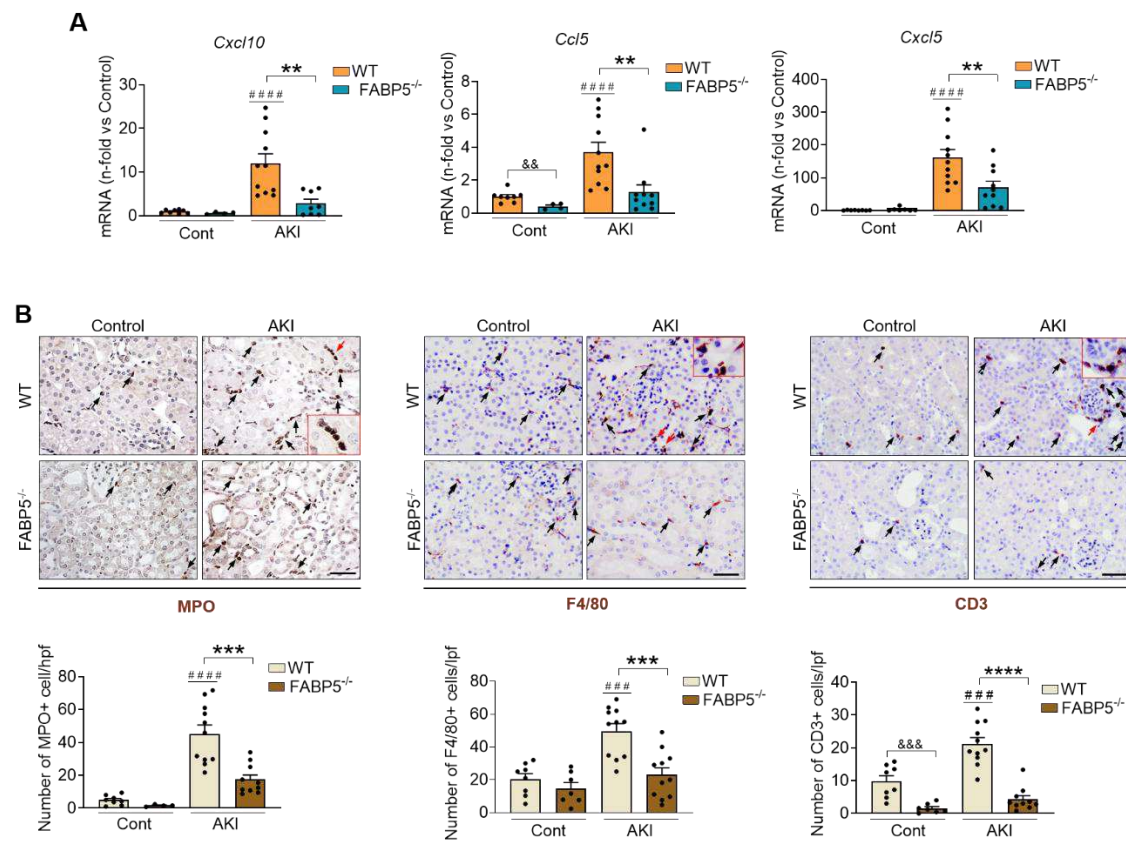


Fig. 6 FABP5 deficiency reduces chemokine expression and recruitment of immune cells in kidneys from AKI mice. **A** Kidney mRNA expression of the proinflammatory chemokines *Cxcl10*, *Ccl5*, and *Cxcl5* at 48 hours post-AKI, measured by RT-qPCR. &&p < 0.01 vs Cont WT, #####p < 0.00001 vs Cont WT; ** p < 0.01 vs AKI WT. **B** Immune cell infiltration in kidney tissue sections was analyzed by immunohistochemistry at 48 hours post-AKI induction by detecting MPO (neutrophils), F4/80 (macrophages), and CD3 (lymphocytes) positive cells. Representative images from pictures taken at an original magnification of 400×/Scale bar 50 μm for MPO; 200×/Scale bar 100 μm for F4/80 and CD3. Black arrows indicate positive-stained cells, and red arrows indicate the regions shown at higher magnification. Quantification is presented as the number of stained cells per high (MPO) or low (F4/80, CD3) power field (at least 10 randomly chosen fields). Data are presented as mean ± SEM. Statistical significance is indicated as follows: &&p < 0.001 vs Cont WT; #####p < 0.0001 and #####p < 0.0001 vs Cont WT; ***p < 0.01 and ****p < 0.0001 vs AKI WT.

Oxidative and mitochondrial stress are also hallmarks of AKI. In WT mice, AKI upregulated *Nrf2* and its target *Hmox1* but downregulated other antioxidant responses (*Cat*, *Sod1*). In AKI FABP5^{-/-} mice, these transcriptional changes were more pronounced, with greater downregulation of *Cat* and *Sod1* and stronger upregulation of *Nrf2* and *Hmox1* than in AKI WT mice (**Fig. S5**) (**Fig. 7A**). These results suggest that FABP5^{-/-}

mice exhibit a higher oxidative stress burden, together with a more dysregulated and insufficient antioxidant response. Indeed, lipid peroxidation was increased in kidneys from AKI FABP5^{-/-} mice, consistent with more severe oxidative stress (**Fig. 7B**). Additionally, *Ppargc1a* and several mitochondrial target genes (*Tfam*, *Ndufs1*, *Sdha*) were downregulated in WT AKI and to a greater extent in AKI FABP5^{-/-} mice (**Fig. S5**) (**Fig. 7C**), in line with evidence of decreased mitochondrial mass assessed by reduced expression of the mitochondrial marker TOMM22 in AKI FABP5^{-/-} mice (**Fig. 7D**). FABP5^{-/-} mice also displayed more severe downregulation of the *Pink1/Parkin* pathway (**Fig. S5**) (**Fig. 7E**), suggesting impaired mitophagy on top of reduced mitochondrial biogenesis, a mechanism known to exacerbate FA-AKI [56].

AKI in WT mice downregulated lipid metabolism-related gene programs, among them key FAO inducers (*Ppara*, *Ppargc1a*) and effectors (*Acs1l*, *Cpt1b*, *Acadm*, *Acox1*, *Ehhadh*), as well as genes involved in lipid degradation (*Ces1d/Ces1f*), and synthesis (*Dgat2*). The only induced genes were *Plin2*, a major structural and coat protein of LD. On the other hand, non-significant changes in the glycolytic genes *Hkl* and *Pfkfb3* were observed in WT AKI mice, whereas *Pkm* was upregulated (**Fig. S5**). In FABP5^{-/-} control mice, the basal expression of several genes related to lipid metabolism (*Dgat1*, *Ces1f*, *Acox1*, *Ehhadh*) was downregulated, while *Cpt1b*, *Acadm*, and the glycolytic genes *Hkl*, *Pfkfb3* and *Pkm* were upregulated, suggesting overall transcriptional remodeling in response to FABP5 deficiency (**Fig. S5**). In support of these findings, isolated PTEC from FABP5^{-/-} mice exhibited comparable gene profiles showing lower basal levels of FAO genes (*Cpt1a*, *Acox1*, and *Ehhadh*) and *Ces1f* (**Fig. S6B**).

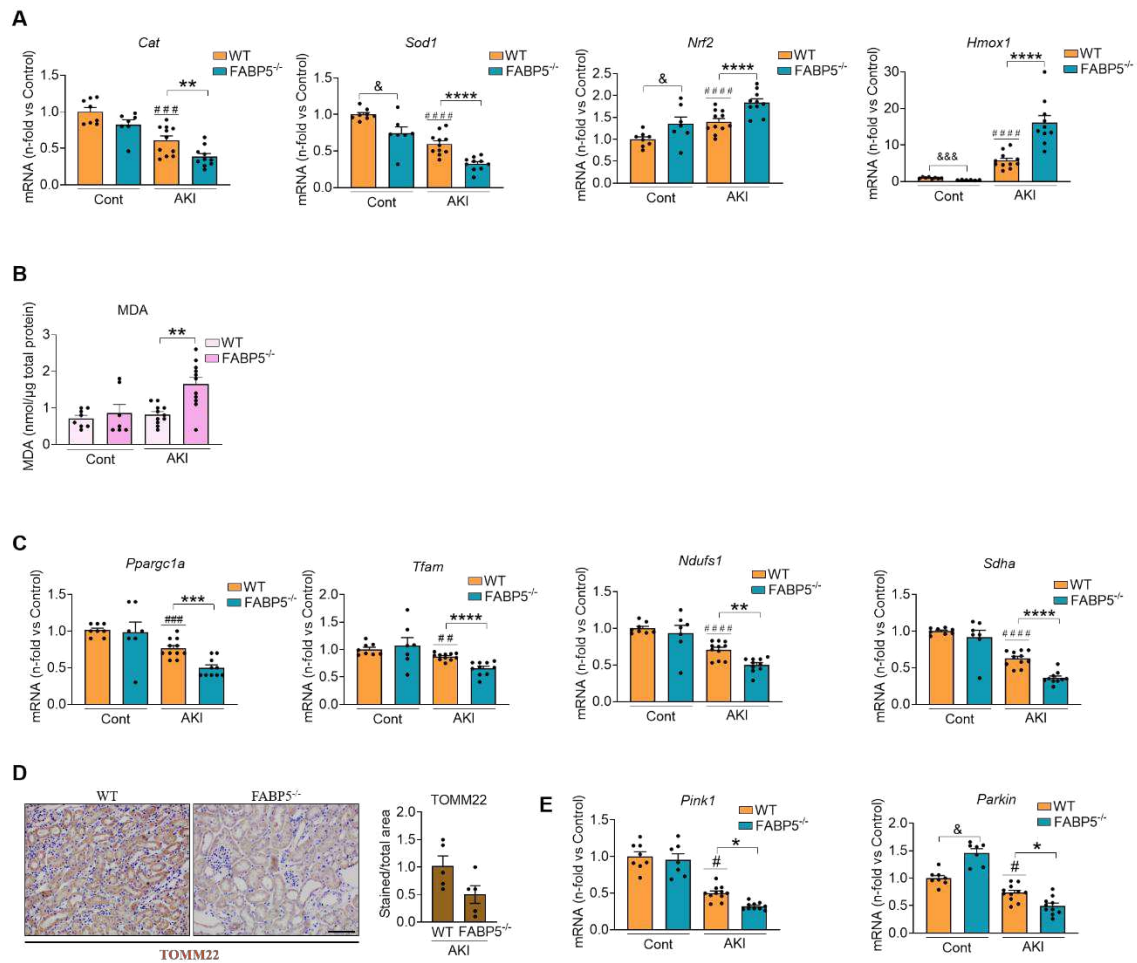


Fig. 7 FABP5 deficiency increases the severity of oxidative stress and impairs mitochondrial homeostasis. **A** Kidneys from WT or FABP5^{-/-} mice were analyzed 48 h after AKI induction. RT-qPCR of antioxidant enzymes (*Cat*, *Sod1*) and oxidative stress-response genes (*Nrf2*, *Hmox1*). **B** Malondialdehyde (MDA) levels in kidney tissue were measured using a TBARS assay kit. **C** Kidney mRNA expression of genes involved in mitochondrial biogenesis, mtDNA stability and respiratory chain function (*Pparg1a*, *Tfam*, *Ndufs1*, *Sdha*) measured by RT-qPCR. **D** Mitochondrial mass in kidney sections analyzed by immunohistochemistry of TOMM22. Representative images, original magnification 200×, with scale bars of 50 μm. Quantification was performed based on the area of TOMM22 staining and normalized to AKI WT. **E** Kidney mRNA expression of the mitophagy-related genes *Pink1* and *Parkin* measured by RT-qPCR. Data quantification and significance throughout all the quantification bar graphs are indicated as follows: ##p < 0.01, ###p < 0.01, ####p < 0.0001 vs Cont WT; *p < 0.01, ***p < 0.001, ****p < 0.0001 vs AKI WT; &p < 0.05 vs Cont WT.

During FA-AKI, genes of lipid metabolism were predominantly downregulated in both WT and FABP-deficient mice (**Fig. S5**). In AKI of other causes, this pattern has been associated with increased lipid deposition and lipotoxicity driven by impaired FAO and lipolysis [57]. However, several of these genes, including *Pparg1a*, (**Fig. 7C**), *Dgat1*,

Ces1d, *Ces1f*, *Ehhadh*, and *Acadm*, were further downregulated in AKI FABP5^{-/-} mice, while *Plin2* was further upregulated (**Fig. S5; Fig. 8A**). Moreover, the higher basal levels of glycolytic enzymes (*Hkl1*, *Pfkb3*, *Pkm*) in FABP5^{-/-} mice were not maintained during AKI, except for *Pkm*, which decayed to levels similar to AKI WT mice (**Fig. S5**). Consistent with this scenario, a detectable lipid accumulation was observed in FABP5^{-/-} AKI mice upon Bodipy staining (**Fig. 8B**). Collectively, these results suggest that FABP5 deficiency increases susceptibility to AKI by further impairing lipid metabolism, without overt compensatory responses in core glycolytic enzymes.

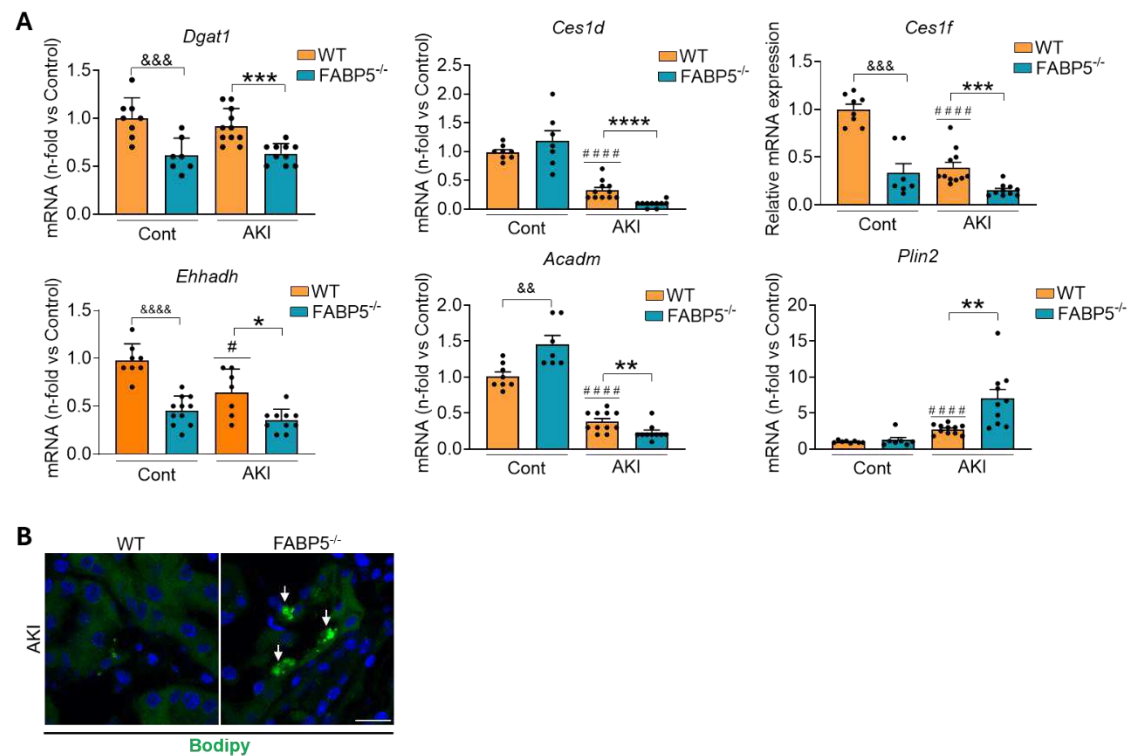


Fig. 8 FABP5 deficiency alters lipid metabolism in FA-AKI kidneys. **A** RT-qPCR of genes involved in lipid metabolism differentially expressed in FABP5^{-/-} mice at 48 hours following AKI induction, including triglyceride synthesis and storage (*Dgat1*, *Plin2*), lipolysis (*Ces1d*, *Ces1f*), and fatty acid oxidation (*Ehhadh*, *Acadm*). Data are presented as mean ± SEM and normalized to the control (Cont) WT group. Statistical significance is indicated as follows: &&&p < 0.01, &&&p < 0.001 and &&&&p < 0.0001 vs Cont WT; #p < 0.05 and #####p < 0.0001 between AKI WT and Control WT; *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 vs AKI KO. **B** Lipid accumulation in the kidney of FABP5^{-/-} mice after 48 hours post-AKI assessed by Bodipy staining. Representative images are shown at 630× magnification (scale bar 25 μm).

Since FABP5 colocalized with cells in the renal microvasculature, we assessed the expression of key genes involved in pericyte-endothelial crosstalk. In WT mice, AKI reduced *Vegfa* and upregulated *Vcam1* mRNA expression (**Fig. S5; Fig. 9A**) as well as prominently vascular VCAM1 protein expression (**Fig. 9B**). In FABP5^{-/-} mice with AKI, *Vegfa* was lower than in AKI WT mice, suggesting additional vascular instability. However, VCAM1 upregulation was prevented, likely limiting both the angiogenic response (**Fig. S5; Fig. 9A,B**) and immune cell recruitment, as shown above in **Fig. 6B**. FABP5 deficiency also increased α SMA⁺ cells (**Fig. 9C**). Some of these cells were identified as pericytes based on concurrent PDGFR expression (**Fig. 9D**). In agreement with in vivo findings, FABP5 inhibition induced α SMA expression in cultured C3H/10T1/2 pericyte-like cells (**Fig. S6C**), which may be related to reduced levels of FAO-associated genes (*Acadm*, *Ehhadh*, *Cpt1a*) (**Fig. S6D**), like in cells exposed to TGF β 1 or the FAO inhibitor etomoxir, both of which suppress FAO and promote phenotypic transition [58]. Consistent with FABP5 inhibition-mediated FAO impairment and ATP depletion, pharmacological inhibition of FABP5 also activated AMPK in this cell type (**Fig. S6E**).

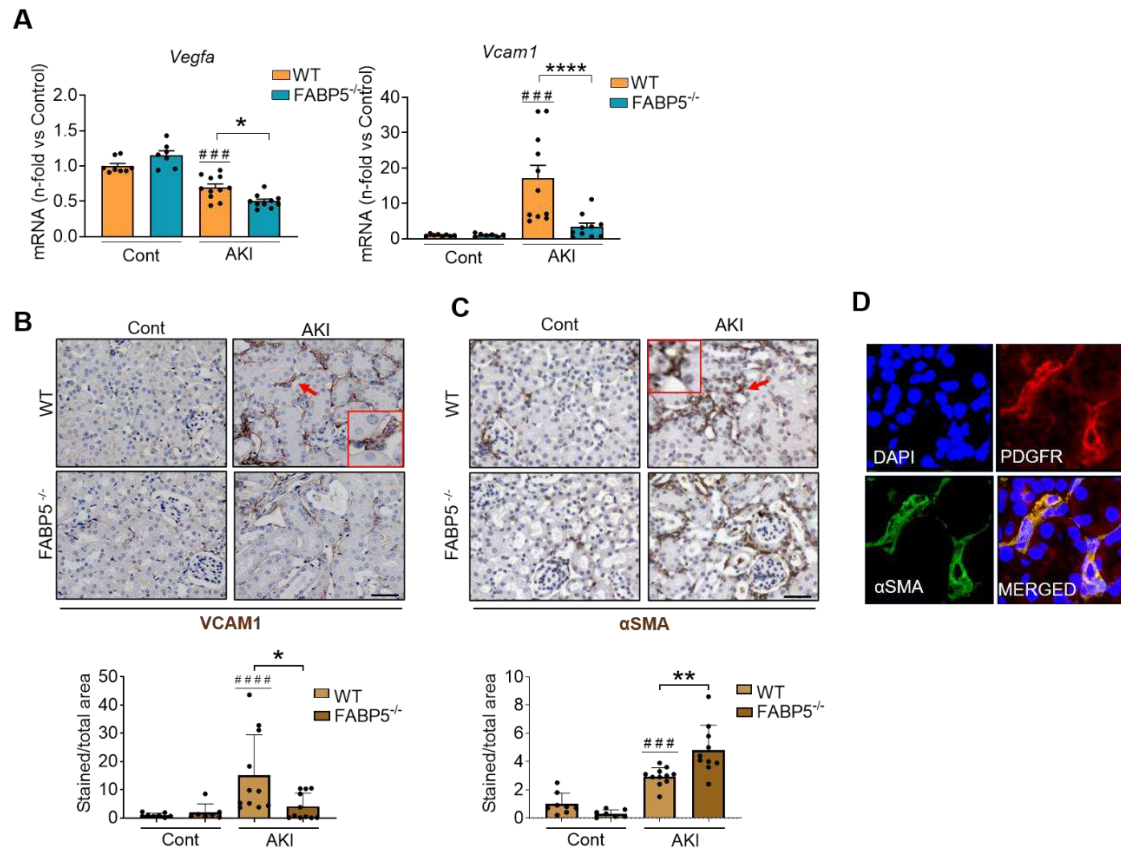


Fig 9. FABP5 regulates endothelial and mesenchymal cell responses during AKI. **A** RT-qPCR of the endothelial activation markers *Vcam1* and *Vegfa* at 48 hours post-AKI in kidneys from WT and FABP5^{-/-} mice; ###p ≤ 0.001 vs Cont WT; *p < 0.05 and ****p < 0.0001 vs AKI WT. **B-C** VCAM1 expression (**B**) and myofibroblast accumulation assessed by αSMA staining (**C**) were analyzed by immunohistochemistry. Representative microphotographs (upper panels) and graph charts quantification (lower panels) representing the Mean ± SEM of the relative stained area (stained/total) normalized to the control. Original magnification 200×; red arrows indicate the regions shown in the higher-power views. Scale bars, 100 μm. ###p < 0.001 and ####p < 0.0001 vs Cont WT; *p < 0.05 and **p < 0.01 vs AKI WT. **D** Immunofluorescence of kidney sections from WT mice showing nuclei (DAPI, blue fluorescence), PDGFR (red fluorescence), and αSMA (green fluorescence). Colocalization of PDGFR and αSMA highlights the pericyte-derived myofibroblast population. Representative images. Original magnification 200×. Scale bars, 100 μm.

Pharmacological inhibition of FABP5 increases the severity of AKI

To pharmacologically confirm the nephroprotective function of FABP5, we administered SBF1 to WT mice before FA-AKI induction (**Fig. 10A**). Both AKI and SBF1 independently reduced kidney *Fabp5* mRNA expression; however, the combination of SBF1 and AKI further decreased expression compared with AKI alone, suggesting

additional mechanisms driving FABP5 downregulation during AKI (**Fig. 10B**). SBFI increased the severity of AKI in WT mice as assessed by worse renal function (**Fig. 10C**), as well as both increased expression of the tubular stress/damage marker *Ngall* (**Fig. 10D**) and cell death (**Fig. 10E**). Despite the increased severity of AKI, leukocyte infiltration was lower than in vehicle-treated AKI mice (**Fig. 10F**), similar to that observed in FABP5^{-/-} mice with AKI (**Fig. 6B**). Furthermore, SBFI-treated AKI mice exhibited a transcriptional profile similar to that of FABP5^{-/-} AKI mice, characterized by increased tubular damage, reduced chemokine production, and decreased vascular responses, mitochondrial biogenesis/mitophagy, and lipid metabolism, along with minor alterations in glycolysis and dysregulated oxidative stress responses (**Fig. 10G**).

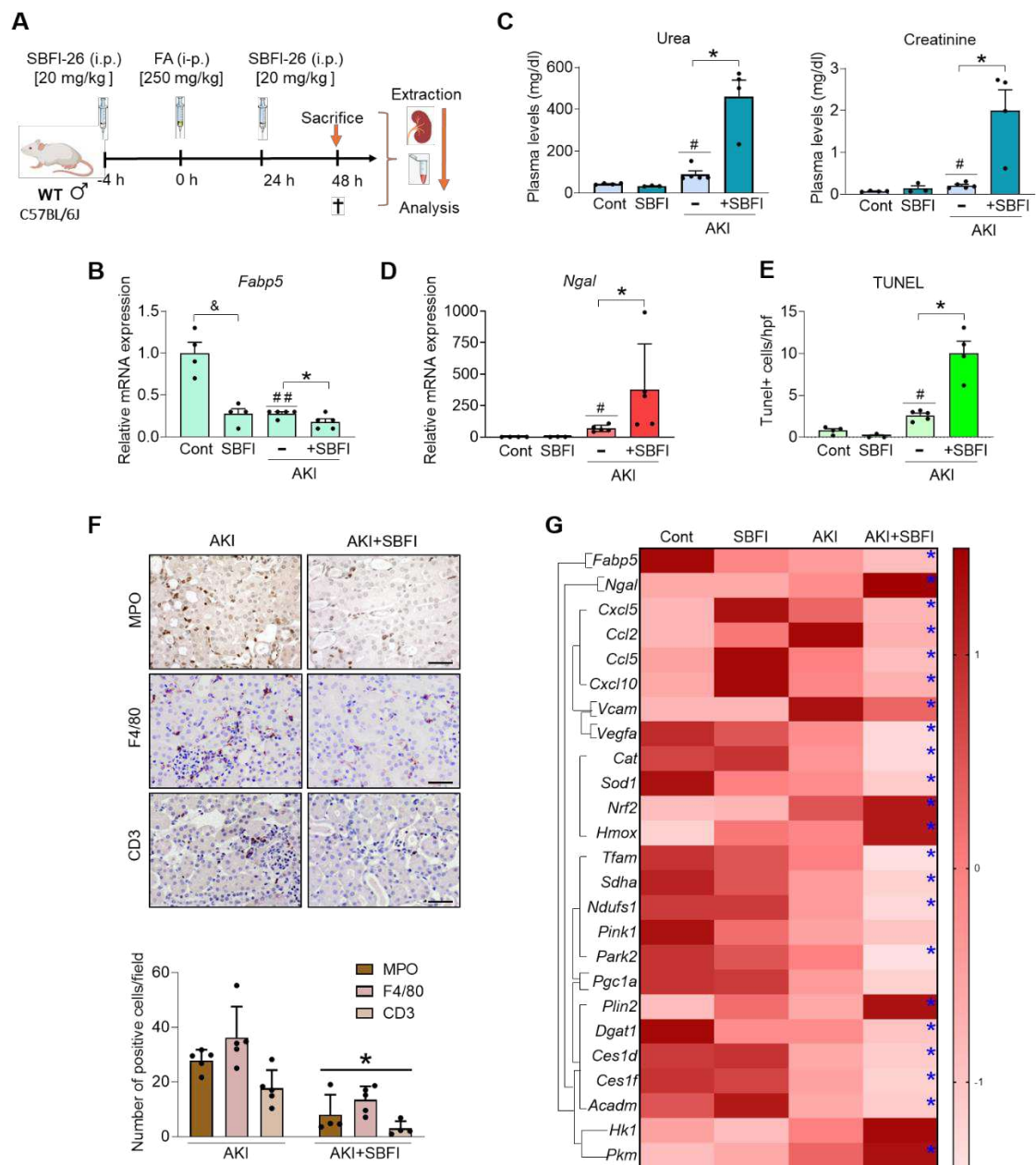


Fig. 10 Pharmacological inhibition of FABP5 increased FA-AKI severity. **A** Experimental design of the FA-AKI model developed in WT mice treated with the FABP5 inhibitor SBFI-26 (SBFI). **B** Transcription levels of FABP5 in the kidney of WT mice with FA-AKI and FABP5 inhibition in comparison with WT mice with AKI. &p < 0.05 SBFI vs control (Cont); #p < 0.05 AKI vs Cont; *p < 0.05 AKI + SBFI vs. AKI. **C-E** SBFI-treated AKI mice exhibited increased plasma urea and creatinine levels (**C**), higher transcription of the tubular stress/damage marker *Ngal* assessed by q-RT-PR (**D**), and increased cell death detected by TUNEL assay (**E**) when compared to AKI mice. #p < 0.05 AKI vs Cont; *p < 0.05 AKI + SBFI vs. AKI. **F** Immune cell recruitment in the kidney diminished in AKI FABP5^{-/-} mice. Leukocyte subsets were assessed by immunohistochemistry of MPO (neutrophils), F4/80 (mononuclear phagocytic cells, macrophages), and CD3 (lymphocytes). Representative micrographs and the corresponding quantification are shown. Original magnification/Scale bar (200x/100 μ m for MPO; 400x/50 μ m for F4/80 and CD3); *p < 0.05 vs AKI. **G** Gene expression profile obtained by q-RT-PCR in mice with AKI and mice with AKI treated with SBFI-26 (AKI

+ SBFI). Expression values were z-score-normalized for each gene. Grouping of genes by function and participation in AKI-related damage pathways was performed manually, with higher-order groupings indicated by brackets that connect related gene sets. * $p < 0.05$ AKI + SBFI vs AKI.

Overall, these findings suggest that pharmacological inhibition of FABP5 activity impairs the kidney's ability to respond effectively to AKI. These results closely match results obtained in FABP5-deficient mice, indicating that the acute loss of FABP5 activity, rather than long-term adaptive changes derived from FABP5 deletion, decreases the kidney resilience to AKI.

FABP5 deficiency does not modulate the AKI-to-CKD transition

To assess whether the increased severity of AKI in FABP5^{-/-} mice modulates the AKI-to-CKD transition, we examined AKI kidneys on day 15 (**Fig. S7A**). In WT mice, FABP5 mRNA and protein levels returned to baseline levels (**Fig. S7B**). In both WT and FABP5^{-/-} mice, plasma urea and creatinine decreased to similar levels, albeit they remained higher than in controls (**Fig. S7C**). There were no longer differences between WT and FABP5^{-/-} mice in MPO⁺ and F4/80⁺ cell infiltration, although CD3⁺ cell infiltration remained lower in FABP5^{-/-} mice with AKI (**Fig. S7D**). Increased collagen deposition (**Fig. S7E**) and most gene expression profiles (**Fig. S7F**) were also similar between WT and FABP5^{-/-} AKI mice. However, although *Hkl* expression did not change substantially, *Pfkfb3* and *Pkm* were higher in FABP5^{-/-} mice, suggesting a greater metabolic adaptation towards glycolysis due to FABP5 deficiency, which was unlikely to involve lactic fermentation given the reduced *Ldh* gene expression. Additionally, some fibrogenic mediators (*Fnl*, *Pail*, *Ctgf*, *Colla2*) remained upregulated in FABP5^{-/-} mice as compared to AKI WT mice (**Fig. S7F**). These results suggest that profibrotic mechanisms operate independently of

FABP5 deficiency and occur alongside the recovery of the inflammatory response after the acute phase of AKI.

Discussion

In this study, we identified FABP5 as a critical metabolic regulator in renal tubular cells with functional relevance to increasing the resilience to kidney injury. FABP5 expression was markedly reduced during nephrotoxic AKI. Mechanistically, FABP5 deficiency or inhibition compromised fatty acid handling, energy metabolism, and mitochondrial homeostasis in tubular cells. Moreover, FABP5 deficiency or inhibition was linked to tubular, inflammatory, and vascular responses in AKI and increased severity of AKI despite decreasing inflammatory cell infiltration. Collectively, these findings highlight FABP5 as a key component of the renal metabolic program and the kidney resilience response that sustains tubular cell viability, affording nephroprotection under conditions of acute stress.

In cultured proximal tubular cells, FABP5 was required to maintain energy metabolism and prevent immediate cell death induced by serum deprivation stress, i.e., induced by fatty acid scarcity and loss of survival and trophic factors that can replenish tricarboxylic acid (TCA) intermediates and may prevent cells from undergoing apoptosis or regulated necrosis [7,59-63]. Even in the presence of serum, FABP5 inhibition disrupted lipid transfer from LDs to mitochondria, leading to delayed cell death and revealing a functional disconnect between gene activation and metabolic output, a defect that was not rescued by compensatory glycolysis. This is consistent with prior observations of the inability of glycolysis to rescue tubular cells [4,9,64].

During starvation, ATGL-mediated lipolysis may fuel mitochondria while autophagy replenishes LDs [38]. Consistently, FABP5 inhibition depleted mitochondrial

fatty acids in cultured tubular cells, activated ATGL transcription, and likely lipolytic enzyme-dependent lipolysis and autophagy. However, reaccumulation of LDs was not observed in the short term, suggesting that FABP5 could also be a fatty acid transport-limiting step required for lipogenesis in tubular cells. Excess release of free fatty acid from LDs that are not oxidized by mitochondria may promote lipotoxicity [65]. Consistent with this observation, kidneys from FABP5^{-/-} mice with AKI accumulated lipids within 48 h. However, FABP5 inhibition also resulted in enhanced lipid efflux in tubular cells, which may be an adaptive response to limit cytoplasmic fatty acid accumulation as previously described in mouse embryonic fibroblasts [38].

The FABP family facilitates the intracellular transport and delivery of FAs to mitochondria and peroxisomes, thereby promoting FAO and ATP production while maintaining mitochondrial integrity [22,66]. In primary mouse cardiac fibroblasts, FABP5 silencing impaired mitochondrial respiration and increased mitochondrial oxidative stress, suggesting inhibition of FAO, while simultaneously inducing α -SMA expression, a hallmark of fibroblast activation [23]. In vivo, FABP5 deficiency exacerbated cardiac injury and fibrosis [23]. Despite evidence linking FABP5 to these metabolic processes in other tissues, the role of FABP5 in the kidney had not been previously addressed. However, in tubular cells and pericytes, inhibition of FAO, a potential consequence of FABP5 deficiency, promoted a profibrotic phenotype when induced by TGF β 1 or by CPT1 inhibition with Etomoxir [9,58]. A decrease in FAO and mitochondrial dysfunction contributes to acute and chronic kidney damage [67,68]. Indeed, CPT1 or PGC-1 α deficiency reduces mitochondrial mass and increases kidney injury, while restoring FAO through PPAR α or PGC-1 α agonism improves mitochondrial function and decreases kidney injury [16,17,69,70].

We now report that FABP5 inhibition in tubular cells resulted in disrupted fatty acid transport and decreased basal respiration and mitochondrial ATP production, leading to rapid mitochondrial membrane depolarization and increased mitochondrial ROS and lipid peroxidation. This redox imbalance likely resulted from incomplete FAO and electron leakage in the respiratory chain, together with impaired peroxisomal antioxidant activity [71,72]. Consistent with *in vitro* findings showing cellular distress in the absence of FABP5 activity, *in vivo* FABP5 deficiency or inhibition increased the severity of nephrotoxic AKI. This phenotype was characterized by more severe kidney dysfunction, enhanced tubular cell injury, dysregulated lipid metabolism, increased oxidative stress, and mitochondrial instability. Notably, this occurred without a corresponding increase in proliferative responses, in the presence of milder inflammatory cell recruitment, and without any residual impact on kidney recovery. Regarding these changes, there was evidence of more limited redox buffering capacity in FABP5-deficient mice, including further downregulation of catalase, the major peroxisomal antioxidant enzyme. In parallel, FABP5 deficiency impaired mitochondrial biogenesis, as assessed by more severe downregulation of *Ppargc1a* gene expression, PGC-1 α -regulated genes, and mitochondrial DNA copy number. Finally, from gene and transcriptomic analysis, there was evidence of more marked recruitment of the unfolded protein response (UPR) and Atf6 activation, which by itself can directly suppress FAO and promote oxidative stress [73]. In this regard, we observed that FABP5 inhibition dysregulated the expression of FAO genes in tubular and pericyte-like cells. In pericyte-like cells, FABP5 inhibition drove their transition into a SMA-expressing myofibroblast phenotype and suppressed the expression of FAO genes. Therefore, the microvascular localization of FABP5 raises the possibility of a role in pericyte-endothelium crosstalk.

The milder inflammatory phenotype was unexpected in the setting of more severe AKI. Inflammatory cells contribute both to the initial wave of injury and to subsequent kidney repair [74-77]. However, FABP5 inhibitors were previously reported to have anti-inflammatory actions in murine psoriatic skin models [78]. Our data suggest that tissue injury should also be evaluated together with inflammation when assessing the therapeutic potential of FABP5 inhibitors, as they may be dissociated. Mechanistically, FABP5 deficiency was associated with reduced induction of specific chemokines and decreased leukocyte infiltration, which may limit leukocyte-mediated damage during the acute phase but also impair subsequent adaptive and reparative immune responses. In this regard, FABP5 deficiency was further associated with downregulation of endothelial VCAM1, which is consistent with its well-characterized role in leukocyte recruitment, and correlates with reduced renal immune infiltration. Nonetheless, both intrarenal immune cell trafficking and VCAM1 expression recovered after 15 days in FABP5^{-/-} mice, strongly suggesting that altered cell migration is independent of FABP5 activity in immune cell subsets, and sustained VCAM1 expression may contribute to worsened AKI-to-CKD progression, as previously reported [79]. A reduction in *Vegfa* gene expression, a major endothelium trophic factor, was also observed. This could have further limited tissue perfusion and leukocyte recruitment.

Some limitations should be acknowledged. This is an experimental study, and no maneuvers to increase FABP5 and kidney resilience were explored. However, the results are clinically relevant as FABP5 inhibitors are being developed clinically to treat malignancy, inflammation, and pain [80,81]. ART26.12 from Artelo Biosciences has already undergone phase 1 clinical trials [82]. The risk of decreasing kidney resilience and increasing the nephrotoxicity of chemotherapeutic agents should be considered when designing phase 2 and 3 clinical trials of FABP5 inhibitors.

Finally, despite the more severe AKI, functional and histologic recovery was similar in WT and FABP5-deficient mice, suggesting that FABP5 primarily modulates the acute phase of injury rather than the repair process. However, in addition to potentially accelerating CKD progression, an AKI episode is clinically associated with other adverse long-term outcomes, such as higher risks for recurrent AKI, cardiovascular events, and mortality [83]. Overall, these data add to the literature on the pathophysiologic relevance of kidney FABP5 activity, as FABP5 was recently reported to mediate part of Ptgcs2-driven kidney injury under diabetic conditions [84].

Conclusion

Overall, cell culture and in vivo findings identify FABP5 as a kidney protective molecule in the setting of AKI, linking fatty acid trafficking, redox balance, and microvascular integrity to maintenance of mitochondrial integrity and renewal (**Figure 11**). Moreover, FABP5 downregulation during AKI joins a growing number of kidney protective factors that are downregulated (PGC1 α , KLOTHO, NLRP6) or insufficiently activated (GDF15, FOSL1) during AKI, thereby negatively impacting kidney resilience [85-90]. Together with PGC1 α , it is directly involved in maintaining mitochondrial health and appears to be an upstream regulator of PGC1 α [85]. These observations suggest that maintaining FABP5 activity, alongside other nephroprotective pathways, may contribute to preserving kidney function and resilience during AKI and should guide the design of clinical trials of FABP5 inhibitors to account for this potential risk.

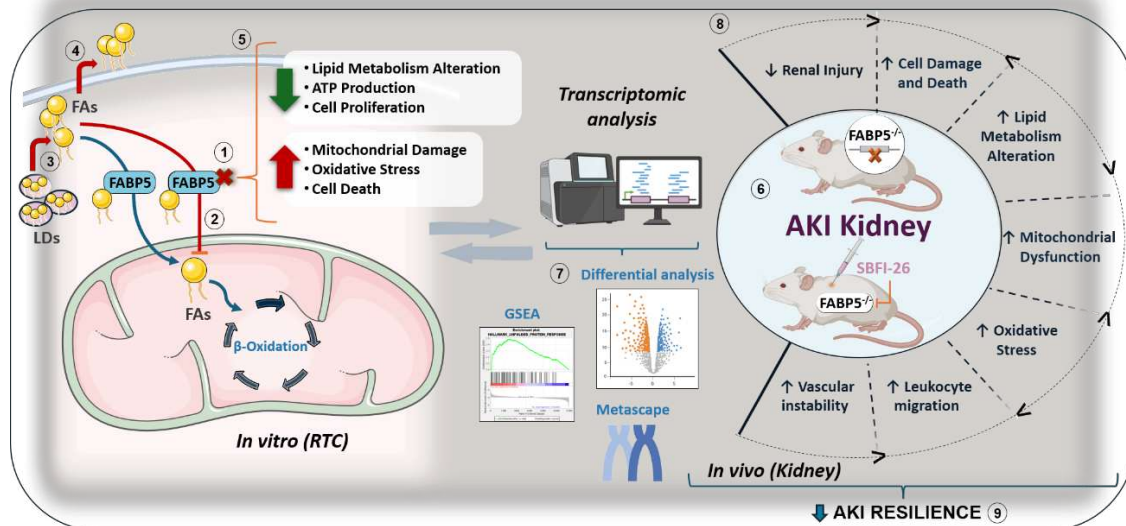


Fig. 11 Graphical summary showing main findings in renal tubular cells and kidney tissue with inhibited FABP5 activity or expression. FABP5 inactivation in cultured renal tubular cells (1) prevents fatty acid entry into the mitochondria (2), increases lipid droplet (LD) degradation (3) and fatty acid exportation (4), and induces pathological responses (5) that support the AKI FABP5^{-/-} mouse phenotype (6). In FABP5^{-/-} mice with AKI, renal transcriptomic analysis (7) predicted dysregulation of pathways linked to injury and maladaptive repair, which were further validated at the gene, protein, and functional levels, characterized by increased severity of kidney dysfunction and damage compared to the AKI wild-type phenotype (8). Collectively, these results identify FABP5 as a nephroprotective factor that promotes resilience during AKI (9).

Abbreviations

AKI Acute kidney injury

BSA Bovine serum albumin

CKD Chronic kidney disease

DEGs Differentially expressed genes

FA Folic acid

FA-AKI Folic acid-induced acute kidney injury

FABP Fatty acid binding protein

FABP5 Fatty acid binding protein 5

1089	FAO Fatty acid oxidation
1090	Fer Ferrostatin
1091	GO Gene Ontology
1092	GSEA Gene Set Enrichment Analysis
1093	KEGG Kyoto Encyclopedia of Genes and Genomes
1094	LD Lipid droplets
1095	NEC-1s Necrostatin 2 racemate
1096	NES Normalized enrichment scores
1097	OCR Oxygen consumption rate
1098	ORO Oil Red O
1099	OXPHOS Oxidative phosphorylation
1100	PPAR Peroxisome proliferator-activated receptors
1101	PTEC Primary proximal tubular epithelial cells
1102	ROS Reactive oxygen species
1103	Rot/AA Rotenone/antimycin A
1104	SEM Standard error of the mean
1105	SGLT2 Sodium-glucose cotransporter
1106	TBARS Thiobarbituric acid reactive substances
1107	TCA Tricarboxylic Acid Cycle
1108	TUNEL Terminal deoxynucleotidyl transferase dUTP nick end labeling

1109 UPR Unfolding protein response

1110 z-VAD z-Val-Ala-DL-Asp(OMe)-fluoromethylketone

1111

1112 **Declarations**

1113

1114 **Ethics approval and consent to participate**

1115 Not applicable

1116

1117 **Consent for publication**

1118 Not applicable

1119

1120 **Availability of data and materials**

1121 The datasets generated during the current study are publicly available in the Gene
1122 Expression Omnibus (GEO) repository, under the accession number GSE329236
1123 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE329236>). Results from the
1124 functional analysis of transcriptomics datasets are included in supplementary information
1125 files/tables.

1126

1127 **Competing interests**

1128 AO has received consultancy or speaker fees or travel support from Astellas, Astrazeneca,
1129 Bioporto, Boehringer Ingelheim, Fresenius Medical Care, GSK, Bayer, Sanofi-Genzyme,
1130 Sobi, Menarini, Lilly, Chiesi, Otsuka, Novo-Nordisk, Sysmex and CSL-Vifor and
1131 Spafarma. Other authors declare no competing interests.

1132

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Author’s contributions

NMC, GCD: conducted most of the research, analyzed and visualized the results; NMC: contributed to manuscript writing and figure design; FMS analyzed and visualized transcriptomic datasets and contributed to manuscript writing; JGG, JLC: developed specific in vitro experimentation. SCN assisted with animal models and tissue preparation and performed histological and immunohistochemical techniques; OL contributed to the interpretation of the results and the discussion; BFF contributed to the interpretation of the results and the discussion, and gave financial support; AO provided expert nephrology review, contributed to the discussion of the results, provided financial support, and wrote

the manuscript; AMR: conceived and directed the research, planned the experiments, analyzed and discussed the results, designed the figures, provided financial support, wrote the manuscript, and managed submission. All authors read and approved the final version of the manuscript.

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Author information

Noelia Molina Cazallas and Gina Cordoba-David contributed equally to this work.

Supplementary Information

Additional file 1.xlsx

Title: Supplementary table 1.

Excel file containing tables from functional enrichment analyses (GSEA and Metascape).

The dataset includes results for all comparisons between non-diseased and diseased wild-type (WT) and FABP5 knockout (KO) mice, as well as genotype comparisons under both conditions.

Additional file 2.docx

Title: Supplementary Figures and Figure Legends.

Description: Word document containing Supplementary Figures S1–S7 and their corresponding figure legends.

Additional file 3.docx

Title: Supplementary Original Images (Not for Publication)

Description: Word document containing the original, unprocessed Western blot images used to generate the figures.

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Supplementary Figures

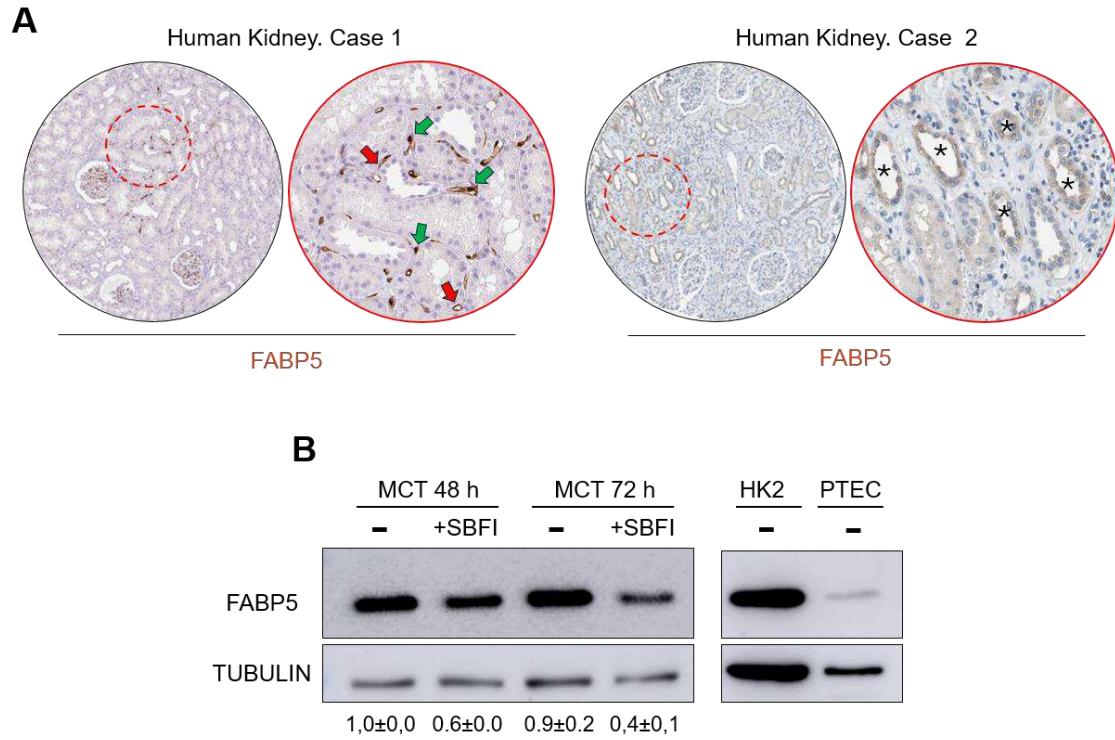


Fig. S1 Expression of FABP5 in human tissue and in cultured human and mouse cells. **A** FABP5 expression in normal human kidney tissue. The images obtained from the Human Protein Atlas show tubulointerstitial expression of FABP5 in cells morphologically consistent with microvascular endothelial cells (red arrows) and pericytes (green arrows) (Case 1, Patient id: 1859), as well as in tubular structures (asterisks) (Case 2, Patient id 3229); <https://www.proteinatlas.org/ENSG00000164687-FABP5/tissue/kidney#>, detected using different antibodies. Circles in the left panels indicate areas shown at higher magnification in the corresponding right panels. **B** FABP5 expression was assessed by Western blot in murine tubular MCT cells treated with vehicle or 50 μ M of the FABP inhibitor SBFI-26 (SBFI) for 48 or 72 h (left), and in human tubular HK2 (HK2) and primary murine tubular epithelial cells (PTEC) (right). Tubulin was used as a loading control. Representative Western blots are shown. Relative FABP5/tubulin values indicated below the corresponding lanes were quantified to assess the inhibitory effect of SFBI on FABP5 expression.

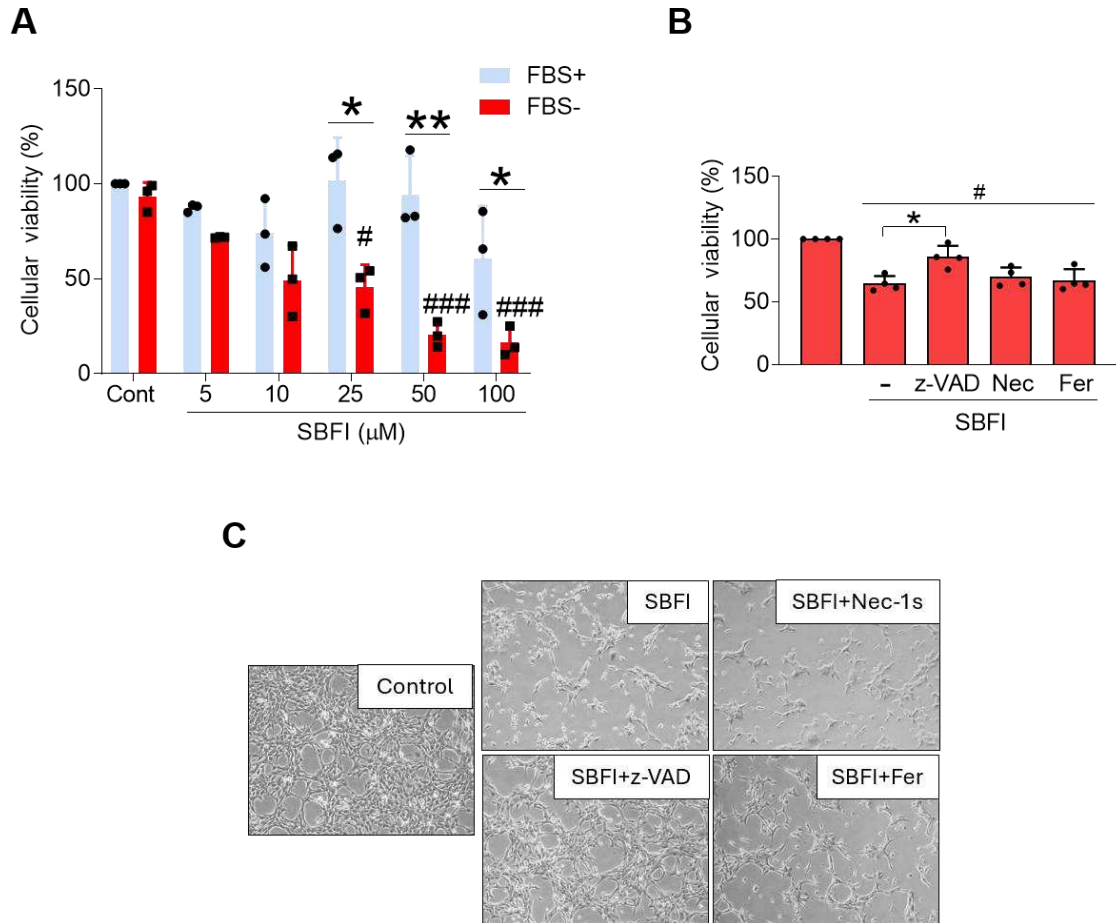
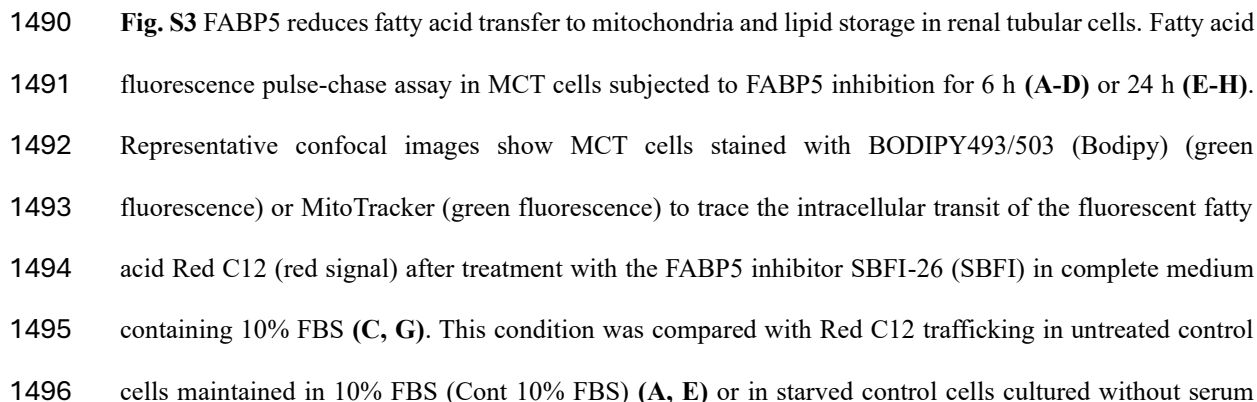


Fig. S2 Pharmacological inhibition of FABP5 reduces cell viability in vitro. **A** Cell viability of MCT cells was measured using the CCK-8 assay after treatment with increasing concentrations of SBFI under culture conditions with fetal bovine serum (FBS+) or without it (FBS-). Data presented as mean $\pm$ SEM from three independent experiments. * $p < 0.05$ and ** $p < 0.01$ SBFI vs. the same SBFI treatment in serum-containing medium; # $p < 0.05$ and ### $p < 0.001$ vs. control untreated cells in serum-free medium (Cont). **B** Cell viability of MCT cells treated with 50 μ M SBFI alone or combined with the cell death inhibitors z-VAD-fmk (z-VAD), necrostatin (Nec-1s) and ferrostatin (Fer) in conditions of FBS depletion was assessed by the CCK assay. Data are presented as mean $\pm$ SEM from four independent experiments. # $p < 0.05$ vs. control (Cont); * $p < 0.05$ vs. SBFI alone. **C** Representative phase-contrast micrographs of MCT cells treated under the same conditions described in C.

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(0% FBS) (**B, F**). Signal colocalization between Red C12 and Bodipy or MitoTracker was quantified using Manders' coefficient analysis. Data are expressed as mean $\pm$ SEM. * $p < 0.05$; # $p < 0.05$. The color semiquantitative scale is intended to help interpret colocalization data (**D, H**). **I** Lipid content was evaluated by Oil Red O staining in human (HK2) and murine primary tubular epithelial cells (PTEC) treated with 50 μ M SBFI and cultured in 10% FBS medium. Quantification of OIL Red O-stained area relative to the total area (normalized to cell number per field). Images were captured at 600 $\times$ magnification; scale bars, 50 μ m. Data are shown as a bar chart of the mean $\pm$ SEM (n=3 independent experiments). * $p < 0.05$ vs control (Cont). **J** Experimental design to assess the extracellular release of fatty acids in cells subjected to FABP5 inhibition. MCT cells were treated with SBFI for 48 h, after which the conditioned medium (CM) was collected and transferred to starved cells with depleted fatty acid stores. Lipid uptake from the CM was visualized by Oil Red O staining in starved cells and interpreted as fatty acid exported because of FABP5 inhibition. **K** Oil Red O staining of lipid droplets in MCT cells treated according to the experimental design described in panel J. Images were captured at 600 $\times$ magnification; scale bars, 50 μ m. Data are presented as mean $\pm$ SEM from five independent experiments. Statistical significance: * $p < 0.05$ CM-treated cells CM) vs control non-CM-treated cells (-).

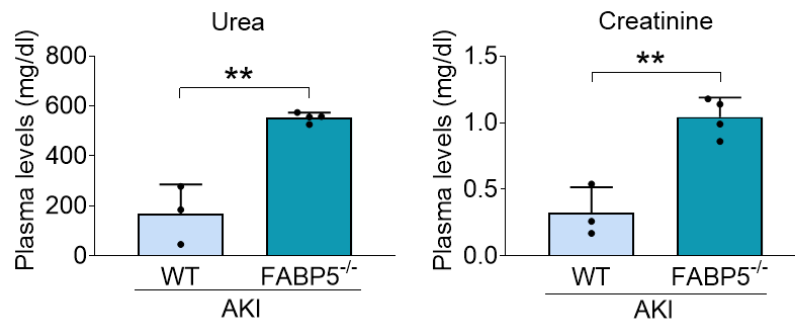
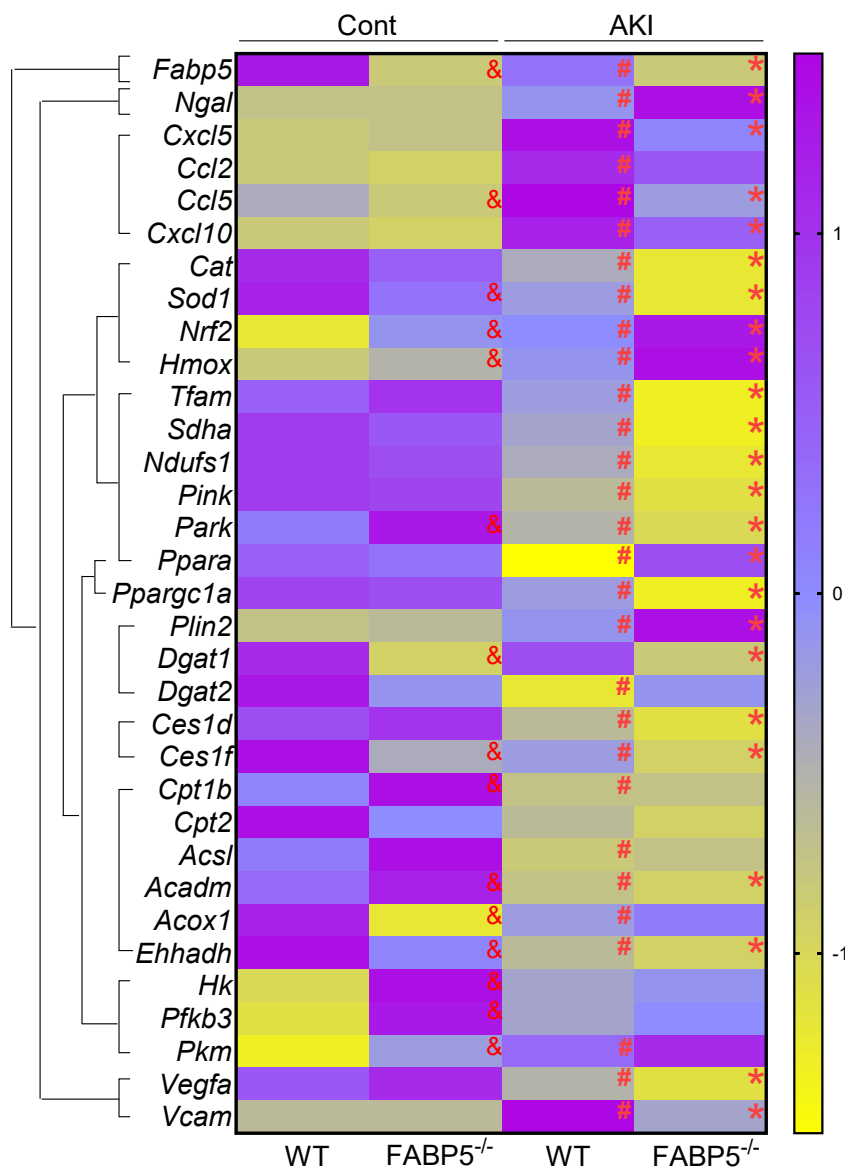


Fig. S4 Assessment of renal function in female FABP5-deficient mice. AKI was induced in female FABP5^{-/-} or WT C57BL/6 mice by folic acid administration (250 mg/kg), and renal function markers were measured at 48 h after AKI induction. **p < 0.05 vs AKI WT.

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1554 **Fig. S5** Heatmap of kidney gene expression in WT and FABP5^{-/-} mice after FA-AKI induction. Kidney gene
1555 expression in WT and FABP5^{-/-} mice 48 hours after FA-AKI. Relative mRNA expression in kidney tissue
1556 from control (Cont) and FA-AKI (AKI) groups was quantified by RT-qPCR. Genes shown are involved in
1557 inflammation, oxidative stress, mitochondrial function, lipid metabolism, and glycolysis. Expression values
1558 were z-score-normalized for each gene, with purple indicating higher expression and yellow indicating
1559 lower expression. Genes are manually classified based on their functional associations and established roles
1560 in AKI-related pathways, with higher-order functional organization indicated by connecting brackets.
1561 *Fabp5* is positioned at the top of this hierarchy, as alterations in its mRNA expression modulate genes across
1562 all pathways shown. Symbols denote statistically significant differences between groups (p < 0.05), &Cont
1563 FABP5^{-/-} vs Cont WT; #AKI WT vs Cont WT, *AKI FABP5^{-/-} vs AKI WT.

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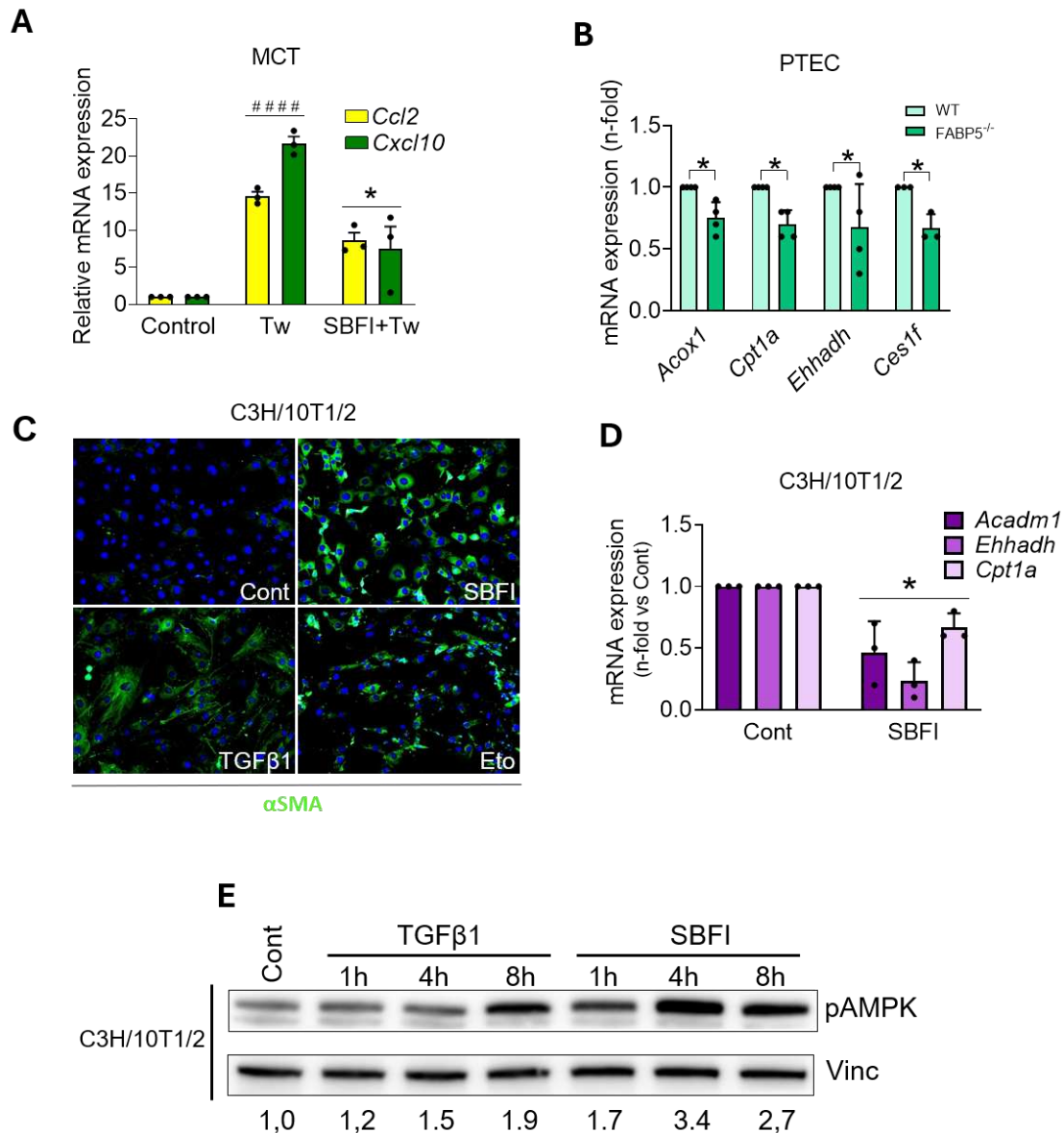


Fig. S6 Pharmacological inhibition of FABP5 in cultured tubular and pericyte-like cells reproduces findings from the *FABP5*^{-/-} AKI model. **A** Pharmacological inhibition of FABP5 in murine tubular cells. MCT cells were exposed to 100 ng/ml TWEAK (Tw) for 6 h, either alone or following pretreatment with 50 μ M of the FABP5 inhibitor SBFI-26 (SBFI). q-RT-PCR assessed TWEAK-induced *Ccl2* and *Cxcl10* mRNA expressions. Data are presented as mean $\pm$ SEM from three independent experiments. #*p* < 0.05 vs Cont; **p* < 0.05 vs Tw. **B** Comparative analysis of the basal expression of key enzymes of fatty acid metabolism assessed by q-RT-PCR in WT or FABP5-deficient primary tubular epithelial cells (PTEC); **p* < 0.05 vs WT. **C-E** Pharmacological inhibition of FABP5 in pericyte-like C3H/10T1/2 cells. α SMA immunofluorescence in cells treated with 50 μ M SBFI for 48 h (**C**). q-RT-PCR of FAO-associated genes after 24 h of FABP5 inhibition with SBFI; **p* < 0.05 vs Cont (**D**). Western blot of pAMPK in cells treated with SBFI in

comparison with 10 nM TGFβ1. A representative experiment with band quantification at the bottom of the image is shown (E).

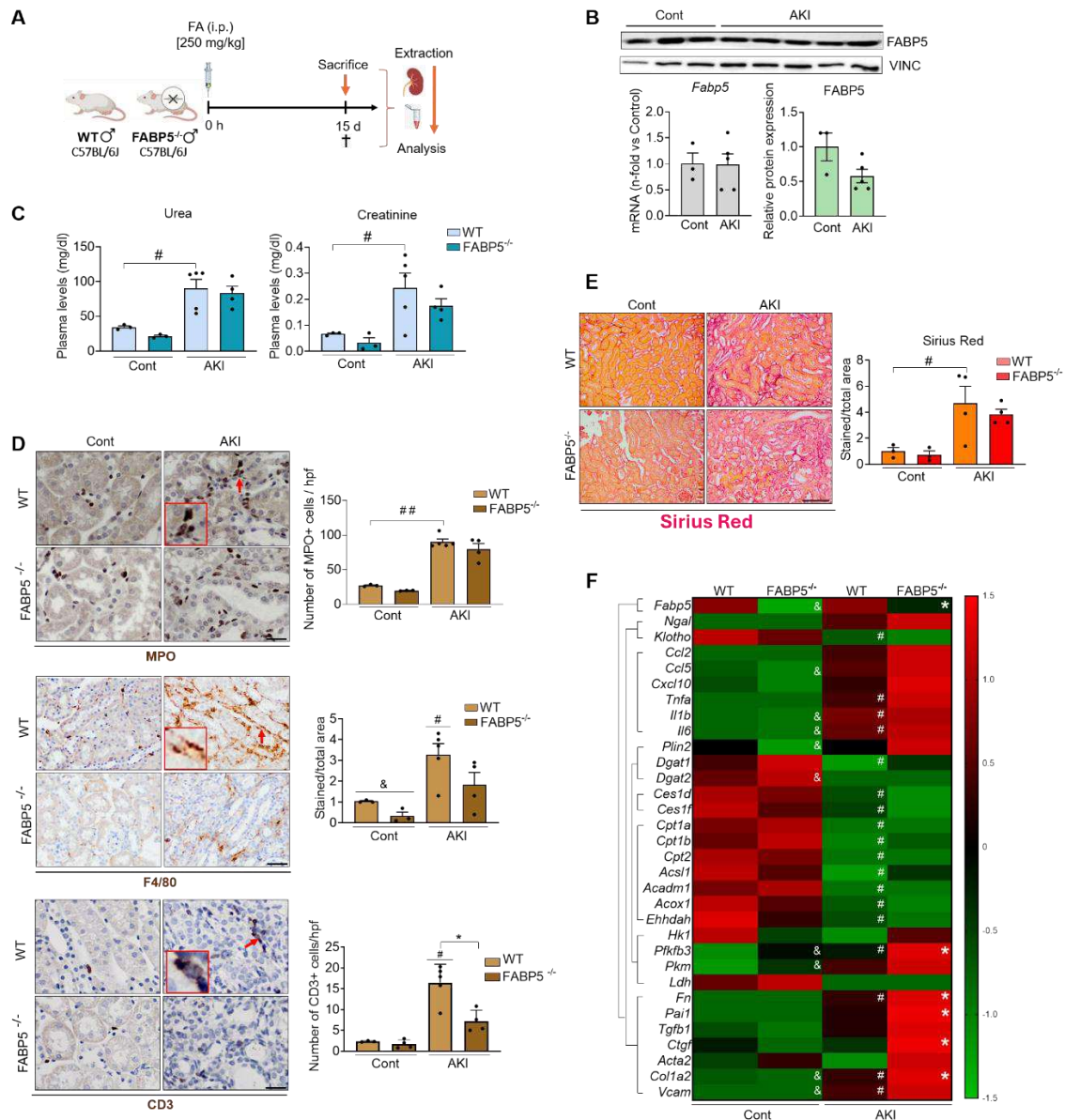


Fig. S7 FABP5 does not participate in AKI to CKD progression. **A** FA-induced AKI with 15 days of follow-up was induced in FABP5^{-/-} and WT C57BL/6 mice. **B** Kidney mRNA and protein expression of FABP5 were measured by RT-qPCR and Western Blot, respectively. **C** Renal function evaluated by the plasma levels of urea (left) and creatinine (right); #p < 0.05 vs. Cont. **D** Immune cell infiltration in kidney sections analyzed by immunohistochemistry for MPO, F4/80, and CD3. Representative images from pictures taken at an original magnification of 400x/Scale bar 50 μ m for MPO; 200x/Scale bar 100 μ m for F4/80 and CD3. Quantification is presented as the number of stained cells per high (MPO) or low (CD3) power field, and as the stained area for F4/80; &p < 0.05 Cont FABP5^{-/-} vs Cont WT, #p < 0.05 AKI vs Cont WT, ##p < 0.01 AKI vs Cont WT, *p < 0.05 vs AKI-WT. **E** Sirius Red histochemical assessment of collagen deposited in

kidney sections. Representative images from different experimental groups. Original magnification 200×. Scale bar 100 μm. Quantification is presented as the Sirius Red-positive area. # $p < 0.05$ vs Cont. **F** Heatmap of kidney gene expression in WT and FABP5^{-/-} mice after 15 days of FA-AKI induction. Kidney mRNA expression of selected genes was quantified by qRT-PCR in the indicated experimental groups. Expression values were z-score normalized for each gene, with red indicating higher expression and green indicating lower expression. Genes were manually grouped based on functional associations and known involvement in AKI-related injury pathways, with higher-order groupings indicated by brackets linking functionally related gene sets. Altered *Fabp5* mRNA expression modulated the expression of genes across all pathways shown. Symbols denote statistically significant differences between groups ($p < 0.05$), &Cont FABP5^{-/-} vs Cont WT; #AKI WT vs Cont WT, *AKI FABP5^{-/-} vs AKI WT.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarytable1.xlsx](#)