Supplementary Methods

Proximal tubule–intrinsic complement shapes epithelial stress responses in rhabdomyolysis-induced acute kidney injury

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Human samples analyses

Collection of human tissues - Kidney biopsies (FFPE) and clinical data from 7 patients with RIAKI (Supplementary Table 1) (5/7 reported previously[1], and re-used in this study) and 3 kidney peritumoral tissues. All patients gave informed consent for the use of part of the biopsy for scientific purposes. Control liver FFPE tissue was ordered from France Tissue Bank.

P	Sex	Age	AHT	Diabetes	Smoke	CPK at admission	Circumstances	Initial creatinine	AKI
A	M	21	No	No	No	10100	Muscle compression	130	Yes
B	M	42	Yes	No	Yes	15000	Muscle compression	450	Yes
C	M	56	No	No	No	1500	Muscle compression	210	Yes
D	M	72	Yes	No	No	1698	NA	780	Yes
E	M	69	No	No	No	461000	Dermatomyositis	412	Yes
F	M	75	Yes	No	yes	13200	Muscle Compression	398	Yes
G	F	65	No	No	Yes	55000	NA	280	Yes

Supplementary Table 1: Clinical data when available for the RIAKI patients with kidney biopsies used for Multiplexed sequential Immunofluorescence. AHT: arterial hypertension, AKI: acute kidney injury, CPK: creatine phosphokinase (in UI/L), Creatinine in µmol/l, NA: not available.

Sequential immunofluorescence (seqIF) and microscopy - Sequential immunofluorescence (seqIF™) protocol was performed on 4 micrometer FFPE sections of kidney biopsies from 7 patients with RIAKI (**Supplementary Table 1**), in comparison to three peri-tumoral tissues from clear cell renal cell carcinoma (ccRCC), using the COMET™ platform (Lunaphore Technologies) for the spatial detection of *14* markers including both cell-specific markers and complement antigens derived from our in situ Complementomics panel for human biopsies [2] (**Supplementary Table 2**). The OME-TIFF output contains DAPI (Lunaphore) for nuclear staining, two autofluorescence channels for background subtraction, and the *14* single marker channels which were used for downstream image analysis. All antibodies were validated using conventional immunohistochemistry (IHC) and

immunofluorescence (IF) staining in conjunction with corresponding fluorophores. Secondary antibodies Alexa Fluor 555 (A32727, ThermoFisher Scientific) and Alexa Fluor 647 (A32733, ThermoFisher Scientific) were used at dilutions of 1:200 and 1:400, respectively. Acquired images were analyzed using Halo Image Analysis software (Indica Labs) with highplex FL module to quantify tubular complement stainings. The detailed method is described in Rivest et al [3] and Boudhabhay I et al [2]

Supplemental Table 2: Sequential Immunofluorescence in patients

Antibody	Species	Clone	Supplier	Dilution
Megalin (Proximal tubular cells)	Rabbit	Polyclonal	Abcam	1:2000
VCAM-1 (Injured tubules)	Rabbit	EPR5047	Abcam	1:100
CD34 (Endothelial cells)	Mouse	QBend-10	Dako	1:100
C3c	Rabbit	Polyclonal	Dako	1:500
C3d	Rabbit	Polyclonal	Dako	1:1000
FH	Rabbit	Polyclonal	ProteinTech	1:100
FB	Rabbit	Polyclonal	ProteinTech	1:100
C1q	Mouse	7H8	Abcam	1:100
C5aR1	Rat	8D6	Santa Cruz	1:100
CD11b	Rabbit	EPR1344	Abcam	1:500
CD3	Rabbit	Polyclonal	Dako	1:50
CD20	Mouse	L26	Dako	1:100
CD68	Mouse	KP1	Dako	1:50
CD163	Mouse	10D6	BD Biosciences	1:50

Spatial multi-omic combining seq-IF and RNA scope - In one patient (Patient F), a fully-automated protease-free protocol was performed on COMET™ with RNA detection followed by protein detection and image pre-processing, combining the RNAscope™ HiPlex Pro Assay and sequential immunofluorescence (seqIF™ [3]) assays. A panel of *2* RNAs (C3, RNA scope HIPlex CS Probe - Hs-C3-T4 Biotechne #430707-T4 and FB, RNA scope HIPlex CS Probe - Hs-CFB-T2 Biotechne #402107-T3) and *14* proteins (**Supplemental Table 2**) was detected. The OME-TIFF output file(s) contains images of DAPI for nuclear counterstaining, two autofluorescence channels for optimal RNA and protein background subtraction, and the *16* single marker channels that were used for downstream image analysis.

Exploration of the transcriptomic signature of human PT expressing *C3* or *CFB* - Single nuclei RNA-seq (snRNA-seq) comprising normal adjacent tissue data from two different ccRCC patients was taken from Hu et al [4]. Processed data were downloaded from https://zenodo.org/records/8063124, file normal_snRNA.rds. Data were analyzed using R (R

version 4.3.2) and the "Seurat" package (Seurat version 5.0.3). Dimensionality reduction, integration and cell annotations were as provided by the authors. The presto R package (version 1.0.0) was used to obtain differentially expressed genes between C3 or CFB positive and negative cells from the proximal tubular cell cluster. For the gene set enrichment analysis (GSEA), all genes were ranked by their estimated log2 fold changes within the positive C3 or CFB group. The resulting list was used as an input for the "GSEA()" function from the "clusterProfiler" package [5] with the "fgsea" method. The 50 hallmark gene sets from the molecular signature database[6] were employed for these analyses and results were visualized using ggplot2 (***ggplot2: Elegant Graphics for Data Analysis***). Ligand–receptor (L/R) interactions were inferred using the SignalR R package, based on curated ligand–receptor interaction databases. For each ligand–receptor pair, expression values of the ligand and corresponding receptor were independently z-scored across samples, and an L/R activity score was defined as the arithmetic mean of these standardized values. Interactions for which either component was not expressed were excluded from downstream analyses. The resulting L/R activity matrices were scaled per interaction, hierarchically clustered, and visualized using the ComplexHeatmap R package, with significant interactions prioritized based on adjusted p-values and correlation metrics.

Animal experimentation

Mice - Wild type, male C57BL/6 mice at 6- to 10-weeks of age were purchased from Charles River Laboratories. $C3^{-/-}$ mice from C57Bl/6 background were purchased from Charles River and backcrossed for 4 generations, $Cfb^{-/-}$ mice from C57Bl/6 background were kindly provided by Prof Steven Sacks (King's college, London) for this study. Due to Covid-19 pandemic, we could receive only 6 *Cfb*$^{-/-}$ mice, which were used for this study. Experimental protocols were approved by the Charles Darwin ethical committee (Paris, France) and by the French Ministry of Agriculture (Paris, France number APAFIS#2148 2019091015099240v1). All the experiments were conducted in accordance with their recommendations for care and use of laboratory animals. Male mice were preferred, as female mice have weaker complement activation capacity and develop less severe complement-mediated kidney diseases [7-9] as well as RIAKI. The selected mouse strain and experimental design are standard and reliably reproduce key features of human pathology.

Glycerol-induced rhabdomyolysis model and mouse treatment - In order to induce rhabdomyolysis, mice were injected intramuscularly in the left quadriceps by 200 µL of glycerol 50% as previously described [1, 10]. For glycerol injection and sacrifice, mice were anesthetized with 2–3% isoflurane. Any exclusions from analysis were due solely to technical issues during sample collection or handling. For phosphate-buffered saline (PBS) or glycerol injections, mice were randomly assigned by administering each treatment at random and housing animals from both groups together to minimize cage effects. One person prepared the mice and syringes, while another, blinded to syringe contents, performed the injections. Experiments were conducted in the morning; animals were monitored at midday and late afternoon for the 24-hour experiments, and daily for the 7-day experiment. Mice were housed in groups of 2–5 in Type IIL cages providing adequate space, with individually ventilated racks maintained at 22 ± 2 °C, under a 12-hour light/dark cycle (lights on from 7 a.m. to 7 p.m.), with automatic watering and environmental enrichment. Humane endpoints included moribund,

unresponsive, or non-ambulatory states, inability to access food or water, and recumbency. All mice were sacrificed by cervical dislocation 1 day after glycerol administration. 1,2 mg/mouse of factor B inhibitor CPX14 (similar to LNP023, provided by Novartis) was introduced by gavage 90 minutes before induction and 10h30 after the induction of the rhabdomyolysis, after reconstitution with the excipient (sterile water + 0,5% methylcellulose + 0,5% Tween 80) and compared to the excipient alone as recommended by the provider [11]. The efficacy of muscle injury was assessed in mice by the elevation of ASAT, more reliable parameter in mice compared to CK. Whole blood was collected retro-orbitally with heparinized capillary tube in microtubes with 10 µL EDTA. Microtubes were centrifuged at 12,000 G for 10 min at room temperature to recover plasma. Plasma was directly frozen at -80°C. Kidneys were immediately snap frozen in liquid nitrogen for immunofluorescence (IF), molecular biology and freshly recovered by flow cytometry analysis. For glycerol injection and sacrifice, mice were anesthetized with isoflurane 2-3%.

Evaluation of the biochemical parameters of mice and patients - For mice, kidney function and muscle injury were evaluated by plasmatic Blood Urea Nitrogen, Creatinine, Creatine Kinase (CK) and Aspartate AminoTransferase (ASAT) coupled with the liver injury marker Alanine AminoTransferase (ALAT) using Konelab equipment. Likewise, creatinine levels were measured in patients' urine by Konelab Chemistry Analyzer equipment (Thermo Scientific).

Histology - The histology was assessed by hematoxylin/eosin staining on 5 µm tick sections of paraffin-embedded kidneys. The slides were scanned with a Nanozoomer whole slide scanner (Hamamatsu Photonics) and blindly quantified. Tubular dilatation and casts were given a score between 0 and 3, which described the percentage of the total cortical area of the biopsy (0 = 0–2%, 1 = >2–25%, 3 = >25–50%, and 4 > 50%).

Immunofluorescence (IF) and immunochemistry (IHC) in mice - Frozen kidneys were cut at 5µm thick sections with a Cryostat at -20°C (Leica AS-LMD, Leica Biosystem) and fixed in acetone on ice for 10 minutes. 4-µm-thick sections of paraformaldehyde-fixed and paraffin-embedded kidneys were cut with a Microtome (Thermo Scientific Microm HM 340E) for IHC. After dewaxing in Clearene (Leica, Clearene 5L 3803600E) and rehydration with gradual concentration of ethanol (100%, 90%,70% and 50%), hematoxylin and eosin staining was performed by routine procedures and Picrofuschine staining (Sigma-Aldrich) according to the manufacturer. Endogenous peroxidases were blocked with 3% H_2O_2 (Gilfrer, 10603051) and non-specific staining by protein block (Dako, X0909). A rat anti mouse C3b/iC3b antibody (HM1065, Dako, 1µg/ml) was incubated for 30 minutes and revealed by a chicken anti rat IgG H+L AF647 (A21247, Invitrogen) or a Chicken anti-rat IgG H+L AF488 (A21470, Invitrogen). A double immunostaining was performed with an anti CD31 (NCL- CD31- 1A10, Leica, 36µg/L), revealed by an anti-mouse Polyview Plus AP and AF 647 Tyramid reagent Invitrogen, B40958. Mounting was performed with ProLong antifade reagent (Invitrogen). TUNEL assay was performed with the DeadEND TM Fluometric TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling) system G3250 kit from Promega, according to manufacturer's recommendations. Slides for IF were scanned with Axio Scan™ Z1 (Zeiss, Oberkochen, Germany). Images were analyzed and staining were quantified using Halo software (Indica Labs).

Estimation of myeloid infiltrate in mouse kidney by mMCP-counter - To evaluate the proportion of immune cells infiltrating the kidney, we used the mouse Microenvironment Cell Population counter (mMCP-counter), which is based on highly specific transcriptomic markers that accurately quantify immune (and stromal) murine cell populations [12] (https://github.com/cit-bioinfo/mMCP-counter). The results of monocyte/macrophage population, corresponding to the inflammatory monocytes [1] are shown, detected in the RNAseq datasets as *Fcgr1* proportion.

In vitro experimentation

Cell lines - Human Renal Proximal Tubule Epithelial Cells (RPTEC, CC-2553, Lonza) were cultured in REGM medium (REGM Medium Bullet Kit, CC-3190, Lonza) at 37°C, 5% CO2, and used until passage 4. The proximal tubular cell line HK2 cells, derived from human immortalized PTECs of kidneys, and the renal cancer cell line Caki-1, which could be used as a model for PT[13], were purchased from ATCC in 2023 and 2016 respectively, and cultured in a humidified atmosphere of 5% CO2 at 37°C until 90% confluence. HK2 cells were cultured in Dulbecco's modified Eagle medium/Nutrient Mixture F-12 (DMEM/F12; Gibco, Thermo Fisher Scientific) containing 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific) and 1% penicillin/streptomycin (MilliporeSigma). Caki-1 cells were cultured in McCoy's medium (Gibco, cat# 16600082) with 10% heat-inactivated FCS (Biowest, cat# S1810-500) and 1X penicillin/streptomycin (Gibco, cat# 10378016). All cell lines were routinely tested for Mycoplasma with a kit (Ozyme, cat# LT07-710) and used only if negative. The cells were authenticated by morphology before each experiment

Cytokine stimulation: Cytokine stimulation in the three cell lines was performed in duplicate with either 10 ng/mL of TNFα (R&D Systems, catalog no. 210-TA), 10 ng/mL of IL1β (Preprotech, catalog no. 200-01B) or 10ng/mL of IL-6 (Preprotech, catalog no. 200-06). Gene expression was measured by a 19-plex Quantigene panel containing genes from the complement system including C3, CFB, C3AR1 alongside four reference genes (HPRT1, GUSB, RPL19, POLR2A). Signal detection was performed with Luminex FLEXMAP 3D Instrument System (ThermoFisher). Target genes were submitted to targeted hybridization and signal amplification according to the recommendation of the manufacturer. Streptavidin phycoerythrin signal was detected by Luminex equipment (Luminex Corporation). Blank well fluorescence was subtracted from median fluorescence and housekeeping genes validated regarding their standard deviation to mean ratio. Analysis was performed with Graphpad Prism® software after normalization of mean fluorescence values on housekeeping gene expression and comparison with gene expression of pooled PBS treated mice, according to the manufacturer's instructions.

Western Blot - For in vitro studies, Caki-1 and HK-2 cells were cultivated in OptiMEM (Thermofisher Scientific) for 48 hours, after which the supernatant was collected. Supernatants were concentrated at 200µg/mL. All samples were prepared using NuPAGE® LDS sample buffer (4X) from Thermo Fisher Scientific, along with a reducing agent (DTT 1M), and subsequently denatured at 90°C for 10 minutes. Protein separation was carried out on a NuPAGE 10% Bis-Tris gel (Thermo Fisher Scientific). The proteins were then transferred onto

a nitrocellulose membrane using the iBlot system from Invitrogen. Following this, the membranes were subjected to overnight incubation with primary antibody anti-C3 (anti C3c, Dako, A0062; anti-C3d, Dako, A0063 and anti C3, Calbiochem, 294869), which also exhibits cross-reactivity with human complement C3 and Rabbit polyclonal IgG anti human FB (ProteinTech, PT10170-1). For the cell signaling, the membranes were revealed with rabbit anti-pan Akt (Cell signalling #4691) 1/1000, rabbit anti-phospho-Akt (Ser473) (Cell signalling #9271), 1/1000; rabbit anti-phospho-S6 Ribosomal Protein (Ser235/236) (Cell signalling #2211), 1/1000; or mouse anti-beta actin monoclonal (Thermo, #MA-140) 1/10000. Subsequently, they were incubated with a secondary antibody (rabbit anti-goat IgG HRP, Thermo Fisher Scientific, #31402 for C3 and goat anti-rabbit IgG HRP, Thermo Fisher Scientific, #31460) or anti-mouse IgG HRP (Thermo, #31430) 1/10000. Visualization was achieved through chemiluminescence using a substrate for HRP (SuperSignal™ West Dura Luminol, Thermo Fisher Scientific, #1856145), detected using the iBright Western Blot Imaging System (iBright FL1500, Thermo Fisher Scientific). Band intensity was quantified with the iBright software.

Protein extraction and C3a, Ba and Bb quantification – Caki-1 cells were lysed in RIPA buffer to obtain a total cell lysate. Ba and Bb concentrations in cell supernatants and cell lysates were measured using ELISA kits (C3a, Ba and Bb fragment EIA MicroVue, QuidelOrtho)

ELISA for C3 detection - C3 concentrations in cell supernatants and cell lysates were measured using a homemade sandwich ELISA. In brief, native antibody [polyclonal C3 (Calbiochem, cat#204869)] was coated on a Nunc MaxiSorp ELISA 96-well and 1% PBS-BSA was used for the blocking. Supernatant or cell lysate was added to the plate and incubated for 1 hour at room temperature. After washing, the plate was incubated with an in-house biotinylated version of the anti-C3 (biotinylation with EZ link NHS Biotin was performed according to the manufacturer's instructions (Thermo Fisher Scientific, cat# 20217) for 1 hour at room temperature. After additional washes, streptavidin coupled with HRP (Dako, cat#P039701-2) was added for 1 hour at room temperature. Binding was revealed using SureBlue TMB Microwell Peroxidase Substrate (Sera Care, cat# 5120-0075), and 2 mol/L sulfuric acids stopped the reaction. Multiskan Ex (Thermo Fisher Scientific) was used to read the optical density at 450 nm. The results are expressed in mg/mL according to the standard curve, made using commercial purified C3 (Complement Technology, cat# A113).

Immunofluorescence on cells - Cells were seeded onto sterilized round glass coverslips in 24-well plates and cultured to ~80% confluence. After a PBS (1×) wash, cells were fixed in 4% paraformaldehyde (PFA) for 30 min at room temperature, then washed three times with PBS. Permeabilization was carried out with 0.1% Triton X-100 for 10 min, followed by three PBS washes. Non-specific binding was blocked with 1% BSA in PBS for 1 h at room temperature. Primary antibodies were applied overnight at 4 °C in the dark, with plates sealed in parafilm to prevent evaporation. Antibodies included anti-C3d (Dako, A0063) anti-Ki-67 (Mouse, Dako, X0931), anti-LAMP1 (Mouse, Cell Signaling, #DB401S), and isotype controls (Dako FLEX negative control mouse and rabbit cocktail, catalog no IR750 and IR600 respectively). After three PBS washes, Alexa Fluor 555-conjugated Goat anti-Mouse IgG (Invitrogen, #A-21422) and Alexa Fluor 647-conjugated Goat anti-Rabbit IgG (Invitrogen, #A32733TR) were applied for 1 h at room temperature. Cells were then washed once with PBS, stained with Phalloidin-iFluor 488 (Abcam, #ab176753) for 30 min protected from light, and counterstained with DAPI

(Thermo Fisher, #62248) for 20 min, followed by two PBS washes. Coverslips were mounted in ProLong™ Diamond Antifade Mountant (Thermo Fisher, #P36961) and stored at 4 °C protected from light until imaging. Slides were scanned with ZEISS Axio Scan.Z1. Image analysis and figure preparation were performed using HALO® software.

Silencing - A 30-minute pre-incubation of 3 siRNAs against CFB (Qiagen, Hs_CFB_1 FlexiTube siRNA, cat# SI03067365; Qiagen, Hs_BF_2 FlexiTube siRNA, cat# SI00311780; Qiagen, Hs_BF_4 FlexiTube siRNA, cat# SI00311794), or 4 siRNAs against C3 (Qiagen, Hs_C3_6 FlexiTube siRNA, cat# SI02622872; Qiagen, Hs_C3_8 FlexiTube siRNA, cat# SI03041871; Qiagen, Hs_C3_9 FlexiTube siRNA, cat# SI03076689, Qiagen, Hs_C3_11 FlexiTube siRNA, cat# SI05095902), or siRNAs against CFH ((Qiagen Hs_CFH_3_Flexitube siRNA SI00003983 and Hs_CFH_4_Flexitube siRNA SI00003990) or siRNA control (Qiagen AllStars negative control siRNA, cat# SI03650318) at 50 nM with lipofectamine RNAimax (ThermoFisher, cat# 13778030) in OptiMEM (Gibco, cat# 31985070) was performed before adding this mixture to cells at 50% confluence in 10 cm² petri dishes or T75 flasks seeded with HK2 (siC3) or Caki-1 cells (siC3, siFB, siFH). The transfection was stopped after 24 h by replacing the medium containing siRNA with the standard medium in which the cells were routinely cultured without antibiotics. Functional assays were performed on the silenced cells 72h post-transfection. Cells treated with siRNA against *CFB* and C3 are designated siFB and siC3, respectively, and the cells treated with a control siRNA against an irrelevant sequence were designated siC.

Proliferation and Mortality - Cells were washed with PBS before adding CFSE reagent 1:1000 (Invitrogen, cat# C34554) for 20 minutes at 37°C. The addition of the complete culture medium stopped staining. The cells were then washed and cultured in 6-well plates for 48 hours in the presence of the standard culture medium of each cell. Supernatants containing dead and adherent cells were recovered and stained for DAPI before acquisition by flow cytometry. Proliferation was assessed by the intensity of CFSE staining by flow cytometry (BD Fortessa) and analyzed using FlowJow software. Dead cells were excluded from the proliferation analysis. To explore whether the phenotype change upon knockdown of C3 or FB is due to intracellular action of the proteins or autocrine effect, purified BSA as a control or C3 (CompTech, catalog no. A113) at 10µg/ml or FB (CompTech, catalog no. A135) at 10µg/ml was added to the culture medium at the same time of CFSE staining.

Spheroids - 24h after stopping the silencing (see Silencing), Caki-1 cells were detached with trypsin and counted with the TC20 automate cell counter (Biorad, cat# 145-0101). 10 000 cells were resuspended in 50µl of their cell culture medium and put in 96 wells cell-repellent plates (Greiner Bio One, cat# 650970) overnight at 37°C, in a 5% CO2 incubator. 24 hours later, 150µL of culture cell medium are added to each well. Spheroid behavior was evaluated by videoimaging over 72 hours (Celloger Mini).

Metabolomic analyses

Cells were washed once with ice-cold PBS. A methanol/distilled water solution (9:1, v/v), pre-chilled to –20°C, was added directly to the cells. The cells were then scraped, collected, and transferred to tubes kept on ice. Samples were stored at –80°C until shipment to the Metabolomic Platform at the Gustave Roussy Institute for targeted metabolomic analysis. Metabolite comparisons were performed using the Wilcoxon rank-sum test. Due to the limited sample size and high number of metabolites, no p-value adjustment was applied. Metabolites

identified as statistically significant were converted into Human Metabolome Database (HMDB) identifiers for pathway enrichment analysis.
Pathway enrichment was conducted using the MetaboAnalyst platform, specifically employing over-representation analysis (ORA) against the SMPDB database (Homo sapiens). Background correction was not applied, as the number of detectable metabolites in the assay was limited. All statistical analyses were performed using R version 4.4.2.

Cell Fractionation - Subcellular fractionation was carried out using the Cell Fractionation Kit (Abcam, #ab109719) following manufacturer's instructions. Briefly, 15 × 10^6 cells were used per 1 mL buffer. Cytoplasmic, organelle, and nuclear fractions were collected, aliquoted, and stored at -80°C. Fraction purity was validated by Western blot using GAPDH, Cytochrome C, and Histone 3 as respective markers. The images of the used fractions purity validation are previously published in our study related to FH [14].

Bulk RNA-seq for mouse kidney and cells

Preparation of the mouse kidneys for bulk RNAseq: Thirty µm-thick frozen kidney sections were cut as above and homogenized in 200 µL of 1-Thioglycerol/Homogenization Solution (Maxwell® 16 LEV simplyRNA Tissue Kit Promega AS1280). The quality and quantity of mRNA were evaluated using a 2100 bioanalyzer with TNA 6000 NanoKits (all Agilent Technologies, Palo Alto, CA, USA). RNA Integrity Numbers superior to 7 were eligible for subsequent reverse transcription into cDNA. 3 of the control mice from the experiment comparing them with $Cfb^{-/-}$ mice did not develop AKI and were not processed for RNAseq. The RNAseq was performed by the GenomIC Cochin Institute INSERM U1016.

Preparation of the cells for bulk RNAseq: After the transfection, the isC and siFB or siC3 cells were kept in normal medium for 5 days before detaching and pelleting the cells. The cell pellet was processed with the Maxwell® 16 LEV Simply RNA Tissue Kit, according to the manufacturer's instructions. The quality of the extracted RNA was evaluated with the Agilent 2100 Bioanalyzer system and 2100 Expert software. RINs for all samples were above 8. The samples were prepared in quadruplicates.

Samples processing for bulk RNAseq: The RNAseq was performed by the GenomIC Cochin Institute INSERM U1016. After RNA extraction, RNA quality (RNA integrity number) was estimated. 1µg of high-quality total RNA sample (RIN >7) was processed to build up the libraries, using TruSeq Stranded mRNA kit (Illumina) according to manufacturer instructions. Briefly, purified poly-A containing mRNA molecules were fragmented and reverse-transcribed using random primers. Replacement of dTTP by dUTP during second strand synthesis allowed us to achieve strand specificity. Addition of a single A base to the cDNA was followed by ligation of Illumina adapters.
Libraries were quantified by qPCR using KAPA Library Quantification Kits for Illumina Libraries (KapaBiosystems, Wilmington, MA). Library profiles were assessed using DNA High Sensitivity LabChip kits on an Agilent Bioanalyzer. Libraries were sequenced on an Illumina Nextseq 500 instrument using 75 base-lengths read V2 chemistry in a paired-end mode.

Data processing for bulk RNAseq: The quality of the FASTQ files was verified using FASTQC (version 0.11.5) and aligned using the STAR algorithm (version 2.5.2b), the quality of the alignment was checked with Picard Tool (version 2.8.1). The differential expression analyses were performed on R studio (version 4.1.3) using the DESeq2 package. Genes were considered differentially expressed in two conditions if they presented a log2-fold change of more than 1.5 and an adjusted p-value < 0.05 (Benjamin Hochberg method). The differentially expressed genes were sorted by the adjusted p-value and represented on volcano plots (EnhancedVolcano version 1.12.0). Gene set enrichment analysis was performed by pre-ranked GSEA 4.3.3 [15], using ordered genes according to their previously calculated Wald test statistic calculated using the DESeq2 package, value and the h.all.v2023.2.Hs.symbols.gmt MSigDB Hallmarks gene sets database and the the 50 hallmark gene sets from the molecular signature database[6].

Given the limited sample size (n=4 per condition), the analysis was performed according to GSEA recommendations using the following parameters: permutation type set to "gene_set," enrichment statistic set to "weighted," ranking metric set to "Signal2Noise," and exclusion of gene sets containing fewer than 15 or more than 500 genes. Results were filtered using a threshold of normalized enrichment score (NES) > 1.5 and false discovery rate (FDR) < 0.25, as recommended by GSEA guidelines for exploratory analyses aimed at identifying enriched signatures with potential biological relevance. Redundancy between gene sets were controlled by hierarchical clustering based on pathway name's similarities using cutree (h = 0.2).
As three datasets were generated for the WT PBS vs GLY as controls of different experiments, each corresponding control dataset was used to evaluate the *C3*$^{-/-}$ and *Cfb*$^{-/-}$ or FB inhibitor mouse phenotype.

RNAseq datasets accession numbers:
The RNAseq dataset comparing kidney transcriptomes from normal C57Bl/6 WT and C3-/- mice, treated or not with gycerol 21 supporting the findings of this study are openly available in repository GEO at accession number GSE153507. Human control peritumoral kidney snRNAseq data was taken from Hu et al 29. Processed data were downloaded from https://zenodo.org/records/8063124, file normal_snRNA.rds. The newly generated mouse kidney RNAseq datasest of the C57Bl/6 WT and Cfb-/- mice treated with glycerol or the C57Bl/6 WT mice, injected or not with FB inhibitor CPX014 are available at 10.5281/zenodo.18644294. The cell lines RNAseq datasest of the siC and siC3 HK2 cells or Caki-1 cells as well as siC and siCFB Caki-1 cells are available as follows: at 10.5281/zenodo.13891307 - HK2 siC and siC3; at 10.5281/zenodo.18644314 – Caki-1 siC and siC3 and 10.5281/zenodo.13891430 - Caki1 siC and siFB.

Statistical Analyses.

Results were analyzed using the statistical software GraphPad Prism 9 (La Jolla, USA) and with R for Kruskall Wallis. Two continuous variables were compared using the Mann-Whitney test. Comparisons between more than 2 groups of mice were performed using Kruskal Wallis

test with Benjamini, Krieger and Yekutieli correction for multiple comparisons. Statistical significance was defined as $p<0.05$, with bilateral tests.

Supplementary References

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