Defective glucose metabolism in polycystic kidney disease identifies a new therapeutic strategy

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Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder characterized by bilateral renal cyst formation1. Recent identification of signaling cascades deregulated in ADPKD has led to the initiation of several clinical trials, but an approved therapy is still lacking2,3. Using a metabolomic approach, we identify a pathogenic pathway in this disease that can be safely targeted for therapy. We show that mutation of PKD1 results in enhanced glycolysis in cells in a mouse model of PKD and in kidney samples from patients with ADPKD.

Glucose deprivation resulted in lower proliferation and higher apoptotic rates in PKD1-mutant cells than in nondeprived cells. Notably, two distinct PKD mouse models treated with 2-deoxyglucose (2DG), to inhibit glycolysis, had lower kidney weight, volume, cystic index and proliferation rates as compared to nontreated mice. These metabolic alterations depend on the extracellular signal-related kinase (ERK) pathway acting in a dual manner by inhibiting the liver kinase B1 (LKB1)–AMP-activated protein kinase (AMPK) axis on the one hand while activating the mTOR complex 1 (mTORC1)-glycolytic cascade on the other. Enhanced metabolic rates further inhibit AMPK. Forced activation of AMPK acts in a negative feedback loop, restoring normal ERK activity. Taken together, these data indicate that defective glucose metabolism is intimately involved in the pathobiology of ADPKD. Our findings provide a strong rationale for a new therapeutic strategy using existing drugs, either individually or in combination.

ADPKD is a chronic progressive disease1. Cysts originate from any segment of the renal tubule in only 1–5% of the nephrons, a condition that should be compatible with a normal renal function1,4. However, the gradual expansion of cysts compresses and eventually replaces the segment of the renal tubule in only 1–5% of the nephrons, a condition that should be compatible with a normal renal function1,4. However, the gradual expansion of cysts compresses and eventually replaces the normal tissue, causing end-stage renal disease in a majority of affected individuals1,4. Thus, therapeutic interventions targeting cyst expansion are currently being tested in multiple clinical trials to delay renal disease progression3,5.

The disease is caused by loss-of-function mutations in either PKD1 or PKD2 (refs. 1,2). To study alterations caused by defective PKD1 function, we isolated mouse embryonic fibroblasts (MEFs) from littermate Pkd1+/+ and Pkd1−/− embryos6, and using these cells we identified a new pathogenic process. During routine culture, Pkd1−/− cells acidified the medium faster than wild-type cells, whereas the opposite was true in cells overexpressing PKD1 (Supplementary Fig. 1). We found the same effect in growth-arrested cells (100% density), suggesting an intrinsic, proliferation-independent metabolic increase in Pkd1−/− cells. Indeed, Pkd1−/− cells had much higher ATP content as compared to wild-type cells (Fig. 1a). To determine which metabolic pathways were altered in these cells, we performed a metabolic profiling of the conditioned extracellular medium of wild-type and Pkd1−/− cells using nuclear magnetic resonance (NMR) spectroscopy7,8 (Fig. 1b). An unsupervised statistical analysis revealed that the metabolic profile of Pkd1−/− cells differed significantly from that of wild-type cells (Supplementary Fig. 2 and Supplementary Table 1), with the most prominent alteration being lower glucose concentrations and higher lactate concentrations (Fig. 1c,d and Supplementary Fig. 2).

These data suggest that Pkd1−/− cells use aerobic glycolysis as a source of energy. Indeed, glucose deprivation abrogated the higher ATP content of Pkd1−/− cells (Fig. 1e). We generated similar results using a MEF cell line that was conditionally inactivated for Pkd1 as a result of its harboring a Pkd1 floxable allele and treatment with Cre (Fig. 1d and Supplementary Fig. 1).

As glucose metabolism is the main source of energy generated by oxidative phosphorylation, which occurs in the mitochondria9, we analyzed the mitochondrial membrane potential in wild-type and Pkd1−/− cells using two independent assays and demonstrated no significant difference between the two cell lines (Fig. 1f,g). In line with this, treatment with oligomycin, an inhibitor of mitochondrial ATP synthase, elevated the ATP content in wild-type cells but had only a minor effect in Pkd1−/− cells (Fig. 1h).

These data suggest that an alternative metabolic pathway that is glucose dependent is the source of the differential ATP content between the two cell lines. Previous studies demonstrated that transcriptional changes can regulate the glycolytic response in cells10,11. In line with this, real-time PCR analysis revealed that Pkd1−/− cells showed a transcriptional signature of metabolic genes that should be compatible with a normal renal function1,4. However, the gradual expansion of cysts compresses and eventually replaces the normal tissue, causing end-stage renal disease in a majority of affected individuals1,4. Thus, therapeutic interventions targeting cyst expansion are currently being tested in multiple clinical trials to delay renal disease progression3,5.

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glycolytic enzyme4,13,14 (Fig. 1i). We conclude that Pkd1−/− cells preferentially rely on aerobic glycolysis for their energy demands, a process similar to the Warburg effect that is observed in cancer cells4,13,14. We thus tested whether higher glucose metabolism contributes to the deregulation of this balance. Indeed, glucose deprivation restored the proliferation index of Pkd1+/+ and Pkd1floxflox; Cre cells, respectively (Fig. 2a). In addition, whereas the Pkd1+/+ cells deprived of glucose activated cell autophagy to survive, Pkd1−/− cells could not activate this response (Fig. 2b,c and Supplementary Fig. 3) and instead had higher apoptotic rates than Pkd1+/+ cells (Fig. 2d,e). We found similar results in the Pkd1floxflox; Cre cells (Fig. 2b,c and Supplementary Fig. 1). Consistent with previous studies, we also found that this effect was in part dependent on mTORC1 (refs. 10,15). Treatment of Pkd1−/− cells with rapamycin partially restored autophagy (Fig. 2c), cell survival under glucose deprivation (Fig. 2e) and downregulated metabolic rates (Supplementary Fig. 4). Furthermore, we found mTORC1-dependent upregulation of hypoxia inducible factor 1, α (HIF1-α) in Pkd1−/− cells as compared to wild-type cells, which was probably responsible for the transcriptional changes observed (Supplementary Fig. 4)10,11.

Consistent with the high ATP content, we also found lower levels of AMPK phosphorylation in Pkd1−/− cells as compared to wild-type cells (Fig. 2f). In a previous study, we showed that the enhanced mTORC1 activity in Pkd1−/− cells is driven for the most part by ERK upregulation16. In line with this, ERK inhibitors restored the amounts of phosphorylated AMPK (Fig. 2g). Furthermore, a recent study reported that the serine/threonine protein kinase B-raf regulates AMPK activity through ERK-dependent phosphorylation of LKB1 (ref. 16). We therefore tested whether a similar mechanism might be involved here. Indeed, LKB1 was strongly phosphorylated at ERK-specific sites in Pkd1−/− cells compared to that seen in Pkd1+/+ cells, and ERK inhibitors reverted this effect (Fig. 2h). Thus, we propose a dual role for ERK in renal ciliogenesis: on the one hand, they regulate LKB1, causing inhibition of AMPK16, and on the other hand, they affect mTORC1 activity, which in turn switches on aerobic glycolysis, increases the amount of ATP and further inhibits AMPK. Treatment of Pkd1−/− cells with metformin or 5-aminomimidazole-4-carboxamide ribonucleoside (AICAR), both of which increase AMPK activity, inhibited ERK (Fig. 2i). These data suggest the existence of a negative feedback loop whereby AMPK can downregulate ERK activity toward the basal conditions (Fig. 2j).

We next asked whether the glycolytic switch observed in cells occurs in vivo as a result of inactivation of Pkd1 in the kidney.
To test this, we used Ksp-Cre; Pkd1flox/flox mice, which develop early and severe PKD1 (referred to here as cystic mice) (Fig. 3a). Compared to kidneys from noncystic control mice (Ksp-Cre; Pkd1floxc−x or Pkd1floxi/i, which we used interchangeably), we found that the kidneys from cystic mice had higher amounts of ATP (Fig. 3b), biochemical alterations similar to those observed in vitro (Fig. 3c) and transcriptional deregulation of key glycolytic enzymes (Fig. 3d), consistent with a switch to glycolysis in vivo14. To experimentally test whether enhanced glycolysis could be observed in these kidneys, we subcutaneously injected uniformly labeled 13C-glucose into Ksp-Cre; Pkd1floxi/i mice or littermate controls (Ksp-Cre; Pkd1floxc−x) and tracked the amounts of 13C-glucose or 13C-lactate using 13C-NMR spectroscopy (Fig. 3e). Forty minutes after injection, the kidneys from the cystic mice showed a significantly higher uptake of 13C-glucose, which was more efficiently converted to 13C-lactate, as compared to kidneys from control noncystic mice (Fig. 3e; n = 5). These data demonstrate that the kidneys from cystic mice are characterized by aerobic glycolysis in vivo.

We next asked whether this is a general feature of kidneys from subjects with ADPKD. We examined the gene expression profiles of both glauconeogenesis and glycolytic pathways using a previously established microarray database derived from renal cysts of patients with known PKD1 mutations (compared to minimally cystic cortical tissues from the same kidneys or to normal tissue as controls)18. We found that many enzymes that are involved in gluconeogenesis and glycolysis were differentially expressed in the renal cysts. A detailed analysis revealed that most of the genes encoding enzymes involved in gluconeogenesis were downregulated and several genes encoding enzymes involved in glycolysis were upregulated in renal cysts as compared to minimally cystic or normal tissue (Fig. 3f and Supplementary Table 2). Overall, these data suggest that higher glucose consumption and enhanced glycolysis are probably features of human ADPKD, as further supported by the presence of lactate in the cyst fluid from subjects with ADPKD19.

On the basis of the data described above, we hypothesized that interfering with glucose metabolism might present a new strategy to retard the progression of ADPKD.
cyst expansion. To test this, we treated the Ksp-Cre; Pkd1fllox−/− mice with 2DG, a glucose analog that cannot be metabolized, and compared the results with littermate Ksp-Cre; Pkd1fllox−/− mice treated with vehicle only (NaCl) and with littermate controls (Ksp-Cre; Pkd1fllox+/− or Pkd1fllox−/− interchangeably) treated with NaCl or 2DG. We found that 2DG treatment led to a lower percentage of kidney weight to total body weight (Fig. 4a–c) in the Ksp-Cre; Pkd1fllox−/− mice as compared to vehicle treatment and had no effect on the weights of other organs or total body weight (Supplementary Fig. 5). Histological evaluation of the kidneys revealed that 2DG led to a lower cystic index as compared to vehicle treatment (Fig. 4d), probably by interfering with the cellular proliferation rates of the kidneys from the cystic mice (Fig. 4e).

Analysis of key metabolic parameters (blood insulin and glucose concentrations and liver glycogen concentrations) showed that the effect of 2DG is not secondary to a general effect on metabolism (Fig. 4f). Notably, using the uniformly labeled 13C-glucose strategy described above, we demonstrated that 2DG acts by causing lower glycolysis in kidneys from Ksp-Cre; Pkd1fllox−/− mice as compared to control mice treated with 2DG, a glucose analog that cannot be metabolized, and compared the results with littermate controls (Ksp-Cre; Pkd1fllox+/− or Pkd1fllox−/− interchangeably) treated with NaCl or 2DG. We found that 2DG treatment led to a lower percentage of kidney weight to total body weight (Fig. 4a–c) in the Ksp-Cre; Pkd1fllox−/− mice as compared to vehicle treated mice (Fig. 4g; n = 3). To determine the efficacy of 2DG treatment in a second, less aggressive model of PKD, we used the previously described Pkd1flox/VV model28 and found that 2DG resulted in a lower percentage of kidney weight to total body weight and ameliorated the histology and cystic index of kidneys from Pkd1flox/VV mice as compared to the same mice treated with vehicle only (Fig. 4h–j).

Thus, we show that the use of glucose analogs, such as 2DG, may be a promising therapeutic approach to ADPKD. The kidney is an organ with high functional redundancy, and loss of a small percentage of nephrons is not typically sufficient to cause functional loss of the entire organ. As outlined above, renal failure in ADPKD is caused by the progressive renal cyst expansion affecting only a minority of nephrons1,4. Therefore, the use of a molecule that is able to selectively reduce the viability or proliferation of cells lining the cysts would probably be an effective therapy. Thus, 2DG might serve this purpose, as it is a well-tolerated molecule that is already in use in humans for some forms of cancer12,21.

Experimental treatment with rapamycin reduces renal cyst expansion in mouse ADPKD22,23. However, results in humans are more controversial for reasons possibly related to the side effects of the drug24–26. Our data highlight the possibility of combination therapy with several drugs to exploit their synergistic effects while reducing potential side effects5. For example, metformin is a well-tolerated drug used for the treatment of type 2 diabetes mellitus12 and has been shown to effectively reduce cyst expansion in a PKD mouse model27,28. Our current study provides the mechanistic explanation for this finding and further suggests that the use of metformin or AICAR (to enhance AMPK activity) in combination with 2DG might be an effective combination therapy, as has been suggested for cancer29.
In summary, our finding of a metabolic switch to glycolysis in the absence of functional polycystin-1 signaling provides the rationale for a new therapeutic approach in ADPKD. We also speculate that this might represent a paradigm in which several drugs that are expected to work synergistically can be exploited to enhance both the efficacy and the tolerance of pharmaceutical compounds used in the treatment of this disease.

**METHODS**

Methods and any associated references are available in the online version of the paper.

*Note: Supplementary information is available in the online version of the paper.*

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**AUTHOR CONTRIBUTIONS**

I.R. designed and performed the experiments, interpreted them and wrote the manuscript. M.C. designed and performed the in vivo experiments with 2DG treatment and 13-C glucose injections and interpreted the results. V.U. performed experiments in vitro on autophagy and signaling. M.P. generated the Ksp-Cre; Pkd1 mice, analyzed the kidneys biochemically and performed quantitative RT-PCR. A.B. designed the studies, supervised the work and collaborations and wrote the manuscript. V.M. prepared samples for metabolomic analysis, acquired and analyzed NMR spectra and performed statistical analysis. G.Q. acquired NMR spectra and performed statistical analysis. S.M. acquired samples for metabolomic analysis, acquired and analyzed NMR spectra and performed statistical analysis. R.M. performed biochemical analysis, interpreted the results and wrote the manuscript. M.C. designed and performed the experiments, interpreted them and wrote the manuscript.
H.X. and F.Q. designed and carried out the 2DG treatment experiment of Pkd1V/Neu mice and analyzed and interpreted the resulting data.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Antibodies, reagents and inhibitors. We used antibodies to pAkt (Ser473) #9271, Akt #9272, pAMPK (Thr172) #4326, AMPK #5823, p56R (Ser235 and Ser236) #2211, S6RP #2217, PERK (Thr202 and Tyr204) #9101, ERK #9102, p4EBP1 (Ser65) #9451, 4EBP1 #9452, pKL1 (Ser428) #3051 and p70S6K (Thr389) #2900 from Cell Signaling Technology at 1:1,000; antibodies to LKB1 (N-19) #sc-8185 from Santa Cruz at 1:1,000; to PACC (Ser 79) #07-303 and ACC #05-1098 from Millipore at 1:500 and 1:300, respectively; antibodies to LC3 NB100 #2331 from Novus Biologicals at 1:1,000; antibodies to actin #A5441 and tubulin #T5168 from Sigma Aldrich at 1:5,000; and antibodies to Ki67 #NCL-L-Ki67-MM1 from Novacstra at 1:1,000; and antibodies to pAkt (Ser473) at a final concentration of 2 mM and oligomycin (Sigma-Aldrich) at a final concentration of 2 mM.

For glucose starvation experiments, the composition of the medium was 2.3 g l−1 basic DMEM (Gibco), 3.7 g l−1 sodium bicarbonate (Gibco), 0.584 g l−1 t-glutamine, 10% serum (Euroclone) and 1% penicillin and streptomycin (Gibco) with 4.5 g l−1 (equivalent to 25 mM) high glucose (Sigma) or 1 mM glucose for starvation.

TAT-Cre treatment in vitro. For conditional inactivation of the Pkd1fl/− mice, we incubated cells twice in the presence of a recombinant TAT-Cre protein at 1 μM (Excellgen) for 2 h with 100 μM of chloroquine (Sigma-Aldrich).

NMR and metabolic profiling. For NMR analysis of the extracellular medium, we mixed 530 μl of cell culture medium with 60 μl of deuterated PBS solution containing 4.4 dimethyl-4-silapentane-1-sulfonic acid (DSS) as a chemical shift reference for both proton and carbon dimensions and 10 μl of 1.2% Na3citrate water solution. The final sample volume was 600 μl and contained 50 mM PBS, 0.02% NaN3 and 90 μM DSS. Further details on NMR sample preparation, spectra acquisition, metabolite recognition and statistical analyses are described in the Supplementary Methods.

13C-glucose experiments. We injected cystic (Ksp-Cre; Pkd1fl/−) and noncystic (Ksp-Cre; Pkd1fl/+ or Pkd1fl/fl) littermate mice at P8 intracerebrally with 1,000 mg per kg body weight of 13C-glucose (Cortecnet). After 40 min, we euthanized the mice, washed the kidneys in PBS and snap froze them in liquid nitrogen. For 2DG treatment, we injected Ksp-Cre; Pkd1fl/− littermate mice intracerebrally with 2DG at 500 mg per kg body weight or NaCl at P7 and P8, and the second injection was followed by an injection with 1,000 mg per kg body weight of 13C-glucose; we then collected the kidneys as described above. Sample preparation and detection of 13C-glucose and 13C-lactate by 13C-NMR spectroscopy are described in the Supplementary Methods.

Glucose, glycogen and insulin quantification. We determined serum glucose concentrations using the BioVision Glucose Assay Kit, liver glycogen concentrations with the BioVision Glycogen Assay Kit and serum concentrations of insulin using the Millipore Rat/Mouse insulin ELISA kit following the manufacturer’s recommendations.

DT/cystic index. To quantify the number of dilated tubules and cysts per kidney, we applied a grid of squares 13.625-μm large to sections of kidneys stained with H&E. We marked each cross with a dot and counted the number of dots inside the lumen on three litters (2.99 mm2 for each). We determined the degree of dilatation according to criteria previously established in Bastos et al.20. Briefly, one dot indicated normal tubules, two dots indicated dilated tubules and three or more dots indicated cysts.

ATP and lactate quantification. For ATP content evaluation, we prepared whole-cell extracts of control and treated MEFs by suspending pellets in lysis buffer as described26. We measured intracellular ATP quantification of lysates on 250 ng of protein by luciferase activity as shown in the standard protocol in the ATP Determination kit (Invitrogen).

We used the medium from control and treated MEFs after 24 h at 100% cell density. We determined the concentration of lactate using the EnzyChrom L-lactate Assay Kit (BioAssay Systems) and quantified the final number of cells.

Mitochondrial transmembrane potential (Ψm) assay. We assessed the mitochondrial transmembrane potential (Ψm) using TMRRM (Invitrogen) and analyzed cells by time-lapse imaging and cytofluorimetrically. For FACS analysis, 24 h after plating, we resuspended the cells in phenol-red free HBSS with 10 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES) and 20 mM tetramethylrhodamine methyl ester (TMRRM) in the presence of 2 μM of the multidrug resistance pump inhibitor cyclosporine-H and incubated them for 30 min at 37 °C. In parallel, we incubated the cells with an uncoupling agent, 4 μM FCCP, to measure the specific mitochondria staining. We measured the TMRRM fluorescence by FACS analysis. For quantitative real-time analysis of mitochondrial transmembrane potential, we incubated the cells for 30 min at 37 °C in phenol-red free HBSS ( Gibco) with 10 mM HEPES ( Gibco), 20 nM TMRRM, 2 μM cyclosporine-H and 2 μg/ml Hoechst 33342. We acquired images with an IN Cell Analyser 1000 (LKT laboratories) before and after 4 μM FCCP was injected in a motorized way, and sequential images were taken for TMRRM and Hoechst 33342 in different regions of interest every 3 min. Images were automatically analyzed with IN Cell Investigator Analysis software (GE Healthcare) to define the TMRRM intensity.

Proliferation and apoptosis assays. For proliferation assays, we carried out immunostaining using an antibody against Ki67, followed by analysis. For cells, we measured fluorescence on triplicates of 300 cells using the microscope Axiopt (Zeiss). For kidney sections, we took images at x20 using the camera Axio MRc5 (Zeiss) with the microscope Axioplan 2 (Zeiss), and we counted positive cells on six sections for each group for each experiment using the ImageJ software.

For apoptosis assays, we analyzed the cells using the DeadEnd Fluorometric transferase-mediated dUTP nick-end labeling (TUNEL) system kit (Promega) following the manufacturer’s instructions.

Generation and 2DG treatment of Ksp-Cre; Pkd1fl/+ and Pkd1fl/VV mice. We previously described the generation of the Ksp-Cre; Pkd1fl/fl mice31. Briefly, we crossed Pkd1fl/fl mice (ref. 32) and Pkd1fl/+; Ksp-Cre mice33, each in a pure C57BL/6 genetic background (backcrossed over nine times). For treatments, we subcutaneously injected 2DG (Sigma-Aldrich) or vehicle (NaCl) daily from P6 until P8 at 500 mg per kg body weight. We previously described the generation of Pkd1fl/V mice34. For treatments, we subcutaneously injected 2DG (Sigma-Aldrich) or vehicle (NaCl) daily from P5 until P7 at 500 mg per kg body weight. For all animal work, the female to male ratio was 1:1, and we randomized which mice were used in each experiment. All animal care and experimental protocols were conducted with approval of a specific protocol (IACUC-401) by the institutional care and use ethical committee at the San Raffaele Scientific Institute. Pkd1fl/V animal care and experimental protocols were conducted with approval of a specific protocol (MO10M387) by the institutional care and use ethical committee at the Johns Hopkins University School of Medicine.

Histology, immunohistochemistry and immunofluorescence. After euthanasia, we removed the kidneys, washed them in PBS, weighed them and fixed them in 4% paraformaldehyde (PFA). After incubation in a sucrose-in-PBS gradient scale from 10% to 30%, we incubated the samples in 10% glycerol (Sigma) in a mixture of optimal cutting temperature medium (OCT) (BIO-OPTICA) and 30% sucrose and then embedded them in OCT. We air-dried cryostat sections for 1 h, rehydrated them in PBS, incubated them in 1:10 Harris Hematoxylin (Sigma-Aldrich) for 2 min, washed them, incubated them in Eosin G (BIO-OPTICA) for 7 min, and then washed and dehydrated them and mounted them in DEPEX (Sigma).

For immunohistochemistry, we washed 10-μm cryosections in TWEEN-0.1% in PBS (like all washes), fixed them in 4% PFA, permeabilized them in 0.1% Triton X-100 in PBS, incubated them in 0.3% hydrogen peroxide for 30 min, blocked them for 1 h at room temperature with 5% normal goat serum (Sigma Aldrich) and 3% BSA (Sigma) in PBS and incubated them overnight at 4 °C with...
We obtained renal cysts of different sizes from five polycystic kidneys. Informed consent was obtained from all study patients, and the Institutional Review Board of the hospital where the nephrectomy was performed approved the research protocol used for this study. We obtained an MCT, which might have contained a few microscopic cysts from the renal cortex, as PKD control tissue from the same kidneys. We used noncancerous renal cortical tissue from three nephrectomized kidneys with isolated renal cell carcinoma as normal control tissue. We previously described the surgical technique, RNA extraction, purification, quality control, microarray hybridization, profiling, and quality assessments. In brief, after extraction with Absolutely RNA RT-PCR Miniprep Kit (Stratagene), we labeled 50–100 ng total RNA and hybridized it onto the GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix) according to the manufacturer’s protocol. We processed scanned raw data images with GeneChip Operating Software (GCOS) 1.4. We extracted probe set signal intensities and normalized them using the robust multiarray average algorithm, which can be found in the R package affy that can be downloaded from the Bioconductor project website (http://www.bioconductor.org/). Microarray data are available at GEO website (accession number GSE7869).

**Western blot analysis.** For western blot analysis, we lysed the cells in lysis buffer (150 mM NaCl, 20 mM NaH2PO4/NaH4PO4, 10% glycerol, 1% Triton X-100, pH 7.2, complete protease inhibitors (Roche)) and phosphatase inhibitors (1 mM final concentration of glycerophosphate, sodium orthovanadate and sodium fluoride). We quantified total lysates and added Laemmli buffer. We resolved proteins in an SDS-PAGE gel and transferred them onto polyvinylidene fluoride (PVDF) membranes. Next, we used 5% milk in Tris-buffered saline and Tween 20 (TBS-T) for blocking and secondary antibody incubations, and 3% BSA in TBS-T was used for incubations with primary antibodies. We visualized horseradish peroxidase (HRP)-conjugated secondary antibodies (anti-rabbit HRP linked (# NA934V) 1:10,000 and anti-mouse HRP linked (# N9931) 1:10,000 from GE Healthcare) using the ECL System (Amersham) after mixing with Signal West Femto Maximum sensitivity substrate from Thermo Scientific when necessary.

**Real-time PCR analysis.** We isolated total RNA from cells or whole kidneys using the RNAspin kit (GE Healthcare) and obtained complementary DNA using oligo(dT) primers (Invitrogen) and Superscript II Reverse Transcriptase (Invitrogen). We performed quantitative real-time PCR in duplicates using LightCycler480 (Roche Molecular Diagnostics) using SYBR Green I master mix. The complete sequence of primers that we used is provided in the Supplementary Methods.

**Statistical analyses.** For statistical analysis of the NMR data, we performed principal components analysis using R statistical open source software (http://www.r-project.org/) with an in-house statistical package called MUMA (free available upon request). For further details, see the Supplementary Methods.

**Additional methods.** Detailed methodology is described in the Supplementary Methods.

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