Supplementary experimental procedures:

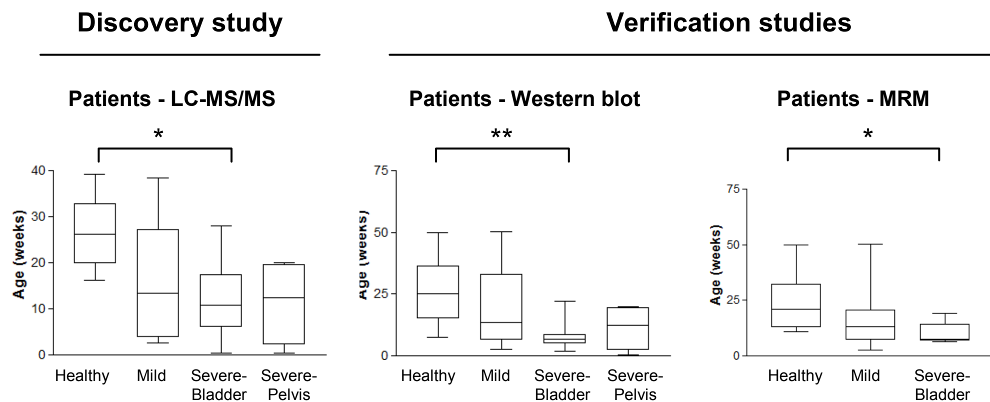
Patients classification

The “Mild” group was composed of patients with grade 1 or 2 hydronephrosis and a renal pelvic diameter between 5 and 15 mm. These patients were followed up with ultrasonography every 3 months during the first year of life, every 6 months the second year, followed by one ultrasonography once a year. In general hydronephrosis in these patients is decreasing during the first 2 years of life. However, patients presenting increased (transitory) pelvic dilatation during this period underwent a MAG3-scan. When this exam confirmed a non-obstructive washout pattern, the patients were kept in the “Mild” group. The “Severe” group was composed of patients scheduled for pyeloplasty with a pelvic dilatation of at least 20 mm and grade 3 and 4 hydronephrosis. Renographies were performed as soon as possible after birth, generally between week 3 and 6 to establish baseline differential renal function (DMSAscan) and washout pattern (MAG3-scan). Indications for surgery were: differential renal function (DRF) >10%, grade 4 hydronephrosis, obstructive washout pattern in diuretic renography with eliminated activity at 30 mn <30% (or drainage half time (T1/2) < 20 min); interval necessary for half of the tracer to be eliminated after the administration of diuretic (furosemide), sustained increase in hydronephrosis, and progressive deterioration of DRF (>5%).

Urine from patients was collected in the morning during 30 min using a sterile pediatric urine collection pouch (B. Braun, Boulogne, France) during hospital consultation. Samples from healthy controls were both collected in a hospital setting (from newborns with heart murmur) and at home using the same sterile collection bags and a pair of gloves. Care was taken to not take the first morning urine. After collection, all urines were frozen within the hour at -20°C both in the hospital (dedicated -20°C freezer in the clinic) and at home. Transport was done using ice blocks in both cases and the samples were finally stored at -80°C in the laboratory. For standardization of urine samples, protein content in each sample was determined by the Bradford method at the end of each mode of preparation (see urinary sample preparation sections in paragraphs below).

The sample collection was restricted to only urine from boys less than 1 year old. Healthy newborns were slightly more aged than the patients due to the difficulty to collect early urine from healthy newborns while affected children are monitored from birth in the hospital. The boxplots show the distribution of the age of the newborns included in the discovery and verification studies (Values are represented as medians ± SD). A Kruskal-Wallis test was performed and revealed that this difference is statistically different between the "Healthy" and

the "Severe-Bladder" group in the discovery phase (*p<0.05) as well as in the verification phase (**p<0.01 in western blot and *p<0.05 in MRM study).



Label-free discovery study

Urinary sample preparation for the label-free discovery study (adapted FASP method)

Each sample (5-15 ml of urine) was deposited onto a vivaspin 2 filter unit with a cut-off of 10 kDa (Sartorius Stedim Biotech, Goettingen, Germany) and centrifuged at 6000*g* at 4°C to reach a volume of ~50-100 µL. Two mL of 10 mM DTT, 8 M urea, 0.1 M Tris-HCl, pH 8.5 were added to the concentrated solution, mixed, and incubated 30 min at room temperature to reduce proteins, followed by centrifugation at *6000 g* at room temperature. Subsequently, 1 mL of 0.05 M of iodoacetamide, 8 M urea, 0.1 M Tris-HCl, pH 8.5 was added to the filters and samples were incubated in the dark for 30 min at room temperature, and then centrifuged at 6000*g* at room temperature. The resulting concentrated solution was diluted with 1 mL of 8 M Urea, 0.1 M Tris-HCl, pH 8 and concentrated again. This step was repeated 2 times and the protein content of the final concentrated solution was determined using the Bradford method (Biorad Protein Assay, Bio-Rad, Marnes-la-Coquette, France), using bovine serum albumin as standard. All samples were adjusted to a final volume of 30 µL at a final concentration of 1 µg/µL with 8 M Urea, 0.1 M Tris-HCl, pH 8. Samples were transferred onto a vivaspin 500 filter unit with a cut-off of 10 kDa (Sartorius Stedim Biotech, Aubagne, France), diluted to 150 µL by addition of 50 mM NH4HCO3, and subjected to proteolytic digestion with trypsin (Promega, Madison, WI, USA) at an enzyme to protein ratio of 1:100. After overnight digestion at room temperature, peptides were collected by centrifugation of the filter unit at 12000 g for 45 min at 4°C. The filter device was rinsed with 20 µl of 0.5 M NaCl and centrifuged again at 12000 g for 45 min at 4°C. The combined filtrates were acidified with trifluoroacetic acid 0.1% prior to nanoLC-MS/MS analysis.

Analysis by nanoLC-MS/MS

Twenty µL of each sample was loaded onto a C18 precolumn (300 µm ID x 5 mm, Dionex) at 20 µL/min in 2% acetonitrile, 0.05% trifluoroacetic acid. After 10 min of desalting, the precolumn was switched online with the analytical C-18 column (75 µm ID x 15 cm), packed in-house with Reprosil C18-AQ Pur 3 µm resin (Proxeon Biosystems, Odense, Denmark) and equilibrated in solvent A (5% acetonitrile, 0.2% formic acid). Peptides were eluted using a 0 to 50 % gradient of solvent B (80% acetonitrile, 0.2% formic acid) during 90 min at a 300 nL/min flow rate. The mass spectrometer was operated in data dependent acquisition mode with the XCalibur software. Survey full scan MS spectra (m/z mass range 300-2000) were acquired in the Orbitrap with the resolution set to a value of 60 000 at m/z 400 (target value of 1e6 charges in the linear ion trap). Then the twenty most intense ions per survey scan were targeted for MS/MS fragmentation using collision-induced dissociation (CID) and the resulting fragments were analyzed in the linear ion trap (LTQ, parallel mode, target value 1e4). Collision energy was set to 35% for MS/MS. The lock mass option was used for internal recalibration in real time. Dynamic exclusion was employed within 60 seconds to prevent repetitive selection of the same peptide.

Database search and protein verification

In order to automatically extract peak lists from Xcalibur raw files, the Extract_msn.exe macro provided with Xcalibur (version 2.1, Thermo Fisher Scientific, Waltham, MA USA) was used through the Mascot Daemon interface. The following parameters were set for creation of the peak lists: parent ions in the mass range 400-4500, no grouping of MS/MS scans, and threshold at 1000. Data were searched against the international protein index (IPI) human database (20100207, 87061 sequences). Carbamidomethyl cysteine was selected as fixed modification, and oxidized methionine and proline, protein N-acetylation and deamidation of asparagines and glutamine were searched as variable modifications. Specificity of trypsin digestion was set for cleavage after K or R except before P, and two missed trypsin cleavage sites were allowed. The mass tolerances were set to 5 ppm and 0.5 Da for MS and MS/MS respectively, and the instrument setting was specified as “ESI-Trap”. Mascot results were parsed with the in-house developed software MFPaQ version 4.0 (Mascot File Parsing and Quantification) (http://mfpaq.sourceforge.net/) and protein hits were automatically validated if they satisfied one of the following criteria: identification with at least one top ranking peptide of minimal length of 6 amino acids and with a Mascot score higher than the identity threshold at p=0.001 (99.9% probability), or at least two top ranking peptides each of minimal length of 6 amino acids and with a Mascot score higher than the identity threshold at p=0.05 (95% probability). In order to calculate the False Discovery Rate (FDR), the search was performed using the “decoy” option in Mascot, and MFPaQ used the same criteria to validate decoy and

target hits. The FDR was calculated at the protein level (FDR = number of validated decoy hits / (number of validated target hits + number of validated decoy hits) x 100) using the validation criteria described above. Clustering of proteins was performed based on peptide sharing, by grouping together all protein sequences matching the same set of peptides (only top ranking peptides with a Mascot score higher than the identity threshold at p=0.05 were considered).

Quantification from raw MS data

For each run, the label-free quantitative module of the software MFPaQ version 4.0 uses the validated identification results and extracts ion chromatograms (XIC) of the identified peptide ions in the corresponding raw nanoLC-MS files based on their experimentally measured retention time (RT) and monoisotopic m/z values. The time value used for this process is retrieved from Mascot result files, based on an MS2 event matching to the peptide ion. If several MS2 events were matched to a given peptide ion, the software checks the intensity of each corresponding precursor peak in the previous MS survey scan. The time of the MS scan which exhibits the highest precursor ion intensity is attributed to the peptide ion and used for XIC extraction, and the time corresponding to the apex of the extracted XIC is then used for the alignment process. Peptide ions identified in all samples to be compared were used to build a retention time matrix in order to align LC-MS runs. If peptide ions were sequenced by MS/MS and validated only in some of the samples to be compared, their XIC signal was extracted in the nanoLC-MS raw file of the other samples using a predicted RT value calculated from this alignment matrix by a linear interpolation method.

Protein relative quantification was performed by pairwise comparisons of different groups, containing each 5 samples analyzed in triplicate. For each peptide ion, an intuitive method was developped for missing intensity values imputation. If the XIC of the peptide ion was missing in only one of the three replicates, a linear regression was performed to fill this missing value, based on the two available values. In the case of two missing values, they were filled only if the third available intensity value was low enough, based on the assumption that the XIC could not be extracted because the peptide ion was of low intensity. To this aim, box plots were performed on the whole population of peptide ions for each replicate, and a threshold value was defined as the lowest whisker in each analysis. If the unique available value for the peptide ion was below the threshold value, the missing values were replaced by the threshold value of the related replicate.

Targeted Mass Spectrometry analysis-Multiple Reaction Monitoring (MRM)

Urinary sample preparation for MRM analysis

A new cohort of patients (31 samples) including "Healthy" (n=10), and "Mild" (n=13), and "Severe-Bladder" (n=8) UPJ obstruction samples for verification of the markers identified during the discovery phase of the study was used. Due to the lower amount of urine generally available in this second cohort, samples were processed using a different protocol than in the discovery phase, including concentration by 1D-SDS PAGE and in-gel digestion of proteins. Thus, urine samples (3-30 ml) were desalted on PD10 columns (GE Healthcare Europe GmbH; Freiburg, Germany) and concentrated by lyophilisation. The amount of urinary protein collected was determined with the Bradford method (Biorad Protein Assay, Biorad). Ten µg of protein urinary sample spiked with 5 ng of two exogenous yeast proteins (alcohol dehydrogenase and enolase, Sigma–Aldrich) used as internal standard, were reduced in Laemmli buffer (final composition 25 mM DTT, 2% SDS, 10% glycerol, 40 mM Tris pH 6.8), 5 min at 95°C. Proteins were alkylated with 90 mM iodo-acetamide for 30 min at room temperature in the dark. Proteins were concentrated on a home-made 12% acrylamide SDS-PAGE gel (the migration was stopped as soon as the protein sample entered the separation gel). Proteins were visualized by colloidal Coomassie Blue staining and a single band containing the whole sample was cut out of the gel. Proteins were in-gel digested by 1 µg of modified sequencing grade trypsin (Promega) in 50 mM Ammonium bicarbonate over night at 37°C. The resulting peptides were extracted from the gel by incubation in 50 mM ammonium bicarbonate for 15 min at 37°C followed by two incubations in 10% formic acid, acetonitrile (1:1) for 15 min at 37°C. These three extractions were pooled with the initial digestion supernatant and dried in a Speed-Vac before analysis by nanoLC-MRM.

Setup of MRM assays

MRM assays were developed and optimized for seven selected urinary candidates proteins as well as for the 2 spiked exogenous yeast proteins, using synthetic peptides containing on their C-terminus an ^{15}N and ^{13}C-labeled arginine or lysine residue (non-purified PEPotec MRM peptides or purified AQUA Ultimate peptides, Thermo Scientific). The selection of the signature peptides of each target protein was based on the initial nanoLC-MS/MS discovery experiment on the LTQ-Orbitrap Velos (for the human proteins) and the already existing proteomics information reported in proteomic data repositories PeptideAtlas (1) or GPM Proteomics Database (2). A maximum of 4 proteotypic peptides from human and yeast proteins were selected. The synthetic peptides were diluted in 2% acetonitrile, 0.05% trifluoroacetic acid and mixed (~250-500 fmol crude peptide/µL or 200 fmol purified peptide/µL). The peptide mixture was analyzed on a hybrid triple quadrupole-ion trap mass spectrometer 5500 Qtrap (AB Sciex) equipped with a nanoelectrospray ion source coupled to an Ultimate3000 system (Dionex, Amsterdam, The Netherlands) for chromatographic peptide separation using a 22 min gradient from 0 to 50 % of solvent B (80% acetonitrile, 0.2% formic

acid) at a flow rate of 300 nL/min. Spray voltage was set at 2800V, curtain gas at 30 psi, nebuliser gas at 10 psi, interface heater temperature at 150°C, and pause time at 3 ms. Peptides were loaded onto a C18 precolumn (300 µm ID x 5 mm, Dionex) at 20 µL/min in 2% acetonitrile, 0.05% trifluoroacetic acid. After 7 min of desalting, the precolumn was switched online with the analytical C-18 column (75 µm ID x 15 cm) packed in-house with Reprosil C18-AQ Pur 3 µm resin, (Proxeon Biosystems, Odense, Denmark) and equilibrated in solvent A (5% acetonitrile, 0.2% formic acid). Collision energies (CEs) were determined according to the following equations: CE = 0.036 x (*m/z*) + 8.857 and CE = 0.0544 x (*m/z*) - 2.4099 (*m/z*, mass to charge ratio of the precursor ion) for double- and triple-charged precursor ions, respectively. The mass spectrometer was first operated in IDA mode with the Analyst software (version 1.5.1, AB Sciex, Foster City, CA, USA). MS/MS spectra were used to extract optimal fragment ions (with the most intense signal in MS) for MRM analyses. The best 2 to 4 peptides and 3 transitions were selected and their CE were optimized with four additional steps (+/-3V, +/-6V) around the predicted CE in MRM mode with Q1 and Q3 set at unit resolution (fwhm 0.7 Da). The final optimized assay consisted of 25 peptides corresponding to the 7 urinary candidate proteins plus the 2 yeast proteins and a total of 150 transitions (Sup. Data 1 and Sup. Table 1). To adjust the amounts of internal standards to the endogenous levels of each candidate biomarkers, the mixture of peptides was then spiked in increasing amount in a test urinary sample. From these results, an adjusted mixture of heavy peptides was prepared, and 14µL of this mixture was subsequently used to resuspend digested clinical samples (10µg total material loaded on gel and in-gel digested). Five µL of this solution was then injected on the system for nanoLC-MRM analysis (corresponding to 3.57µg theoretical total material on column). Regarding proteins monitored with AQUA peptides, the corresponding amount on column of heavy standard peptide was 10fmol for ARG1, LRRC15, VERSICAN and HSPA5 peptides, 20fmol for EGF and CDH13 peptides, and 250fmol for PTGDS peptides.

Assessment of the repeatability of the experimental workflow used for the MRM verification study.

In a first test, triplicate identical aliquots obtained from a unique healthy urinary sample from the cohort of the verification study were processed in parallel (10 µg total protein concentrated on SDS-PAGE, in-gel digestion and peptide extraction) and submitted to LC-MRM analysis using the method described above, monitoring the 150 transitions corresponding to the peptides from 7 candidate biomarkers proteins (63 transitions) plus the 2 spiked yeast standard proteins (12 transitions), and associated spiked heavy peptides. Data were analyzed with the Skyline software (http://proteome.gs.washington.edu/software/skyline) and manually inspected to ensure

correct peak detection and integration. The AuDIT (Automatic Detection of Inaccurate and Imprecise transitions) module was used to assess the quality of the measurements for each transition (3, 4). This algorithm allows to automatically calculate the ratio of the peak areas for any 2 transitions of the same precursor, and to compare it with that obtained for the internal heavy standard. Based on the p-value measuring the similarity between the light peptide and heavy standard for relative ratios, all measured transitions were classified as "good" (p-value>10-5). Based on the CVs calculated across the triplicate measurements, 10 out of the 75 transitions were classified as "bad" (CV>20%). Sup. Data 2 and Sup. Table 2 show the boxplots for these CVs, calculated for all measured transitions (75 transitions of the candidate biomarker proteins and spiked yeast standard proteins), or only for the 65 transitions qualified as « good » after AuDIT analysis. CVs across the triplicate experiments are shown for the light target peptides (median value 15%), the heavy internal standards (median value 15%), and for the peak area ratio calculated for light/heavy pairs (median value 7%).
In order to assess the repeatability of the experimental protocol on a larger series of urinary samples and LC-MRM measurements, an additional test was performed in which 10 aliquots from a unique pooled urinary sample from healthy donors were processed in parallel as described above and submitted to LC-MRM analysis (triplicate injections). The best responding endogenous transitions corresponding to the 7 candidate biomarkers protein were monitored (10 transitions). Sup. Data 3 shows the plot of raw peak area values for these transitions (mean raw peak area across triplicate injections, error bars showing standard deviation across triplicate injections). CVs across the 10 replicate experiments are in the range 6-24%, median value 12% for the 10 monitored transitions.

Calibration curves, LOD/LLOQ calculation, and estimation of endogenous protein amounts
The isotopically labeled AQUA peptides TIGIIGAPFSK (from ARG1), NQVTPLDILSK and YPANVAVDPVER (from EGF), AQGFTEDTIVFLPQTDK (from PTGDS), LLASDAGLYR (from VER), NWLLLNQPR (from LRRC15), TWNDPSVQQDIK (from HSPA5), VNSDGGLVALR and YEVSSPYFK (from CDH13) were spiked in increasing amounts in a pooled urine sample from healthy donors. Samples spiked with different amounts of the AQUA peptides were processed in parallel according to the analytical protocol used for the verification study (SDS-PAGE and in-gel digestion), and 3.57µg theoretical total material was injected for nanoLC-MRM analysis (resulting in the range: 0,05; 0,1; 0,5; 1; 10; 20; 50; 100; 250; 500 fmol of peptide on column). Three transitions were monitored for each peptide (listed in Sup. Data 1). Results were processed with the QuaSAR software (4) to generate response curves (raw peak area of the heavy peptide against spiked amount in fmol) for

each transition. The LOD was estimated by the software as a raw peak area value, and was then converted to an amount of AQUA peptide on column using the equation of the linear regression line for each transition (Sup. Table 3). Skyline views of the overlaid MRM chromatograms of the 3 monitored transitions are shown in Sup. Data 4 and 5 for the YPANVAVDPVER (from EGF) and TIGIIGAPFSK (from ARG1) respectively, together with the response curves generated by QuaSAR for these peptides.

Quality control and assessment of the experimental repeatability during the LC-MRM analysis of the clinical samples

Assessment of the performances of the LC-MRM instrumental platform during the verification study was performed through repeated injection of a quality control external standard, consisting in a commercial digest of beta-galactosidase (AB Sciex). Ten fmol of this sample were injected regularly every 5 clinical samples in the time course of the analytical sequence. Eighteen transitions corresponding to 6 peptides of the digest were monitored. CVs obtained for raw peak area values across the 8 quality control runs acquired during the study are shown in Sup. Data 6 (range 15-28% for the 18 transitions, median value 21%), as well as the CVs for full width at half maximum, and retention time drift, calculated from Skyline exported values.

In addition, the repeatability of the MRM analysis in the verification study was also assessed through the monitoring of the two exogenous yeast proteins (ADH_1 and enolase1) spiked at constant concentration into all the clinical samples to be measured. These proteins were monitored using several transitions for targeted peptides (listed in Sup. Data 1), and the measured signals were normalized using the isotopic heavy forms of the yeast peptides (PEPotec peptides, Thermo Scientific) just as the candidate biomarker proteins, to correct for MS signal variations from run to run. The results obtained on the spiked-in proteins during the verification phase, before and after normalization with the heavy standards are shown in Sup. Data 7. Across the 31 urine samples measured by MRM in the verification cohort, the CVs measured for corrected peak areas of the transitions of these yeast proteins were typically 20% (median value 23%, range 18- 25%).

Relative quantification of the candidate proteins across clinical samples using MRM data

MRM data were used for relative quantification of protein candidates in the verification cohort, to confirm differential excretion of some of them. Isotopic labeled peptides (AQUA or partially purified PEPotec peptides) were used 1/ to ensure proper identification of the transitions (manually checked in Skyline for all the samples) and 2/ to correct the variability of the LC-MRM analysis and ensure proper relative quantification of the endogenous light peptides. All transitions described in Sup. Data 1 were monitored. Data were processed with

QuaSAR for quantitative analysis. To assess the quality of the transitions detected in the clinical study, the AuDIT module was used. All transitions tagged as "bad" based on the calculated p-value (measuring the similarity of transitions ratios between light and heavy peptides in each clinical group) were not considered for further quantitative analysis. For the 9 peptides monitored together with an AQUA heavy standard, we used for final quantification the transition showing the best LOD in the calibration curve experiments described above. For the remaining peptides monitored together with a PEPotec partially purified standard, we selected the transition giving the best CV in the preliminary triplicate test experiment shown in Sup. Data 2. Endogenous protein amounts were estimated by QuaSAR from the calculation of peak area ratio (PAR) values between light and heavy peptides, and from the known spiked amounts of the heavy internal standard peptides (approximate amounts were used in the case of partially purified PEPotec peptides). Relative quantification and statistical analysis between sample groups was performed using the endogenous amounts calculated by the software. For each sample group comparison (Mild/Healthy, Severe-Bladder/Healthy and Severe-Bladder/Mild), statistical analysis was performed by unpaired *t* test assuming unequal variances (ttest pvalue). Pvalues were corrected for multiple testing using the Benjamini-Hochberg procedure (22 pvalues corresponding to the 22 quantified peptides per comparison) (*<0,05; **<0,01; ***<0,001). Adjusted pvalues (pvalues BH) less than 0.05 were considered statistically significant (Sup. Data 8).

Unilateral Ureteral Obstruction (UUO)

Briefly, under oxygen-isoflurane anesthesia and through a longitudinal, left abdominal incision, the ureter was exposed and ligated with a 6/0 nylon thread at the uretero-pelvic junction. Mice were maintained on standard mouse chow and tap water. Mice were subjected to UUO during 3 or 8 days. At the end of the protocol, control and 3- or 8-days obstructed mice (n=8/group) were sacrificed. The kidneys were removed and divided in different parts according to the different protocols employed. For mRNA and protein extraction, kidney sections were snap frozen in liquid nitrogen and stored at -80°C until extraction. For histology, kidney sections were fixed in Carnoy's solution for 24 h, dehydrated by respectively 5 and 3 successive baths with absolute ethanol and acetone and embedded in paraffin.

Human and mouse protein sample preparation for Western blot analyses

Urine samples were desalted on PD10 columns (GE Healthcare Europe GmbH, Freiburg, Germany), concentrated by lyophilisation and stored at −80°C. Renal mouse tissues were homogenized in ice-cold RIPA cell lysis buffer (10 mM Tris·HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1% SDS, 1% Np40) containing a cocktail of protease inhibitors

(Complete mini, Roche Diagnostics, Meylan, France) using an homogenizer Ball Mill MM301 (Retsch, Haan, Germany) at the frequency of 30 shakes/sec during 3 min. Homogenized tissues were then centrifuged at 13,000 *g* during 15 min at 4°C.The resulting supernatants were stored at −80°C and used as tissue extracts for experiments. Protein concentrations were determined by Bradford assay (Bio-Rad).

Western Blot analyses

For Western blot analysis, equal amounts of urinary proteins (10 µg) or tissue extracts (20 µg) were separated on 10% SDS-PAGE gels and transferred on nitrocellulose. Membranes were stained with Ponceau Red (Sigma-Aldrich, Lyon, France) and blocked 30 min in 40 mM Tris-HCl, pH 8, 150 mM NaCl, 0.05% Tween 20, 5% powder skimmed milk, prior to incubation with the primary antibodies: rabbit polyclonal anti-Arginase1 (Sigma-Aldrich, 0,5µg/ml), rabbit polyclonal anti–arginase2 (Santa Cruz Biotechnology, 1:500) and goat monoclonal anti-Gapdh (Santa Cruz Biotechnology, 1:250). The antigen-antibody complex was detected with peroxydase-conjugated anti-rabbit or anti-goat antibodies. Immunoreactivities were revealed with the ECL™ Western blotting kit (GE Healthcare) by autoradiography. The reactivity of the anti-ARG1 antibody was first tested on a liver extract as a positive control. Densitometric analysis of autoradiographies was performed using the Total Lab v 11.5 analysis Software (FSVT ScienceTec, Courbevois, France). Values of anti-ARG1 immunoreactivities on human urine samples (10 µg) were also normalized by the quantity of albumin present in each sample that was quantified based on the Ponceau coloration. Densitometric values from murine ARG1 and ARG2 antibodies on kidney extracts were normalized by the anti-GAPDH signal intensity. Relative intensities from different gels were also normalized using the signal obtained from a control sample loaded on all gels.

Measurement of arginase activity

Briefly, 25 µl of activation buffer containing manganese chloride (40mM Tris-HCl, 10 mM $MnCl_2$) was added to 50 µl of tissue homogenate (160 µg/ml) followed by heating of the samples at 56°C for 10 min to activate the arginases. Twenty-five µl of 1 M L-arginine (pH 9.7) was then added to each tube followed by incubation of the samples at 37°C for 1 h to hydrolyze the L-arginine. The reaction was stopped by adding 400 µl of an acid mixture containing sulfuric acid, phosphoric acid and water in a ratio of 1:3:7. The urea concentration was then measured by a colorimetric assay as follows. Fifty microliters of 9% α-isonitrosopropiophenone (ISPF) dissolved in ethanol was added to the samples followed by heating of the tubes at 100°C for 30 min. The color was developed by keeping the tubes in the dark at room temperature for 10 min. Aliquots of 200 µl were transferred to a 96-well plate, and absorbance at 540 nm was measured in a microplate reader. Values were

corrected for basal urea levels obtained in the absence of L-arginine. Each assay was run in duplicate. The activity was completely inhibited when renal extract was previously boiled confirming that the produced urea was the result of the arginase activity. Values of arginase activity of each sample were expressed in UE (µmol urea/h/mg protein) related to a control value of 100%.

Quantification of Gene Expression by Real-Time Quantitative PCR

Total RNA from mouse kidney samples was isolated using the QIAGEN RNeasy Mini kit (Qiagen, Courtaboeuf, France) and eluted in 20 µl of RNase-free water and treated by HL-dsDNase (ArticZymes, Tromsø, Norway) according to the manufacturer's protocol. The sample RNA concentration was measured on a NanoDrop instrument (ND-1000 spectrophometer) and RNA purity was determined by the A260/A280 and A260/A230 ratios. For RT-PCR, 500 ng of total RNA were used for cDNA synthesis by Superscript II Reverse Transcriptase (Invitrogen, Life Technologies SAS, St Aubin, France) in a volume of 20 µl following the manufacturer's protocol. PCR amplification was performed in a total volume of 20 µl containing 5 µl (12.5 ng) of diluted cDNA sample, 1 µl of each of forward and reverse primer (final concentration of 500 nM) and 10 µl of SsoFast EvaGreen Supermix (Bio-Rad) using an ABI StepOnePlus apparatus. Acquisition and data analyses were performed using the StepOnePlus v2.0 software.

The linear efficacy of each pair of primers was previously validated by qPCR on a dilution range of mixed cDNA samples. Primers used are listed in Sup. Table 5. Gene of hypoxanthine-guanine phosphoribosyltransferase *(Hprt*), an ubiquitously and equally expressed gene that is free of pseudogenes, was defined as the best reference gene in our experiments compared to other housekeeping genes like glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), Glucuronidase b (*Gusb*) and RNA Polymerase II (*RpII*) and was used for normalization. The integrity of mRNA samples was also determined by RT-qPCR with the “3’:5’ assay” described by Nolan et al., (5) using Hprt mRNA as the targeted sequence. Briefly, the integrity of the Hprt mRNA was taken as representative of the integrity of all mRNA in the samples. So, levels of three targeted Hprt amplicons spatially separated in the sequence (one towards the 5 ‘end, the second towards the center and the third towards the 3 ‘end) was determined by RT-qPCR. In all samples, the 3’:5’ amplicons ratio was comprised between 0.7 and 1 reflecting the success of the Reverse Transcriptase to proceed along the entire length of the transcript during cDNA synthesis and indicating absence of mRNA degradation.

Gene expression of targeted genes Cationic amino acid transporter (*Cat1, -2*), Arginine succinate synthase (*Ass*), Arginine succinate lyase (*Asl*), Ornithine decarboxylase (*Odc*), Ornithine aminotransferase (*Oat*), P5C synthase (*P5cs*), P5C reductase (*P5cr*), P5C

dehydrogenase (*P5cd*) and Proline dehydrogenase (*Prodh*) was measured in kidney from control and obstructed mice. The relative transcript abundance was expressed in cycle threshold (ΔCt) values corresponding to $\Delta Ct = Ct^{target} - Ct^{Hprt}$. Relative changes in transcript levels compared with controls were expressed as ΔΔCt values ($\Delta\Delta Ct = \Delta Ct^{treated} - \Delta Ct^{control}$). All ΔΔCt values were considered to correspond to the binary logarithm of the fold change. Primers used are listed in Sup. Table 5.

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