Supplementary methods:

Adult CKD Patients from the NephroTest study

In all patients, GFR was measured (mGFR) using ^{51}Cr-EDTA renal clearance [1]. Plasma FGF23 levels were measured for 397 patients. For these 397 patients, we analyzed biological and clinical data recorded on the day blood samples were drawn. We measured PTH concentration using a second-generation two-site immunoradiometric assay (Allegro-Intact PTH and then the Allegro-calibrated Intact PTH Advantage assay; Nichols Institute Diagnostics, San Clemente, CA) [2]. We measured plasma phosphate concentration by colorimetry (phosphomolybdate assay) and microalbuminuria by immuno-turbimetry (AU680 Beckman Coulter).

Patients with acute nephrotic syndrome

Children were first examined by a physician. Blood and urine collections were fist performed during the acute nephrotic phase and then after remission. Mean age of patients was 12 years +/- 4.8 years. The patients were receiving the same treatment during the acute proteinuric and the remission phases in order to avoid potential interferences between immunosuppressive drugs and renal phosphate handling. Patients with low eGFR (calculated according to Schwartz's formula) were excluded from the study. Eight consecutive children were included and their respective therapies is displayed in the table below. TmG/GFR was calculated as previously described[3, 4].

Treatment	Prednisone	Mycophenolate mofetil	Cyclosporin
Patient 1 NP	0.1 mg/kg/d	2*250mg/d	
Patient 1 R	0.1 mg/kg/d	2*250 mg/d	
Patient 2 NP	0.1 mg/kg/d	2*250mg/d	
Patient 2 R	0.1 mg/kg/d	2*250 mg/d	
Patient 3 NP	2mg/kg/d		3mg/kg/d
Patient 3 R	2mg/kg/d		3mg/kg/d
Patient 4 NP	2mg/kg/d		
Patient 4 R	2mg/kg/d		
Patient 5 NP	2mg/kg/d		

Patient 5 R	2mg/kg/d
Patient 6 NP	0
Patient 6 R	0
Patient 7 NP	0.2 mg/kg/d
Patient 7 R	0.2 mg/kg/d
Patient 8 NP	2mg/kg/d
Patient 8 R	2mg/kg/d

Rat kidney immunohistochemistry and immunofluorescence

Rat kidneys were fixed by perfusion with 4% paraformaldehyde. Kidneys were removed, dehydrated and paraffin-embedded. Kidney sections of 5 µm in thickness were used. Immunohistochemistry was performed using the citrate buffer (0.1 mol/l, pH6) microwave-based antigen target retrieval technique and the EnVision Flex kit (Dako). For immunohistochemistry, slides were counterstained with Haematoxylin for the nuclei staining. Immunofluorescence analysis on rat kidney sections was performed as previously described [5]. Confocal imaging was performed on a Leica TCS SP5 MP confocal microscope.

RNA extraction and Real-Time PCR

Total RNA was extracted from tissues using Nucleospin RNA II Kit (Macherey-Nagel) according to the manufacturer's instructions and total RNA was extracted using RNeasy micro kit (QIAGEN) according to the manufacturer's instructions. Five hundred ng of RNA was used to synthesize cDNA using the qScript cDNA SuperMix (Quanta Biosciences). Real-time PCR analysis was performed as previously described [6]. Primers used are described in Table 2. Mouse or rat acidic ribosomal phosphoprotein P0 were used as internal standards.

Western blotting

Kidney tissue proteins were extracted as previously described [7]. Protein concentration was determined by the Bradford assay (Biorad). Loading of the gels was checked by Coomassie

blue staining. Proteins were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore) as previously described [7].

Phosphate uptake

Opossum kidney (OK) cells were cultured to confluence in DMEM/Ham's F12 (vol/vol) supplemented with 7% defined fetal bovine serum (Hyclone, Perbio Science, USA), 5 nM sodium selenite, 0.03 nM insulin, under a 5% CO2-containing atmosphere. Twenty four hours before the experiment, cells were starved in serum free medium. Albumin 10 gr/l was added or not 12 hours before the experiment. For phosphate uptake experiments, cells were incubated for 2 hours in the presence of 10 Nm PTH or diluent (H2O). Radiolabeled phosphate uptake was measured by incubating cells for 10 min in transport medium (mM: NaCl 137, KCl 5.4, MgSO4 1.2, CaCl2 2.8, KH2PO4 0.1 and Tris-Hcl 14, pH 7.4) containing 0,5 uCi/ml 32P. Cells were then washed 3 times with 137 mM NaCl cold medium (pH 7,4) and solubilized with NaOH. The radioactivity of the lysate was measured by liquid scintillation counting. Each assay was performed in triplicate and total protein content was measured by the Bradford assay (Biorad). Results were calculated using the following formula: (cpm sample - cpm blank) / (specific activity of ^{32}Pi x time in min). Values were then divided by the protein concentration in μg/ml. To obtain relative values, we then divided each normalized value by the mean value obtained in control samples.

Antibodies

Anti-albumin FITC-conjugated (F0117) was purchased from DAKO, anti-Klotho was purchased from Transgenic Inc (KM 2076), Rabbit polyclonal anti-NaPi-IIa and NaPi-IIc antibodies were a kind gift of J. Biber (University of Zurich, Switzerland), rabbit polyclonal anti-NCC antibodies were a kind gift of J. Loffing (University of Zurich, Switzerland). NHE3

monoclonal antibody was purchased from Millipore, ant-FRS2 from R and D and p-FRS2 were purchased from Cell Signaling. NHERF-1 antobody was from Santa Cruz.

PTH in rats was measured using rat bioactive intact PTH kit from Immunotopics and FGF23 using Kainos human intact FGF23 ELISA kit.

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3. Bijvoet, OL, Morgan, DB, Fourman, P: The assessment of phosphate reabsorption. *Clin Chim Acta,* 26**:** 15-24, 1969.
4. Walton, RJ, Bijvoet, OL: Nomogram for derivation of renal threshold phosphate concentration. *Lancet,* 2**:** 309-310, 1975.
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6. Hasler, U, Vinciguerra, M, Vandewalle, A, Martin, PY, Feraille, E: Dual effects of hypertonicity on aquaporin-2 expression in cultured renal collecting duct principal cells. *J Am Soc Nephrol,* 16**:** 1571-1582, 2005.
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Supplementary Table 1: Characteristics of patients with or without FGF23 dosage

	No FGF 23 N=1341	FGF 23 N=397	p-value
Age, years	59.3 ± 15.1	60.6 ± 14.8	0.2
Male sex	65.6(880/1341)	72.0(286/397)	0.02
BMI, kg/m²	26.7 ± 5.2	26.6 ± 5.4	0.6
Sub saharian origin	14.4(184/1280)	11.1(44/397)	0.09
SBP, mm Hg	137 ± 20	135 ± 20	0.1
DBP, mm Hg	75 ± 12	74 ± 12	0.2
Diabetes	27.7(370/1335)	24.2(96/397)	0.2
Cardiovascular disease	18.7(246/1313)	19.3(76/393)	0.8
Nephropathy			
Diabetic	10.7(143/1341)	8.8(35/397)	<.0001
Glomerular disease	13.3(178/1341)	16.6(66/397)	
Vascular disease	26.5(355/1341)	33(131/397)	
PKD	6.5(87/1341)	4.3(17/397)	
Interitial disease	6.5(87/1341)	15.1(60/397)	
Others	36.6(491/1341)	22.2(88/397)	
mGFR, ml/min/1.73m²	39.2 [26.6-55.1]	34.4 [23.3-46.5]	<.0001
ACR mg/mmol	9.7 [1.7-48.8]	10.4 [1.7-62.4]	0.4
Albuminuria mg/mmol			
<3	32.89(441/1341)	33.5(133/397)	0.3
3-30	34(456/1341)	29.97(119/397)	
≥ 30	33.11(444/1341)	36.52(145/397)	
Albuminemia, g/L	39.3 ± 4.6	38.1 ± 3.8	<.0001
Ca2+, mmol/L	1.21 ± 0.06	1.23 ± 0.07	<.0001
25(OH)D, ng/ml	18 [11-27]	21 [14-28]	<.0001
1,25(OH)2D3, pg/ml	27 [18-38]	28 [19-38]	0.3
PTH intacte, pg/ml	60.0 [38.0-104.0]	66.0 [41.0-94.0]	0.2
Hemoglobin, g/dL	12.6 ± 1.6	12.8 ± 1.7	0.05
Ferritin, µg/L	115.0 [61.0-209.0]	121.0 [68.0-199.0]	0.7
Phosphates, mmol/L	1.09 ± 0.22	1.09 ± 0.23	0.4
Treatments			
Phrosphocalcic regulato	27.9(353/1264)	39.1(155/396)	<.0001
Vitamin D (native)	13.7(173/1264)	18.4(73/396)	0.02
Calcitriol	7.3(92/1264)	17.4(69/396)	<.0001
Phophate binder	1.4(18/1264)	4.0(16/396)	0.001

mean ± sd or median [IQR] or % (n/N)

Supplementary Table 2. Multivariate analysis of phosphatemia according to albuminuria to creatinine ratio (ACR) in 3 classes.

	ACR in mg/mmol	DFGm < 45 ml/min/1.73m²		DFGm ≥ 45 ml/min/1.73m²	
All patients		**n=1092**		**n=646**	
Model 0	< 3	0 (ref)		0 (ref)	
	3 – 30	0.0525±0.0189	p=0.005	-0.0101±0.0157	p=0.5
	≥ 30	0.1479±0.0180	p<0.0001	0.0387±0.0190	p=0.04
Model 1	< 3	0 (ref)		0 (ref)	
	3 – 30	0.0006±0.0164	p=1.0	-0.0052±0.0136	p=0.7
	≥ 30	0.0485±0.0173	p=0.005	0.0273±0.0173	p=0.1
Patients with FGF-23 measurements		**n=285**		**n=112**	
Model 0	< 3	0 (ref)		0 (ref)	
	3 – 30	0.0248±0.0363	p=0.5	0.0114±0.0371	p=0.8
	≥ 30	0.1453±0.0336	p<0.0001	-0.0477±0.0416	p=0.3
Model 1	< 3	0 (ref)		0 (ref)	
	3 – 30	-0.0115±0.0321	p=0.7	0.0131±0.0317	p=0.7
	≥ 30	0.0640±0.0326	p=0.05	-0.0456±0.0373	p=0.2
Model 2	< 3	0 (ref)		0 (ref)	
	3 – 30	-0.0070±0.0280	p=0.8	0.0176±0.0313	p=0.6
	≥ 30	0.0348±0.0287	p=0.2	-0.0430±0.0368	p=0.2

Estimated regression coefficient (β ± sd, p-value)

Model 0: crude model
Model 1: adjustments for gender, sub-Saharan origin, diabetes, age, body mass index, mGFR, mean blood pressure, log $1,25(OH)_2D_3$, ferritin, fractional excretion of phosphate, center, log PTH and 24-hr protein intake
Model 2: Model 1 + FGF-23

Supplementary Table 3. Multivariate analysis of phosphatemia (mmol/L) according to log-transformed albuminuria to creatinine ratio (mg/mmol), stratified by mGFR levels.

	mGFR< 30 ml/min/1.73m²		**mGFR ≥ 30 ml/min/1.73m²**	
All patients	**n=596**		**n=1142**	
Model 0	0.0343±0.0055	p<0.0001	0.0099±0.0028	p=0.0003
Model 1	0.0151±0.0053	p=0.005	0.0072±0.0028	p=0.01
Patients with FGF23 measurements	**n=160**		**n=237**	
Model 0	0.0402±0.0107	p=0.0002	0.0026±0.0055	p=0.6
Model 1	0.0242±0.0111	p=0.03	0.0035±0.0051	p=0.5
Model 2	0.0200±0.0098	p=0.04	0.0019±0.0048	p=0.7

Estimated regression coefficient (β ± sd, p-value)

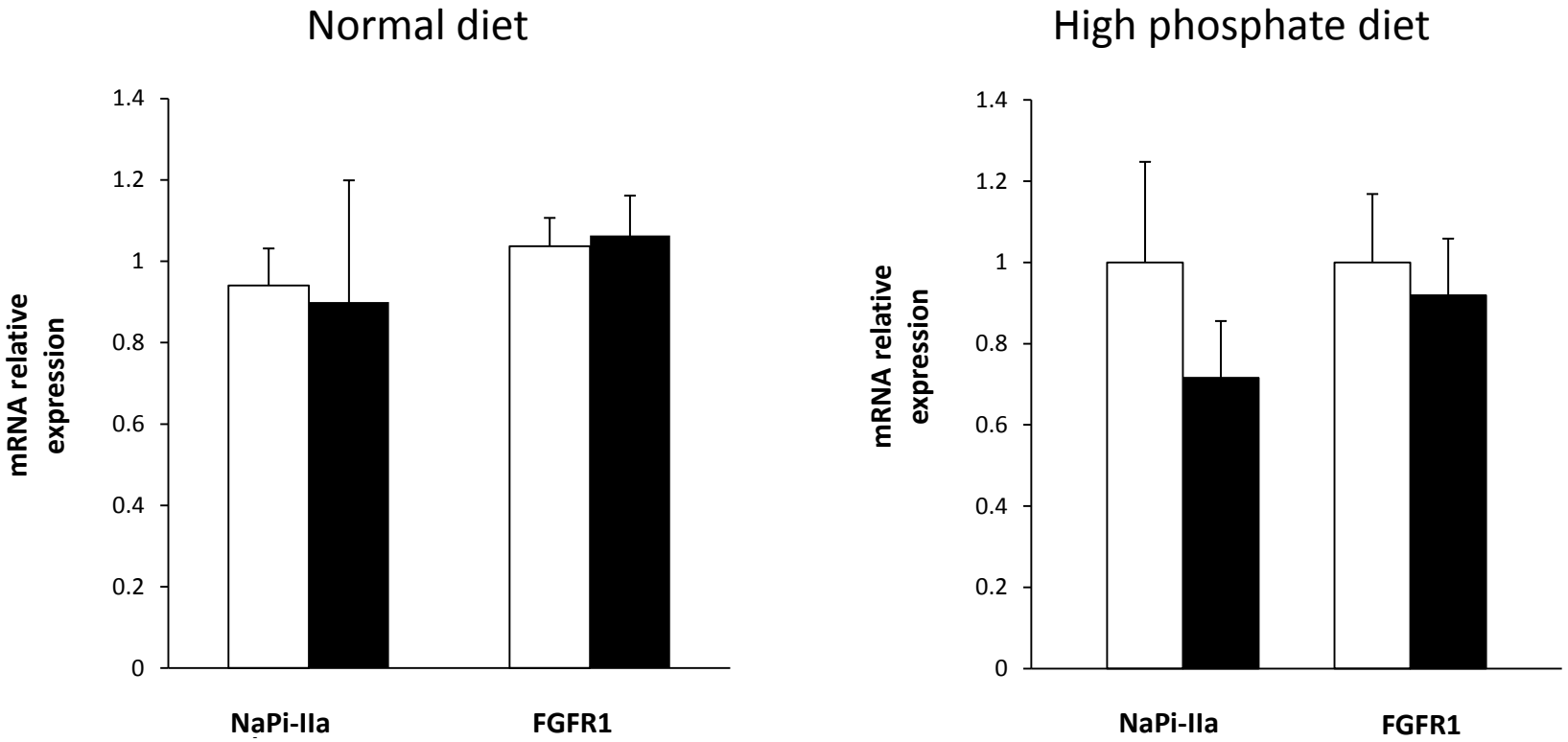
Model 0: crude model
Model 1: adjustments for gender, sub-Saharan origin, diabetes, age, body mass index, mGFR, mean blood pressure, log $1,25(OH)_2D_3$, ferritin, fractional excretion of phosphate, center, log PTH and protein intake.
Model 2: Model 1 + FGF-23

Supplementary Table 4: Biochemichal data of rats treated by puromycin after 15 days

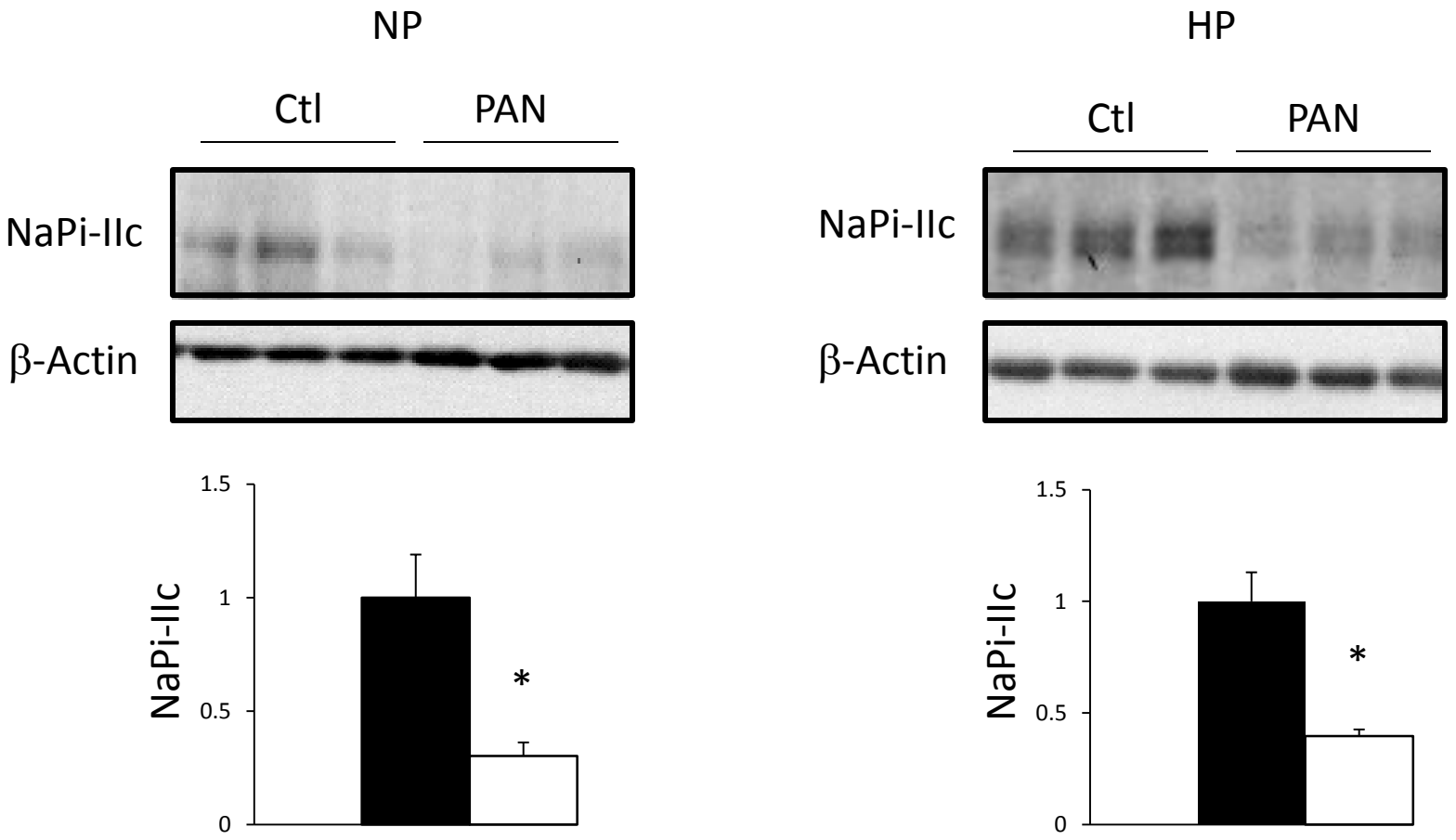
Data	Ctl rats(6)	PAN rats (6)	P value
Na (mmol/L)	139±1	138±1	Ns
K (mmol/L)	4.6±0.4	5.4±0.34	Ns
Calcium(mmol/L)	2.52±0.03	2.5±0.04	Ns
Phosphate (mmol/L)	2.44±0.08	2.84±0.33	Ns
Creatinine (umol/L)	23.63±0.4	30±4	Ns
Creatinine clearance (ml/min)	2.28±0.15	1.4±0.44	0.03
Urine volume (ml/24 hours)	8.9±0.8	7.6±1.6	Ns
Phosphate excretion (mmol/24 hours)	1.30±0.1	0.93±0.14	0.05
Fractional excretion of phosphate (%)	18.6±1.7	18.7±1.6	Ns
FGF23 (pg/ml)	331±35	499±65	0.05
PTH (pg/ml)	81.7±25.1	86.7±15.9	Ns
Proteinuria/creatininuria(g/mmol)	0.04±0.01	2.5±0.3	0.01
Food intake (g/last day)	23.8±0.6	21.2±1.9	Ns

Supplementary table 5: Biochemical data of Puromycin treated rat at 15 days with high phosphate diet

Data	HP Ctl rats (9)	HP PAN rats (9)	P value
Na (mmol/L)	139±1.4	139±0.8	Ns
K (mmol/L)	6.5 ±0.8	5.5±0.3	Ns
Calcium(mmol/L)	2.31±0.04	2.22±0.08	Ns
Phosphate (mmol/L)	3.1±0.2	3.7±0.2	0.08
Creatinine (umol/L)	18.8±1.4	13.8±1.2	Ns
Creatinine clearance (ml/min)	2.32±0.16	1.53±0.6	Ns
Urine volume (ml/24 hours)	11.3±1	20.22±1.9	Ns
Phosphate excretion (mmol/24 hours)	5.490±0.544	5.894±0.593	Ns
Fractional excretion of phosphate (%)	68±12	89±17	Ns
FGF23(pg/ml), n=4/4	681±74	>800	0.03
PTH (pg/ml)	330±75	1253±174	0.001
Proteinuria/creatininuria (g/mmol)	0.25±0.05	7.7±5.6	0.01
Food intake (g/24h)	20.4±0.9	21.2±0.7	Ns



Supplementary Figure 1: mRNA expression levels of NaPi-IIa and FGFR1 receptor were unchanged in proteinuric rats
mRNA expression levels of NaPi-IIa and FGFR1 were assessed by RT-PCR in control (Ctl) and puromycinaminonucleoside-induced nephrotic (PAN) rats. Results are expressed as mean relative expression compared to control ± SEM from 6 animals for all group except Ctl High phosphate diet (n=5).

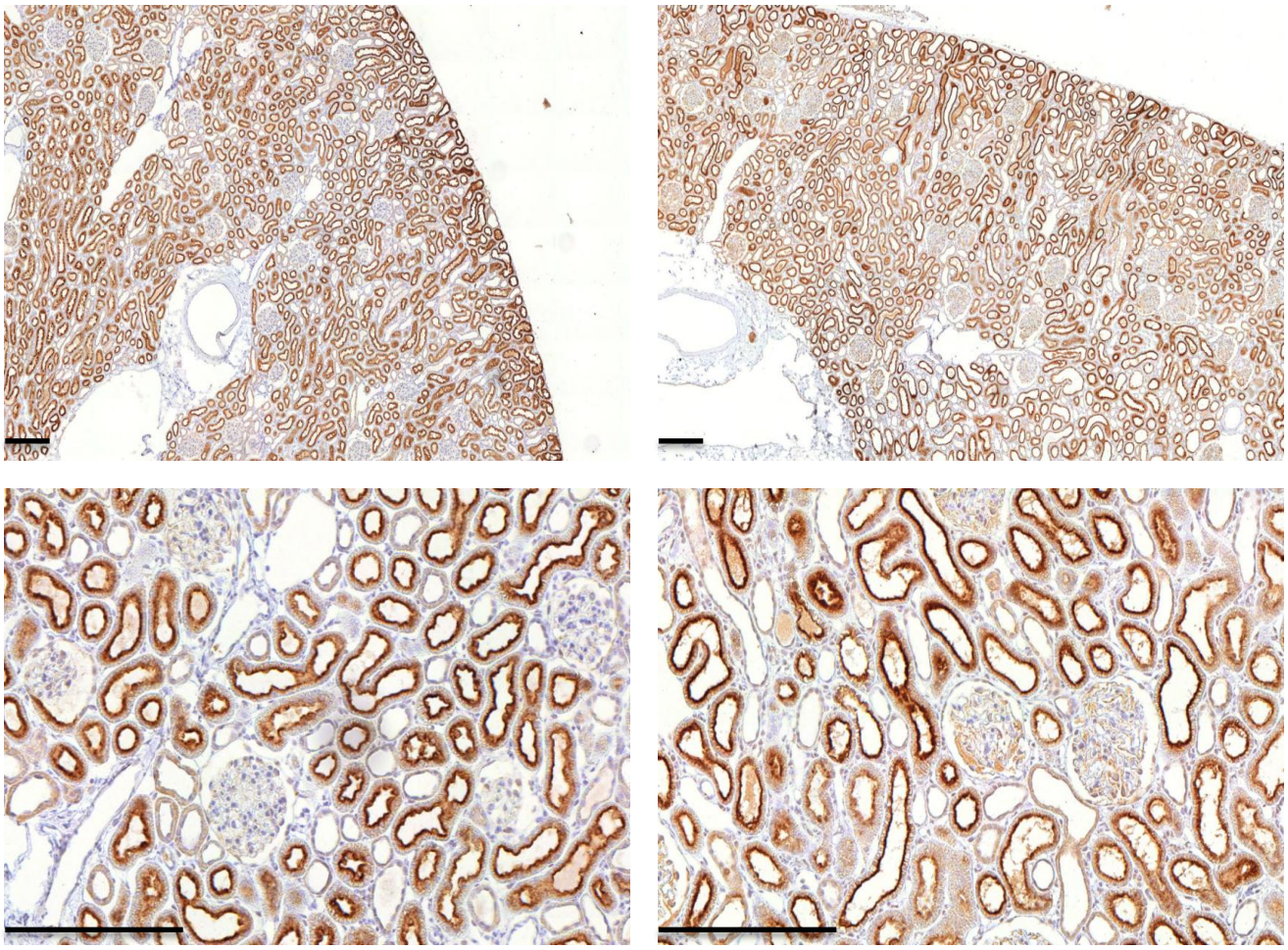


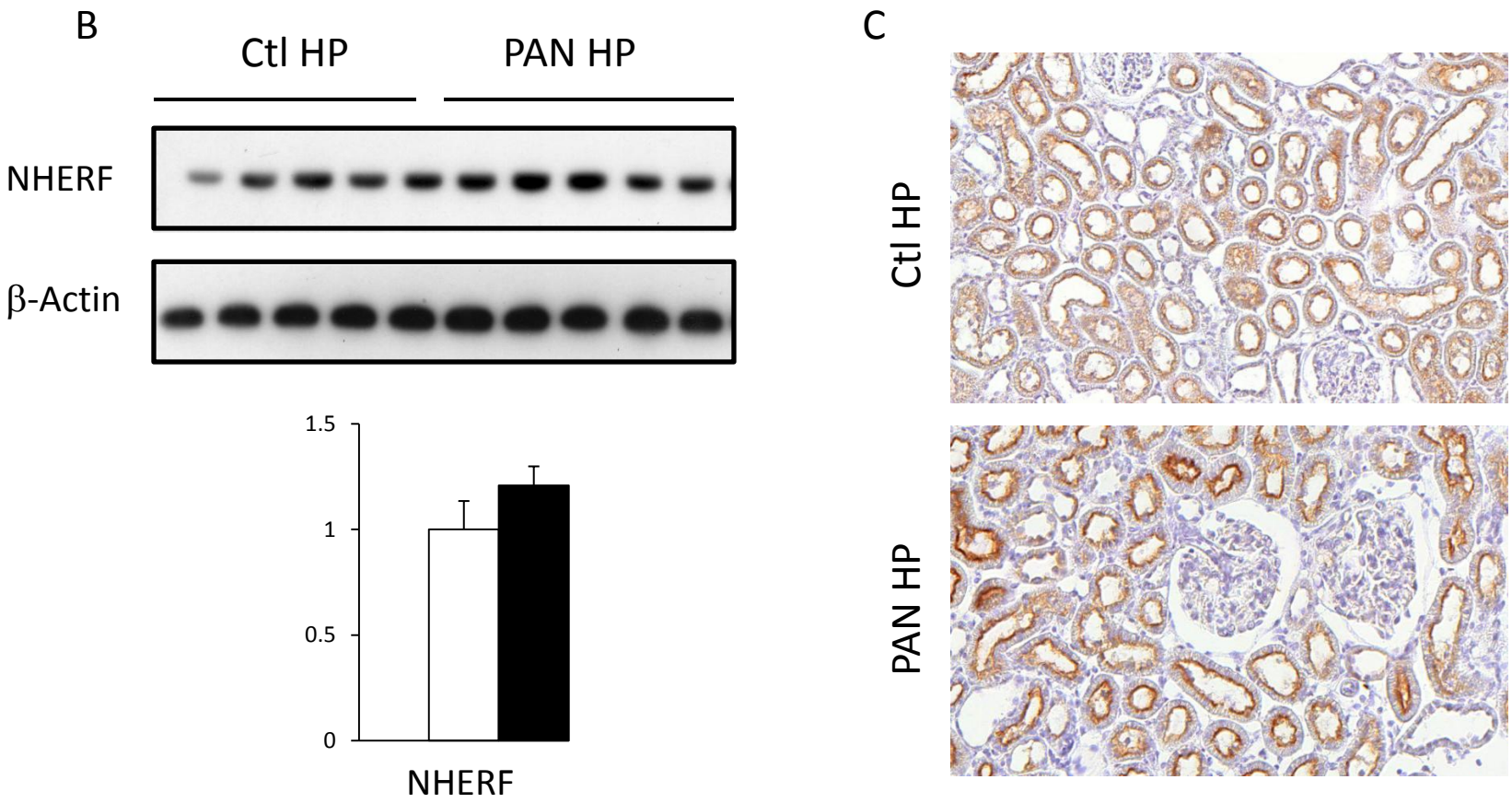
Supplementary Figure 2: NaPi-II c is downregulated in proteinuric rats fed a normal diet (NP) or a high phosphate diet (HP): NaPi-IIc protein was detected by Western blot of kidney lysates in Ctl and PAN rats fed a normal (NP) or a high phosphate (HP) diet. Representative Western blots of NaPi-IIc from three animals are shown on top. Quantification of Western blots (n = 5 and 5) are shown. Results are expressed as the mean ratio of individual values over the mean value obtained in Ctl ± SEM. *$p<0.05$

A

Ctl HP

PAN HP

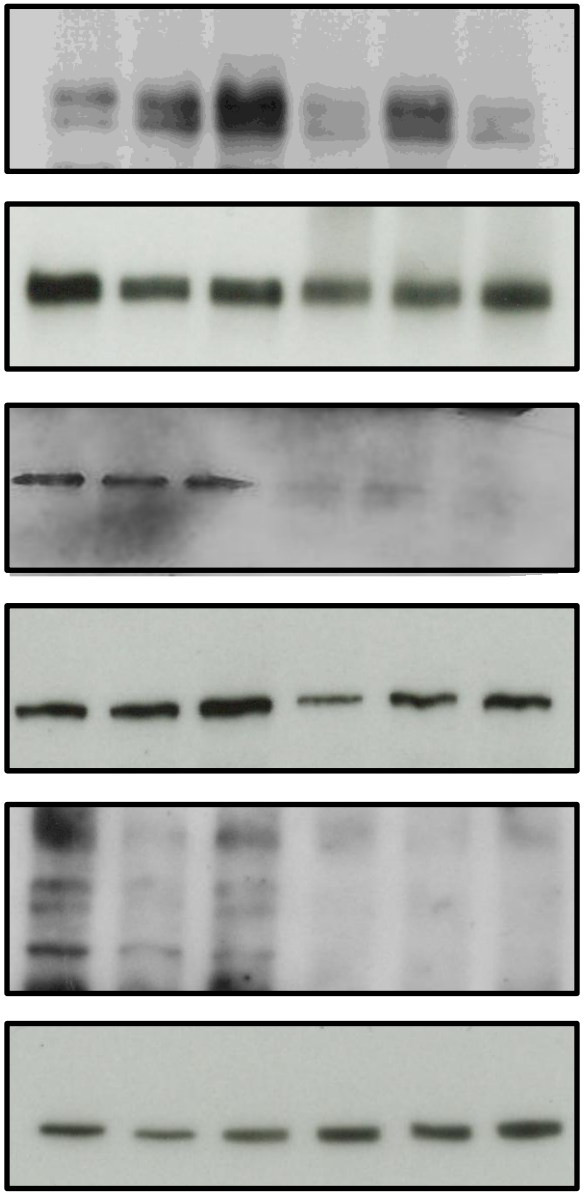


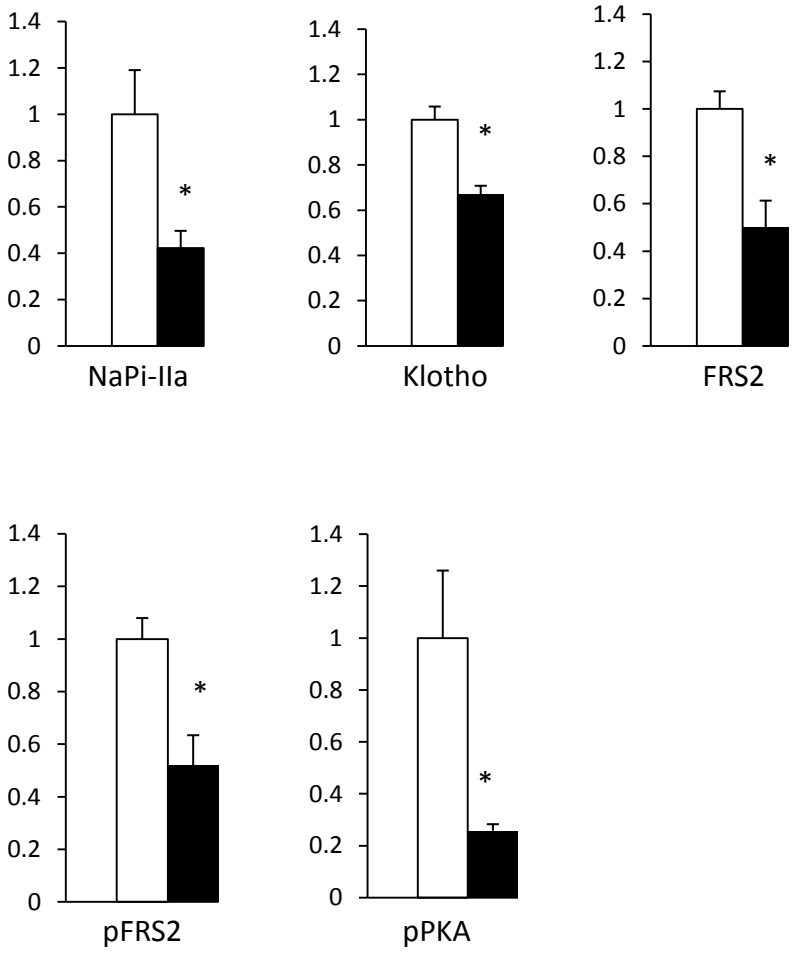


Supplementary figure 3: Megalin and NHERF expression and subcellular repartition are similar in control and proteinuric rats

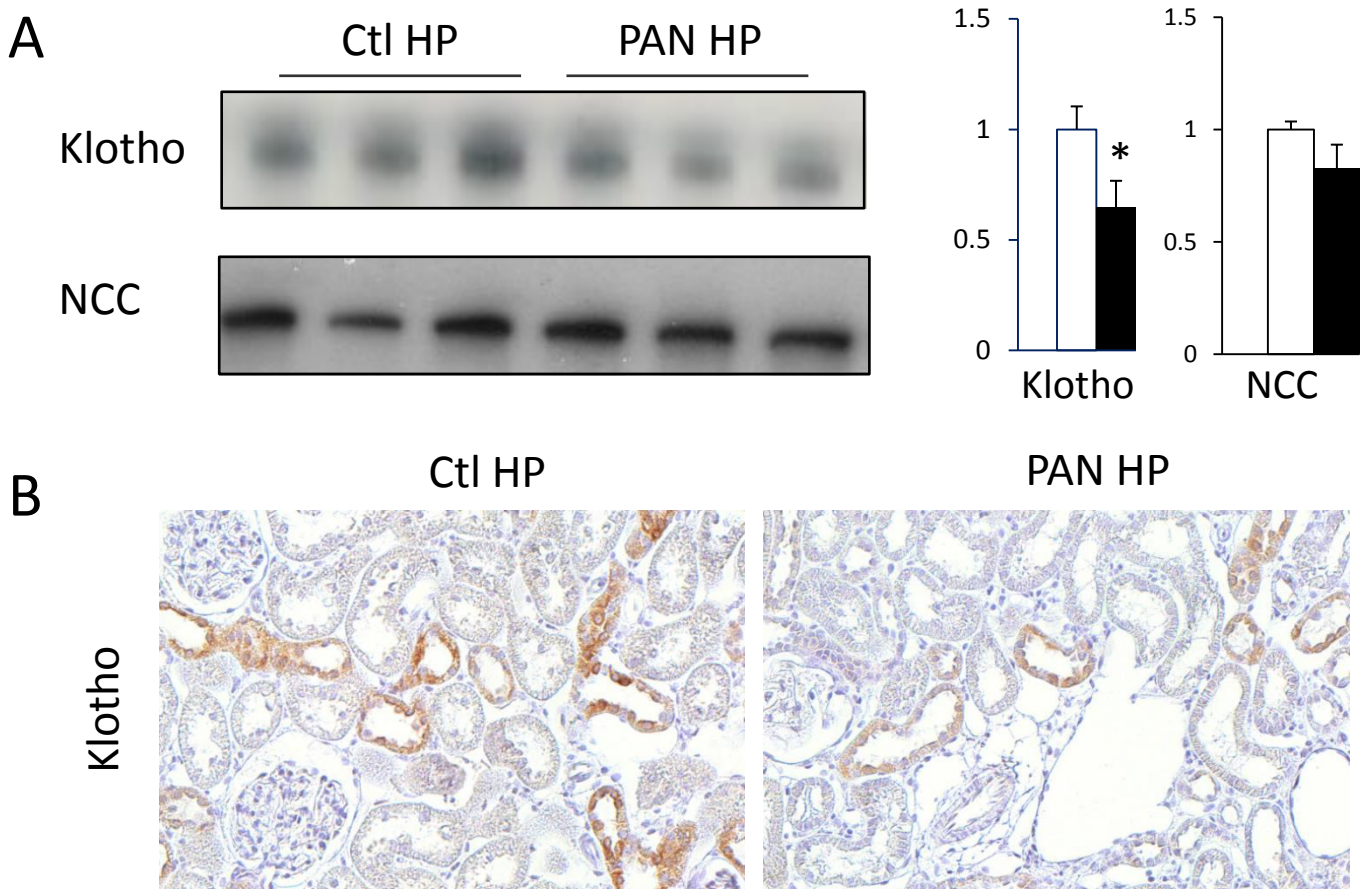
Megalin (A) and NHERF (C) expression was assessed by immunohistochemistry in control (Ctl) and puromycinaminonucleoside-induced nephrotic (PAN) rats exposed to a high phosphate diet. NHERF-1 expression in cortical lysate from Ctl and PAN rats exposed to a high phosphate diet (B). Expression and subcellular localization of NHERF and Megalin were similar under both conditions.

Supp. Fig. 4





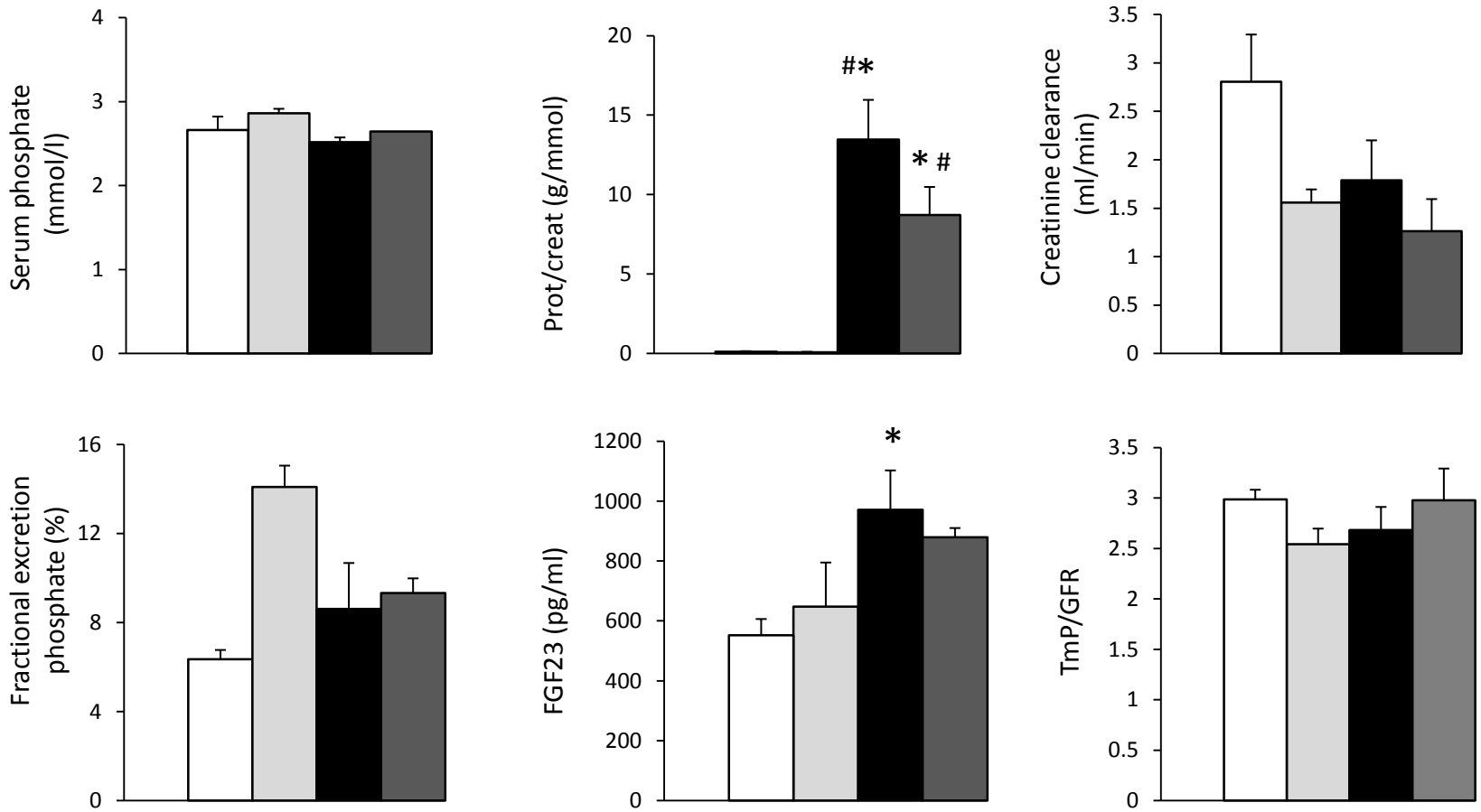
Supplementary Figure 4: Effect of a high phosphate diet: NaPi-IIa, Klotho, FRS2α and its phosphoryled form (p-FRS2α) and p-PKA were detected by Western blot of kidney lysates in Ctl fed a normal diet (open bars) or fed a high phosphate diet (closed ars). Representative Western blots of NaPi-IIa, Klotho, FRS2α, p-FRS2α (3 animals per group) are shown. Bars (Right panel) show densitometric quantification of Western blots (n = 6 and 6). Results are expressed as the mean ratio of individual values over the mean value obtained in Ctl ± SEM. *p<0.05. An important caveat for this observations is that rats subjected to control normal diet and high phosphate diet were not handled at the same time.



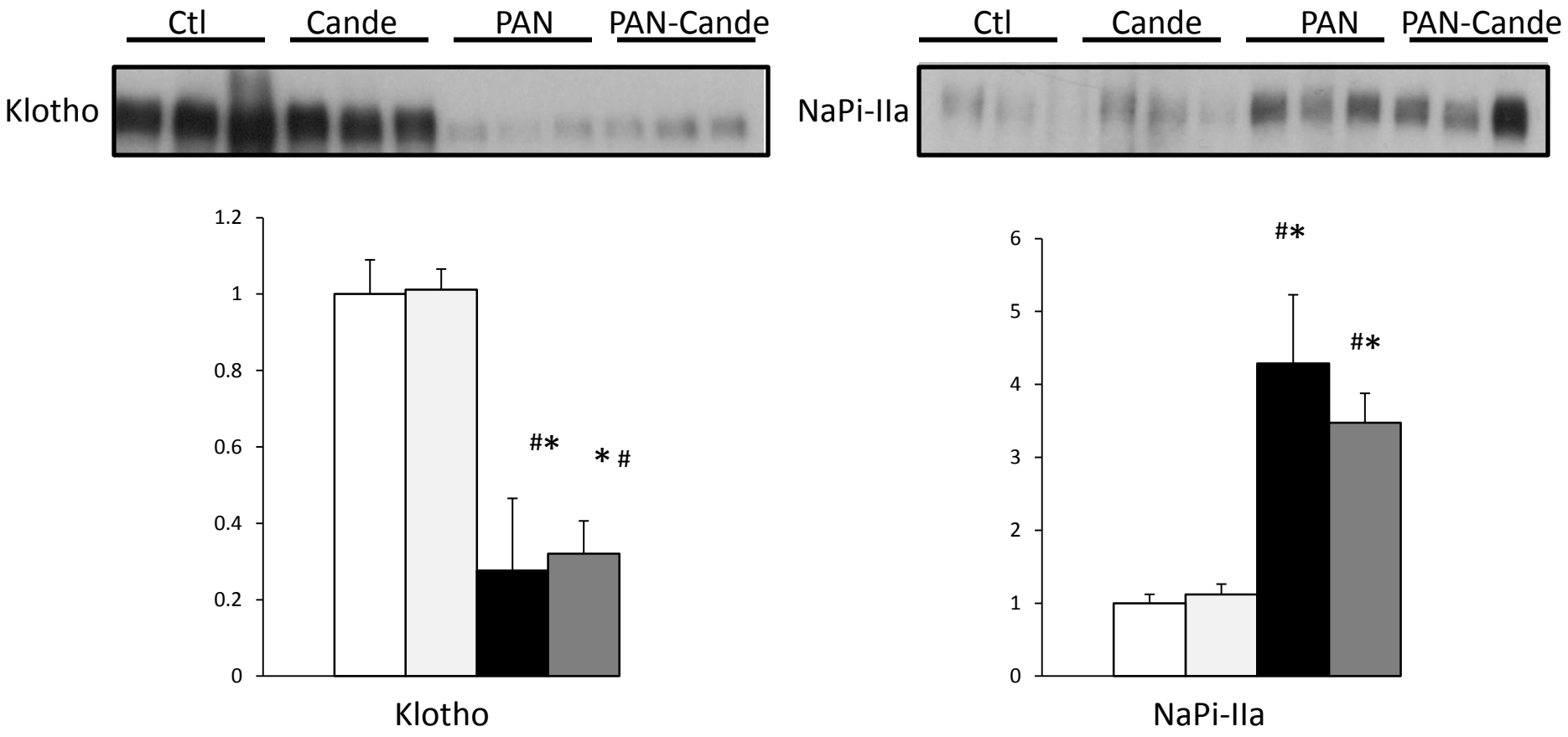
Supplementary Figure 5: Klotho expression is decreased in proteinuric rats given a high phosphate diet
Klotho and NCC expression were assessed by Western blotting in cortical lysates from control (Ctl) and puromycin-aminonucleoside-induced nephrotic (PAN) rats under a high phosphate diet (A-B) A. Representative Western blot of Klotho and NCC (Left panel: 3 animals per group). Beta-actin was used as loading control. Bars (Right panel) show densitometric quantification of Western blots (n = 9). Results are expressed as the mean ratio of individual values over the mean value obtained in Ctl ± SEM. B. Klotho expression was analyzed by immunohistochemistry. Decreased Klotho expression is apparent in PAN rats. *p<0.05

Supp. Fig. 6

A


Ctl
Candesartan
Puromycin
Puromycin-Candesartan
Serum phosphate (mmol/l)
Prot/creat (g/mmol)
#*
* #
Creatinine clearance (ml/min)
Fractional excretion phosphate (%)
FGF23 (pg/ml)
*
TmP/GFR

B



Supplementary Figure 6: Candesartan does not reduce proteinuria in PAN rats: Graphical representation of last day physiological data in PAN rat without (closed bars) and with (dark grey bars) Candesartan treatment and control without (open bars) and with (light grey bars) candesartan treatment (n=5; 5; 5, 6) (A). Kidney cortex were analyzed 14 days after PAN injection. C and D. Representative Western blot of NaPi-IIa and Klotho (Left panel: 3 animals per group). Bars (Right panel) show the densitometric quantification of Western blots (n = 5;5;5;6) . Results are expressed as the mean ratio of individual values over the mean value obtained in Ctl ± SEM. *p<0.05 compared to Ctl, # p<0.05 compared to Candesartan