Supplementary materials and methods

Isolation of single tubular fragments using the complex object parametric analyzer biosorter (COPAS)

To prepare single renal tubular fragments, mice were anesthetized with Ketamin (Narketan 10, 80 mg/kg body wt; Chassot, Belp, Switzerland) and Xylazine (Rompun, 33 mg/kg body wt; Bayer, Leverkusen, Germany). Mice were perfused through the heart with 10ml of cold PBS then with 10ml of digestion solution (1mg/ml, Worthington ; 1mg/ml hyaluronidase, Sigma ; 0.1mg/ml DNaseI, SIGMA prepared in ice-cold KREBS : 145 mM NaCl, 10 mM HEPES, 5 mM KCl, 1 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.8 mM $MgSO_4$, 5 mM glucose, pH 7.3). Renal cortex from both kidneys was dissected under a stereomicroscope. Samples were finely minced and digested in 10 ml of fresh digestion solution at 37°C for 15 minutes. The tubular digest was first filtered through 250- and 212-µm nylon sieves. The flow-through was then filtered with a 100-µm and a 40-µm cell strainer (Becton Dickinson Labware, Franklin Lakes, NJ). The tubules retained by the 40-µm cell strainer were diluted with ice-cold Krebs to a total volume of 50 ml. All sortings were performed with a complex object parametric analyzer and sorter (COPAS) instrument (Union Biometrica, Somerville, MA). As already described for sorting fluorescent renal collecting ducts[1], the following instrument settings were used: delay 8, width 5, photomultiplier tube (PMT) 700, sheath fluid pressure 4.2-4.5. The sample fluid pressure was set to maintain a sort frequency of 20–40 events/s (sample pressure ~5.40–5.90). The mixer speed was 80%. Sorted tubules were collected directly into ice-cold KREBS containing 0.05% BSA (Fluka). They were centrifuged at 800g for 10minutes and the pellet was used for RNA and protein expression analysis.

Preparation of total RNA and cDNA from sorted tubules

RNA extraction was performed with RNAqueous-Micro extraction kit (Applied Biosystems/Ambion, Switzerland). Immediately after sorting, pelleted tubules were resuspended in 50 µl of elution buffer provided by the manufacturer. RNA isolation and DNase treatment was performed according to the manufacturer's recommendations.

Gene expression profiling – Agilent Microarray hybridization and analysis

The quality of the isolated RNA was determined with a NanoDrop ND 1000 (NanoDrop Technologies,Delaware, USA) and a Bioanalyzer 2100 (Agilent, Waldbronn, Germany). Total RNA samples (600ng) were reverse-transcribed into

double-stranded cDNA in presence of RNA poly-A controls, RNA Spike-In Kit, One-Color (Agilent p/n 5188-5282). The double-stranded cDNA were in vitro transcribed in presence of Cy3-labelled nucleotides using a Low RNA Input Linear Amp Kit+Cy dye, one-color kit (Agilent P/N 5188-5339, Waldbronn, Germany). The Cy3-labeled cRNA was purified using a RNeasy mini kit, Qiagen (p/n. 74104 or 74106) and its quality and quantity was determined using NanoDrop ND 1000 and Bioanalyzer 2100. Only cRNA samples with a total cRNA yield higher than 2 µg and a dye incorporation rate between 9 pmol/ µg and 20 pmol/ µg were considered for hybridization. Cy3-labeled cRNA samples (1.65 µg) were mixed with a Agilent Blocking Solution, subsequently randomly fragmented to 100-200 bp at 65°C with Fragmentation Buffer, and resuspended in Hybridization Buffer using a Gene Expression Hybridization Kit (Agilent p/n 5188-5242). Target cRNA Samples (100µl) were hybridized to Whole mouse Genome 4x44k OligoMicroarrays (Agilent) for 17h at 65°C. Arrays were then washed using Agilent GE Wash Buffers 1 and 2, according to the manufacturer instructions. An Agilent Microarray Scanner (Agilent p/n G2565BA) was used to measure the fluorescent intensity emitted by the labeled target.

Array Data Processing and quality control

Raw data processing was performed using the Agilent Scan Control and the Agilent Feature Extraction Software Version 10. Quality control measures were considered before performing the statistical analysis. These included, inspection of the array hybridization pattern (absence of scratches, bubbles, areas of non hybridization), proper grid alignment, performance of the spike in controls (linear dynamic range between 5 orders of magnitude) and number of green feature non-uniformity outliers (below 100 for all samples). Expression values were imported into Gene-spring 10 (Agilent Technologies, USA).

Real-Time PCR

cDNA was synthesised by reverse transcription of 200 ng of RNA using random primers and ImpromII Reverse Transcription Kit (Promega, Madison, WI) following instructions of the manufacturer. Real-time PCR was performed on a ABI Prism 7700 cycler (Applied Biosystems) with the iQ SYBR Green Supermix (Biorad), 2 ng of reverse transcribed RNA per well and the following primers were used to measure gene expression of NCC: forward 5’ TGACCTGCATTCATTCCTCA 3’, reverse 5’ GAAGCGAACAGGTTCTCCAG 3’; GAPDH: forward 5’CCTGCTTCACCACCTTCTT-GA3’, reverse 5’ CATGGCCTTCCGTGTTCCTA 3’; NKCC2: forward 5’

GGATCCAACCAATGACATCC 3', reverse 5' TTTGCAATGGCAATGAGAAGG 3'; WNK1 Kidney Specific [2]: forward 5' TGCTGCTGTTCTCAAAAGGATTGTA 3', reverse 5' TTCAGGAATTGCTACTTTGTCAAAACTG 3'; WNK4 [2]: forward 5' GCGGAGGAGGTGGCT 3', reverse 5' GCCACTGGCTGGTAGTCA 3'; Renin: forward 5' ATCTTTGACACGGGTTCAGC 3', reverse 5' TGATCCGTAGTGGA-TGGTGA. Relative mRNA gene expression was measured after normalization to GAPDH gene expression: Ratio = $2^{Ct\ (GAPDH–Target)}$

Immunohistochemistry

Mouse kidneys were fixed with 3% paraformaldehyde in phosphate buffer perfused through the descending aorta as previously described[3]. Cryosections were pretreated with blocking solution (Normal Goat Serum 10% in PBS with 0.1% bovine serum albumin (BSA)) for 60 min. Sections were incubated overnight at 4°C with primary antibodies diluted in PBS with 0.1% BSA then washed in PBS for 15 min. and incubated with secondary antibody for 2 hours at room temperature. After washing with PBS, sections were coverslipped using DAKO-Glycergel (Dakopatts) containing 2.5% 1,4-diazabicyclo[2.2.2]octane (Sigma) as a fading retardant. For characterization of the phospho-NKCC2 antibody, paraffin-embedded kidneys of mice and rats were processed for immunohistochemistry according to standard procedures. Immunohistochemistry images were acquired with a Leica DFC490 charged-coupled device camera attached to a Leica DM 6000 fluorescence microscope (Leica, Wetzlar, Germany).

Sample preparation and analysis for immunoblots

COPAS sorted tubules were centrifuged at 750 g for 5 min. The pellet was lysed in 10 µl SDS loading buffer (10 mM Tris·HCl, pH 6.8, 1% SDS, 2% dithiothreitol, 13% glycerol, and bromophenol blue) and stored at -20°C. Kidneys were homogenized in icecold K-HEPES buffer (200 mM mannitol, 80 mM HEPES, 41 mM KOH, pH 7.5) containing phosphatase and protease inhibitor cocktails (Roche), centrifuged at 2000g for 15 min at 4°C and the pellet was resuspended in K-HEPES buffer (200 mM mannitol/80 mM K-HEPES/41 mM KOH/pH 7.5 supplemented with antiproteases (Complete tablets from Roche). To prepare kidney membrane fraction, the kidney lysate was centrifuged at 100000g for 1h at 4°C. The pellet was then resuspended in K-HEPES. After measurement of protein concentration (Optima), electrophoresis was initially carried out (Mini Protean Tetra Cell, Biorad) on a 8% polyacrylamide gel for either sorted tubules (400 tubules per lane) or 30µg of protein from whole lysates or membrane fractions from total mouse kidney membrane fraction on 8%

polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane (Whatman, Bottmingen, Switzerland) with a BioRad Mini-Trans-Blot Cell system (100V, 2 hours). After transfer, membranes were blocked with Odyssey blocking buffer (LI-COR Biosciences) and incubated overnight at 4°C with prediluted antibodies in Odyssey blocking buffer. After washing the primary antibodies, Goat anti mouse Alexa Fluor 680-conjugated IgG (dilution 1:15000 in blocking buffer, LI-COR Biosciences) or goat anti rabbit Alexa Fluor 800CW-conjugated IgG (dilution 1:15000 in blocking buffer LI-COR Biosciences) was added for 1 h at room temperature before visualization and analysis by the Odyssey IR imaging system (LI-COR Biosciences). Densitometric quantifications were also performed with the Odyssey IR imaging system. Densitometric values were first normalized to their respective actin values. This ratio was then normalized to the mean of the control group which was arbitrarily set to 100. Results are expressed as mean ± standard error (S.E).

Characterization of antibodies against NCC and SPAK

Lysates from isolated DCTs or whole kidneys from wild type and NCC deficient animals[4] were resolved by immunoblotting as described above. To confirm the specificity of the NCC and SPAK antibodies for their phosphosites, kidney protein lysates were treated with Calf Intestinal Phosphatase (NEB) for 1 h at 37°C.

Legends

Supplementary figure 1: I-1 in human kidney

Representative images of immunostainings of cryosections from human kidneys stained for NCC (A) or I-1 (B). I-1 is highly abundant in NCC-positive DCTs (D). I-1 is also expressed in TALs (T). Bar ~40 µm.

Supplementary figure 2: Characterization of phosphoform-specific antibodies against NKCC2

(A) Dotblotting of the pNKCC2 antibody demonstrated high affinity to the pNKCC2 peptide (QT^{P}FGHNT^{P}MC), but weak affinity to the non-phosphorylated NKCC2 peptide (QTFGHNTMC) or the closely related pT53/pT58 NCC peptide (MRT^{P}FGYNT^{P}ID). (B) Western blotting of rat and mouse kidney samples detected a glycosylated smear of approximately 170kDa representing pNKCC2. These protein bands were not observed following pre-incubation of the antibody with the

phosphorylated immunizing peptide. (C) Immunohistochemistry on paraffin sections of rat and mouse kidney demonstrated strong labeling at the apical pole of thick ascending limb cells. No labeling of similar sections was detected following pre-incubation of the antibody with the phosphorylated immunizing peptide. Images were taken at low magnification from the border between inner stripe of outer medulla to the inner medulla (ISOM/IM), and at higher magnifications from the inner stripe of outer medulla (ISOM). Immunohistochemistry on paraffin sections of mouse brain demonstrated that the pNKCC2 antibody does not detect NKCC1 in the choroid plexus, which can be readily displayed with an antibody against NKCC1. Overviews with higher magnifications shown as inserts.

Supplementary figure 3: Characterization of phosphoform-specific antibodies against NCC

Peptides against the NCC epitopes CLYMR**T**FGYN (pT53NCC), CFGYN**T**IDVV (pT58NCC), CHYAN**S**ALPGE (pS71NCC) and CADLH**S**FLKE (pS89NCC) were raised in rabbits and plasma antibodies were affinity purified against the corresponding phospho- and non-phosphopeptide (Pineda)[5]. To confirm the specificity of the antibodies for NCC, kidney protein lysates from Wild-Type (WT) and NCC deficient mice ($NCC^{-/-}$) were resolved by immunoblotting using the purified antibodies. Protein lysates were also incubated (+) with Calf Intestinal Phosphatase (CIP ; New England Biolabs) for 1h at 37°C. Controls without CIP (-) were incubated at 37°C or at room temperature. The phosphoform-specificity of the antibodies was confirmed when the specific bands for NCC disappeared in the phosphatase-treated samples. The band detected by the antibody against total NCC was not sensitive to the phosphatase treatment. Detection of β-actin served as loading control.

Supplementary figure 4: Characterization of phosphoform specific antibody against SPAK and against total SPAK

(A) To confirm the specificity of the antibody for phospho SPAK (pSPAK), total kidney (TK) protein lysates from wild type mice were incubated with (+) or without (-) Calf Intestinal Phosphatase (CIP; New England Biolabs) for 1h at 37°C and resolved by immunoblotting. COPAS sorted DCTs (400 tubules) were also blotted to confirm the strong expression of pSPAK in this segment. (B) Immunoblots using SPAK and pSPAK antibodies with total kidney lysates (TK) and sorted DCT were performed in parallel using the same membrane. Both antibodies recognized the same band at the expected height of SPAK (arrows) both in TK and DCT. The arrowhead indicates the

line along which the membrane was cut into two pieces that were incubated either with the SPAK or the pSPAK antibodies.

Supplementary Table 1: Gene expression of renal tuble markers in EGFP$^+$, EGFP$^-$ and all tubules sorted by COPAS

Gene expression of renal tubule markers in EGFP$^+$ tubules, all tubules (ALL) and EGFP$^-$ tubules were extracted from the total list of gene identified after array data processing in Genespring 10. Datas are shown as average normalized intensities for each group (n=4 for each group). Comparative ratio analyses of gene expression for ALL versus EGFP$^-$, EGFP$^+$ versus EGFP$^-$ and EGFP$^+$ versus ALL allowed to identify highly enriched genes in the DCT.

Supplementary Table 2: Highly enriched gene products in the EGFP$^+$ (DCT) tubules.

Datas are shown as the average normalized intensities for each gene product comparing the EGFP$^+$ tubules, all kind of tubules (ALL) and EGFP$^-$ tubular gene expression (n=4 for each group). Comparative ratio analysis of gene expression for ALL versus EGFP-, EGFP+ versus EGFP- and EGFP+ versus ALL allowed to identify highly enriched gene products in the DCT. The first hundred most enriched genes were ordered according to the magnitude of the EGFP+ versus EGFP- ratio. A few genes were represented on the microarray with more than one spot. Those genes are listed only once and for the spot that appeared first on the list of the most enriched gene products.

Supplementary Table 3: Densitometric quantification of immunoblots

Immunoblot analysis of kidney membrane fractions from WT and I-1$^{-/-}$ mice were used to quantify renal sodium transporter abundances. Densitometric analysis from the immunoblot presented in figure 4F, was performed with the LICOR Odyssey infrared-scanner detection system. Each band was normalised to β-actin protein levels. The average for each protein was set to 100% for WT and expressed for I-1$^{-/-}$ mice in percent of the WT values. Statistical analyses were performed with unpaired student t-test comparing the two groups. (mean ± SE, n = 7 per group) * $p < 0.05$, *** $p < 0.001$ vs control.

Supplementary Table 4: Renal gene expression in I1-/- mice

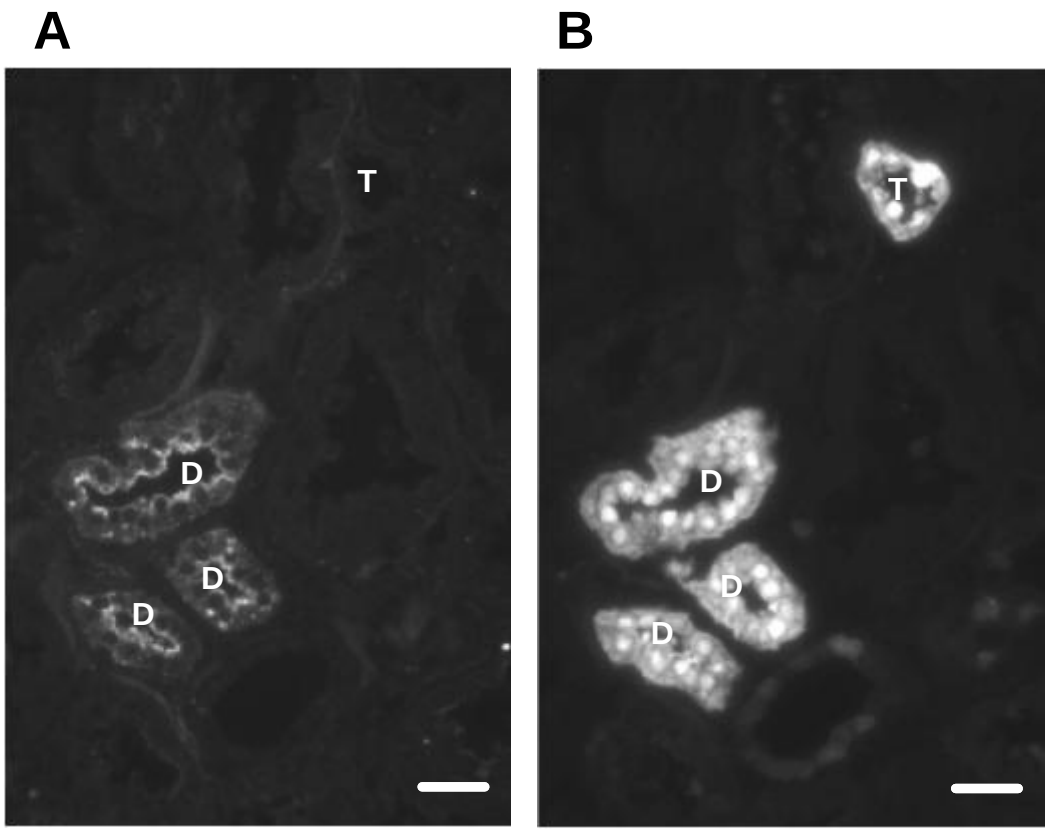
Gene expression of NKCC2, NCC, Renin, kidney-specific WNK1, and WNK4 were assessed by real time PCR on cDNA from WT and I-1$^{-/-}$ mice (n = 6 per group). Gene

expression was normalised to GAPDH expression. No statistical difference was found comparing gene expression from WT and I-1$^{-/-}$ mice.

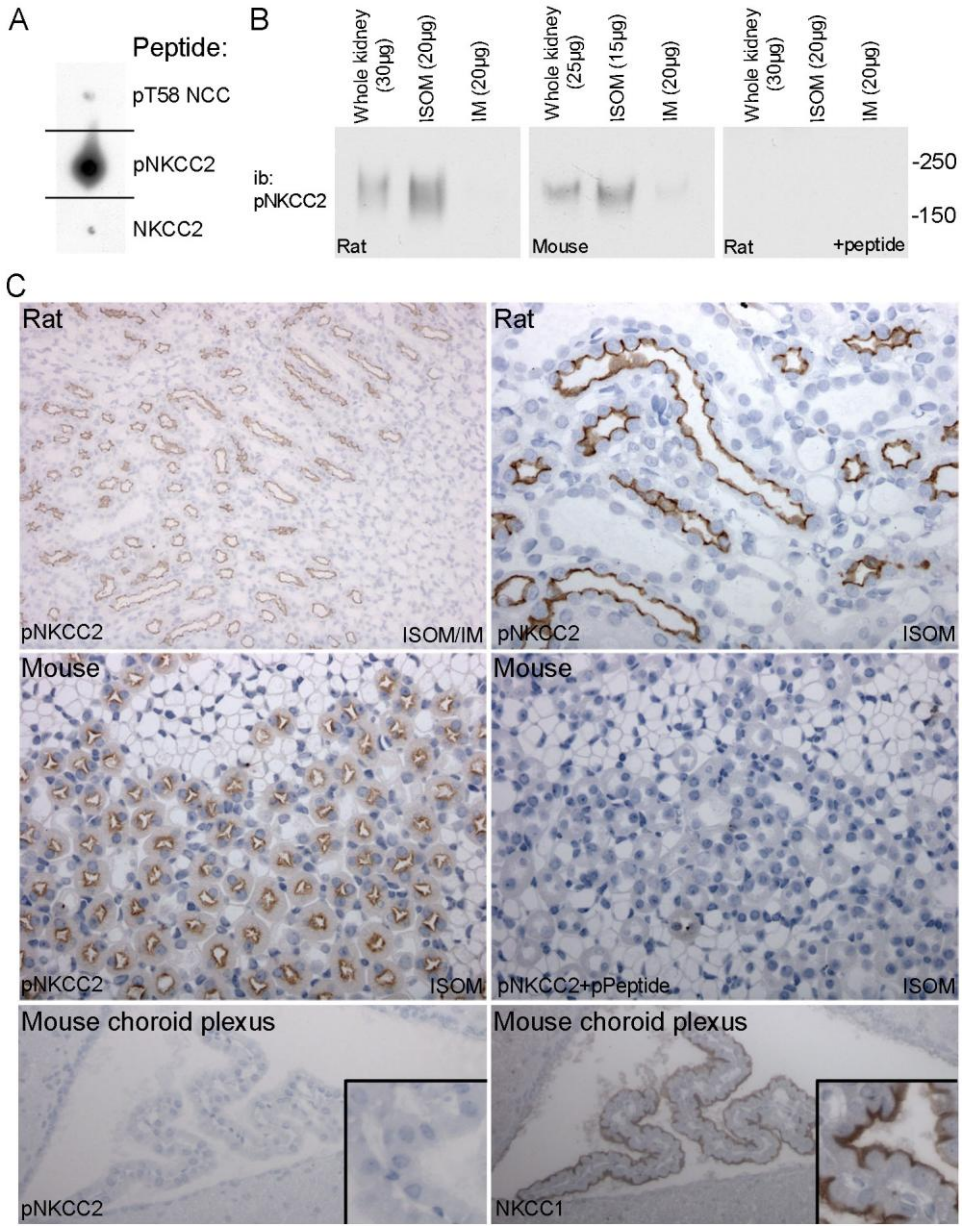
References

1. Miller, RL, Zhang, P, Chen, T, Rohrwasser, A, Nelson, RD: Automated method for the isolation of collecting ducts. *Am J Physiol Renal Physiol,* 291**:** F236-245, 2006.
2. O'Reilly, M, Marshall, E, Macgillivray, T, Mittal, M, Xue, W, Kenyon, CJ, Brown, RW: Dietary electrolyte-driven responses in the renal WNK kinase pathway in vivo. *J Am Soc Nephrol,* 17**:** 2402-2413, 2006.
3. Wagner, CA, Loffing-Cueni, D, Yan, Q, Schulz, N, Fakitsas, P, Carrel, M, Wang, T, Verrey, F, Geibel, JP, Giebisch, G, Hebert, SC, Loffing, J: Mouse model of type II Bartter's syndrome. II. Altered expression of renal sodium- and water-transporting proteins. *Am J Physiol Renal Physiol,* 294**:** F1373-1380, 2008.
4. Schultheis, PJ, Lorenz, JN, Meneton, P, Nieman, ML, Riddle, TM, Flagella, M, Duffy, JJ, Doetschman, T, Miller, ML, Shull, GE: Phenotype resembling Gitelman's syndrome in mice lacking the apical Na+-Cl- cotransporter of the distal convoluted tubule. *J Biol Chem,* 273**:** 29150-29155, 1998.
5. Sorensen, MV, Grossmann, S, Roesinger, M, Gresko, N, Todkar, AP, Barmettler, G, Ziegler, U, Odermatt, A, Loffing-Cueni, D, Loffing, J: Rapid dephosphorylation of the renal sodium chloride cotransporter in response to oral potassium intake in mice. *Kidney international,* 83**:** 811-824, 2013.

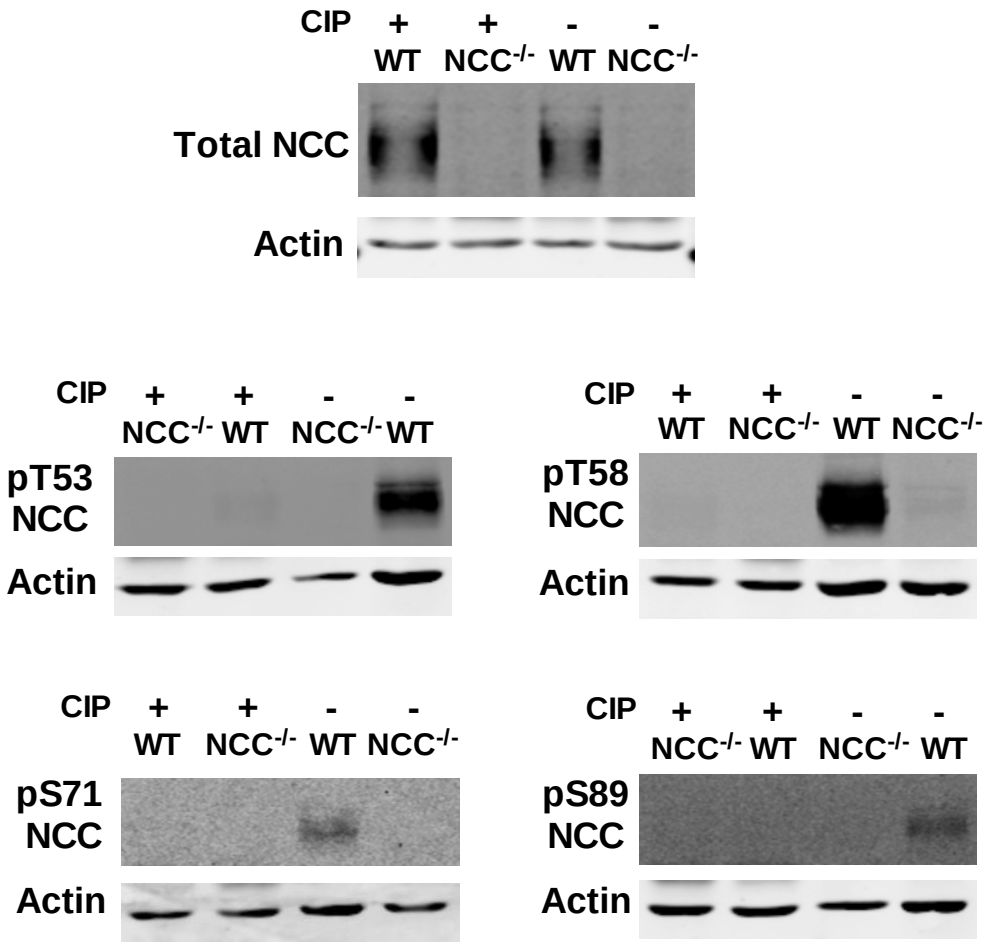
Supplementary figure 1: I-1 in human kidney



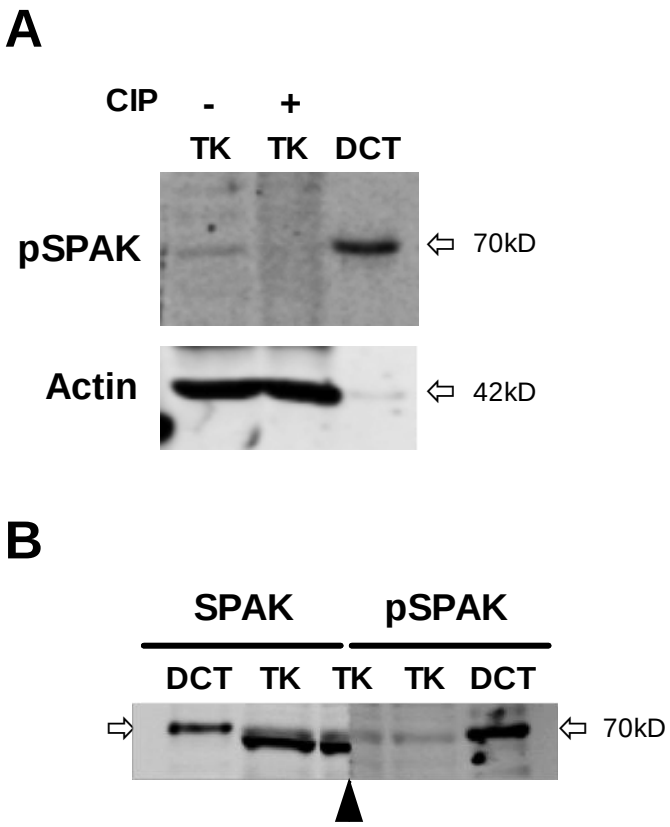
Supplementary figure 2: Characterization of phosphoform-specific antibody against NKCC2



Supplementary figure 3: Characterization of phosphoform-specific antibodies against NCC



Supplementary figure 4: Characterization of antibodies against SPAK



Supplementary Table 1: Gene expression of renal tuble markers in EGFP$^+$, EGFP$^-$ and all tubules sorted by COPAS

			Average Raw Intensity			RATIOS		
Description	**Gene Symbol**	**GenBank ID**	**All**	**EGFP-**	**EGFP+**	**All/EGFP-**	**EGFP+/EGFP-**	**EGFP+/All**
aquaporin 1	Aqp1	NM_007472	84866,1	93721,4	3251,6	0,91	0,03	0,04
low density lipoprotein receptor-related protein 2 (megalin)	Lrp2	XM_130308	249372,3	267185,4	12660,9	0,93	0,05	0,05
solute carrier family 34, member 1(NaPi-IIa)	Slc34a1	NM_011392	206126,2	216446,1	9562,4	0,95	0,04	0,05
solute carrier family 12, member 1 (NKCC2)	Slc12a1	NM_183354	25033,4	27476,1	45187,2	0,91	1,64	1,81
uromodulin (Tamm-Horsfall glycoprotein)	Umod	NM_009470	128325,9	98263,3	374249,7	1,31	3,81	2,92
parvalbumin	**Pvalb**	**NM_013645**	**2860,0**	**35,8**	**33507,1**	**79,84**	**935,35**	**11,72**
solute carrier family 12, member 3 (NCC)	**Slc12a3**	**NM_019415**	**36663,5**	**961,9**	**300176,2**	**38,12**	**312,08**	**8,19**
transient receptor potential cation channel, subfamily M, member 6	**Trpm6**	**NM_153417**	**927,2**	**50,3**	**12394,7**	**18,43**	**246,36**	**13,37**
potassium inwardly-rectifying channel, subfamily J, member 1 (ROMK)	Kcnj1	NM_019659	74288,2	27920,0	303140,6	2,66	10,86	4,08
sodium channel, nonvoltage-gated, type I, alpha (αENaC)	Scnn1a	NM_011324	5444,6	3366,5	29019,4	1,62	8,62	5,33
sodium channel, nonvoltage-gated 1 beta (βENaC)	Scnn1b	NM_011325	1698,9	1632,6	3375,2	1,04	2,07	1,99
sodium channel, nonvoltage-gated 1 gamma (γENaC)	Scnn1g	NM_011326	5785,5	5421,4	8529,2	1,07	1,57	1,47
transient receptor potential cation channel, subfamily V, member 5	Trpv5	AK085479	699,3	425,2	3305,2	1,64	7,77	4,73
solute carrier family 26, member 4 (pendrin)	Slc26a4	NM_011867	45439,5	48686,0	20500,7	0,93	0,42	0,45
aquaporin 2	Aqp2	NM_009699	7760,8	9031,4	754,0	0,86	0,08	0,10

Supplementary Table 2: Highly enriched gene products in the EGFP$^+$ (DCT) tubules.

				Average Raw Intensity			RATIOS		
Probe ID	Description	Gene Symbol	GenBank ID	All	EGFP-	EGFP+	All/EGFP-	EGFP+/EGFP-	EGFP+/All
A_51_P500156	parvalbumin	Pvalb	NM_013645	2860.0	35.8	33507.1	79.84	935.35	11.72
A_51_P445985	solute carrier family 12, member 3	Slc12a3	NM_019415	36663.5	961.9	300176.2	38.12	312.08	8.19
A_52_P184322	transient receptor potential cation channel, subfamily M, member 6	Trpm6	NM_153417	927.2	50.3	12394.7	18.43	246.36	13.37
A_51_P412966	leucine rich repeat protein 1, neuronal	Lrrn1	NM_008516	709.0	44.7	9062.1	15.86	202.74	12.78
A_52_P197722	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase-like 1	Hmgcll1	NM_173731	307.0	21.1	4165.9	14.53	197.17	13.57
A_52_P425317	RIKEN cDNA 4933406C10 gene	4933406C10Rik	AK016682	147.9	138.2	26843.8	1.07	194.30	181.48
A_52_P405655	sal-like 3 (Drosophila)	Sall3	AK045051	1054.5	80.0	13758.4	13.18	172.00	13.05
A_51_P433989	mitogen activated protein kinase 10	Mapk10	NM_009158	211.8	14.4	2439.6	14.75	169.83	11.52
A_52_P137678	ankyrin 2, brain	Ank2	NM_178655	198.9	16.3	2760.4	12.22	169.60	13.88
A_52_P577484	SRY-box containing gene 9	Sox9	NM_011448	323.3	28.6	4511.6	11.32	157.91	13.96
A_51_P301537	Mus musculus RIKEN cDNA 4933413N12 gene (4933413N12Rik), mRNA [XM_128561]	4933413N12Rik	XM_128561	111.7	102.2	15239.3	1.09	149.17	136.48
A_51_P487487	spermatogenesis associated glutamate (E)-rich protein 4d	Speer4d	NM_025759	257.9	18.0	2515.6	14.37	140.12	9.75
A_51_P290626	gulonolactone (L-) oxidase	Gulo	NM_178747	183.0	15.2	2086.8	12.03	137.16	11.40
A_51_P361448	scavenger receptor class A, member 5 (putative)	Scara5	NM_028903	168.8	14.6	1975.2	11.52	134.83	11.70
A_52_P146513	Unknown	NAP102411-1	null	151.5	12.1	1608.8	12.53	133.04	10.62
A_52_P474089	calpain 6	Capn6	NM_007603	126.6	11.0	1452.8	11.52	132.27	11.48
A_52_P531731	hypothetical protein 6430537F04	6430537F04	AK032394	129.0	13.8	1748.5	9.34	126.54	13.55
A_52_P330868	Mus musculus similar to spermatogenesis associated glutamate (E)-rich protein 4b; sperm-associated glutamate (E)-rich protein 4b; spermatogenic-associated glutamate (E)-rich protein 4b (LOC380883), mRNA [XM_354792]	XM_354792	XM_354792	117.1	10.1	1269.0	11.63	126.02	10.84
A_52_P220933	predicted gene, ENSMUSG00000033219	ENSMUSG000000	NM_198666	133.6	12.4	1353.1	10.77	109.08	10.13
A_51_P182131	RIKEN cDNA 5330417C22 gene	5330417C22Rik	BC022655	84.0	9.1	969.0	9.18	105.94	11.54
A_52_P563908	prospero-related homeobox 1	Prox1	AK038396	378.7	49.6	5009.2	7.63	100.89	13.23
A_52_P564706	BC024495 secreted frizzled-related sequence protein 1 {Mus musculus}, complete [TC1002499]	TC1002499	null	345.8	42.8	4291.0	8.08	100.23	12.41
A_51_P283507	ATP-binding cassette, sub-family A (ABC1), member 13	Abca13	AK044229	4885.7	573.4	53386.6	8.52	93.10	10.93
A_51_P511000	Mus musculus RIKEN cDNA C130090K23 gene (C130090K23Rik), mRNA.	C130090K23Rik	NM_145560	3803.4	422.8	38506.3	9.00	91.08	10.12
A_51_P240476	dynein, axonemal, heavy chain 1 1	Dnahc11	NM_010060	240.0	31.9	2842.7	7.53	89.15	11.84
A_51_P368210	phytanoyl-CoA hydroxylase interacting protein	Phyhip	NM_145981	260.4	26.4	2288.7	9.88	86.85	8.79
A_51_P211786	carbohydrate (chondroitin 4) sulfotransferase 13	Chst13	AK004401	127.6	16.8	1365.1	7.61	81.41	10.70
A_51_P352296	secreted frizzled-related protein 1	Sfrp1	NM_013834	8523.7	1039.0	81385.9	8.20	78.33	9.55
A_51_P295621	ADAMTS-like 3	Adamtsl3	AK081635	34.9	8.5	661.1	4.10	77.75	18.96
A_52_P642005	calmodulin binding transcription activator 1	Camta1	AK122383	693.6	112.6	8012.3	6.16	71.15	11.55
A_51_P515046	Unknown	A_51_P515046	null	91.4	16.0	1033.6	5.72	64.64	11.31
A_52_P851214	Mus musculus adult male hippocampus cDNA, RIKEN full-length enriched library, clone:C630035I16 product:unclassifiable, full insert sequence.	Clstn2	AK049981	9.5	13.4	864.4	0.71	64.46	90.90
A_52_P1020368	Mus musculus 0 day neonate cerebellum cDNA, RIKEN full-length enriched library, clone:C230070P17 product:unclassifiable, full insert sequence.	1700049G17Rik	AK048799	81.3	65.4	3917.2	1.24	59.90	48.19
A_51_P419286	basic leucine zipper transcription factor, ATF-like 3	Batf3	NM_030060	716.4	113.4	6727.4	6.32	59.35	9.39
A_52_P452667	prominin 2	Prom2	NM_178047	2549.5	412.0	24255.6	6.19	58.87	9.51
A_51_P505967	sperm associated antigen 6	Spag6	NM_015773	167.4	34.6	2030.9	4.83	58.64	12.13
A_51_P322473	RIKEN cDNA 2310042D19 gene	2310042D19Rik	NM_172417	473.9	85.3	4928.7	5.56	57.79	10.40
A_51_P466857	V-set and transmembrane domain containing 2A	Vstm2a	NM_145967	882.5	164.1	9181.2	5.38	55.93	10.40
A_52_P97699	RIKEN cDNA D430019H16 gene	D430019H16Rik	BC058677	661.0	128.7	6969.7	5.14	54.16	10.54
A_52_P153019	prostaglandin F receptor	Ptgfr	NM_008966	3112.8	620.2	32934.1	5.02	53.10	10.58
A_52_P529660	Mus musculus leucine rich repeat containing 52 (Lrrc52), mRNA.	Lrrc52	XM_136373	45.5	12.0	629.1	3.78	52.28	13.81
A_51_P147942	insulin-like growth factor 1	Igf1	NM_010512	143.6	27.4	1428.3	5.24	52.15	9.95
A_51_P264934	matrilin 4	Matn4	NM_013592	260.6	44.2	2204.9	5.90	49.94	8.46
A_51_P341543	defensin beta 7	Defb7	NM_139220	7.2	10.4	502.1	0.70	48.32	69.35
A_52_P91720	SLIT and NTRK-like family, member 4	Slitrk4	NM_178740	90.4	8.3	402.1	10.83	48.17	4.45
A_52_P144818	protein phosphatase 1, regulatory (inhibitor) subunit 1A	Ppp1r1a	NM_021391	2544.2	519.5	24174.5	4.90	46.53	9.50
A_52_P585339	hypothetical protein 9830132L24	9830132L24	AK036546	48.3	11.1	514.0	4.35	46.39	10.65
A_51_P175988	5-hydroxytryptamine (serotonin) receptor 3A	Htr3a	NM_013561	40.7	10.0	462.4	4.07	46.17	11.36
A_52_P536434	insulin-like growth factor 1	Igf1	NM_184052	122.3	26.8	1191.7	4.56	44.48	9.75
A_51_P465082	TOX high mobility group box family member 3	Tox3	NM_172913	2993.6	646.2	28597.5	4.63	44.25	9.55

				Average Raw Intensity			RATIOS		
Probe ID	Description	Gene Symbol	GenBank ID	All	EGFP-	EGFP+	All/EGFP-	EGFP+/EGFP-	EGFP+/All
A_51_P184508	spermatogenesis associated glutamate (E)-rich protein 5, pseudogene 1	Speer5-ps1	AK005695	230.7	49.7	2157.1	4.65	43.44	9.35
A_51_P220317	cell division cycle associated 7 like	Cdca7l	NM_146040	103.0	23.5	1020.6	4.37	43.35	9.91
A_51_P172085	Rho GDP dissociation inhibitor (GDI) gamma	Arhgdig	NM_008113	135.9	26.2	1115.6	5.19	42.61	8.21
A_51_P335146	histocompatibility 60	H60	AF084643	10.2	11.5	473.9	0.89	41.26	46.46
A_51_P302566	monoamine oxidase B	Maob	NM_172778	1169.1	286.3	11744.3	4.08	41.03	10.05
A_51_P433237	cadherin 3	Cdh3	NM_007665	202.3	59.0	2389.4	3.43	40.50	11.81
A_51_P264527	RIKEN cDNA B230317C12 gene	B230317C12Rik	NM_019833	799.6	166.3	6721.4	4.81	40.41	8.41
A_52_P426740	RAB27A, member RAS oncogene family	Rab27a	NM_023635	1065.8	271.4	10746.5	3.93	39.59	10.08
A_51_P380078	Fc fragment of IgG binding protein	Fcgbp	BC026653	44.9	10.1	396.7	4.46	39.35	8.83
A_51_P104977	expressed sequence C77713	C77713	C77713	34.9	12.1	473.4	2.88	39.09	13.57
A_52_P16482	calcium channel, voltage-dependent, beta 4 subunit	Cacnb4	NM_146123	743.3	220.1	8288.2	3.38	37.66	11.15
A_51_P385974	receptor-associated protein of the synapse	Rapsn	NM_009023	272.7	67.0	2501.1	4.07	37.35	9.17
A_51_P273170	nucleolar protein 3 (apoptosis repressor with CARD domain)	Nol3	NM_030152	2201.3	541.8	19634.3	4.06	36.24	8.92
A_52_P305539	kininogen 2	Kng2	NM_201375	61.0	16.4	592.3	3.71	36.01	9.71
A_51_P227785	kelch-like 3 (Drosophila)	Klhl3	AK033046	1913.4	491.3	17584.5	3.89	35.79	9.19
A_52_P87804	major facilitator superfamily domain containing 4	Mfsd4	NM_172510	2772.1	733.3	25844.8	3.78	35.24	9.32
A_52_P566653	WNK lysine deficient protein kinase 1	Wnk1	AK044849	950.4	273.1	9503.3	3.48	34.79	10.00
A_52_P207314	Mus musculus HtrA serine peptidase 4 (Htra4), mRNA.	Htra4	XM_284398	151.1	46.8	1606.9	3.23	34.31	10.64
A_51_P125383	ankyrin repeat and SOCS box-containing protein 11	Asb11	NM_026853	221.7	59.1	2025.4	3.75	34.29	9.14
A_52_P638337	RIKEN cDNA 5031410I06 gene	5031410I06Rik	NM_207657	42.2	9.3	317.5	4.52	34.06	7.53
A_51_P466633	solute carrier family 16 (monocarboxylic acid transporters), member 7	Slc16a7	NM_011391	13644.9	3199.6	108758.6	4.26	33.99	7.97
A_51_P469688	synaptotagmin XVII	Syt17	NM_138649	307.5	87.8	2962.4	3.50	33.72	9.63
A_52_P280448	Unknown	TC1045172	null	385.6	109.4	3677.0	3.52	33.60	9.54
A_52_P94482	tweety homolog 1 (Drosophila)	Ttyh1	NM_021324	70.6	16.3	533.5	4.32	32.68	7.56
A_51_P206225	urocanase domain containing 1	Uroc1	NM_144940	4328.1	1096.3	35765.4	3.95	32.62	8.26
A_52_P519943	fatty acid desaturase domain family, member 6	Fads6	BC044804	156.7	49.2	1603.1	3.19	32.61	10.23
A_52_P434073	ligand of numb-protein X 1	Lnx1	BC040367	84.0	18.3	594.3	4.58	32.39	7.07
A_52_P322181	adrenergic receptor, beta 1	Adrb1	AK039569	577.8	189.7	6120.2	3.05	32.26	10.59
A_51_P436269	glutamate receptor, ionotropic, NMDA2C (epsilon 3)	Grin2c	NM_010350	215.8	48.1	1549.3	4.48	32.18	7.18
A_52_P423810	metallothionein 1	Mt1	BC027262	1653.2	574.8	18262.7	2.88	31.77	11.05
A_51_P212592	RIKEN cDNA E430002G05 gene	E430002G05Rik	NM_173749	2309.7	672.4	20865.4	3.43	31.03	9.03
A_52_P489495	G87803 protein K04F10.5 [imported] - Caenorhabditis elegans, partial (3%) [TC975904]	TC975904	null	1300.8	376.6	11400.7	3.45	30.27	8.76
A_52_P154918	RIKEN cDNA C230095G01 gene	C230095G01Rik	NM_178768	1817.9	551.1	16503.6	3.30	29.95	9.08
A_51_P302398	RIKEN cDNA 1700028J19 gene	1700028J19Rik	AK006462	736.2	218.3	6486.0	3.37	29.71	8.81
A_52_P707535	Mus musculus 16 days embryo head cDNA, RIKEN full-length enriched library, clone:C130006F20 product:unclassifiable, full insert sequence.	Tiam2	AK047848	102.9	28.9	851.7	3.56	29.50	8.28
A_52_P419455	adenosine A2b receptor	Adora2b	NM_007413	344.4	111.6	3200.1	3.09	28.66	9.29
A_52_P88007	solute carrier family 6 (neurotransmitter transporter, L-proline), member 7	Slc6a7	NM_201353	44.7	11.2	321.1	3.97	28.58	7.19
A_52_P321140	defensin beta 1	Defb1	NM_007843	17432.9	4517.5	128391.1	3.86	28.42	7.36
A_51_P299149	glutathione peroxidase 6	Gpx6	NM_145451	2312.2	750.7	21225.8	3.08	28.28	9.18
A_52_P351116	IGFB_MOUSE Insulin-like growth factor IB precursor (IGF-IB) (Somatomedin). [Mouse] {Mus musculus}, complete [TC1060680]	TC1060680	null	46.1	15.9	449.3	2.90	28.23	9.75
A_52_P939420	Mus musculus 16 days neonate thymus cDNA, RIKEN full-length enriched library, clone:A130096P14 product:weakly similar to L1 REPETITIVE SEQUENCE-ENCODED ORF-2 (FRAGMENT) [Mus musculus], full insert sequence.	Ap3b1	AK038350	8.1	9.6	267.6	0.84	27.90	33.20
A_51_P247883	collagen, type V, alpha 2	Col5a2	NM_007737	913.5	329.4	9177.9	2.77	27.86	10.05
A_51_P199168	cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	Cidea	NM_007702	209.2	17.2	479.7	12.15	27.86	2.29
A_51_P233153	Ca2+-dependent activator protein for secretion 2	Cadps2	NM_153163	1226.9	389.8	10834.4	3.15	27.79	8.83
A_51_P138496	Mus musculus adult male corpora quadrigemina cDNA, RIKEN full-length enriched library, clone:B230337C21 product:hypothetical protein, full insert sequence.	Tdrd3	AK046037	47.3	14.9	413.1	3.17	27.69	8.73
A_51_P363338	protein kinase C, mu	Prkcm	NM_008858	1576.2	504.0	13936.0	3.13	27.65	8.84
A_51_P309854	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2	Kcnn2	NM_080465	315.5	115.5	3119.7	2.73	27.02	9.89
A_52_P420608	gene model 1631, (NCBI)	Gm1631	NM_201366	36.4	16.0	428.9	2.28	26.81	11.78
A_51_P351062	outer dense fiber of sperm tails 4	Odf4	NM_145746	254.4	70.0	1860.2	3.64	26.59	7.31
A_52_P182298	relaxin 1	Rln1	Z27088	175.8	45.4	1198.1	3.88	26.42	6.82

Supplementary Table 3: Densitometric quantification of immunoblots

	WT	**I-1$^{-/-}$**
Total NCC	100 ± 4.5	111.5 ± 6.3
pT53 NCC	100 ± 6.1	41.7 ± 3.7 ***
pT58 NCC	100 ± 12.7	44.5 ± 5.8 ***
pS71 NCC	100 ± 8.5	51.9 ± 8.7 ***
pS89 NCC	100 ± 13.9	54.4 ± 6.1 *
NaPi2a	100 ± 5.0	101.5 ± 16.3
NKCC2	100 ± 5.6	86.2 ± 14.7
αENaC (90kD)	100 ± 7.3	100.0 ± 6.7
αENaC (30kD)	100 ± 12.5	123.6 ± 11.9
βENaC	100 ± 10.8	79.6 ± 4.2
γENaC (85kD)	100 ± 5.8	107.9 ± 6.4
γENaC (70kD)	100 ± 7.8	103.4 ± 2.5
Pendrin	100 ± 6.1	119.1 ± 2.8 *
NDCBE	100 ± 4.1	106.6 ± 2.8

Supplementary Table 4: Renal gene expression in I1$^{-/-}$ mice

	WT	**I-1$^{-/-}$**
NKCC2	0.0399 ± 0.0009	0.0404 ± 0.0043
NCC	0.554 ± 0.017	0.601 ± 0.018
Renin	0.0386 ± 0.0032	0.0455 ± 0.0036
WNK1 Kidney-Specific	0.0482 ± 0.0018	0.0531 ± 0.0027
WNK4	0.0511 ± 0.0020	0.0516 ± 0.0029