Supporting Information

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SI Materials and Methods

Generation of the Targeting Vector. A BAC containing part of the mouse *With No lysine (K) 1* (*WNK1*) locus was identified by PCR and Southern blot screening of the Mouse BAC Library (Life Technologies) constructed from CJ7/129SV ES cells by the California Institute of Technology (CITB). The chosen BAC contains exons 1–21 of *mWNK1*, 4 kb sequence upstream of the first exon and 360 bp sequence downstream of exon 21. All strains and protocols for BAC modifications were described previously (1, 2). The sequence of the primers used to amplify all of the homology arms by PCR is given in Table S1. The BAC construct was checked at each step by DNA digestion and direct sequencing of the targeted regions. Briefly, the loxP site in the BAC backbone was deleted and replaced by an ampicillin resistance gene, a *pgk-DTA* cassette for negative selection of the ES cells clones, and an *MluI* restriction site for linearization using a modified pTAmp plasmid (1). One loxP site and a *pgk-neo*^{r} resistance cassette flanked by two flippase recognition target (FRT) sites were inserted 66 bp downstream of *mWNK1* exon 1 using the pL451 vector (2). A second loxP site and a *pgk-puro*^{r} resistance cassette flanked by two FRT3 sites (5′-GAAGTTCCTATACTATTTGAGAATAGGAACTTC-3′) were inserted 93 bp upstream of *mWNK1* exon 2.

Generation of the Mouse Model for Familial Hyperkalemic Hypertension.

Generation of ES cells clones. The modified BAC was linearized by enzymatic restriction with *MluI* and purified by phenol-chloroform and ethanol precipitation. Its integrity was checked by electrophoresis; 129S2/SvPas mouse ES cells were electroporated with 25 μg linearized vector. After a 2-wk selection with both G418 and puromycin, individual clones were picked. Genomic DNA was extracted and then submitted to enzymatic restriction with *PvuII* to identify by Southern blotting clones (Fig. S1).

Generation of* WNK1$^{+/\text{Familial Hyperkalemic Hypertension}}$ *animals. *WNK1*$^{+/i1loxneo}$ mice were generated by microinjecting ES cells into blastocysts using standard techniques. Chimeric founders were crossed with C57BL/6N animals to establish the *WNK1*$^{+/i1loxneo}$ line. These animals were then crossed with mice expressing the flippase FLP recombinase under the control of the promoter of the human actin beta gene (hACTB) (3) to delete the *pgk-neo*^{r} and *pgkpuro*^{r} selection cassettes. Resulting heterozygous *WNK1*$^{+/i1lox}$ females were then bred with *pgk-Crem* transgenic males (4) expressing the Cre recombinase in the germ line to obtain genetically identical *WNK1*$^{+/i1lox}$ and *WNK1*$^{+/\text{FHHt}}$ animals. Mice were genotyped by PCR (Fig. S1 and Table S1).

Telemetry. Experiments were performed on seven *WNK1*$^{+/i1lox}$ and six *WNK1*$^{+/FHHt}$ male mice. The catheter of the blood pressure telemeter (model TA11PA-C10; Data Sciences International) was inserted into the aorta through the left common carotid artery (5). The telemetric transmitter probe was positioned s.c. on the flank. After the mice had recovered from the anesthesia in a warm (36 °C) box, they were housed in individual cages placed on top of the telemetric receivers in a light/dark-cycled recording room (7:00 AM to 7:00 PM). After a 1-wk recovery period, recordings of cardiovascular parameters and locomotor activity were obtained continuously during 24 h in the freely moving mice in their home cages every 15 min for 60 s. Each recording was visualized to select one segment without erratic fluctuations of enough duration (51.2 s) every 15 min (four segments per hour) for 24 h (i.e., 96 segments for each animal per day). The values for each recording segment were averaged over a 12-h period (7:00 AM to 7:00 PM for daytime and 7:00 PM to 7:00 AM for nighttime).

1. Lee EC, et al. (2001) A highly efficient Escherichia coli-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. *Genomics* 73(1):56–65.
2. Liu P, Jenkins NA, Copeland NG (2003) A highly efficient recombineering-based method for generating conditional knockout mutations. *Genome Res* 13(3):476–484.
3. Rodríguez CI, et al. (2000) High-efficiency deleter mice show that FLPe is an alternative to Cre-loxP. *Nat Genet* 25(2):139–140.
4. Lallemand Y, Luria V, Haffner-Krausz R, Lonai P (1998) Maternally expressed PGK-Cre transgene as a tool for early and uniform activation of the Cre site-specific recombinase. *Transgenic Res* 7(2):105–112.
5. Butz GM, Davisson RL (2001) Long-term telemetric measurement of cardiovascular parameters in awake mice: A physiological genomics tool. *Physiol Genomics* 5(2):89–97.

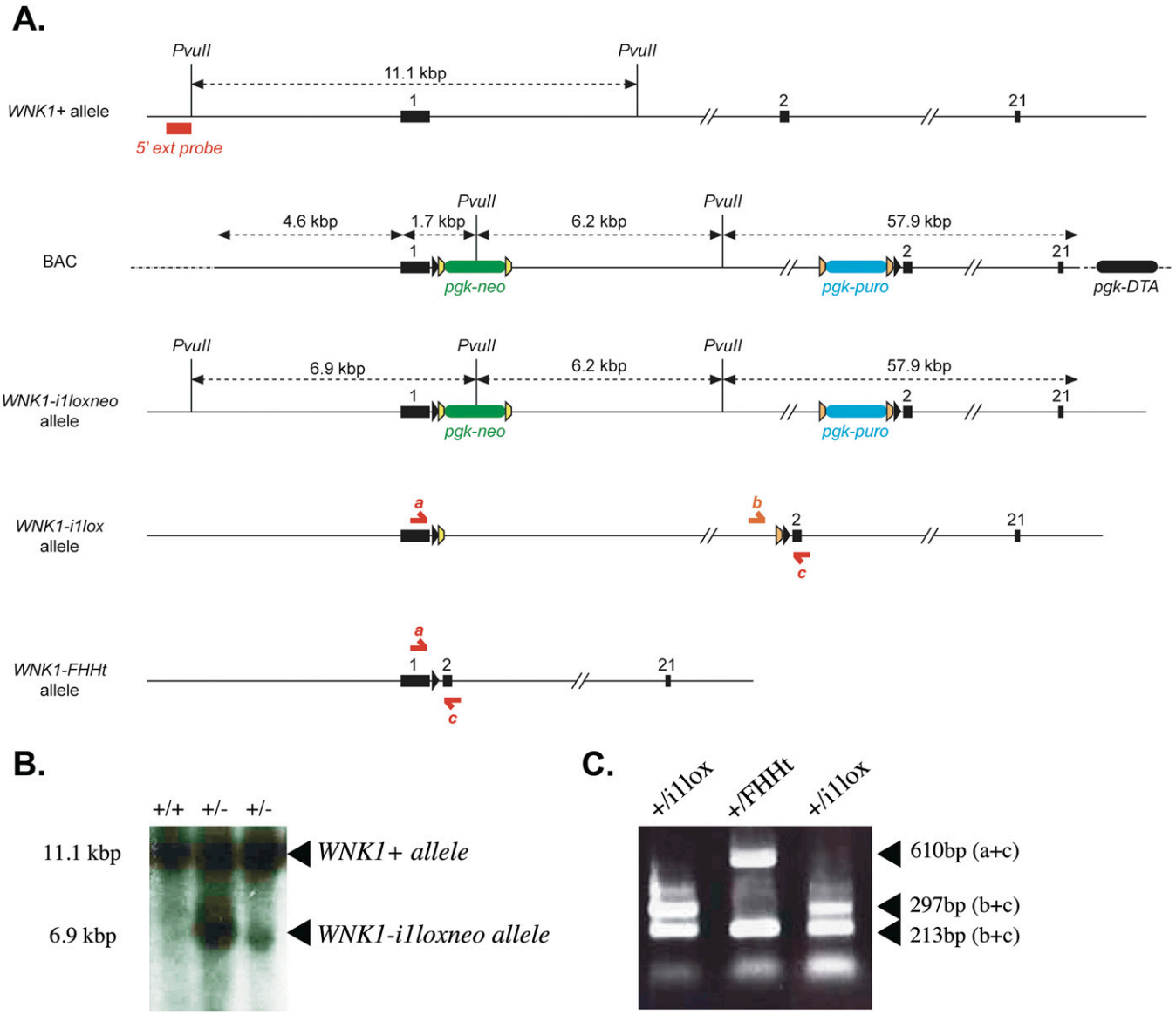
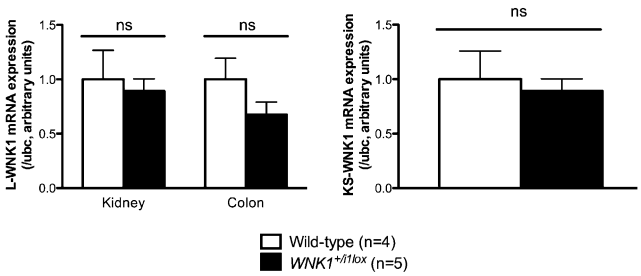


Fig. S1. Generation of WNK1$^{+/FHHt}$ mice. (*A*) Targeting strategy for generating *WNK1*$^{+/FHHt}$ mice. The diagram shows the WT *mWNK1* locus, the targeting construct (BAC), and the targeted locus before (*WNK1-i1loxneo* allele) and after (*WNK1*^{i1lox} allele) FLP recombination and after Cre recombination (*WNK1*^{FHHt} allele). The black triangles represent the loxP sites, and the yellow and orange trapezoids correspond to the WT and mutated (FRT3) FRT sites, respectively. (*B*) Verification of homologous recombination by Southern blotting of PvuII-digested genomic DNA of the selected ES cell clones; the probe (5′ext probe) is shown as a red rectangle in *A*. The 6.9-kbp band corresponds to the insertion of recombination sites and selection cassette near exon 1. (*C*) Genotyping of the *WNK1*^{i1lox} and *WNK1*^{FHHt} alleles by PCR on genomic DNA derived from mouse tail. The localization of the primers used are shown in *A* (red and orange arrows above and below the schematic representation of the *WNK1*^{FHHt} allele). Primer a, mWNK1-i1-5LAs; primer b, mWNK1-i15′puro2-s; primer c, mWNK1-i13′puro-as. An example of a representative PCR is given: the 213-bp band corresponds to the WT allele, the 297-bp band corresponds to the *WNK1*^{i1lox} allele, and the 610-bp band corresponds to the *WNK1*^{FHHt} allele.

A.

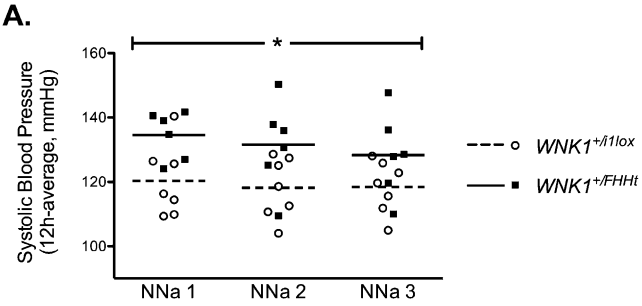


B.

	Wild-type (n=5)	$WNK1^{+/i1lox}$ (n=5)
Na^+ (mmol/L)	148.0 ± 0.5	147.2 ± 0.4
K^+ (mmol/L)	4.8 ± 0.2	4.8 ± 0.2
Cl^- (mmol/L)	111.0 ± 0.4	110.2 ± 1.1
pH	7.33 ± 0.04	7.35 ± 0.02
HCO_3^- (mmol/L)	22.08 ± 0.77	22.18 ± 0.61
Hematocrit	36.2 ± 1.2	35.4 ± 1.6

Fig. S2. Introduction of loxP sites at each extremity of *WNK1* first intron does not affect *WNK1* expression or blood electrolytes. (*A*) Long WNK1 (L-WNK1) and kidney-specific WNK1 (KS-WNK1) expression are similar in the tissues of $WNK1^{+/i1lox}$ ($n = 5$) and WT ($n = 4$) female mice. (*B*) Na^+, K^+, and Cl^- plasma concentrations are similar in $WNK1^{+/i1lox}$ ($n = 5$) and WT ($n = 5$) male mice. ns, not significant.

A.



B.

	$WNK1^{+/i1lox}$			$WNK1^{+/FHHt}$		
	NNa1	NNa2	NNa3	NNa1	NNa2	NNa3
Number of animals	7	7	7	6	6	6
Mean	120.3	118.2	118.4	134.5	131.6	128.3
St. dev.	11.16	9.4	8.2	7.4	13.7	13.0
s.e.m.	4.2	3.5	3.1	3.0	5.6	5.3
Lower 95%CI	110	109.5	110.8	126.8	117.2	114.7
Upper 95% CI	130.7	126.9	125.9	142.3	146	142

Fig. S3. Systolic blood pressure is increased in $WNK1^{+/FHHt}$ mice. (*A*) Systolic blood pressure of $WNK1^{+/i1lox}$ ($n = 7$; white circles) and $WNK1^{+/FHHt}$ ($n = 6$; black squares) mice recorded over 3 d in basal conditions. Individual values (12-h mean of the night period) are shown; the lines correspond to the mean. * $P < 0.05$ (repeated measures ANOVA). (*B*) Mean, SD, SEM, and confidence interval (CI) for each baseline day. NNa, control diet.

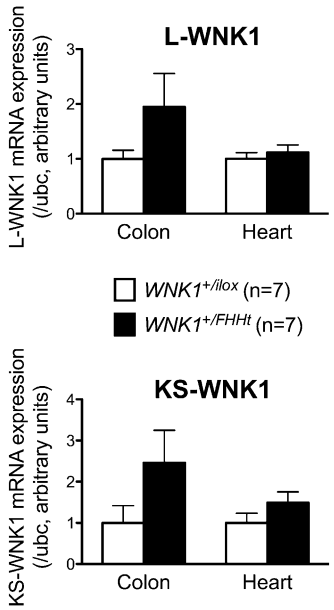


Fig. S4. Extrarenal expression of L- and KS-WNK1 on deletion of *WNK1* first intron. Quantification of the expression level of (*Upper*) L-WNK1 and (*Lower*) KS-WNK1 transcripts in the cortical segments of the nephron of $WNK1^{i1lox}$ ($n = 7$) and $WNK1^{FHHt}$ ($n = 7$) mice by real-time quantitative RT-PCR showed that expression of both isoforms is not modified by the intronic deletion in the heart and colon. Results (mean ± SEM) are expressed in arbitrary units relative to the expression of ubiquitin c (ubc).

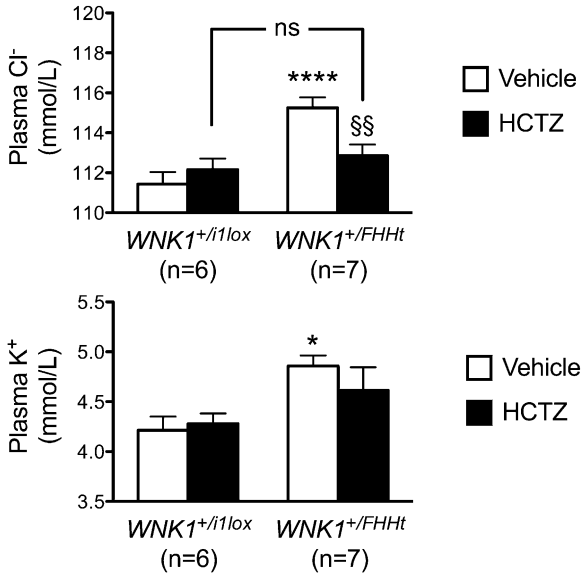


Fig. S5. Plasma Cl^- but not plasma K^+ is normalized by a single injection of hydrochlorothiazide (HCTZ) in $WNK1^{+/FHHt}$ mice. Vehicle (0.9% NaCl) or HCTZ (12.5 mg/mL in 0.9% NaCl) was injected i.p., and mice were placed in metabolic cages for 3 h to collect urine and measure food and water intake. Blood was then sampled from the rero-orbital sinus of mice anesthetized with isoflurane, and plasma Cl^- and K^+ were measured with a Radiometer ABL80 Flex. Plasma Cl^- and K^+ are not modified by the HCTZ injection in $WNK1^{+/i1lox}$ mice ($n = 6$), whereas plasma Cl^- but not K^+ was reduced in $WNK1^{+/FHHt}$ mice to a level comparable with the level of control mice. $^*P < 0.05$ and $^{****}P < 0.0001$ compared with $WNK1^{+/i1lox}$ mice injected with vehicle; $^{§§}P < 0.01$ compared with $WNK1^{+/FHHt}$ mice injected with vehicle (Bonferroni multiple comparisons test after a one-way ANOVA); ns, not significant.

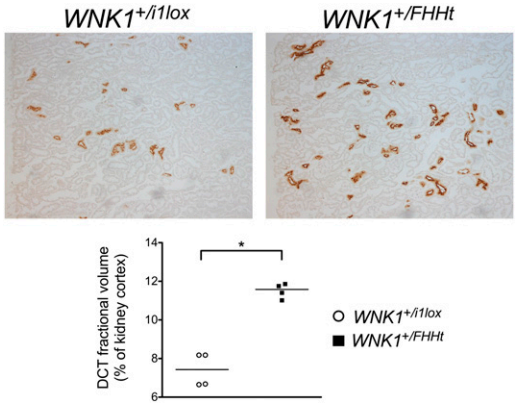


Fig. S6. Increased distal convoluted tubule (DCT) fractional volume in WNK1$^{+/FHHt}$ mice. Representative images of Na^+–Cl^- cotransporter (NCC) immunostaining in (*Upper Left*) WNK1$^{+/i1lox}$ and (*Upper Right*) WNK1$^{+/FHHt}$ kidney sections. (*Lower*) DCT fractional volume, quantified as the number of intersections between DCTs (identified as NCC-positive nephron segments) and a 50-μm grid, is increased in *WNK1$^{+/FHHt}$ mice*. Individual data are plotted, and the bar corresponds to the median. *$P < 0.05$ (Mann–Whitney nonparametric ranking test).

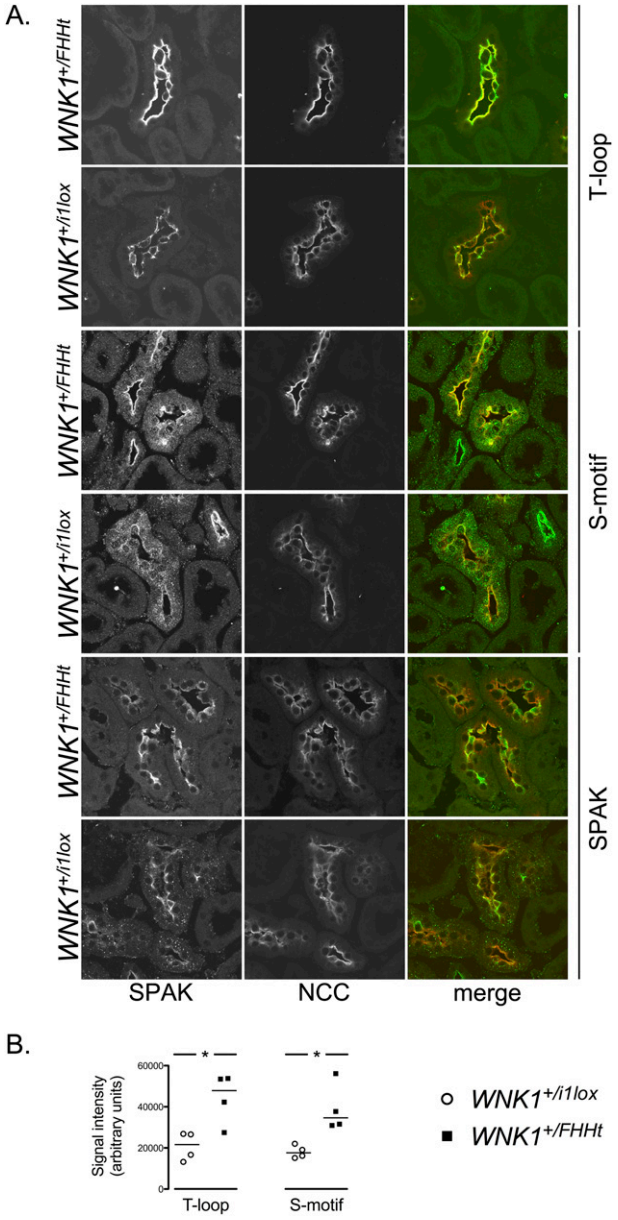


Fig. S7. The abundance of phosphorylated Ste20-related proline-alanine rich kinase (SPAK) is increased in the subapical compartment of DCT cells in *WNK1*$^{+/FHHt}$ mice. (*A*) Representative immunolabeling of SPAK, phosphorylated SPAK T loop, or phosphorylated SPAK S motif and double staining for NCC in the renal cortex of *WNK1*$^{+/i1lox}$ and *WNK1*$^{+/FHHt}$ mice. (*B*) Signal intensities obtained by confocal evaluation of the fluorescence signals using ZEN software. Individual data are plotted, and the bars correspond to the median. Statistical analysis was performed using a nonparametric Mann–Whitney ranking test. *$P < 0.05$.

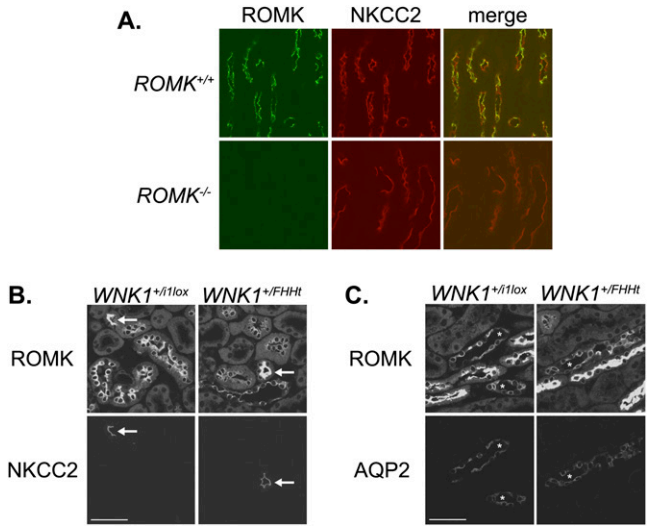


Fig. S8. Renal outer medullary potassium (K) channel (ROMK) expression is not modified in the collecting ducts of *WNK1*$^{+/FHHt}$ mice. (*A*) Representative images of *ROMK*$^{+/+}$ and *ROMK*$^{-/-}$ kidneys after colabeling for ROMK and the Na^+-K^+-$2Cl^-$ cotransporter NKCC2. No signal is detected with the ROMK antibody in *ROMK*$^{-/-}$ kidney, thereby showing its specificity. (*B*) Representative images of *WNK1*$^{+/i1lox}$ and *WNK1*$^{+/FHHt}$ kidneys after double labeling for ROMK and NKCC2. (Scale bar: 50 μm.) The signal detected in the thick ascending limb of Henle's loop (arrows) is much stronger than in the adjacent segments, thereby precluding the quantification of ROMK expression in the DCT, connecting tubule, and collecting duct (CD) by immunoblot on cortex homogenates. (*C*) Representative images of the medullary rays of *WNK1*$^{+/i1lox}$ and *WNK1*$^{+/FHHt}$ kidneys after double labeling for ROMK and AQP2. (Scale bar: 50 μm.) Thick ascending limb was identified by the typical strong expression of ROMK (asterisks), and CDs were identified by presence of significant AQP2 signal. ROMK expression is more similar in *WNK1*$^{+/FHHt}$ CD, identified by the presence of AQP2 signal, than *WNK1*$^{+/i1lox}$ CDs.

Table S1. Administration of HCTZ for 3 d completely corrected the metabolic disorders of *WNK1*$^{+/FHHt}$ mice

	HCTZ (0.24 mg/g per day in food)	
Plasma	*WNK1*$^{+/i1lox}$ ($n = 7$)	*WNK*$^{+/FHHt}$ ($n = 6$)
Na^+ (mmol/L)	147 ± 0.4	145.8 ± 0.6
K^+ (mmol/L)	4.5 ± 0.3	4.7 ± 0.6
Cl^- (mmol/L)	109.7 ± 0.9	109.2 ± 0.6
pH	7.37 ± 0.02	7.37 ± 0.01
HCO_3^- (mmol/L)	23.4 ± 0.5	24.7 ± 0.5

Table S2. Sequence of primers used for PCR amplification and cloning of the homology arms used to modify the BAC, mice genotyping, and real-time quantitative RT-PCR

	Sequence
Homology arms subcloning	
N-i15′LA-s	ataagaatgcggccgcCCAGCAGTACCAGCAAAGACC
Bg-i15′LA-as	gaagatctAAACTGGCTTAACAGAGCCAAA
E-i15′RA-s	cggaattcTGTCGAGTTCACTCTTTACTTG
X-i15′RA-as	ccgctcgagAATTCCCAAAGGCTGAGGATA
E-i13′LA-s	gcgaattcAGCTCCTGTAAAGGCACCAA
X-i13′LA-as	Ccgctcgagatggtgacaatctcagaagtcag
Sp-lox-i13′RA-s	gcACTAGTATAACTTCGTATAGCATACATTATACGAAGTTATGTGCCATCTTGACCAGCTTATT
Nh-i13′RA-as	gctagcAGAGGGCAACCTTGACATTG
Genotyping	
mWNK1-i15′Las	CCAGCAGTACCAGCAAAGACC
mWNK1-i15′puro2-s	TACCTGACTTCTGAGATTGTCACC
mWNK1-i13′puro-as	CGAACAATGTTGGGATGTTG
Real-time quantitative RT-PCR (SyBr Green)	
mWNK1-qPCR-ex2s	Cgtctggaacacttaaaacgtatct
mWNK1-qPCR-ex3as	Caccagcttcttaaaactttgattt
mWNK1-qPCR-ex4a-s	Ttgttattgtaaattctcattgctg
mWNK1-qPCR-ex5as	AGGAATTGCTACTTTGTCAAAACTG
mNCC-s	CTGGAGAACCTGTTCGCTTC
mNCC-as	GACTTGACCTTGCCATTGGT
Renin-F	ATGAAGGGGGTGTCTGTGGGG
Renin-R	ATGTCGGGGAGGGTGGGCACCTG
mUbc-s	AGCCCAGTGTTACCACCAAG
mUbc-as	ACCCAAGAACAAGCACAAGG

In the sequence of the subcloning primers, the lowercase letters correspond to the sequence of the restriction site used for subcloning. In the sequence of Sp-lox-i13′RA-s, the underlined sequence is the loxP site.