**Severe hyperkalemia is rescued by low potassium diet in renal βENaC-deficient mice**

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Running head: PHA1 in mice lacking βENaC

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**Abstract**

In adulthood, an induced nephron-specific deficiency of αENaC (Scnn1a) resulted in Pseudohypoaldosteronism type 1 (PHA-1) with sodium loss, hyperkalemia and metabolic acidosis, that is rescued through high sodium/low potassium ($HNa^+/LK^+$) diet. In the present study, we addressed whether renal βENaC expression is required for sodium and potassium balance or can be compensated by remaining (α and γ) ENaC subunits using adult nephron-specific knockout ($Scnn1b^{Pax8/LC1}$) mice. Upon induction, these mice present a severe PHA-1 phenotype with weight loss, hyperkalemia and dehydration, but unlike the $Scnn1a^{Pax8/LC1}$ mice without persistent salt wasting. This is followed by a marked downregulation of STE20/SPS1-related proline-alanine-rich protein kinase (SPAK) and $Na^+/Cl^-$ cotransporter (NCC) protein expression and activity. Most of the experimental $Scnn1b^{Pax8/LC1}$ mice survived with a $HNa^+/LK^+$ diet that partly normalized NCC phosphorylation, but not total NCC expression. Since salt loss was minor, we applied a standard sodium/$LK^+$ diet that efficiently rescued these mice resulting in normokalemia and totally normalization of NCC phosphorylation, but not total NCC expression. A further switch to $LNa^+$/standard $K^+$ diet induced again a severe PHA-1-like phenotype, but with only transient salt wasting indicating that low $K^+$ intake is critical to decrease hyperkalemia in a NCC-dependent manner. In conclusion, while the βENaC subunit plays only a minor role in sodium balance, severe hyperkalemia results in downregulation of NCC expression and activity. Our data demonstrated the importance to primarily correct the hyperkalemia with a low potassium diet that normalizes NCC activity.

**Introduction**

Two different forms of PHA-1 exist, the renal and the systemic form. The renal form (PHA-1) results from mutations in the mineralocorticoid receptor gene [17]. Mutations in one of the three ENaC (α or β or γ) subunits are causative for the systemic form of pseudohypoaldosteronism type 1 (PHA-1), a salt-losing syndrome described by Cheek and Perry [47, 7]. The majority (80%) of human PHA-1 mutations are observed in the exon 8 of the SCNN1A (αENaC) gene and only 20% in the SCNN1B (βENaC) or SCNN1G (γENaC) gene [47]. ENaC is expressed at the apical side in tight epithelia of the distal colon, in respiratory epithelium, in principal cells of the distal tubule and in the collecting duct, and the duct of several excretory ducts [34]. ENaC channel has a predominant role in sodium/potassium homeostasis and blood pressure regulation. *In vivo*, the constitutive knockout of each of the three ENaC subunits (α, β, γ) revealed the importance of each subunit for survival: these knockouts die soon after birth from a combined lung and renal phenotype [19, 4, 24]. Scnn1a$^{-/-}$ neonates failed to clear the lungs of liquid and presented a life-threatening hyperkalemia [19]. Scnn1b$^{-/-}$ neonates did not develop severe respiratory distress but die from hyperkalemia. Scnn1g$^{-/-}$ neonates died from hyperkalemia, but transiently presented problems to clear their lungs of liquid [4]. Hyperkalemia was common in all three mouse models and most likely the major cause of death. Persistent and symptomatic hyperkalemia was recently also reported in a PHA1 patient [27].

We previously performed a genetic dissection of Scnn1a gene function along the nephron [34]. Using promoters driving the expression in CD (Hoxb7) [35], in CNT (Atp6v1b1) [30] or CD (aquaporin 2) [9], αENaC subunit expression was conditionally deleted in a nephron segment-specific manner. Surprisingly, the activity of αENaC in CD was dispensable and likely compensated by ENaC expression within CNT and/or DCT2 [35]. When αENaC-

mediated sodium reabsorption was deleted in CNT and CD (aquaporin 2 promoter) [9], the phenotype was mild and no perinatal lethality was observed thus raising the question of the importance of DCT2 with the remaining nephron segment still expressing ENaC.

*In vitro*, ENaC activity in oocytes is dependent on the expression of the αENaC subunit. When the αENaC subunit was expressed alone, a small but significant amiloride-sensitive current was recorded, that is 1-2% of the maximum activity measured when the three subunits (α, β, γ) were co-expressed [6]. Co-expression of aβ and αγ subunit might produce 5-10% of maximal activity whereas βγ subunits had no or barely detectable activity [5]. These observations stressed the importance of αENaC as chaperone protein to bring β, γ or βγ subunits to the plasma membrane. This prediction was fully supported *in vivo* by the inducible deletion of αENaC along the entire adult nephron and the collecting duct (excluding the glomerulus) using a doxycycline-inducible system [29]. This caused a severe salt losing syndrome with life threatening hyperkalemia if not rescued by a high sodium and low potassium diet. PHA-1 patients with βENaC mutation are currently treated with such a diet, and upon severe hyperkalemia cation exchange resins are applied [1]. Based on *in vitro* data, we predicted that the kidney without the βENaC subunit still confers a certain percentage of ENaC activity which may lead to a less severe phenotype in comparison to αENaC knockout mice.

In the present study, we addressed whether ENaC channels consisting of αγ (lacking the βENaC subunit) along the nephron generate sufficient ENaC activity to prevent life-threatening hyperkalemia, salt wasting, and lethality characteristics of systemic PHA-1. We took advantage of an inducible nephron-specific βENaC knockout mouse model that lacks the βENaC subunit along the nephron in adulthood. Our data imply that low potassium diet alone is necessary and sufficient to avoid severe hyperkalemia.

## Methods

### Generation and induction of nephron-specific Scnn1b$^{Pax8/LC1}$ mice

To inactivate the *Scnn1b* gene loci along the nephron, conditional triple-transgenic *Scnn1b$^{lox/lox}$;Pax8::rTA$^{0/tg}$;TRE::LC-1$^{0/tg}$* (βENaC, *Scnn1b$^{Pax8/LC1}$*) mice were obtained by interbreeding of *Scnn1b$^{lox/lox}$* mice [25] and single-transgenic for *Pax8::rTA$^{0/tg}$* or *TRE::LC-1$^{0/tg}$* [40] giving rise to control (*Scnn1b$^{lox/lox}$;Pax8::rtTA$^{0/tg}$; Scnn1a$^{Pax8}$*) and nephron-specific (*Scnn1b$^{lox/lox}$;Pax8::rtTA$^{0/tg}$; TRE::LC-1$^{0/tg}$; Scnn1b$^{Pax8/LC1}$*) mice. Genotyping of the mice was performed by PCR-based DNA analysis from ear biopsies using the following primers: Pax8-rtTA_ST1: 5'-CCATGTCTAGACTGGACAAGA-3', Pax8-rtTA_ST2: 5'-CTCCAGGCCACATATGATTAG-3'; LC-1 Cre3: 5′-TCGCTGCATTACCGGTCGATGC-3′; LC-1 Cre4: 5′-CCATGAGTGAACGAACCTGGTCG-3′. The recombination of the floxed *Scnn1b* allele was investigated in kidney, liver, colon, and heart by PCR analysis using the three following primers (*Scnn1b* allele): 35as, 5' CTG CTC TGG GAT TAC AGG 3'; β-1as, 5' GAT AAG GTG GGA AGA GCT GG 3'; and 36_S-2 (s), 5' CAC TCA GGC ACA TGA TAG ACA GG 3'. If not otherwise stated, 35 cycles were run, and each run consisted of 1 min. each at 94°C, 56°C, and 72°C. The *Pax8::rtTA* and the *TRE::LC1* transgene were detected by PCR using primers and protocol as previously described [29].

Animals were housed in individually ventilated cages at 23±1°C with 14 hours day and 10 hours night (breeding) and 12 hours day and 12 hours night cycle (experimental conditions), respectively, and with free access to food (standard diet, 0,17% sodium and 0.97% potassium, ssniff Spezialdiäten GmbH, Germany) and tap water if not otherwise stated. Animal maintenance and experimental procedures were in agreement with the federal guidelines and were approved by local authorities.

**Induction with doxycycline**

Following doxycycline induction (at day 0) for 2-4 days (standard diet; *protocol A*, **Supplemental Figure 1A**), parameters like water and food consumption, urine volume and feces output, and urinary electrolytes were collected and measured. Parameters like e.g. food intake, as well as urine volume and feces weight were measured 24 hours following induction. Body weight is presented as percentage of Δ body weight changes with respect to timepoint of reference weight. Plasma aldosterone and the hematocrit values were determined at day 3, and plasma electrolytes were analyzed at day 4. We further applied the following diets (**Supplemental Figure 1**). High $Na^+$ ($HNa^+$) and low $K^+$ ($LK^+$) protocols *(protocol B and C)*: After doxycycline induction, Scnn1b control and experimental mice were individually placed into metabolic cages (Tecniplast, Buguggiate, Italy) and fed a standard diet (0,17% sodium and 0.97% potassium) for 3 consecutive days followed by a switch to a high $Na^+$ (3.5% sodium) and low $K^+$ (< 0.1% potassium) diet, supplemented with 0.2% potassium in drinking water ($HNa^+/LK^+$ diet). Food was given as powder (ssniff Spezialdiäten GmbH, Germany) either up to 30 hours (acute $HNa^+/LK^+$ diet, *protocol B*, **Supplemental Figure 1B**) or up to 34 days (long-term $HNa^+/LK^+$ diet, *protocol C*, **Supplemental Figure 1C**). $LK^+$ protocols *(protocol D)*: Following 3 consecutive days under a standard diet, Scnn1b mice were fed with a standard $Na^+$ (3.5% sodium), but low $K^+$ diet (0.17% sodium; potassium < 0.1% given as powder, ssniff Spezialdiäten GmbH, Germany), supplemented with 0.2% potassium in drinking water ($LK^+$ diet) up to 25 days (*protocol D*, **Supplemental Figure 1D**). *Protocol E*, **Supplemental Figure 1E**: one series of Scnn1b control and experimental mice were switched back to a standard diet (0,17% sodium and 0.97% potassium) at day 32 after 29 days under $HNa^+/LK^+$ diet, and physiological parameters were further determined every 24 hours during five consecutive days (day 29 – 34). $LNa^+$ protocol (*protocol F*, **Supplemental Figure 1F**): Following 3 consecutive days under a standard diet, Scnn1b mice were fed with $LK^+$ diet for

16 days (between day 3 and day 19) and then were switched to a low $Na^+$ (< 0.1% sodium) and normal $K^+$ (0.97% potassium) diet ($LNa^+$ diet). The physiological parameters were determined every 24 hours during 4 consecutive days (day 19 – 23). Body weight, water and food consumption, urine volume and feces output were assessed every 6 hours (*protocol B*) or 24 hours at 6 am (*protocols A, C, D, E, and F*). $Na^+$ and $K^+$ intake were determined as the quantity of food intake multiplied by the concentration of $Na^+$ or $K^+$ containing in the diet and normalized by the mouse body weight. At the end of the experiments, and following anesthesia with 1% isoflurane, plasma samples were collected from the retro-orbital venous plexus, followed by cervical dislocation and collection of tissues (kidney, liver, colon and heart) that were frozen by liquid nitrogen.

**Analysis of urinary and plasma electrolytes and aldosterone**

(*Protocol A-F*, **Supplemental Figure 1**) Urinary and plasma $Na^+$ and $K^+$ collected in metabolic cages were measured using a flame photometer (Cole-Parmer Instrument). Food and water intake, feces output, urine volume, and $Na^+$ and $K^+$ intake and loss were normalized to the body weight of the corresponding experimental day. Sodium balance was calculated as the ratio of sodium intake normalized to the sodium output in urine and feces. Plasma aldosterone levels were measured following standard procedures using a RIA (Coat-A-Count RIA kit; Siemens Medical Solutions Diagnostics, Ballerup, Denmark) (*protocol A, C, and D* **Supplementary Figure 1; Table 1**). Plasma creatinine was measured at days 2 and 3 at the end of urine collections (*protocol A,* **Supplementary Figure 1**). (*Protocol A*, **Supplemental Figure 1**) Urinary and serum creatinine as well as serum urea and glucose concentrations were measured at the Zurich Integrative Rodent Physiology (ZIRP) platform (University of Zurich, Switzerland). The plasma osmolality was calculated as following; (2x [serum $Na^+$]) + (2x [serum $K^+$]) + [serum glucose] + [serum urea] (**Table 2**) [22]. The free water clearance

($C_{H2O}$) was calculated as following; $C_{H2O} = V - (U_{osm}*V)/ P_{osm}$, where V is urine volume (ml/min), $U_{osm}$ is urine osmolality and $P_{osm}$ is plasma osmolality. The fractional excretion of sodium and potassium was assessed as described [12]. Plasma creatinine was measured at the end of urine collections. Plasma aldosterone concentration was measured using an RIA (Coat-A-Count RIA kit; Siemens Medical Solutions Diagnostics, Ballerup, Denmark). Mouse samples with values >1200 pg/ml were further diluted using a serum pool with a low aldosterone concentration (<50 pg/ml).

**Analysis of protein expression**

Frozen tissues were homogenized and protein extracted as described [2]. Anti-βENaC and pT53-NCC antibodies were obtained and used as described [36]. Anti-pSPAK antibody was obtained from Millipore. Antibodies pSPAK was diluted 1/500. Antibodies βENaC, and pNCC (T53) were diluted 1/1000. Anti-β-actin antibody (Sigma-Aldrich) was used as loading control. Protein samples of kidney lysates from SPAK KO mice were used as controls (kindly provided by O.Staub) [11, 23].

**Immunofluorescence**

Cryosections (5 $\mu$m) of kidneys were incubated overnight at 4°C with primary antibodies against Cre (1:10'000) [20], βENaC (1:40'000) [45], or CaBP D28k (1:10'000) (Swant, Marly, CH). Binding sites of primary antibodies were detected by fluorescence-labelled secondary antibodies (goat-anti-rabbit-CY3 (1:1000), goat anti mouse-FITC (1:100); Jackson ImmunoResearch Laboratories, West Grove, PA). Images were acquired with a fluorescence microscope (Leica DM6000 B; Leica Microsystems, Buffalo Grove, IL) and processed by Leica Application Suite software before importing into Adobe Photoshop CS3 (Adobe Systems, Inc., San Jose, CA) and Powerpoint for image arrangement and labeling.

**Statistical analysis**

All values are presented as mean ± standard error of the mean (SEM) %. The data were analyzed using unpaired 2-tailed Student's t test. Values expressed as a function of time were analyzed using multiple comparisons by two-way ANOVA (Bonferroni), Scnn1b$^{Pax8/LC1}$ versus controls mice. A $p$ value less than 0.05 was considered statistically significant; *, $P< 0.05$ ; **, $P< 0.01$ ; ***, $P< 0.001$.

**Results**

**Adult nephron-specific Scnn1b-deficient mice develop a severe PHA-1 without persistent salt wasting**

To determine the role of the βENaC subunit in adult kidney, Scnn1b deletion was induced with doxycycline in four week-old mice under a standard diet (*protocol A*, **Figures 1, Supplementary Figure 1**). Using semiquantitative PCR, we identified efficient gene targeting in kidney but not in heart of Scnn1b$^{Pax8/LC1}$ mice (**Figure 1A,** lane 1 and 4). Surprisingly, we also detected recombination in liver and colon. In kidney, Western blot analyses revealed, already after 2 days of induction, a significant decrease of βENaC (92% of control, $P<0.01$) (**Figure 1B**). This was further confirmed by immunohistochemistry (**Figure 1D**). Four days following doxycycline induction, Scnn1b$^{Pax8/LC1}$ mice presented with clinical symptoms of severe PHA1 characterized by significant loss of body weight (**Figure 2A**), lower plasma sodium and life-threatening hyperkalemia (**Figure 2B**), accompanied by a more than tenfold increase of plasma aldosterone levels (**Table 1**). The 24-hours $Na^+$ excretion did not change between control and experimental group (**Figure 2C**), coherent with the $FE_{Na}$ that did not differ amongst the groups at day 2 and day 3 (0.36% in experimental versus 0.35% in

control, and 0.89% in experimental versus 0.95% in control mice, respectively). Contrary to the sodium data at day 2, there was no change of 24 h $K^+$ excretion but a significant decrease of potassium excretion at day 3 and 4 (**Figure 2D**). The $FE_K$ was significantly decreased at day 2 and 3 (22.7% in experimental versus 33.2% in control mice, $P<0.05$, and 33.1% in experimental versus 66.8% in control mice, $P<0.001$, respectively).

Body weight loss was accompanied by progressive decrease in food (**Supplementary Figure 2A**) and water intake (**Supplementary Figure 2B**), and experimental animals presented with decreased feces (**Supplementary Figure 2C**) and urine output (**Supplementary Figure 2D**). Consistent with the dramatic decrease in food intake (**Supplementary Figure 2A**), the 24-hours $Na^+$ (**Supplementary Figure 2E**) and $K^+$ intake (**Supplementary Figure 2F**) was significantly decreased at 3 and 4 days after doxycycline induction. $Scnn1b^{Pax8/LC1}$ mice presented a severe pseudohypoaldosteronism phenotype with severe dehydration, significantly increased hematocrit and plasma osmolalities, and increased plasma urea values whereas plasma glucose, and urine volume and osmolality were not different between the groups (**Table 2**). The ratio of urine to plasma osmolality ($U/P_{osm}$) and the creatinine clearance were not significantly altered. The free water clearance was negative in both control and experimental mice i.e., knockout mice are still able although less efficient to concentrate the urine than control littermates (**Table 2**).

**Scnn1b knockout mice are rescued by high $Na^+$ and low $K^+$ diet.**

To assess if the severe hyperkalemia in $Scnn1b^{Pax8/LC1}$ mice can be rescued, mice were induced by doxycycline and kept for three days under a standard diet, and then switched to a high $Na^+$ and low $K^+$ diet. Plasma and urinary electrolytes were collected to assess the physiological parameters (*protocol B,* **Supplementary Figure 1**). $Scnn1b^{Pax8/LC1}$ mice significantly lost weight after the second day (**Figure 3A**), but the high $Na^+$ and low $K^+$ diet

stabilized the loss of body weight (**Figure 3A**). Plasma $K^+$ concentration was not affected, whereas the experimental mice presented a slight but significant lower plasma sodium concentration (**Figure 3B**). Upon this diet, Scnn1b$^{Pax8/LC1}$ mice nearly normalized their food and water consumption, feces output, urine volume and sodium and potassium excretion as well as their sodium and potassium intake (**Supplementary Figures 3**).

We further followed the Scnn1b$^{Pax8/LC1}$ mice upon a prolonged high $Na^+$ and low $K^+$ diet (*protocol C,* **Supplementary Figure 1**). Scnn1b$^{Pax8/LC1}$ mice stabilized their body weight (**Supplementary Figures 4A**), became normokalemic (5 mmol/l) although their plasma sodium concentration remained slightly but significantly lower (**Supplementary Figure 4B**). 24 hours urinary $Na^+$ and $K^+$ excretion as well as $Na^+$ and $K^+$ intake did not differ (**Supplementary Figure 4C-F**). Physiological parameters like food and water intake, feces weight, and urine volume did not differ between the groups (**Supplementary Figure 5**). Under both standard and $HNa^+/LK^+$ diets, protein expression of both α- and γ-ENaC did not differ between control and experimental mice (**Supplementary Figure 6**). Switch to a standard diet (*protocol E,* **Supplementary Figure 1**) reinitiated the severe hyperkalemic PHA-1 phenotype without persistent salt-wasting (data not shown). In summary, the life-threatening hyperkalemia is normalized in Scnn1b$^{Pax8/LC1}$ mice upon a high sodium and low potassium diet, and nearly 80% (20 of 24) of the experimental mice are rescued. No salt wasting was observed under high sodium and low potassium diet in both groups.

**Hyperkalemia determines NCC expression in Scnn1b$^{Pax8/LC1}$ mice**

To analyze whether deletion of the βENaC subunit in adult kidney might have any effect on NCC regulation, we further tested total and phosphorylated NCC in kidney cortex extracts from Scnn1b$^{Pax8/LC1}$ mice kept under standard (*protocol A*) and $HNa^+/LK^+$ diet (*protocol B*) (**Figure 3**). Under standard diet, Scnn1b$^{Pax8/LC1}$ mice show a striking down-regulation of both,

the total and the phosphorylated (T53) form of NCC expression (**Figure 3C and D**). Upon $HNa^+/LK^+$ diet, the pT53-NCC protein expression from $Scnn1b^{Pax8/LC1}$ mice is normalized but not total NCC protein expression (**Figure 3E and F**). The phosphorylation of SPAK (**Supplementary Figures 7**) followed the same expression pattern as that of pT53-NCC (**Figure 3**).

Since we did not observe persistent salt wasting, $HNa^+$ may not be necessary to rescue the $Scnn1b^{Pax8/LC1}$ mice. Interestingly, a $LK^+$ diet (with standard $Na^+$) (*protocol D,* **Supplementary Figure 1**) efficiently normalized all physiological parameters, like body weight (**Figure 4A**), plasma electrolyte concentrations (**Figure 4B**) as well as food and water intake, feces output and urinary volume (**Supplementary Figure 8**). The daily $Na^+$ and $K^+$ excretion (**Figure 4C and D**) did not differ between control and experimental mice. Standard $Na^+$ but $LK^+$ diet rescues the phosphorylated form of NCC but not the total NCC protein expression (**Figure 4E and F**). Only kayexalate (potassium binder) with a $K^+$-deficient diet normalizes both total and phosphorylated NCC protein expression (**Figure 4G and H**).

Whereas standard ($Na^+$ and $K^+$) diet decreased phosphorylated SPAK levels (**Supplementary Figures 7A**), standard $Na^+$ but low $K^+$ ($LK^+$) diet normalized phosphorylated SPAK expression in 21 of 24 $Scnn1b^{Pax8/LC1}$ experimental mice, which were efficiently rescued (**Supplementary Figures 9**). Severe hyperkalemia thus correlates with an aldosterone-independent decrease of NCC protein expression. Low potassium diet alone is necessary and sufficient to avoid severe hyperkalemia and to rescue the phosphorylated form of NCC protein expression.

**Scnn1b knockout mice present a transient salt wasting phenotype under $LNa^+$ diet**

To further determine the role of the β ENaC subunit in sodium balance, control and experimental Scnn1b mice were rescued with standard sodium but low potassium ($LK^+$) diet

for 16 days, and then switched to a standard potassium but low sodium ($LNa^+$) diet (*protocol F*, **Supplementary Figure 1, Figures 5**). This reinitiated the PHA-I phenotype with body weight loss (**Figures 5A**), lower plasma sodium concentration and hyperkalemia (**Figures 5B**), but only a transient salt wasting that is normalized at day 2 and 3 after the switch (**Figures 5C**). The 24 h $K^+$ excretion was significant decreased at day 3 after the switch to $LNa^+$ diet (day 23) (**Figures 5D**). Consistent with the body weight loss, a decrease of food intake was observed at day 3 after the switch to $LNa^+$ diet (day23) (**Supplementary Figure 10A**). The water intake, feces weight, and urine volume did not differ between the groups (**Supplementary Figure 10B-D**). Consistent with the decrease in food intake (**Supplementary Figure 10A**), the 24-hours $Na^+$ (**Supplementary Figure 10E**) and $K^+$ intake (**Supplementary Figure 10F**) were significantly decreased at day 3 after the switch to $LNa^+$ diet (day 23). A switch to a low $Na^+$ and standard $K^+$ diet (*protocol F,* **Supplementary Figure 1**) reinitiated the downregulation of NCC total and pT53-NCC protein expression levels in $Scnn1b^{Pax8/LC1}$ mice (**Figure 6**). The 24-hours fecal $Na^+$ loss did not differ between control and experimental group (at day 23) (0.5(±0.23) µmol in experimental versus 0.84(±0.32) µmol in control mice). While sodium output (urine and feces) did not differ between control and experimental group, the sodium intake was severely decreased in KO animals (**Figure 7**). In summary, the $Scnn1b^{Pax8/LC1}$ mice present hyperkalemia and a transient salt wasting when challenged with a low $Na^+$ and standard $K^+$ diet.

## Discussion

### Acute nephron-specific gene deletion of the βENaC subunit induces life-threatening hyperkalemia without persistent salt wasting

*Efficient deletion of tubular βENaC subunit* is observed already after 2 days of doxycycline treatment with a residual protein pool of about 8% (**Figure 1**). This is consistent with few cells still expressing βENaC (**Figure 1**), and may reflect the short *in vitro* half-life of total ENaC protein as measured in MDCK cells [37]. Adult nephron-specific Scnn1b$^{Pax8/LC1}$ mice developed a life-threatening PHA-I similar to the Scnn1a$^{Pax8/LC1}$ mice [29], although without persistent salt wasting under standard sodium concentration in the diet (**Figure 2**). A salt-dependent PHA-I even under low sodium condition is also observed in mice exhibiting a constitutive and decreased βENaC protein expression [31]. The primary event is seen when Scnn1b$^{Pax8/LC1}$ mice increase $Na^+$ loss on the first day under low salt (day 21, **Figure 5C**) diet that is accompanied by decrease of food intake (**Figure 8 and Supplemental Figure 9**). Furthermore, as in the Scnn1a$^{Pax8/LC1}$ mice [29], our data clearly demonstrate that an acute genetic deletion of the βENaC subunit cannot be compensated by residual ENaC activity through αγENaC channels (**Figure 2**). Mice exhibit a dramatic weight loss upon standard diet and the βENaC-induced PHA-I phenotype is reinitiated by returning to either a standard diet (*data non shown*) or a standard potassium but low sodium diet (**Figure 5**). While KO mice presented normal diuresis the third day after induction (**Table 2**), the fourth day showed almost anuric mice (**Figure 2**). Indeed, Scnn1b$^{Pax8/LC1}$ mice got rapidly and severely sick, consistent with the loss in body weight and the decrease in food intake (**Supplementary Figure 2**). Likewise, acute pharmacological blockade of ENaC by amiloride on a low salt diet was life-threatening in rats confirming no compensatory mechanisms when ENaC is pharmacologically blocked along the ASDN [14]. These data revealed a severe hyperkalemia,

a severe decrease of sodium intake with an impaired of water homeostasis in the experimental mice.

**Standard sodium but low potassium diet efficiently corrects the kalemia of Scnn1b$^{Pax8/LC1}$ mice in a pNCC-dependent manner.**

*Scnn1b$^{Pax8/LC1}$ mice are rescued by high or standard sodium /low potassium diet.* It is conceivable that high plasma potassium concentration as found in Scnn1b$^{Pax8/LC1}$ mice plays a determinant role either by its depolarizing effect on membrane potential in heart or on skeletal muscle [13, 16]. Hyperkalemia in humans suffering from PHA-1 is life-threatening leading to cardiac arrhythmia and ultimately cardiac arrest [27, 32]. Furthermore, we asked the question whether low potassium (and standard sodium) diet would be sufficient to rescue the βENaC-induced PHA-I phenotype. Indeed, a low potassium diet alone was sufficient to rescue the Scnn1b$^{Pax8/LC1}$ mice for more than 25 days (**Figure 4, Supplemental Figure 8**), highly suggesting a differential role of sodium and potassium in NCC regulation. It has been demonstrated that potassium loading itself induces a PHA-1 phenotype in a mouse model with reduced αENaC expression within the CNT [30], where baseline ENaC-mediated sodium transport was the highest and largely aldosterone-independent [26].

*Severe hyperkalemia correlates with the decrease in phosphorylation of the SPAK-NCC pathway.* While the total NCC protein expression is normalized in Scnn1a$^{Pax8/LC1}$ mice treated with a $HNa^+/LK^+$ diet [29], total NCC is significantly downregulated in Scnn1b$^{Pax8/LC1}$ mice independently of sodium- and potassium-containing diets (**Figure 3 and 4**) but dependent of potassium binder (kayexalate) treatment. Thus, the regulation of total NCC is diet-independent whereas its phosphorylation is diet-sensitive in Scnn1b$^{Pax8/LC1}$ mice (**Figure 3, 4 and 6**). Interestingly, the two higher values of plasma potassium concentration under $HNa^+/LK^+$ diet (**Figure 8B**) originate from the two Scnn1b$^{Pax8/LC1}$ mice that present a severe

reduction of phosphorylated NCC protein expression (**Figure 3E**). This is not consistent with obtained data from the Scnn1a$^{Pax8/LC1}$ mice [29], since all Scnn1a$^{Pax8/LC1}$ mice normalized both the total and the phosphorylated form of NCC. This suggests a different role of α- and β-ENaC subunits in the NCC regulation. Furthermore, the observed decrease in pNCC under standard diet is linked to severe hyperkalemia in Scnn1b$^{Pax8/LC1}$ mice (**Figure 8A**). Switching from a standard $K^+$ diet to a low $K^+$ diet showed that plasma potassium drove changes in pNCC abundances (**Figure 4**). While kayexalate treatment allowed the normalization of both total and phosphorylated NCC protein expression (**Figure 4**). This reveals an aldosterone-independent and potassium-dependent pNCC regulation. Interestingly, $LK^+$ diet normalizes plasma potassium concentration and the pNCC protein expression of Scnn1b$^{Pax8/LC1}$ mice (**Figure 8C**). In addition, while the ratio between pNCC to NCC is decreased under standard diet, a $HNa^+/LK^+$ diet normalize and a standard $Na^+/LK^+$ increases this ratio (*data not shown*). Switch to a $LNa^+$ but standard $K^+$ diet reinitiates a negative relationship between the plasma potassium concentration and the abundance of the pNCC expression (**Figure 8D**). This was also demonstrated through an acute oral $K^+$ loading in aldosterone-deficient mice [36] or potassium loading in a mouse model with reduced αENaC expression in the CNT [30]. Recently, several groups [28, 39] have demonstrated a negative linear relationship between the plasma potassium concentration and the abundance of the phosphorylated form of NCC. As a consequence, hyperkalemia increased the distal delivery of sodium by decreasing NCC activity allowing secretion of potassium through ROMK [3]. Overall, the observed rescue of Scnn1b$^{Pax8/LC1}$ mice through a standard sodium but low $K^+$ diet (**Figure 4, Supplemental Figure 8**) is consistent with reports showing that a high sodium diet might rather further decrease NCC phosphorylation [8], and that a low potassium diet presents a strong stimulus to increase total and phosphorylated NCC [15, 41], even in $Na^+$-repleted mice [43]. It has been proposed that the accompanying anions of sodium, like chloride or citrate furthermore

regulate pNCC in response to high $K^+$ diet, and thus acidic high $K^+$ diet may reduce urinary $K^+$ loss [10, 46]. We thus do not exclude a possible role of chloride in the regulation of NCC activity in our βENaC-deficient model. Interestingly, in wildtype mice high sodium and low potassium (Western) diet results in an aldosterone and angiotensin-independent increase of NCC activity via the with-no-lysine kinase (WNK)-SPAK pathway [43], although other kinases may be involved [44]. Consistent with published data [21, 33, 42], we have shown a reduction of the phosphorylation of SPAK that follows the downregulation of pNCC (**Supplemental Figure 7 and 9**). Our data confirm that SPAK-NCC regulation is tightly regulated through potassium diet.

In summary, our study demonstrates that adult βENaC subunit expression in DCT2/CNT/CD is required for primarily modulating the daily potassium homeostasis. We found a minor and transient role of the βENaC subunit in sodium homeostasis that may only be needed when chronically challenged upon low salt diet. In human, systemic PHA-I only ameliorates after 6 years of continuous treatment, whereas renal PHA-I rapidly improves after infancy [18]. Our data further unveiled the need of rapidly plasma potassium correction to ensure survival. It may be interesting to follow whether compensatory mechanism develop in the long term (months) and our mice may provide a suitable model to address this issue. For human PHA-1 patients, it may be considered to include in addition to high salt intake (up to 30g/day) a low potassium diet and/or potassium chelation either acutely (e.g., through kayexalate [38]) or chronically through newly developed potassium binders (e.g., patiromer). The daily control of plasma potassium concentration is thus mandatory.

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**Disclosures**

None.

**Ethical approval**

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution. Protocols followed the Swiss federal guidelines and were approved by the veterinarian local authorities (« Service de la consommation et des affaires vétérinaires ») of the Canton de Vaud, Switzerland.

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**Figures legends**

**Figure 1 Recombination of βENaC in the kidneys of inducible nephron-specific Scnn1b$^{Pax8/LC1}$ mice.** (**A**) DNA-based genotyping using (**A**) on extracts from kidney, liver, and heart from Scnn1b controls (Ctr., lanes 2 and 3) and experimental (Scnn1b$^{Pax8/LC1}$) mice (Exp., lanes 1 and 4); note the absence of recombination in controls (**A,** lanes 2 and 3) and the presence of recombination in liver (**A**, lanes 1 and 4). (**B**) Western blot analysis of whole kidneys and their quantification (**C**) 2 days after induction in Scnn1b control and experimental mice. Protein levels were normalized to β-actin and expressed in percentage of control. (D) Immunofluorescence detection of βENaC, $Ca^{2+}$-binding protein (CaBP), and Cre-recombinase (Cre) in kidney sections from Scnn1b control or experimental (Scnn1b$^{Pax8/LC1}$) mice following two days of standard diet.

Results are presented as mean ± SEM and data were analyzed by unpaired t test. *P* values <0.05 were considered statistically significant; Ctr., control, and Exp., experimental group.

**Figure 2 Adult Scnn1b$^{Pax8/LC1}$ mice develop a severe PHA type 1 phenotype. (A)** Body weight changes (Δ body weight), BW in percentage of initial BW during 4 consecutive days following doxycycline induction upon standard diet (*protocol A*); Scnn1b control (n=20) and experimental (Scnn1b$^{Pax8/LC1}$) mice (n=8). (**B**) Measurement of plasma electrolytes, namely $Na^+$ (left) and $K^+$ (right panel, mmol/l) in Scnn1b control (n=21) and experimental (n=7) mice. (**C**) 24hours urinary $Na^+$ and (**D**) $K^+$ excretion (mmol/24/gBW) in control (n=20) and experimental (n=8) mice.

**Figure 3 Switch to high $Na^+$ and low $K^+$ diet rescues plasma electrolyte concentration in Scnn1b$^{Pax8/LC1}$ mice.** (**A**) Body weight changes (Δ body weight, BW) of control and experimental mice (each group, n=6) in percentage of initial BW upon standard and short term high $Na^+$ and low $K^+$ diet (*protocol B*). (**B**) Measurements of plasma $Na^+$ and $K^+$

concentrations (mmol/l) in control (n=6) and experimental mice (n=5) at 30 hours following diet switch. (**C, and E**) Representative Western blot analyses of NCC, pNCC and actin on kidney cortex extracts from control and experimental Scnn1b mice upon standard diet (**C**) *protocol A,* control, n=5, and experimental mice, n=6; (**E**) upon short-term high $Na^+$ and low $K^+$ diet, *protocol B,* in control, n=5, experimental mice, n=5. (**D and F**) Quantification of proteins from corresponding Western blot analyses. Protein levels are normalized to actin and expressed in percentage of control. Results are presented as mean ± SEM and data were analyzed by unpaired t test. *P* values <0.05 were considered statistically significant.

**Figure 4 Low $K^+$ diet normalizes the body weight and the plasma electrolytes of Scnn1b$^{Pax8/LCI}$ mice independent of the $Na^+$ diet** (**A**) Body weight changes (Δ body weight, BW) in percentage of initial BW day 21 following doxycycline induction, and (**B**) plasma $Na^+$ and $K^+$ concentrations (mmol/l) under low $K^+$ diet (*protocol D*) in controls and experimental mice (each group, n=5). Daily urinary (**C**) $Na^+$ and (**D**) $K^+$ excretion (mmol/24h/gBW), in control and experimental mice (each group, n=6). Representative Western blot analyses of total NCC, pNCC and actin levels (**E**) in kidney cortex extracts from control and experimental Scnn1b mice upon low $K^+$ diet (*protocol D*, control, n=5, experimental mice, n=5). (**F**) Quantification of proteins from corresponding Western blot analyses. Protein levels are normalized to actin and expressed in percentage of control. (**G**) Representative Western blot analyses of total NCC, pNCC and actin levels in kidney extracts from control and experimental Scnn1b mice treated for 2weeks upon doxycycline and $K^+$-deficient diet with kayexalate (poly (sodium 4-styrenesulfonate) (Sigma Aldrich) mixed with the food (0.96µl/day/grBW)) (each group, n=4). (**H**) Quantification of proteins from corresponding Western blot analyses. Protein levels are normalized to actin and expressed in percentage of

control. Results are presented as mean ± SEM and data were analyzed by unpaired *t* test. *P* values <0.05 were considered statistically significant.

**Figure 5 Adult Scnn1b$^{Pax8/LC1}$ mice develop a transient sodium wasting phenotype when challenging with low $Na^+$ diet.** (**A**) Body weight changes (Δ body weight, BW) of control and experimental mice in percentage of initial BW upon low $K^+$ and low $Na^+$ diets (*protocol F*); Scnn1b control, n=6 and experimental mice: n=4. (**B**) Measurements of plasma $Na^+$ and $K^+$ concentrations (mmol/l) in control (n=6) and experimental mice (n=4) at 4 days following diet switch. (**C**) 24hours urinary $Na^+$ and (**D**) $K^+$ excretion (mmol/24/gBW) in Scnn1b control (n=6) and experimental (n=4) mice. Values were normalized to the body weight. **P*<0.05, ***P*<0.01, ****P*<0.001.

**Figure 6 Low $Na^+$ in combination with standard $K^+$ diet decreases total NCC and pNCC in Scnn1b$^{Pax8/LC1}$ mice.** Representative Western blot analyses of total NCC, pNCC and actin levels (**A**) in kidney extracts from control and experimental Scnn1b mice upon low $Na^+$ diet (*protocol F,* control, n=6 and experimental mice, n=4). (**B**) Quantification of proteins from corresponding Western blot analyses. Protein levels are normalized to actin and expressed in percentage of control. Results are presented as mean ± SEM and data were analyzed by unpaired t test. *P* values <0.05 were considered statistically significant.

**Figure 7 Decrease of sodium intake in Scnn1b$^{Pax8/LC1}$ mice under low sodium diet.** Sodium balance under low $Na^+$ diet is illustrated as the ratio of sodium intake grey, normalized to the sodium output in urine (white) or feces (black bar) at day 23. Data were taken from mice under low $Na^+$ diet in experiments (sodium intake; Supplemental Figure 10, (fecal sodium; result section), and (urinary sodium Figure 5).

**Figure 8 Relation of pNCC protein expression and the severe hyperkalemia in Scnn1b$^{Pax8/LCI}$ mice.** Phosphorylated NCC protein expression (y axis) and the plasma potassium concentration (mmol/l) of the corresponding mouse (x axis) under standard diet (protocol A) (A), HNa/LK diet (protocol B) (B), LK diet (protocol D) (C), and LNa diet (protocol F) (D). The standard value for the plasma potassium concentration is indicated in grey.

**Supplementary Figures**

**Supplemental Figure 1 Schema of the experimental protocols.** Representative setup used to determine physiological parameters in Scnn1b$^{Pax8/LCI}$ mice placed either in standard (thin line) or metabolic cages (bold line) and induced by doxycycline (Dox) under standard diet (**A**), a short term high $Na^+$ and low $K^+$ diet (**B**), a long-term high $Na^+$ and low $K^+$ diet (**C**), and a long-term low $K^+$ diet (**D**), back to standard diet after one month under $HNa^+/LK^+$ diet (**E**), and back to $LNa^+$ diet after 16days under $LK^+$ diet (**F**). Arrows indicate the end of the experiment, and the time point of serum and organs recovery.

**Supplemental Figure 2 Physiological parameters as measured following induction under standard diet.** Measurement of daily 24 hours (**A**) food (g/24h/gBW), (**B**) water intake (ml/24h/g BW), (**C**) feces (g/24h/gBW), (**D**) urine volume output (ml/24h/gBW), and measurement of (**E**) $Na^+$ and (**F**) $K^+$ intake (mmol/24/gBW) in Scnn1b control (n=20) and experimental mice (n=8), *protocol A.* Values were normalized to the body weight.

**Supplemental Figure 3 Physiological parameters as measured following induction under acute High $Na^+$ and low $K^+$ diet.** (**A**) Food intake (g/gBW), (**B**) water intake (ml//gBW), (**C**)

feces output (g/gBW), (**D**) urine volume (ml/gBW), and measurement of daily urinary $Na^+$ (**E**) and $K^+$ (**F**) excretion (mmol/24h/gBW), and (**G**) $Na^+$ and $K^+$ (**H**) intake (mmol/24h/gBW), in Scnn1b control (n=6) and experimental mice (n=8) following doxycycline treatment upon standard diet (24-hour measurement) and high $Na^+$/low $K^+$ (6-hour measurement); *protocol B.* Values were normalized to the body weight.

**Supplemental Figure 4 Long term high $Na^+$ and low $K^+$ diet normalizes all physiological parameters in Scnn1b$^{Pax8/LC1}$ mice.** Measurement of (**A**) body weight changes (Δ body weight, BW) in percentage of initial BW, and (**B**) plasma $Na^+$ and $K^+$ concentration (mmol/l), (**C**) 24hours urinary $Na^+$ and (**D**) $K^+$ excretion (mmol/24h/gBW), and (**E**) $Na^+$ and (**F**) $K^+$ intake (mmol/24h/gBW) in control (n=8) and Scnn1b$^{Pax8/LC1}$ mice (n=6), (*protocol C*). *P* values <0.05 were considered statistically significant.

**Supplemental Figure 5 Long term high $Na^+$ and low $K^+$ diet normalizes food and water intake, feces weight and urinary volume excretion in Scnn1b$^{Pax8/LC1}$ mice.** 24 hours (A) food (g/24h/gBW) and (B) water intake (ml/24h/gBW), (C) feces output (g/24h/gBW), and (D) urine volume (ml/24h/gBW), in control (n=8) and Scnn1b$^{Pax8/LC1}$ mice (n=6), (*protocol C*). Results are presented as mean ± SEM and data were analyzed by unpaired t test. *P* values <0.05 were considered statistically significant.

**Supplemental Figure 6 α- and γ-ENaC protein expression is unchanged in Scnn1b$^{Pax8/LC1}$ mice.** Representative Western blot analyses of α-, γ-ENaC and actin on kidney from Scnn1b control and experimental mice upon standard diet (*protocol A*) (**A**); controls, n=6 and experimental Scnn1b mice: n=6; (**B**) Acute high $Na^+$ and low $K^+$ diet (*protocol B*); control and experimental mice, each group, n=6; (**C**) Long term high $Na^+$ and low $K^+$ diet (*protocol C*); control (n=8) and experimental mice (n=6). Left panel, quantification of proteins from corresponding Western blots analyses. Protein levels were normalized to actin

and expressed in percentage of control. Results are presented as mean ± SEM and data were analyzed by unpaired t test. *P* values <0.05 were considered statistically significant.

**Supplemental Figure 7 High $Na^+$ and low $K^+$ diet normalizes pSPAK in Scnn1b$^{Pax8/LC1}$ mice.** Representative Western blot analyses of phosphorylated SPAK and actin on kidney cortex extracts from Scnn1b control and experimental mice upon standard diet (*protocol A*) (**A**); controls, n=6 and experimental Scnn1b mice: n=5; (**C**) high $Na^+$ and low $K^+$ diet (*protocol B*); in control and experimental mice, each group, n=5. (**B and D**) Quantification of proteins from corresponding Western blot analyses. Protein levels were normalized to actin and expressed in percentage of control. Results are presented as mean ± SEM and data were analyzed by unpaired t test. *P* values <0.05 were considered statistically significant.

**Supplemental Figure 8 Long-term low $K^+$ diet and standard $Na^+$ restores food and water intake, feces weight and urinary volume excretion in Scnn1b$^{Pax8/LC1}$ mice.** Daily (A) food (g/24h/gBW) and (B) water intake (ml/24h/gBW), (C) feces output (g/24h/gBW), and (D) urine volume (ml/24h/gBW), in control (n=6) and experimental mice (n=6; *protocol D*). Results are presented as mean ± SEM and data were analyzed by unpaired t test. *P* values <0.05 were considered statistically significant.

**Supplemental Figure 9 Long-term low $K^+$ diet normalizes pSPAK in Scnn1b$^{Pax8/LC1}$ mice.** Representative Western blot analyses of phosphorylated SPAK and actin on kidney cortex extracts from Scnn1b control and experimental mice upon standard $Na^+$ and low $K^+$ diet (**A**); *protocol D*), each group, n=5. (**B**) Quantification of proteins from corresponding Western blot analyses. Protein levels were normalized to actin and expressed in percentage of control. Results are presented as mean ± SEM and data were analyzed by unpaired t test. *P* values <0.05 were considered statistically significant.

**Supplemental Figure 10 Physiological parameters as measured under both standard $Na^+$/low $K^+$ and standard $K^+$/low $Na^+$ diet in $Scnn1b^{Pax8/LC1}$ mice.** (**A**) Food intake (g/gBW), (**B**) water intake (ml//gBW), (**C**) feces output (g/gBW), (**D**) urine volume (ml/gBW), and measurement of (**E**) $Na^+$ and $K^+$ (**F**) intake (mmol/24h/gBW), in control (n=6) and experimental mice (n=4) under low $K^+$ diet and following $LNa^+$ diet (24-hour measurement); *protocol F.* Values were normalized to the body weight.

**Table 1**

**Serum aldosterone concentration in Scnn1b$^{Pax8/LC1}$ mice**

| Plasma aldosterone (nM) | | |
|---|---|---|
| | **Ctr.** | **Exp.** |
| **Standard diet** | 2.9±0.4 (12) | 46.3±13.0 (3) *** |
| **HNa$^+$/LK$^+$ diet** | 2.4±0.8 (4) | 18.7±5.6 (4) * |
| **LK$^+$ diet** | 1.4±0.2 (5) | 6.8±3.3 (5) n.s |

Values are given as mean ± SEM; Number of mice indicated in parentheses. Plasma aldosterone was measured as described in Methods. * P<0.05, **p<0.01, ***p<0.001. For each group, experimental mice were compared to their diet-matched control mice; Ctr., control and Exp., experimental animals.

**Table 2**

**Parameters of whole body fluid balance**

| Parameters | Standard diet, protocol A (+3days) | |
|---|---|---|
| | Scnn1b | |
| | Ctr. | Exp. |
| **Hematocrit (%)** | 47.4±1.2 (5) | 63.3±1.6 (4) *** |
| **Plasma $Na^+$ (mmol/l)** | 148±0.7 (6) | 144.6±1.3 (5) n.s |
| **Plasma $K^+$ (mmol/l)** | 4.6±0.2 (6) | 6.6±0.3 (5) *** |
| **Plasma urea (mmol/l)** | 22.5±2 (6) | 46.7± (6) * |
| **Plasma glucose (mmol/l)** | 11.7±0.6 (6) | 11.5±0.8 (6) n.s |
| **Plasma Osm (calculated)** | 339.2 (5) | 362.2 (6) |
| **Plasma Osmolality (mOsm/$H_2O$Kg)** | 321.6±1.4 (5) | 364.3±10.3 (6) ** |
| **Urine osmolality (mOsm/$H_2O$ Kg)** | 3888.8±780 (5) | 3062±512.4 (6) n.s |
| **U/P osm** | 12.1±2.7 (5) | 8.4±1.4 (6) n.s |
| **Volume urine (ml/24h)** | 0.7±0.2 (6) | 0.9±0.2 (5) n.s |
| **Urine creatinine (mg/dL)** | 39.2±6.7 (6) | 31.8±5.5 (5) n.s |
| **Plasma creatinine (mg/dL)** | 0.15±0.02 (6) | 0.13±0.02 (5) n.s |
| **Creatinine clearance (µl/min)** | 106.1±25.5 (6) | 147.5±36 (5) n.s |
| **Free water clearance (ml/min) (calculated)** | -0.0054 (5) | -0.0047 (5) |

Values are given as mean ± SEM; Number of mice indicated in parentheses. * P<0.05, ** P<0.01, *** P<0.001. For each parameter, experimental mice were compared to their

corresponding control littermates; Ctr., control and Exp., experimental animals. n. s, not significant.

Figure 1

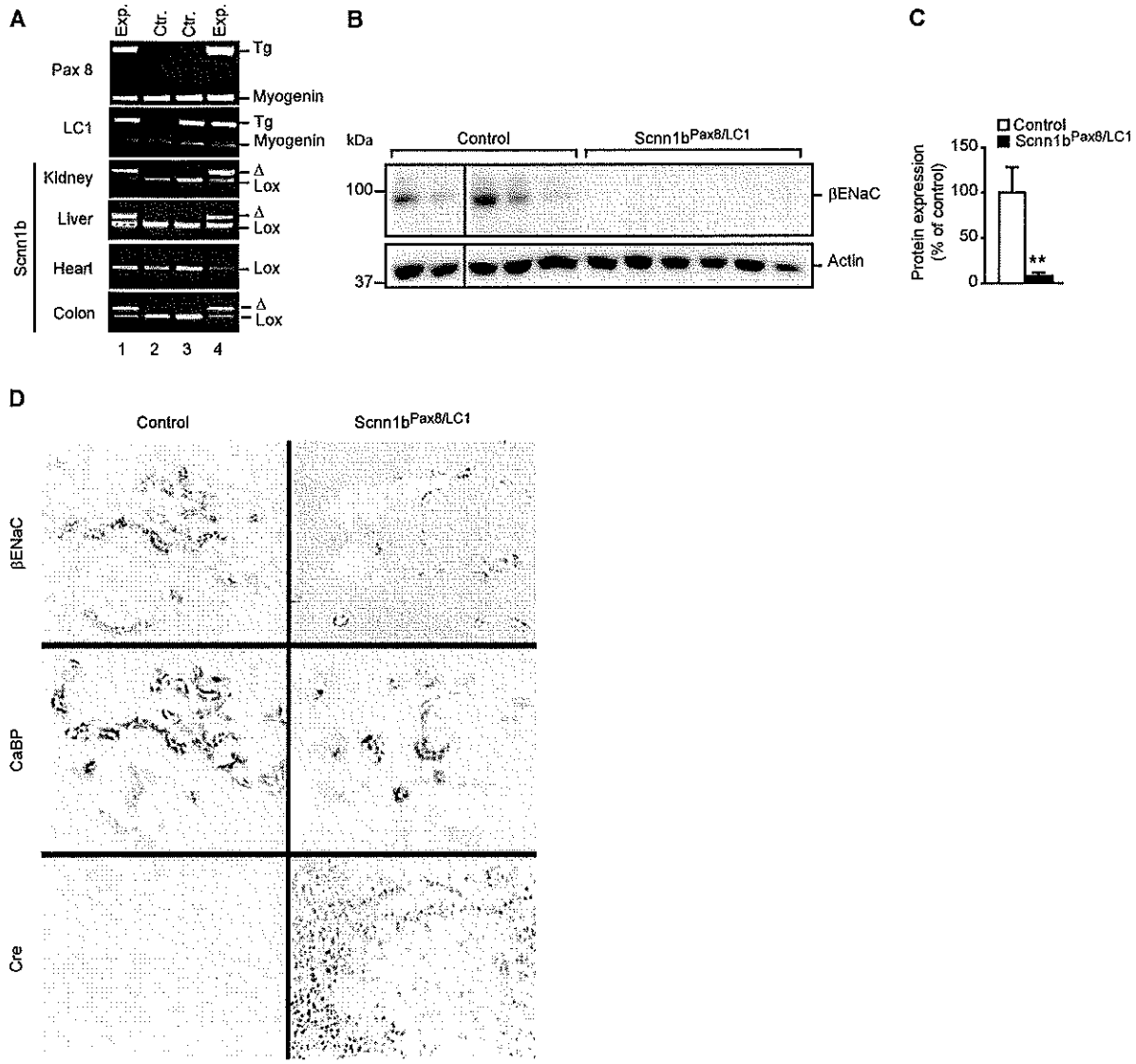

A
Exp.
Ctr.
Ctr.
Exp.
Pax 8
Tg
Myogenin
LC1
Tg
Myogenin
Scnn1b
Kidney
Δ
Lox
Liver
Δ
Lox
Heart
Lox
Colon
Δ
Lox
1 2 3 4
B
kDa
Control
Scnn1b Pax8/LC1
100
βENaC
37
Actin
C
Control
Scnn1b Pax8/LC1
Protein expression (% of control)
150
100
50
0
**
D
Control
Scnn1b Pax8/LC1
βENaC
CaBP
Cre

Figure 2

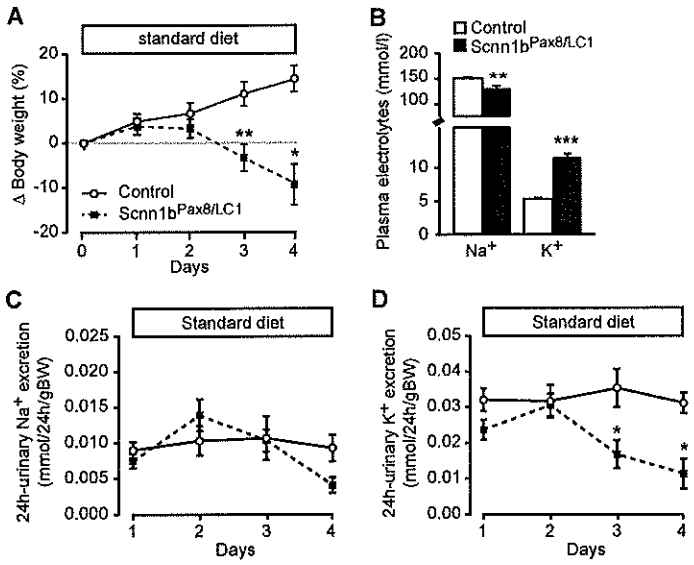

A
standard diet
Δ Body weight (%)
Control
Scnn1b Pax8/LC1
Days
B
Control
Scnn1b Pax8/LC1
Plasma electrolytes (mmol/l)
Na+
K+
C
Standard diet
24h-urinary Na+ excretion (mmol/24h/gBW)
Days
D
Standard diet
24h-urinary K+ excretion (mmol/24h/gBW)
Days

Figure 3

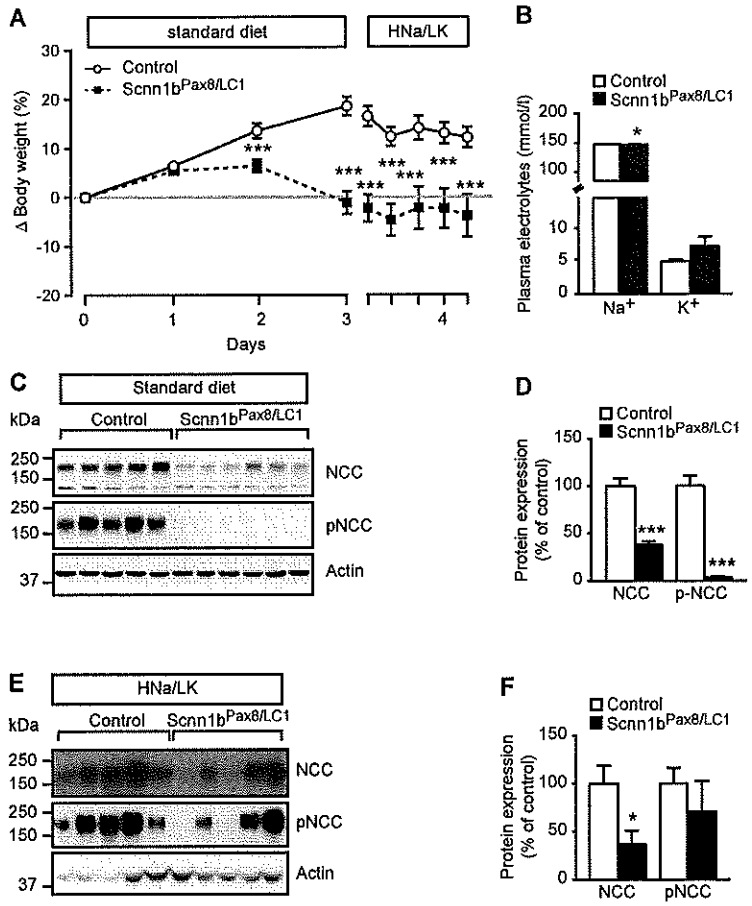

A
standard diet
HNa/LK
Control
Scnn1b Pax8/LC1
Δ Body weight (%)
Days
B
Control
Scnn1b Pax8/LC1
Plasma electrolytes (mmol/l)
Na+
K+
C
Standard diet
kDa
Control
Scnn1b Pax8/LC1
NCC
pNCC
Actin
D
Control
Scnn1b Pax8/LC1
Protein expression (% of control)
NCC
p-NCC
E
HNa/LK
kDa
Control
Scnn1b Pax8/LC1
NCC
pNCC
Actin
F
Control
Scnn1b Pax8/LC1
Protein expression (% of control)
NCC
pNCC

Figure 4

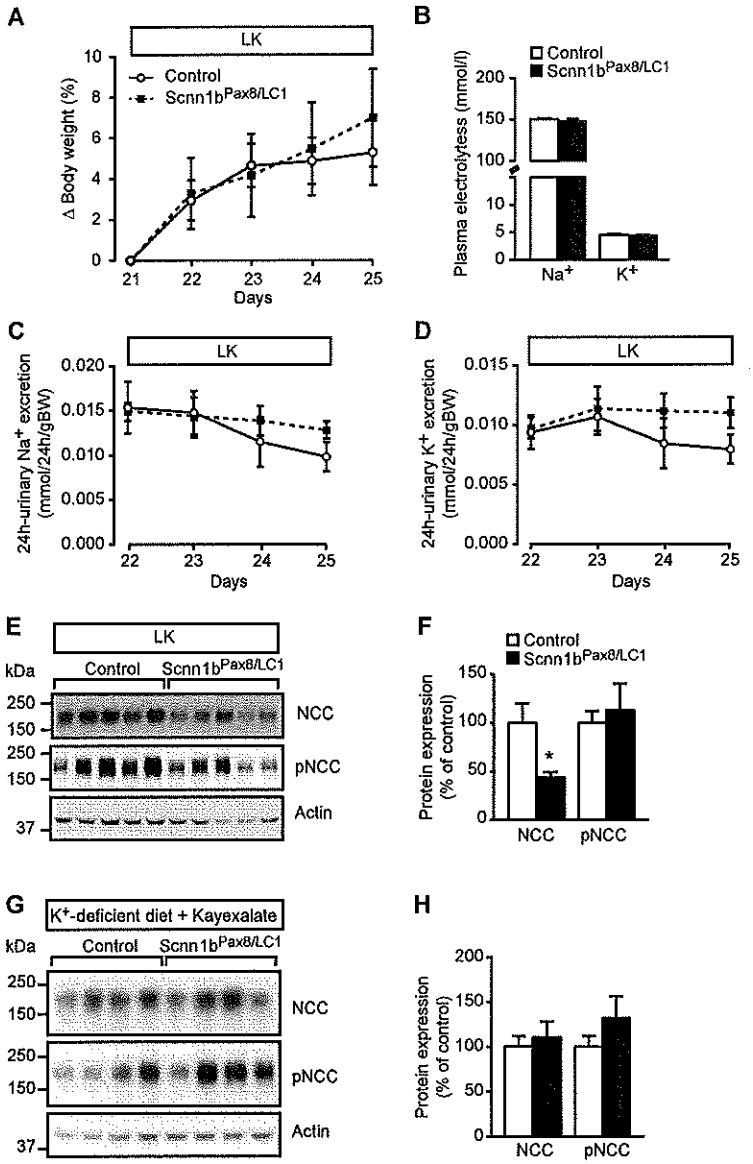

A
LK
Control
Scnn1b Pax8/LC1
Δ Body weight (%)
Days
B
Control
Scnn1b Pax8/LC1
Plasma electrolytess (mmol/l)
Na+
K+
C
LK
24h-urinary Na+ excretion (mmol/24h/gBW)
Days
D
LK
24h-urinary K+ excretion (mmol/24h/gBW)
Days
E
LK
Control
Scnn1b Pax8/LC1
kDa
NCC
pNCC
Actin
F
Control
Scnn1b Pax8/LC1
Protein expression (% of control)
NCC
pNCC
G
K+-deficient diet + Kayexalate
Control
Scnn1b Pax8/LC1
kDa
NCC
pNCC
Actin
H
Protein expression (% of control)
NCC
pNCC

Figure 5

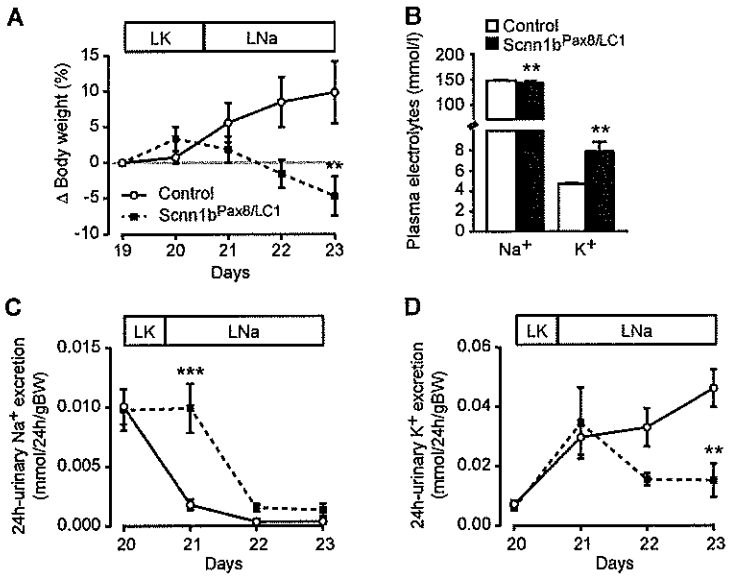

A
LK
LNa
Δ Body weight (%)
15
10
5
0
-5
-10
19
20
21
22
23
Days
Control
Scnn1b^Pax8/LC1
**
B
Control
Scnn1b^Pax8/LC1
Plasma electrolytes (mmol/l)
200
150
100
8
6
4
2
0
**
**
Na+
K+
C
LK
LNa
24h-urinary Na+ excretion (mmol/24h/gBW)
0.015
0.010
0.005
0.000
***
20
21
22
23
Days
D
LK
LNa
24h-urinary K+ excretion (mmol/24h/gBW)
0.06
0.04
0.02
0.00
**
20
21
22
23
Days

Figure 6

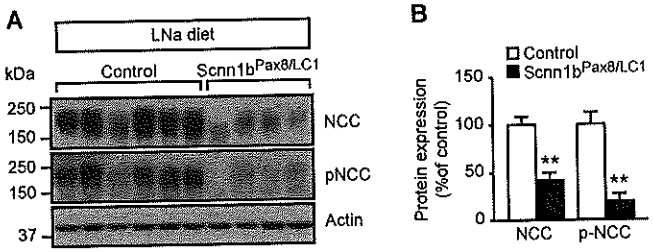

A
LNa diet
kDa
Control
Scnn1b Pax8/LC1
250
150
NCC
250
150
pNCC
37
Actin
B
Control
Scnn1b Pax8/LC1
Protein expression (%of control)
150
100
50
0
**
**
NCC
p-NCC

Figure 7

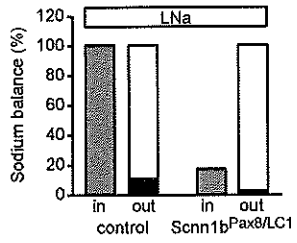
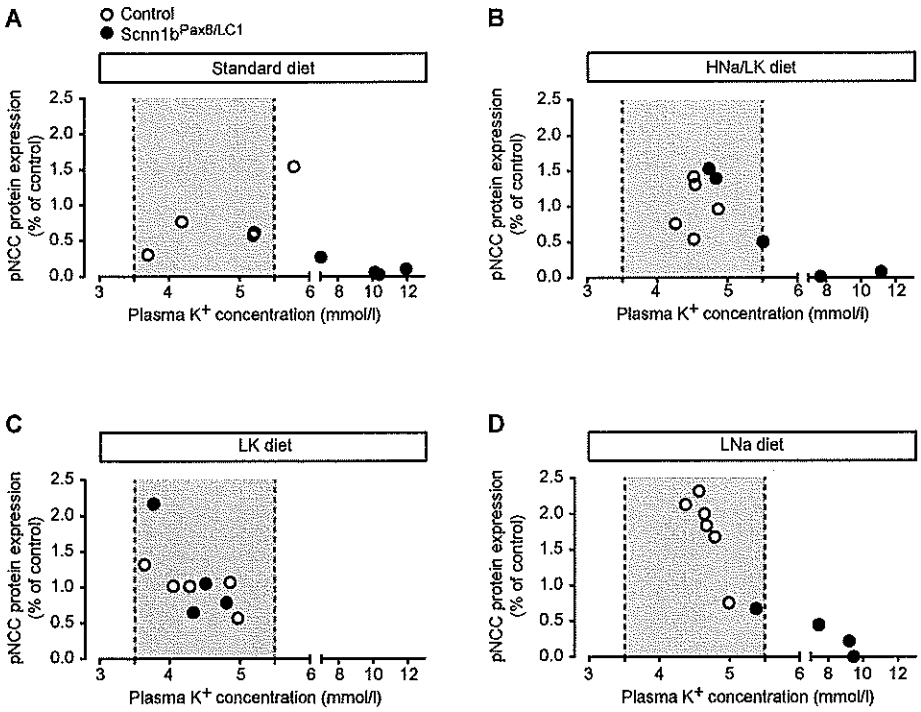
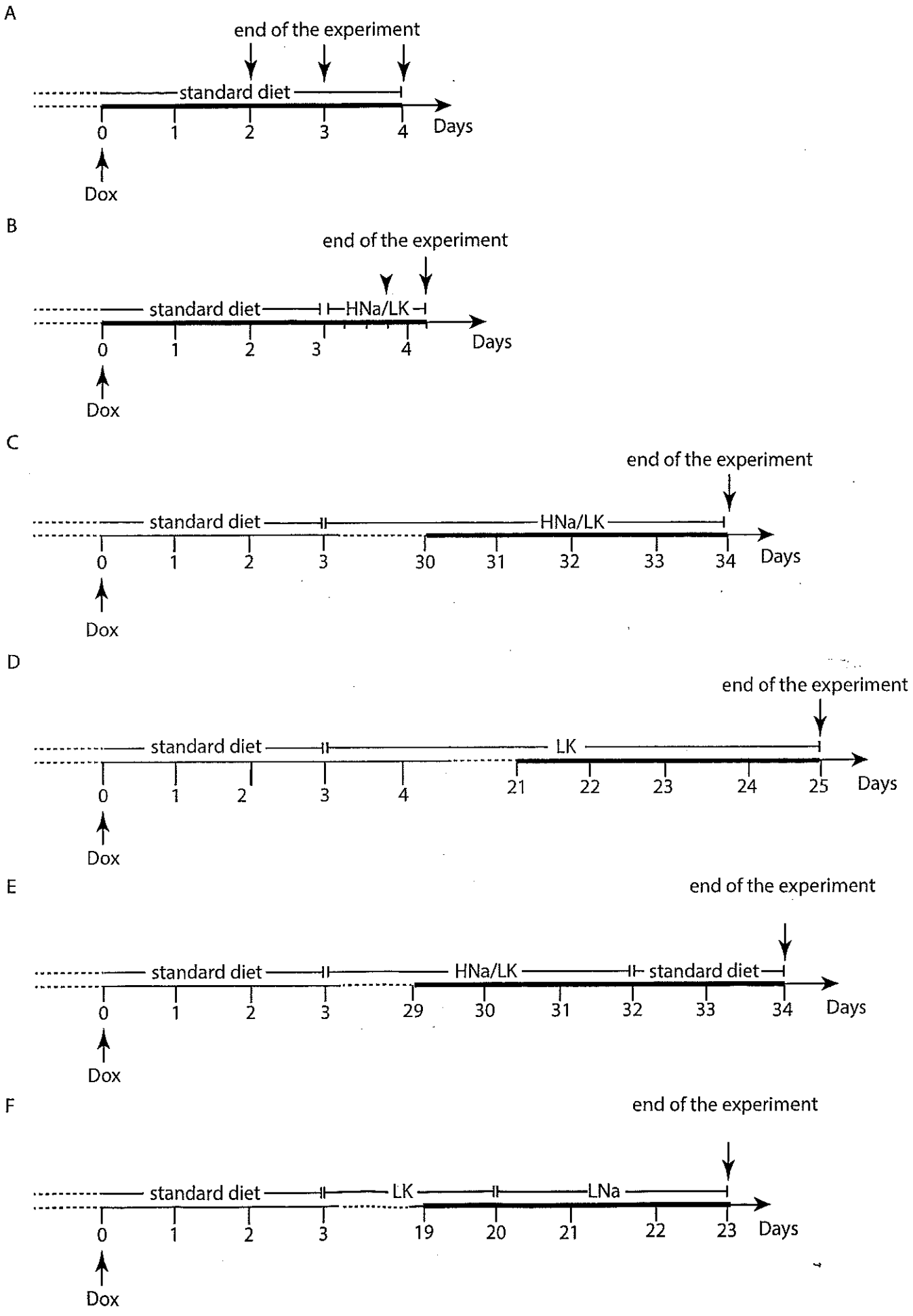

LNa
120
100
80
60
40
20
0
Sodium balance (%)
in
out
in
out
control
Scnn1b Pax8/LC1

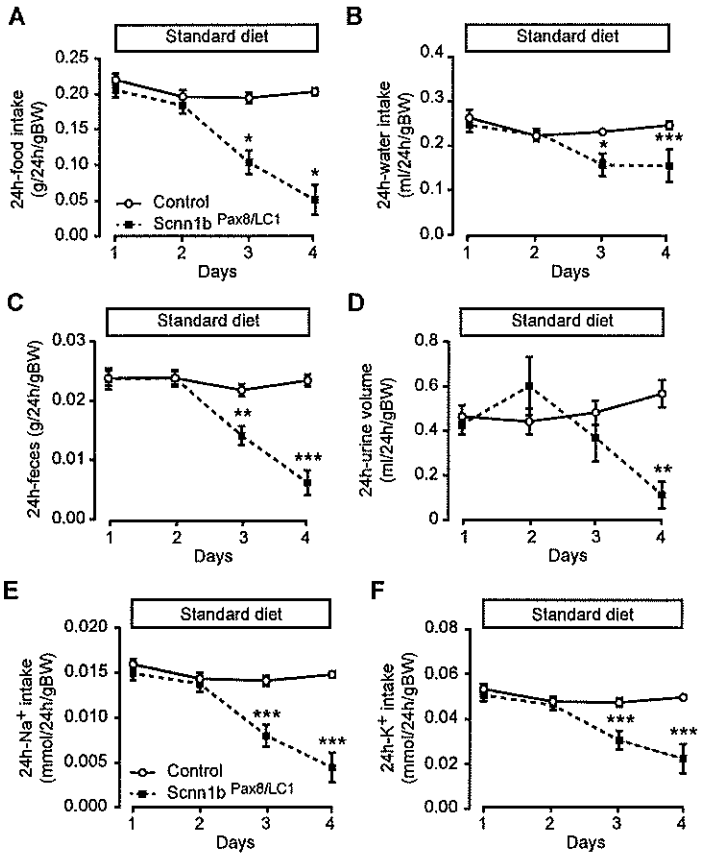
Figure 8


A
Control
Scnn1b Pax8/LC1
Standard diet
pNCC protein expression (% of control)
Plasma K+ concentration (mmol/l)
B
HNa/LK diet
C
LK diet
D
LNa diet

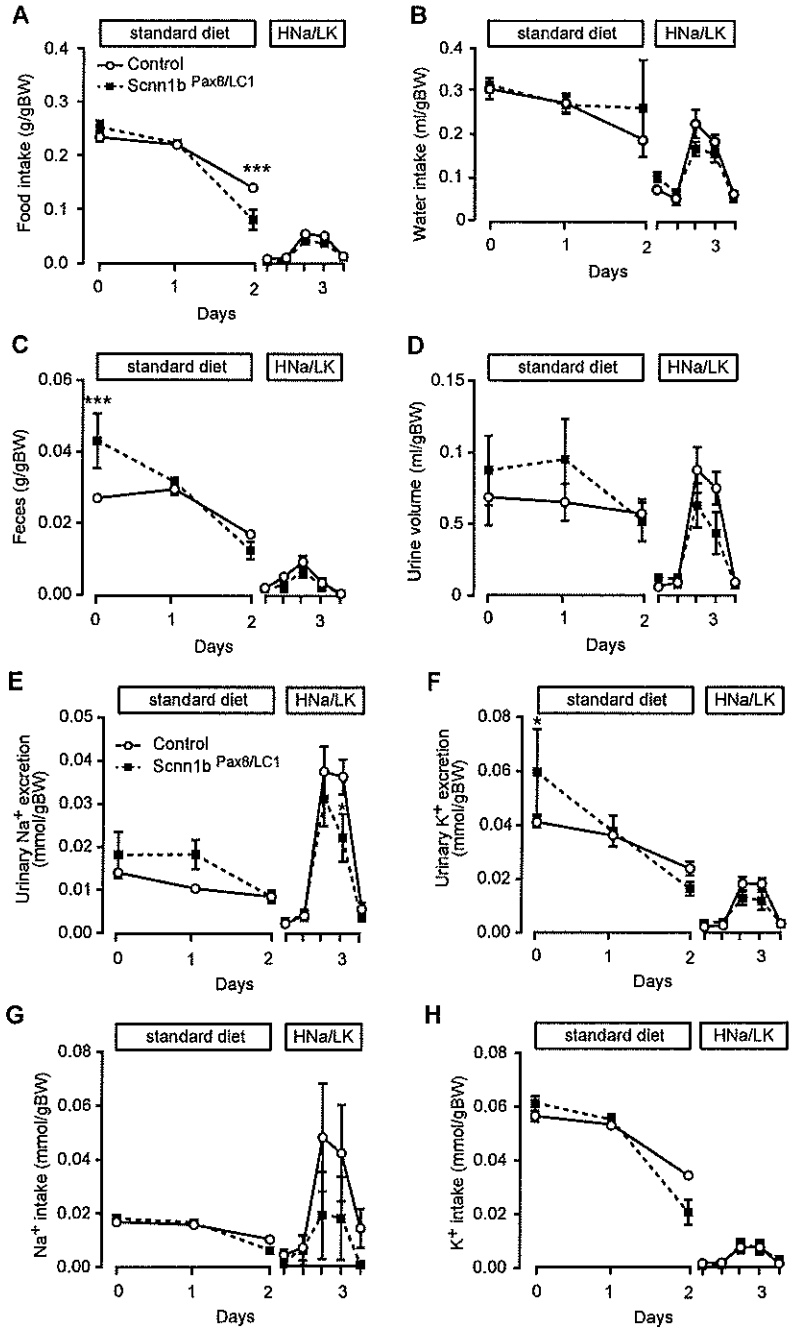
Supplemental Figure 1



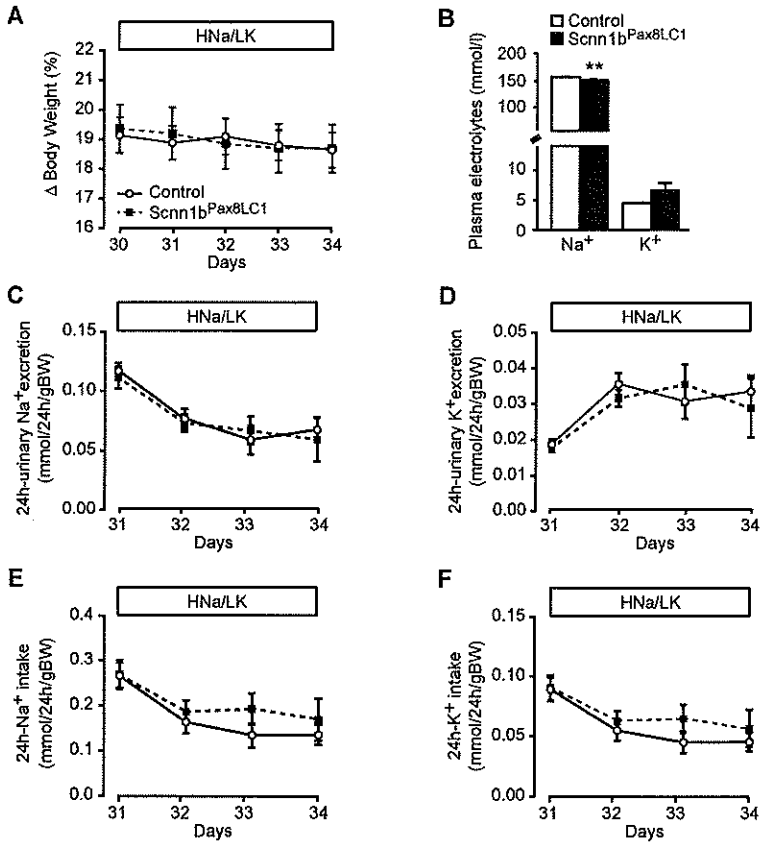
Supplemental Figure 2



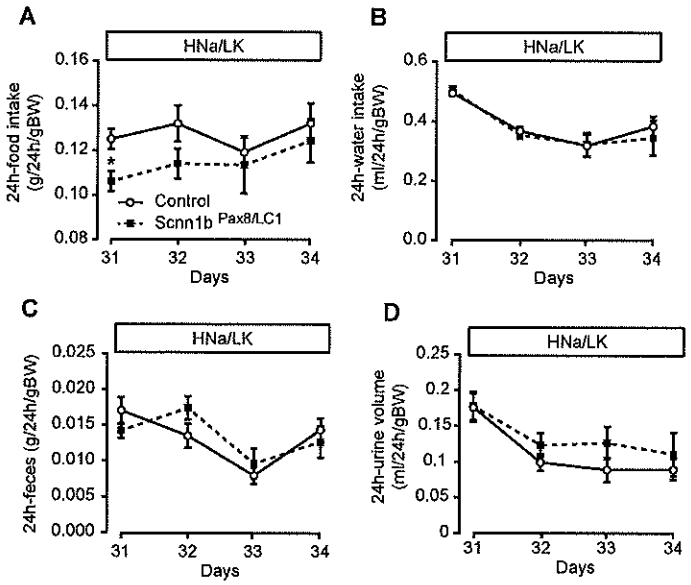
Supplemental Figure 3



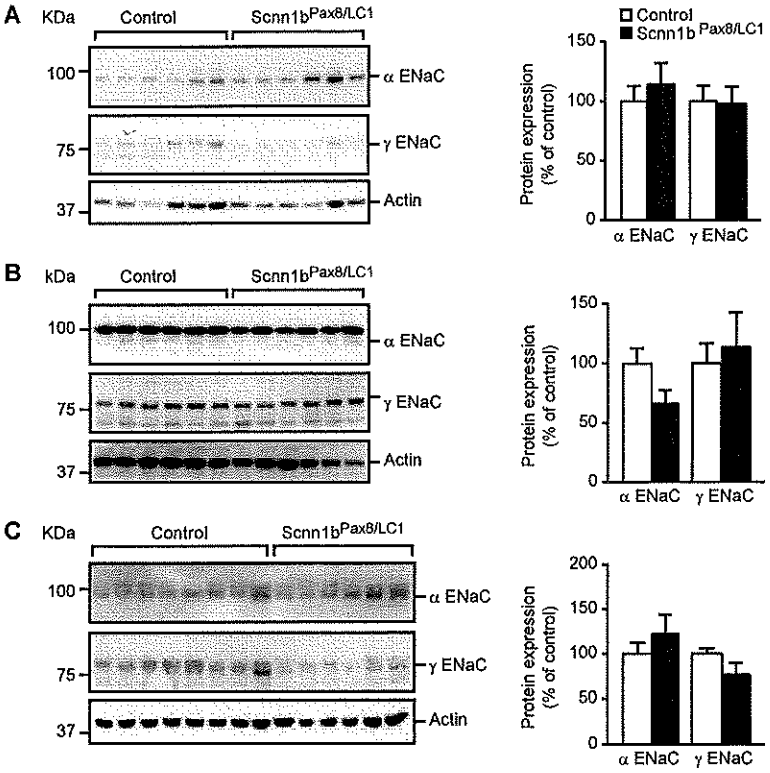
Supplemental Figure 4



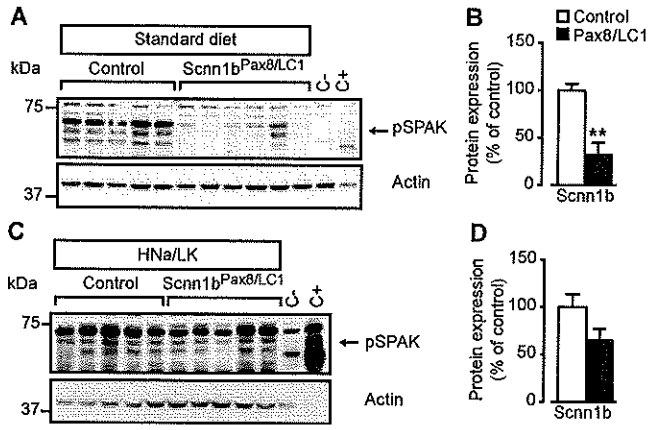
Supplemental Figure 5



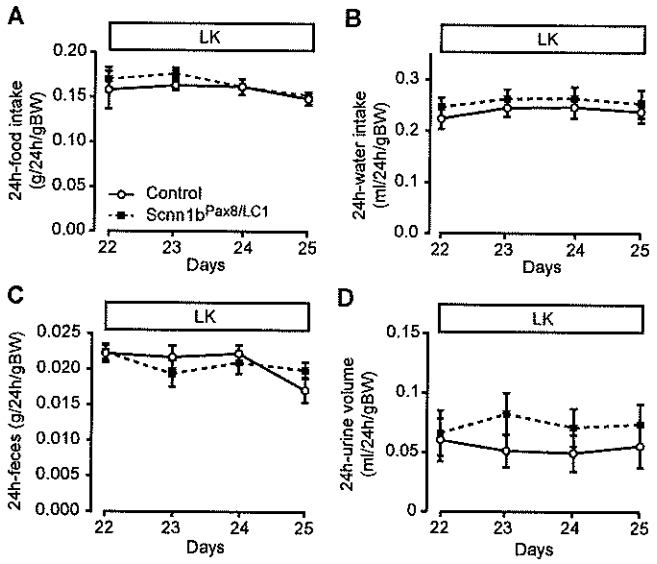
Supplemental Figure 6



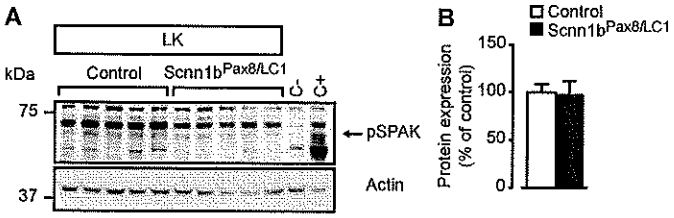
Supplemental Figure 7



Supplemental Figure 8



Supplemental Figure 9



Supplemental Figure 10