Supplemental data

Plasma Potassium determines NCC Abundance in Adult Kidney-specific γENaC Knockout

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Table of contents

Supplemental Figure 1 **Schema of the experimental protocols.** Representative setup used to determine physiological parameters in control and γENaC-deficient ($Scnn1g^{Pax8/LC1}$) 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 short term low K^+ diet (**C**), a K^+-deficient diet +/- supplemented with a K^+ chelator (kayexalate) (**D**), and (**E**) following a 6 days treatment with a K^+-deficient diet at day 0 of Dox induction. Arrows indicate the end of the experiment, and the time point of serum and organ recovery.

Supplemental Figure 2 **Physiological parameters measured following induction under standard diet in control and γENaC-deficient mice.** Measurement of daily 24 hours (**A**) food (g/24h/gBW), and (**B**) water intake (ml/24h/g BW), (**C**) feces (g/24h/gBW), and (**D**) urine volume output (ml/24h/gBW), (**E**) Na^+ and (**F**) K^+ intake (mmol/24/gBW) in control and experimental ($Scnn1g^{Pax8/LC1}$) (each group, n=5) mice; *protocol A*. Values were normalized to the body weight. **P<0.01, ***P<0.001.

Supplemental Figure 3 **Acute high Na^+ and low K^+ diets do not restore food and water intake, feces output and urinary volume excretion in γENaC-deficient mice.** (**A**) Food intake (g/gBW), (**B**) water intake (ml/gBW), (**C**) feces output (g/gBW), and (**D**) urine volume (ml/gBW), in control (n=8) and experimental ($Scnn1g^{Pax8/LC1}$) (n=9) mice 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. *P<0.05, **P<0.01, ***P<0.001.

Supplemental Figure 4 Acute low K^+ diet does not normalize food and feces weight in γENaC-deficient mice. (**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 ($Scnn1g^{Pax8/LC1}$) (n=6; *protocol C*). Values were taken normalized to the body weight. *P<0.05.

Supplemental Figure 5 No difference in protein abundances of NCC, pNCC, SPAK, and pSPAK in γENaC-deficient mice before Dox induction. Representative Western blot analysis (**A**) of NCC and pNCC, (**D**) SPAK and pSPAK on whole kidney protein extracts from Scnn1g control and deficient mice upon standard diet (each group, n=4). (**B, E**) Quantification of proteins and (**C, F**) ratio of (**C**) pNCC to total NCC abundances and (**F**) pSPAK to total SPAK abundances from the 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 6 $Scnn1g^{Pax8/LC1}$ mice stabilize their body weight upon K^+-deficient diet supplemented with sodium kayexalate. Body weight changes (Δ body weight, BW) of Scnn1g control treated with kayexalate (n=6) or without (n=3) and experimental mice treated with kayexalate ($Scnn1g^{Pax8/LC1}$, n=7) in percentage of initial BW upon standard Na^+ and K^+-deficient diet ± kayexalate following Dox induction (at day 0, *protocol D*). Results are presented as mean ± SEM and data were analyzed by unpaired t test. P values <0.05 were considered statistically significant. *P<0.05, **P<0.01, ***P<0.001.

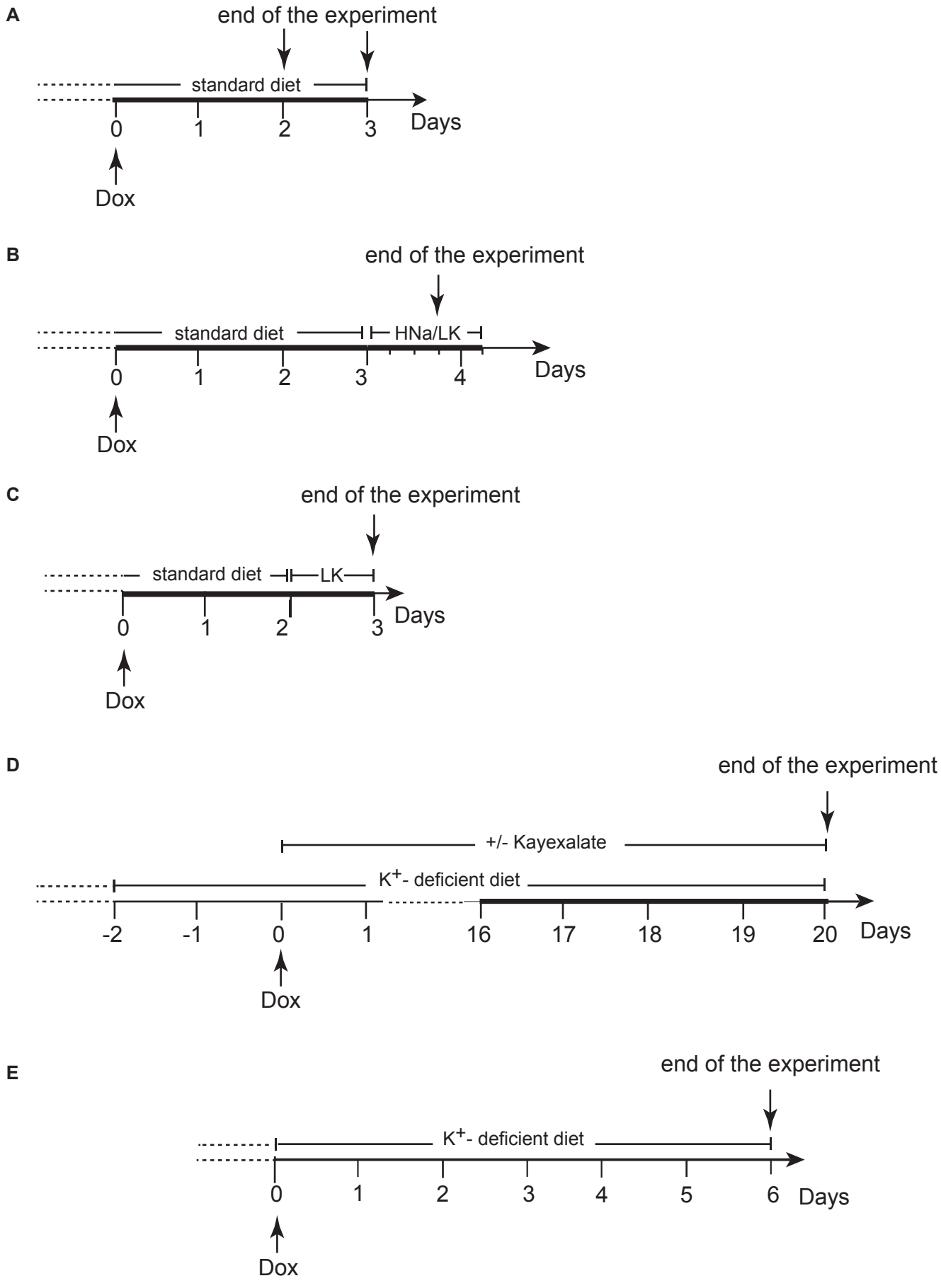
Supplemental Figure 7 Adult γENaC-deficient mice still present salt wasting under K^+-deficient diet with kayexalate treatment. (**A**) Body weight changes (Δ body weight, BW) of Scnn1g control treated (Scnn1g control with kayexalate, n=6) or without kayexalate (Scnn1g control without kayexalate, n=3) and experimental mice treated with kayexalate ($Scnn1g^{Pax8/LC1}$ with kayexalate, n=7) in percentage of initial BW upon K^+-deficient diet (*protocol D*). 24 hours urinary (**B**) Na^+ and (**C**) K^+ excretion (mmol/gBW), and 24 hours (**D**) Na^+ and (**E**) K^+ intake (mmol/gBW), (**F**) 24 hours food intake (g/gBW) and (**G**) water intake (ml//gBW), (**H**) feces output (g/gBW), and (**I**) urine volume (ml/gBW), in control without kayexalate (n=3) and with kayexalate (n=6), and experimental mice with kayexalate (n=7) ($Scnn1g^{Pax8/LC1}$) following doxycycline treatment and upon K^+-deficient diet; *protocol D*. Values were normalized to the body weight. *P<0.05, **P<0.01, ***P<0.001.

Supplemental Figure 8 γENaC-deficient mice normalize plasma sodium and potassium concentration under K^+-deficient diet with kayexalate treatment. Measurements of plasma (**A**) Na^+ and (**B**) K^+ concentrations (mmol/l) in Scnn1g control without (n=3) and with kayexalate (n=6) and experimental $Scnn1g^{Pax8/LC1}$ mice with kayexalate (n=7) following doxycycline treatment upon K^+-deficient diet; *protocol D*. Representative Western blot analyses of (**C**) total and (**D**) phosphorylated NCC and actin on kidney from control without (n=3) and with kayexalate (n=4) and experimental Scnn1g mice treated with kayexalate (n=4) upon K^+-deficient diet (*protocol D*). (**E** and **F**) Quantification of proteins and (**G**) ratio of pNCC to total NCC abundances 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, *P<0.05, **P<0.01, ***P<0.001.

Supplemental Figure 9 Increased urinary sodium loss in γENaC-deficient mice treated with K^+-deficient diet supplemented with kayexalate. 24 hours fecal (**A**) sodium and (**B**) potassium concentration and (**C**) sodium and (**D**) potassium balance in Scnn1g control (n=6) and experimental ($Scnn1g^{Pax8/LC1}$) mice (n=7) following 20 day of doxycycline treatment and upon K^+ -deficient diet supplemented with kayexalate*; protocol D*. The mean sodium or potassium intake (gray column) is compared with both urinary (white) and fecal (black column) sodium and potassium loss on K^+-deficient diet complemented with kayexalate; *protocol D*. ***P<0.001.

Supplemental Figure 10 Loss of apical α and βENaC subunit localization in cortical collecting duct cells from γENaC-deficient mice. Immunofluorescence detection of α-, β–, and γENaC in consecutive kidney sections from control or knockout ($Scnn1g^{Pax8/LC1}$) mice following two days of standard diet; *protocol A*; filled arrows, residual γENaC expressing cell that exhibits a apical membrane trafficking of the α– and βENaC subunit (filled arrows) and of cells that exhibit a disturbed αENaC subunit expression (open arrows).

Supplemental Figure 1



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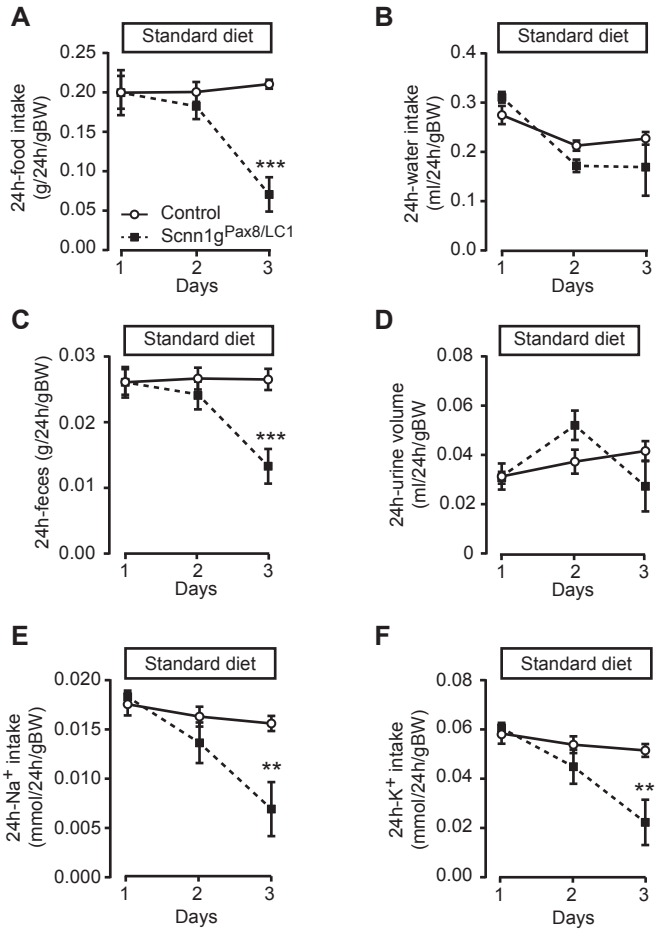

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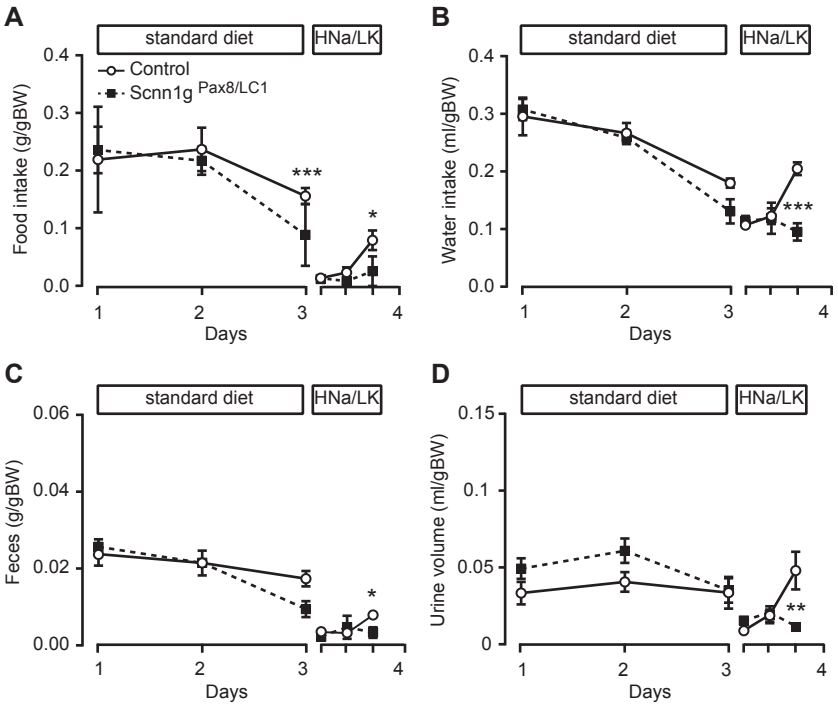

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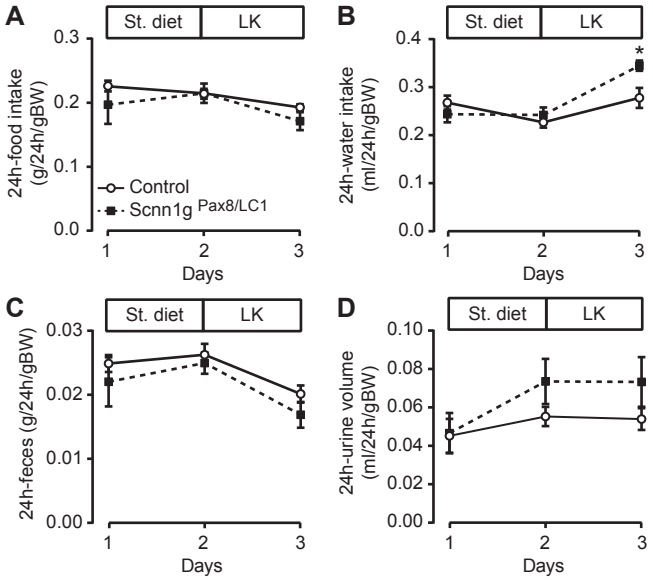

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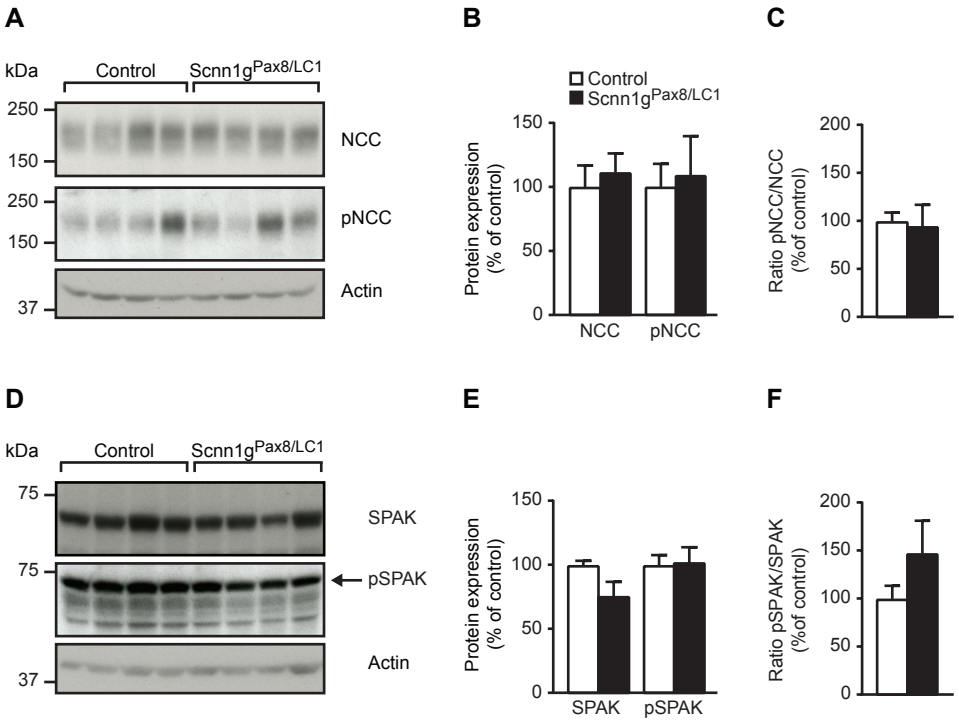

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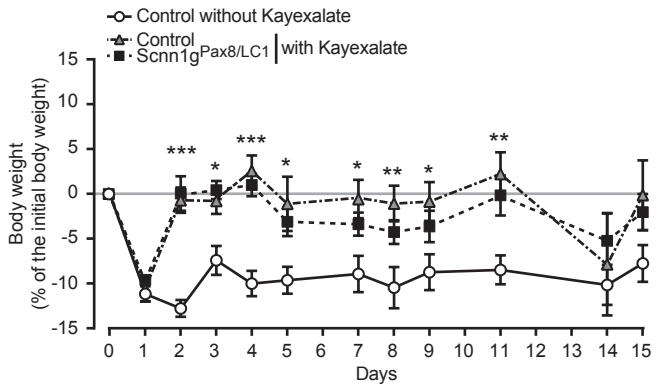

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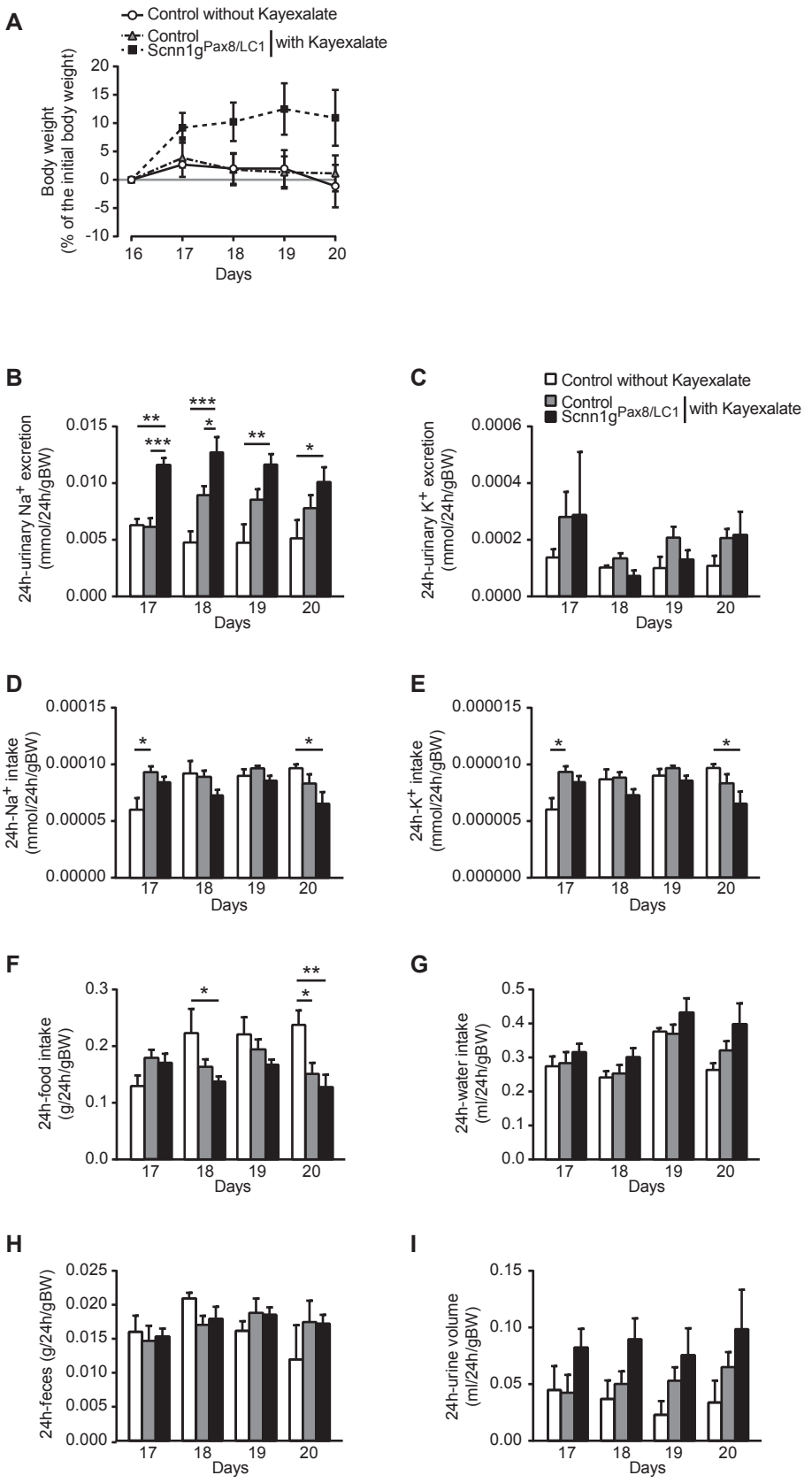

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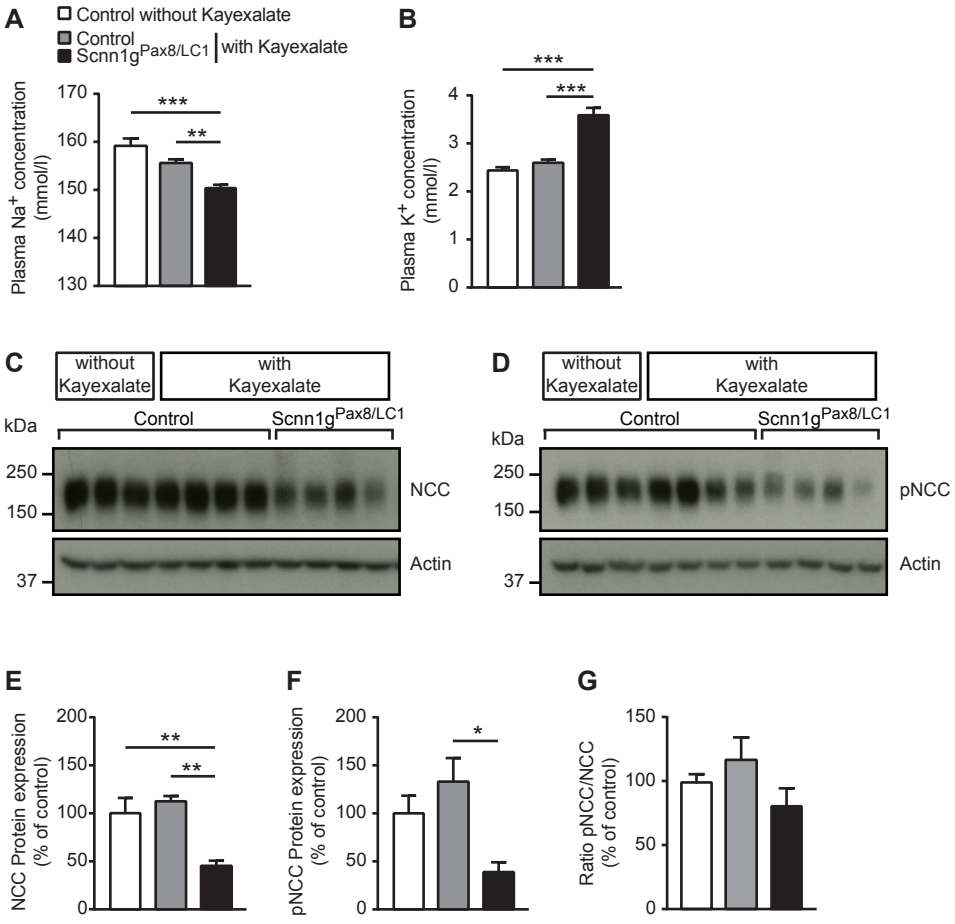
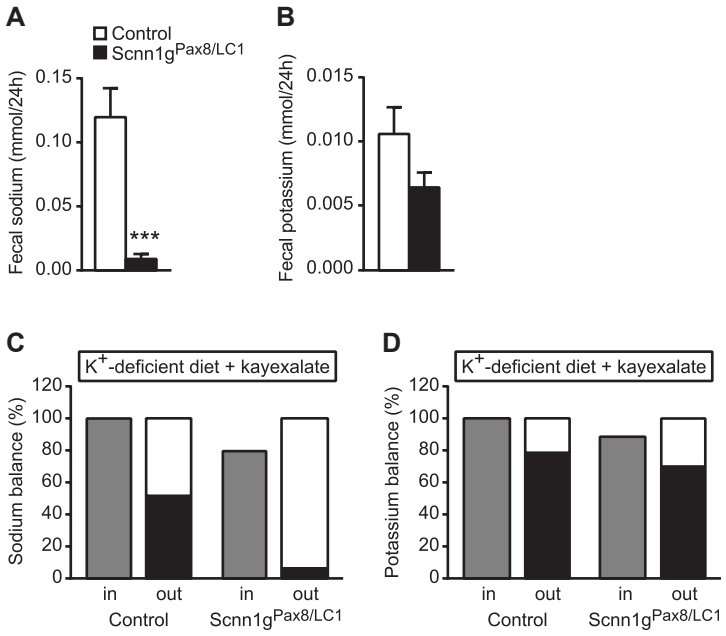


Supplemental Figure 9



Supplemental Figure 10