SUPPLEMENTAL MATERIAL AND METHODS

Experimental protocol for combined high Na^+ and high K^+ loading (2% Na^+ + 5% K^+ diets). Standard rodent chow GLP 3433 (PROVIMI KLIBA, Kaiseraugst, Switzerland) in powdered form was supplemented with NaCl and NaCl + KCl to reach either a final 2% Na^+ or a 2% Na^+ + 5% K^+ concentration. The food was then mixed with deionized milliQ water in 1:1 proportion (50 g food + 50 ml water) to ensure homogeneous soaking with Na^+ and K^+. Male mice were then kept individually in metabolic cages and first all fed for 48 h with the high salt (2% Na^+) diet. Afterwards, the mice were distributed in two groups and mainted for additional 48 h either on the same 2% Na^+ diet or switched to the 2% Na^+ + 5% K^+ diet. Daily food, water intake and body weights were measured and 24 hours urine was collected. At the end of the experiment, mice were anesthetized and heparinized blood was collected from the abdominal vena cava for electrolyte measurements. Plasma and urinary electrolytes (Na^+, K^+) were measured with a flame photometer (Eppendorf EFOX 5053, Hamburg, Germany). Urine creatinine was measured with the Jaffe method[1].

Muscle K^+ content: Female $AS^{+/+}$ and $AS^{-/-}$ mice were kept in standard cages. Mice were fed with 2%K^+ diet for two days. At the end of the experiment, mice were anesthetized and the gastrocnemius muscle was dissected immediately from right and left hindlimb. Muscle K^+ content was determined as described[2]. Briefly, fresh muscles were weighed and then homogenised in distilled water. Proteins were precipitated by 10% trichloroacetic acid. After centrifugation at 10,000x*g* for 10 min at 4°C, supernatant was collected and the K^+ content in the supernatant was measured with a flame photometer (Eppendorf EFOX 5053, Hamburg, Germany) and expressed as a value normalized against muscle weight.

Quantitative real-time PCR: 400ng total RNA from whole kidneys of $AS^{+/+}$ and $AS^{-/-}$ mice was used as a template for reverse transcription using the GoScript™ Reverse Transcriptase Kit (Promega, Madison, WI). Quantative real-time PCR was performed on a Roche LightCycler® 480 II (Roche Diagnostics, Rotkreuz, Switzerland) using the following primers for α-, β- and γ-ENaC subunits and glyceraldehydes 3-phosphate dehydrogenase (GAPDH): α-ENaC

forward 5´-CCC TCC AAG TAC ACA CAG CA-3´ and reverse 5´-ACC CTT GGG CTT AGG GTA GA-3´. β-ENaC forward 5´-GAC CAT CTC CAT GGC TGA CT-3´ and reverse 5´-CCT TCC TGC TCA GGG TGA TA-3´. γ-ENaC forward 5´-GTG CTT GTC CAT CAG CAG AA-3´ and reverse 5´-CCT CTG TGC ACT GGC TGT AA-3´. GAPDH forward 5´-AAT GGG GTG AGG CCG GTG CT-3´ and reverse 5´-CAC CCT TCA AGT GGG CCC CG-3´. Real time PCR reactions were performed using the LightCycler 480 SYBR Green I Master reaction mix. Briefly, 1 µl cDNA (10ng), 1 µl of each primer (10 µM), 7 µl RNase free water, 10 µl LightCycler 480 SYBR Green I Master reaction mix reached 20 µl final reaction volume. Reaction conditions were: denaturation at 95^{o}C for 10 min followed by 45 cycles of denaturation at 95^{o}C for 15 s and annealing 60 ^{o}C for 15 s and elongation at 72^{o}C for 20 s followed by dissociation stage (95^{o}C for 5 s, 60^{o}C for 1 min followed by slow ramp to 97^{o}C). All reactions were run in triplicate. The expression of gene of interest was calculated in relation to HPRT. Relative expression ratios were calculated as $R = 2^{[Ct(GAPDH- Ct(test\ gene)]}$.

References

1. Seaton, B, Ali, A: Simplified manual high performance clinical chemistry methods for developing countries. *Med Lab Sci,* 41**:** 327-336, 1984.
2. Meneton, P, Schultheis, PJ, Greeb, J, Nieman, ML, Liu, LH, Clarke, LL, Duffy, JJ, Doetschman, T, Lorenz, JN, Shull, GE: Increased sensitivity to K+ deprivation in colonic H,K-ATPase-deficient mice. *The Journal of clinical investigation,* 101**:** 536-542, 1998.

SUPPLEMENTAL FIGURE LEGENDS

Suppl. figure 1. Expression of the epithelial sodium channel (ENaC) and renal outer medullary potassium channel (ROMK) in kidneys of $AS^{+/+}$ and $AS^{-/-}$ mice kept on 0.8% K^+ (control) diet. Detection of α-, β- and γ-ENaC subunits and ROMK by immunoblotting using total membrane fractions of whole kidney homogenates. Data were normalized against β-actin. Bar graphs represent densitometric data. n = 5 mice per group. Values are mean ± SEM. * $p \leq 0.05$.

Suppl. figure 2. Expression and subcellular localization of the epithelial sodium channel (ENaC) and renal outer medullary potassium channel (ROMK) in kidneys of $AS^{+/+}$ and $AS^{-/-}$ mice kept for 48h on 5% K^+ diet. (A) Detection of α-, β- and γ-subunit of ENaC and ROMK by immunoblotting using total membrane fractions of whole kidney homogenates. Data were normalized against β-actin. Bar graphs represent densitometric data. n = 5 mice per group. Values are mean ± SEM. * $p \leq 0.05$, *** $p \leq 0.001$. (B) Detection of β-ENaC and ROMK in the late distal convoluted tubule (DCT) by immunofluorescence on kidney cryosections. Tubules were identified by co-staining for calbindin D28K (not shown) as described in materials and methods. Bar ~25 µm.

Suppl. figure 3. Response of $AS^{+/+}$ mice (open bars); and $AS^{-/-}$ mice (filled bars) to feeding either a 2% Na^+ or a 2% Na+ + 5% K^+ diet. Mice were placed first for 48h on the 2% Na^+ diet and then maintained for additional 48h on 2% Na^+ or switched to the 2% Na^+ + 5% K^+ diet. Mice were kept in metabolic cages to record food intake (A), body weight (B), water intake (C), urine volume (D), plasma [K^+] (E), plasma [Na^+] (F), total urinary K^+ excretion (G), total Na^+ excretion (H), 24h urine [K^+] / [creatinine] (I), and 24h urine [Na^+] / [creatinine] (J) ratios. Food intake is reported for the last 48h of the feeding period. Changes in body weight were recorded over the last 24 hours of the experiment and expressed as the difference (Δ) to the body weight of the mice before the experiment. Water intake and urinary volumes were reported over the last 24 hours of the feeding period. Blood and urinary ion concentrations (E

to J) were measured at the end of the experiment. n = 4-8 mice per group. Values are mean ± SEM. ** $p \leq 0.01$, *** $p \leq 0.001$.

Suppl. figure 4. Skeletal muscle K^+ content in $AS^{+/+}$ and $AS^{-/-}$ female mice kept on 2% K^+ diet for 48h. The concentration of K^+ is expressed as per gram of wet muscle (gastronemicus) from right and left hind limb of each mouse. n = 5-6 mice per group.

Suppl. figure 5. Effect of 2% K^+ diet on the abundance of the renal outer medullary potassium channel (ROMK) and the epithelial sodium channel (ENaC) in kidneys of $AS^{+/+}$ mice kept for 48h on control (open bars) and 2% K^+ (filled bars) diets. Detection of ROMK and α-, β- and γ-subunit of ENaC by immunoblotting using total membrane fractions of whole kidney homogenates. Data were normalized against β-actin. Bar graphs represent densitometric data. n = 5 mice per group. Values are mean ± SEM. * $p \leq 0.05$.

Suppl. figure 6. Effect of 2% K^+ diet on the abundance of renal outer medullary potassium channel (ROMK) and the epithelial sodium channel (ENaC) in kidneys of $AS^{-/-}$ mice kept for 48h on control (open bars) and 2% K^+ (filled bars) diets. Detection of ROMK and α-, β- and γ-subunit of ENaC by immunoblotting using total membrane fractions of whole kidney homogenates. Data were normalized against β-actin. Bar graphs represent densitometric data. n = 5 mice per group. Values are mean ± SEM. * $p \leq 0.05$.

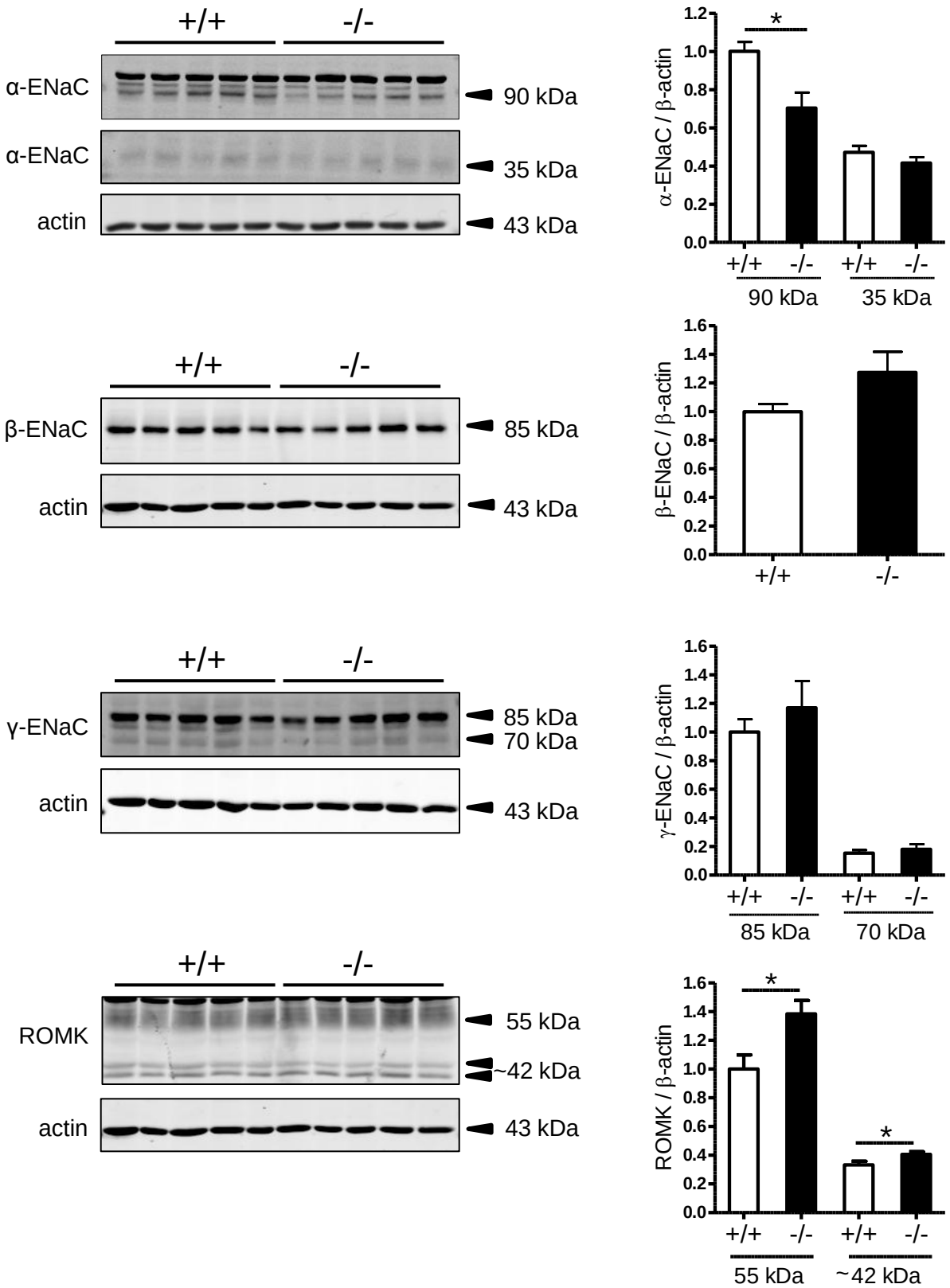
Suppl. figure 7. Expression and subcellular localization of renal outer medullary potassium channel (ROMK) and β- subunit of the epithelial sodium channel (ENaC) in kidneys of $AS^{+/+}$ and $AS^{-/-}$ mice kept for 48h on control and 2% K^+ diet. Detection of ROMK and β-ENaC in the late distal convoluted tubule (DCT2), connecting tubules (CNT) and cortical collecting ducts (CCD) by immunofluorescence on kidney cryosections; asterisks – ROMK-positive thick ascending limbs (TAL). Tubules were identified by co-staining for calbindin D28K (not shown) as described in materials and methods. Bar ~25 µm.

Suppl. figure 8. Transcription of ENaC in kidneys of $AS^{+/+}$ and $AS^{-/-}$ mice kept for 48h on 2% K^+ diet. Detection of α-, β- and γ-subunit of ENaC mRNA levels were assessed by real-time RT-PCR. n = 7 mice per group. Values are mean ± SEM. * $p \leq 0.05$.

Suppl. figure 9. Effect of 2% K^+ diet on expression and phosphorylation of the thiazide-sensitive sodium chloride cotransporter (NCC) in kidneys of $AS^{+/+}$ and $AS^{-/-}$ mice kept for 48h on the respective diets. Detection of total NCC and NCC phosphorylated at threonine 53 (pT53 NCC) and threonine 58 (pT58 NCC) by immunoblotting on using total membrane fractions of whole kidney homogenates in $AS^{+/+}$ (A) and $AS^{-/-}$ mice (B). Data were normalized against β-actin. Bar graphs represent densitometric data. n = 5 mice per group. Values are mean ± SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

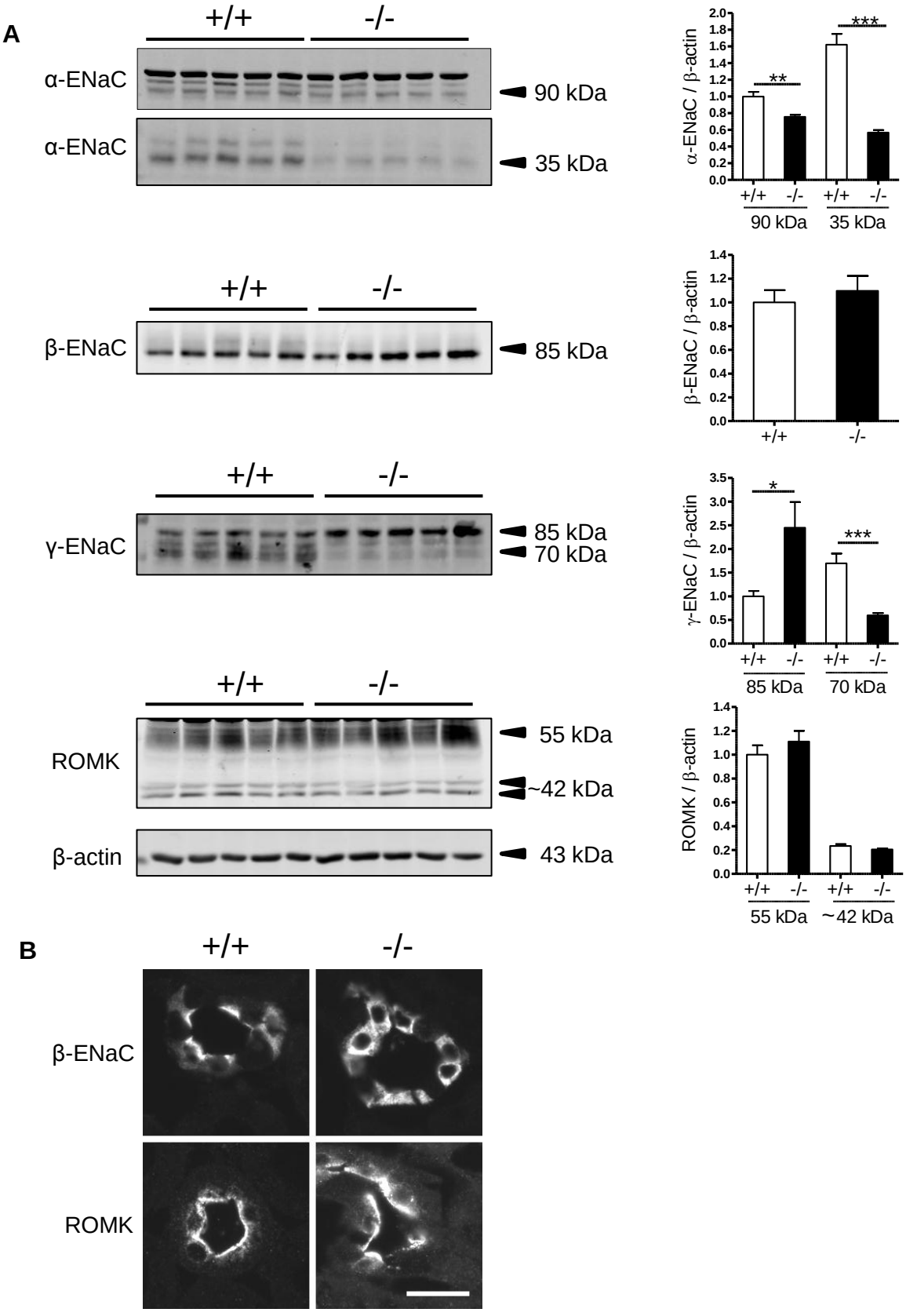
Suppl. figure 1:

Control diet (0.8% K^+)



Suppl. figure 2:

High K^+ diet (5% K^+)



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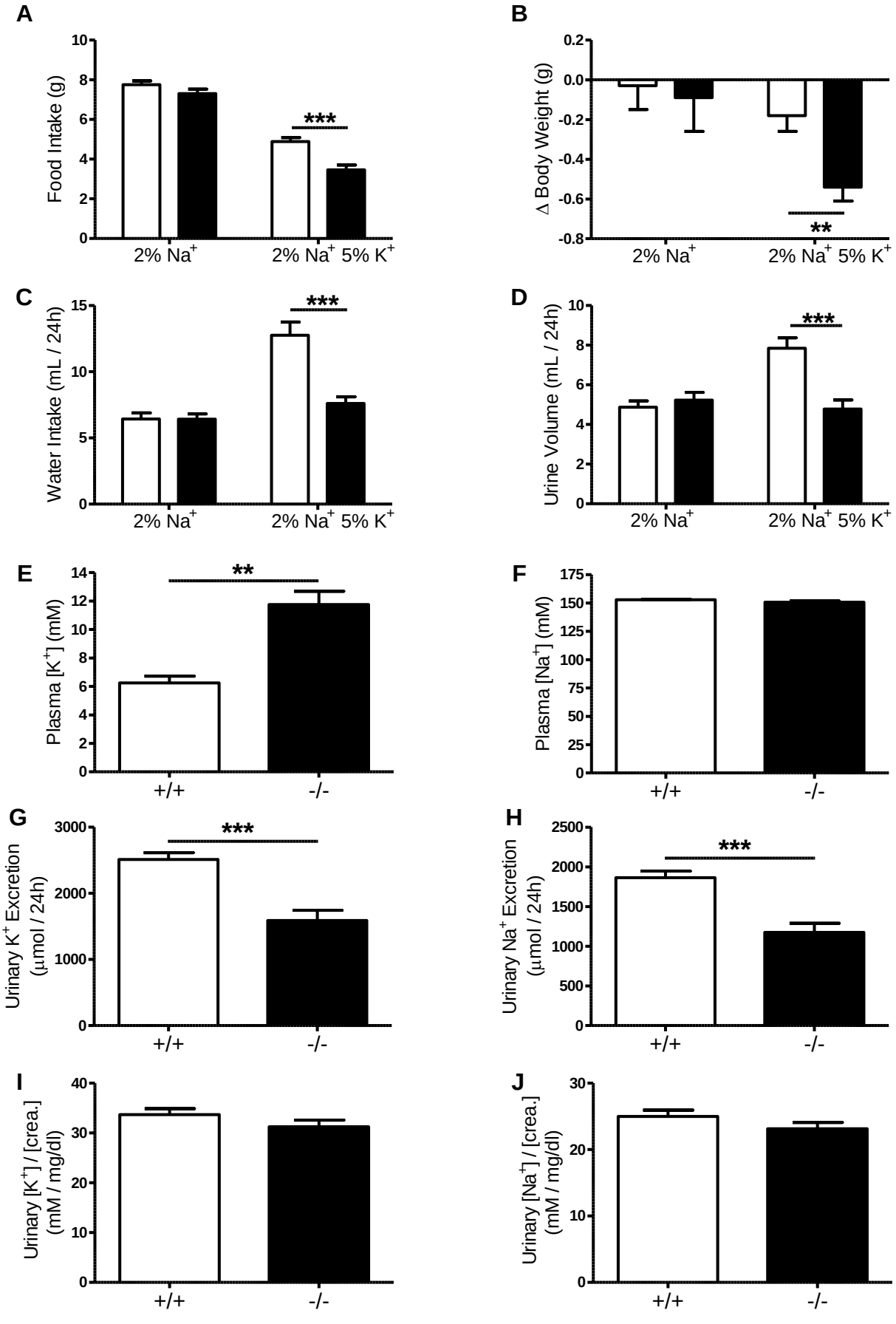
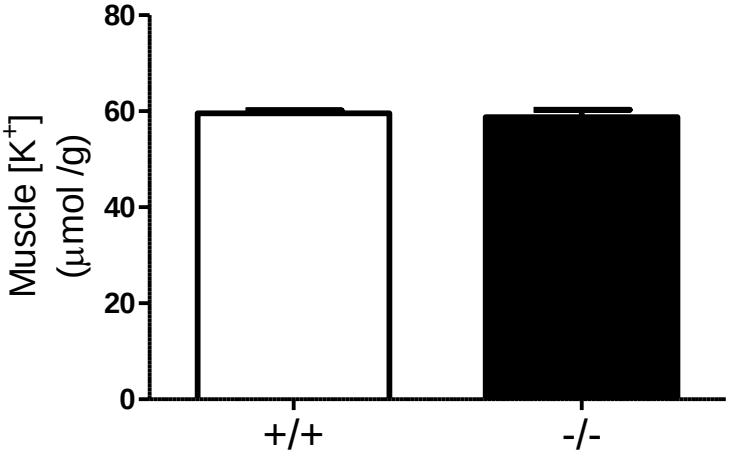


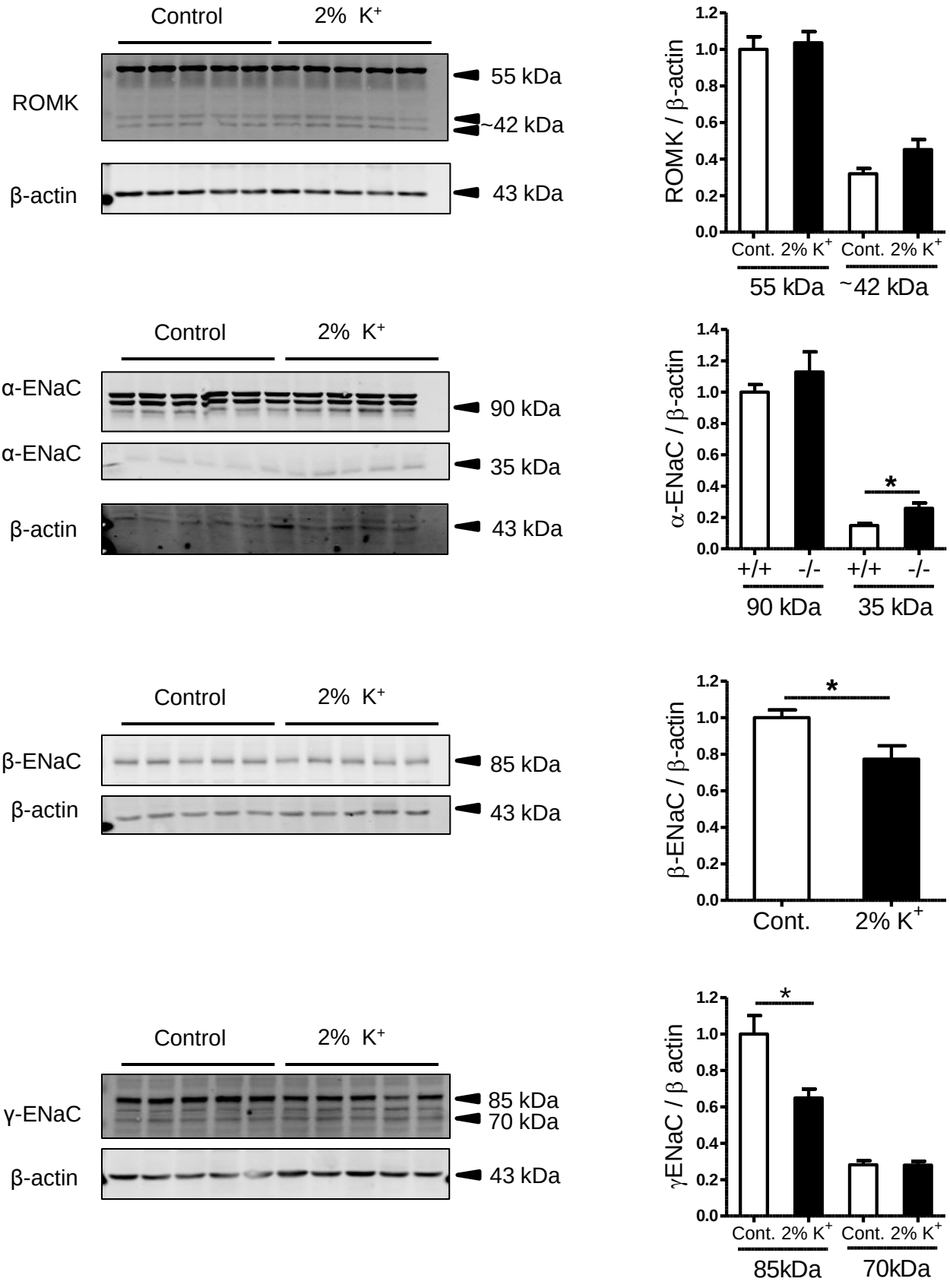
Suppl. figure 4:

High K^+ diet (2% K^+)



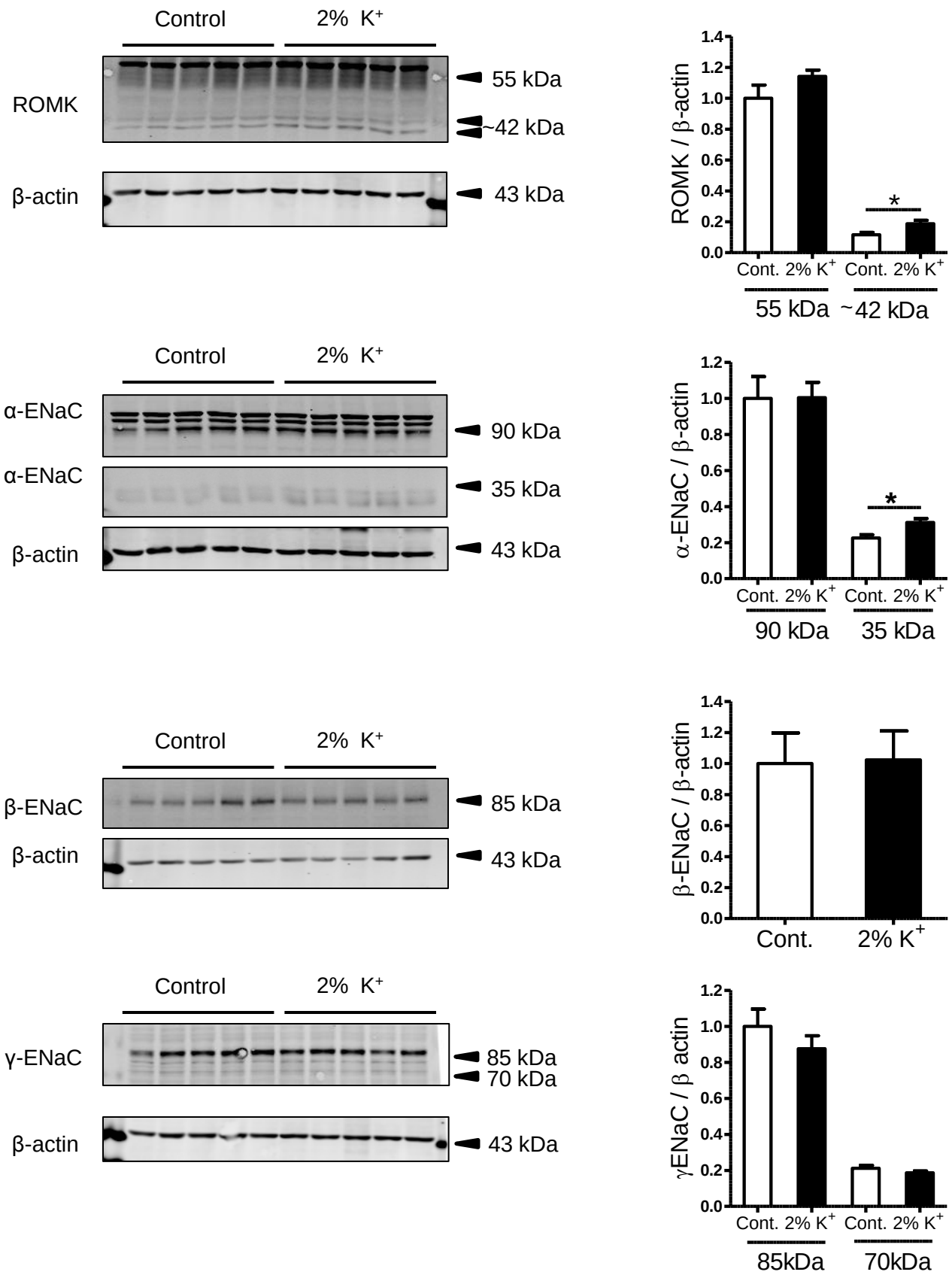
Suppl. figure 5:

AS$^{+/+}$ mice: Control (0.8% K$^+$) vs. 2% K$^+$



Suppl. figure 6:

AS$^{-/-}$ mice: Control (0.8% K$^+$) vs. 2% K$^+$



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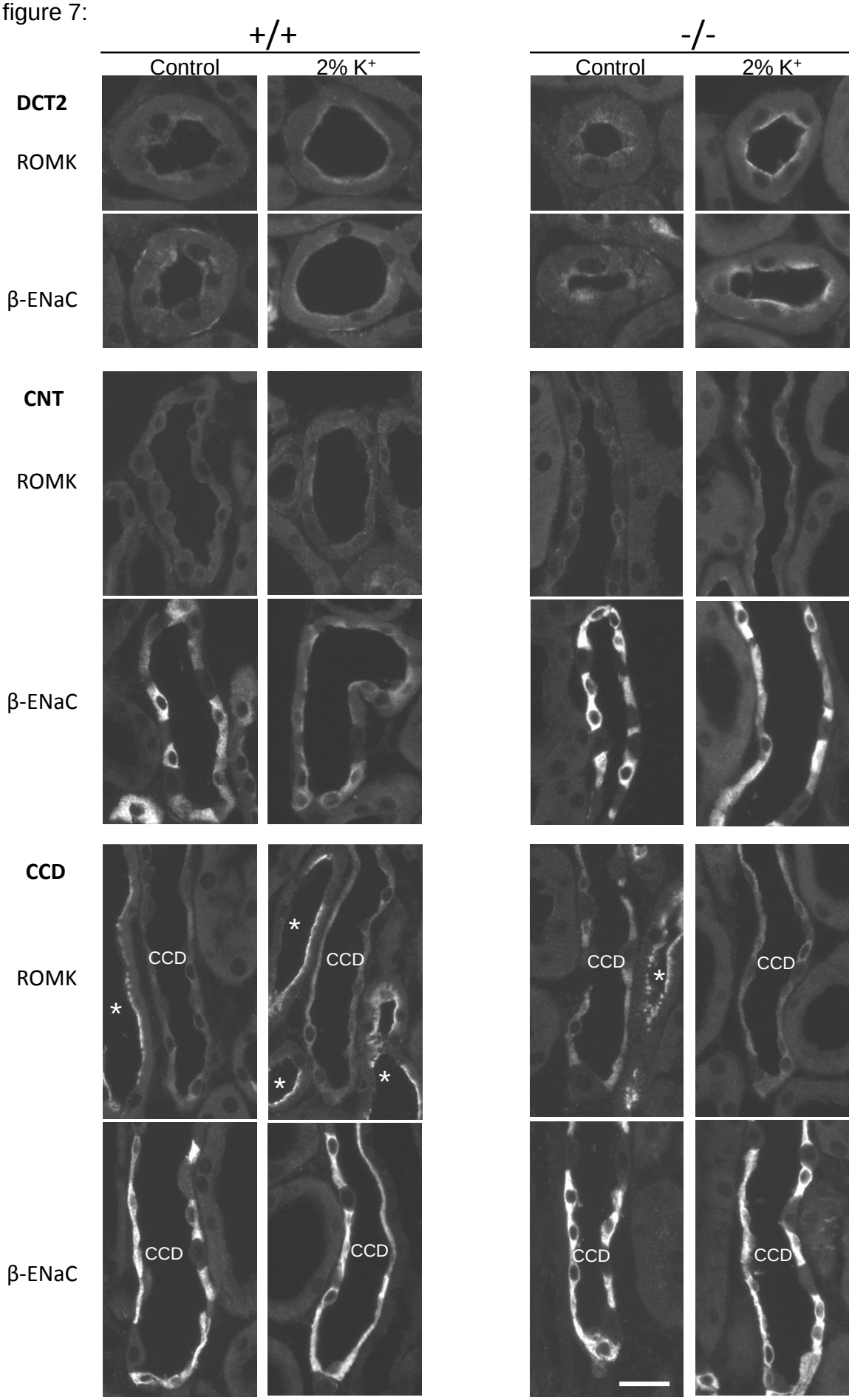
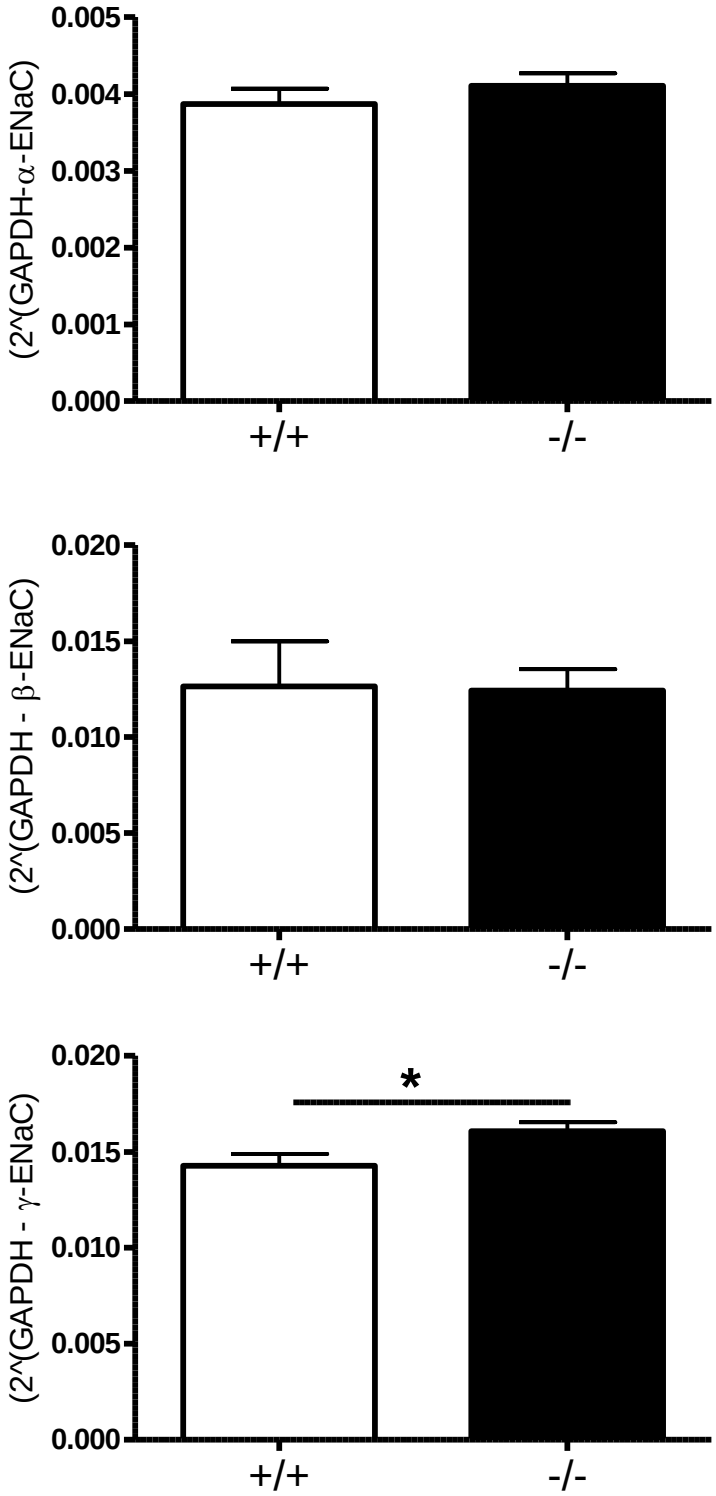


Suppl. figure 8:

High K^+ diet (2% K^+)



Suppl. figure 9: