Af17 Deficiency Increases Sodium Excretion and Decreases Blood Pressure

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ABSTRACT
The putative transcription factor AF17 upregulates the transcription of the epithelial sodium channel (ENaC) genes, but whether AF17 modulates sodium homeostasis and BP is unknown. Here, we generated Af17-deficient mice to determine whether deletion of Af17 leads to sodium wasting and low BP. Compared with wild-type mice, Af17-deficient mice had lower BP (11 mmHg), higher urine volume, and increased sodium excretion despite mildly increased plasma concentrations of aldosterone. Deletion of Af17 led to increased dimethylation of histone H3 K79 and reduced ENaC function. The attenuated function of ENaC resulted from decreased ENaC mRNA and protein expression, fewer active channels, lower open probability, and reduced effective activity. In contrast, inducing high levels of plasma aldosterone by a variety of methods completely compensated for Af17 deficiency with respect to sodium handling and BP. Taken together, these data identify Af17 as a potential locus for the maintenance of sodium and BP homeostasis and suggest that a particular histone modification is directly linked to these processes. Af17-mediated regulation of BP is largely, but not exclusively, the result of modulating ENaC, suggesting it has potential as a therapeutic target for the control of BP.


Abnormal regulation of BP leads to hypertension or hypotension. Hypertension is a major risk factor for cardiovascular diseases such as heart attack, congestive heart failure, stroke, peripheral vascular disease, and ESRD. Hypotension can deprive the brain and other vital organs of oxygen and nutrients, leading to shock, a life-threatening condition.

Enhanced Na+/H+ reabsorption is long thought to increase blood volume and thus systemic arterial BP. Molecular characterization of monogenic forms of hypertension has confirmed this idea. These disorders are exemplified by mineralocorticoid excess, glucocorticoid-remedial aldosteronism, pseudohypoaldosteronism type II (PHAII), and Liddle’s syndrome. They result from inactivated 11β-HSD2,1 fused 11-hydroxylase/aldosterone (aldo) synthase (AS),2 gain-of-function mutations in the mineralocorticoid receptor (MR),3 or in the with-no-lysine (K) kinases WNK1 and WNK4,4,5 or gain-of-function mutations in β and γ subunits of the epithelial sodium channel (ENaC),6,7 respectively. All of these mutations impinge on salt...
absorptive mechanisms in the kidney. WNK1 and WNK4 have been shown to regulate ENaC.

A number of inherited disorders characterized by salt wasting and hypertension are caused by loss-of-function mutations impairing salt homeostasis. Examples include Bartter’s and Gitelman’s syndromes, with defects in six genes encoding renal tubular transporters and ion channels (reviewed in ref. 10), and autosomal recessive PHAI, with loss-of-function mutations in the three subunits of ENaC.

However, the etiology of abnormal BP is not completely defined. First, BP is a complex trait determined by the interactions of multiple genetic and environmental factors. Epigenetic regulation through chromatin modifications should be important in BP control. To date, a direct link between a particular gene impinging histone modifications to BP has not been clearly established. Second, numerous mouse models lacking ENaC and their direct or indirect regulators such as AS, angiotensinogen (Agt), angiotensin-converting enzyme (ACE), MR, Sgk1, WNK1, WNK4, and Period have been reported. However, the role of these models in showing the epigenetic control of BP is still unclear. This is because, to our knowledge, the function of these genes in chromatin regulation remains unknown. Finally, ENaC is tightly regulated at multiple levels by aldosterone. Most studies concerning ENaC and aldosterone focus on posttranslational events (reviewed in 21), leaving a gap in knowledge between chromatin modifications and regulation of ENaC transcription, Na+ retention and BP.

We have described that aldosterone activates ENaC transcription in part by inhibiting Dot1a-Af9-mediated histone H3 K79 methylation in IMCD3 cells and that ALL-1 partner at 17q21 also decreases H3 K79 methylation to upregulate these genes. Decreased BP observed in several of these knockout mice under the same dietary conditions (see Discussion).

Active Channel Number, Open Probability, and Effective Activity of ENaC Were Lower in Af17−/− than Af17+/+ Mice on the Normal Na+ Diet

To determine whether the Na+ disturbance is a result of attenuated ENaC function, we performed cell-attached experiments to directly assess the basal ENaC activity on the apical plasma membrane of principal cells in split-open aldosterone-distal nephron. Figure 2A contains representative current traces. In parallel with dramatically decreased ENaC open probability (Po) and the number of active channels within a patch (N), Af17−/− mice on the normal Na+ diet have markedly reduced effective ENaC activity (INPo), which is defined as NPo multiplied by the probability of observing patches with at least one active channel (f) (Figure 2, B–D). Although the decrease in N can be attributed to the impaired ENaC transcription (see below), the decrease of the Po points to an unexpected new role of Af17 in regulating ENaC activity.

Af17−/− Mice Fed the Normal Na+ Diet Display Impaired ENaC Expression and Increased H3 K79 Methylation in Whole Kidney Lysate

To investigate whether the decreased active channel number of ENaC results from impaired ENaC expression in kidney, we performed real-time RT-qPCR and Western blot analyses. Af17−/− mice displayed decreased expression of α, β, and γENaC to about 19, 67, and 76% of control at the mRNA level and to about 19, 42, and 37% of control at the protein level, respectively (Figure 3). As predicted, histone H3 K79 dimethylation (me2K79) was increased to 157% of control, and H3 K9 dimethylation (me2K9) remained constant with Af17 deletion (Figure 3).

Af17 Is Expressed in Aqp2-Expressing Renal Collecting Duct Principal Cells

To test whether Af17 is expressed in aldosterone-sensitive distal nephron, we analyzed the expression of β-geo reporter driven
by the Af17 promoter in the Af17−/− kidney,25 with Aqp2 as a marker of principal cells. X-gal staining was detected in Aqp2-expressing and many other cells (Figure 4). Therefore, Af17 is expressed in the principal cells, providing a molecular basis for directly regulating ENaC expression and activity.

Af17−/− Mice Fed a Normal Na+ Diet Display H3 K79 Hypermethylation at the αENaC Promoter

To show that Af17 regulates αENaC transcription through modulation of H3 K79 methylation in vivo, chromatin immunoprecipitation (ChIP) assay coupled with real-time qPCR was conducted with primers specific for amplification of the αENaC promoter regions (Figure 5A and refs. 22,27,28). ChIP with anti-me2K79 detected no substantial signals in Ra and R1 regions but robust signals from R0, R2, and R3 in wild-type (WT) mice (Figure 5B). Despite no difference in Ra, Af17−/− mice significantly enhanced me2K79 in all four other regions, with >170-fold in R1 versus WT control (Figure 5B). No obvious differences in total H3 or H3 me2K9 between the two groups over the entire αENaC 5′-flanking region were observed (Figure 5C). Furthermore, ChIP with normal rabbit IgG yielded barely detectable background signals. In brief, deletion of Af17 causes specific changes in H3 me2K79 associated with the αENaC promoter, but not a generalized effect on histone H3 in vivo in mouse kidney, indicating that Af17 upregulates αENaC mRNA expression by modulating H3 me2K79 at the αENaC promoter.

Aldo-Perfused Af17−/− Mice Display Similar Renal Physiology and BP to Af17+/+ Control

To directly test the hypothesis that high levels of aldo antagonizes the effect of Af17 deletion on H3 K79 methylation and thus ameliorates the renal disfunction of Af17−/− mice, Af17+/+ and Af17−/− (n = 10 to 11 mice/genotype) animals

Figure 1. Af17−/− mice fed the normal Na+ diet showed impaired renal function and decreased BP, despite mildly increased plasma aldo. Af17+/+ (n = 34) and Af17−/− (n = 37) mice fed the normal Na+ diet in metabolic cages were analyzed for the parameters as indicated. For BP measurement, n = 57 mice for Af17+/+ and n = 65 mice for Af17−/−. For all other parameters, n = 6 to 37 mice/group. For additional parameters, see Supplemental Figure S1. In all cases, *P < 0.05 versus Af17+/+.
Aldo perfusion (Figure 6). Other urinary values ([Na\(^+\)], [K\(^+\)], [creatinine], [Na\(^+\)]/[creatinine], and [K\(^+\)]/[creatinine]) were similar at all of these three time points (Supplementary Figure S2). The plasma [Na\(^+\)], [K\(^+\)], and [aldo] at day 8 showed no significant difference between the mutants and WT, each with >1600 pg/ml of plasma aldo (Figure 6E and Supplementary Figure S2).

Compared with the normal Na\(^+\) diet, aldo perfusion significantly increased the number of active channel, Po, and effective activity of ENaC in both sets of mice except WT Po to various degrees ranging from 16 to about 600% (Figure 2, A–D). However, all of these parameters were no longer significantly different between the two groups. Consistently, no significant difference in protein expression of α, β, and γENaC and H3 m2K79 was observed between Af17\(^{-/-}\) and Af17\(^{+/+}\) mice (Supplementary Figure S3).

On a Low Na\(^+\) Diet, Af17\(^{-/-}\) Mice Exhibited Similar Renal Physiology and BP to Af17\(^{+/+}\) Control in a Time-Dependent Manner

Low Na\(^+\) diet is known to increase [aldo]. To test whether low Na\(^+\) diet-induced aldo excess also rescues Af17\(^{-/-}\) phenotype, we challenged Af17\(^{+/+}\) and Af17\(^{-/-}\) (n = 6 to 20 mice/group) animals with a low Na\(^+\) diet (0.02% Na\(^+\)). Urine was collected at 12 hours and then every 24 hours. After 72 hours, urinary Na\(^+\) was barely detectable. Therefore, additional urinary analyses beyond this time point were omitted. BP was measured for 7 days. At day 8, mice were terminated for further analyses. All mice survived the treatment, eliminating the possibility of biased data because of differences in the animal tolerance to the challenge.

Both groups had greatly decreased urine volume and excretion of Na\(^+\), K\(^+\), and creatinine to levels ranging from 17 to 69% of their pretreatment values at 12 hours. For these parameters, Af17\(^{-/-}\) mice maintained significantly higher levels than control (Figure 7 and Supplementary Figure S4). However, such differences became nonsignificant as the treatment proceeded. No differences were also observed in urinary [Na\(^+\)], [K\(^+\)], [creatinine], [Na\(^+\)]/[creatinine], and [K\(^+\)]/[creatinine], except [Na\(^+\)] at 12 hours and [creatinine] at 12 and 24 hours (Supplementary Figure S4). The diastolic, systolic, and mean BPs in the Af17\(^{-/-}\) were normalized in all days examined (Figure 7 and Supplementary Figure S4). Similar to aldo perfusion, dietary salt re-

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**Figure 2.** Impairment and restoration of ENaC activity in Af17\(^{-/-}\) mice. (A) Representative current traces from cell-attached patches monitoring ENaC activity under conditions as indicated. Dashed lines indicate the respective current state with a c denoting the closed state. (B–D) Summary graph of ENaC Po (B), active channels within a patch (C), and effective ENaC activity (D). In all cases, *P < 0.05 versus Af17\(^{+/+}\).
striction for 8 days caused a pronounced elevation of ENaC activity for both genotypes compared with those on the normal Na\(^+\) diet (Figure 2). Importantly, we no longer observed an inhibitory effect of Af17 deletion on ENaC Po, the functional channel numbers, and the effective ENaC activity (fNPo) for Af17\(^{+/+}\) and Af17\(^{-/-}\), respectively. The plasma [Na\(^+\)], [K\(^+\)], and [aldo] were statistically indistinguishable between the two genotypes (Figure 7 and Supplementary Figure S4). However, the [aldo] was 750 pg/ml in Af17\(^{-/-}\) and 650 pg/ml in Af17\(^{+/+}\) mice (Figure 7E), which was significantly higher than that on the normal Na\(^+\) diet (Figure 1J).

**Figure 3.** Af17 regulates mRNA and protein expression of ENaC. (A) Real-time RT-qPCR for expression of ENaC genes in kidney of mice fed the normal Na\(^+\) diet, with \(\beta\)-actin as internal control.

**DISCUSSION**

Strong evidence suggests the presence of BP loci at 17p11–21 around marker D17S250 on human chromosome 17.\(^{24,31}\) Mutations in WNK4 within this interval are the causative factor for PHAII.\(^3\) Af17 also lies in this segment only 290 kb away

\(n = 3\) mice/group. (B) Western blots for expression of proteins as indicated, with \(\beta\)-actin as internal control. For \(\alpha\)ENaC, the approximately 30-kD band was excluded in data analyses. me2K79 and me2K9: histone H3 dimethylated K79 and K9, respectively. \(n = 4\) mice/group. In all cases, *\(P < 0.05\) versus Af17\(^{+/+}\).
Af17 is expressed in aldose-sensitive distal nephron. (A and B) Sections of Af17+/− kidney were stained with X-gal and an antibody against the principal cell marker Aqp2. Boxed regions in A and B were amplified in C and D, respectively. The arrowheads indicate cells apparently expressing Aqp2 and β-geo reporter driven by the Af17 promoter.

Figure 4. Af17 deletion causes H3 K79 hypermethylation at the αENaC promoter in kidney. (A) Diagram of the αENaC promoter. ChIP assay showing increased H3me2K79 associated with R0-R3, but not with Ra subregion of the αENaC promoter. Kidney chromatin was prepared from four WT and four mutant mice, pooled into two groups according to genotype, and analyzed by ChIP with the antibodies indicated, and followed by real-time qPCR with primers amplifying the subregions of αENaC promoter as shown in A. Relative H3me2K79 abundance was set to 1 in R0 from WT kidneys and was calculated accordingly for all other samples. *P < 0.05 versus Af17+/+. n = 4 mice/group (B). Representative agarose gel analyses of the final qPCR products were shown to verify the specificity of qPCR for each sample (C).
to see whether mutations of Af17 occur and account for BP variation in the general population.

The observed renal phenotype in Af17−/− mice is not simply caused by a defect in feeding and drinking behavior, but at least partially results from decreased ENaC activity. Under basal conditions, Af17 may downregulate H3me2K79 and up-regulate ENaC by multiple mechanisms, ranging from expression of mRNA, protein, and active channels to Po (Figure 9). Although the mechanism is elusive at this moment, identification of Af17 as an ENaC Po regulator is a surprise and points to another feature not limited to Af17 mice is that the changes in Na+ reabsorption are not accompanied by an inverse effect on K+, indicating that the impaired ENaC function can not account for the complete panoply of the renal dysfunction. Indeed, such coupling was not observed in Sgk−/− and Sgk−/+ mice. 38

Af17−/− mice share the low BP phenotype on a normal Na+ diet with Ren1−/−, AS−/−, Agt−/−, and WNK1−/− mice. Ren1, AS, Agt, and Ace are key components of the renin-angiotensin-aldosterone system and thus are upstream regulators of ENaC. WNK1 and WNK4 can upregulate ENaC activity through SGK1 and Nedd4. Although the BP phenotype of Period−/− mice has not been reported, these mice fed the standard diet also had higher Na+ excretion and lower αENaC expression than WT controls.

Another feature not limited to Af17−/− mice is that the urinary Na+ excretion and lower αENaC expression than WT controls.

Figure 6. Aldo perfusion largely compensated for the loss of Af17 function in renal physiology. Twenty-four-hour urine volume, urinary electrolyte excretion, and BP were examined before (day 0) and after aldosterone perfusion as indicated. n = 10 to 11 mice/genotype. Dark bar: Af17−/+ . Gray bar: Af17−/−. For additional parameters, see Supplemental Figure S2. In all cases, *P < 0.05 versus Af17+/−.
plasma $[K^+]$ was significantly different. Moreover, the significantly increased Na$^+$ excretion was not coupled with markedly decreased K$^+$ excretion and was instead paralleled by increased K$^+$ excretion during the early phases of Na$^+$ restriction in Sgk1$^{-/-}$ versus Sgk1$^{+/+}$ mice.17,37 Similarly, significantly elevated urinary [Na$^+$]/[creatinine] and plasma [K$^+$] in βENaC$m/-$ mice on a low Na$^+$ diet were not accompanied with the expected changes in the urinary excretion of Na$^+$, K$^+$, and plasma [Na$^+$].39

The most striking feature differing $Af17^{-/-}$ from others identified thus far lies in its response to aldosterone. The renal physiology and BP phenotype of $Af17^{-/-}$ mice show similar renal physiology and BP in a time-dependent manner on the low Na$^+$ diet. $Af17^{+/+}$ (solid line or dark bar) and $Af17^{-/-}$ (dotted line or gray bar) mice were fed the low Na$^+$ diet (0.02% Na$^+$) in metabolic cages and analyzed for the parameters before (day 0) and after treatment at various time points as indicated. For BP measurement, $n = 17$ to 21 mice/group. For all other parameters, $n = 4$ to 14 for $Af17^{-/-}$ mice and $n = 6$ to 14 for $Af17^{-/-}$ mice. For additional parameters, see Supplemental Figure S4. In all cases, $^*P < 0.05$ versus $Af17^{+/+}$.

**CONCISE METHODS**

**Reagents**
Antibodies against dimethyl histone H3 K79 and dimethylated histone H3 K9 were purchased from Millipore. Antibodies specific for α, β, or γENaC were either purchased from SantaCruz or kindly provided by Dr. Ryoichi Teruyama, who purified these antibodies originally generated by Dr. Mark Knepper’s group. The chicken anti-Aqp2 antibody is a generous gift from Dr. James Wade (University of Maryland, College Park, MD).

**Serum, Plasma, and Urine Measurements**
The concentrations of Na$^+$, K$^+$, and creatinine in urine or serum were measured with an analyzer (Roche Cobas Integra 400 plus) in the Clinical Pathology Laboratory, Department of Veterinary Medicine and Surgery, University of Texas MD Anderson Cancer Center. This analyzer measures Na$^+$ and K$^+$ using ion selective potentiometry and creatinine by absorbance photometry. Plasma aldosterone was specifically measured using a 125I-Coat-A-Count RIA kit (Siemens, Los Angeles, CA).42 Because the aldosterone-specific antibody used in this kit was generated in rabbit, mouse
samples can be directly measured, eliminating large variations and possible aldosterone loss created from additional extraction steps. Plasma and urine osmolarity was measured by vapor pressure (Wescor Vapro Vapor Pressure Osmometer 5520; Scimetrics, Houston, TX).

Metabolic Balance Studies
Two- to 4-month-old Af17+/+ and Af17−/− mice and their mutant littermates were used for the study. They were acclimated for 3 to 7 days in Tecniplast metabolic cages with free access to water and normal laboratory diet containing 0.4% Na+ for up to 5 days in metabolic cages and analyzed for the parameters before (day 0) and after treatment as indicated. n = 8 to 26 for Af17+/+ mice and n = 7 to 25 for Af17−/− mice. For additional parameters, see Supplemental Figure S5. In all cases, *P < 0.05 versus Af17+/+.

Figure 8. On the high K+ diet, Af17−/− and Af17+/+ mice had similar renal physiology and BP on the high K+ diet. Af17+/+ and Af17−/− mice were fed the high K+ diet (6% K+) for up to 5 days in metabolic cages and analyzed for the parameters before (day 0) and after treatment as indicated. n = 8 to 26 for Af17+/+ mice and n = 7 to 25 for Af17−/− mice. For additional parameters, see Supplemental Figure S5. In all cases, *P < 0.05 versus Af17+/+.

Electrophysiology, Real-Time RT-qPCR, X-gal Staining, and IHC
These assays have been described previously22,25,44 and are detailed in Supplemental Information.
Figure 9. Model for impairment and restoration of Na⁺ balance and BP in Af17⁻/⁻ mice. Under basal conditions such as the normal Na⁺ diet, deletion of Af17 leads to significantly increased H3 K79 methylation, and subsequently impaired ENaC activity, disturbance of Na⁺ balance, and decreased BP. The mildly increased plasma [aldo] is not sufficient to antagonize the effect of Af17 loss on H3 K79 methylation and thus fails to rescue the Af17⁻/⁻ phenotype. Under aldo excess such as aldo perfusion or Af17⁻/⁻ mice. For clarity, detailed mechanisms by which Af17 and aldo inhibit H3 K79 methylation are not shown. The dotted line indicates unknown mechanisms.

Statistical Analysis
Quantitative data were presented as mean ± SEM. A t test was used for all comparisons, with the statistical significance set at P < 0.05.

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DISCLOSURES
None.

REFERENCES


Supplemental Information.

Impaired sodium balance and decreased blood pressure in Af17<sup>-/-</sup> mice

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Materials and Methods.

**X-gal staining and immunohistochemistry.** Adult mouse kidneys were fixed in freshly prepared 4% paraformaldehyde at room temperature for 30-60 minutes, rinsed in rinsing buffer (PBS with 0.02% NP-40, 0.01% sodium deoxycholate, and 2mM MgCl₂) and then incubated in staining solution (1 mg/ml X-gal plus 5 mM potassium ferricyanide and 5 mM potassium ferrocyanide in rinsing buffer) overnight at 37°C. Immunohistochemical labeling was subsequently performed with the chicken anti-Aqp2 antibody which was prediluted with PBS plus 5% normal serum. The incubation was conducted overnight at 4°C. HRP labeled secondary antibodies, Elite ABC kit, and DAB staining kit (all from Vector Laboratories) were used for subsequent immunohistochemical staining procedures according to the manufacturer’s instructions.

**Electrophysiology.** Isolation of the aldosterone-sensitive distal nephrons (ASDNs) containing connecting tubule (CNT) and cortical collecting duct (CCD) suitable for electrophysiology has been described previously.¹⁻³ Briefly, mice were sacrificed by CO₂ administration followed by cervical dislocation with kidneys immediately removed. Kidneys were cut into thin slices (< 1mm) with slices placed into ice-cold physiologic saline solution buffered with HEPES (pH 7.4). The ASDN was identified as merging of CNT into CD and was mechanically isolated from cortical sections of kidney slices by micro-dissection using watchmaker forceps under a stereomicroscope. Isolated ASDN was allowed to settle onto 5 x 5 mm cover glass coated with poly-L-lysine. Cover glass containing ASDN was placed in a perfusion chamber mounted on an inverted Nikon Eclipse Ti microscope and perfused with room temperature HEPES buffered (pH 7.4) saline solution. ASDN were split-open with two sharpened micropipettes controlled with
different micromanipulators to gain access to the apical membrane and were used within 1-2 hr of isolation.

ENaC activity in principal cells was determined using patch clamp electrophysiology in cell-attached configuration under voltage-clamp conditions (-V_p = -60 mV).^2^3 Recording pipettes had resistances of 8-12 megaohms. Typical bath and pipette solutions were (in mM): 150 NaCl, 5 KCl, 1 CaCl_2_, 2 MgCl_2_, 5 glucose and 10 HEPES (pH 7.4); and 140 LiCl, 2 MgCl_2_ and 10 HEPES (pH 7.4), respectively. For each experimental condition, at least 6 ASDNs from 2-3 different mice were assayed. Gap-free single channel current data from gigaohm seals were acquired (and subsequently analyzed) with an Axopatch 200B (Axon Instr.) patch clamp amplifier interfaced via a Digidata 1440 (Axon Instr.) to a PC running the pClamp 10.2 suite of software (Axon Instr.). Currents were low-pass filtered at 100 Hz with an eight-pole Bessel filter (Warner Instr.). Events were inspected visually prior to acceptance. For presentation, current data from some cell-attached patches were subsequently software filtered at 50 Hz and slow baseline drifts were corrected. Channel activity, defined as NP_o, was calculated using the following equation: NP_o = (t_1 + 2t_2 + … + nt_n), where N and P_o are the number of ENaC in a patch and the mean open probability of these channels, respectively, and t_n is the fractional open time spent at each of the observed current levels. P_o was calculated by dividing NP_o by the number of active channels within a patch as defined by all-point amplitude histograms. The frequency of observing ENaC (f) for a given condition was calculated as the ratio of the number of patches containing at least one active ENaC to the total number of patches made on the apical membrane of principal cells.
Supplemental Figures.

Fig. S1. Additional metabolic balance data in Af17+/+ and Af17−/− mice on the normal Na⁺ (0.4%) diet. Af17+/+ and Af17−/− mice fed the normal Na⁺ diet were placed in metabolic cages and analyzed as indicated. n = 34-37 per data point except osmolarity. For osmolarity, n=6 mice/genotype. *: < 0.05 vs. Af17+/+. BW: body weight (g). No significance was detected for all parameters at P < 0.05 vs. Af17+/+. 
Fig. S2. Aldosterone excess largely compensated for the loss of Af17 function in renal physiology: analyses of additional parameters. Af17+/+ (dark bar) and Af17−/− (light gray bar) mice were fed the normal Na+ diet and perfused with aldosterone via s.c. implanted mini pumps at a dose of 10 µg/100 g BW/day in metabolic cages. 24-h urinary analyses were performed before (Day 0) and after aldosterone administration for 4-8 days as indicated. n=10-11 mice for each genotype. *: P < 0.05 vs. Af17+/+. 
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Fig. S3. Aldo excess blunts the differences in ENaC protein expression and H3K79 methylation between $Af17^{+/+}$ and $Af17^{-/-}$ mice. $Af17^{+/+}$ and $Af17^{-/-}$ mice were perfused with aldo for 8 days. Kidneys were analyzed by immunoblotting with the antibodies as indicated. n=4 mice/group. *P < 0.05 vs. $Af17^{+/+}$.

A. Urinary [Na$^+$] (mEq/L) at 0h, 12h, 24h, 48h, 72h

B. Urinary [K$^+$] (mEq/L) at 0h, 12h, 24h, 48h, 72h

C. Urinary [Cre] (mg/dl) at 0h, 12h, 24h, 48h, 72h

D. Urinary [Na$^+$]/[Cre] at 0h, 12h, 24h, 48h, 72h

E. Urinary [K$^+$]/[Cre] at 0h, 12h, 24h, 48h, 72h

Fig. S4. On the low Na$^+$ diet, $Af17^{+/+}$ and $Af17^{-/-}$ mice had similar renal physiology at most time points: analyses of additional parameters. $Af17^{+/+}$ (solid line) and $Af17^{-/-}$ (dotted line) mice were fed the low Na$^+$ diet (0.02% Na$^+$) in metabolic cages. 24-h urinary analyses were performed before (Day 0) and after treatment at various time points as indicated. n=4-14 for $Af17^{+/+}$ mice and n=6-14 for $Af17^{-/-}$ mice. In all cases, *P < 0.05 vs. $Af17^{+/+}$.
Fig. S5. On the high K⁺ diet, Af17⁻/⁻ and Af17⁺/+ mice had similar renal physiology and BP: analyses of additional parameters. Af17⁺/+ (dark bar) and Af17⁻/⁻ (light grey bar) were fed the high K⁺ diet (6% K⁺) for up to 5 days in metabolic cage and analyzed for the parameters before (Day 0) and after treatment for 2-5 days as indicated. n=8-26 for Af17⁺/+ mice and n=7-25 for Af17⁻/⁻ mice. In all cases, *P < 0.05 vs. Af17⁺/+.

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