

Renal Corin Is Essential for Normal Blood Pressure and Sodium Homeostasis

Supplementary Figures and Table

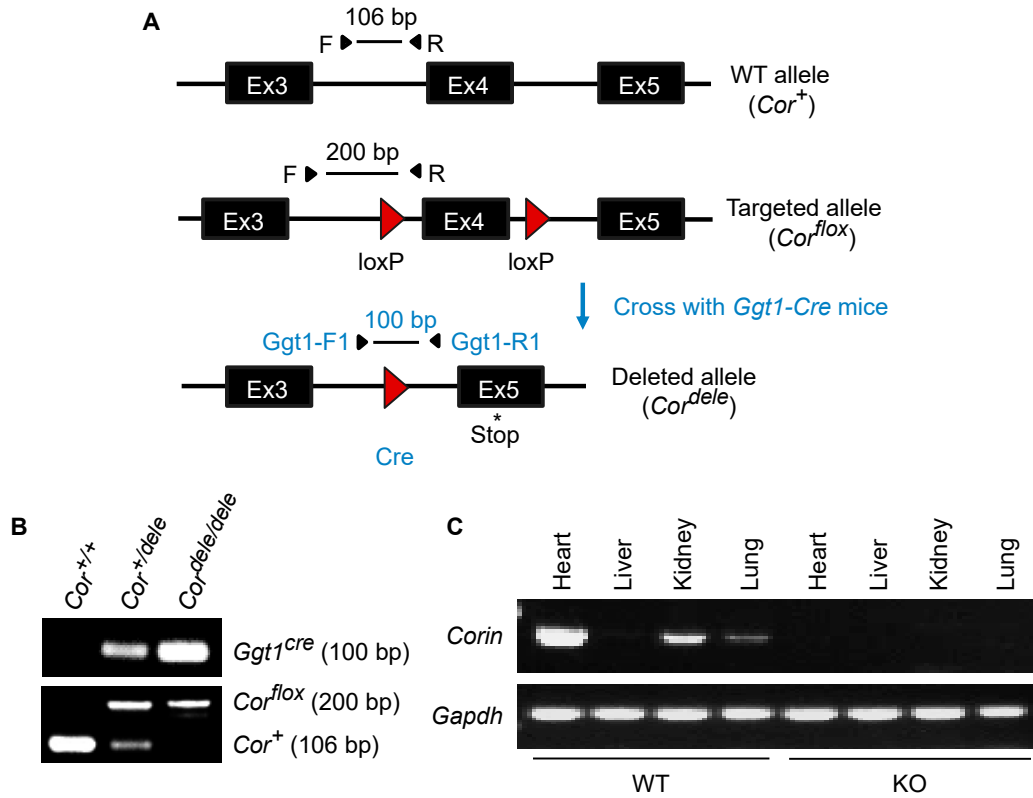


Figure S1. Generation of corin kcKO mice. (A) Schematic illustration of *Corin* WT allele (Cor^+), Cor^{lox} allele with two loxP loci flanking exon 4 in the *Corin* gene, and Cor^{dele} allele generated by crossing Cor^{lox} and *Ggt1-Cre* mice. Oligonucleotide primers used in PCR genotyping and expected sizes of PCR fragments are indicated. *Stop codon. (B) PCR genotyping in WT mice and heterozygous and homozygous mice with Cor^{dele} allele. (C) RT-PCR analysis of *Corin* mRNA expression in tissues from WT mice and corin global KO mice generated by crossing Cor^{lox} mice and *CMV-Cre* mice expressing *Cre* ubiquitously.

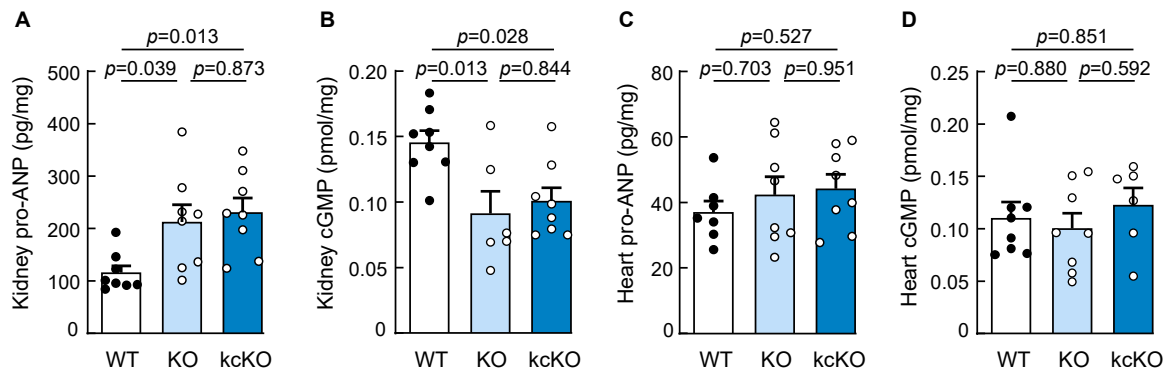
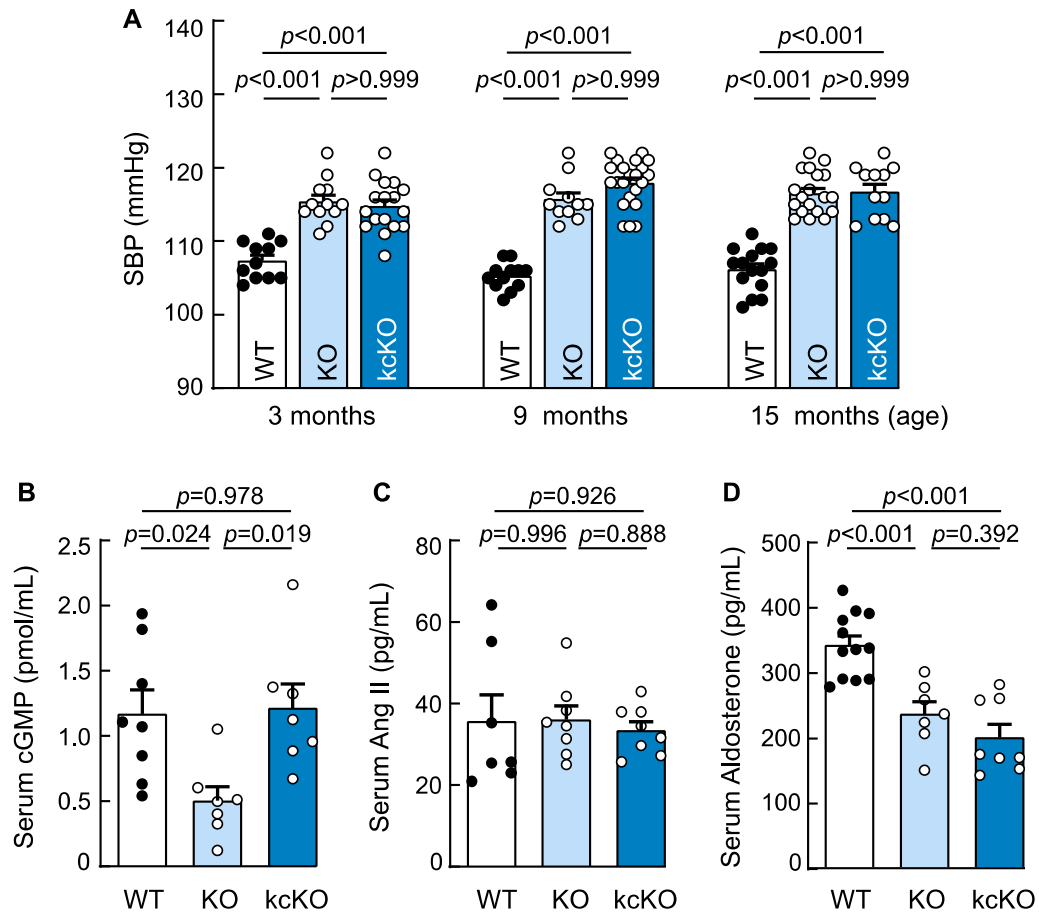


Figure S2. Pro-ANP and cGMP levels in the kidney and heart from female WT, corin KO, and kcKO mice. Kidney pro-ANP (A) and cGMP (B) and heart pro-ANP (C) and cGMP (D) levels in WT (black dots), corin KO and kcKO (white dots) mice. $n = 6-8$ per group. Data shown are mean \pm SEM. p Values were analyzed by one-way ANOVA.



dots), corin KO, and kcKO (white dots) mice were measured by ELISA (**B,D**) or Enzyme Immunoassay (**C**). $n = 7-12$ per group. Data shown are mean \pm SEM. p Values were analyzed by one-way ANOVA.

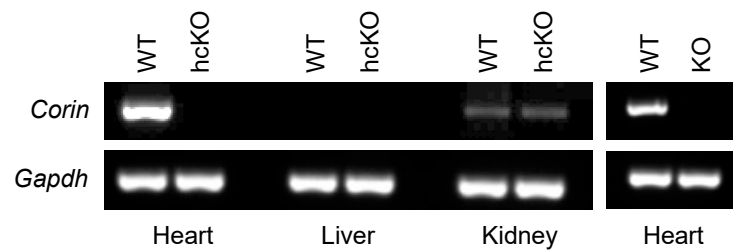


Figure S4. RT-PCR analysis of *Corin* mRNA expression in corin hcKO mice. *Corin* mRNA expression in the heart, liver (negative control), and kidney from WT, corin hcKO, and KO mice was examined by RT-PCR. *Gapdh* mRNA expression was used as a control. Data are representative of at least three experiments.

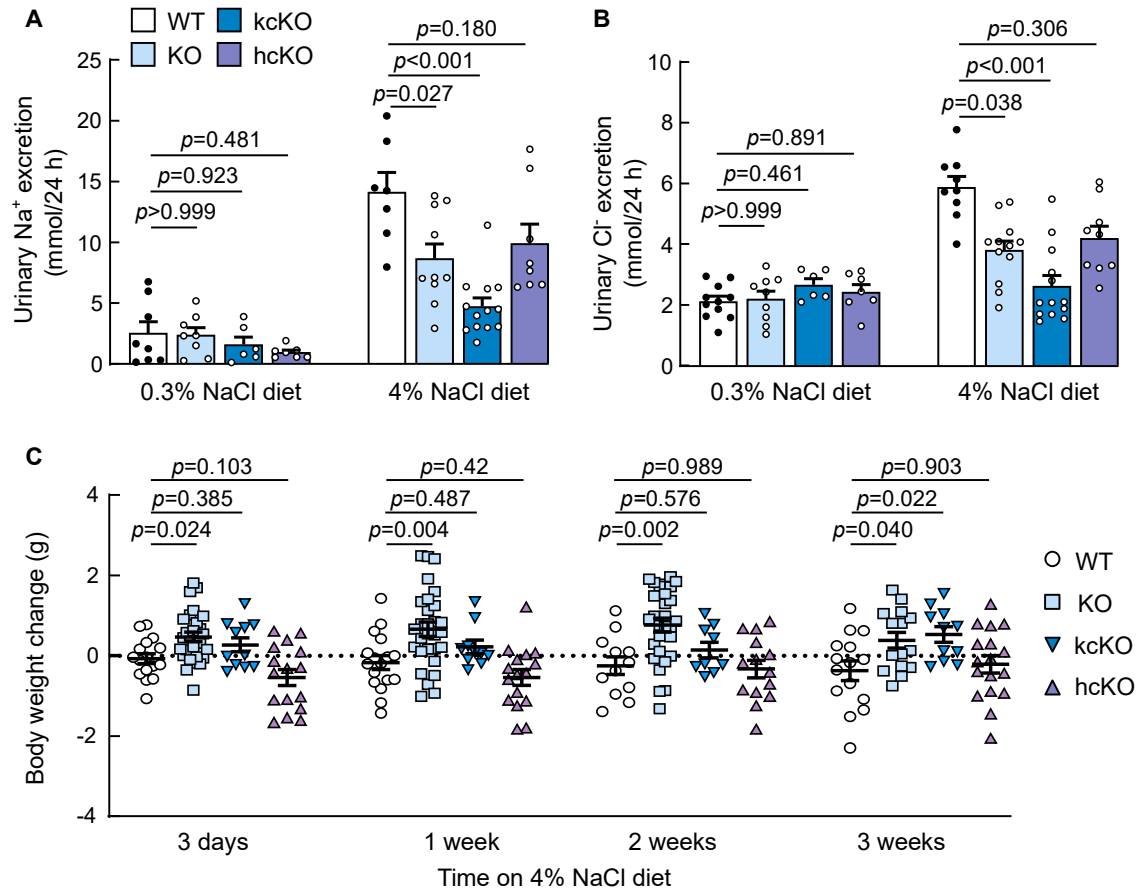


Figure S5. Urinary salt excretion and body weight change in female corin kcKO mice. (A,B) Urinary Na^+ (A) and Cl^- (B) excretion in female WT (black dots), corin KO, kcKO, and hcKO (white dots) mice on 0.3% and 4% NaCl diet were measured. $n = 6\text{--}13$ per group. (C) Body weight changes in female WT, corin KO, kcKO, and hcKO mice when 0.3% NaCl diet was switched to 4% NaCl diet. $n = 9\text{--}29$ per group. Data are mean \pm SEM. p Values were analyzed by one-way ANOVA (A,C) or Kruskal-Wallis test (B).

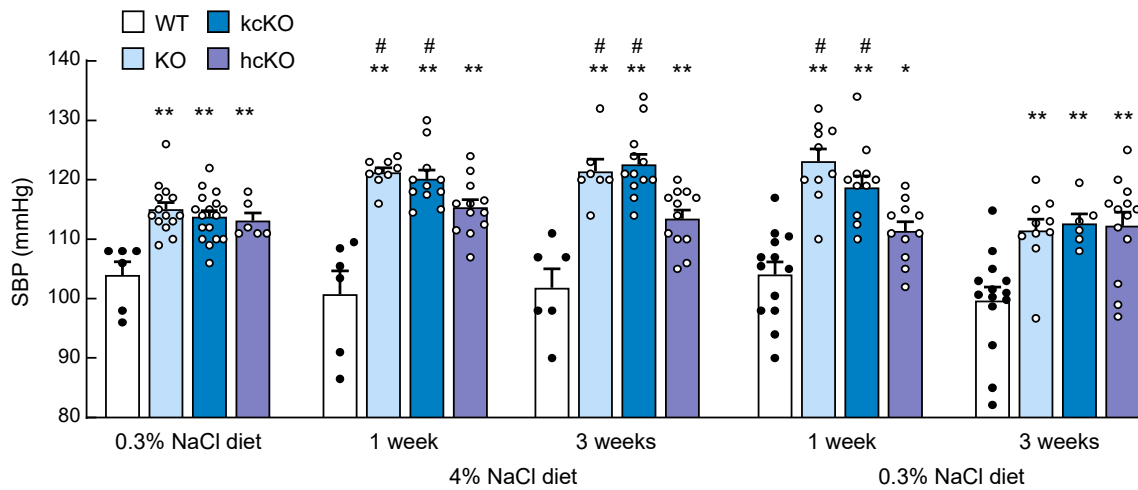


Figure S6. Salt-exacerbated hypertension in female corin KO and kcKO mice. Systolic blood pressure (SBP) was measured in female WT (black dots), corin KO, kcKO, and hcKO (white dots) mice on 0.3% NaCl diet, then 4% NaCl diet for one and three weeks, and back to 0.3% NaCl diet for one

and three weeks. * $p < 0.05$; ** $p < 0.01$ vs. WT of the same group. # $p < 0.05$ vs. the same genotype on initial 0.3% NaCl diet. $n = 6-17$ per group. Data shown are mean \pm SEM. p Values were analyzed by one-way ANOVA.

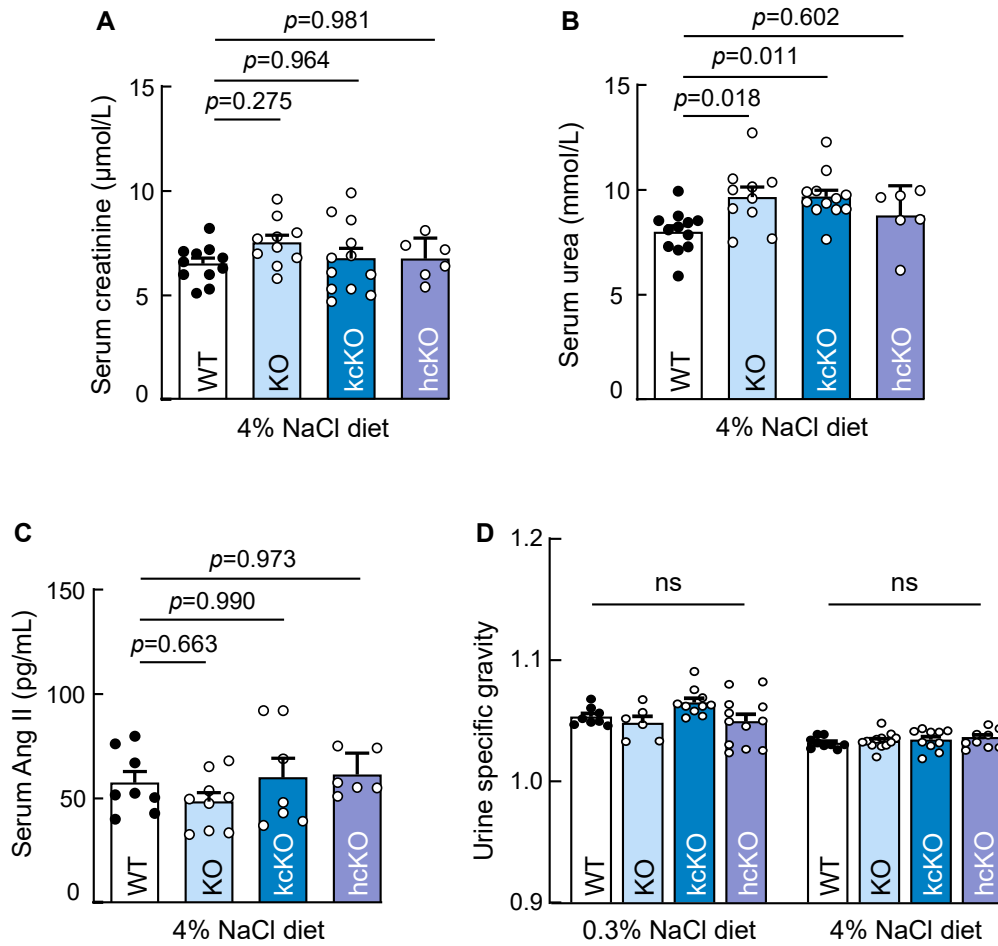


Figure S7. Serum creatinine and urea levels and urine specific gravity in male WT, corin KO, kcKO, and hcKO mice. (A–C) Serum creatinine (A), urea (B), and Ang II (C) levels in WT (black dots), corin KO, kcKO, and hcKO (white dots) mice on 4% NaCl diet were measured. $n = 6-12$ per group. (D) Urine specific gravity was measured in samples from WT, corin KO, kcKO, and hcKO mice on 0.3% NaCl and 4% NaCl diets. $n = 6-12$ per group. Data shown are mean \pm SEM. p Values were analyzed by one-way ANOVA. ns, not significant vs. WT.

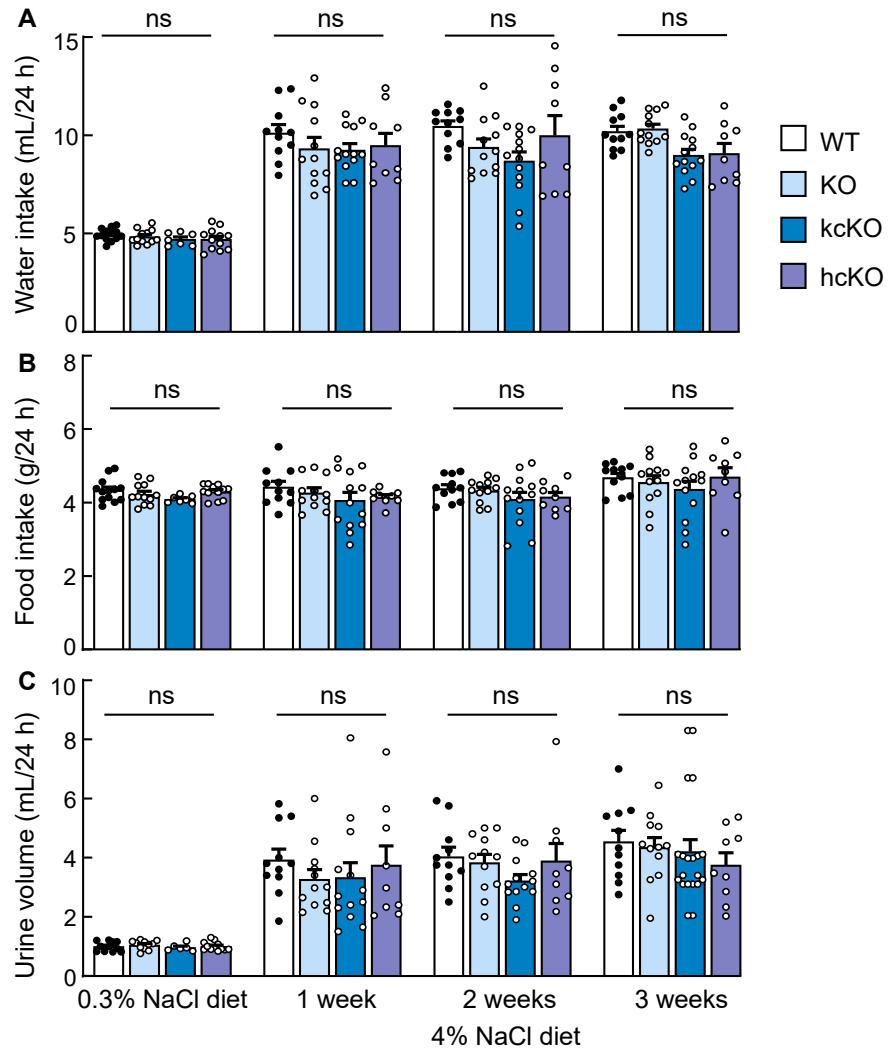


Figure S8. Water and food intakes and urine volume in female corin KO, kcKO, and hcKO mice. (A–C) Water (A) and food (B) intakes and urine volume (C) were measured in female WT (black dots), corin KO, kcKO, and hcKO (white dots) mice on 0.3% NaCl diet and 4% NaCl diet for one to three weeks. $n = 6–20$ per group. Data shown are mean \pm SEM. p Values were analyzed by one-way ANOVA (A,B) or Kruskal-Wallis test (C). ns, not significant.

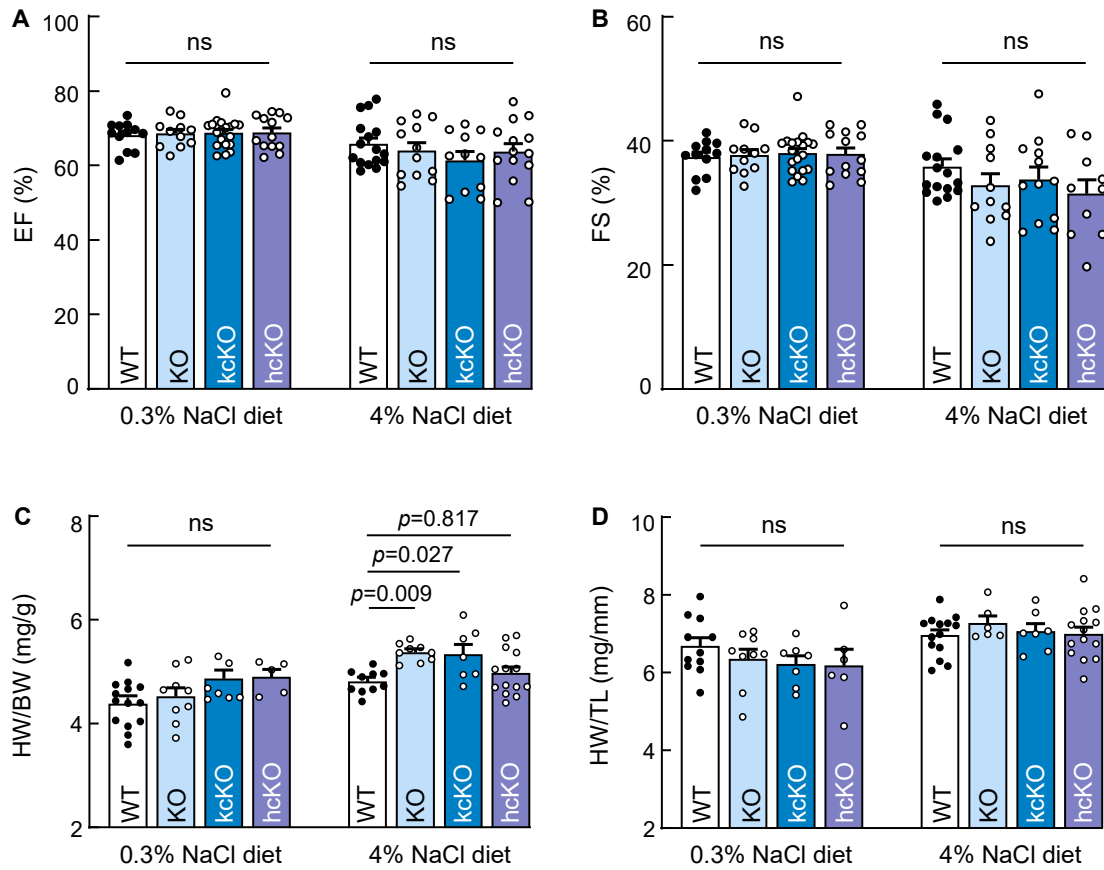


Figure S9. Cardiac hypertrophy in female corin KO and kcKO mice on 4% NaCl diet. (**A,B**) Cardiac function, indicated by ejection fraction (EF) (**A**) and fraction shortening (FS) (**B**), was measured by echocardiography in female WT, corin KO, kcKO, hcKO mice on 0.3% NaCl diet and 4% NaCl diet for three weeks. $n = 10-19$ per group. (**C,D**) Cardiac hypertrophy was assessed by ratios of heart weight (HW) to body weight (BW) (**C**) or tibia length (TL) (**D**) in female WT, corin KO, kcKO, hcKO mice on 0.3% NaCl diet and 4% NaCl diet for three weeks. $n = 5-14$ per group. Data shown are mean \pm SEM. p Values were analyzed by one-way ANOVA. ns, not significant.

Table S1. Sequences of oligonucleotide primers used in PCR and RT-PCR.

| Gene | Locus | Primer | Sequence | Size (bp) |
|-------|----------------|---------|----------------------------|---|
| Corin | NM_016869.3 | forward | ATCACTCACAGCCAGTGTCAA | 390 |
| | | reverse | TCCACAGAGTGATTGCTTTCCAT | |
| Corin | NM_016869.3 | forward | TCAATTATGTGTGGCCTTCGGGGTG | <i>Cor⁺</i> 106 <i>Cor^{flox}</i> 200 |
| | | | GTTTT | |
| | | reverse | TCTATCTTATTGAAAAGTCTCTGTTT | |
| Gapdh | NM_001289726.1 | forward | TGTCCTACCCCCAATGTGT | 138 |
| | | reverse | GGTCCTCAGTGTAGCCCAAG | |
| Ggt1 | NZ_CP070291.1 | forward | GCGGTCTGGCAGTAAAACTATC | 100 |
| | | reverse | GTGAAACAGCATTGCTGTCACTT | |