Supplemental Data

# Knockout of the circadian clock protein PER1 exacerbates hypertension and increases kidney injury in Dahl salt-sensitive rats

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## Supplementary Methods

*RT-PCR and Sequencing.* Total liver RNA was extracted and converted to cDNA using random hexamer primers as previously described (Douma et al.). A total of 20ng of cDNA was used in PCR with the rPER1_RNA_FWD and rPER1_RNA_REV primers using a 53*C annealing temperature. The 461 base pair products were excised from agarose gel and purified using a gel extraction kit (Qiagen, Germantown, MD, USA). Purified samples were then subjected to Sanger sequencing using the rPER1_RNA_SEQ primer. RT-PCR primers and the primer used for sequencing are listed below.

| | |
|---|---|
| rPER1_RNA_FWD | GCCTCTTCACCTTCCCTGTTTTG |
| rPER1_RNA_REV | ATGAGTTCTTTCTGGGTCCTGGC |
| rPER1_RNA_SEQ | AGATGAACTGTGAGAACTCCGCTG |

*Western Blot*. Total liver protein homogenates were prepared and subjected to gel electrophoresis and Western blotting similar to previously described (Crislip et al.). In brief, membranes were stained with Ponceau for 5 min and imaged before washing with PBS. Blots were blocked in 3% non-fat milk in PBS-Tween for 8 hours and the primary anti-PER1 antibody (Cat No. GTX128974, Genetex, Irvine, CA, USA; RRID: AB_2885855) was diluted 1:500 in 1% non-fat milk in PBS-Tween overnight in 4$^{o}$. After washing, blots were incubated with horseradish-peroxidase-conjugated anti-rabbit secondary antibody. After an additional wash step, detection was performed using SignalFire ECL reagent (Cell Signaling Technology) with a 60 second exposure.

## Supplemental References

Douma LG, Costello HM, Crislip GR, Cheng KY, Lynch IJ, Juffre A, Barral D, Masten S, Roig E, Beguiristain K, Li W, Bratanatawira P, Wingo CS, Gumz ML. Kidney-specific KO of the circadian clock protein PER1 alters renal Na+ handling, aldosterone levels, and kidney/adrenal gene expression. Am J Physiol Renal Physiol. 2022 Apr 1;322(4):F449-F459. doi: 10.1152/ajprenal.00385.2021.

Crislip GR, Wohlgemuth SE, Wolff CA, Gutierrez-Monreal MA, Douglas CM, Ebrahimi E, Cheng KY, Masten SH, Barral D, Bryant AJ, Esser KA, Gumz ML. Apparent Absence of BMAL1-Dependent Skeletal Muscle-Kidney Cross Talk in Mice. Biomolecules. 2022 Feb 5;12(2):261. doi: 10.3390/biom12020261.

**Supplemental Table S1. Cosinor Analysis of Heart Rate**

| Treatment Group | Mesor | | | Amplitude | | | Robustness | | |
|---|---|---|---|---|---|---|---|---|---|
| | Average | SEM | Two-Way ANOVA | Average | SEM | Two-Way ANOVA | Average | SEM | Two-Way ANOVA |
| **SS Normal salt** | 416.2 | 8.3 | p-value genotype 0.7524<br>p-value diet 0.0548<br>p-value interaction 0.6316 | 45.1 | 1.8 | **p-value genotype 0.0011**<br>p-value diet 0.0574<br>p-value interaction 0.1834 | 73.0 | 3.9 | **p-value genotype 0.0137**<br>p-value diet 0.0667<br>p-value interaction 0.0681 |
| **SS High salt** | 390.5 | 7.9 | | 42.4 | 2.6 | | 72.9 | 5.6 | |
| **SS$^{Per1-/-}$ Normal salt** | 408.1 | 9.2 | | 34.0 | 3.4 | | 66.1 | 6.2 | |
| **SS$^{Per1-/-}$ High salt** | 392.2 | 15.0 | | 19.7* | 7.0 | | 33.3* | 14.1 | |

Data were evaluated by two-way ANOVA to compare effects of diet and genotype. *P<0.05, diet effect within KO; N=4 SS, N=5 SS$^{Per1-/-}$

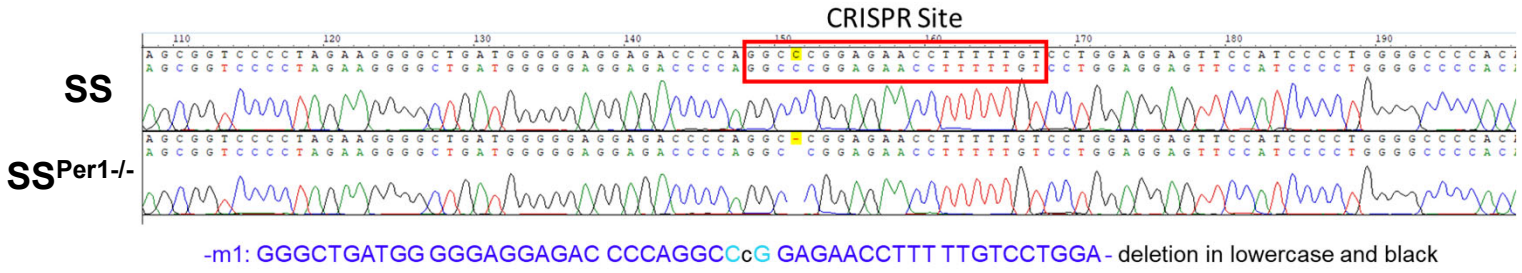
**Supplemental Table S2. Cosinor Analysis of Activity**

| Treatment Group | Mesor | | | Amplitude | | | Robustness | | |
|---|---|---|---|---|---|---|---|---|---|
| | Average | SEM | Two-Way ANOVA | Average | SEM | Two-Way ANOVA | Average | SEM | Two-Way ANOVA |
| **SS Normal salt** | 4.6 | 0.5 | **p-value genotype 0.039***<br>p-value diet 0.078<br>p-value interaction 0.221 | 3.5 | 0.5 | **p-value genotype 0.030***<br>p-value diet 0.066<br>p-value interaction 0.351 | 51.6 | 4.7 | p-value genotype: 0.816<br>p-value diet: 0.370<br>p-value interaction: 0.308 |
| **SS High salt** | 4.4 | 0.4 | | 3.1 | 0.4 | | 52.4 | 5.8 | |
| **SS$^{Per1-/-}$ Normal salt** | 4.2 | 0.3 | | 2.9 | 0.3 | | 56.6 | 5.7 | |
| **SS$^{Per1-/-}$ High salt** | 2.9* | 0.6 | | 1.8* | 0.5 | | 44.5 | 9.0 | |

Data were evaluated by two-way ANOVA to compare effects of diet and genotype. *P<0.05, diet effect within KO; N=4 SS, N=5 SS$^{Per1-/-}$

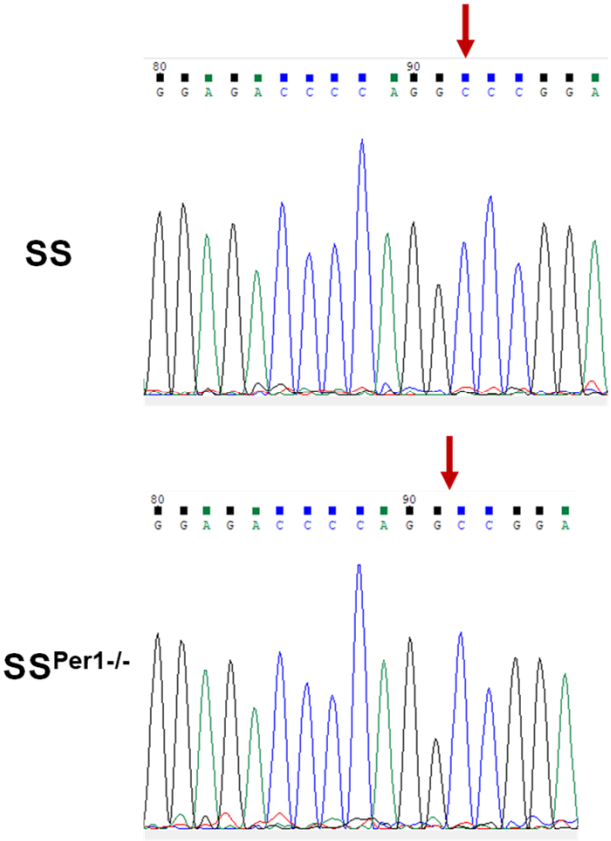
| **Supplemental Table S3. Plasma Electrolytes 21 Days After 4% NaCl** | | |
|---|---|---|
| Electrolyte | **SS (N=5)** | **SS$^{Per1-/-}$ (N=5)** |
| **Potassium (mmol/L)** | 3.6 ± 0.1 | **3.0 ± 0.1*** |
| **Sodium (mmol/L)** | 143 ± 1 | 144 ± 1 |
| **Calcium (mmol/L)** | 2.49 ± 0.01 | **2.30 ± 0.01**** |
| **Chloride (mmol/L)** | 103 ± 1 | 100 ± 3 |
| **Creatinine (mg/dL)** | 0.32 ± 0.02 | **0.46 ± 0.01*** |
| Data were evaluated by Student's t-test | | |

## A. Genomic DNA Sequence

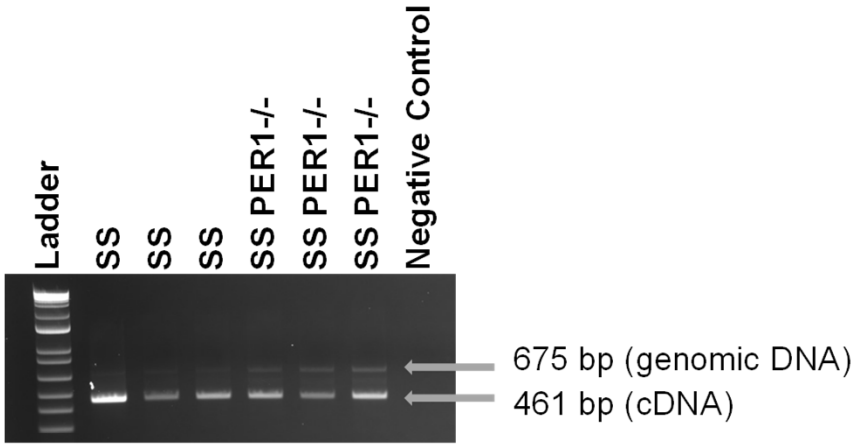



-m1: GGGCTGATGG GGGAGGAGAC CCCAGGCCcG GAGAACCTTT TTGTCCTGGA - deletion in lowercase and black

## B. cDNA Sequence

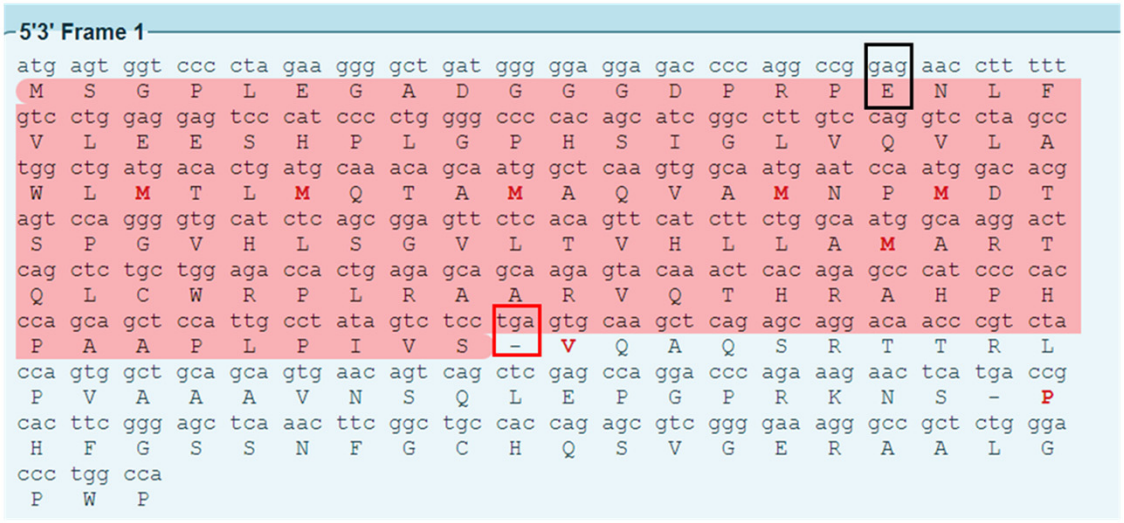



## C. RT-PCR Products for Sequencing

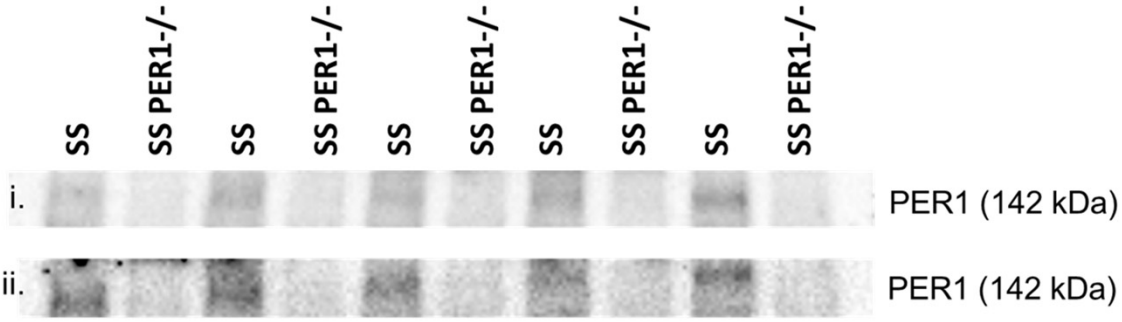



**Supplemental Figure S1. Genomic and cDNA Sequence Demonstrates a Single Nucleotide Deletion in SS$^{Per1-/-}$ Rats.** A. Genomic DNA sequencing following CRISPR (performed at MCW). B. Representative chromatogram from SS and SS$^{Per1-/-}$ rats demonstrates a single nucleotide deletion in exon 1. C. RT-PCR products: 461 bp cDNA bands were gel purified and sent for sequencing. Presence of higher molecular weight 675 bp band indicates some genomic DNA contamination of the original RNA samples. Primers were designed to include an intron so that cDNA would be distinguishable from genomic DNA.

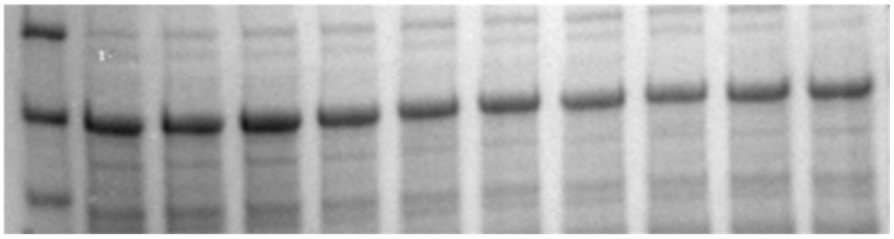
## A. Predicted Protein Sequence



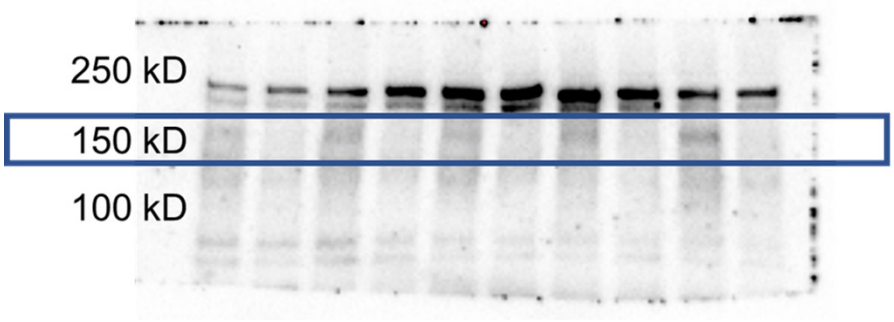

## B. Western Blot



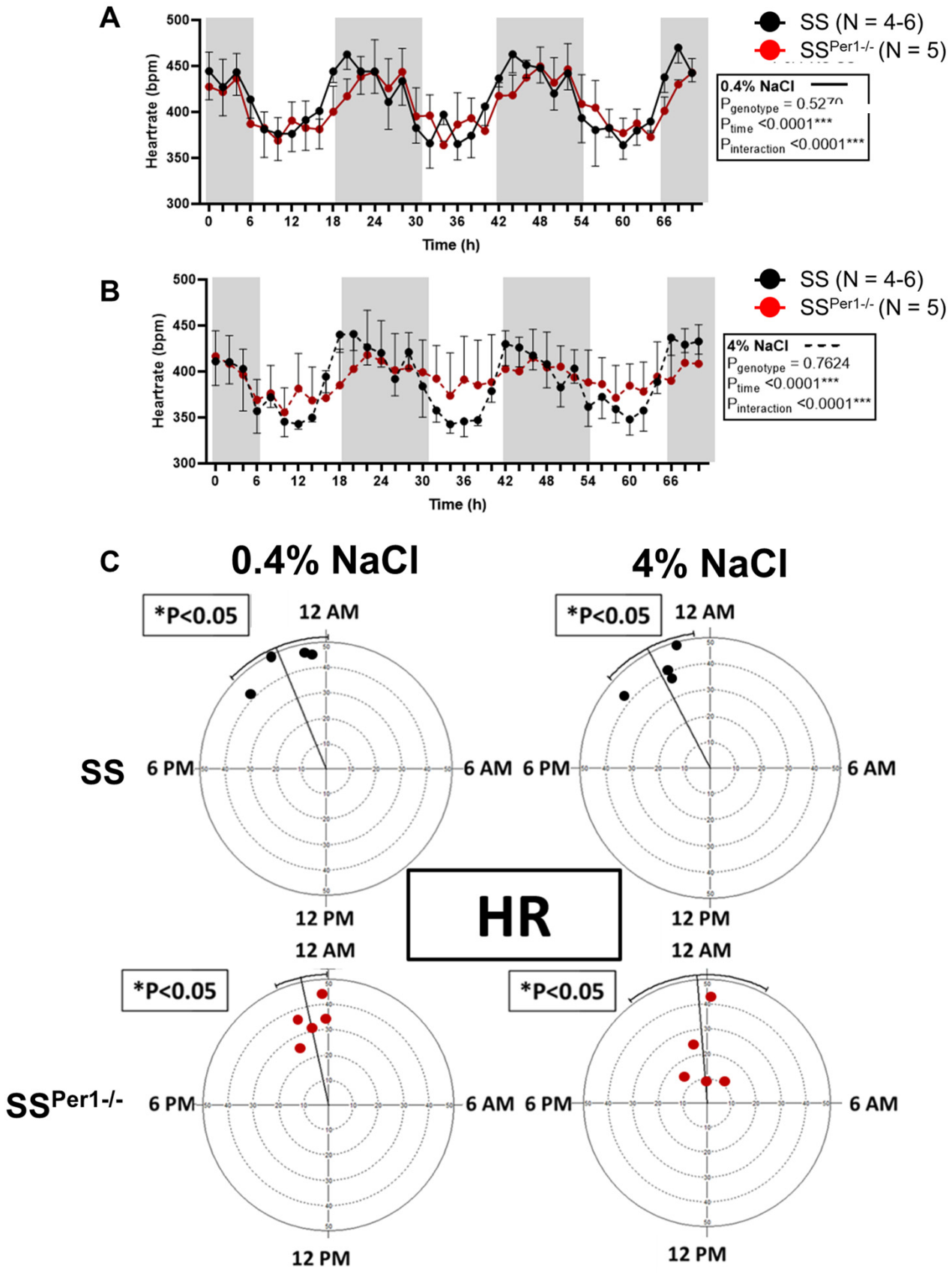

## C. Ponceau Stained Blot



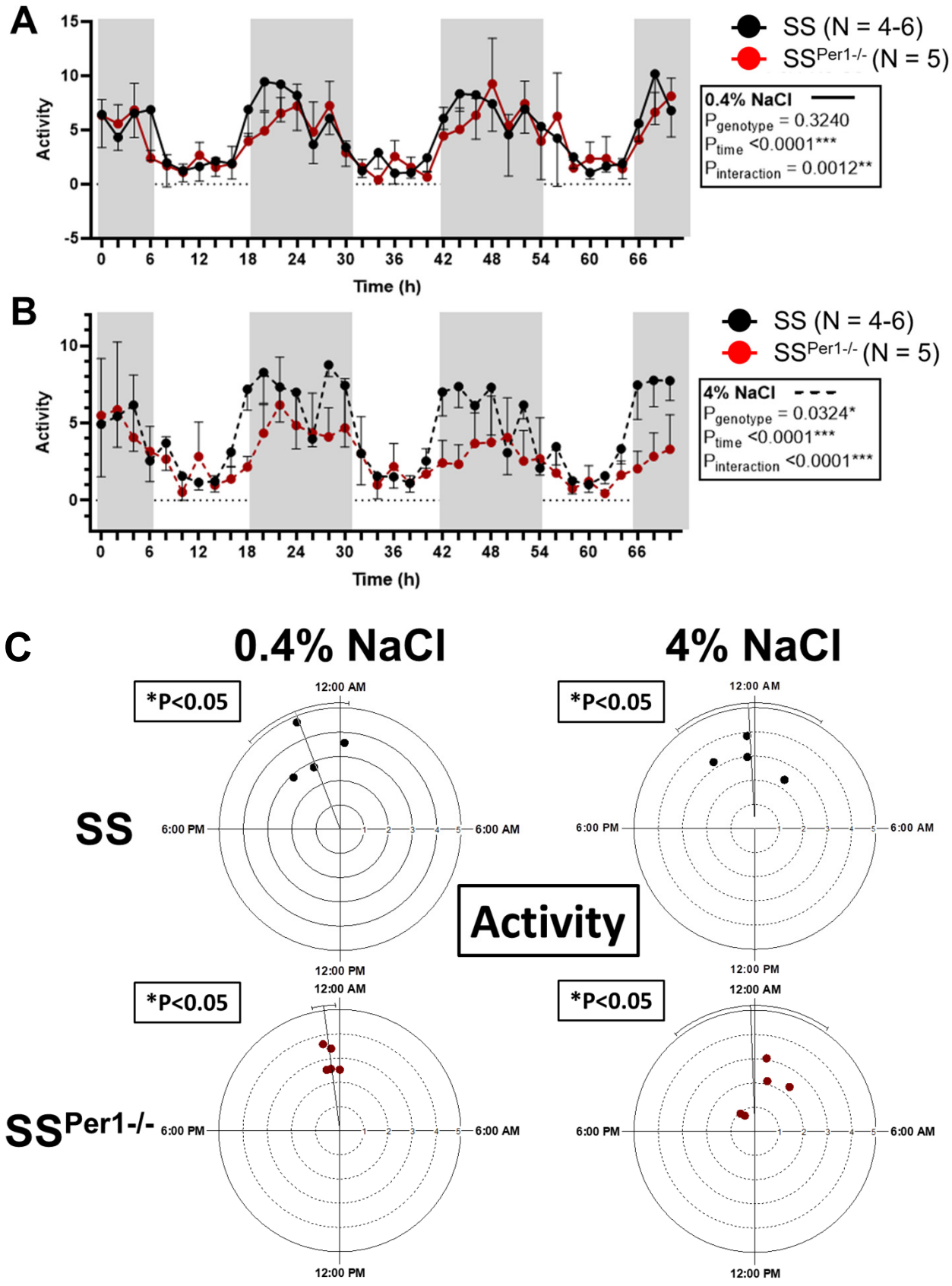
## D. Uncropped Blot (30 µg protein per well)



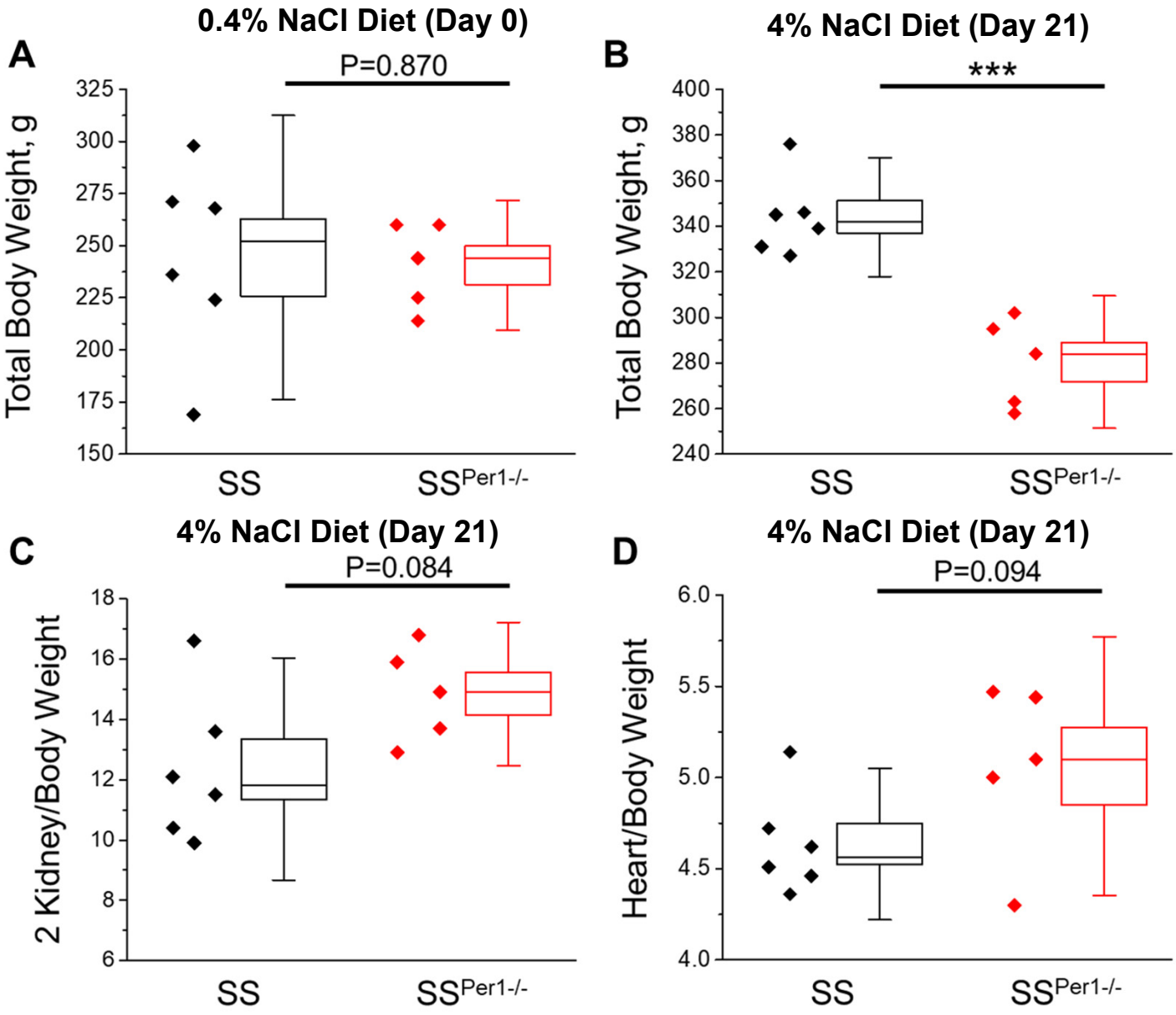

**Supplemental Figure S2. Predicted Protein Sequence and Loss of PER1 Protein in SS$^{Per1-/-}$ Rats.**
(A) Nucleotide sequence from the mutant SS$^{Per1-/-}$ rat was used to predict the protein sequence following deletion of a single g nucleotide. https://web.expasy.org/translate/ was used to generate the predicted amino acid sequence aligned with the nucleotide sequence above. The black box indicates the first amino acid that changes from the wild type protein. The red box indicates a premature stop codon that results in a truncated 109 amino acid protein. B. Western blot of liver lysates probed with an anti-PER1 antibody (Cat No. GTX128974 Genetex) shows loss of protein in SS$^{Per1-/-}$ rats. Blots were run with 30 µg (i) or 40 µg protein (ii) per well. (C) Ponceau S stained blot as a loading control. D. Uncropped blot; blue box indicates cropped area in Panel (A).




**Supplemental Figure S3. Heart rate rhythms in SS and SS$^{Per1-/-}$ rats.** Heart rate measures from the telemetry data were averaged every two hours during (A) the last three days of the normal salt 0.4%NaCl diet or (B) the last three days of the high salt 4% NaCl diet in SS and SS$^{Per1-/-}$ rats. Data in A and B were evaluated using two-way RM ANOVA, data are mean plus or minus SEM. (C) Rayleigh plots depict acrophase on a 24hr clock where distance from the origin represents amplitude and the time of peak is indicated. Error bars represent the 95% confidence interval, Rayleigh plots, n=4 SS and n=5 SS$^{Per1-/-}$




**Supplemental Figure S4. Activity rhythms in SS and SS$^{Per1-/-}$ rats.** Activity counts from the telemetry data were averaged every two hours during (A) the last three days of the normal salt 0.4% NaCl diet or (B) the last three days of the high salt 4% NaCl diet in SS and SS$^{Per1-/-}$ rats. Shaded regions in A and B indicate the active period when lights are off in the animal facility. Data in A and B were evaluated using two-way RM ANOVA, data are mean plus or minus SEM. (C) Rayleigh plots depict acrophase on a 24hr clock where distance from the origin represents amplitude and the time of peak is indicated. Error bars represent the 95% confidence interval, Rayleigh plots, n=4 SS and n=5 SS$^{Per1-/-}$




**Supplemental Figure S5. Effect of PER1 knockout on body and organ weight.** (A) Total body weight of animals before switching to the 4% NaCl diet. (B) Total body weight of animals after three weeks on the 4% NaCl diet. (C) Weight of both kidneys collected from animals normalized to their body weight after three weeks on the 4% NaCl diet. (D) Weight of heart collected from animals normalized to their body weight after three weeks on the 4% NaCl diet. ***P < 0.001, n = 6 and 5 for SS and $SS^{Per1-/-}$ rats, respectively.