**ONLINE SUPPLEMENT**

# Renal Collecting Duct NOS1 Maintains Fluid-Electrolyte Homeostasis and Blood Pressure

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Detailed Methods

*Genotyping*

DNA was extracted from mouse tail snips using MasterPure DNA Purification Kit (Epicentre Biotechnologies, Madison WI) and probed for the presence or absence of the AQP2-CRE transgene with the following primers: mAQP2 F: 5'- CTC TGC AGG AAC TGG TGC TGG -3', creTag R: 5'- GCG AAC ATC TTC AGG TTC TGC GG -3'. For the determination of the wild type or mutant (flox) NOS1, two PCRs were run with the following primers: Int5-1: 5'- GAC GTG TCT GCA ACT TCA GC -3', and 3.5B: 5'- GAT ACG TGT AGA GGG CAA ATG -3'. Or the INT5-1 forward with XK-U: 5'- CTA GGA AGG GGT CGG TAC -3' as the reverse. Wild type NOS1 gene results in a single band of 1.47 kbp. Heterozygous expression of the flox NOS1 results in a band of 1.47 and 1.4 kbp in the first PCR. Finally, a homozygous flox NOS1 results in a 1.47 kbp band in the first PCR and a 1.4 kbp band in the second PCR. Mice that were positive for AQP2-CRE and homozygous for flox NOS1 are the CDNOS1KO. Mice that were negative for AQP2-CRE and homozygous for flox NOS1 are the flox control mice.

Table S1: Antibodies used in the study.

| Antibody | Sequence | Amino Acids | Host | Application | Concentration | Company | Location |
|---|---|---|---|---|---|---|---|
| NOS1 | rat | C-terminus | rabbit polyclonal | IB | 20 µg/10ml | Santa Cruz | Santa Cruz, CA |
| NOS1 | human | 1414-1434 | rabbit polyclonal | IB, IHC | 1.4 µg | Biomol | Plymouth Meeting, PA |
| NOS3 | human | 1025-1203 | Mouse monoclonal | IB | 12.5 µg/ 10ml | BD Biosciences | San Jose, CA |
| CD3-ε | mouse | C-terminus | goat polyclonal | IHC | 0.2 µg | Santa Cruz | Santa Cruz, CA |
| F4/80 | mouse | ? | rat monoclonal | IHC | 0.1 µg | AbD Serotech | Raleigh, NC |

IB- immunoblot
IHC - immunohistochemistry

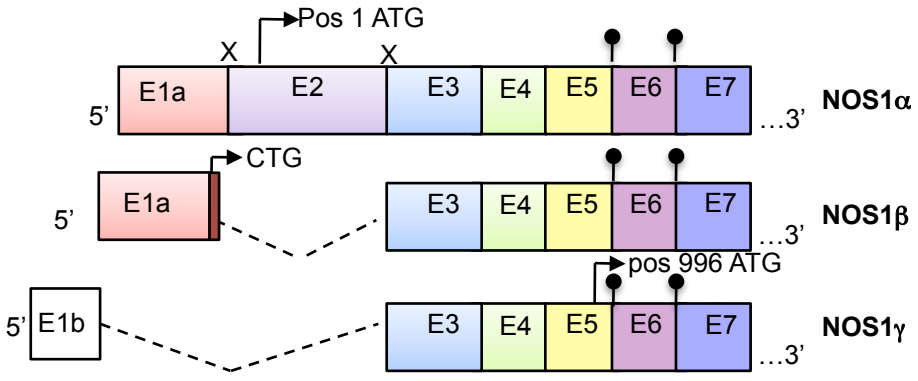


**Figure S1.** Diagram of the NOS1 splice variant (A) mRNA and (B) protein. A) The first 7 exons of the mouse NOS1 splice variants with predicted start sites (ATG) listed. The dotted line represents the spliced exons. ⚲ represents the loxP sites inserted to flank exon 6. X represents where the neomycin cassette was inserted using homologous recombination to delete exon 2 in the NOS1α knockout mouse from Jackson Laboratories (Huang et al. 1993).. B) The predicted protein structure of the NOS1 splice variants. NOS1β and NOS1γ lack the PDZ domain that is encoded by exon 2, and form N-truncated proteins. The approximate location of the N-terminus and C-terminus antibodies used in our studies are indicated. Note this diagram is drawn to approximate size. Adapted from Eliasson et al. 1997.

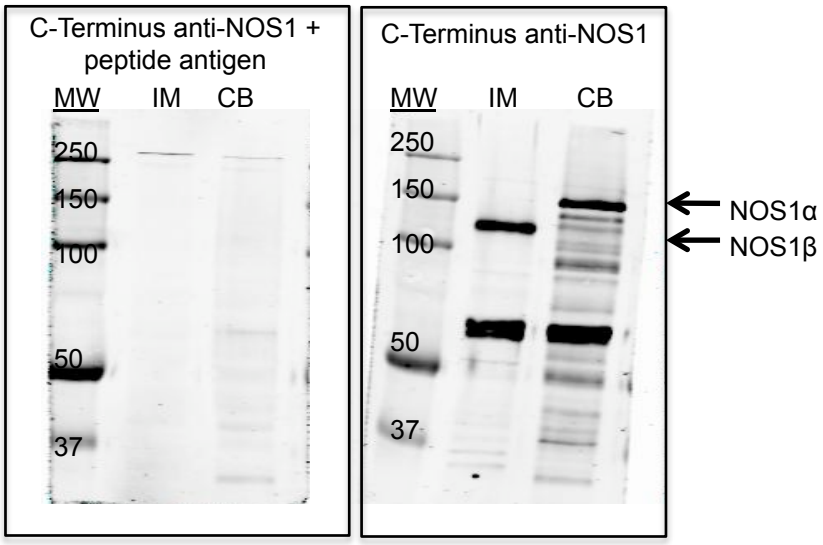


**Figure S2.** Representative immunoblot (IB) of 10 µg of inner medullary (IM) or cerebellar (CB) homogenate from a wild type mouse. The left blot was incubated in C-terminus anti-NOS1 preincubated with the peptide antigen. The right blot was incubated in C-terminus anti-NOS1 and note that the peptide blocks all binding of anti-NOS1.

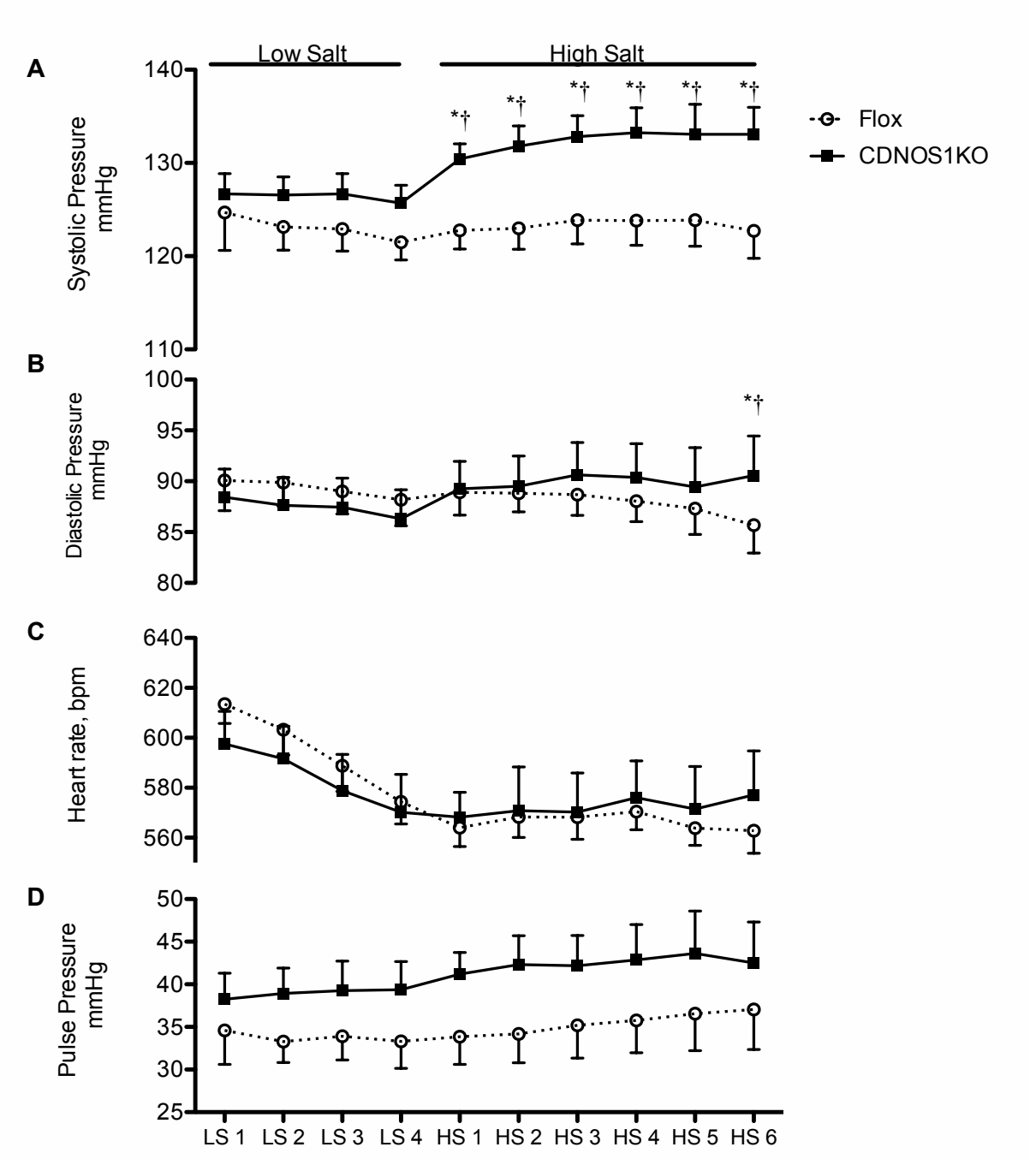


**Figure S3.** Systolic, diastolic and heart rates of flox and CDNOS1KO mice on low salt (LS) and six days of high salt (HS) diet. (A) CDNOS1KO have a significantly higher systolic blood pressured compared to flox control mice while on a HS diet. (B) On day 6 of HS diet, CDNOS1KO mice had a significantly higher diastolic pressure than flox mice. (C) There were no differences in heart rate between flox and CDNKOS1KO mice, although both heart rate. (D) pulse pressure was also not different between the

genotypes of mice on LS or day 6 of HS. * P < 0.05 between flox and CDNOS1KO mice. † P < 0.05 LS compared to HS.

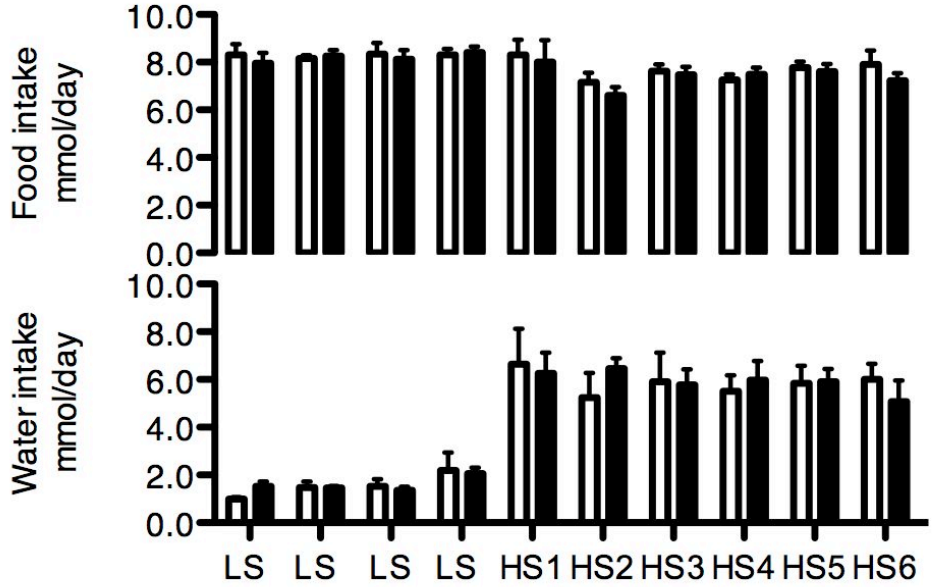


**Figure S4.** Food and water intake were similar between flox and CDNOS1KO mice on low salt (LS) and high salt (HS) diets. Water intake was significantly increased in both genotypes while on a HS diet (Genotype p = 0.94, Diet p < 0.001, interaction p = 0.90, N = 6)