

SUPPLEMENTARY MATERIAL

Slc4a8 in the Kidney: Expression, Subcellular Localization and Role in Salt Reabsorption

Jie Xu Sharon Barone Kamyar Zahedi Marybeth Brooks Manoocher Soleimani

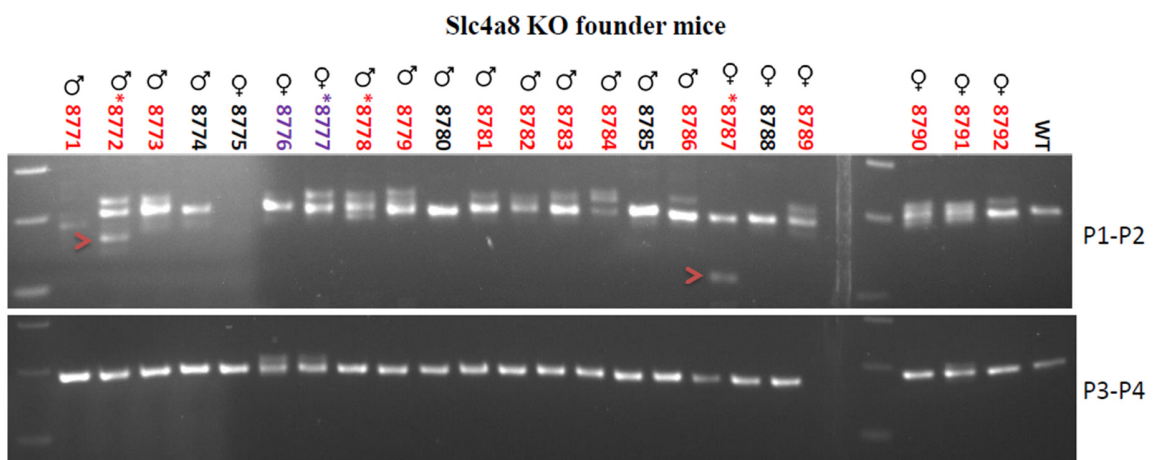
Center on Genetics of Transport and Epithelial Biology, Department of Medicine, University of Cincinnati, Research Services, Veterans Affairs Medical Center, Cincinnati, USA

Supplementary Materials:

Fig. S1. Generation of Slc4a8 KO mice

Fig. S1A. Generation of Slc4a8 KO mice by CRISPR/Cas9.

Description. Two gRNAs targeting the coding sequences of exon 3 and 4 were designed and injected along with Cas9 mRNA into cell embryos from C57 mice (Experimental Procedures).



Red: Editing in E3
Purple: Editing in both E3 and E4
* P1-P2 PCR products sequenced

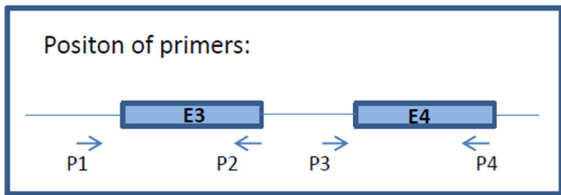
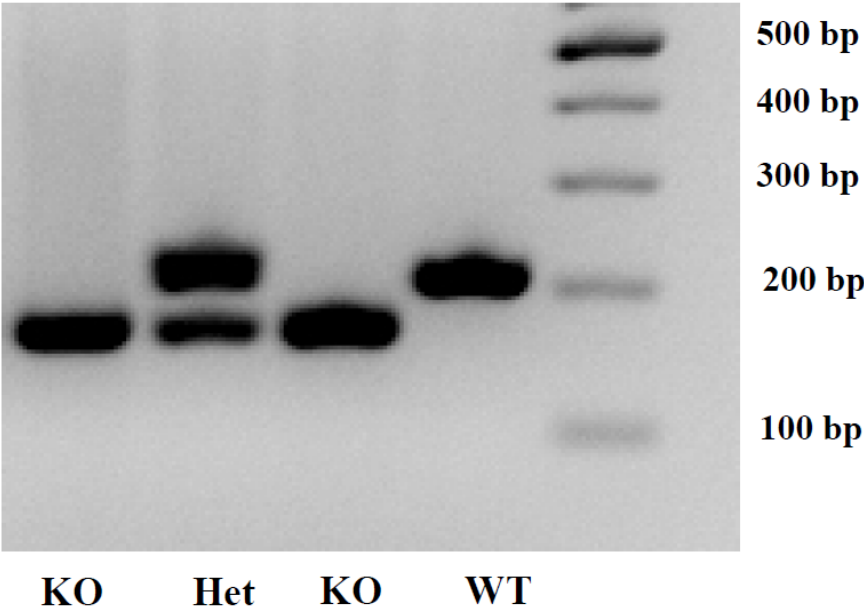


Fig. S1B. Tail DNA genotyping. Identification of Slc4a8 deficient mice.



Genotyping

The primers used for tail genotyping by PCR are depicted below:

P1(VS4360):acaccctttgtggaaggaattc

P2(VS4361): GTTACCGTGGGCCAAGGCTTC

Fig. S2. Expression of pendrin and NCC in kidneys of Slc4a8 KO mice.

Fig. S2A. Effect of Slc4a8 deletion on mRNA expression and protein abundance of pendrin.

Left panel: Northern hybridization. Right panel: Western blot. Northern and Western blots show comparable mRNA levels and protein abundance in WT and Slc4a8 KO mice.

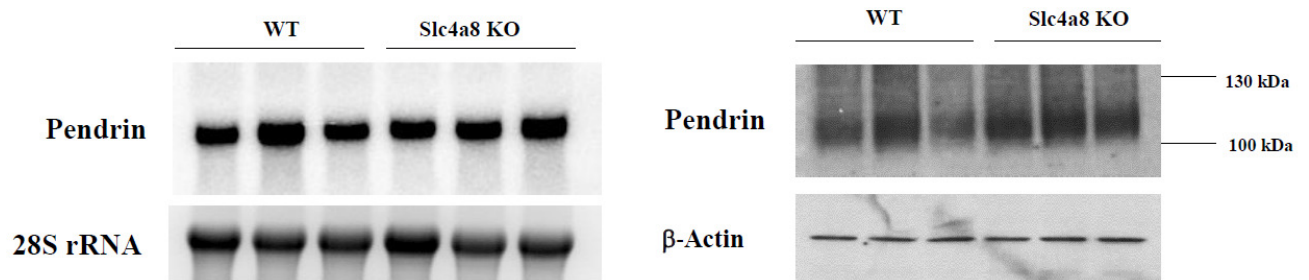


Fig. S2B. Effect of Slc4a8 deletion on mRNA expression and protein abundance of NCC. Left panel: Northern hybridization. Right panel: Western blots of total and phosphorylated NCC (top and middle panels). Northern and Western blots show comparable mRNA levels and protein abundance in WT and Slc4a8 KO mice.

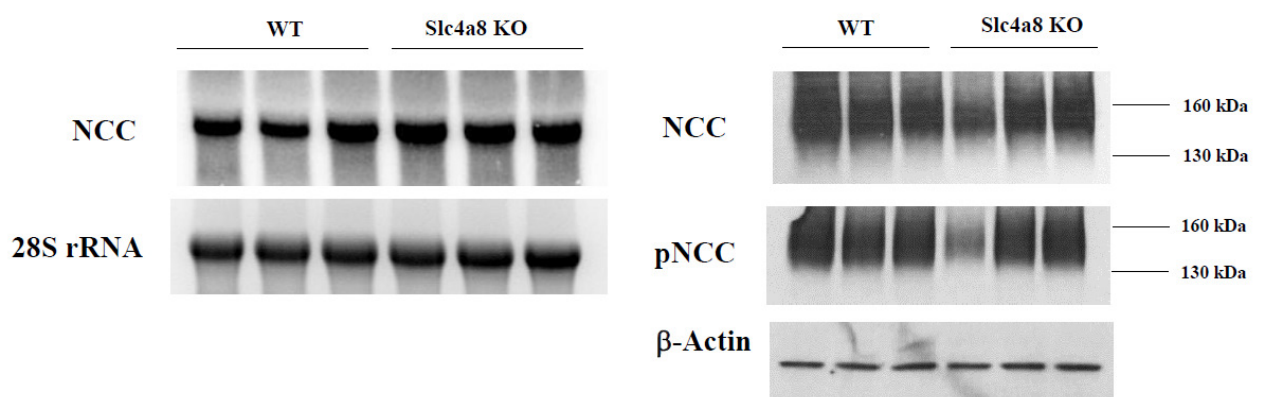


Fig. S3. Expression of Slc4a8 in medulla of animals subjected to water deprivation. A representative northern blot on combined RNAs from two separate medullas depicts the effect of enhanced medullary tonicity on Slc4a8 expression in the medulla in water deprivation. Four wild type animals were subjected to water deprivation for 24 hrs and compared to control group (n=4 per each group). Expression of Slc4a8 in the medulla increased in water deprived animals by 44% (p<0.05 vs. control, n=4).

