

# Inactivation of Notch signaling in the mammalian renal collecting duct causes nephrogenic diabetes insipidus

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**Supplemental Table 1.** Primers used for quantitative real-time RT-PCR

Gene	Forward	Reverse
<b>Mib1</b>	CCT ACG ACC TGC GTA TCC TG	ACC TTT CCT CTA CGC CCA TT
<b>HeyL</b>	CAG GGC ACA TCA CAT CAA C	GGA GCG AGC AAG AAA TAA GA
<b>AQP1</b>	AAG TGG CAA GGA AGG GAT AG	GGA AGG CTG TGT GTG TGA A
<b>Cbd-D28k</b>	CAC CTC TGT GCT GCT TCT ATC	TCT GGC TAC CTT CCC TTA CC
<b>AQP2</b>	CGC TCC TTT TCG TCT TCT TT	ACA GTC ACA GCA GGG TTG AT
<b>AQP3</b>	ATG CGT GTG CCT ACT GTT TT	CCC CTC CCA ATG TCT ATC TT
<b>AQP4</b>	GAC TCA AGC CAG GGA AGG	GTC CAC AGG TAA GGG GTT TT
<b>V2R</b>	GAG GAG AAT AGG CAA CAG AGG	GAG GGA GGA ATG ACA GGA AG
<b>H<sup>+</sup>-ATPase</b>	GGC TGG TGA AGA AAT CCA A	CAC ACT GGT AGG CAA GGA AC
<b>AEI</b>	GAG CAA ACA GCC ACA GAC TAC	ACC CCA TAC ACC ATC CTC TC
<b>Pendrin</b>	GAG AAA GTG AAT GTC CCA AAG	TTG TCA TCA AAG AAC CCA CA
<b>Foxi1</b>	AGT CCA GCC CAA ATA CCC	TGA CCT CTG TCT CTG CTC AA

## Supplemental Methods

### *Histology and Immunohistochemistry*

The newborn mouse kidneys were fixed in 4% paraformaldehyde overnight at 4°C, paraffin-embedded, sectioned (thickness, 4 µm), and stained with hematoxylin-eosin. Paraffin sections were incubated with rabbit anti-NHE3 (Santa Cruz Biotechnology), rabbit anti-NKCC2, rabbit anti-NCC (1), or mouse anti-Calbindin D-28k (Swant) antibodies, and then were visualized with Alexa-594-conjugated secondary antibody (Molecular Probes).

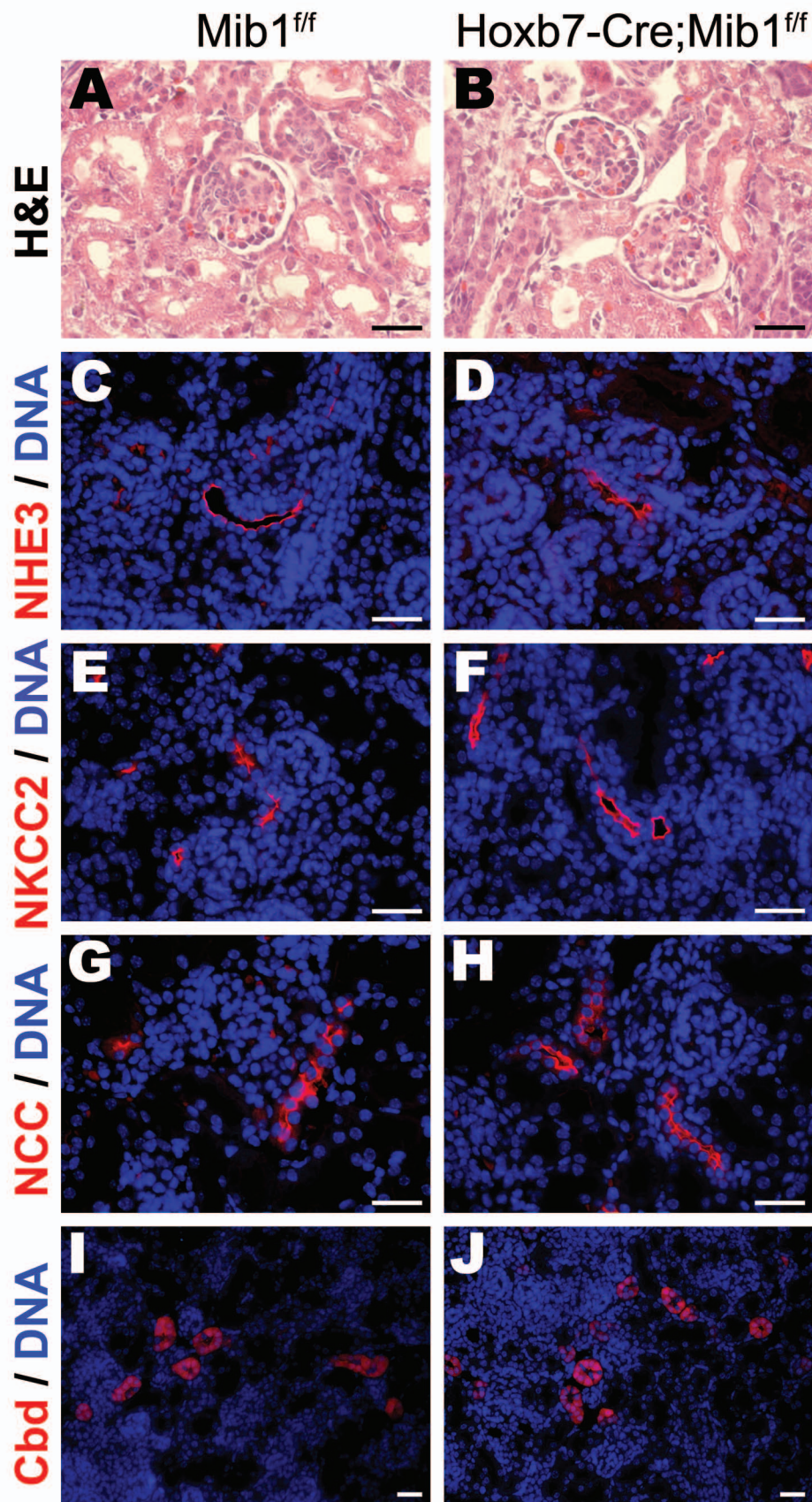
## Supplemental Figure legends

### Figure S1

Morphology of nephron structures in newborn mouse kidneys **(A-B)** H&E staining showed normal structure and morphology of glomeruli in the newborn Hoxb7-Cre;Mib1<sup>f/f</sup> mouse kidneys. **(C-D)** One of the five plasma membrane Na<sup>+</sup>/H<sup>+</sup> exchangers, NHE3, is expressed on apical membranes of renal proximal tubule. Immunohistochemical staining showed the normal expression of NHE3 in the newborn Hoxb7-Cre;Mib1<sup>f/f</sup> mouse kidneys. **(E-F)** The detection of bumetanidesensitive type2 Na-K-2Cl cotransporter (NKCC2) showed normal structures of the thick ascending limb of Henle in the Hoxb7-Cre;Mib1<sup>f/f</sup> mouse kidneys. **(G-H)** The expression of thiazidesensitive Na-Cl cotransporter (NCC) was unaffected in the distal convoluted tubules of the Hoxb7-Cre;Mib1<sup>f/f</sup> mouse kidneys. **(I-J)** The vitamin D-dependent Ca<sup>2+</sup>-binding protein, calbindin-D28k (Cbd), localized in the distal convoluted and connecting tubules showed normal expression in the Hoxb7-Cre;Mib1<sup>f/f</sup> mouse kidneys. Scale bars: 30  $\mu$ m.

### Supplemental references

1. Jeon, U.S., Han, K.H., Park, S.H., Lee, S.D., Sheen, M.R., Jung, J.Y., Kim, W.Y., Sands, J.M., Kim, J., and Kwon, H.M. 2007. Downregulation of renal TonEBP in hypokalemic rats. *Am J Physiol Renal Physiol* 293:F408-415.



**Figure S1. Jeong *et al.***