

**Supplemental Material:**

**Title: *Hnf4a* is required for the development of Cdh6-expressing progenitors into proximal tubules in the mouse kidney**

Sierra S. Marable, Eunah Chung, and Joo-Seop Park

Division of Pediatric Urology and Division of Developmental Biology, Cincinnati  
Children's Hospital Medical Center, Cincinnati, OH 45229, USA  
University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA

Short title: *Hnf4a* in proximal tubule development

Corresponding author:

Joo-Seop Park  
Cincinnati Children's Hospital Medical Center  
Location R1566, ML7007  
3333 Burnet Avenue  
Cincinnati, OH 45229  
TEL: 513-803-7871  
Email: [joo-seop.park@cchmc.org](mailto:joo-seop.park@cchmc.org)

## Supplemental Material

Supplemental Figure 1. Deletion of *Hnf4a* does not affect the formation of the S-shaped body.

Supplemental Figure 2. Deletion of *Hnf4a* does not affect the formation of other nephron segments in the newborn kidney.

Supplemental Figure 3. Mature proximal tubule markers are absent in the *Hnf4a* mutant kidney.

Supplemental Figure 4. The glomerulus is connected to *Cdh6*<sup>+</sup> tubules in the *Hnf4a* mutant and control kidneys.

Supplemental Figure 5. Deletion of *Hnf4a* does not affect the formation of other nephron segments in the postnatal kidney.

Supplemental Figure 6. *Cdh6* expression persists postnatally in the *Hnf4a* mutant and control kidneys.

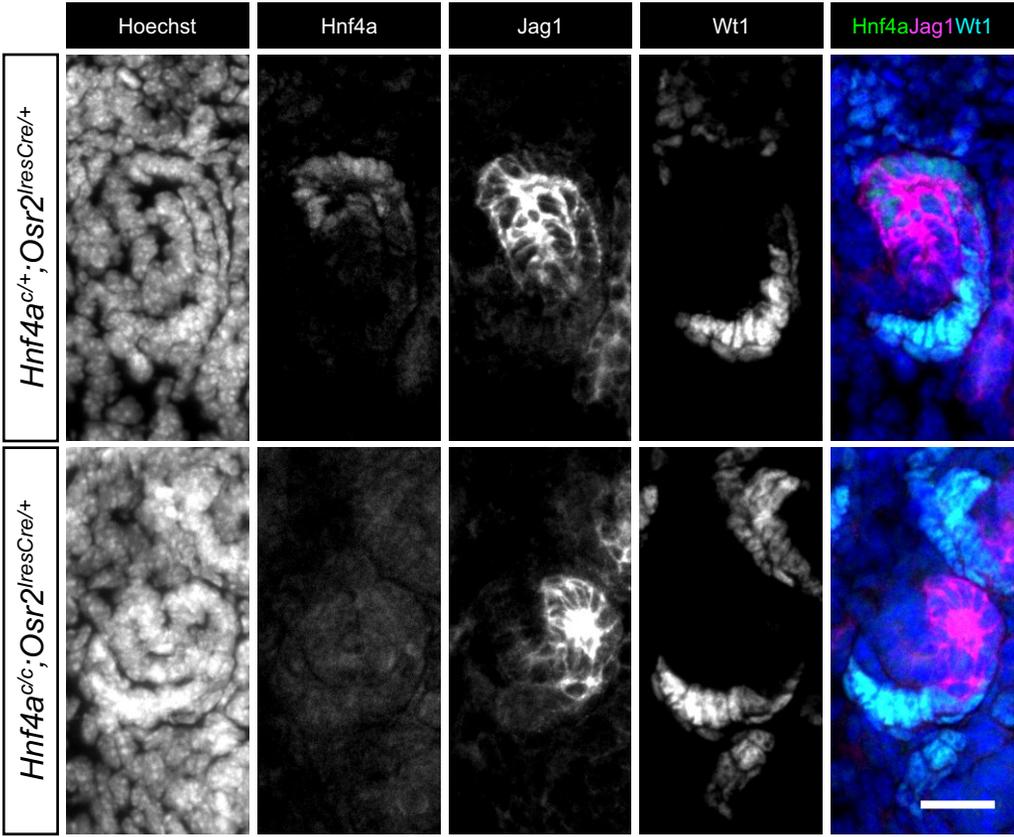
Supplemental Figure 7. Expression of top marker genes that are enriched in early and mature proximal tubules.

Supplemental Table 1. Genome-wide mapping of *Hnf4a* binding sites in the mouse kidney at P0 (ChIP-seq)

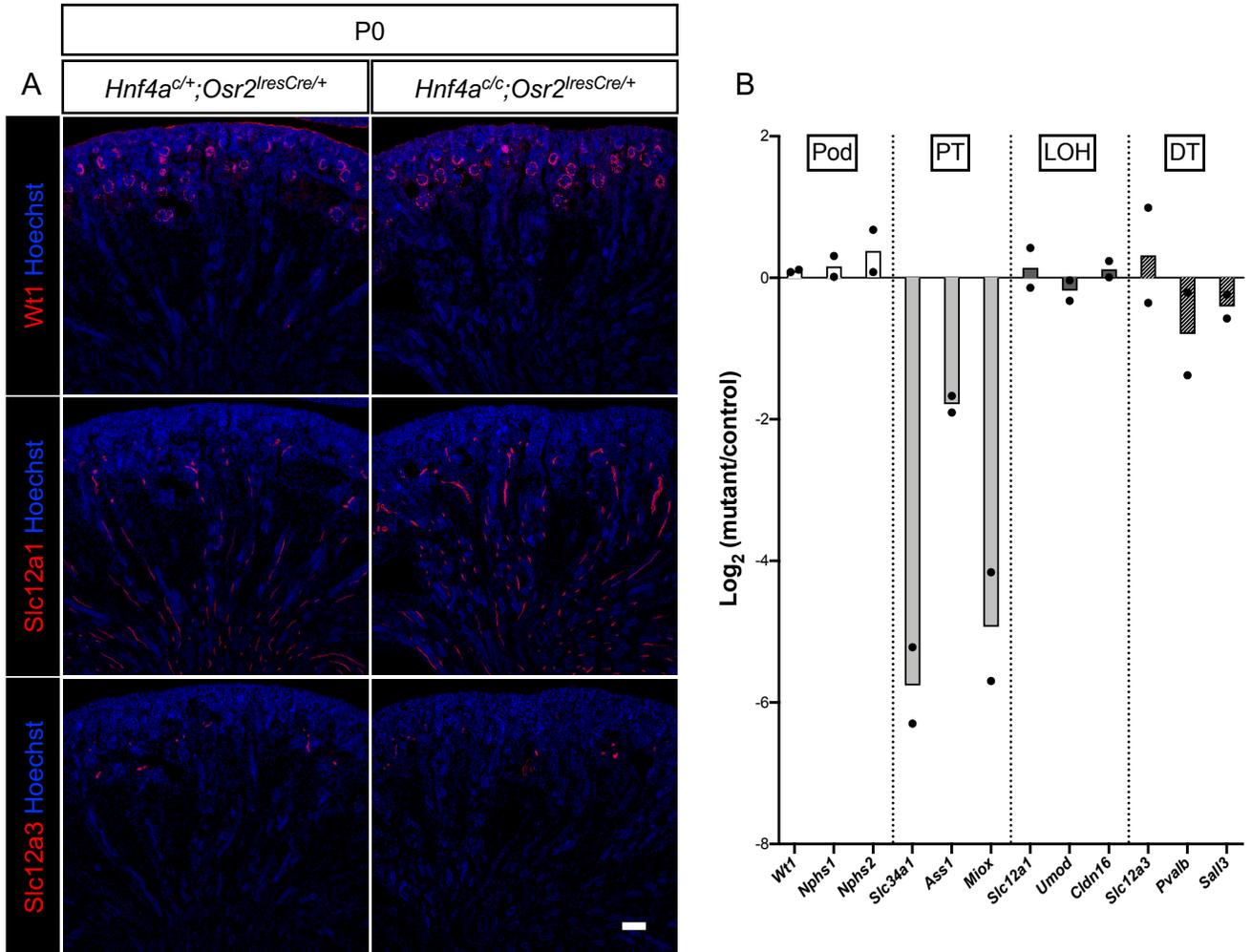
Supplemental Table 2. Differential gene analysis of the *Hnf4a* mutant kidney at P0 (RNA-seq)

Supplemental Table 3. Intersection of ChIP-seq and RNA-seq

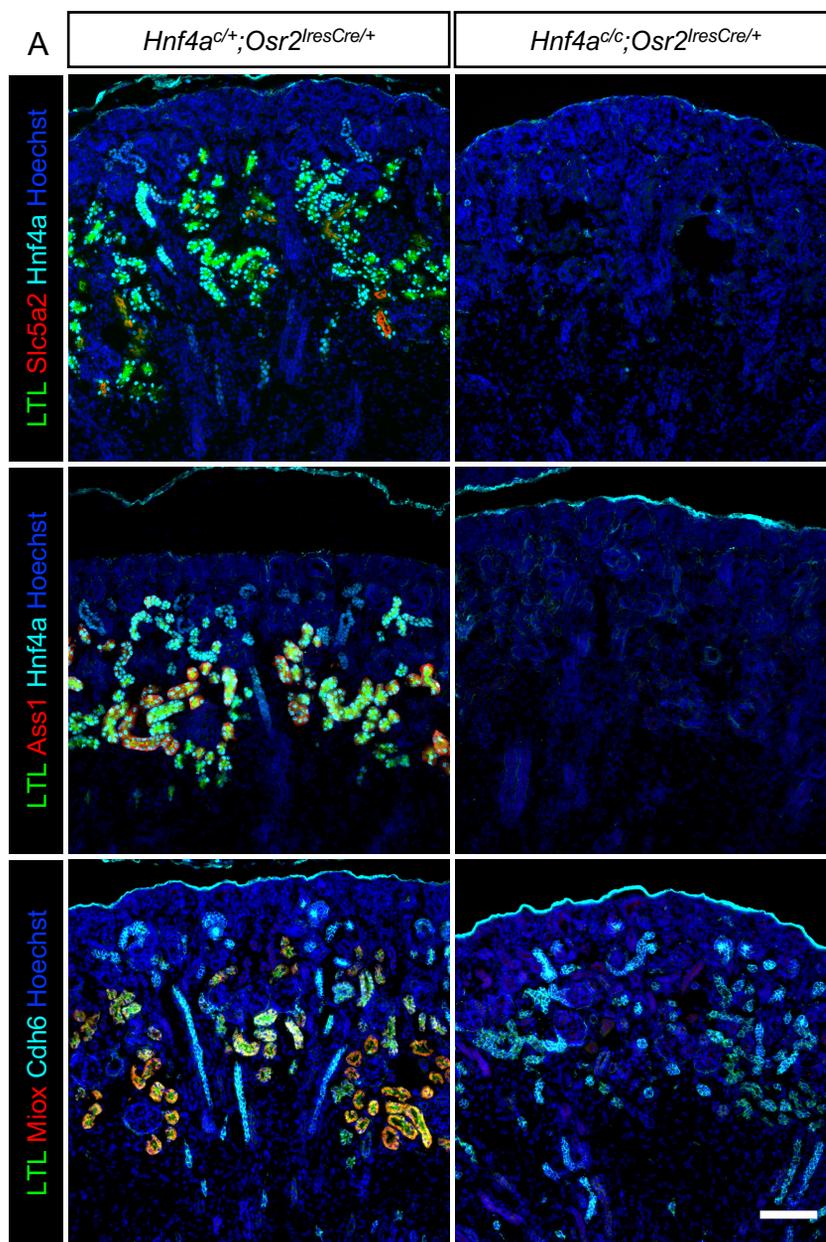
**Supplemental Figure 1.** Deletion of *Hnf4a* does not affect the formation of the S-shaped body. Representative image of  $n=3$ . Stage E18.5. Scale bar, 25 $\mu$ m.



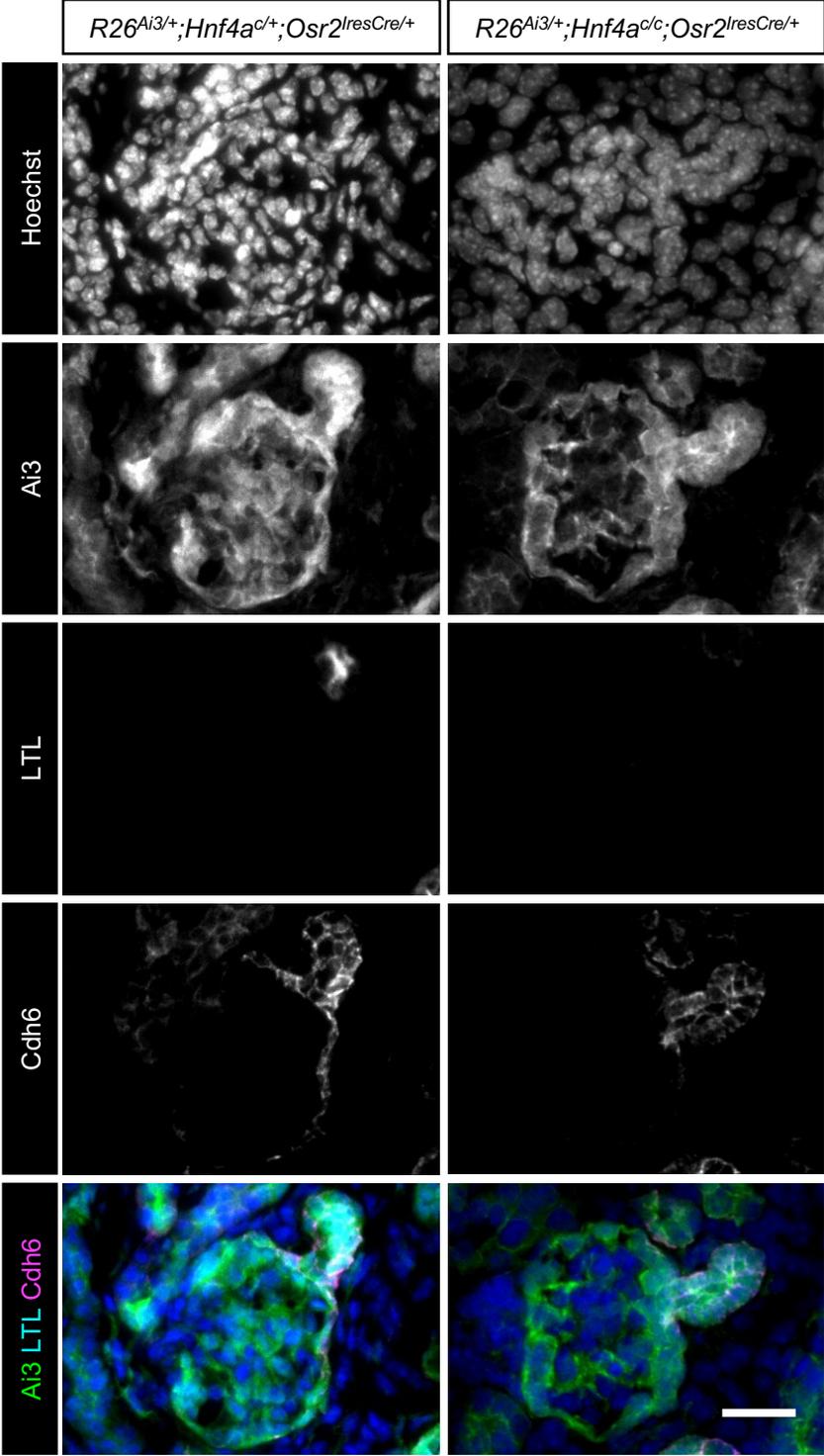
**Supplemental Figure 2.** Deletion of *Hnf4a* does not affect the formation of other nephron segments in the newborn kidney. (A) Immunofluorescence staining on podocytes (*Wt1*), loops of Henle (*Slc12a1*), and distal tubules (*Slc12a3*) shows no difference between mutant and control. Representative image of  $n=3$ . Stage P0. Scale bar, 100 $\mu$ m. (B) RNA-seq analysis of *Hnf4a* mutant kidneys at P0 showed a significant decrease in expression of PT genes but not podocyte (Pod), loop of Henle (LOH), nor distal tubule (DT) genes.



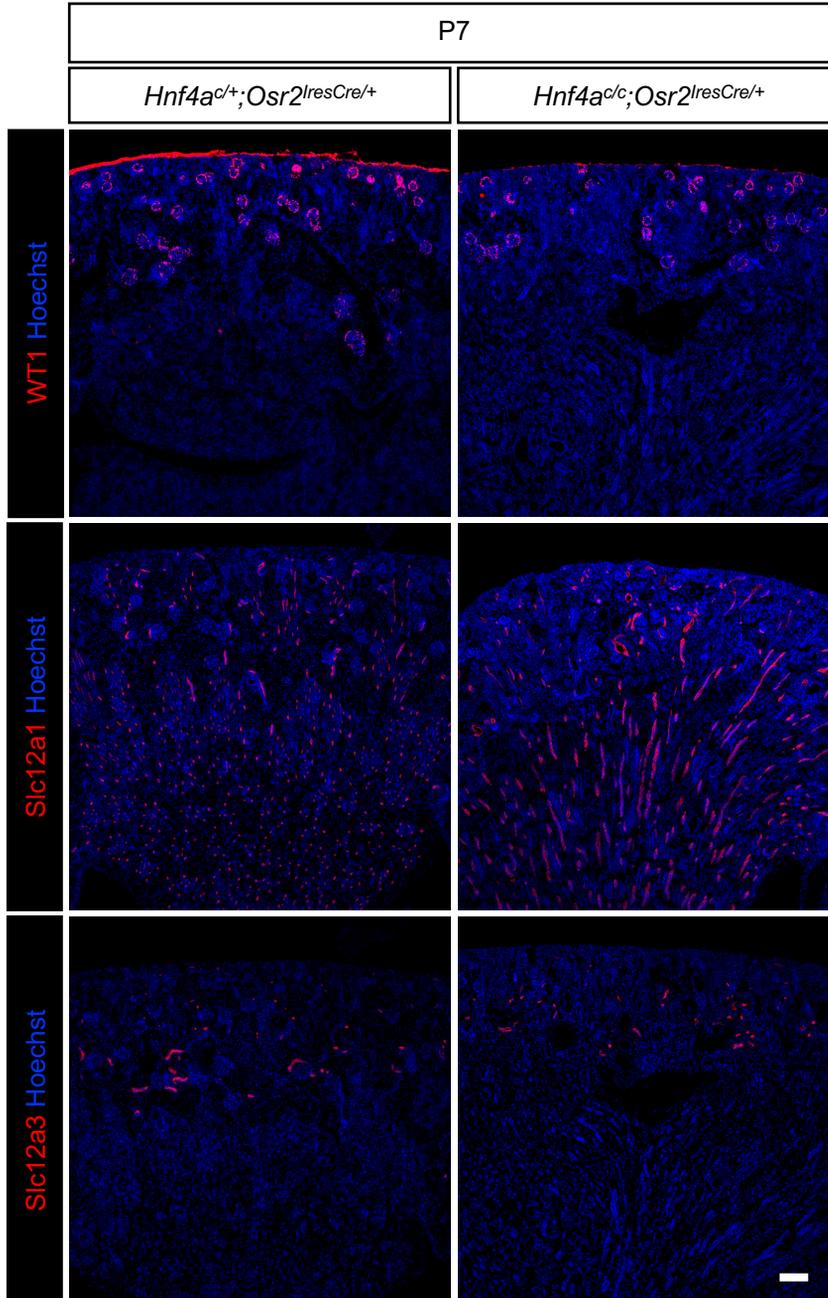
**Supplemental Figure 3.** Mature proximal tubule markers are absent in the *Hnf4a* mutant kidney. Representative image of  $n=3$ . Stage P0. Scale bar, 100 $\mu$ m.



**Supplemental Figure 4.** The glomerulus is connected to Cdh6<sup>+</sup> tubules in the *Hnf4a* mutant and control kidneys. Representative image of *n*=3. Stage E18.5. Scale bar, 25μm.



**Supplemental Figure 5.** Deletion of *Hnf4a* did not affect the formation of other nephron segments in the postnatal kidney. *Wt1*, *Slc12a1*, and *Slc12a3* mark podocytes, loops of Henle, and distal tubules, respectively. Representative image of  $n=3$ . Stage P7. Scale bar, 100 $\mu$ m.



**Supplemental Figure 6.** *Cdh6* expression persists postnatally in the *Hnf4a* mutant and control kidneys. Representative image of  $n=3$ . Scale bar, 100 $\mu$ m.

