Title Page

Title: Hedgehog signaling is required for the maintenance of mesenchymal nephron progenitors

Eunah Chung,[1,2,3] Patrick Deacon,[3,*] Yueh-Chiang Hu,[4,6] Hee-Woong Lim,[5,6] and Joo-Seop Park[1,2,3,4,7]

[1]Division of Nephrology and Hypertension, Northwestern University Feinberg School of Medicine, Chicago, Illinois
[2]The Feinberg Cardiovascular and Renal Research Institute, Chicago, Illinois
[3]Division of Pediatric Urology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
[4]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
[5]Division of Biomedical Informatics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
[6]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio
[7]Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, Ohio

[*]Current address: Asklepios BioPharmaceutical, Inc., 20 TW Alexander Dr., Research Triangle Park, North Carolina

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Corresponding author:

Joo-Seop Park

303 E. Superior St.

SQBRC 8-406

Chicago, IL 60611

TEL: 312-503-0105

Email: jooseop.park@northwestern.edu

ABSTRACT

Background Mesenchymal nephron progenitors (mNPs) give rise to all nephron tubules in the mammalian kidney. Since premature depletion of these cells leads to low nephron numbers, high blood pressure, and various renal diseases, it is critical to understand how mNPs are maintained. While Fgf, Bmp, and Wnt signaling pathways are known to be required for the maintenance of these cells, it is unclear if any other signaling pathways also play roles.

Methods To test the potential role of Hedgehog signaling in mNPs, we conditionally deleted *Shh* from the collecting duct and *Smo* from the nephron lineage. To identify the genes regulated by Hedgehog signaling in mNPs, we performed RNA-seq analysis from mNPs with different Smo doses. To test if the upregulation of Notch signaling mimics loss of Hedgehog signaling, we performed *Jag1* gain-of-function study in mNPs.

Results We found that loss of either Shh or Smo resulted in premature depletion of mNPs. Our transcriptional profiling data from *Smo* loss- and gain-of-function mutant mNPs suggested that Hedgehog signaling inhibited the activation of Notch signaling and upregulated the expression of Fox transcription factors such as *Foxc1* and *Foxp4*. Consistent with these observations, we found that ectopic expression of *Jag1* caused the premature depletion of mNPs as seen in the *Smo* mutant kidney. We also found that Foxc1 was capable of binding to mitotic condensed chromatin, a hallmark of a pioneer factor.

Conclusions Our study demonstrates a previously unappreciated role of Hedgehog signaling in preventing premature depletion of mNPs by repressing Notch signaling and likely by activating the expression of pioneer factors.

INTRODUCTION

In the developing mammalian kidney, *Six2*-expressing mesenchymal nephron progenitors (mNPs) are multipotent because they have the ability to develop into distinct nephron segments,[1-3] each responsible for unique physiological functions to serve as blood filtration units. Premature depletion of these cells leads to low nephron numbers, increasing the risk of high blood pressure and various renal diseases.[4] Hence, it is critical to understand how mNPs are maintained. It is known that Fgf, Bmp, and Wnt signals are required for the maintenance of these cells[5-16] and this knowledge made it possible to establish *in vitro* culture systems of mNPs.[17-20] However, it is unclear if any other signaling pathways also play roles in preventing the premature depletion of mNPs.

The Hedgehog signaling pathway plays crucial roles in embryonic development and tissue homeostasis. This pathway can be activated by any of three ligands: Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh). In the absence of the Hedgehog protein, a transmembrane receptor called Patched (Ptch1 and Ptch2) inhibits another transmembrane protein called Smoothened (Smo), preventing the activation of downstream signaling events. The binding of the Hedgehog protein to Ptch relieves its inhibition on Smo, whose activation eventually results in the stabilization of full-length Gli transcription factors (Gli1, Gli2, and Gli3) and Gli-mediated transcriptional activation. *GLI3* mutations producing truncated GLI3 causes renal malformations in human patients with Pallister-Hall syndrome and in a mouse model.[21-23] While it has been shown that Hedgehog signaling regulates various aspects of kidney development,[24-30] its role in mNPs has been elusive. Here we show that Hedgehog

signaling is required for the maintenance of mNPs. We also show that, in mNPs, Hedgehog signaling represses Notch signaling and upregulates the expression of *Fox* transcription factors including *Foxc1*, a pioneer transcription factor.

METHODS

Mice

Foxc1 conditional knock out (flox) allele (*Foxc1*^{c}) was made via a same-day sequential loxP insertion approach. Briefly, the 5' and 3' single-guide RNAs (target sequence: GTGGAGAGCAAATGTGATGT and CTCTAAGAGTGCCGGGAATA, respectively) were selected according to the on- and off-target scores from the web tool CRISPOR[31] and synthesized from Integrated DNA Technologies (IDT). To form the ribonucleoprotein complex (RNP), individual sgRNA (60ng/µl) was mixed with Cas9 protein (IDT; 80ng/µl) in Opti-MEM (ThermoFisher) and incubated at 37°C for 15 minutes. The donor oligo (Ultramer from IDT) with asymmetrical homologous arm design[32] and the loxP sequence was added to the respective RNP at the final concentration of 500ng/µl. The zygotes from superovulated female mice on the C57BL/6 background were first electroporated with 7µl RNP/donor mix targeting the 5' site on ice using a Genome Editor electroporator (BEX; 30V, 1ms width, and 5 pulses with 1s interval). Two minutes after electroporation, zygotes were moved into 500µl cold M2 medium (Sigma), warmed up to room temperature, and then cultured in KSOM (CytoSpring) containing 2µM M3814 (AOBIOUS) in a CO_2 incubator. Four hours later, the zygotes were electroporated again to target the 3' site and subsequently transferred into the oviductal

ampulla of pseudopregnant CD-1 females for birth. The offspring carrying 5' and 3' loxP in cis were identified by PCR and Sanger sequencing. *Foxc1*^{c} was genotyped with two oligonucleotides (TGGGAATAGTAGCTGTCAGATGGC and TCTGTTCGCTGGTGTGAGGAATC, wild type = 229bp, flox = 269bp).

Other mouse alleles used in this study have been previously published: *Rosa26*^{Sun1},[33] *Shh*^{Cre},[34] *Calb1*^{Cre},[35] *Shh*^{c},[36] *Smo*^{c},[37] *Six2GFPcre*,[1, 3] *Smo*$^{-}$,[38] *Rosa26*^{SmoM2},[39] *Rosa26*^{NuTrap},[40] *Rosa26*^{Jag1}.[41] All experiments were performed in accordance with animal care guidelines and the protocols were approved by the Institutional Animal Care and Use Committee of Cincinnati Children's Hospital Medical Center or Northwestern University. We adhere to the NIH Guide for the Care and Use of Laboratory Animals.

Immunofluorescence Staining

Kidneys were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 minutes, incubated overnight in 10% sucrose/PBS at 4°C, and imbedded in OCT (Fisher Scientific). Cryosections (8-9µm) were incubated overnight with primary antibodies in 5% heat-inactivated sheep serum/PBST (PBS with 0.1% Triton X-100). We used primary antibodies for GFP (1:500, Aves GFP-1020), Wt1 (1:100, Santa Cruz sc-7385), Cytokeratin (1:200, Sigma C2562), and Six2 (1:200, Abcam ab277942). Fluorophore-labeled secondary antibodies were used for indirect visualization of the target. Images were taken with a Nikon Ti-2 inverted widefield microscope equipped with an Andor Zyla

camera and Lumencor SpectraX light source housed at the Confocal Imaging Core (CIC) at Cincinnati Children's Hospital Medical Center (CCHMC).

Nephron Counts

Glomeruli counts were done as previously described (Cebrian 2014). Briefly, postnatal day 14 kidney was minced with a razor blade and incubated in 2.5ml 6N HCl for 60 minutes followed by repeated pipetting to disrupt further. To the suspension, 10ml of deionized water was added, mixed, and incubated overnight at 4°C. A set volume of macerate (0.1ml to 1ml) was moved to a gridded 6-well dish (Pioneer Scientific PSMP6G) and the experimenter scored the glomeruli blind to the genotype.

Fluorescence activated cell sorting (FACS) and RNA-seq

Six2GFPcre+ embryonic kidneys were dissociated using TrypLE Select Enzyme 10X (Gibco) and mechanical disruption by repetitive pipetting. Cells were washed in PBS containing 10% fetal bovine serum (FBS), then resuspended in PBS containing 1% FBS and 10mM EDTA and resuspended cells were filtered through 40µm Nylon Cell Strainer (BD Falcon) and kept on ice until FACS. The GFP+ cells were isolated using BD FACSAria II or BD FACSAria Fusion (BD Biosciences) device at the Research Flow Cytometry Core at Cincinnati Children's Hospital. Collected GFP+ cells were spun down and cell pellet was stored in -80°C until RNA extraction. Bulk RNA-seq was performed as previously described.[42] Total RNA from FACS isolated cells were prepared using Single Cell RNA Purification Kit (Norgen). From total RNA, mRNA was isolated using

NEBNext Poly(A) mRNA Magnetic Isolation Module (E7490, New England Biolabs) following manufacturer's instructions. Fragmentation of RNA followed by reverse transcription and second strand cDNA synthesis was done using NEBNext RNA First Strand Synthesis Module (E7525L) and NEBNext RNA Second Strand Synthesis Module (E6111L) following manufacturer's instructions. The resulting double-stranded cDNA's were further processed to DNA sequencing libraries using ThruPLEX DNA-seq 12S Kit (R400428, Clontech Laboratories). Libraries were size-selected by gel purification for an average size of 350bp. Each purified library was quantified using a Qubit fluorometer (Life Technologies) and equal amounts of each library were pooled and submitted for sequencing on the Illumina NextSeq 500 by the DNA Sequencing and Genotyping Core at CCHMC. All RNA-seq reads were aligned to UCSC mouse genome 10 mm using STAR aligner.[43] Only uniquely aligned reads were used for downstream analysis. Raw read counts for each gene were measured using FeatureCounts in the subread package with an option, "-s 2 -O --fracOverlap 0.8".[44] Differential gene expression analysis was performed using edgeR.[45] Genes with fold-change > 1.5 and FDR < 0.05 were selected as differentially expressed genes. Hierarchical clustering was performed using Pearson correlation coefficient as a similarity measure under Ward's criterion. Linear regression analysis of the gene copy was performed using R, and p-values were adjusted to FDR to identify significantly correlated genes. The data were deposited to Gene Expression Omnibus (GSE237179).

Transcription factor binding on mitotic chromosomes

293A cells were plated on a chambered coverslip (µ-Slide 8 Well Glass Bottom, ibidi #80827) and transfected the next day with 0.8µg plasmid DNA expressing GFP-tagged HoxA11 (p1050), FoxA1 (p1031),[46] or Foxc1 (p1104) using 2µl Lipofectamine 2000. The next day, transfection media was replaced with fresh media containing nocodazole at 330nM final concentration. We set up confocal imaging to start at 15 hours after nocodazole treatment and finish by 18 hours. Hoechst 33342 was added just before microscopy. We imaged live cells on a Nikon A1R inverted LUNV confocal laser scanning microscope equipped with a heated stage insert providing CO_2 and humidity (Tokai Hit incubator) housed at the Confocal Imaging Core (CIC) at CCHMC. We looked for condensed mitotic chromosomes and captured those images with a 60x oil-immersion objective lens and determined if GFP fusion proteins were colocalized.

RESULTS

Loss of Shh causes renal hypoplasia

It was reported that, in the developing mouse kidney, *Shh* is expressed in the medullary collecting ducts and the ureter.[24, 47] Consistent with this, we found that *Shh*^{Cre}, whose Cre expression is controlled by the *Shh* locus, activated the *Rosa26*^{Sun1} reporter mainly in the medullary collecting ducts and the ureter (Figure 1A). To investigate the roles of Shh in kidney development, we conditionally deleted *Shh* from the kidney using *Calb1*^{Cre}.[35] Similar to *Hoxb7*^{cre},[24] *Calb1*^{Cre} targets the collecting duct and the ureter in the kidney (Supplemental Figure 1). The *Shh* mutant kidney was considerably smaller than the control kidney (Figure 1B). Six2 immunostaning showed that Six2+ mNPs were

absent at multiple positions at the cortex of the mutant kidney (Figure 1C, while arrowheads), raising an interesting possibility that Hedgehog signaling is required for the maintenance of mNPs.

Loss of Smo in the nephron lineage causes premature depletion of mNPs

In order to test if Hedgehog signaling is required for the maintenance of mNPs, we conditionally deleted *Smo*, the gene encoding an essential component of Hedgehog signaling (Smoothened), using transgenic *Six2GFPcre*, which targets mNPs and their descendants.[1, 3] *Six2GFPcre*-mediated deletion of *Smo* would inhibit Hedgehog signaling in the entire nephron lineage including mNPs. Similar to the *Shh* mutant kidney, we found that the *Smo* mutant kidney was considerably smaller than the control kidney with weaker *Six2GFPcre* expression (Figures 2A & 2B). Six2 immunostaning showed that fewer Six2+ mNPs were present in the *Smo* mutant embryonic kidneys by *Six2GFPcre* (Figure 2C). We found that the *Smo* mutant kidney was still smaller than the control kidney at 2 weeks with almost 50% reduction in nephron number (Figures 2D & 2E). Taken together, these results show that loss of Smo causes premature depletion of mNPs and reduced nephron endowment, supporting the idea that Hedgehog signaling is required for the maintenance of mNPs.

To examine how increased Hedgehog signaling affects mNPs, we carried out a gain-of-function study of *Smo* employing *Six2GFPcre* and *Rosa26-SmoM2* allele, which expresses a constitutively active form of Smoothened upon Cre-mediated recombination.[39] To test if *SmoM2* expression leads to the persistent presence of mNPs

by inhibiting the depletion of these cells, we attempted to examine Six2+ mNPs at P4 when mNPs are normally depleted. However, we were unable to obtain live *SmoM2* mutant pups beyond P3 due to postnatal lethality. We found that *Six2GFPcre*-mediated activation of *SmoM2* caused the mutant kidney to be considerably smaller than the control kidney (Figure 2F), suggesting that *SmoM2* expression failed to increase nephron endowment. Interestingly, while the control kidneys apparently started to show signs of loss of mNPs at P3, the kidneys from the surviving *SmoM2* mutant pups had more Six2+ mNPs with stronger Six2 signal (Figure 2F), suggesting that constitutive activation of Hedgehog signaling inhibits the depletion of mNPs.

Identification of Hedgehog-responsive genes in mNPs

In order to determine which genes are regulated by Hedgehog signaling in mNPs, we FACS-isolated Six2GFPcre+ mNPs from both loss- and gain-of-function mutants of *Smo* and their control kidneys at E18.5 (Figure 3A), and performed RNA-seq analyses. Since both loss- and gain-of-function mutants have their own corresponding controls, our datasets contain transcriptional profiling data from mNPs with four different doses of Smo (Figure 3B). While unsupervised clustering of transcriptome identified eight groups of differentially expressed genes, we focused on Groups III and VIII, which are upregulated and downregulated by Hedgehog signaling, respectively (Figure 3B and Supplemental Table 1). *Ptch1*, the gene encoding a critical component of Hedgehog signaling, is one of the best-known Hedgehog-responsive genes.[48, 49] We found that there was a striking linear correlation between *Ptch1* expression and *Smo* dosage

(Figure 3C). Similarily to *Ptch1*, we found that Hedgehog signaling upregulated the genes encoding Fox transcription factors such as *Foxc1*, *Foxd2*, and *Foxp4* in mNPs (Figure 3C).

Our RNA-seq data showed that *Jag1*, *Hey1*, *Lhx1*, and *Pax8* were upregulated by loss of *Smo* (Figure 3D). *Jag1* encodes the major Notch ligand in the nephron lineage[50] and *Hey1* is a well-known Notch target gene.[51] *Lhx1* is also likely a Notch target gene because constitutive activation of Notch signaling causes ectopic expression of *Lhx1* in mNPs.[52] Microarray analysis from mNPs showed that *Pax8* activation was accompanied by activation of *Hey1* and *Lhx1*.[10] Given that Notch is the major signal that leads to the differentiation of MNPs,[2, 52, 53], this result raised an interesting possibility that Hedgehog signaling maintains mNPs by repressing Notch signaling.

Roles of Hedgehog responsive genes in nephron progenitors.

A previous report suggested that Foxc1 and Foxc2 are required for the maintenance of mNPs.[54, 55] Since our RNA-seq data suggest that *Foxc1* was upregulated by Hedgehog signaling while *Foxc2* was not (Figure 3C and Supplemental Table 1), we tested how loss of *Foxc1* alone would affect mNPs. We found that the *Foxc1* mutant kidney by *Six2GFPcre* showed weaker *Six2GFPcre* expression at P2 (Figure 4A). However, loss of Foxc1 did not significantly reduce the nephron numbers (data not shown), suggesting that this is a subtle phenotype. Interestingly, we found that Foxc1 was capable of binding to mitotic chromatin, a characteristic of a pioneer factor such as Foxa1,[46]

(Figure 4B), suggesting that Foxc transcription factors may act as pioneer factors in kidney development.

Since our RNA-seq data suggested that loss of Smo caused the upregulation of *Jag1*, we tested if ectopic expression of *Jag1* would phenocopy the *Smo* loss-of-function mutant kidney by employing *Six2GFPcre* and *Rosa26-Jag1* allele, which expresses *Jag1* upon Cre-mediated recombination. We discovered that the *Jag1* gain-of-function mutant kidney had considerably fewer Six2+ mNPs, mimicking loss of Smo. This result is consistent with previous reports that Notch signaling triggers the differentiation of mNPs.[2, 52, 56]

Taken together, our results suggest that Hedgehog signaling prevents premature depletion of mNPs by activating the expression of a pioneer factor (*Foxc1*) and repressing Notch signaling.

DISCUSSION

It was reported that forced expression of a truncated form of Gli3 with *Six2GFPcre* causes premature depletion of mNPs.[28] While this result was consistent with the fact that the truncation of GLI3 causes renal malformations in human patients with Pallister-Hall syndrome, the phenotype was thought to be caused by a ligand-independent action of the truncated GLI3 and not considered as evidence for active Hedgehog signaling in mNPs.[57] Our loss-of-function studies of *Shh* and *Smo* mutant kidneys strongly suggest that Hedgehog signaling is active in mNPs and that Shh acts as the ligand although we cannot rule out the possibility that Shh may act redundantly with Ihh, which is expressed

in straight proximal tubules.[47] Consistent with the notion that Hedgehog signaling is required for the maintenance of mNPs, forced expression of *SmoM2* inhibited depletion of mNPs (Figure 2F). Further investigation will be required to determine how SmoM2 expression prolongs nephrogenesis.

Our transcriptome analysis suggests that Hedgehog signaling upregulates *Fox* genes and inhibits Notch signaling in mNPs. It has been shown that Hedgehog signaling regulates expression of *Fox* genes in diverse biological contexts.[39, 48, 49, 58-60] Considering that Hedgehog signaling upregulates multiple *Fox* genes in mNPs, it comes as no surprise that the loss of Foxc1 alone did not significantly reduce nephron numbers. Nevertheless, our discovery that Foxc1 can bind to mitotic chromatin suggests that Foxc1, and potentially other Fox factors, can act as a pioneer factors in the developing kidneys. Our finding that forced expression of *Jag1* phenocopies loss of *Smo* is consistent with previous reports that Notch activation causes premature depletion of mNPs.[2, 56] Ectopic Notch activation in mNPs may be responsible for cessation of nephrogeneis and inhibition of Notch signaling may be an underlying mechanism for how *SmoM2* expression prolongs nephrogenesis.

In summary, our study demonstrates a previously unappreciated role of Hedgehog signaling in preventing premature depletion of mNPs. In conjunction with other signaling pathways known to be required for mNP maintenance, such as Fgf and Bmp, employing Hedgehog signaling pathway is likely to improve our ability to promote self-renewal of mNPs *in vitro* without losing multipotency.

Author contributions

E.C. performed most of the experiments. P.D. performed glomerular counting. Y.C.H. generated *Foxc1* flox mice. H.W.L. performed bioinformatic analyses. E.C. and J.P. designed the experiments, analyzed the data, and co-wrote the manuscript. J.P. made the figures. All authors approved the final version of the manuscript.

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Disclosures

None.

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FIGURE LEGENDS

Figure 1. *Shh* is expressed in the medullary collecting duct in the developing mouse kidney and its deletion causes renal hypoplasia accompanied by loss of Six2+ nephron progenitors. (A) Lineage analysis of Shh+ cells in the mouse kidney at E17.5. *ShhCre* activates the Rosa26 Sun1 reporter mainly at the medullary collecting duct. Scale bar, 100µm. (B) Conditional deletion of *Shh* from the collecting duct with *Calb1Cre*. The *Shh* mutant kidney is smaller than the control kidney at P0. Scale bar, 1mm. (C) In the newborn control kidney, Six2+ cells are present throughout the cortex of the kidney. The cortex of the *Shh* mutant kidney has several regions, where Six2+ cells are absent (white arrowheads). Scale bar, 100µm. Image is representative of *n*=3.

Figure 2. Conditional deletion of *Smo* with *Six2GFPcre* leads to loss of Six2+ nephron progenitors. (A) Conditional deletion of *Smo* from the nephron lineage with *Six2GFPcre*. The *Smo* mutant kidney is smaller than the control kidney at P0. Scale bar, 1mm. (B) *Six2GFPcre* expression is weaker in the *Smo* mutant kidney at P0. Scale bar, 1mm. (C) Fewer Six2+ cells are present in the *Smo* mutant kidney at E16.5 and E18.5. Scale bar, 100µm. (D) The *Smo* mutant kidney is smaller at 2 weeks. Scale bar, 1mm. (E) Nephron endowment is reduced by about 50% in the *Smo* mutant kidney. (F) While the *SmoM2* gain-of-function mutant is smaller than the control kidney, the mutant kidney has more Six2+ cells with stronger Six2 signal. Stage, P3. Scale bar, 100µm. Images are representative of *n*=3.

Figure 3. Identification of the genes responding to Hedgehog signaling in the nephron progenitors. (A) FACS isolation of Six2GFPcre+ nephron progenitors (upper, negative control with no GFP; lower, Six2GFPcre+ cells are shown in green) (B) Unsupervised clustering of transcriptome of Six2GFPcre+ nephron progenitors with four different Smo doses. (KO=*Smo*$^{c/-}$*;Six2GFPcre*, Het=*Smo*$^{c/+}$*;Six2GFPcre*, Wt=*Smo*$^{+/+}$*;Rosa26*$^{+/+}$*;Six2GFPcre*, Tg=*Smo*$^{+/+}$*;Rosa26*$^{SmoM2/+}$*;Six2GFPcre*). Group III (marked by the dark green bar) represents genes exhibiting upregulated expression in response to Hedgehog signaling, while Group VIII (marked by the orange bar) represents genes displaying downregulated expression in response to Hedgehog signaling. (C) Representative genes from Group III (D) Representative genes from Group VIII

Figure 4. Roles of Hedgehog responsive genes in nephron progenitors. (A) The *Foxc1* mutant kidney shows weaker *Six2GFPcre* expression at P2. Scale bar, 1mm. (B) Foxa1-GFP fusion protein can bind to mitotic chromatin while Hoxa11-GFP fusion protein cannot. Similar to Foxa1-GFP, Foxc1-GFP fusion protein can bind to mitotic chromatin. Hoxa11-GFP signals were observed only in interphase nuclei. Scale bar, 10µm. (C) Ectopic expression of *Jag1* with *Six2GFPcre* causes premature depletion of Six2+ nephron progenitors. The Rosa26 NuTrap reporter serves as a lineage tracer for *Six2GFPcre*. Scale bar, 100µm

Figure 1

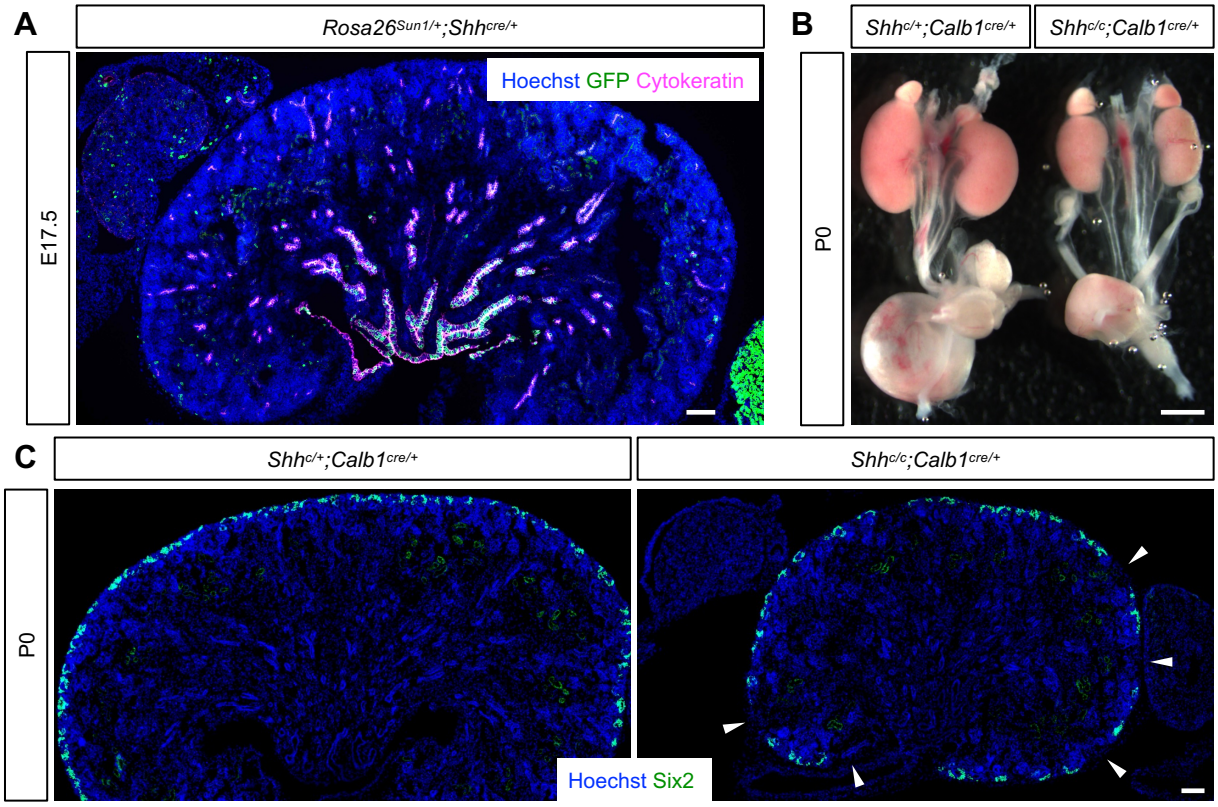

A
Rosa26Sun1/+;Shhcre/+
E17.5
Hoechst GFP Cytokeratin
B
Shhc/+;Calb1cre/+
Shhc/c;Calb1cre/+
P0
C
Shhc/+;Calb1cre/+
Shhc/c;Calb1cre/+
P0
Hoechst Six2

Figure 2

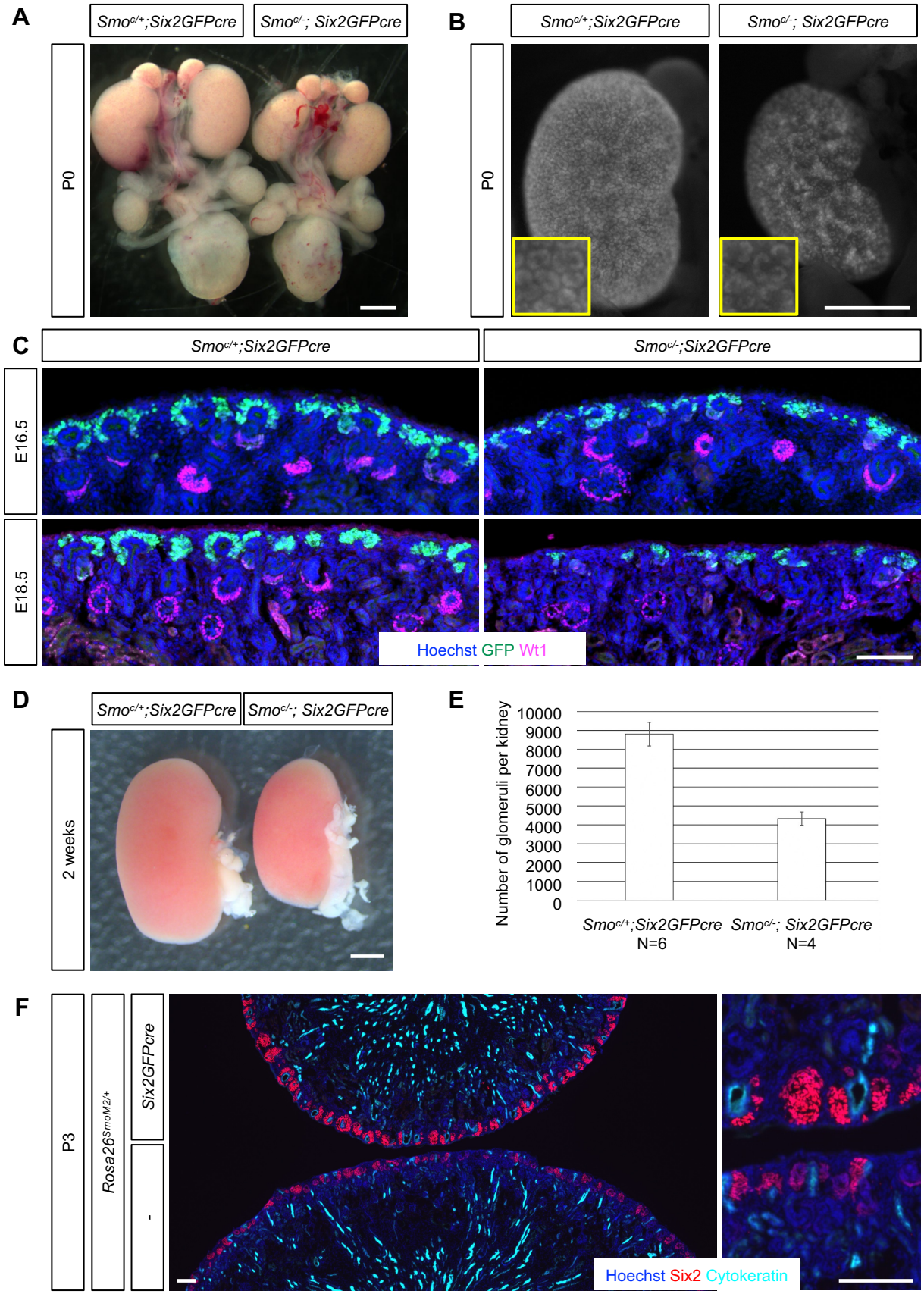

A
Smo$^{c/+}$;Six2GFPcre
Smo$^{c/-}$; Six2GFPcre
P0
B
Smo$^{c/+}$;Six2GFPcre
Smo$^{c/-}$; Six2GFPcre
P0
C
Smo$^{c/+}$;Six2GFPcre
Smo$^{c/-}$;Six2GFPcre
E16.5
E18.5
Hoechst GFP Wt1
D
Smo$^{c/+}$;Six2GFPcre
Smo$^{c/-}$; Six2GFPcre
2 weeks
E
Number of glomeruli per kidney
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Smo$^{c/+}$;Six2GFPcre
N=6
Smo$^{c/-}$; Six2GFPcre
N=4
F
P3
Rosa26$^{SmoM2/+}$
Six2GFPcre
-
Hoechst Six2 Cytokeratin

Figure 3

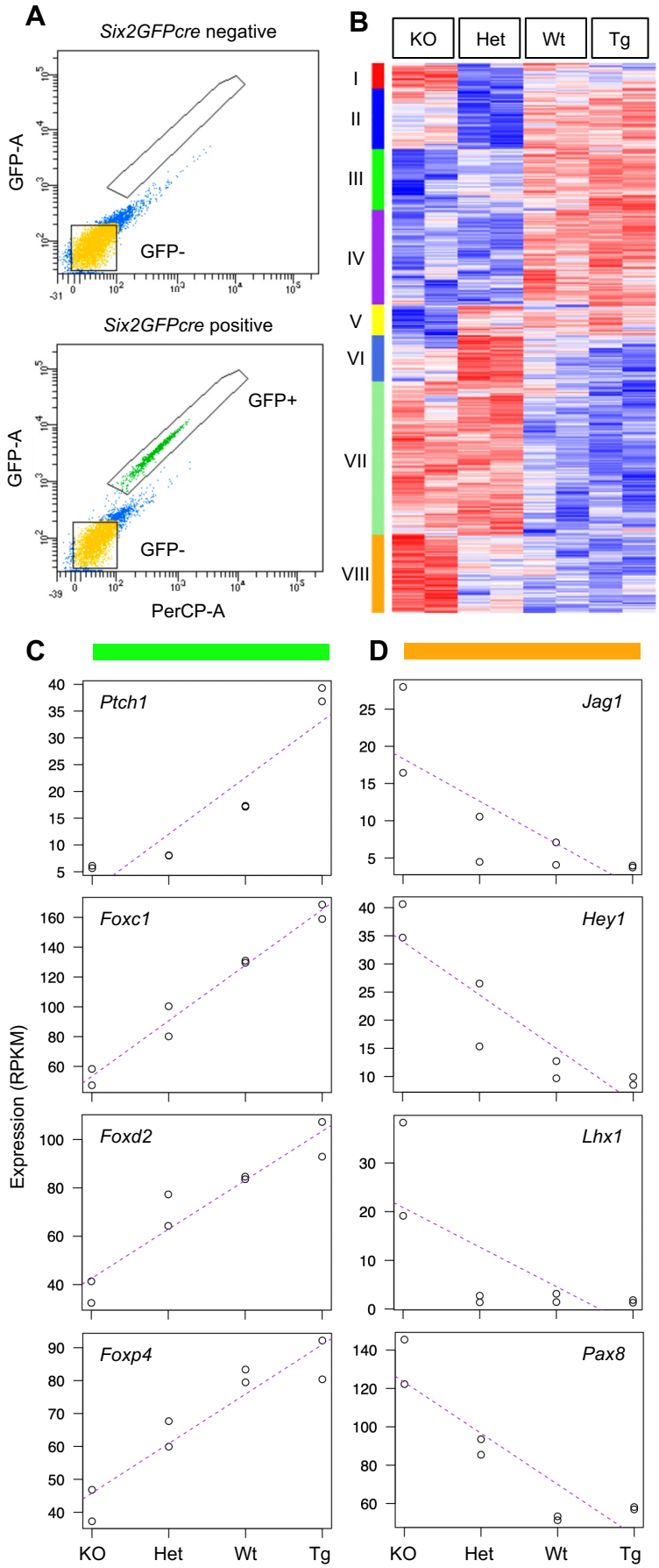
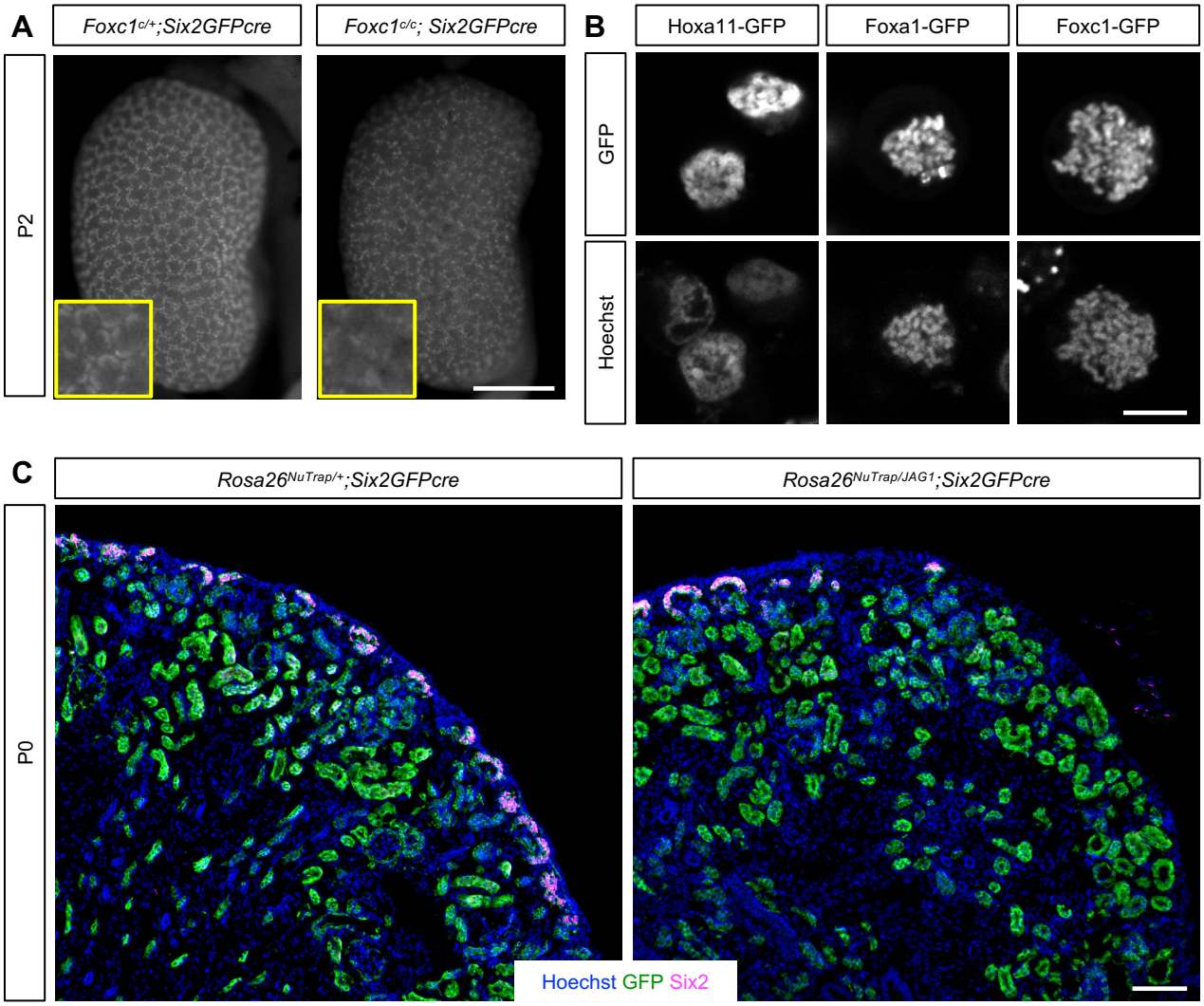

A
Six2GFPcre negative
GFP-A
GFP-
Six2GFPcre positive
GFP+
GFP-
PerCP-A
B
KO
Het
Wt
Tg
I
II
III
IV
V
VI
VII
VIII
C
D
Ptch1
Jag1
Foxc1
Hey1
Foxd2
Lhx1
Foxp4
Pax8
Expression (RPKM)
KO
Het
Wt
Tg

Figure 4


A
Foxc1c/+;Six2GFPcre
Foxc1c/c; Six2GFPcre
P2
B
Hoxa11-GFP
Foxa1-GFP
Foxc1-GFP
GFP
Hoechst
C
Rosa26NuTrap/+;Six2GFPcre
Rosa26NuTrap/JAG1;Six2GFPcre
P0
Hoechst GFP Six2