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Hydronephrosis in the Wnt5a-ablated kidney is caused by an abnormal ureter-bladder connection

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Abstract

The Wnt5a null mouse is a complex developmental model which, among its several posterior-localized axis defects, exhibits multiple kidney phenotypes, including duplex kidney and loss of the medullary zone. We previously reported that ablation of Wnt5a in nascent mesoderm causes duplex kidney formation as a result of aberrant development of the nephric duct and abnormal extension of intermediate mesoderm. However, these mice also display a loss of the medullary region late in gestation. We have now genetically isolated duplex kidney formation from the medullary defect by specifically targeting the progenitors for both the ureteric bud and metanephric mesenchyme. The conditional mutants fail to form a normal renal medulla but no longer exhibit duplex kidney formation. Approximately 1/3 of the mutants develop hydronephrosis in the kidneys either uni- or bilaterally when using Dll1Cre. The abnormal kidney phenotype becomes prominent at E16.5, which approximates the time when urine production begins in the mouse embryonic kidney, and is associated with a dramatic increase in apoptosis only in mutant kidneys with hydronephrosis. Methylene blue dye injection and histologic examination reveal that aberrant cell death likely results from urine toxicity due to an abnormal ureter-bladder connection. This study shows that Wnt5a is not required for development of the renal medulla and that loss of the renal medullary region in the Wnt5a-deleted kidney is caused by an abnormal ureter-bladder connection.

Keywords

Wnt5a; kidney development; hydronephrosis; nephric duct; metanephric mesenchyme; renal medulla

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Conflict of Interest statement

None declared.

1. Introduction

The kidney arises from intermediate mesoderm and is driven by reciprocal interactions between the epithelial nephric duct (ND) and mesenchymal cells in the overlying nephrogenic cord. Ureteric bud (UB) cells bearing c-RET receptors extend from the ND when induced by surrounding GDNF-expressing metanephric mesenchyme (MM) to form the collecting ducts and ureter. Loss of GDNF/c-RET signaling during early kidney development results in abnormal UB budding or kidney agenesis (Dressler, 2009); whereas, disruptions of signaling molecules, such as Wnt7b, Wnt9b, or Fat4, later in kidney development may instead cause cyst formation or a complete loss of the renal medulla (Karner et al., 2009; Saburi et al., 2008; Yu et al., 2009).

The renal medullary region comprises the inner most portion of the kidney, including the medullary collecting ducts, loops of Henle, vasa recta, and interstitium (Little et al., 2007), and plays a prominent role in concentrating urine through water recovery. Disruption of medullary development can lead to renal failure and has been attributed to a variety of factors. For example, Wnt7b regulates formation of the corticomedullary axis by controlling the orientation of cell division, and loss-of-function mutants fail to form the medullary region but without affecting kidney size or cortical development (Yu et al., 2009). Obstructive uropathy also causes the loss of the renal medulla. Hydronephrosis and hydroureter of murine kidneys at E18.5 can arise from a congenital blockage of normal urine outflow, such as ureterovesical junction obstruction (UVJO) or ureteropelvic junction obstruction (UPJO). In the E18.5 mouse, such obstructions involving kidney-bladder connectivity first manifest as ureter distention with a loss of medullary definition and eventually lead to kidney atrophy. Most genes associated with UVJO are involved in UB budding during early kidney development and also yield duplex or multiple ureters (Rasouly and Lu, 2013).

Wnt5a is a secreted ligand typically associated with the non-canonical Wnt pathway and can function through either the planar cell polarity (PCP) or calcium-dependent pathways to regulate tissue morphogenesis (Endo et al., 2015; Kikuchi et al., 2012). Deletion of Wnt5a in the mouse disrupts proper tissue outgrowth of several caudal structures, as well as the limbs and face, where it is highly expressed (Yamaguchi et al., 1999). These mutants show striking similarity to patients with Robinow syndrome, and, in fact, mutations in Wnt5a have been reported in patients with the autosomal dominant form (Person et al., 2010; Roifman et al., 2015). These patients manifest not only skeletal dysplasia but also abnormalities in the renal medulla, which include hydronephrosis and less frequently cystic dysplasia of the kidney (Patton and Afzal, 2002; Wiens et al., 1990).

The Wnt5a null mouse exhibits multiple kidney phenotypes such as double ureters, medullary loss, renal agenesis, and horseshoe kidneys (Huang et al., 2014a; Nishita et al., 2014; Pietila et al., 2016). We have also observed duplex kidney formation in conditional Wnt5a mutants using mesoderm-specific TCre. This phenotype was associated with an abnormal extension of the intermediate mesoderm and was dependent upon ablation in early mesoderm prior to UB budding (Yun et al., 2014). However, the role of Wnt5a later during metanephric development itself is unclear. Here, we investigate its potential role using

various tissue-limited Cre lines to genetically dissect the phenotypic complexity presented by the Wnt5a null mouse. Only Cre lines which can delete Wnt5a in both UB and MM progenitors induce hydronephrosis and hydroureter either uni- or bilaterally without duplex kidney formation. The discernible kidney abnormalities begin at E16.5 with an expanded pelvic region and ureter. Furthermore, we establish that the phenotype is caused by an abnormal bladder-ureter connection. These findings indicate that Wnt5a likely does not play a critical role in kidney medullary development but rather is required for proper bladder-ureter connectivity.

2. Materials and Methods

2.1. Animal and tissue culture

Generation, maintenance and genotyping of Wnt5a flox/flox ($Wnt5a^{tm1.1Tpy}$) (Miyoshi et al., 2012), AP2Cre (Nelson and Williams, 2004), Rarb2Cre (Kobayashi et al., 2005), Dll1Cre (Wehn et al., 2009), and Hoxb7/myr-Venus (Chi et al., 2009) mouse lines have been described previously (Kitagaki et al., 2011). Noon on the day of vaginal plug detection was designated E0.5. Mice were managed according to NIH guidelines for the care and use of laboratory animals and studied under a protocol approved by the NCI-Frederick Animal Care and Use Committee.

2.2. Histopathological and TUNEL assay

Kidneys were dissected and fixed in 4% paraformaldehyde/PBS solution (Electron Microscopy Sciences) overnight, after which they were embedded in paraffin according to standard procedures. Serial 4-μm paraffin sections were collected and stained with hematoxylin/eosin (H&E). Also, for TUNEL assays, embryos were fixed in 4% paraformaldehyde/PBS at 4°C overnight, soaked overnight in 30% sucrose, embedded in OCT, and cryosectioned at 16-μm. TUNEL assay was performed using the In Situ Cell Death Detection Kit (Roche) according to manufacturer's instructions. The images were photographed by confocal microscopy (Zeiss LSM 710).

2.3. Calbindin staining, LacZ staining and vibratome sectioning

Calbindin staining of kidneys at E13.5 was performed as previously described (Yun et al., 2014). Also, X-gal staining was performed as previously described (Nakaya et al., 2005). Vibratome sections (200 μm) from E15.5 kidneys expressing the Hoxb7/myr-Venus ND/UB reporter were photographed by confocal microscopy (Zeiss LSM 710).

2.4. Methylene blue injection

Methylene blue (10 mg/mL) in normal saline solution was injected into the kidneys of isolated whole urogenital systems using a sterile scalp vein set (27GX3/4", Exelint). Hydrostatic pressure was then applied to push the ink through the ureter to the bladder.

3. Results

3.1. Wnt5a deletion in both UB and MM progenitors is necessary to generate the loss-of-renal-medulla phenotype

The Wnt5a null mouse exhibits multiple renal phenotypes such as duplex kidney and loss of the renal medullary region (Figure 1A & 1B). Previously, we reported duplex kidney formation and a defect in axis extension when Wnt5a was ablated during ND formation and prior to UB budding using TCre ((Yun et al., 2014) and Supplementary Figure 1A–1D). Loss of the renal medulla, however, is caused during metanephric development either by disruption of cortico-medullary axis formation, as demonstrated in Wnt7b mutants (Yu et al., 2009), or by hydronephrosis. To understand the role of Wnt5a during medullary development, we utilized mouse lines containing AP2Cre for UB progenitor-specific deletion (Figure 2A) and/or Rarb2Cre for MM progenitor-specific deletion (Figure 2B). Wnt5a deletion using either AP2Cre (n=9, Figure 2D & 2G) or Rarb2Cre individually (n=7, Figure 2E & 2H) did not show loss of the renal medulla or double ureters. Wnt5a is expressed in both the UB and MM even though the level of Wnt5a expression is extremely low (Yun et al., 2014). Therefore, we examined the kidney phenotype by producing a mouse line bearing both AP2Cre and Rarb2Cre alleles to delete Wnt5a throughout the kidney and its progenitors (Figure 2C) without affecting axis extension in the embryo as observed in Wnt5a f/−;TCre mutants (Supplementary Figure 1A–1J). With Wnt5a ablation, these mice developed uni- (n=3) or bilateral (n=2) hydronephrosis and hydroureter but without formation of double ureters (n=5, Figures 2F & 2I). These results indicate that Wnt5a deletion in both UB and MM progenitors is sufficient to generate the loss-of-renal-medulla phenotype.

3.2. Loss of the renal medullary zone begins at E16.5 in mutant kidneys

Due to the difficulty in generating Wnt5a fl/−;AP2Cre;Rarb2Cre mutant mice, especially the small litter sizes, we replaced the Cre alleles with Dll1Cre, which can also delete Wnt5a from the MM and UB in the kidney ((Wehn et al., 2009), Figure 3A). Henceforth, “mutant” refers to embryos in which Wnt5a ablation is achieved using Dll1Cre. These mutants showed hydronephrosis and hydroureter (Figure 3B) in approximately 1/3 of embryos (Table 1), which differs from the 100% penetrant phenotype reported for Wnt7b mutants (Yu et al., 2009). To explore the cause of renal medullary loss, we first evaluated a temporal series of sectioned normal and mutant kidneys (Figure 4A–4J). Histologically, both were similar developmentally up to E15.5 (n=6, Figure 4B & 4D), after which we first observed some loss of the medullary zone. The mutant medullary zone was considerably smaller (n=4) than that of normal kidneys at E16.5 (Figure 4E & 4F). Whereas normal kidneys at E16.5 and beyond showed an increased medullary region with developmental progression (black bars), hydronephrotic mutant kidneys exhibited progressively reduced medullary regions (n=4, Figure 4H & n=3, Figure 4J). UB/collecting duct formation appeared normal in these mutants (Figure 4K & 4L), unlike Wnt5a null mutants, which contain dilated collecting ducts and Bowman’s capsule lumens (Pietila et al., 2016) and are subjected to the earlier extension defect in intermediate mesoderm, or Wnt7b mutants, which manifest dilated collecting duct epithelia in the prospective medullary region (Yu et al., 2009). At higher magnification, the epithelial tubules in our mutants were elongated as single cell layers

(Figure 4N) typical of those in the normal kidney (Figure 4M). However, mutants showed loss of the renal medulla with accompanying hydroureter beginning at E16.5, when passive urine filtration is initiated (Rasouly and Lu, 2013), suggesting that hydronephrosis is caused by an accumulation of urine in the kidney due to an abnormal ureter-bladder connection.

3.3. An abnormal ureter-bladder connection causes the loss of the renal medulla

Another possible role for Wnt5a involves the regulation of medullary tubule formation through planar cell polarity (PCP) signaling. Mutations in PCP signaling molecules such as Fat4 (Saburi et al., 2008) or Wnt9b (Karner et al., 2009) result in polycystic kidney formation due to the abnormal orientation of cell division during tubule formation. To address this possibility in Wnt5a mutants, we evaluated mutant kidneys, which exhibited a normal renal medulla, for cyst formation (Figure 5) and found no evidence of cystic abnormalities (Figure 5D, 5G, 5J, 5K, 5M, & 5N) even at 3 months of age. Mutant kidneys expressing Hoxb7/myr-Venus contain collecting ducts populated with single layers of epithelial cells typical of normal kidneys (Figure 4M & 4N; Figure 5F–5H), suggesting that Wnt5a does not play a role in renal medullary tubule development as is the case for PCP mutants. However, the ducts are frequently dilated in the hydronephrotic mutant kidney in which the renal medulla is lost compared with a similar site in the normal kidney (Supplementary Figure 2A & 2B). Moreover, apoptosis was increased only in hydronephrotic mutant kidneys where this occurred (n=3), suggesting the possibility of toxicity from the accumulation of urine in the kidney subsequent to a malformation of the ureter-bladder connection. To confirm this, we injected methylene blue dye into the medulla-deficient mutant kidney. The dye flowed freely into the bladder from the normal kidney (Figure 6A); whereas the dye was not found in the bladder lumen of the hydronephrotic mutants (n=4, Figure 6B). Serial coronal sections through the lower urinary tract of E18.5 mutant embryos with unilateral hydronephrosis revealed an enlarged ureter wedged against the bladder wall (BW) with no evidence of ureterbladder connectivity on the side where hydronephrosis is evident (n=3, Figure 6D, Supplementary Figure 3B). Normal embryos and mutant urinary tracts without hydronephrosis display typical ureter-bladder connections (Figure 6C, Supplementary Figure 3A & 3B). All of these data suggest that Wnt5a does not play a significant role in kidney medullary development but is important for proper ureter-bladder connectivity.

4. DISCUSSION

Wnt5a null mice show several tissue abnormalities associated with outgrowth including truncation of the A-P axis (Yamaguchi et al., 1999). The resulting compressed tissue spacing manifest in these mutants may physically disrupt proper tissue interactions during development, leading to congenital defects. Because of the complexity and plethora of abnormalities in Wnt5a mutants from body structure to organ shape, it has been difficult to establish the actual causes of each abnormality. Kidneys in the Wnt5a null mouse also reveal multiple abnormalities such as double ureters, loss of renal medulla, small kidneys, horseshoe kidneys, and even no kidneys (Huang et al., 2014a; Nishita et al., 2014; Pietila et al., 2016). Deletion of Wnt5a using a Wnt5a floxed allele with a tissue-specific Cre allows us to limit this complexity. In this regard, Wnt5a deletion using either UB progenitor-

specific AP2Cre or MM progenitor-specific Rarb2Cre individually yielded neither duplex kidney formation nor loss of the renal medulla. Also, we did not observe renal agenesis or otherwise abnormal kidney development in these mutants, suggesting that loss of Wnt5a from either renal progenitor (UB or MM) is insufficient to cause renal malformations. However, Wnt5a deletion using the combined AP2Cre and Rarb2Cre alleles induced loss of the renal medullary region but without other phenotypes seen in the Wnt5a null kidney, suggesting that the loss-of-renal-medulla phenotype arises independently from expression of Wnt5a in the metanephros. Indeed, we previously demonstrated that duplex kidney formation is initiated in the early embryo during the genesis of the ND (Yun et al., 2014). Here, using tissue-specific deletion, we also have been able to genetically isolate duplex kidneys from hydronephrosis. Moreover, we were able to determine that the actual cause of the loss of the renal medulla in Wnt5a mutants lies not in the development of the kidney but rather in the insertion of the ureter into the bladder (Figure 6B & 6D).

For proper ureter-bladder connectivity, the cells populating the most posterior ND play a key role (Batourina et al., 2005). During development, the ND tip reaches and inserts into the cloacal epithelium (Chia et al., 2011; Weiss et al., 2014), forming the common nephric duct (CND). The CND is then eliminated by apoptosis to facilitate ureter insertion into the bladder. The ND cells, from which the UB emerges, originate in the intermediate mesoderm (Staack et al., 2003). Moreover, AP2Cre targets Wnt5a expression in the ND, while Rarb2Cre deletes Wnt5a from the nephrogenic cord (mesenchyme surrounding the ND from which the MM progenitors arise). Thus, by using both Cre lines, Wnt5a expression would be lost from both the posterior end of the ND and the surrounding mesenchyme, eliminating this secreted signaling ligand throughout that domain. Such a loss is consistent with a possible role of these progenitors in mediating proper ureter-bladder interactions. The fact that hydronephrosis is induced only with the combination of AP2Cre and Rarb2Cre also suggests that Wnt5a signaling in and around the ND tip is required for formation of a normal ureter-bladder connection. Dll1Cre should delete Wnt5a in this region as well but seems somewhat less efficient than AP2Cre/Rarb2Cre. Dll1Cre induced hydronephrosis in about 33% of Wnt5a mutants; whereas deletion of Wnt5a using AP2Cre/Rarb2Cre yielded 100% penetrance albeit with smaller numbers. The expression of Wnt5a in and around the ND tip and comparisons of Cre alleles used and their respective targeted regions may provide clues to understanding the role of Wnt5a in the ureter insertion process. The precise mechanism, however, remains under investigation. One possibility may be that Wnt5a is associated with ND tip migration and insertion into the cloacal epithelium. In this regard, ND insertion is thought to be a prerequisite for formation of a proper ureter-bladder connection (Chia et al., 2011; Weiss et al., 2014). Moreover, Wnt5a plays a major role in the directional migration of cells in several tissues during development and also in the invasion and metastasis of cancer cells (Endo et al., 2015; Kikuchi et al., 2012). Alternatively, Wnt5a may facilitate apoptosis in the CND, and in its absence, the persistent CND prevents proper insertion of the ureter into the bladder.

Mutation of some PCP-associated genes such as Fat4 (Saburi et al., 2008) or Wnt9b (Karner et al., 2009) induces cystic kidney formation, and Wnt5a has been implicated as a non-canonical Wnt/PCP molecule (Endo et al., 2015; Kikuchi et al., 2012). Moreover, some patients with Robinow syndrome have cystic kidneys, and Wnt5a deletion in zebrafish also

produces cystic kidneys. Thus, we expected to observe cyst formation in the Wnt5a mutant kidney as well (Huang et al., 2014b). Surprisingly, however, we did not observe any cyst formation even later in adult kidneys, suggesting that Wnt5a expression in the murine kidney is not required for proper patterning of the collecting ducts. This finding is actually consistent with the extremely low levels of Wnt5a expression in the developing mouse kidney (Yun et al., 2014) (GUDMAP:10265). Instead, the ducts might be dilated as a sequela of the failed vesicoureteral connection and subsequent urine accumulation. This may indicate that a similar process is operative in patients with Robinow syndrome or even in the zebrafish model and that their phenotypes may also reflect, to some extent, hydronephrosis caused by an abnormal ureter-bladder connection.

In contrast with cyst formation in the zebrafish, a recent study using the Wnt5a null mouse kidney at E16.5 describes anomalies in the basement membrane of UB-derived epithelial cells, which resulted in dilated ducts and the Bowman's capsule lumen (Pietila et al., 2016). We observed that loss of the medulla begins at E16.5 (Fig 4F), when the murine embryonic kidneys initiate passive urine filtration (Rasouly and Lu, 2013). Therefore, the accumulation of urine may induce the dilation observed in the collecting ducts and capsule lumens. This phenotype is likely exacerbated as the mice age and are exposed to sustained urine accumulation, which would explain the increased apoptosis and progressive loss of the medullary regions (Figure 4F–4J). The reported anomalies in the basement membrane of UB-derived epithelial cells in E16.5 null kidneys may also result from urine toxicity. In this regard, conditional deletion of Wnt5a using various Cre lines may help address our understanding of the role of the basement membrane in the Wnt5a null.

Finally, our system can provide a unique model for the study of abnormal ureter insertion. Most cases of UVJO occur concurrently with multiple ureters (Rasouly and Lu, 2013). Since we have effectively isolated the medullary phenotype from ureter duplication, this should help us identify the mechanism responsible for abnormal ureter insertion. We are now investigating possible mechanisms.

In conclusion, our results support the position that Wnt5a does not play a crucial role in renal medullary development in the mouse embryonic kidney, but it is critical for forming a proper ureter-bladder connection. Our model now allows us to focus specifically on the events associated with this single phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

MM	metanephric mesenchyme
ND	nephric duct
PCP	planar cell polarity
UB	ureteric buds
UPJO	ureteropelvic junction obstruction
UVJO	ureterovesical junction obstruction

References

Batourina E, Tsai S, Lambert S, Sprenkle P, Viana R, Dutta S, Hensle T, Wang F, Niederreither K, McMahon AP, Carroll TJ, Mendelsohn CL. Apoptosis induced by vitamin A signaling is crucial for connecting the ureters to the bladder. Nature genetics. 2005; 37:1082–1089. [PubMed: 16186816]

Chi X, Hadjantonakis AK, Wu Z, Hyink D, Costantini F. A transgenic mouse that reveals cell shape and arrangement during ureteric bud branching. Genesis. 2009; 47:61–66. [PubMed: 19111008]

Chia I, Grote D, Marcotte M, Batourina E, Mendelsohn C, Bouchard M. Nephric duct insertion is a crucial step in urinary tract maturation that is regulated by a Gata3-Raldh2-Ret molecular network in mice. Development. 2011; 138:2089–2097. [PubMed: 21521737]

Dressler GR. Advances in early kidney specification, development and patterning. Development. 2009; 136:3863–3874. [PubMed: 19906853]

Endo M, Nishita M, Fujii M, Minami Y. Insight into the role of Wnt5a-induced signaling in normal and cancer cells. International review of cell and molecular biology. 2015; 314:117–148. [PubMed: 25619716]

Huang L, Xiao A, Choi SY, Kan Q, Zhou W, Chacon-Heszele MF, Ryu YK, McKenna S, Zuo X, Kuruvilla R, Lipschutz JH. Wnt5a is necessary for normal kidney development in zebrafish and mice. Nephron Exp Nephrol. 2014a; 128:80–88. [PubMed: 25412793]

Huang L, Xiao A, Wecker A, McBride DA, Choi SY, Zhou W, Lipschutz JH, et al. A possible zebrafish model of polycystic kidney disease: knockdown of wnt5a causes cysts in zebrafish kidneys. J Vis Exp. 2014b

Karner CM, Chirumamilla R, Aoki S, Igarashi P, Wallingford JB, Carroll TJ. Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. Nature genetics. 2009; 41:793–799. [PubMed: 19543268]

Kikuchi A, Yamamoto H, Sato A, Matsumoto S. Wnt5a: its signalling, functions and implication in diseases. Acta Physiol (Oxf). 2012; 204:17–33. [PubMed: 21518267]

Kitagaki J, Ueda Y, Chi X, Sharma N, Elder CM, Truffer E, Costantini F, Lewandoski M, Perantoni AO. FGF8 is essential for formation of the ductal system in the male reproductive tract. Development (Cambridge, England). 2011; 138:5369–5378.

Kobayashi A, Kwan KM, Carroll TJ, McMahon AP, Mendelsohn CL, Behringer RR. Distinct and sequential tissue-specific activities of the LIM-class homeobox gene Lim1 for tubular morphogenesis during kidney development. Development. 2005; 132:2809–2823. [PubMed: 15930111]

Little MH, Brennan J, Georgas K, Davies JA, Davidson DR, Baldock RA, Beverdam A, Bertram JF, Capel B, Chiu HS, Clements D, Cullen-McEwen L, Fleming J, Gilbert T, Herzlinger D, Houghton D, Kaufman MH, Kleymenova E, Koopman PA, Lewis AG, McMahon AP, Mendelsohn CL, Mitchell EK, Rumballe BA, Sweeney DE, Valerius MT, Yamada G, Yang Y, Yu J. A high-resolution anatomical ontology of the developing murine genitourinary tract. Gene Expr Patterns. 2007; 7:680–699. [PubMed: 17452023]

Miyoshi H, Ajima R, Luo CT, Yamaguchi TP, Stappenbeck TS. Wnt5a potentiates TGF-beta signaling to promote colonic crypt regeneration after tissue injury. Science. 2012; 338:108–113. [PubMed: 22956684]

Nakaya MA, Biris K, Tsukiyama T, Jaime S, Rawls JA, Yamaguchi TP. Wnt3a links left-right determination with segmentation and anteroposterior axis elongation. Development (Cambridge, England). 2005; 132:5425–5436.

Nelson DK, Williams T. Frontonasal process-specific disruption of AP-2alpha results in postnatal midfacial hypoplasia, vascular anomalies, and nasal cavity defects. Developmental biology. 2004; 267:72–92. [PubMed: 14975718]

Nishita M, Qiao S, Miyamoto M, Okinaka Y, Yamada M, Hashimoto R, Iijima K, Otani H, Hartmann C, Nishinakamura R, Minami Y. Role of Wnt5a-Ror2 signaling in morphogenesis of the metanephric mesenchyme during ureteric budding. Mol Cell Biol. 2014; 34:3096–3105. [PubMed: 24891614]

Patton MA, Afzal AR. Robinow syndrome. Journal of medical genetics. 2002; 39:305–310. [PubMed: 12011143]

Person AD, Beiraghi S, Sieben CM, Hermanson S, Neumann AN, Robu ME, Schleiffarth JR, Billington CJ Jr, van Bokhoven H, Hoogeboom JM, Mazzeu JF, Petryk A, Schimmenti LA, Brunner HG, Ekker SC, Lohr JL. WNT5A mutations in patients with autosomal dominant Robinow syndrome. Developmental dynamics : an official publication of the American Association of Anatomists. 2010; 239:327–337. [PubMed: 19918918]

Pietila I, Prunskaite-Hyyrylainen R, Kaisto S, Tika E, van Eerde AM, Salo AM, Garma L, Miinalainen I, Feitz WF, Bongers EM, Juffer A, Knoers NV, Renkema KY, Myllyharju J, Vainio SJ. Wnt5a Deficiency Leads to Anomalies in Ureteric Tree Development, Tubular Epithelial Cell Organization and Basement Membrane Integrity Pointing to a Role in Kidney Collecting Duct Patterning. PLoS One. 2016; 11:e0147171. [PubMed: 26794322]

Rasouly HM, Lu W. Lower urinary tract development and disease. Wiley Interdiscip Rev Syst Biol Med. 2013; 5:307–342. [PubMed: 23408557]

Roifman M, Marcelis CL, Paton T, Marshall C, Silver R, Lohr JL, Yntema HG, Venselaar H, Kayserili H, van Bon B, Seaward G, Consortium FC, Brunner HG, Chitayat D. De novo WNT5A-associated autosomal dominant Robinow syndrome suggests specificity of genotype and phenotype. Clinical genetics. 2015; 87:34–41. [PubMed: 24716670]

Saburi S, Hester I, Fischer E, Pontoglio M, Eremina V, Gessler M, Quaggin SE, Harrison R, Mount R, McNeill H. Loss of Fat4 disrupts PCP signaling and oriented cell division and leads to cystic kidney disease. Nature genetics. 2008; 40:1010–1015. [PubMed: 18604206]

Staack A, Donjacour AA, Brody J, Cunha GR, Carroll P. Mouse urogenital development: a practical approach. Differentiation; research in biological diversity. 2003; 71:402–413. [PubMed: 12969333]

Wehn AK, Gallo PH, Chapman DL. Generation of transgenic mice expressing Cre recombinase under the control of the Dll1 mesoderm enhancer element. Genesis. 2009; 47:309–313. [PubMed: 19298012]

Weiss AC, Airik R, Bohnenpoll T, Greulich F, Foik A, Trowe MO, Rudat C, Costantini F, Adams RH, Kispert A. Nephric duct insertion requires EphA4/EphA7 signaling from the pericloacal mesenchyme. Development. 2014; 141:3420–3430. [PubMed: 25139858]

Wiens L, Strickland DK, Sniffen B, Warady BA. Robinow syndrome: report of two patients with cystic kidney disease. Clinical genetics. 1990; 37:481–484. [PubMed: 2200625]

Yamaguchi TP, Bradley A, McMahon AP, Jones S. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. Development. 1999; 126:1211–1223. [PubMed: 10021340]

Yu J, Carroll TJ, Rajagopal J, Kobayashi A, Ren Q, McMahon AP. A Wnt7b-dependent pathway regulates the orientation of epithelial cell division and establishes the cortico-medullary axis of the mammalian kidney. Development. 2009; 136:161–171. [PubMed: 19060336]

Yun K, Ajima R, Sharma N, Costantini F, Mackem S, Lewandoski M, Yamaguchi TP, Perantoni AO. Non-canonical Wnt5a/Ror2 signaling regulates kidney morphogenesis by controlling intermediate mesoderm extension. Hum Mol Genet. 2014; 23:6807–6814. [PubMed: 25082826]

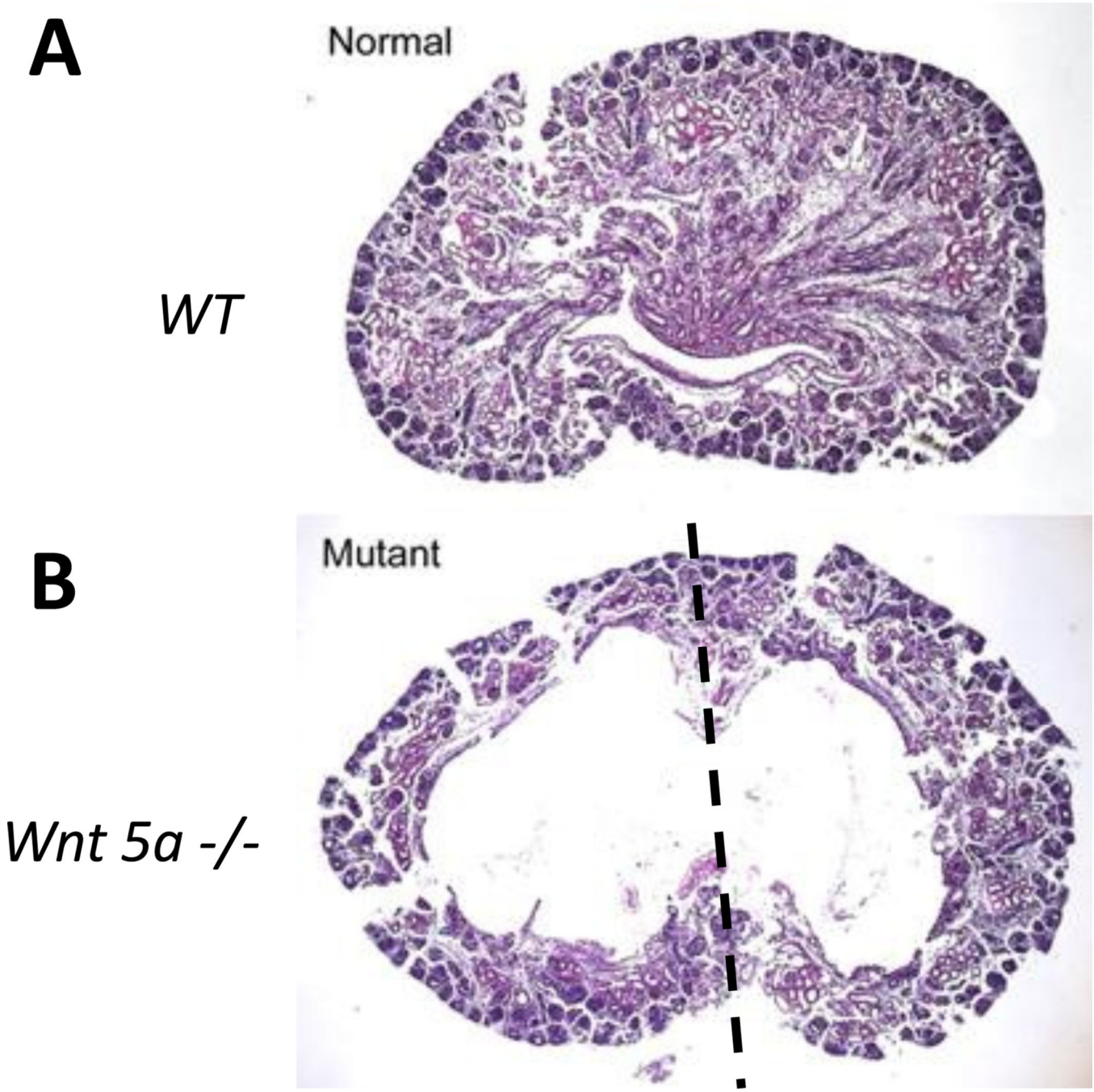

Fig 1. Wnt5a deletion induces loss of the medullary region in the developing kidney

Hematoxylin & Eosin (H&E) staining of sagittal sections of (A) normal and (B) Wnt5a −/− kidney at E18.5. A dotted line bisects the duplex kidney.

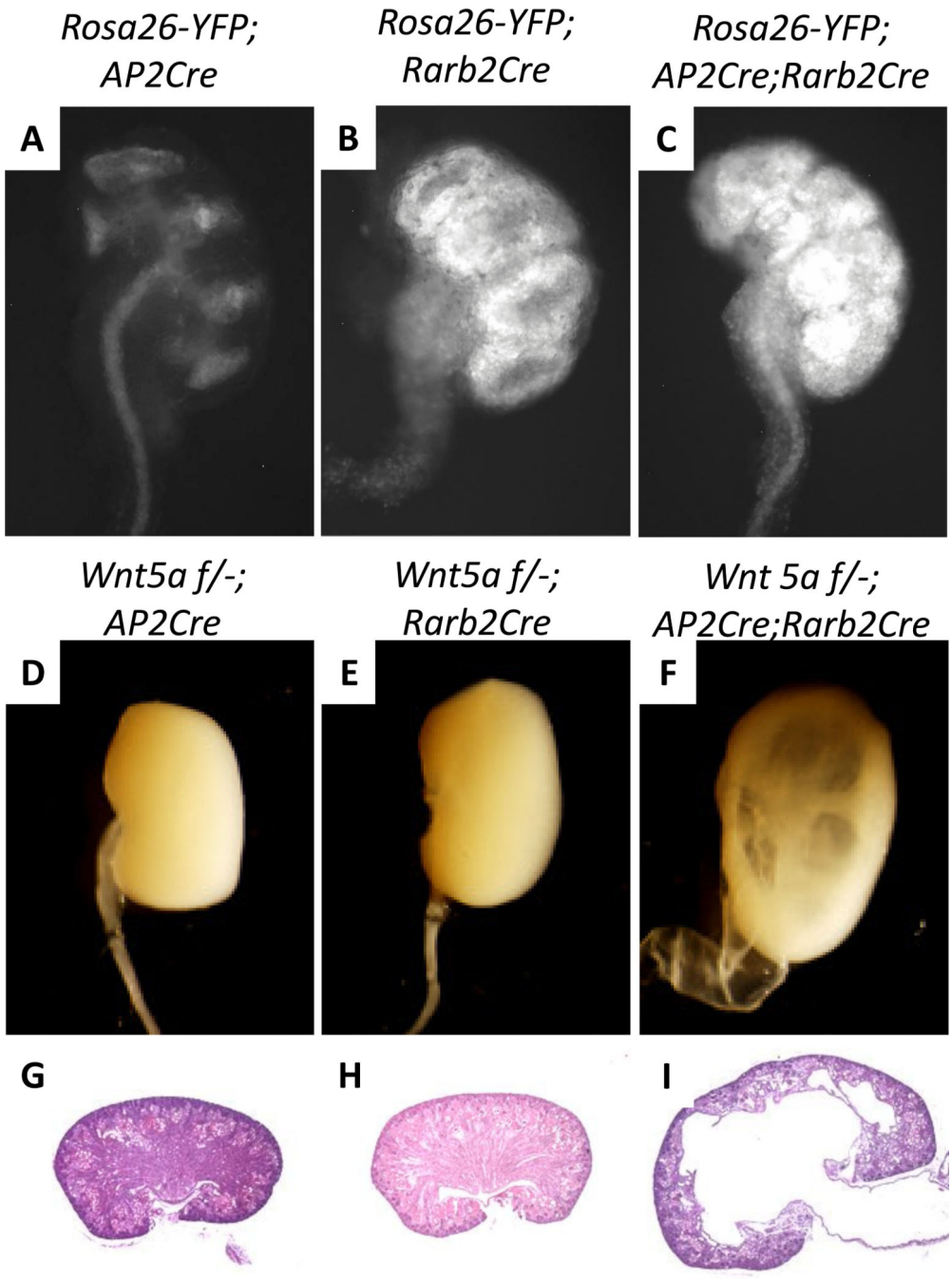

Fig 2. Wnt5a deletion in both UB and MM progenitors is necessary for the loss of the medullary region

(A–C) Rosa26-YFP expression in the kidney at E12.5 for (A) AP2Cre (B) Rarb2Cre (C) combined AP2Cre and Rarb2Cre. (D–F) Kidney at E18.5 of (D) Wnt5a f/−;AP2Cre, (E) Wnt5a f/−;Rarb2Cre and (F) Wnt5a f/−;AP2Cre;Rarb2Cre. (GI) H&E staining of sagittal sections of (D–F).

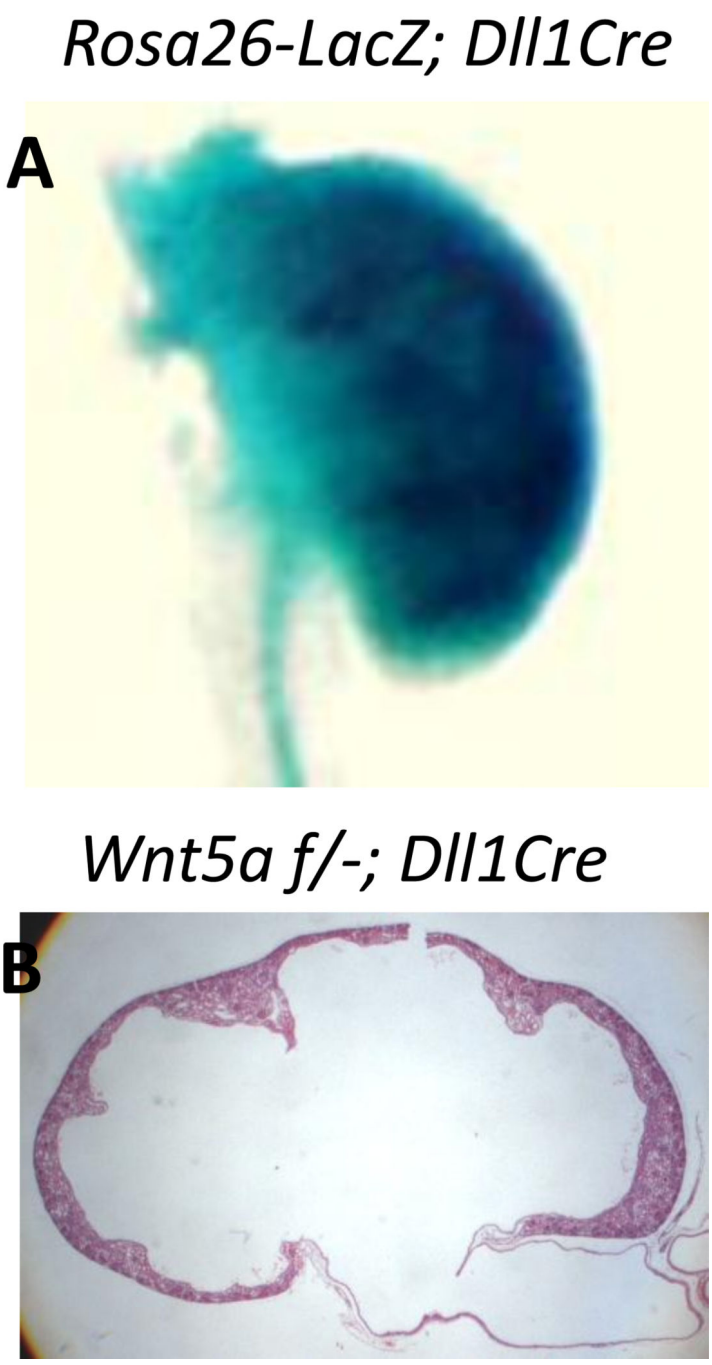

Fig 3. Wnt5a deletion using Dll1Cre induces the same kidney phenotype as observed with the combination of AP2Cre and Rarb2Cre

(A) Rosa26-LacZ expression in the kidney at E12.5 for Dll1Cre is observed throughout both the UB and MM progenitors. (B) Kidney at E18.5 of Wnt5a f/−;Dll1Cre showing severe loss of the medullary region and hydroureter without duplex kidney formation.

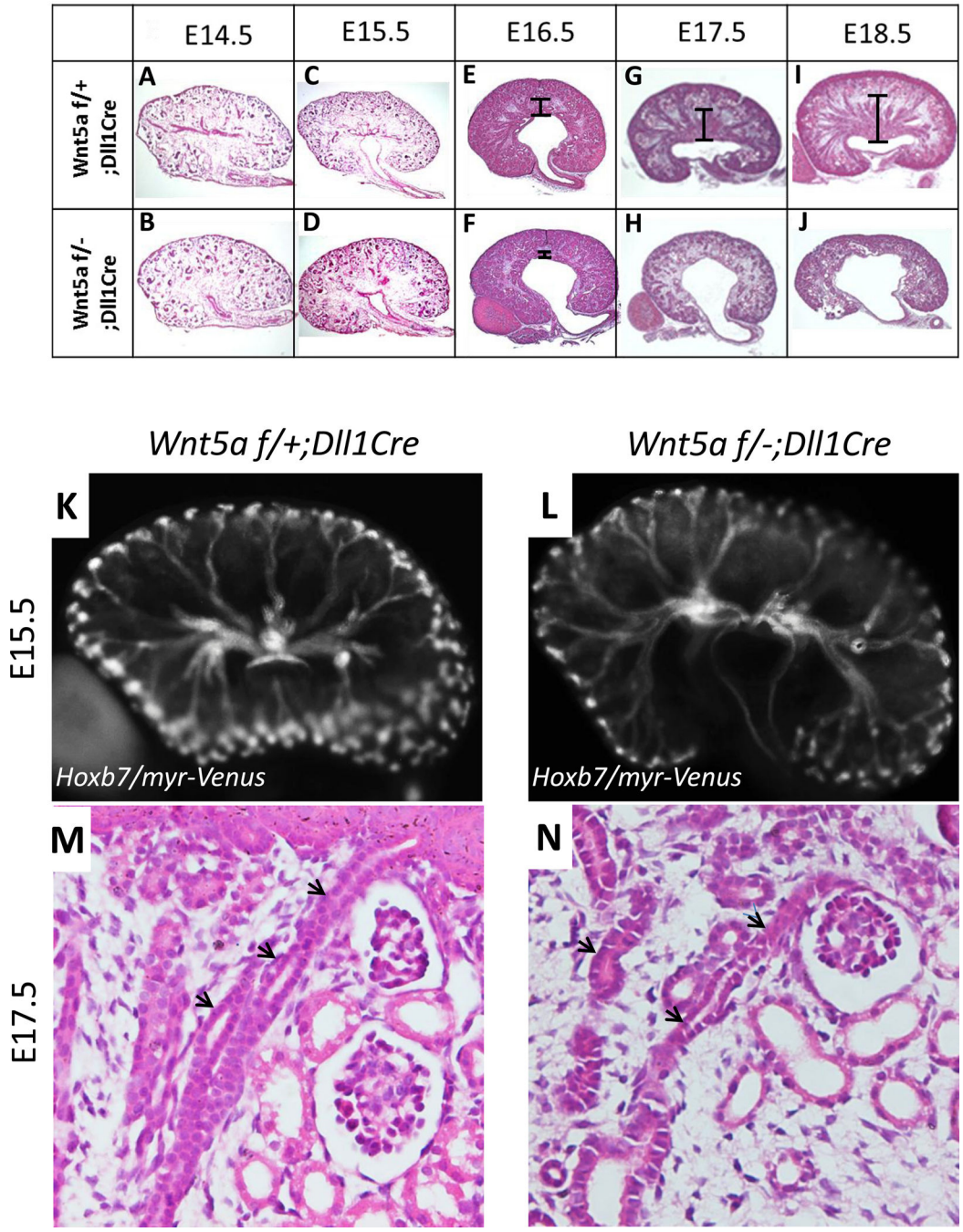

Fig 4. Loss of the medullary region begins at E16.5 in the mutant kidney

(A–J) H&E staining of sagittal sections of (A, C, E, G, I) Wnt5a f/+;Dll1Cre and (B, D, F, H, J) Wnt5a f/−;Dll1Cre kidneys at indicated developmental stages. In the mutants, loss of the medullary region is first apparent at E16.5 and the area involved increases in later stages. (K, L) Vibratome section of (K) Wnt5a f/+;Dll1Cre and Wnt5a f/−;Dll1Cre kidneys at E15.5 containing Hoxb7/myr-Venus for the UB-derived tissue. Collecting duct formation appears normal in the mutant. (M, N) H&E staining of sagittal sections at E17.5 of (M) Wnt5a f/

+;Dll1Cre and (N) Wnt5a f/−;Dll1Cre kidneys. The epithelial tubules in the mutants are elongated as single cell layers, typical of those in the normal kidney.

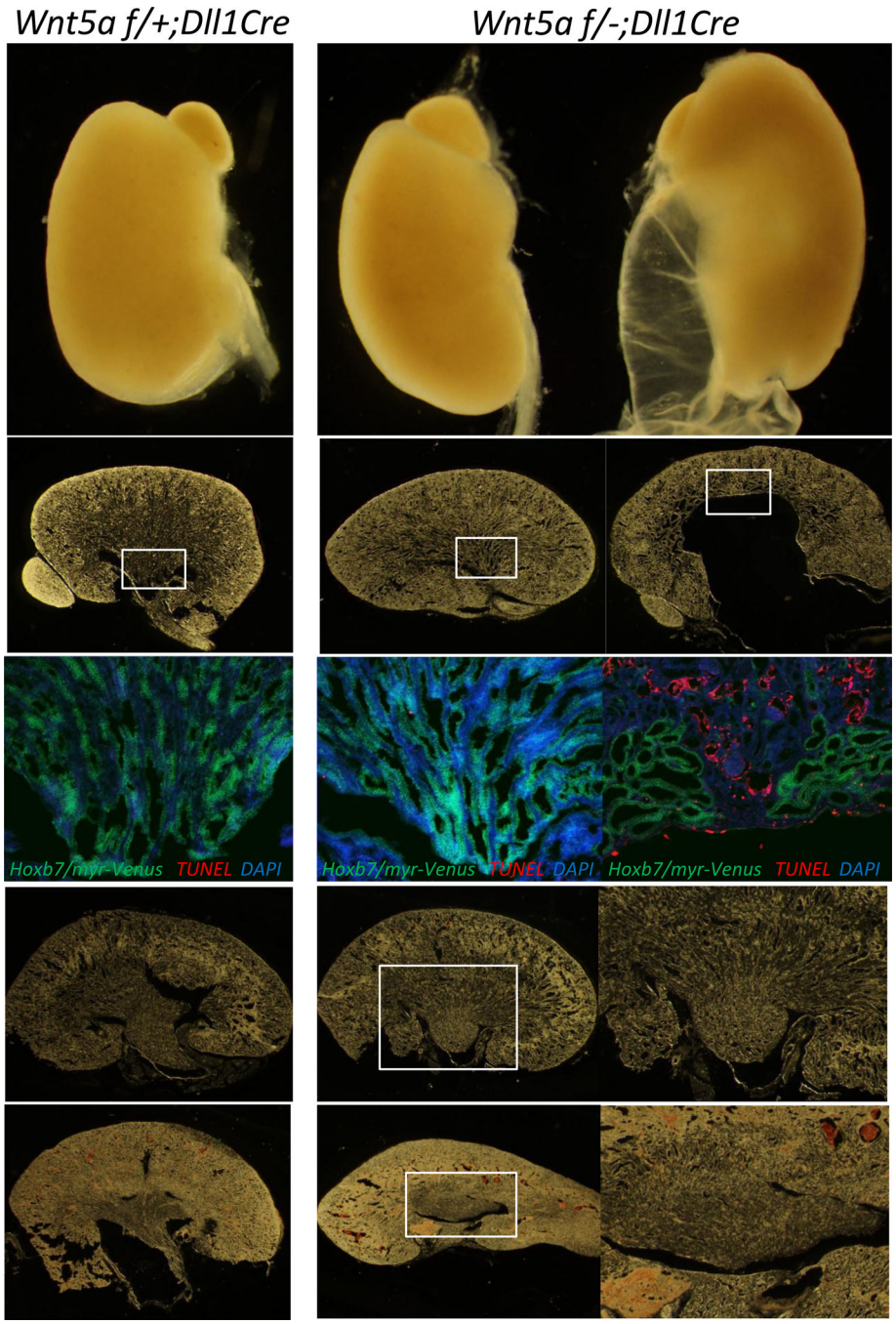

Fig 5. Wnt5a does not play a crucial role during renal medullary development, but apoptosis in the mutant kidney is associated with hydronephrosis

(A–H) Kidneys at P1 of (A) Wnt5a f/+;Dll1Cre and (B) a pair of Wnt5a f/−;Dll1Cre. (C–H) sagittal sections of (A, B). (F–H) TUNEL staining (Red) of sagittal sections of (C–E) medulla. Collecting ducts are marked with the Hoxb7/myr-Venus signal. The collecting ducts are populated with single layers of epithelial cells typical of normal kidneys, and apoptosis precedes the loss of the renal medulla in mutant kidneys. (I–K) sagittal sections of (I) Wnt5a f/+;Dll1Cre and (J, K) Wnt5a f/−;Dll1Cre kidneys at P14. (K) Rectangle area is enlarged to show medulla of (I). (L–N) sagittal sections of (L) Wnt5a f/+;Dll1Cre and (M,

N) Wnt5a f/−;Dll1Cre kidneys from 3 month-old mice. (N) Rectangle area is enlarged to show medulla of (M). The renal medulla develops without cyst formation in mutants, but ducts become dilated with hydronephrosis.

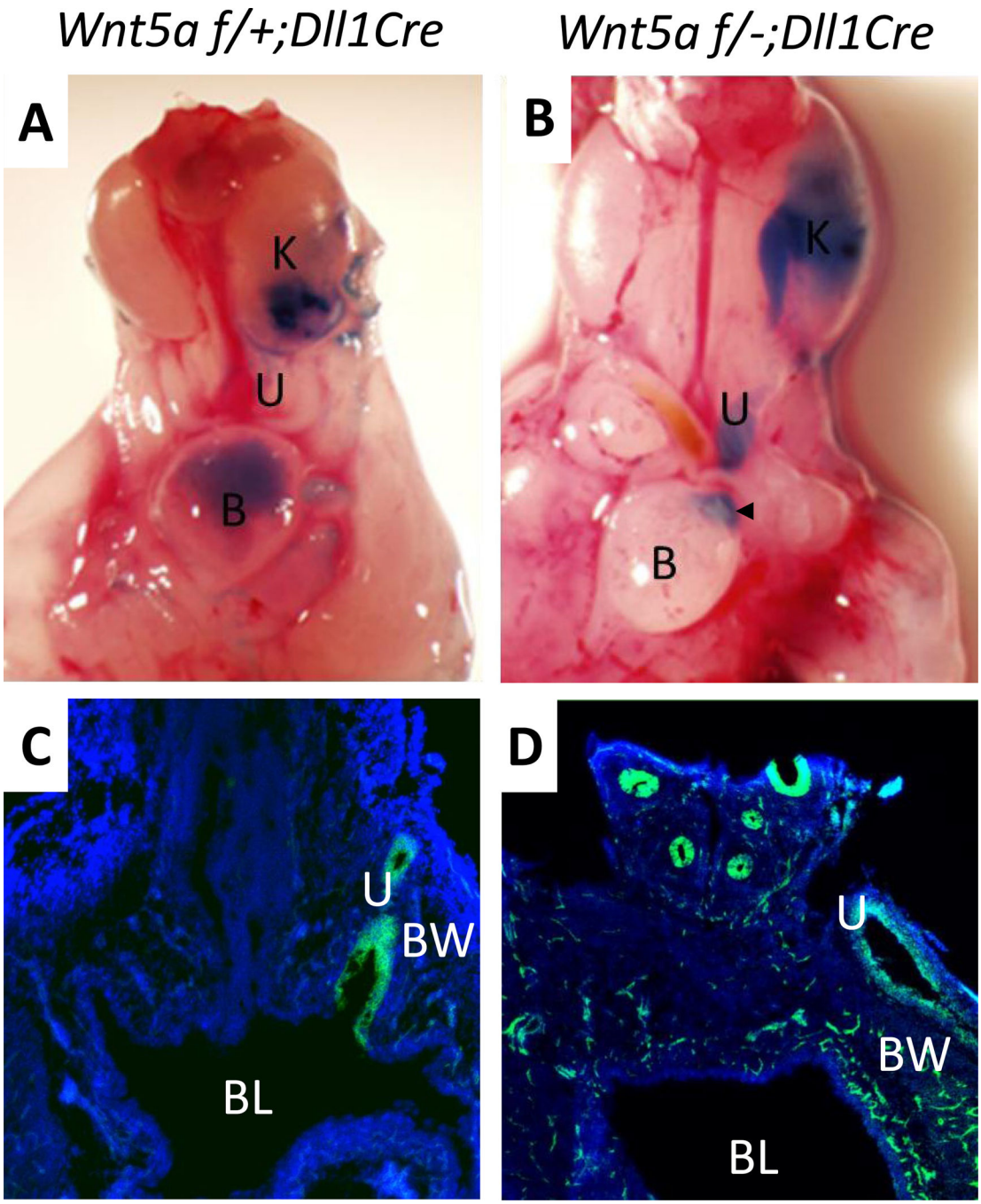

Fig 6. Urine flow is obstructed in Wnt5a mutants with loss of the medullary region

(A, B) Methylene blue dye was injected into the kidneys of embryos at E18.5: The dye flows freely into the bladder lumen from the normal kidney, whereas it does not enter the bladder lumen in mutants with hydronephrosis. (C, D) Representative images of coronal sections through the lower urinary tract. The normal ureter is inserted into the bladder lumen (C), while the side with unilateral hydronephrosis reveals an enlarged ureter wedged against the bladder wall (BW) with no evidence of ureter-bladder connectivity (D). (A, C) Wnt5a f/

+;Dll1Cre and (B, D) Wnt5a f/−;Dll1Cre. The ureter is marked by the Hoxb7/myr-Venus reporter. K – kidney; B – bladder; BL – bladder lumen; BW – bladder wall; U – ureter

Table 1

Frequency of the hydronephrosis phenotype in Wnt5a f/−;Dll1Cre mutants

Genotype	Wnt5a f/+; Dll1Cre	Wnt5a f/−; Dll1Cre	
No. of embryos with hydroureter / No. of total embryos	0/39	Uni-lateral 11/58	bi-lateral 9/58
Percentage (%)	0	19.0	15.5