# Supplemental material

Dionne et al., https://doi.org/10.1083/jcb.201710019

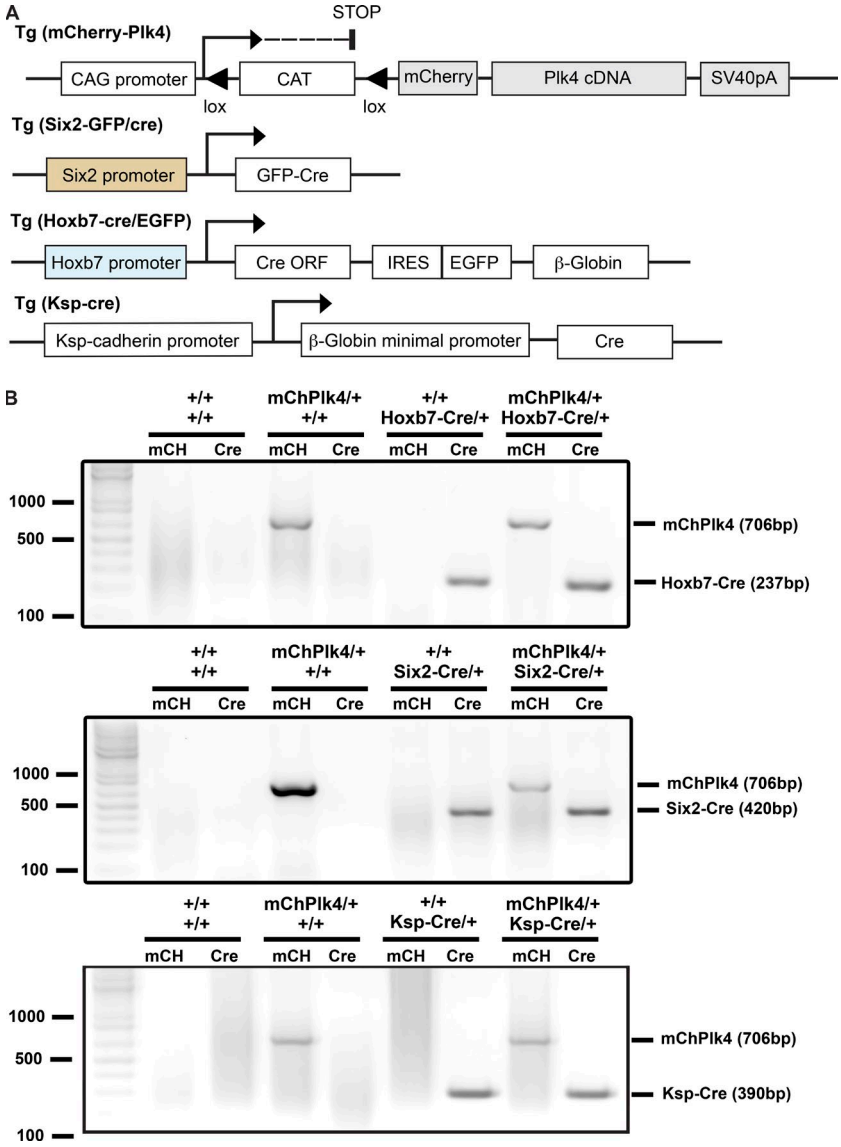



Figure S1. **Mouse models used for inducing CA in renal cells. (A)** Schematic representation of each transgene used in this study. Expression of mChPlk4 is regulated by the chicken β-actin promoter (pCAG) and is prevented by the presence of a Chloramphenicol Acetyl Transferase (CAT) coding sequence (that includes a stop codon), flanked by two loxP sites (black triangles). Three different transgenes were used to induce Cre-mediated expression of Plk4. **(B)** PCR analysis demonstrating the four genotypes obtained from each mating scheme.

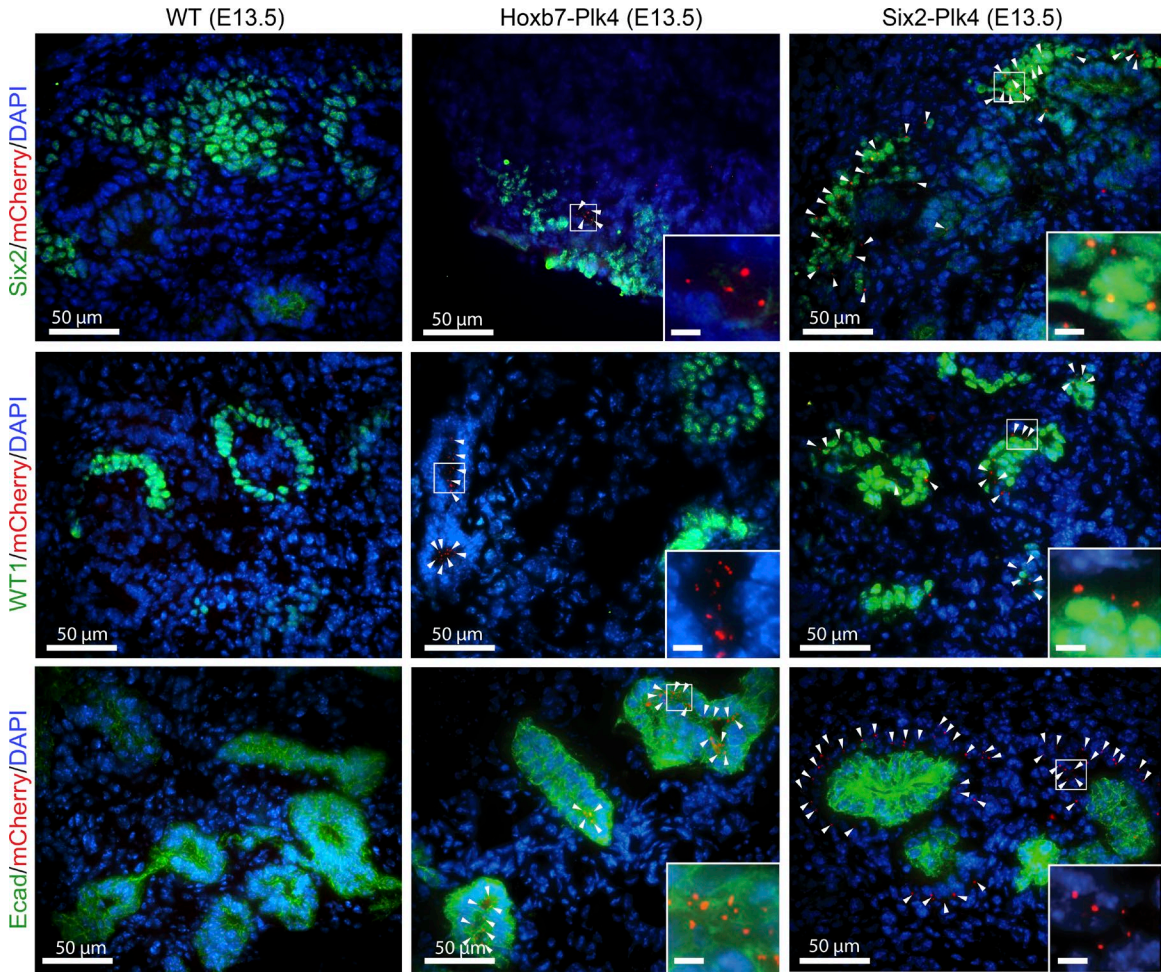



Figure S2. **Conditional Plk4 expression in the developing mouse kidney at E13.5.** Immunofluorescence images of kidney sections from E13.5 mice stained with antibodies against mCherry (to identify mChPlk4), Six2 (MM), WT1 (glomerular precursors), and E-cadherin (UB structures). In Hoxb7-Plk4 animals, foci of mChPlk4 (arrowheads) are specifically detected in E-cadherin–positive UB tubules, but are absent in Six2- or WT1-positive cells. In Six2-Plk4 mice, mChPlk4 (arrowheads) localize to Six2- and WT1-positive cells, adjacent to but not in E-cadherin–positive UB tubules. No mChPlk4 expression was observed in kidneys from control (WT) animals. White boxes depict regions shown at fivefold magnification as inset images. Nuclei were stained with DAPI. Bars (insets), 5 µm.

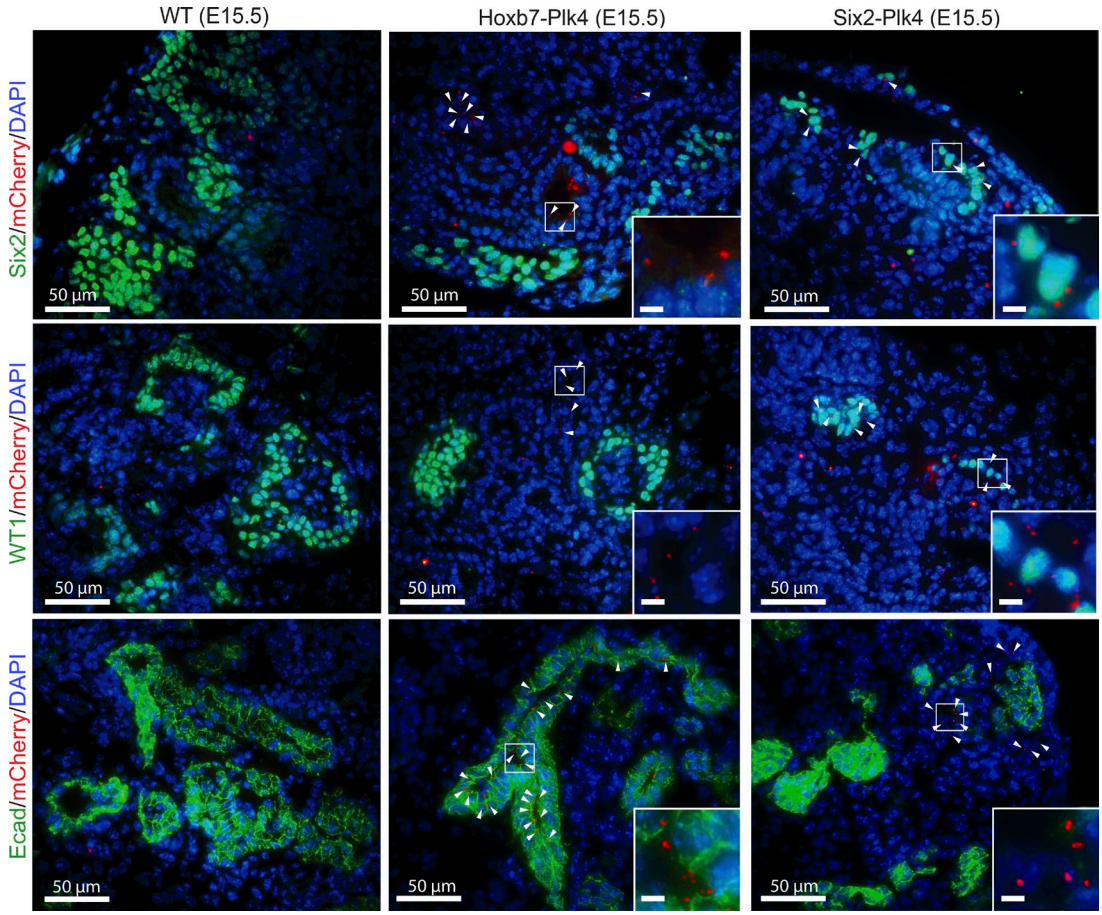



Figure S3. **Conditional Plk4 expression in the developing mouse kidney at E15.5.** Immunofluorescence images of kidney sections stained with antibodies against mCherry (to identify mChPlk4), Six2 (MM), WT1 (glomerular precursors), and E-cadherin (UB structures). In Hoxb7-Plk4 animals, foci of mChPlk4 (arrowheads) are specifically detected in E-cadherin–positive UB tubules, but are absent in Six2- or WT1-positive cells. In Six2-Plk4 mice, mChPlk4 (arrowheads) localize to Six2- and WT1-positive cells, adjacent to but not in E-cadherin–positive UB tubules. No mChPlk4 expression was observed in kidneys from control animals. White boxes depict regions shown at fivefold magnification as insets. Nuclei were stained with DAPI. Bars (insets), 5 µm.

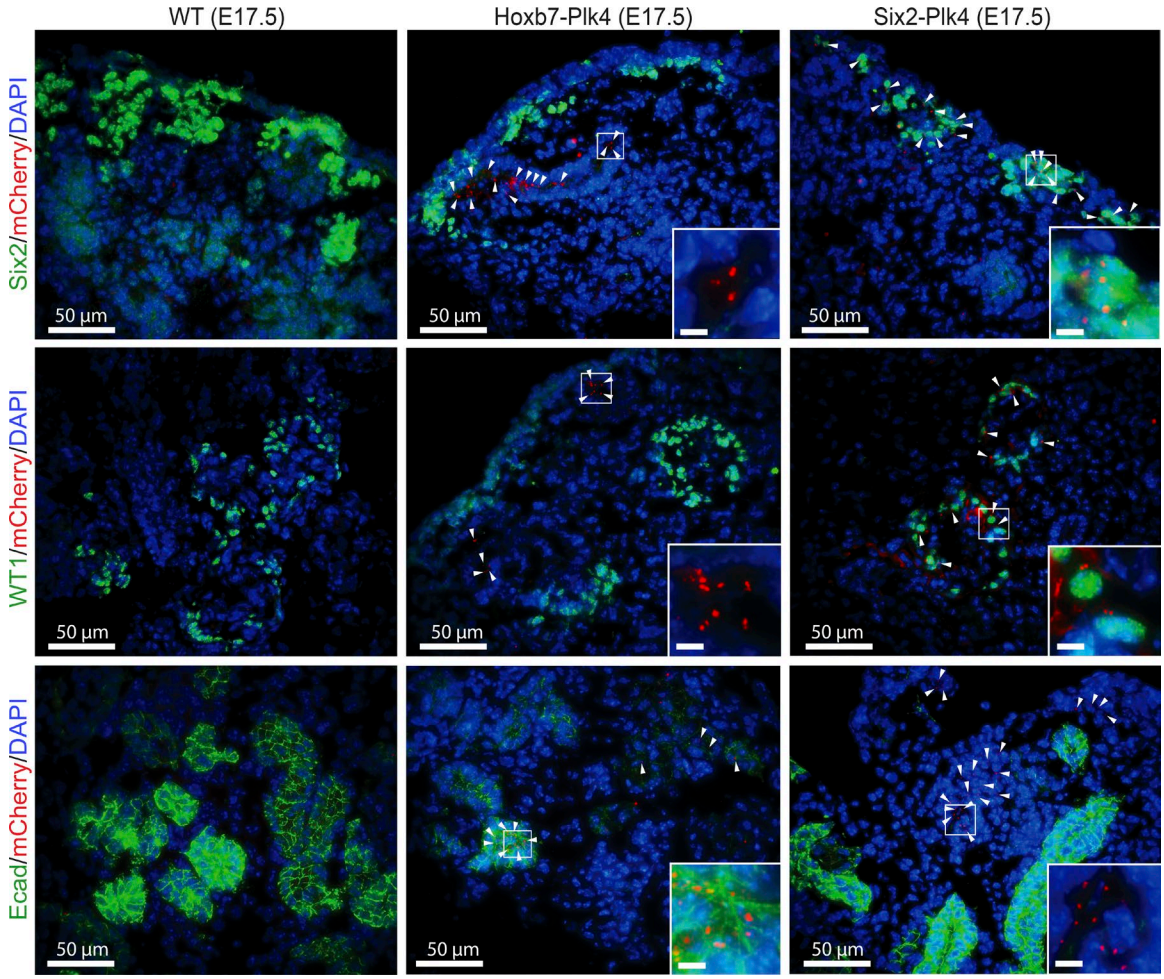



Figure S4. **Conditional Plk4 expression in the developing mouse kidney at E17.5.** Immunofluorescence images of kidney sections stained with antibodies against mCherry (to identify mChPlk4), Six2 (MM), WT1 (glomerular precursors), and E-cadherin (UB structures). In Hoxb7-Plk4 animals, foci of mChPlk4 (arrowheads) are specifically detected in E-cadherin–positive UB tubules, but are absent in Six2- or WT1-positive cells. In Six2-Plk4 mice, mChPlk4 (arrowheads) localize to Six2- and WT1-positive cells, adjacent to but not in E-cadherin–positive UB tubules. No mChPlk4 expression was observed in kidneys of control animals. White boxes depict regions shown at fivefold magnification as insets. Nuclei were stained with DAPI. Bars (insets), 5 µm.

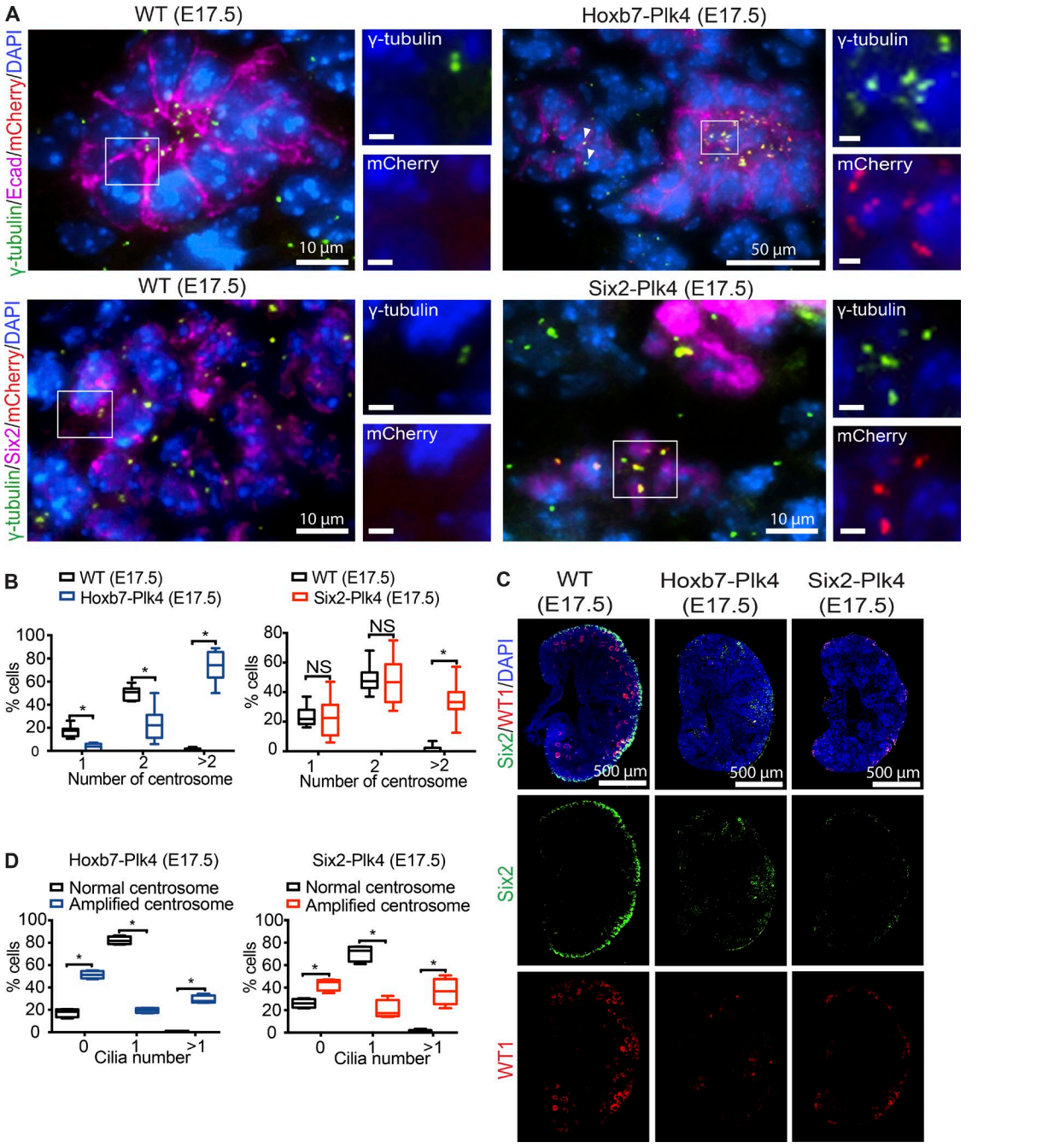



Figure S5. **Plk4 overexpression causes CA and ciliogenesis defects in renal progenitors. (A)** Kidney sections from E17.5 control, Hoxb7-Plk4, and Six2-Plk4 mice immunostained with antibodies targeting mCherry (Plk4), γ-tubulin (centrosomes), E-cadherin (UB tubules), and Six2 (MM). Nuclei were stained with DAPI. Control kidneys lacking mChPlk4 mainly contain one centrosome, whereas supernumerary centrosomes are seen specifically in regions of UB tubules and in MM cells overexpressing mChPlk4 (white box, magnified 1.5-fold). Importantly, adjacent cells within the same kidneys that lack mChPlk4 signal display normal centrosome number (arrowheads). Bars (insets), 2 µm. **(B)** Graphs show the percentage of cells with normal centrosome number (one or two) or CA (more than two centrosomes) in Hoxb7-Plk4 and Six2-Plk4 mice. *n* = 1,154 cells (E17.5 WT control), 518 mChPlk4-positive cells (E17.5 Hoxb7-Plk4), 1,553 cells (E17.5 WT control), and 210 mChPlk4-positive cells (E17.5 Six2-Plk4). A two-tailed unpaired *t* test was performed to determine statistically significant differences between samples (*, $P < 0.05$). **(C)** Immunofluorescence images of kidney sections from E17.5 Hoxb7-Plk4, Six2-Plk4, and control mice stained with antibodies against Six2 (to identify MM cells) and WT1 (glomerular precursors). Nuclei are stained with DAPI. There was a significant decrease in the density of both Six2-positive progenitors and WT1-positive structures compared with control samples. **(D)** Quantification of ciliary assembly defects in Hoxb7-Plk4 and Six2-Plk4 mice at E17.5. The percentage of cells with zero, one, or more than one cilium was determined from cells that contained normal centrosome number compared with cells with CA. *n* = 264 cells (WT; normal centrosomes), 187 cells (Hoxb7-Plk4; amplified centrosomes), 200 cells (WT; normal centrosomes) and 167 cells (Six2-Plk4; amplified centrosomes), from five mice of each genotype per developmental stage. Statistical significance was determined by a two-tailed unpaired *t* test (*, $P < 0.05$).

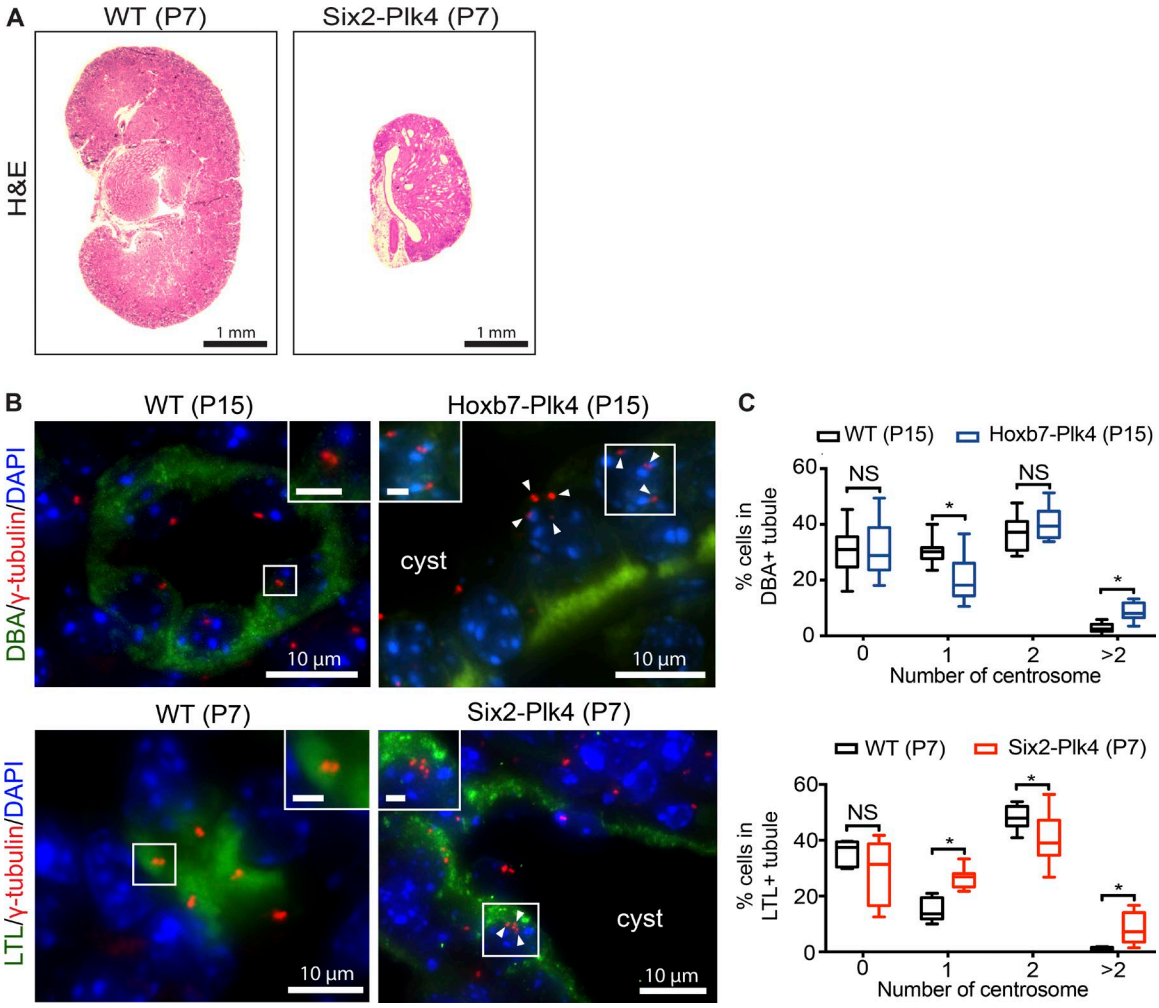



Figure S6. **CA causes cystogenesis. (A)** H&E-stained kidney sections from P7 Six2-Plk4 and control mice. **(B)** Immunofluorescence images of P7 and P15 kidney sections immunostained for γ-tubulin (centrosomes), LTL (marker of proximal tubules), or DBA (marker of collecting ducts). Nuclei were stained with DAPI. CA persisted in cystic epithelial cells of collecting ducts (Hoxb7-Plk4) and proximal tubules (Six2-Plk4), and was absent in control kidneys. White box denotes regions magnified in insets. Arrowheads point to examples of cells containing multiple centrosomes. Bars (insets), 2 µm. **(C)** Quantification of CA in Hoxb7-Plk4 (P15) and Six2-Plk4 (P7). *n* = 1,340 cells (P15 WT), 1,618 cells (P15 Hoxb7-Plk4), 731 cells (P7 WT), and 559 cells (P7 Six2-Plk4). Statistical significance was determined by a two-tailed unpaired *t* test (*, P < 0.05).

Table S1. **Summary of the total number of mice generated and analyzed by mating Tg(mChPlk4)/+ and Hoxb7-Cre$^{GFP}$/+ (A), Tg(mChPlk4)/+ and Six2-Cre$^{GFP}$/+ (B), and Tg(mChPlk4)/+ and Ksp-Cre/+ (C)**

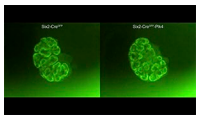
| **A** | **+/+ +/+** | **mChPlk4/+ +/+** | **+/+ Hoxb7-Cre/+** | **mChPlk4/+ Hoxb7-Cre/+** | **Total** |
|---|---|---|---|---|---|
| E13.5 | 4 (17) | 8 (33) | 6 (25) | 6 (25) | 24 |
| E15.5 | 10 (32) | 5 (16) | 9 (29) | 7 (23) | 31 |
| E17.5 | 15 (31) | 11 (22) | 13 (27) | 10 (20) | 49 |
| P0 | 10 (24) | 9 (22) | 10 (24) | 12 (29) | 41 |
| P15 | 15 (33) | 11 (24) | 12 (27) | 7 (16) | 45 |
| **B** | **+/+ +/+** | **mChPlk4/+ +/+** | **+/+ Six2-Cre/+** | **mChPlk4/+ Six2-Cre/+** | **Total** |
| E13.5 | 15 (34) | 11 (25) | 10 (23) | 8 (18) | 44 |
| E15.5 | 7 (26) | 9 (33) | 6 (22) | 5 (19) | 27 |
| E17.5 | 11 (25) | 13 (30) | 12 (27) | 8 (18) | 44 |
| P0 | 15 (27) | 17 (30) | 11 (20) | 13 (23) | 56 |
| P7 | 9 (26) | 11 (31) | 12 (34) | 3 (9) | 35 |
| P15 | 12 (28) | 16 (37) | 14 (33) | 1 (2) | 43 |
| **C** | **+/+ +/+** | **mChPlk4/+ +/+** | **+/+ Ksp-Cre/+** | **mChPlk4/+ Ksp-Cre/+** | **Total** |
| P7 | 1 (12.5) | 2 (25) | 1 (12.5) | 4 (50) | 8 |
| P30 | 3 (13) | 7 (29) | 6 (25) | 8 (33) | 24 |

The percentage of the total for each genotype is presented in parentheses.

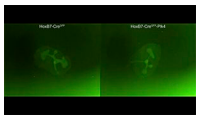
Table S2. **Detailed list of antibodies and other reagents used in this study**

| **Antibodies (clone)** | **Product details** | **Dilution (FFPE/OCT)** |
|---|---|---|
| Mouse anti-centrin (20H5) | EMD Milipore (04-1624) | 1:200/Not used |
| Mouse anti–γ-tubulin (GTU-88) | Sigma-Aldrich (T5326) | 1:500/1:2,000 |
| Mouse anti-acetylated α-tubulin (6-11B-1) | Sigma-Aldrich (T7451) | 1:5,000/1:5,000 |
| Rabbit anti-six2 | Proteintech (11562-1-AP) | 1:100/1:100 |
| Mouse anti-WT1 (6F-H2) | Dako (M 3561) | 1:50/1:50 |
| Goat anti–E-cadherin | R&D Systems (AF748) | 1:200/1:200 |
| Rat anti-mCherry (16D7) | Invitrogen (M11217) | Not used/1:500 |
| Rabbit anti-podocin | Roselli et al., 2002 | 1:5,000/Not used |
| Mouse anti-pHH3 | Cell Signaling (9406) | 1:200/Not used |
| Rat anti–α-tubulin | Santa Cruz Biotechnology (sc-53029) | 1:500/Not used |
| Mouse anti–cytokeratin-8/TROMA I | Developmental Studies Hybridoma Bank (AB-531826) | 1:50/Not used |
| Rabbit anti–CLC-K | Alomone Labs (ACL-004) | 1:1,000/Not used |
| Rabbit anti-Ki67 | Leica (NCL-Ki67p) | 1:1,000/Not used |
| Goat anti-RET | Neuromics (GT15002) | 1:100/Not used |
| Rabbit anti-pERK | Cell Signaling (4370) | 1:100/Not used |
| Rabbit anti-WNT11 | Genetex (GTX105971) | 1:200/Not used |
| Rabbit anti-LEF1 | Cell Signaling (2230) | 1:200/Not used |
| LTL-FITC | Vector Laboratories (FL-1321) | 1:300/Not used |
| DBA-FITC | Vector Laboratories (FL-1031) | 1:300/Not used |

FFPE, formalin-fixed, paraffin-embedded; OCT, Optimal Cutting Temperature compound.



Video 1. **Live-cell imaging of ex vivo metanephric organ cultures from Six2-Cre$^{GFP}$ and Six2-Cre$^{GFP}$-Plk4 embryos.** Kidneys were isolated at E13.5 and imaged at 30-min intervals for 40 h. Video is presented at six frames per second.



Video 2. **Live-cell imaging of ex vivo metanephric organ cultures from Hoxb7-Cre$^{GFP}$ and Hoxb7-Cre$^{GFP}$-Plk4 embryos.** Kidneys were isolated at E12.5 and imaged at 30-min intervals for 40 h. Video is presented at six frames per second.

## Reference

Roselli, S., O. Gribouval, N. Boute, M. Sich, F. Benessy, T. Attié, M.C. Gubler, and C. Antignac. 2002. Podocin localizes in the kidney to the slit diaphragm area. *Am. J. Pathol.* 160:131–139. https://doi.org/10.1016/S0002-9440(10)64357-X