Proximal Tubule Proliferation Is Insufficient to Induce Rapid Cyst Formation after Cilia Disruption

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ABSTRACT

Disrupting the function of cilia in mouse kidneys results in rapid or slow progression of cystic disease depending on whether the animals are juveniles or adults, respectively. Renal injury can also markedly accelerate the renal cyst formation that occurs after disruption of cilia in adult mice. Rates of cell proliferation are markedly higher in juvenile than adult kidneys and increase after renal injury, suggesting that cell proliferation may enhance the development of cysts. Here, we induced cilia loss in the kidneys of adult mice in the presence or absence of a Cux-1 transgene, which maintains cell proliferation. By using this model, we were able to avoid additional factors such as inflammation and dedifferentiation, which associate with renal injury and may also influence the rate of cystogenesis. After induction of cilia loss, cystic disease was not more pronounced in adult mice with the Cux-1 transgene compared with those without the transgene. In conclusion, these data suggest that proliferation is unlikely to be the sole mechanism underlying the rapid cystogenesis observed after injury in mice that lose cilia function in adulthood.


Primary cilia defects cause human disorders called ciliopathies, which have phenotypes ranging from mid-gestation lethality in Meckel–Gruber syndrome, to obesity in Bardet–Biedl syndrome.1 One feature shared by many ciliopathies is kidney cysts. In fact, the most prevalent ciliopathy is autosomal dominant polycystic kidney disease, caused by mutations in the ciliary proteins Polycystin-1 or Polycystin-2.

The core of the primary cilium is the axoneme consisting of microtubules that extend from the basal body. The axoneme serves as a track for kinesin and dynein motors to transport proteins into and out of the cilium through intraflagellar transport (IFT).2 Congenital mutations disrupting IFT, such as mutations in the heterotrimeric kinesin component Kif3a or the IFT complex protein Ift88, result in the absence of cilia, causing embryonic lethality.3–5 In contrast, conditional disruption of these genes in the kidney results in cystic disease.6,7 Several mechanisms have been proposed for how cilia dysfunction causes renal cysts. Cilia are mechanosensors that initiate a polycystin-dependent calcium signal upon flow-induced cilia deflection.8,9 However, support for the mechanosensory model eroded based on data showing that disruption of Polycystin-1 or the ciliogenic genes Ift88 or Kif3a in adult mice did not result in cyst development for nearly half a year.6,7,10,11 Due to this protracted cyst development period, it appears that loss of the mechanosensory function alone is insufficient to drive cyst formation.

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Intriguingly, in Polycystin-1 conditional mutant mice, there is a switch from rapid to slow cystogenesis depending on whether the gene is disrupted before or after postnatal day (P) 12. Similar results were obtained with the Kif3a conditional mutants. This “switch” period corresponds to the normal decrease in proliferation that occurs during kidney maturation, suggestive of a proliferative effect on cyst severity. Furthermore, cilia dysfunction and cystogenesis have been associated with the randomization of cell division orientation. Thus, rapid cyst development could result from altered mitotic spindle positioning when in a highly proliferative environment.

Other studies connecting the rate of cyst formation to proliferation utilized adult-induced cilia mutants that underwent renal ischemia reperfusion (IR). Renal injury results in an increase in cell proliferation, particularly in the proximal tubules followed by rapid cyst development. It is important to note that in addition to proliferation, renal injury causes epithelial dedifferentiation, increased cell death, and inflammation, other factors that may enhance cyst formation.

Additional data in the conditional Polycystin-1 mutants revealed that the kidney is undergoing major transcriptional changes during the switch period (P12–P16), in which genes involved in mature renal function are induced and developmental genes are downregulated. Thus, the differences in rates of cyst formation between adult and juvenile mutants or after renal injury could be related to these changes in gene expression and be independent of proliferation.

The purpose of this work is to assess the importance of proliferation in cyst formation in the absence of confounding effects of IR. Using a genetic approach, we demonstrate that maintaining proliferation in the proximal tubule epithelium is not the sole driving force causing the rapid formation of cysts observed after injury.

**RESULTS**

**Temporal Analysis of Cilia Loss and Cyst Formation in Inducible Ift88 Mutants**

To define when the switch from rapid to slow cyst formation occurs in Ift88 mutants, and to compare our model with the Kif3a and Polycystin-1 models, we injected Ift88 conditional mutants (CAGG-CreER; Ift88floxed/floxed), and control mice with tamoxifen at P2 or at subsequent time points through P14 (Figure 1A). Tamoxifen activates Cre leading to Ift88 disruption and cilia loss in the mutant mice but not controls (Figure 1). Three weeks postinjection, Western blot analysis and immunofluorescence showed a reduction in IFT88 (Figure 1B) and cilia in the kidneys (Figure 1C). A few cilia are present on some cells, likely reflecting the efficacy of Cre activity. The effects of cilia loss on the kidney were analyzed 3 weeks post-Cre induction using histologic and kidney weight and length analyses (Figure 2, A and B). Cyst formation was evident in Ift88 mutants induced on and before but not after P11 (Figures 1C and 2A, subpanels b–d). These data differ slightly from the Polycystin-1 conditional mutant in which the switch period occurred at P14. Cysts were detected in cilia mutant but not control mice induced between P2 and P11 in Dolichos biflorus (DBA)–positive collecting tubules (Figure 2C, subpanels a–c) and Lotus tetragonolobus (LTA)–positive proximal tubules (Figure 2C, subpanels d–f).

**Temporal Proliferation Analyses in the Kidney**

Proliferation has been proposed as a component of rapid cyst formation in both juvenile-induced Kif3a mutants and after IR. To assess whether proliferation correlates with cyst development in the Ift88 model, we analyzed proliferation rates in the normal kidney from P7 through P16 (Figure 3). Proliferation was high between P7 and P14, but decreased sharply by P16 (Figure 3). Proliferative cells were detected throughout the kidney, including the proximal tubules (Figure 3A), collecting tubules (data not shown), as well as in the interstitium. This drop in proliferation and the distribution of proliferative cells are similar to that reported in the Polycystin-1 study (P13–P14), but occurs later than P7 as reported for Kif3a.
Renal Injury and Enhanced Cyst Development in Ift88 Adult-Induced Mutants

IR-induced proliferation is thought to accelerate cyst formation in Kif3a conditional mutants. To compare the models, we analyzed whether adult Ift88 mutants also exhibit accelerated cystogenesis after injury (Figure 4). Cilia/Ift88 loss and cyst formation was induced by tamoxifen injection at P30 and was analyzed in mutant animals by immunofluorescence microscopy, histochemistry, and Western blot (Figure 4, A and B). Three weeks after tamoxifen injection, one kidney was subjected to a 45-minute IR injury, whereas the other served as a sham control. Cyst formation, nephron segment identity, and proliferation were evaluated between 1 and 21 days after surgery. Cysts were detected in the injured kidney of Ift88 mutant mice by 14 days postinjury and were largely in LTA positive proximal tubules and to a lesser extent in DBA collecting tubules (Figure 4A, subpanels d, h, l, and p, and Supplemental Figure 1) but not in sham or controls (Figure 4A, subpanels i–p). Cysts were also detected in non-DBA/LTA tubules. These are likely the Loop of Henle that is also prominently affected by IR injury. As reported in Kif3a mutants and other IR models, proliferation was elevated in both cilia mutant and control IR kidneys as early as 24 hours postsurgery (Figure 4, C–G). This increased proliferation persisted for 14 days, after which it dropped in control IR kidneys to sham levels (Figure 4D, green line). In contrast, proliferation rates remained elevated in the cilia mutant IR kidneys (Figure 4D, purple line). At this point, the mutant kidneys possessed cystic lesions (Figure 4A and Supplemental Figure 1). An elevation in proliferation was not observed in the mutant sham or control sham kidneys (Figure 4D, red and blue lines, respectively). The proliferative response induced by injury occurred mainly in proximal tubules immediately after IR (Figure 4E). Minimal proliferation was observed in the collecting ducts (Figure 4F), although this became elevated in the injured kidneys 3 weeks after IR injury. However, proliferation was not detected in the control sham kidneys (Figure 4D, red and blue lines, respectively). The proliferative response induced by injury occurred mainly in proximal tubules immediately after IR (Figure 4E). Minimal proliferation was observed in the collecting ducts (Figure 4F), although this became elevated in the injured kidneys 3 weeks after IR injury. However, proliferation was not detected in the control sham kidneys (Figure 4D, red and blue lines, respectively). The proliferative response induced by injury occurred mainly in proximal tubules immediately after IR (Figure 4E). Minimal proliferation was observed in the collecting ducts (Figure 4F), although this became elevated in the injured kidneys 3 weeks after IR injury. However, proliferation was not detected in the control sham kidneys (Figure 4D, red and blue lines, respectively). The proliferative response induced by injury occurred mainly in proximal tubules immediately after IR (Figure 4E). Minimal proliferation was observed in the collecting ducts (Figure 4F), although this became elevated in the injured kidneys 3 weeks after IR injury. However, proliferation was not detected in the control sham kidneys (Figure 4D, red and blue lines, respectively). The proliferative response induced by injury occurred mainly in proximal tubules immediately after IR (Figure 4E). Minimal proliferation was observed in the collecting ducts (Figure 4F), although this became elevated in the injured kidneys 3 weeks after IR injury.
A Proliferative Proximal Tubule Environment Is Insufficient for Rapid Cyst Formation

Our findings are consistent with a potential causative role of proliferation in the cystic phenotype. However, a caveat of IR is that it causes additional effects other than proliferation, including loss of epithelial characteristics and dedifferentiation, cell necrosis and apoptosis, and inflammation. Thus, to more specifically analyze proliferation in adult-induced cyst formation, we utilized a transgenic line expressing Cux1 under control of the cytomegalovirus promoter. Cux1 is a homeodomain protein expressed during nephrogenesis, but with minimal expression in mature tubules. It functions as a cell cycle–dependent transcriptional repressor of cyclin kinase inhibitors p21 and p27. The Cux1 transgenic mice develop multiorgan hyperplasia, but do not have renal cysts. To confirm the hyperplasias in Cux1 transgenics and identify where proliferation was occurring, we injected Cux1 transgenic (Cux1Tg) and control mice (Cux1wt) with bromodeoxyuridine (BrdU) at time points between P26 and P55. There was a consistent 4- to 5-fold increase in proliferation in the Cux1Tg proximal tubule epithelium; however, proliferation was only minimally altered in the collecting ducts (Figure 5, A and B, and Figure 6B). The effect of Cux1 in the proximal tubules parallels the proliferation seen after IR injury. One substantial difference between the Cux1 transgenic lines and the IR models is that the Cux1Tg mice maintain proliferation mainly in epithelium with less interstitial cell proliferation (Figures 5D and 6B versus Figure 4C). Previous studies show that Cux1 expression is not associated with apoptosis until late in cystic disease and is likely due to tissue damage. Although we have not yet ruled out a role for Cux1 in inflammation, this does not appear to be the case based on kidney histology. Thus, the Cux1Tg line represents a good model to test the effect of persistent proliferation in proximal tubules on cyst formation. Importantly, in both IR and Cux1Tg lines, the amount of proliferation in proximal tubules is similarly elevated and is 4–5 times higher than their respective background controls (Figure 4E and Figure 5, B–D). The proliferation in Cux1 kidneys is also chronic (Figure 5B) rather than transient as occurs in IR, and thus the cumulative proliferation over time is likely higher in Cux1Tg animals.

To evaluate the effect of elevated and prolonged proliferation in the proximal tubules on cyst development, we induced cilia/Ift88 loss on the Cux1Tg background in adult mice (Figure 6). The renal phenotype and cilia loss were assessed 3 weeks after cilia ablation as done after IR. Surprisingly, no histologic difference in cyst severity was evident between cilia mutants with or without Cux1Tg (Figure 6, C and D). Cux1Tg mice were not protected from cyst formation as determined after IR injury in a parallel cohort of CAGG-CreER, Ift88lox/lox, Cux1Tg animals (Supplemental Figure 2). Thus, the conclusion from this work is that proliferation alone in proximal tubule epithelium of adult-induced cilia mutants is insufficient to drive rapid cystogenesis as observed after renal injury.

DISCUSSION

The clinical importance of primary cilia was established by the discovery that cystic kidney diseases are associated with primary cilia dysfunction. Although the mechanisms involved are unknown, it was proposed that the cilium is a mechanosensor detecting fluid flow in a polycystin-dependent...
Figure 4. AKI induces cyst formation in adult Ift88 conditional mutant mice. (A) Conditional Ift88 mutant mice were injected with tamoxifen at P30 and subjected to unilateral IR injury. Kidneys were analyzed 3 weeks postsurgery. Immunofluorescence analysis of 460
manner. However, if loss of cilia-mediated mechanosensation is the cause of renal cysts, it is surprising that adult-induced mutants show slow and protracted cyst development. This is in contrast to the rapid and severe cystic phenotype observed in juvenile-induced cilia mutants. The protracted rate of cyst formation in adult mutants makes it difficult to reconcile a mechanosensor model. Alternatively, different mechanisms may drive cystogenesis in adults versus juvenile kidneys, or factors in addition to cilia loss may be required.

The rate of cyst formation in mouse models has been shown to switch from rapid to slow modes between P13 and P14 in Polycystin-1 conditional mutants, at around P10 for Kif3a mutants, and between P11 and P12 in the current Ift88 study. Although not tested, we suspect that these differences may reflect Cre activity, protein stability, or the time required for cillum disassembly.

We noted a dramatic decrease in proliferation within the normal developing kidney that correlates with this switch point. In addition, the rate of cyst development in adults can be accelerated by injury, which is also associated with a transient increase in proliferation. In the case of the adult-induced Kif3a mutants, the injury-associated increase in cyst formation was correlated with this proliferative response. The possible association between cyst formation and proliferation is further supported by data showing decreased proliferation rates in the kidney coincide with a decrease in cyst formation.

Proliferation as the basis of cyst initiation in cilia mutants is attractive based on the role of ciliary proteins in regulating mitosis and oriented cell division. In several cystic kidney models, the orientation of cell division is random rather than occurring along the longitudinal axis of the nephron as seen in normal animals. The cilium has been proposed to regulate mitotic spindle orientation by positioning the centrioles. In this scenario, the absence of cilia would disrupt spindle positioning and cause rapid tubule expansion when cilia function is ablated in a proliferative environment. The proliferation increase required for this model would occur after injury, or in the juvenile kidney. In contrast, cyst formation would be protracted in a noninjured/adult kidney where proliferation is low.

Several complications arise in injury-induced cystic models because injury itself causes increased proliferation, cell death, dedifferentiation, inflammation, and developmental reprogramming. Thus, it is not clear whether proliferation or one (or more) of these factors contribute to the rapid cyst progression observed after injury. In fact, analysis of the adult-induced Polycystin-1 mutant mice suggested that proliferation was a consequence of cyst expansion and not an initiating force. Rather, Piontek et al. argue the developmental state of the kidney is critical. Alternatively, inflammation is associated with renal injury and recent studies have demonstrated that depletion of infiltrating macrophages has protective effects on cyst severity and renal function.

The goal of this study was to more specifically test the role of proliferation as a facilitator of rapid cyst formation using an approach that limited confounding factors. We utilized adult-induced Ift88 mutants carrying the Cux1Tg that chronically elevates proliferation in proximal tubules. Importantly, in IR and Cux1Tg mice, the increase in proliferation was similar and proximal tubules were predominantly affected in both cases. Furthermore, the inflammatory response with infiltrating interstitial cells observed after renal injury does not occur in the Cux1Tg mice, although a role for Cux1 in inflammation cannot be formally excluded. Thus, the cilia mutants on a Cux1Tg background appear to be a good model to evaluate whether elevated proliferation in proximal tubule epithelium is sufficient to drive cystogenesis that is observed in adult-induced cilia mutants after injury.

Importantly, whereas the Cux1Tg mice have enlarged kidneys, expression of the transgene in otherwise normal mice does not cause or prevent cysts, nor is there sufficient apoptosis to mask cyst formation (Supplemental Figure 2). Using the Cux1Tg genetic approach to mimic the proliferative environment in the proximal tubules that occurs after IR but without interstitial cell proliferation, we found that cystogenesis

sections with cilia marker anti-Arl13b (red) and basement membrane marker anti-entactin (green) antibodies shows that primary cilia are present on Ift88+/− kidneys (subpanels a and c), whereas cilia are absent on Ift88−/− Cre− kidneys (subpanels b and d), which develop multiple cysts (subpanels d and h; cysts are labeled "cy"). DBA (red) and LTA (green) staining of renal sections is used to identify collecting tubule and proximal tubules, respectively. Corresponding hematoxylin and eosin images are shown at high magnification (subpanels i–l) and at lower magnification (panels m–p). (B) Representative Western blot analysis showing loss of IFT88 protein from tamoxifen-injected Ift88+/− Cre− kidneys. Cre− and WT kidneys are used as controls for IFT88 expression and actin is used for loading control. (C) Analysis of proliferation after IR in injured and sham kidneys. P30 Ift88+/− Cre− mice are injected with tamoxifen and are subjected to unilateral IR after 3 weeks. Proliferation is measured using BrdU in proximal tubules LTA (green), and collecting ducts DBA (red). Nuclei are stained with Hoechst (blue). Arrows indicate proliferative cells located in the interstitium. (D) Proliferation indices are calculated by counting BrdU-positive cells divided by total number of nuclei in a given image field. The location of BrdU-labeled cells is determined by colocalization with LTA (proximal tubules) (E) or DBA (collecting ducts) (F), or is not colocalized (for interstitial cells and unlabeled tubules) (G). The graphs show proliferation at four time points after IR. Open circles in D–G indicate that the 1-day time point had only two BrdU injections due to time constraints of the experimental paradigm. Values are mean ± SEM. Asterisks indicate a significant difference from Cre− sham (*P < 0.05; **P < 0.01; Kruskal–Wallis ANOVA followed by post hoc Bonferroni corrected Mann–Whitney U test). Scale bars, 42 μm in A; 50 μm in C.
that cilia/IFT participates in the inflammation and tissue repair processes. In the kidney of adult cilia mutants, this altered response could manifest as widespread cyst development, as seen after injury, or as slowly forming focal cysts in tubules requiring repair due to the “wear and tear” of normal aging. Interestingly, macrophages infiltrate the kidney in response to inflammatory cytokines expressed by tubule epithelium in Polycystin-1 mutants and chemical-mediated ablation of these macrophages reduced cyst severity and improved renal function. In addition, if one evaluates the effect of antiproliferative therapies on cystogenesis with the immune system in mind, the effects may not be on the renal epithelium but rather the infiltrating immune cells. These combined observations suggest that perhaps polycystic kidney disease is a disease of altered inflammatory process aberrant wound repair. Thus, it will be informative to evaluate whether inhibition or activation of inflammation is able to alter the severity of ciliopathy phenotypes in affected tissues.

**CONCISE METHODS**

**Mice**

The mouse alleles and transgenes used were as follows: Ift88tm1Bky (Ift88fl/fl), CAGG-Cre/Esr1/5Amc/J (CAGG-CreER), and cytomegalovirus: Cux1 transgene (Cux1fl/fl). Animals were maintained in Association for Assessment and Accreditation of Laboratory Animal Care International–accredited facilities in accordance with Institutional Animal Care and Use Committee regulations at the University of Alabama at Birmingham (UAB).

**Cre Induction**

To induce Cre activity in CAGG-CreER Ift88fl/fl animals, a single intraperitoneal injection of tamoxifen at 9 mg/40 g body weight was administered for animals in the P2–P15 age range. Induction of Cre in adult animals was achieved by once-daily injection of tamoxifen (6 mg/40 g body weight) for 3 consecutive days.

**Renal IR Injury**

Three weeks after Cre induction, mice were anesthetized with isoflurane and unilateral IR injury was performed by clamping the left renal pedicle for 45 minutes followed by clamp release to allow reperfusion. Right sham kidneys underwent identical treatment.
without clamping of the renal pedicle. Mice were euthanized and both kidneys were removed for analysis after IR injury surgery. The IR injury procedures were performed by the UAB–University of California San Diego (UCSD) O’Brien Core Center for Acute Kidney Injury Research.

**Immunoblotting and Immunofluorescence**

Samples were processed for immunofluorescence and immunoblotting as previously described.29,30 Primary antibodies were used and diluted as follows: anti-actin 1:1000 (A2066; Sigma-Aldrich), anti-IFT88 1:1000,31 anti-Arl13b 1:1500 (gift from Dr. Tamara Caspary), anti-entactin 1:500 (MAB1946; Chemicon International), and FITC-conjugated anti-BrdU A21303 1:500 (Invitrogen). Secondary antibodies included the following: Alexa Fluor-546 and -488 conjugated goat anti-mouse IgG (A11003, A11001) and Alexa Fluor-546 and -488 conjugated donkey anti-rabbit IgG (A10040, A21206) (Invitrogen). For renal tubule identification, Rhodamine-labeled DBA and FITC-conjugated LTA (Vector Laboratories) were added during secondary antibody incubation. Nuclei were visualized by Hoechst 33342 (Invitrogen).

**Kidney Measurements and Histology**

Hematoxylin and eosin staining and kidney measurements were performed as previously described.6

**Proliferation Analyses**

For analysis of proliferation, three BrdU injections, one every 24 hours, were administered intraperitoneally (30 μl/g body weight, Invitrogen category no. 00–0103) for the 3 days immediately preceding euthanasia of the mouse. For the 1-day postinjury samples, because of time constraints, BrdU was injected 2 hours postsurgery and again approximately 20 hours later. Mice were euthanized and kidneys were harvested 2 hours after the last injection. Kidneys were fixed in 4% paraformaldehyde and cryosections were processed for immunostaining. After permeabilization, sections were incubated in 2 M HCl for 40 minutes at 37°C and then washed in PBS five times for 5 minutes each, followed by blocking in 3% BSA in PBS and then BrdU antibody incubation. At least three animals at each time point were analyzed.

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**Figure 6.** A highly proliferative environment in the proximal tubule epithelium is not sufficient to cause cyst formation in adult-induced cilia mutants. (A) Control (Ift88<sup>fl/fl</sup>; Cre<sup>2</sup>) and cilia mutant (Ift88<sup>fl/fl</sup>; Cre<sup>2</sup>) littermates in the presence (Cux1<sup>Tg</sup>) or absence (Cux1<sup>WT</sup>) of the Cux1 transgene are injected with tamoxifen at P30 to induce cilia loss and their kidneys are isolated 3 weeks later. Immunofluorescence analysis of kidney sections stained for cilia with antibody against Arl13b (red) and proximal tubules (LTA; green) are shown. In Cux1<sup>WT</sup>; Ift88<sup>fl/fl</sup> Cre<sup>2</sup> and Cux1<sup>Tg</sup>; Ift88<sup>fl/fl</sup> Cre<sup>2</sup> controls (subpanels a and b) primary cilia are present throughout the kidney compared with Cux1<sup>WT</sup>; Ift88<sup>fl/fl</sup> Cre<sup>2</sup> and Cux1<sup>Tg</sup>; Ift88<sup>fl/fl</sup> Cre<sup>2</sup> mutant littermates (subpanels c and d) in which ciliogenesis is severely affected. (B) Analysis of proliferation (BrdU; red) in proximal tubule segments of Ift88 cilia mutant mice in the presence and absence of the Cux1 transgene. Note proliferative cells are prominently in the tubule epithelium in Cux1<sup>Tg</sup> mice. (C) Quantification of kidney length and weight shows no apparent difference between control and mutant mice in the presence of the Cux1 transgene. Differences in kidney length and weight between Cux1<sup>WT</sup> and Cux1<sup>Tg</sup> are seen independent of Ift88 genotype and cilia loss. Values are mean ± SEM. Asterisks indicate a significant difference from Cux1<sup>WT</sup>; Cux1<sup>Tg</sup>; Cre<sup>2</sup> (**) (P < 0.01; ANOVA followed by Tukey’s post hoc test). Numbers inside bars indicate number of mice in each group. (D) Representative histologic sections of WT and Ift88 mutant kidneys without the Cux1 transgene (Cux1<sup>WT</sup>) and with the Cux1 transgene (Cux1<sup>Tg</sup>). Scale bar, 21 μm in A and B; 100 μm in D.
DISCLOSURES

None.

REFERENCES


This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2012020154/-/DCSupplemental.
Supplemental Figure 1

Cyst localization in Adult Induced CAGG-CreER; IFT88<sup>F/F</sup> Injured Kidneys
**Supplemental Figure 1:** Ischemia-reperfusion injury of adult induced CAGG-CreER;Ift88\(^{fl/fl}\) cilia mutant mice results in rapid cyst formation predominantly in LTA (green) positive proximal tubules. Cysts are also present in a few DBA (red) positive collecting tubules as well as in tubules that do not label with either DBA or LTA. Hoechst stained nuclei in blue. Scale bar is 60 \(\mu\)m.

**Supplemental Figure 2:** The ectopic expression of Cux1 does not protect kidneys from forming cysts after injury. Control (Ift88\(^{fl/fl}\); Cre-) and cilia mutant (Ift88\(^{fl/fl}\); Cre+) littermates in the presence of the Cux1 transgene were injected with tamoxifen at P30 to induce cilia loss and subjected to unilateral renal ischemia reperfusion injury. Kidneys were harvested 3 weeks post-surgery, sectioned and Haematoxylin and Eosin stained to analyzed cyst development. No cysts develop in the uninjured kidney of Cux\(^Tg\) animals regardless of Ift88 genotype (A and B), despite injury in Cux\(^Tg\); Ift88\(^{fl/fl}\); Cre- animals, no cysts were detected at three weeks (C). Cysts were prevalent in Cux\(^Tg\); Ift88\(^{fl/fl}\); Cre\(^+\) animals after injury (D). Scale bar is 100 \(\mu\)m.