

Cilia to basement membrane signaling is a biomechanical driver in models of autosomal dominant polycystic kidney disease

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CONFLICT-OF-INTEREST STATEMENT

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD), the leading genetic cause of kidney failure, results from loss-of-function mutations in *PKD1*, encoding polycystin-1 (PC1). PC1 localizes to the primary cilium. In the absence of PC1, adverse signaling from the primary cilium orchestrates cyst formation, but the biomechanical underpinnings of this cilia-dependent cyst activation (CDCA) remain unclear. Combining tubule-specific orthologous mouse models with a tubule-on-chip platform, we show that PC1 and cilia govern the composition, mechanical properties and shape of the tubular basement membrane (TBM), the principal rigid determinant of tubule geometry. PC1 loss triggers TBM thinning, heparan sulfate enrichment and deformation, leading to distension, preferentially of the distal nephron. These changes are driven by a cilia-dependent transcriptional program, with GLIS2 — a key CDCA effector — participating as a downstream mediator. Reduction of TBM stiffness amplifies *Pkd1*^{-/-} tubule-on-chip dilation and increases cyst formation in vivo. Conversely, increasing luminal pressure through ureteral obstruction induces disproportionate distension of *Pkd1*-deficient tubules and triggers an irreversible cystogenic program. Together, these findings establish a TBM-centered biomechanical model of ADPKD in which tubule deformation is governed by both basolateral and luminal mechanical factors, and identify the cilium–TBM axis, operating in part through GLIS2, as a central driver of cystogenesis.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disorder, accounting for 5–10% of kidney failure cases. It is characterized by the progressive transformation of a subset of renal tubules into cysts that grow in number and size, increasing renal mass up to 20-fold and progressively impairing kidney function, with most patients reaching kidney failure by the sixth decade (1). Only modest benefit is gained from blood pressure control and the vasopressin V2R antagonist tolvaptan, the sole approved treatment for ADPKD (2).

ADPKD is caused by mutations inactivating the complex formed by polycystin-1 (PC1, encoded by *PKD1*), a large orphan receptor, and polycystin-2 (PC2, encoded by *PKD2*), a cation channel. This complex localizes mainly to primary cilia, filiform organelles protruding from the apical surface of tubular cells that integrate chemical and mechanical cues. Although patients carry heterozygous germline mutations, cystogenesis is triggered only when functional PC1/PC2 falls below a critical threshold, most often through somatic inactivation of the remaining allele (3). Yet how cysts form remains unresolved.

Cell proliferation sustains cyst growth downstream of polycystin inactivation but is insufficient on its own to initiate it: tubular proliferation also rises after nephron reduction, ischemia-reperfusion injury or albuminuria without cyst formation (4–6). Planar cell polarity (PCP) defects have been proposed to cause cysts through loss of oriented cell division or convergent extension (7, 8), but recent evidence indicates that PCP defects are not sufficient (9, 10). Likewise, several pathways promote cyst growth (mTOR, cAMP, metabolic rewiring), yet none appears active during early tubule dilation (11). The primary morphogenetic events causing tubule dilation thus remain only partially understood.

The primary cilium is instrumental in cystogenesis. Ciliary PC1/PC2 is required to prevent cysts (12), and genetic cilia ablation prevents cyst formation in *Pkd1* mutant mice (11, 13),

supporting a “cilia-dependent cyst activation” (CDCA) pathway. GLIS2 was recently identified as a critical transcriptional effector of CDCA, though how it induces tubule dilation is unclear (14). Two other cilia-regulated genes promote cystogenesis—*Ccl2* (13) and *Cdk1* (15), acting in macrophage recruitment and tubular proliferation—but, unlike cilia ablation, their loss slows cyst expansion rather than preventing early dilation of *Pkd1*-deficient tubules (13, 15), implying additional ciliary functions in cystogenesis.

In higher metazoans, the extracellular matrix (ECM) is a key determinant of tissue geometry and mechanics. In the kidney, decellularization shows that the ECM—largely composed of juxtaposed tubular basement membranes (TBM)—retains nephron shape and the tubule compliance that accommodates the luminal pressure required for kidney function (16). The TBM comprises interacting collagen IV and laminin networks linked by heparan sulfate proteoglycans and nidogen (17). Through their hydrophilic carbohydrate chains, heparan sulfate proteoglycans (HS) promote BM hydration and deformability (18), whereas the collagen and laminin networks confer rigidity, further increased by covalent collagen IV crosslinks (19, 20). BM biomechanics are thus largely set by thickness, composition and crosslinking (21).

The role of TBM mechanics in ADPKD has not been systematically investigated. Because intratubular pressure is not raised in cysts of rodent models, early researchers proposed that cysts arise from increased TBM deformability (22); however, early studies—mostly using non-orthologous models—failed to show consistent changes in TBM mechanics, structure or composition (22, 23).

Here, we combined tubule-specific *Pkd1*-deletion mouse models, with or without concomitant cilia ablation, and a tubule-on-chip model with tunable ECM properties to dissect the interplay between cilia, *Pkd1*, TBM and tubular dilation.

RESULTS

***Pkd1* loss triggers cilia-dependent proximal and distal nephron dilation through distinct mechanisms**

To gain insights into the early morphogenic events underlying tubule dilation, we first studied male mice with a post-developmental inactivation of *Pkd1* (*Pkd1*^{Δtub}) with or without simultaneous ablation of primary cilium (*Kif3a* or *Ift20* inactivation: *Pkd1*^{Δtub}; *Kif3a*^{Δtub} and *Pkd1*^{Δtub}; *Ift20*^{Δtub}) at a very early stage of the disease, that is 2 weeks after the completion of doxycycline treatment (*i.e.*, 8 weeks of age), when tubules display only slight dilations (**Figure 1A**). We used the *Pax8*.rtTA system which drives functional Cre expression along the nephron (**Supplemental Figure 1A**), efficiently excises floxed *Pkd1* alleles (**Supplemental Figure 1B**) and achieves near complete cilia loss in *Pkd1*^{Δtub}; *Kif3a*^{Δtub} tubules (**Supplemental Figure 1, C**). Morphometric analysis confirmed that *Pkd1* loss induces an increase in cross-sectional areas of proximal (PT) and distal tubules [consisting of collecting ducts (CD) and distal convoluted tubules (DCT)], which was prevented by concomitant cilia disruption following *Kif3a* or *Ift20* inactivation (**Figure 1, A-C**). Ki67 labelling revealed that PC1 deficiency increased cell proliferation in PT (**Figure 1, A and D**), but not in the distal nephron (**Figure 1, A, B and D**). Instead, we observed increased tubular cell stretching in CD and DCT, as reflected by an increase in the mean distance between two adjacent nuclei (later referred to as internuclear distance; **Figure 1, A, B and E**). Linear regression analysis showed that distension (*i.e.*, increase in internuclear distance) was the main factor explaining distal nephron dilation at this stage (**Figure 1, F-G**). At a later time point, distension and increased proliferation were observed in both proximal and distal *Pkd1*^{-/-} tubules (**Figure 1, H-J**). Genetic cilia ablation (*i.e.*, *Kif3a* or *Ift20* inactivation) rescued PC1-deficient distal nephron distension, tubular cell proliferation and cyst development (**Figure 1, H-J**). Analysis of 18 week-old animals

confirmed that cilia ablation fully rescued tubule deformation in *Pkd1*^{Δ_{tub}} mice (**Supplemental Figure 2**). Collectively, these results identify cilia-driven tubule distension as an important factor responsible for the early dilation of PC1-deficient tubules.

Preferential distal tubule distension correlates with specific changes of the TBM

As shape of the tubule is determined by TBM geometry (16), we reasoned that CD distension could be linked to specific TBM properties. TBM stiffness depends on its thickness and composition: laminin and cross-linked collagen IV provide rigidity, while the sugar moieties of heparan- and chondroitin-sulfate (HS/CS) confer flexibility by increasing BM hydration (18). Studying these parameters in basal conditions, we noticed that CD had thinner TBM compared to PT with higher HS content, suggesting an increased compliance (**Figure 2, A-B**), as historically suggested by pressure-volume measurements on isolated rabbit tubules (16). We further observed that distal nephron distension in *Pkd1*^{Δ_{tub}} mice was associated with prominent TBM thinning (**Figure 2C**). TBM thinning also occurred in PT but to a smaller extent than in CD or DCT (**Figure 2C**). A careful inspection of multiple tubule sections in transmission electron microscopy failed to detect any perforation or tubular cell protrusion through the TBM (**Figure 2C; Supplemental Figure 3**). Cilia ablation abolished the ability of PC1-deficient tubular cells to induce TBM thinning (**Figure 2C**). We further observed a specific increase in HS immunoreactivity of the TBM of PC1-deficient tubules undergoing distension, which was prevented by cilia disruption, without notable changes in collagen IV and laminin labelling (**Figure 2, D-G**). These results indicate that *Pkd1* loss is associated with a cilia-dependent remodeling of the TBM. They also suggest that intrinsic properties of distal nephron TBM may render this segment permissive to distension. Detailed transmission electron microscopy images of TBM from male mice are presented in **Supplemental Figure 3**.

We further examined tubular remodeling in kidneys from *Pkd1*^{Δ_{tub}} female mice, which display slower disease progression, at successive time points to better define the temporal relationship between TBM thinning and tubule distension (**Supplemental Figure 4**). TBM thickness was already decreased in *Pkd1*-deficient collecting ducts at 8 weeks of age (**Supplemental Figure 4, A–E**), whereas an increase in internuclear distance and HS staining was only observed later at 12 weeks (**Supplemental Figure 4, F–J**). TBM thinning persisted at 18 weeks in *Pkd1*-deficient kidneys (**Supplemental Figure 4, K–M**), although multilayering of the TBM was occasionally observed (**Supplemental Figure 4N**). These results identify TBM thinning as an early event that precedes tubule distension and HS enrichment and that persists in overtly cystic kidneys.

TBM remodeling and tubule distension are more prominent features of cystogenesis driven by *Pkd1* loss than by cilia ablation

Single cilia-deficient mouse models typically develop cystic kidney disease resembling the ADPKD phenotype (24, 25). Cyst formation occurs rapidly when cilia are ablated during kidney development (24), but the cystic phenotype is drastically delayed when cilia are ablated at later time points (25). With the strategy that we used for cilia ablation, cysts have only been detected in 9-month-old male mice (26). Indeed, contrary to single *Pkd1* mutant mice and similarly to double *Pkd1*/cilia mutant mice, single *Kif3a* mutant mice did not show any spontaneous tubular distension or TBM thinning at 8 weeks. Proliferative index, internuclear distance, and TBM thickness remained unchanged after *Kif3a* inactivation in both proximal and distal tubules (**Figure 3, A–F**). We next conducted the same analyses in the context of neonatal tubular inactivation of *Pkd1* or *Kif3a*, as cilia ablation in the developing kidney leads to rapid cyst formation (**Figure 4**). Doxycycline feeding of lactating mothers at birth induced explosive and diffuse cystogenesis in 2-week-old *Pkd1*^{Δ_{tub}} neonates with increased tubule distension and

marked HS accumulation in the TBM (**Figure 4, A–D**). In contrast, in single cilia-deleted neonates (*Kif3a*^{Δtub}), the same treatment only led to a modest dilation of outer medullary tubules (OMT), with unchanged internuclear distance (**Figure 4, A–C**). *Kif3a*-deficient dilated tubules showed a mild, yet non-significant increase in HS labelling compared to the outer medullary tubules of control littermates (**Figure 4, A and D**). Collectively, these findings indicate that cyst formation after cilia ablation is not initially associated with tubule distension or marked HS enrichment of the TBM, suggesting that the mechanisms of cyst formation after PC1 or cilia ablation do not fully overlap.

TBM remodeling and CD distension coincide with a specific transcriptional program

Our results suggest that cilia-dependent TBM remodeling promotes cyst formation in ADPKD and is decoupled from cell proliferation. Three non-mutually exclusive mechanisms can cause TBM distension without cell proliferation: (1) ECM digestion by proteases, which allows rapid BM remodeling during embryogenesis (27); (2) incorporation of BM softeners such as hydrating HS/CS, which allows rapid BM remodeling during *C.elegans* development (28); (3) deformation of the BM by traction forces exerted by cells through cytoskeleton motor proteins, as documented during cancer invasion (29, 30). To gain insights into the effectors involved, we profiled the transcriptome of PT and CD micro-dissected from 8-week-old control and *Pkd1*^{Δtub} mice (**Figure 5, A–C, Supplemental Figure 5A**). PT and CD specific gene expression profiles confirmed dissection quality (**Supplemental Figure 5B**). *Pdgfrb* and *Itgam* expressions were undetectable in CD, indicating the absence of contamination of the extracts by fibroblasts or macrophages, respectively. Overall, *Pkd1* inactivation induced a significant dysregulation in the expression of 2151 genes in CD but only 1198 in PT (**Supplemental Figure 5C**). Enrichment analyses showed an enrichment in gene sets related to cell proliferation in PC1-deficient PT, but not in CD (**Figure 5B**), a finding consistent with our immunolabelling

experiments. More importantly, we identified a panel of 26 genes, which are upregulated in PC1-deficient CD but not in PT and are involved in the three aspects of BM remodeling that we prespecified, despite similar reduction in *Pkd1* mRNA with secondary increases in *Pkd2* and *Glis2* (14) expression (**Figure 5C**). These include 4 genes encoding ECM proteases, 3 genes allowing the production of BM softeners (*i.e.*, enzymes involved in HS/CS biogenesis; *Hs3st3a1*, *Chsy1*), 6 additional ECM components and 13 genes involved in cell-BM interactions or cytoskeleton dynamics. Further re-analysis of snRNAseq datasets comparing human ADPKD and healthy kidneys (31) showed that the BM remodeling signature that we identified in distending CD from *Pkd1*^{Δtub} mice was significantly enriched in ADPKD human tubular cells (**Figure 5, D-F**). Of note, in these datasets of terminal human ADPKD kidneys, TBM remodeling gene induction was not restricted to distal segments, suggesting a generalization of the process at late stages.

Primary cilia participate in the induction of BM remodeling genes

To gain insight into the mechanisms by which cilia elicit tubule distension, we first took advantage of the transcriptome dataset derived from ciliated and deciliated *Pkd1*^{-/-} precystic tubules (14). Although BM remodeling genes were mostly induced in the distal nephron, which modestly contributes to the RNA pulled down in this dataset, we were able to replicate our findings for a set of genes, a fraction of which (*e.g.*, *Adamts1*, *Adamts14*, *Chsy1*, *Map6*, *Fgd3*) showed cilia-dependent regulation (**Supplemental Figure 6**).

To complement this approach, we performed RNA-seq on PT and CD micro-dissected from *Pkd1*^{Δtub}; *Ift20*^{Δtub} mice and control littermates (**Figure 6A**). Integration of these data with our previous RNAseq results revealed that cilia ablation counteracted the majority of the transcriptional dysregulation triggered by *Pkd1* ablation in CD and PT (**Figure 6B and Supplemental Figure 7**). Specifically, approximately half of the BM remodeling genes

upregulated upon *Pkd1* ablation were reduced in deciliated CD (**Figure 6C**), suggesting that cilia promote tubule distension through transcriptional regulation.

The transcriptional regulator GLIS2 participates in TBM remodeling and *Pkd1*-deficient tubule distension, downstream of cilia

The transcriptional regulator GLIS2 was recently identified as an important mediator of CDCA (14) and was correspondingly downregulated in deciliated *Pkd1*-deficient PT and CD (**Figure 6D**). To assess the role of GLIS2 in tubule distension, we studied *Pkd1*^{Δtub} and *Pkd1*^{Δtub}; *Glis2*^{Δtub} mice. As previously described, *Glis2* inactivation suppressed the cystic enlargement of *Pkd1*-deficient kidneys (14). Although *Glis2* ablation drastically prevented tubule dilation, blinded examination of kidneys consistently revealed slight focal tubular dilation in the cortex of *Pkd1*^{Δtub}; *Glis2*^{Δtub} kidneys that were not observed in cilia-deficient *Pkd1*^{Δtub} animals (**Figure 6E**). These tubules were generally heterogeneously labelled by a calbindin-specific antibody, suggesting a DCT or early CD origin (**Figure 6F**). Overall, *Glis2* ablation completely restored CD internuclear distance (**Figure 6G**) but only partially mitigated TBM thinning (**Figure 6, H-I**). Of note, in these mice housed in a different animal facility, there was only a trend toward proximal TBM thinning at this time point. Taken together, these results suggest that cilia regulate TBM remodeling through both GLIS2-dependent and GLIS2-independent mechanisms.

Loss of *Pkd1* is associated with altered tubule mechanics

We asked whether remodeling of the TBM alters the physical properties of tubules. We isolated CD and PT from 8-week-old *Pkd1*^{Δtub} mice (with intact TBM) and measured their diameter variations in response to increasing luminal pressure using a dedicated isolated tubule perfusion

setup (**Figure 7A**) with physiologic pressure ranges (32). We observed that PC1-deficient CD, which have undergone distension but not proliferation (*i.e.*, dissected from 8-week-old mice; **Figure 1, A-E**), showed a steep increase in tubule diameter at low pressure (*i.e.*, from 0 to 10 cmH₂O; **Figure 7, B-D**). In contrast, PC1-deficient PT, which have not yet undergone distension at this time point but are nonetheless larger than in control mice because of cell proliferation (**Figure 1, A-E**), showed similar pressure-diameter curves as control PT (**Figure 7, E-F**). Repeating these experiments with PT isolated from 12-week-old animals, a time point where distension has occurred in this segment (**Figure 1H and K**), we found a similar steep increase in tubule diameter as in distended PC1-deficient CD, although for a higher pressure threshold (*i.e.*, from 21 to 31 cmH₂O; **Figure 7, G-I**). Cilia ablation rescued the excessive dilation of PC1-deficient CD (**Figure 7, B-D**) and PT (**Figure 7, G-I**) by transmural pressure. Notably, decreasing pressure in distended PC1-deficient tubules resulted in a decrease of their diameter indicating elastic deformation (**Supplemental Figure 8**). Together, these results demonstrate that tubule distension and TBM remodeling in ADPKD are associated with an alteration of tubule mechanics, characterized by a steep increase in tubule diameter with segment-specific pressure thresholds, which also appears as a cilia-dependent mechanism.

Changes in TBM mechanical properties result in permissive conditions for cystogenesis

Our results regarding the early modifications of TBM in ADPKD suggest that increased ECM deformability favors cystogenesis. To investigate this question *in vitro*, we used an established tubule-on-chip model, whereby immortalized tubular cells form ciliated tubules of physiological dimensions in a deformable ECM scaffold (33). Using edited ciliated clonal lines derived from mouse collecting duct (mIMCD3) (34), we observed only minor differences between control and *Pkd1*^{-/-} clones in the ability to dilate ECM scaffolds with a collagen content of 9.5 g/l (**Figure 8A**). However, by lowering collagen content, thereby reducing stiffness, we

observed that *Pkd1*^{-/-} clones dilated more rapidly the ECM scaffold than the parental line (**Figure 8, B-C**). This increased dilation was not caused by increased cell proliferation but by an increase in internuclear distance (**Figure 8, D-F**). These in vitro results demonstrate that ECM mechanics affect the ability of *Pkd1*^{-/-} cells to deform tubules, so that ECM deformability facilitates dilation. Ciliation of *Pkd1*-deficient cells was unaffected by collagen content (**Figure 8, G-H**), indicating that ECM mechanics do not regulate *Pkd1*^{-/-} tubule dilation by affecting ciliogenesis.

To test this concept in vivo, we inactivated *Pxdn*, which encodes peroxidasin, a collagen IV specific cross-linking enzyme (in which germinal ablation induces a 20% decrease in TBM elastic modulus) (19), in *Pkd1*^{Δtub} mice. *Pxdn*^{-/-} single mutant mice have slightly smaller kidneys than control at baseline and do not spontaneously develop cysts, even in aging (**Figure 9, A-B**). The modest alteration of TBM mechanics caused by *Pxdn* inactivation translated into increased cyst burden in *Pkd1*^{Δtub} mice (**Figure 9, C-E**). At the studied time point, cystogenesis was more pronounced in males compared to females (P-value for gender by two-way ANOVA < 0.001). However, the impact of *Pxdn* inactivation on cyst area was not affected by gender (P-value for interaction between gender and genotype by two-way ANOVA = 0.8). These results are consistent with a model where reduced TBM stiffness facilitates tubule deformation and cystogenesis. While cyst formation was increased in *Pkd1*^{Δtub}; *Pxdn*^{-/-} mice compared to *Pkd1*^{Δtub} mice, this did not translate into an increase in kidney weight over body weight ratio (KW/BW; **Figure 9D**). This discrepancy prompted us to assess tubular cell proliferation, which was overall reduced in the context of *Pxdn* inactivation (**Figure 9, F-G**), in line with the observation that stiffer substrates usually promote cell proliferation (34). The fact that reducing BM stiffness (via *Pxdn* inactivation) promoted tubule distension while reducing cell proliferation,

further supports the view that, besides cell proliferation, BM mechanics and tubule distension are involved in cystogenesis.

Tubule obstruction exacerbates PC1-deficient tubule distension and precipitates cystogenesis

Tubular obstruction from enlarging cysts has been proposed to contribute to further cyst formation and micro-puncture as well as microdissection studies have shown that cysts frequently arise from obstructed tubules (35). We tested in vivo how alterations in TBM biomechanics affect tubule response to obstruction with respect to PC1 and cilia. After a single day of unilateral ureteral obstruction (UUO), *Pkd1*^{Δ_{tub}} mice showed a disproportional increase in kidney weight compared to controls. This was not the case in kidneys with concomitant cilia ablation (**Figure 10, A-B; Supplemental Figure 9A**). The kidney weight increase correlated with enlargement of the CD and DCT, but not PT and was associated with a marked increase in internuclear distance, indicating tubule distension (**Figure 10, C-H; Supplemental Figure 9, B-D**). BrdU labelling in *Pkd1*-deficient CD did not significantly change following obstruction (**Supplemental Figure 9, E-F**), suggesting that early cyst expansion post-UUO occurs largely independently of cell proliferation, though the limited sample size of this analysis warrants caution in interpretation.

Two-way ANOVA confirmed that a significant interaction between genotype and obstruction governs distal nephron distension (**Figure 10, D-E; Supplemental Figure 9, B-D**). Of note, this first set of experiments was conducted on 8-week-old females, which at this age already display a thinner TBM in CD but, contrary to males, have negligible tubule dilation at baseline. Similar results were obtained with male mice (**Supplemental Figure 10**).

Prolonging obstruction resulted in cystic transformation of PC1-deficient kidneys, with a two- and three-fold increase in kidney weight to body weight ratio 4 and 14 days after the surgery,

respectively (**Figure 11, A-D; Supplemental Figure 11, A-B**). Cilia ablation reduced obstructed PC1-deficient kidney enlargement to the mild level observed in cilia-deficient mice with intact *Pkd1* (i.e., *Kif3a*^{Δtub}; **Figure 11, B-D**). Cystic transformation of *Pkd1*-deficient kidneys was mainly the consequence of an increase in DCT and CD cross-sectional area, while tubule internuclear distance remained higher than in control or PC1-deficient DCT and CD lacking cilia (**Figure 12, A-C; Supplemental Figure 11, C-H**). We then asked if the cystic transformation of PC1-deficient tubule could be caused by an excessive proliferative response to obstruction. As expected, labelling cycling cells with PCNA or Ki67 revealed that, with time, obstruction increased the fraction of cycling cells in CD and DCT. However, the magnitude of this increase was similar across genotypes (**Figure 12, D-F, Supplemental Figure 12, A-D**). Consistent with our baseline findings, non-obstructed PT lacking PC1 displayed a higher fraction of cycling cells than control or deciliated PT. Obstruction further increased the cycling cell fraction in all genotypes (**Supplemental Figure 12, C-E**). The proliferation rate did not correlate with PT deformation: obstruction reduced PT cross-sectional area in control animals, indicating atrophy. This compaction of PT was similarly prevented by *Pkd1* or cilia ablation, though cilia alone mutant tended to have overall smaller proximal tubule diameter at baseline (**Figure 12, G-H**).

In summary, the cystogenic response to obstruction corresponded with the observed effect of PC1 and cilia on TBM structure and mechanics: PC1-deficient CD and DCT have a thinner TBM and higher distensibility than PT and correspondingly dilate more strongly, precipitating cystogenesis.

Transient obstruction is sufficient to precipitate irreversible cystogenesis

Previous reports showed that only a minority of PKD cysts are obstructed at any given time (22, 36), suggesting that permanent tubule obstruction is not a frequent driver of cystogenesis.

To study the effect of a transient obstruction on cyst formation, we used a reversible UUO model (R-UUO): *Pkd1*^{Δ^{tub}} mice underwent ligation of the distal ureter, followed 4 days later by either surgical release of the obstruction or a sham operation, and the kidneys were analyzed at 14 days (*i.e.*, 10 days after UUO reversion; **Figure 13A**). Notably, release of the obstruction after 4 days failed to reduce cyst growth at 14 days (**Figure 13, B-D**), indicating that transient obstruction is sufficient to commit tubules to an irreversible cystogenic program.

Altogether, these results establish a TBM-centered model of cystogenesis in which *Pkd1* loss drives cilia-dependent remodeling of TBM shape and mechanics, which, in conjunction with increased luminal pressure, produces tubule dilation in a manner resembling the formation of a tire bulge (**Figure 13E**). In this condition of increased tension, obstruction precipitates cystogenesis independent of cell proliferation.

DISCUSSION

ADPKD is the most common ciliopathy. Although its genetics are well understood, the mechanism of its defining feature—cyst formation—has remained unclear. We show that cilia and PC1 regulate TBM composition and thereby its biomechanical properties, and that disturbance of TBM shape and biomechanics drives tubular dilation and cyst formation, defining a cystogenic mechanism that we believe to be previously unrecognized in ADPKD.

TBM remodeling in cystic epithelia has traditionally been viewed as a secondary, passive event. Earlier reports of TBM thickening in PKD came from chemically induced rat models or terminal human ADPKD kidneys with marked fibrosis (37–39), and ECM stiffening has been proposed to drive proliferation via mechanosensitive YAP/TAZ activation (40). In contrast, our data show that TBM alteration is a very early morphogenic consequence of *Pkd1* inactivation, present at the onset of distal tubule dilation: TBM thinning precedes the increase in internuclear distance that marks the reversible columnar-to-flattened epithelial transition caused by *Pkd1/Pkd2* loss (41). Thinning is accompanied by progressive HS accumulation, and both changes are predicted to lower TBM stiffness. Accordingly, pressure–diameter curves of isolated tubules revealed a strong correlation between TBM thinning, tubule distension *in vivo* and increased distensibility *ex vivo*, and increasing ECM deformability facilitated *Pkd1*^{-/-} tubule-on-chip dilation and cystogenesis in *Pkd1*^{Δtub} mice. These data indicate that TBM remodeling and the resulting change in tubule mechanics play an important role in cyst formation. Because our models use diffuse tubular *Pkd1* inactivation, the kinetics may differ from patients, in whom scattered *Pkd1*-deficient clones must expand before substantially deforming the TBM. Notably, our findings illuminate clinical observations: various collagen mutations have been linked to renal cysts, and combined *COL4A1* and *PKD2* mutations accelerate the ADPKD course (42–44).

TBM remodeling in PC1 deficiency requires cilia: two strategies preventing ciliogenesis—targeting the ciliary motor subunit *Kif3a* and the intraflagellar transport protein *Ift20*—consistently prevented TBM remodeling and tubule distension in PC1-deficient kidneys. Cilia are known to regulate ECM composition in mesenchymal cells such as chondrocytes, where they are embedded in and monitor the matrix. In the tubule, cilia instead protrude into the lumen and are thought to sense urine flow via PC1/PC2. Consistent with cilia matching TBM mechanics to hydrodynamic load, TBM thickness decreases along the tubule in parallel with hydrostatic pressure (45), whereas glomerular hyperfiltration is associated with TBM thickening (46). In some contexts, such as kidney development (24) or—in our study—prolonged obstruction, cilia ablation itself causes tubule dilation, suggesting cilia signaling helps maintain tubule diameter when luminal pressure rises. However, early distension with TBM remodeling and HS accumulation was specific to *Pkd1* deficiency, indicating at least partially distinct mechanisms. BM remodeling and primary cilia both contribute to development and cancer (47), so the cilia–BM connection may have implications beyond the kidney.

Our transcriptomic data point to three non-mutually exclusive mechanisms that may cooperate to drive TBM remodeling upon *Pkd1* loss: upregulation of matrix proteases, a shift toward a more compliant matrix composition, and altered epithelial traction forces. Converging evidence supports each arm: inactivating the TBM protease ADAMTS1 in a *Pkd1* model attenuates cyst growth (48); reducing epithelial traction through ROCK inhibition modestly lowers PKD burden (40, 49); and altering glycosaminoglycan composition by deleting xylosyltransferase 2 (*Xylt2*) is sufficient to induce cystogenesis (50). Increasing collagen IV deformability alone (*Pxdn* inactivation) did not dilate wild-type tubules but amplified cyst formation under *Pkd1* deficiency, mirroring patients with collagen IV mutations that are permissive to, but not

sufficient for, cysts (42–44). Together, these mechanisms likely act cooperatively to lower the mechanical barrier to cyst formation.

We further show that cilia ablation abrogates induction of the TBM transcriptional program, and that GLIS2, a transcriptional effector of cystogenesis downstream of cilia, is partly responsible for TBM thinning. Other ciliary pathways appear unlikely or unresolved contributors: Hedgehog signaling has been experimentally excluded (51–53), WNT and PDGF- β promote fibrosis and TBM thickening rather than thinning (54, 55), and a role for ciliary calcium signaling remains contested (56–59). Thus transcriptional regulation, partly via GLIS2, appears to be the main link between cilia and TBM remodeling, although the upstream ciliary regulators remain to be defined.

Our study also refines the role of cell proliferation in ADPKD. Although abnormal proliferation is integral to ADPKD and drives cyst growth (15, 60), we find it becomes a significant driver later than previously appreciated. Early on, TBM remodeling is the primary morphogenetic event, driving distal nephron distension independently of proliferation; only later do distension and proliferation occur concurrently. UUO experiments dissociated these processes: obstruction increased tubular proliferation across all genotypes, yet initial dilation progressed only in PC1-deficient DCT and CD, where it coincided with TBM remodeling and excessive cell stretching. By enlarging the area available for daughter-cell intercalation, TBM remodeling may both reduce cell crowding—limiting extrusion and apoptosis—and increase stretching, which accelerates the G2/M transition (61).

Cysts frequently arise from obstructed tubules in ADPKD animal models, linking luminal obstruction to cystogenesis (36). Obstruction can stem from intraluminal crystals, particularly calcium oxalate, which accelerates cystogenesis in PKD models (62); granular casts of necrotic

proximal-tubule debris after ischemia–reperfusion injury (63); hyaline casts from dehydration; or compression of adjacent nephrons by expanding cysts—the proposed basis of the “snowball effect”, whereby cysts preferentially emerge near pre-existing ones (64). These obstructive events are usually thought to accelerate cystogenesis by activating injury and inflammatory pathways (64). Our results suggest that the mechanical component may be at least equally important: although tubular injury occurred in all segments, cyst formation was restricted to segments undergoing TBM remodeling and excessive distension. Consistent with this, an extension of our tubule-on-chip shows that both loss of flow and increased intraluminal hydrostatic pressure independently promote *Pkd1*^{-/-} tubule's dilation (65). Together, these findings provide a mechanistic framework for how obstruction drives cystogenesis in ADPKD.

Our animal models recapitulate a feature of human ADPKD—faster cyst growth in distal segments than in the proximal tubule—and provide a mechanical explanation: CD and DCT have thinner, HS-enriched TBM, precociously activate TBM-remodeling genes upon PC1 loss, and undergo early distension with altered biomechanics, culminating in excessive cell stretching and accelerated cystogenesis after obstruction. That reducing matrix stiffness increases cyst formation partially contradicts the prevailing view that elevated ECM stiffness drives tubular proliferation and disease progression (40, 66); that view may still apply to late cyst growth, where peri-cystic fibrosis predominates.

Several limitations apply. Our principal model relies on global tubular *Pkd1* inactivation and does not reproduce the mosaic pattern of human ADPKD, in which biallelic *Pkd1* loss occurs in only a fraction of cells. Because we examined mainly *Pkd1* inactivation, our conclusions may not extend to other polycystic kidney diseases. We did not characterize the effect of obstruction on TBM structure and composition. While a recent study reporting that ADAMTS1—one of the proteases identified in our screen—contributes to cyst growth (48)

provides indirect support for the proteolytic arm, direct causal evidence for the compositional and traction-force arms remains to be established. Finally, we did not analyze double cilia/*Pkd1* mutants beyond 18 weeks and therefore cannot fully distinguish delayed cysts onset from true suppression.

In summary, we show that PC1 and cilia regulate the composition and mechanical properties of the TBM. Our findings reveal what we believe to be a previously unappreciated role for primary cilia in TBM regulation and support a biomechanical model for cyst formation in ADPKD.

METHODS

Sex as a biological variable

Our study examined male and female animals, and sex-dimorphic effects are reported.

Mice

Mice were housed at constant ambient temperature in a free-pathogen facility on a 12-hour day/night cycle and were fed *ad libitum* in an enriched environment. Breeding and genotyping were performed according to standard procedures. All mice were on a C57BL/6 (mixed J/N) background. *Pkd1*^{flox/flox} mice (B6.129S4-*Pkd1*^{tm2Ggg/J}, Jackson Laboratory), *Kif3a*^{flox/flox} mice (*Kif3a*^{tm1Gsn}, C57BL/6 genetic background, kindly provided by Peter Igarashi, Stony Brook University), *Ift20*^{flox/flox} mice (*Ift20*^{tm1.1Gjp}, kindly provided by Gregory Pazour, UMass Chan Medical School) were crossed to Pax8rtTA (Tg[*Pax8*-rtTA2S*M2]1Koes, Jackson Laboratory) and TetO-Cre (Tg[tetO-cre]1Jaw, Jackson laboratory) mice to create mice with inducible tubule-specific *Pkd1* knockout (named as *Pkd1*^{Δtub}), double *Pkd1*; *Kif3a* knockout (named as *Pkd1*^{Δtub}; *Kif3a*^{Δtub}) and double *Pkd1*; *Ift20* knockout (named as *Pkd1*^{Δtub}; *Ift20*^{Δtub}). *Kif3a*^{Δtub} mice were additionally analyzed and compared to *Pkd1*^{Δtub} mice for tubular morphometry and TBM remodeling. *Pxdn*-deficient mice (referred to as *Pxdn*^{-/-}) were kindly provided by Miklos Geiszt (Semmelweis University) and were crossed with *Pkd1*^{Δtub} mice to generate *Pkd1*^{Δtub} mice with or without constitutive *Pxdn* inactivation (named as *Pkd1*^{Δtub}; *Pxdn*^{-/-}). *Pkd1*^{Δtub}; *Glis2*^{Δtub} were kindly provided by Stefan Somlo (Yale School of Medicine). Most animals received doxycycline hyclate (Abcam, ab141091, 2 mg/mL) in drinking water supplemented with 5% sucrose and protected from light, from postnatal day 28 to 42 to induce the inactivation of floxed alleles. In some experiments, doxycycline was administered from postnatal day 0 to 14 to induce neonatal inactivation of *Pkd1* or *Kif3a*. Animals lacking either TetO-Cre or Pax8rtTA

were used as controls. Animals of both sexes were used in experiments. All studied animals were included in the analyses. To assess Cre recombination efficiency, *Pkd1*^{Δtub} mice were crossed with mice carrying the *iSuRe-Cre* reporter allele, which induces the expression of a membrane-localized tandem tomato (MbTomato) fluorescent protein upon cre mediated recombination (67). These mice received doxycycline from postnatal day 28 to 42 and tomato expression in tubules was assessed at postnatal week 8.

Statistics

Data were expressed as means. Differences between the experimental groups were evaluated using one or two-way ANOVA followed, when significant ($P < 0.05$), by the Tukey-Kramer test. When only two groups were compared, unpaired t-test or Mann-Whitney test was used as appropriate. In some experiments (as indicated in the figure legends), *Pkd1*^{Δtub}; *Kif3a*^{Δtub} and *Pkd1*^{Δtub}; *Ift20*^{Δtub} were pooled for analysis, as both genotypes disrupt ciliogenesis and produced indistinguishable phenotypes in our hands. Control and tubule specific mutant were housed in the same cages. No randomization was performed. Determination of sample size was based on laboratory experience. We did not perform a priori sample size calculation. Quantification of TBM thickness and morphometric analysis were performed in a blind fashion. Linear regression was also used to correlate tubule-cross sectional area and internuclear distance in the CD and the DCT at 8 weeks. The statistical analysis was performed using GraphPad Prism V10 Software. Error bars in each quantification figure indicate the mean value.

Study approval

All mice experiments were conducted in accordance to the guidelines of the French government animal welfare policy. Animal procedures were approved by the ethical committee of the

Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (APAFIS agreements #201907041733347, #2020090715389782, #2020111915598007).

Data availability

The transcriptomic data supporting the findings of this study will be openly available in the public domain through the BioStudies database (accession number S-BSST2959).

Additional snRNAseq datasets were derived from the following resources available in the public domain: <https://www.ncbi.nlm.nih.gov/geo/> (GSE185948).

All the mice models used in this study can be obtained upon request to the corresponding author, with the agreement of the scientist who generated the initial transgenic line.

Values for all data points in graphs are reported in the Supporting Data Values file.

A full description of the experimental procedures is provided in the Supplemental material.

AUTHOR CONTRIBUTIONS

M.M, S.C, F.T, A.V, and F.B designed the study; M.M, B.La, R.A, J.V, A.V, M.B, P.H, L.C, G.C, V.S, A.A, M-C.V, B.Le, H.T, K.O, S.C, Ch.S, R.A, VS and F.B performed experiments; T.B and M.K provided mIMCD-3 cells; N.G generated image analysis pipeline; M.R and S.S provided material from *Glis2*-deficient mice and intellectual insights; R.B provided the *iSuRe-Cre* mice and revised related work; M.M, F.B, A.V, R.A, C.C, B.La, S.D. and S.C analyzed data; N.C performed bioinformatic analysis of RNAseq data; S.G performed U-Net segmentation, M.M and F.B drafted and revised the manuscript with the help of A.V, S.C, E.W.K, M.K, S.S, and F.T; all authors approved the final version of the manuscript.

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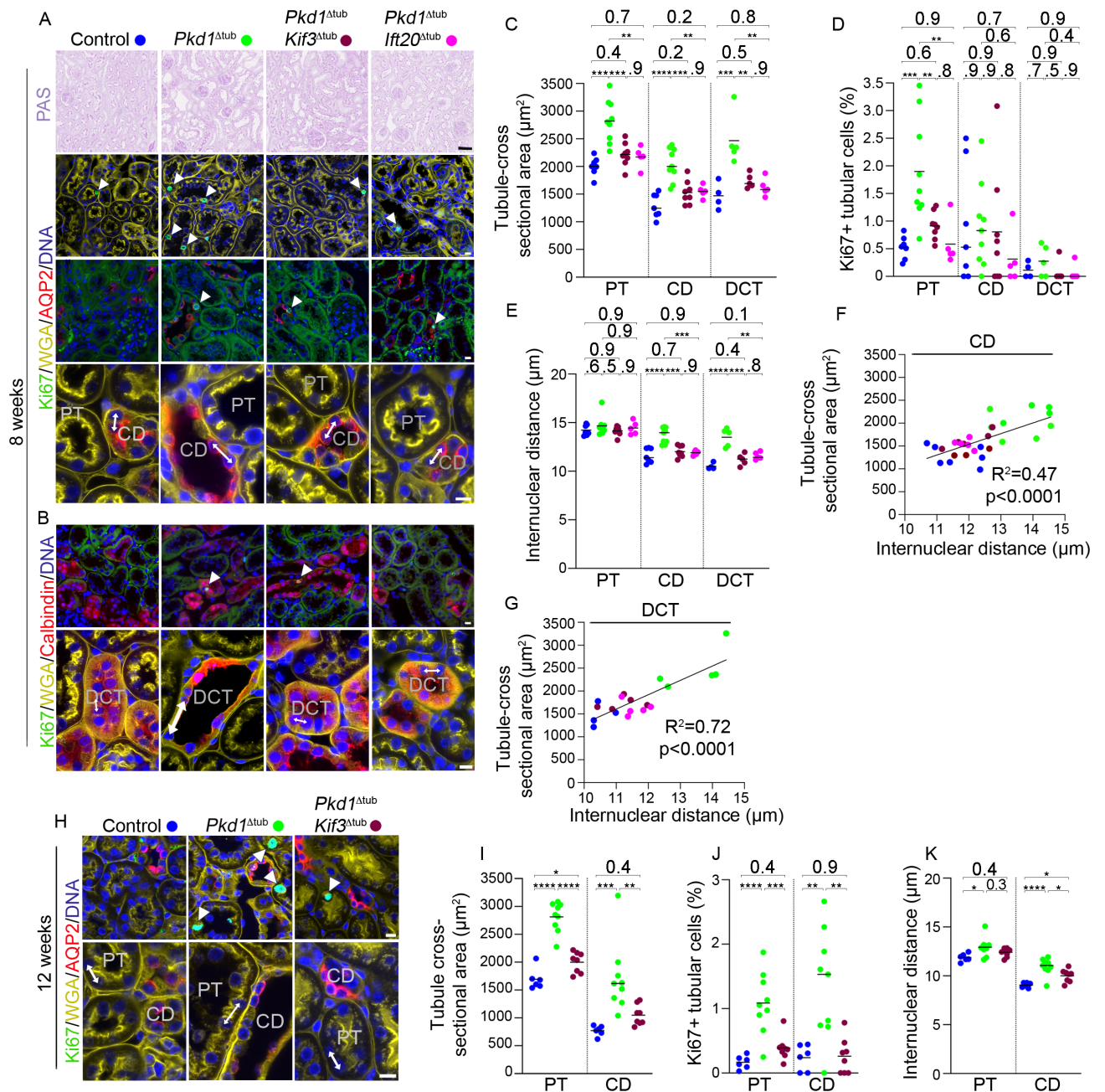


Figure 1. *Pkd1* deletion drives cilia-dependent distal tubule distension independently of cell proliferation. (A and B) Periodic acid Schiff's (PAS) staining and labelling of Ki67 (which stains proliferating cells), DNA, wheat germ agglutinin (WGA, which stains the brush border of proximal tubule [PT] and all basement membranes) and aquaporin 2 (AQP2) a collecting duct (CD) marker (A) or calbindin (a marker of distal convoluted tubules [DCT]) (B) of kidneys from 8-week-old control, *Pkd1*^{Δtub}, *Pkd1*^{Δtub} *Kif3a*^{Δtub} and *Pkd1*^{Δtub} *Ift20a*^{Δtub} male mice. Arrows: examples of internuclear distance measurements. Scale bars: 10 μm. (C-E) Quantification of mean PT, CD and DCT cross-sectional area (C), proliferation index (percentage of Ki67+ cells; D) and internuclear distance (E) in PT, CD and DCT of kidneys from 8-week-old control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub} *Kif3a*^{Δtub} and *Pkd1*^{Δtub} *Ift20a*^{Δtub} mice. (F and G) Linear regression of tubule cross-sectional area and internuclear distance for CD (F) and DCT (G) in the same mice groups at 8 weeks. (H) Labelling of Ki67, DNA, WGA and AQP2 of kidneys from 12-week-old control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub} *Kif3a*^{Δtub} mice. Arrows: examples of internuclear distance measurements. Scale bars: 10 μm. (I and K) Quantification of mean tubule cross-sectional area (I), proliferation index (Ki67+ cells) (J) and internuclear distance (K) in PT and CD of kidneys from 12-week-old control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub} *Kif3a*^{Δtub} mice. Each dot represents one individual male mouse. One-way ANOVA followed by Tukey-Kramer test: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, or the indicated P-value. Control mice consist in littermate *Pkd1*^{flox/flox} and *Pkd1*^{flox/flox} *Ift20a*^{flox/flox} animals lacking cre or rtTA transgene. AU: arbitrary unit. Confirmatory analysis on distal collecting tubules were only performed in the first cohort of mice analyzed.

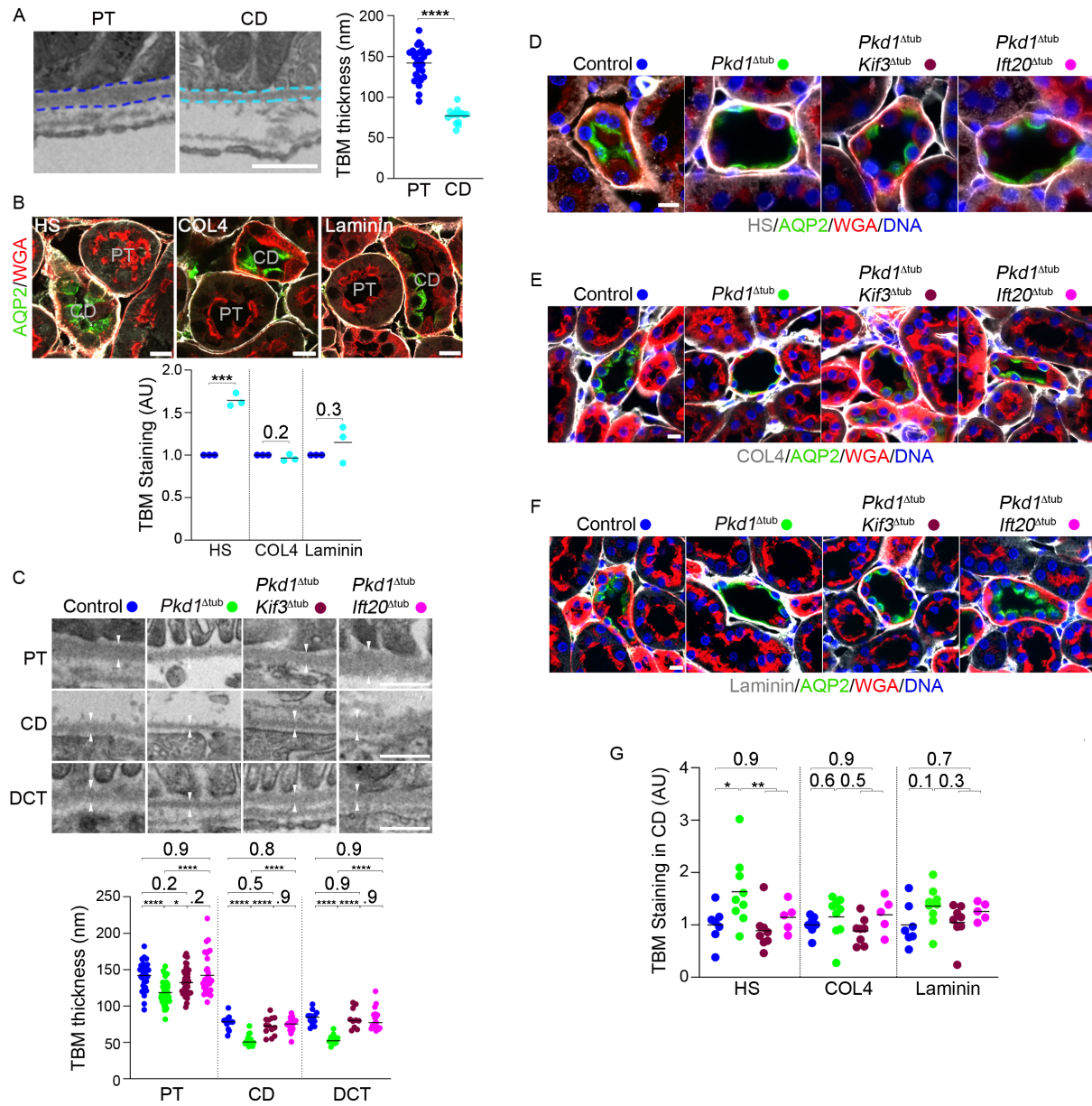


Figure 2. *Pkd1* deletion induces cilia-dependent basement membrane remodeling. (A) Transmission electron microscopy and quantification of proximal tubule (PT) and collecting duct (CD) tubular basement membrane (TBM) thickness in 8-week-old control mice. Dashed lines underline TBM. Each dot represents one individual tubule (mean of 3 to 21 measurements per tubule; $n=3$ mice). Scale bar: 0.5 μ m. Student t-test: **** $P<0.0001$. (B) Labelling of PT brush border (WGA), collecting duct (AQP2) and heparan sulfate (HS; left panel), collagen IV (COL4; middle panel) or laminin (right panel), and quantification of HS, COL4 and laminin signal intensity in 8-week-old control mice. Each dot represents one individual male mouse. Scale bar: 10 μ m. Student t-test: **** $P<0.0001$. (C) Transmission electron microscopy and quantification of TBM thickness of PT, CD and distal convoluted tubules (DCT) from 8-week-old control, $Pkd1^{\Delta tub}$, $Pkd1^{\Delta tub}; Kif3^{\Delta tub}$ and $Pkd1^{\Delta tub}; Ifi20^{\Delta tub}$ mice (i.e., 2 weeks after the end of doxycycline treatment). Each dot represents one individual tubule (mean of 8 to 20 measurements per tubule; $n=4$ to 5 mice per genotype). Scale bar: 0.5 μ m. (D-G) Staining and quantification of heparan sulfate (D,G), COL4 (E,G) and laminin (F,G) in CD labelled with AQP2 from 8-week-old control, $Pkd1^{\Delta tub}$, $Pkd1^{\Delta tub}; Kif3^{\Delta tub}$ and $Pkd1^{\Delta tub}; Ifi20^{\Delta tub}$ mice. Each dot represents one individual male mouse. Scale bars: 10 μ m. One-way ANOVA followed by Tukey-Kramer test: * $P<0.05$, ** $P<0.01$ or the indicated P-value. Data from $Pkd1^{\Delta tub}; Kif3^{\Delta tub}$ and $Pkd1^{\Delta tub}; Ifi20^{\Delta tub}$ mice were pooled for this statistical analysis. Control mice consist in $Pkd1^{lox/lox}$ and $Pkd1^{lox/lox}; Kif3^{lox/lox}$ littermates lacking cre or rtTA transgene. AU: arbitrary unit.

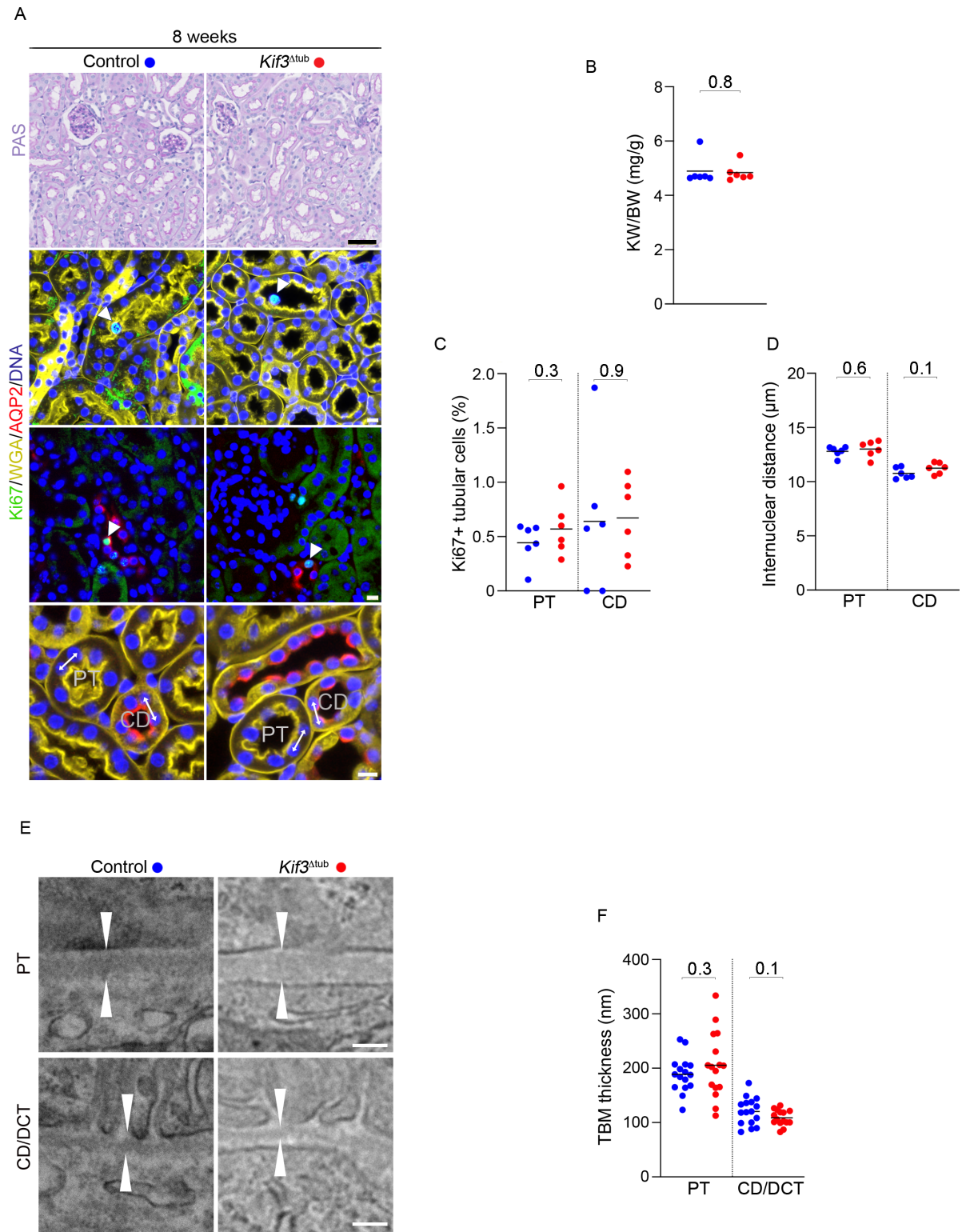


Figure 3. *Kif3a* inactivation alone does not lead to tubule distension. (A) Periodic acid Schiff's (PAS) staining and labelling of Ki67 (which stains proliferating cell; arrowheads), DNA, wheat germ agglutinin (WGA, which stains the brush border of proximal tubule [PT] and all basement membranes) and aquaporin 2 (AQP2, a collecting duct [CD] marker) of kidneys from 8-week-old control or *Kif3a^{Δtub}* mice. Arrows: examples of internuclear distance measurements. Scale bars: 10 μm. (B-D) Quantification of kidney weight (KW) over body weight (BW) ratio (B), proliferation index (percentage of Ki67+ cells; C) and internuclear distance (D) in PT and CD of kidneys from 8-week-old control, or *Kif3a^{Δtub}* mice. (E and F) Transmission electron microscopy (E) and quantification (F) of TBM thickness of PT and CD from 8-week-old control and *Kif3a^{Δtub}* littermate mice (*i.e.*, 2 weeks after the end of doxycycline treatment). Each dot represents one individual tubule (mean of 8 to 20 measurements per tubule; n=3 mice per genotype). Scale bar: 200 nm. P-values are from one-way ANOVA followed by Tukey-Kramer test.

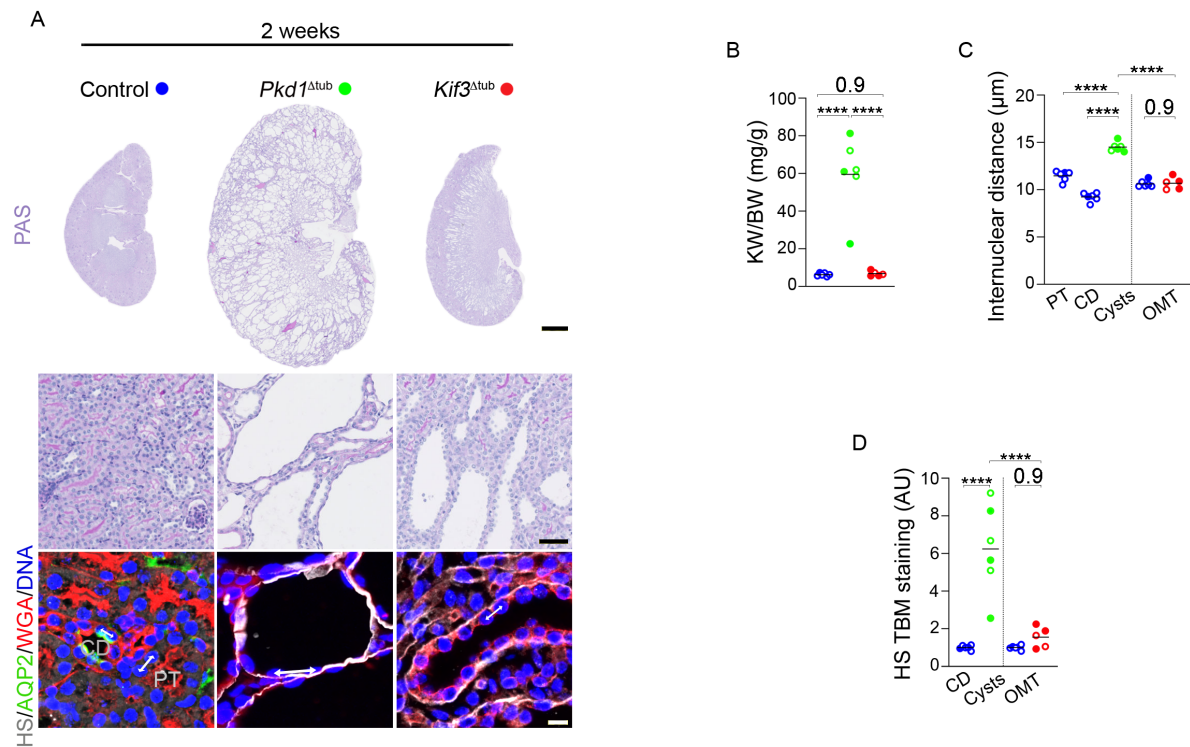


Figure 4. Comparison between neonatal cilia and *Pkd1* loss reveals distinct modes of tubule dilation. (A) PAS staining and labelling of heparan sulfate (HS), DNA, wheat germ agglutinin (WGA) and aquaporin 2 (AQP2) of kidneys from 2-week-old control, *Pkd1*^{Δtub} and *Kif3a*^{Δtub} mice. White arrows: examples of internuclear distance measurements. Scale bars: 1 mm (upper panel), 50 μm (middle panel) and 10 μm (lower panel). (B and D) Quantification of KW/BW (B), internuclear distance (C) and HS staining (D) in the tubular basement membrane (TBM) of proximal tubules (PT), collecting ducts (CD), *Pkd1*^{Δtub} cysts and outer medullary tubules (OMT) in kidneys from the same groups of mice, as indicated. Each dot represents one individual female (closed circle) or male (open circle) mouse. One-way ANOVA followed by Tukey-Kramer test: ***P<0.001, ****P<0.0001 or the indicated P-value. Control mice consist in *Pkd1*^{flox/flox} or *Kif3a*^{flox/flox} littermates lacking cre or rtTA transgene.

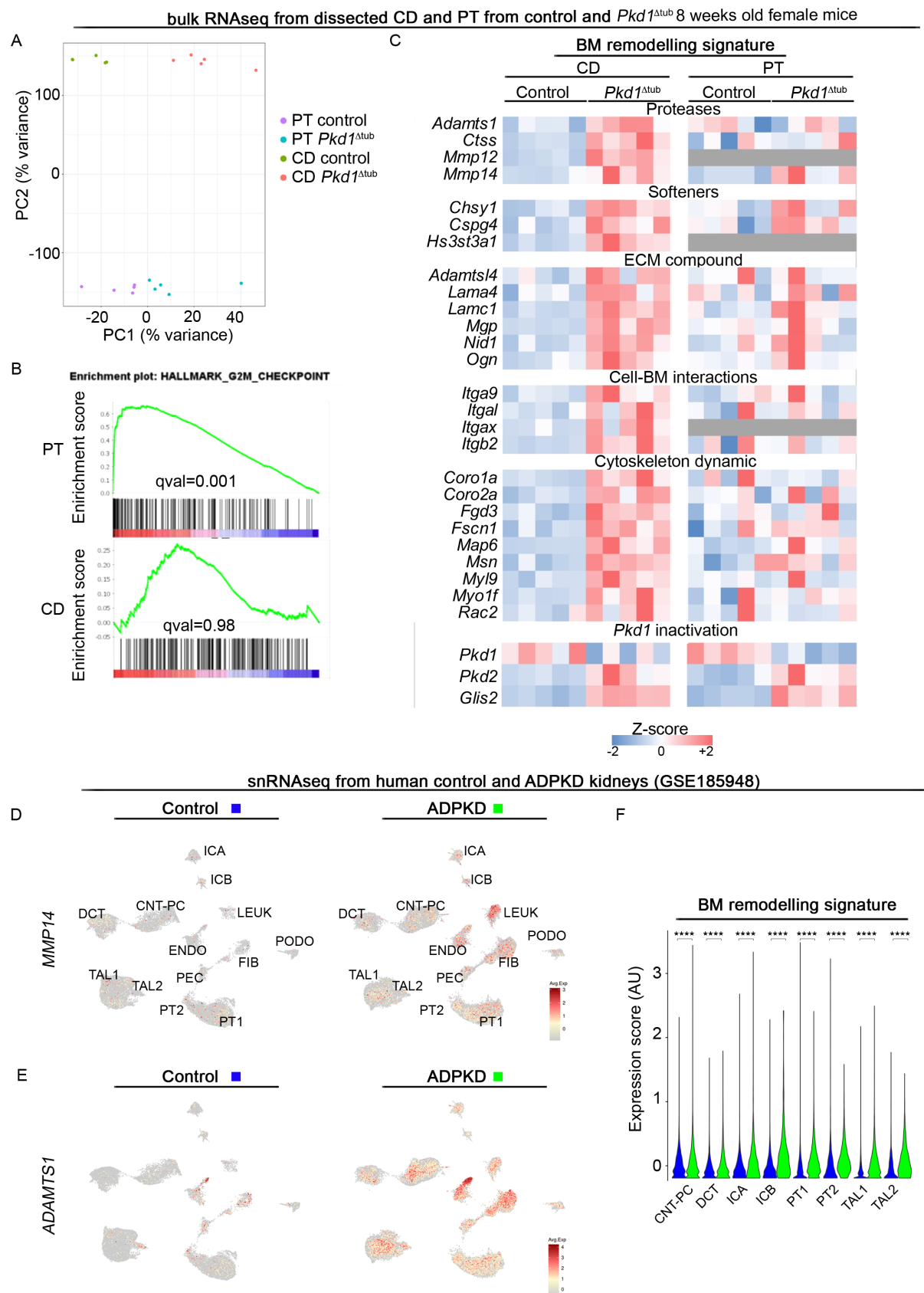


Figure 5. Transcriptomic profiling of micro-dissected proximal tubules (PT) and collecting ducts (CD) identifies a basement membrane (BM) remodeling signature associated with tubule distension. (A) Principal component analysis (PCA) of the transcriptome of proximal tubules (PT) and collecting ducts (CD) micro-dissected from control and *Pkd1*^{Δtub} 8-week-old female mice (*i.e.*, 2 weeks after the end of doxycycline treatment; n=5 mice per genotype, each dot represents a tubule segment from an individual mouse). (B) Gene set enrichment analysis (GSEA) comparing proliferation related gene sets between control and mutant mice in PT or CD. (C) Heatmaps showing the relative expression for TBM remodeling candidate genes in CD and PT micro-dissected from control and *Pkd1*^{Δtub} 8-week-old female mice (n=5 mice per genotype). Grey in the heatmap of PT indicates undetectable expression. (D and E) Expression of *MMP14* (D) and *ADAMTS1* (E) in snRNAseq data of control and ADPKD human kidneys. PT proximal tubule, PEC parietal epithelial cells, TAL thick ascending limb of Henle's loop, DCT distal convoluted tubule, CNT_PC connecting tubule and principal cells, ICA Type A intercalated cells, ICB Type B intercalated cells, PODO podocytes, ENDO endothelial cells, FIB fibroblasts, LEUK leukocytes, URO urothelium. (F) BM remodeling signature expression score in the indicated tubular cell population from control or ADPKD human kidneys. Bonferroni test : ****P<0.0001. Control mice consist in *Pkd1*^{flox/flox} littermates lacking cre or rtTA transgene. AU: arbitrary unit.

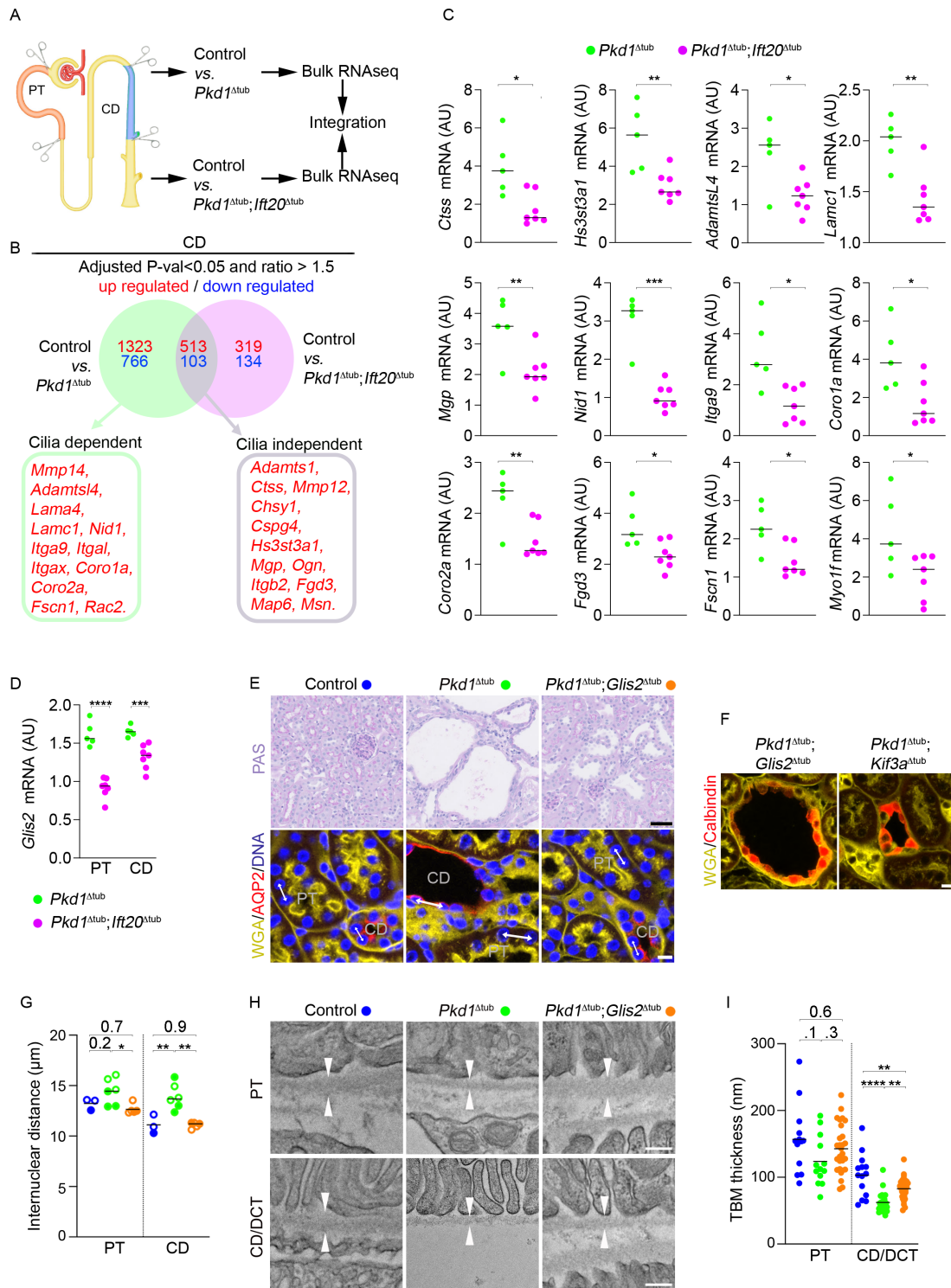


Figure 6. The primary cilium is instrumental in the induction of TBM remodeling genes downstream of PC1 loss and promotes tubule distension through GLIS2. (A) Schematic representation of the pipeline used to analyze cilia-dependent mediators of tubular basement membrane remodeling: bulk RNAseq was performed on collecting duct (CD) and proximal tubule (PT) micro-dissected from control and *Pkd1*^{Δtub}; *Ifi20*^{Δtub} mice. Data were integrated with those obtained in *Pkd1*^{Δtub} (Figure 5C). (B) Venn diagram showing the number of genes with significant variation between *Pkd1* and *Pkd1*^{Δtub}; *Ifi20*^{Δtub} mice and their corresponding littermate controls in CD (upper panel). The cilia-dependent and cilia-independent genes of the basement membrane remodeling signature are indicated in the lower panel. (C) Dot plot showing the relative expression (fold increase compared to the relative controls) their respective controls groups) of the BM remodeling in ciliated and deciliated *Pkd1*-deficient CD; only significant genes are shown. Each dot represents a tubule segment from an individual mouse. Student t-test: *P<0.05, **P<0.01, *** P<0.001. (D) Dot plot of *Glis2* mRNA expression in CD or PT from ciliated or deciliated *Pkd1*-deficient tubules. Each dot represents a tubule segment from an individual mouse. Two-way ANOVA followed by Sidak's test *** P<0.001; ****P<0.0001 (P-value for interaction between genotype and segments = 0.0041). (E) PAS staining and labelling of PT (WGA-positive) and CD (AQP2-positive) of kidneys from 8-week-old control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub}; *Glis2*^{Δtub} mice. Arrows: examples of internuclear distance measurements. Scale bars: 50 μm (upper) and 10 μm (lower). (F) Immunolabelling of calbindin (a marker of distal convoluted tubule (DCT) and early CD) of kidneys from 8-week-old *Pkd1*^{Δtub}; *Glis2*^{Δtub} and *Pkd1*^{Δtub}; *Kif3a*^{Δtub} mice. Scale bar: 10 μm. (G) Quantification of mean internuclear distance in PT and CD of kidneys from 8-week-old control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub}; *Glis2*^{Δtub} mice. Each dot represents one individual male (open circle) or female (closed circle) mouse. (H and I) Transmission electron microscopy (H) and quantification of TBM thickness (I) in PT, CD and DCT in kidneys from the same groups of mice. Each dot represents one individual tubule (mean of 8 to 20 measurements per tubule; n=3 mice per genotype). Scale bar: 200 nm. One-way ANOVA followed by Tukey-Kramer test: *P<0.05, **P<0.01, ****P<0.0001, or the indicated P-value. Control mice consist in littermate *Pkd1*^{fllox/fllox} and *Pkd1*^{fllox/fllox}; *Glis2*^{fllox/fllox} animals lacking cre or rtTA transgene.

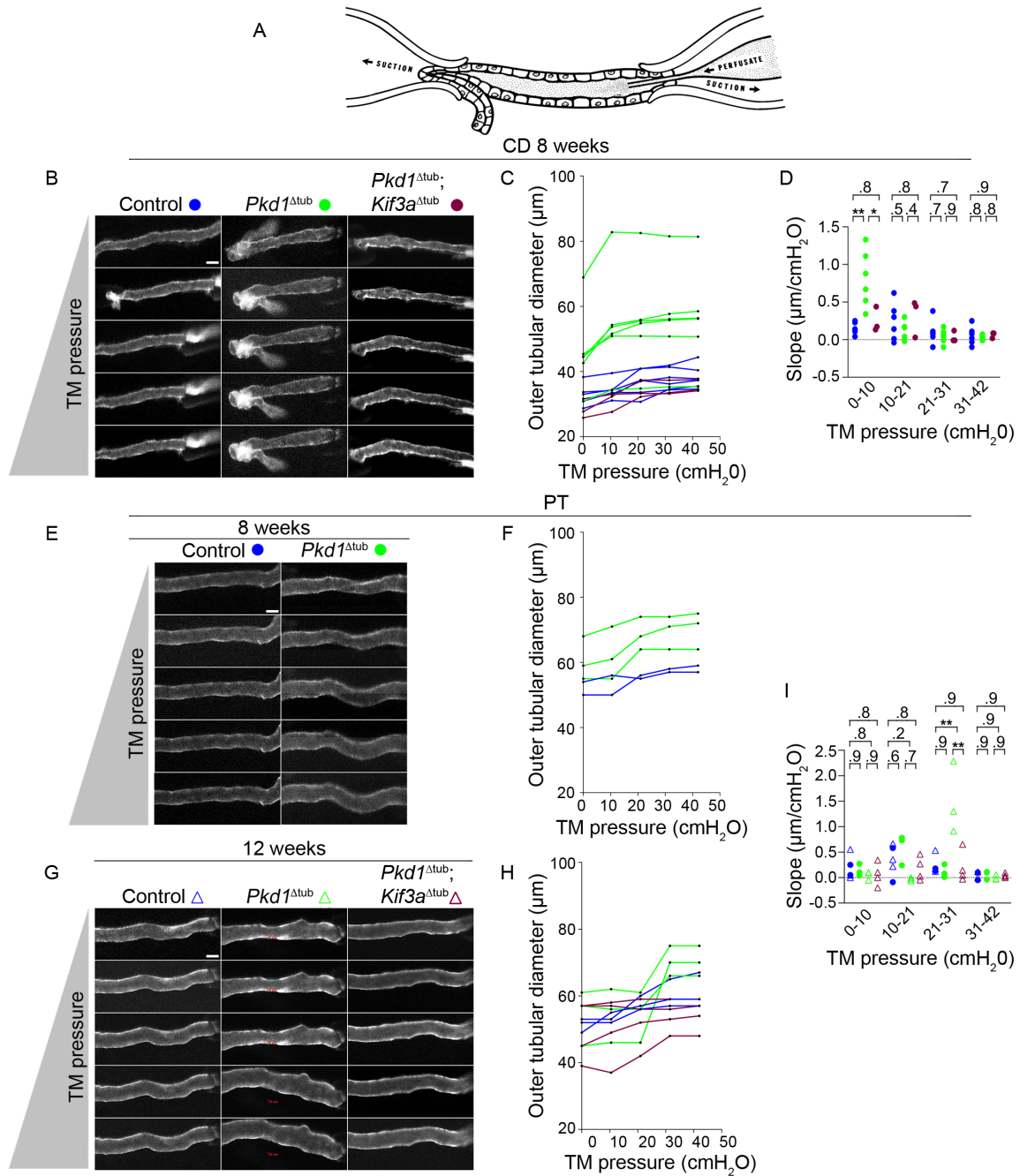


Figure 7. *Pkd1* deletion alters tubule biomechanics. (A) Scheme of isolated intact tubule perfusion setting [Welling 1972]. (B and C) Representative images (B) and quantification of the variation in outer tubule diameter induced by a progressive increase in transmurial pressure (TM; C) in collecting ducts isolated from 8-week-old control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub}; *Kif3a*^{Δtub} male mice. Each dot represents one individual tubule (mean of 10 measurements per tubule) for a pressure increment. (D) Quantification of the slope of the diameter-pressure curves for each pressure increment in collecting ducts isolated from the same groups of mice. Each dot represents one individual tubule. Scale bar: 50 μm. Student t-test: **P<0.01. (E and F) Representative images (E) and quantification of the variation in outer tubule diameter induced by incremental increase in TM pressure (F) in proximal tubules isolated from 8-week-old control and *Pkd1*^{Δtub} mice. Each dot represents one individual tubule (mean of 10 measurements per tubule) for a pressure increment. (G and H) Representative images (G) and quantification of the variation in outer tubule diameter induced by incremental increase in TM pressure (H) in proximal tubules isolated from 12-week-old control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub}; *Kif3a*^{Δtub} mice. Each triangle represents one individual tubule (mean of 10 measurements per tubule) for a pressure increment. (I) Quantification of the slope of the diameter-pressure curves for each pressure increment in PT isolated from 8 (dots) and 12-week-old (triangles) control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub}; *Kif3a*^{Δtub} mice. PT from control animals at 8 (blue dot) and 12 weeks (blue triangle) were pooled in analyses. Each symbol represents one individual tubule. Scale bars: 50 μm. One-way ANOVA followed by Tukey-Kramer test: **P<0.01. Control mice consist in *Pkd1*^{fllox/fllox} littermates lacking cre or rtTA transgene.

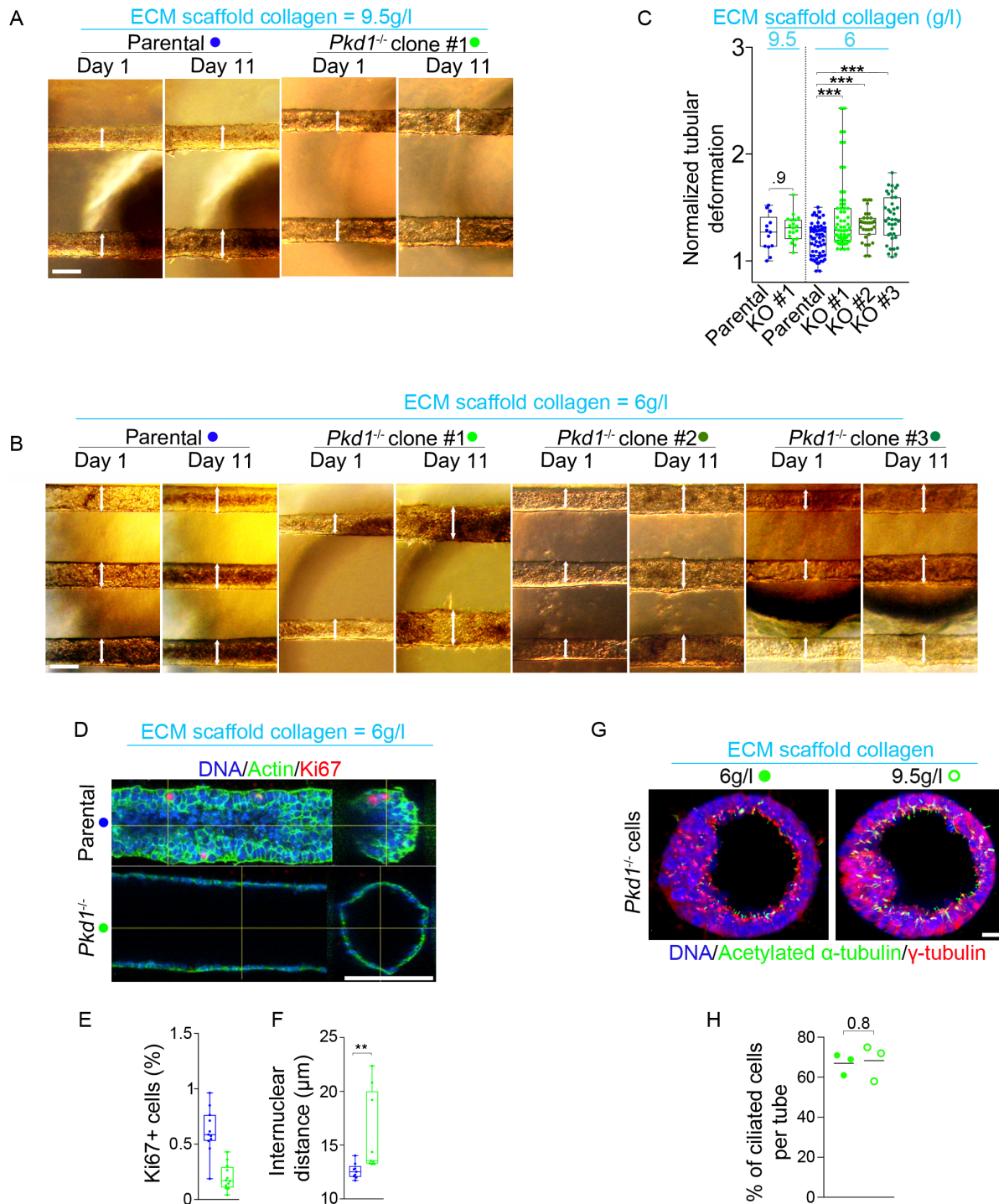


Figure 8. Extracellular matrix mechanics modulates *Pkd1*^{-/-} tubule-on-chip dilation in vitro. (A and B) Representative images of tubules-on-chip formed by parental or *Pkd1*^{-/-} mIMCD-3 clones in scaffolds containing 9.5 (A) or 6 g/l (B) collagen I, 1 and 11 days after confluency. (C) Quantification of the normalized tubule deformation in the corresponding tubules-on-chip at 11 days. Each dot represents one individual tubule from n=8 for parental cells, n=8, 5 and 4 chips for *Pkd1*^{-/-} clone 1, 2 and 3 respectively. Arrows indicate tubule diameter. Scale bar: 100 μm. (D-F) Labelling (D) and quantification of Ki67 (E) and internuclear distance (F) in parental and *Pkd1*^{-/-} tubules-on-chip. Each dot represents one individual tubule (parental cells : N=3 chips/n=9 to 11 tubules ; *Pkd1*^{-/-} cells : N=3 chips/n=9 to 12 tubules). Mann-Whitney test: **P<0.01, ***P<0.001. (G) Representative orthogonal sections of ciliated *Pkd1*^{-/-} tubules-on-chip embedded in 6 or 9.5 g/l collagen I and maintained under static conditions for 5 days. Cilia were identified by immunostaining of acetylated α-tubulin (green) and γ-tubulin (red). Scale bar: 100 μm. (H) Corresponding quantification of percentage of ciliated cells per tubule. Each dot represents one individual tubule (n=3 tubules per condition). Scale bar: 10 μm. The reported P-value was calculated using a Student t-test. The data shown in this figure are derived from at least two different independent cell seeding experiments.

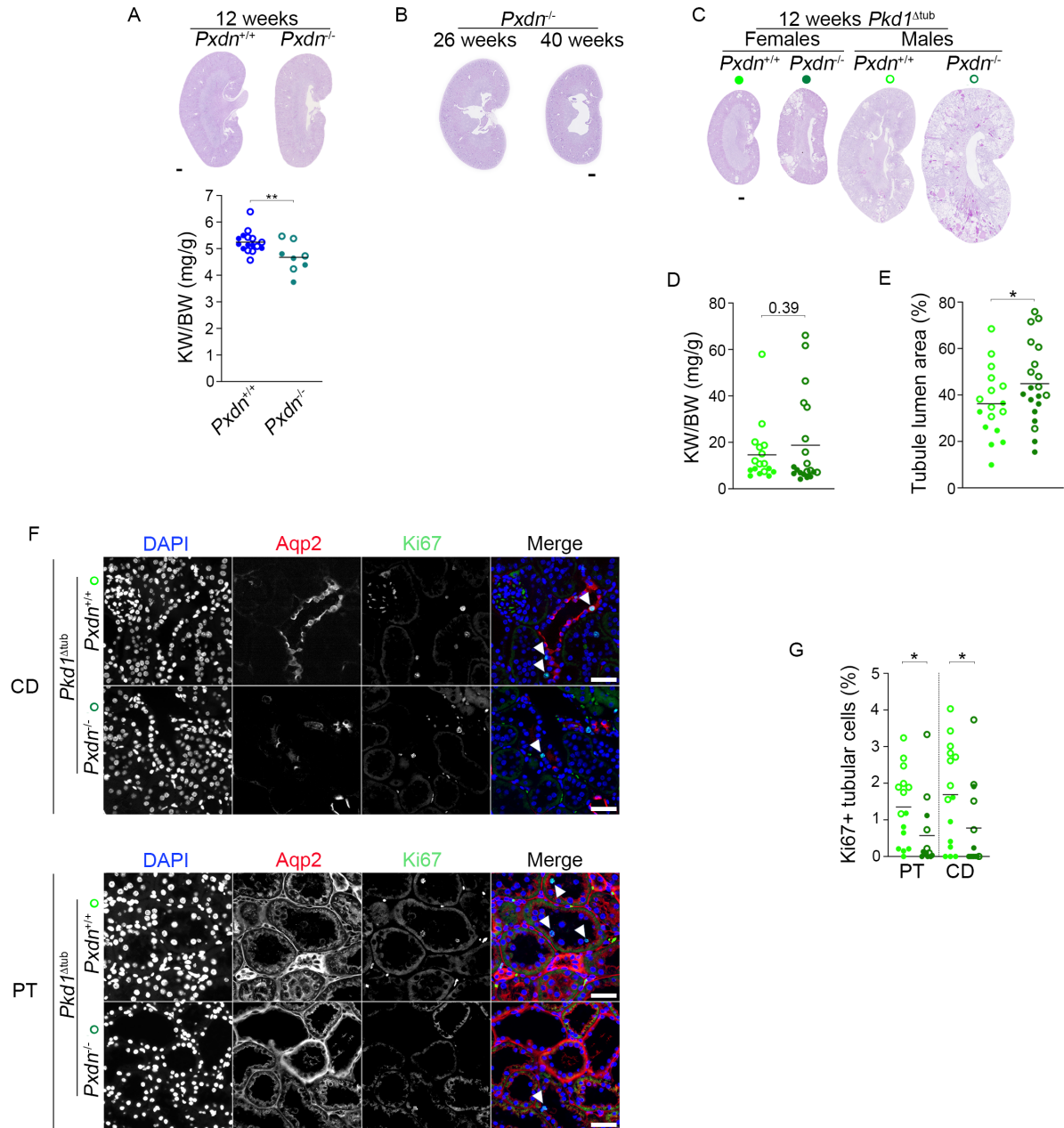


Figure 9. Decrease in tubular basement membrane stiffness promotes cyst formation in vivo. (A) Periodic acid Schiff's (PAS) staining and quantification of kidney weight to body weight ratio (KW/BW) of kidneys from 12-week-old control knock-out female (closed circle) and male (open circle) mice with or without concomitant peroxidasin (*Pxdn*) gene inactivation. Each dot represents one mouse. Scale bar: 0.5 mm. (B) PAS staining of kidney sections from aged *Pxdn*^{-/-} mice showing no cyst development. (C-E) PAS staining (B), quantification of KW/BW (C) and tubular dilations (D) of kidneys from 12-week-old *Pkd1*^{Δtub} female (closed circle) and male (open circle) mice with or without concomitant *Pxdn* gene inactivation. Each dot represents one individual mouse. Scale bar: 0.5 mm. P-value indicates the *Pxdn* genotype effect in Two-way ANOVA (gender and *Pxdn* genotype; P-value for interaction between gender and genotype = 0.8). (F and G) Labelling (F) and quantification of Ki67-positive cells (G) in CD and PT in kidneys from the same groups of mice. Each dot represents one individual mouse. Scale bar: 40 μm. P-values indicate the *Pxdn* genotype effect in Two-way ANOVA (gender and *Pxdn* genotype; P-value for interaction between gender and genotype = 0.24 and 0.15 in PT and CD, respectively ; *P<0.05).

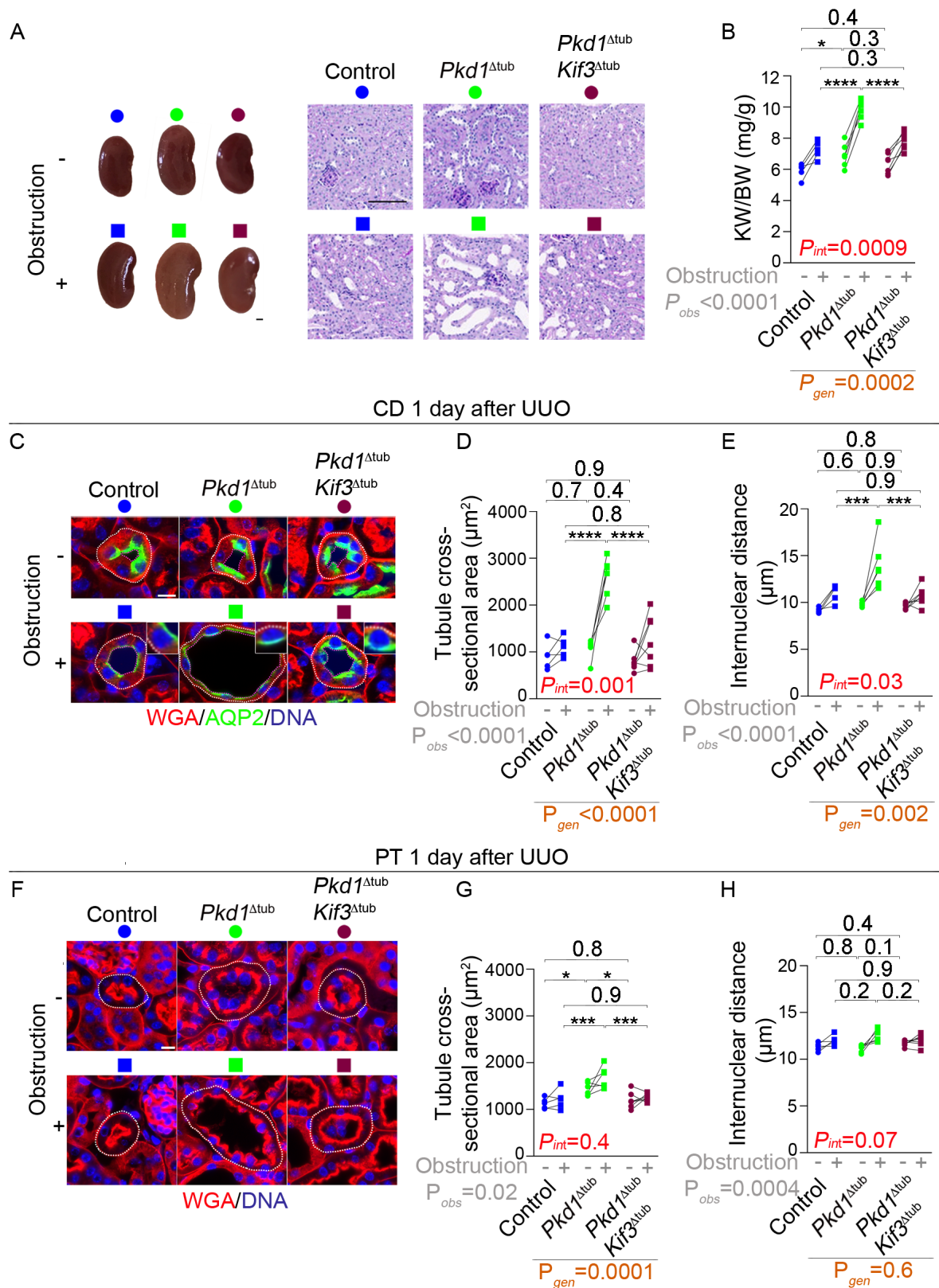


Figure 10. Acute ureteral obstruction accelerates *Pkd1*^{Δtub} distal tubule dilation in a cilia-dependent manner. (A and B) Kidney pictures, Periodic-acid Schiff's (PAS) staining (A) and quantification (B) of kidney weight to body weight ratio (KW/BW) of obstructed and non-obstructed kidneys from 8-week-old control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub}; *Kif3a*^{Δtub} one day after unilateral ureteral obstruction (UUO). Scale bars: 1 mm (left) and 0.1 mm (right). (C-H) Labelling of DNA, wheat germ agglutinin (WGA, which stains the brush border of proximal tubule [PT] and all basement membranes) and aquaporin 2 (AQP2, a collecting duct [CD] marker; C, F), quantification of mean tubule cross-sectional area (D, F) and internuclear distance (E, H) of CD (C-E) or PT (F-H) of kidneys from the same animals. Each pair of linked symbols (dot and square) represents the obstructed (square) and non-obstructed (dot) kidneys of an individual female mouse. Scale bars: 10 μm. Two-way ANOVA, P-value for obstruction (grey; *P*_{obs}), genotype (brown; *P*_{gen}) and their interaction (red; *P*_{int}), followed by Tukey-Kramer test: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 or the indicated P-value.

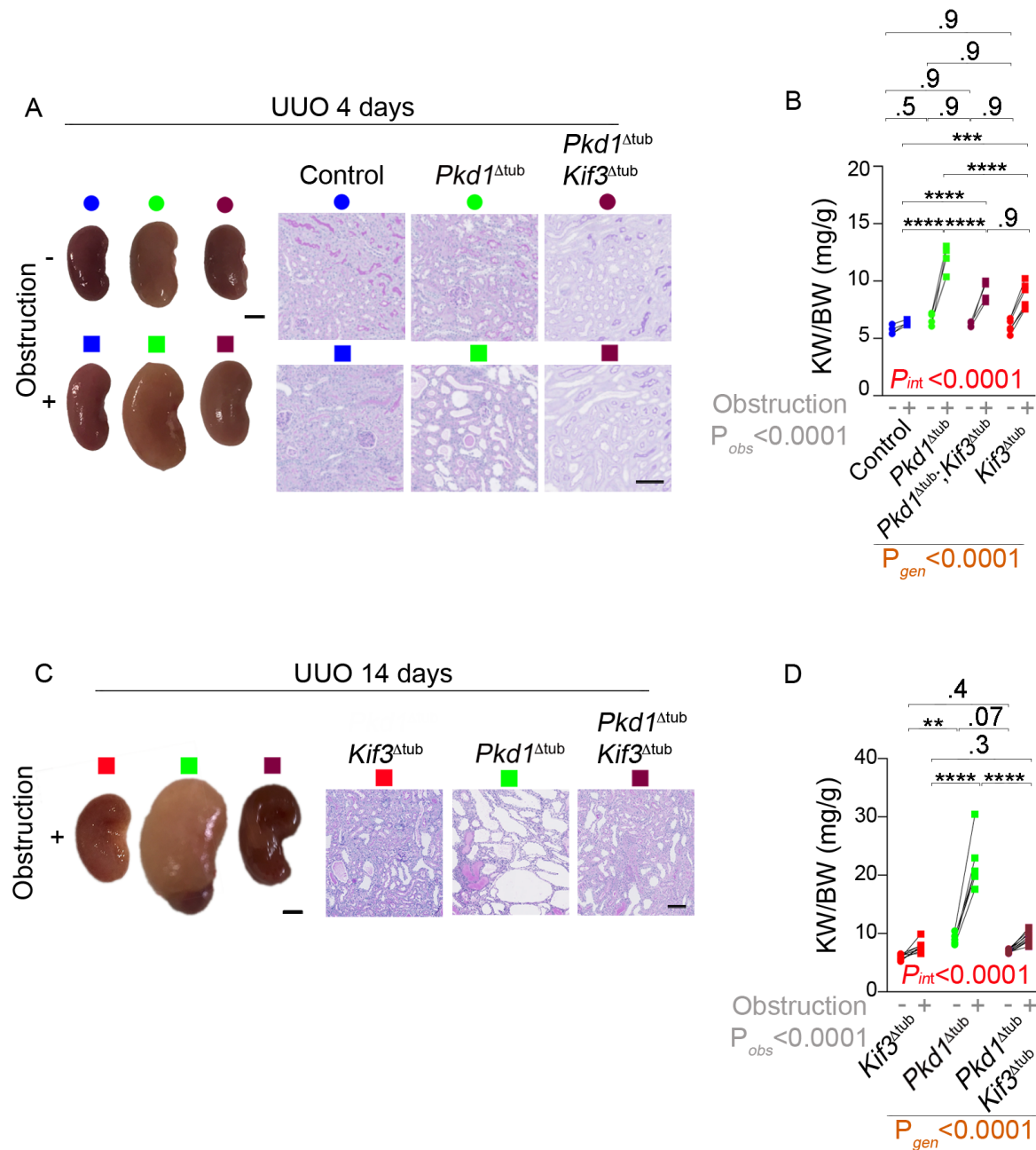


Figure 11. Ureteral obstruction triggers explosive cystogenesis in *Pkd1*^{Δtub} distal tubules in a cilia-dependent manner. (A and B) Kidney pictures and Periodic-acid Schiff[®] (PAS) staining (A) and quantification (B) of kidney weight to body weight ratio (KW/BW) of obstructed and non-obstructed kidneys control, *Pkd1*^{Δtub}, *Pkd1*^{Δtub}; *Kif3a*^{Δtub} and *Kif3a*^{Δtub} mice (in red) four days after unilateral ureteral obstruction (UUO) performed at 8 weeks of age. (C-D) Kidney pictures and PAS staining (C) and quantification (D) of KW/BW of kidneys from *Kif3a*^{Δtub} and *Pkd1*^{Δtub}; *Kif3a*^{Δtub} fourteen days after UUO performed at 8 weeks of age (D). Single *Kif3a* mutant mice (*Kif3a*^{Δtub} mice) are presented to show apparent epistasis of *Kif3a* over *Pkd1* inactivation. Scale bars: 1 mm (kidney pictures) and 0.1 mm (PAS). Each pair of linked symbols (dot and square) represents the obstructed (square) and non-obstructed (dot) kidneys of an individual female mouse. Scale bars: 10 μm. Two-way ANOVA, P-value for obstruction (grey; *P*_{obs}), genotype (brown; *P*_{gen}) and their interaction (red; *P*_{int}), followed by Tukey-Kramer test: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 or the indicated P-value. Control mice consist in *Pkd1*^{flox/flox} or *Pkd1*^{flox/flox}; *Kif3a*^{flox/flox} littermates lacking cre or rtTA transgene.

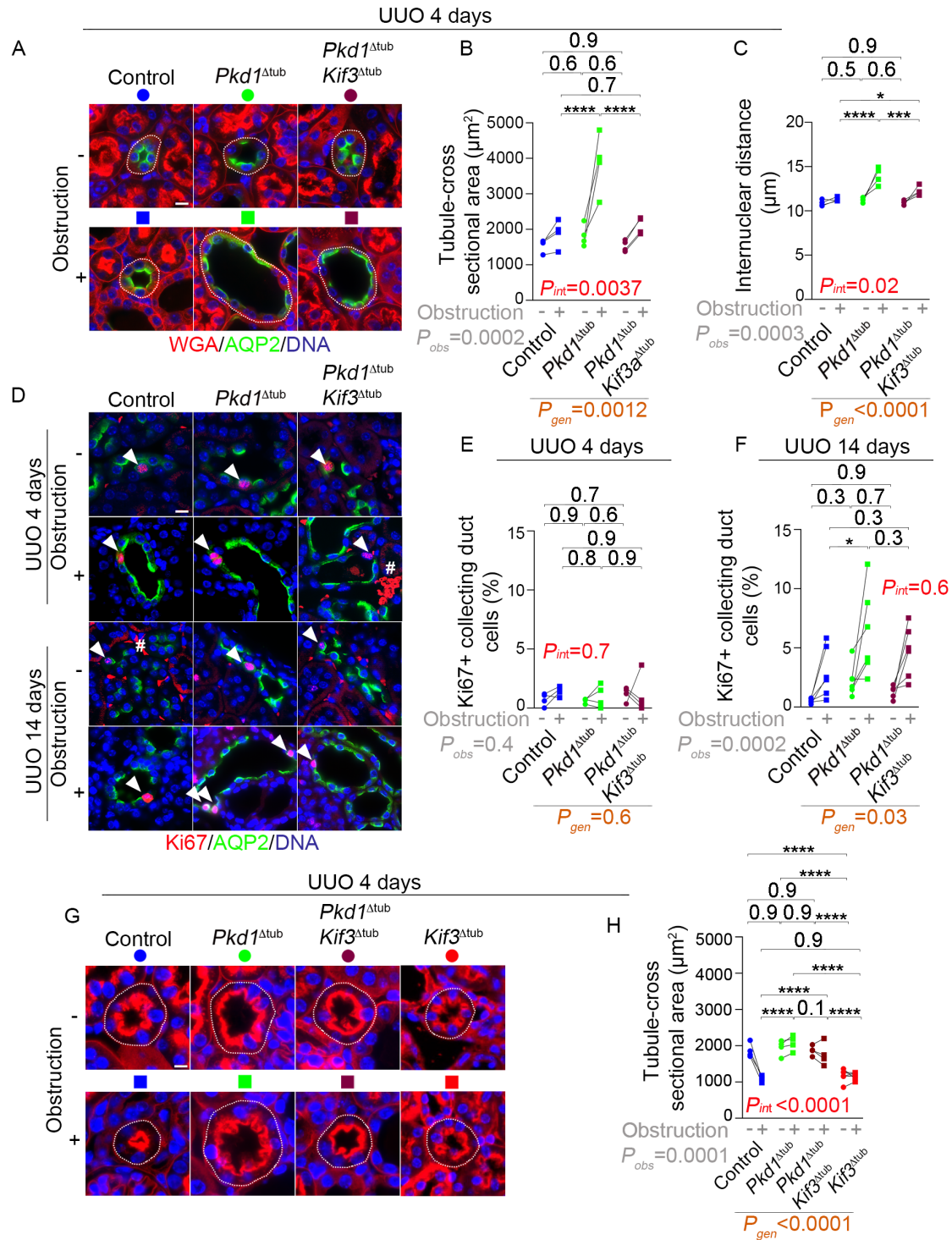


Figure 12. Accelerated cystogenesis after obstruction correlates with tubule distension and not proliferation (A-C) Fluorescent labelling of DNA, wheat germ agglutinin (WGA, which stains the brush border of proximal tubule [PT] and all basement membranes) and aquaporin 2 (AQP2, a collecting duct [CD] marker) and quantification of CD mean tubule cross-sectional area (B) and mean internuclear distance (C) in kidneys from control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub}; *Kif3a*^{Δtub} four days after unilateral ureteral obstruction (UUO) performed at 8 weeks of age. **(D-F)** Fluorescent labelling of DNA, Ki67 and AQP2 (D) and quantification (E-F) of Ki67 staining (white arrowhead; # indicates non-specific red blood cells autofluorescence) in CD of kidneys from control, *Pkd1*^{Δtub} and *Pkd1*^{Δtub}; *Kif3a*^{Δtub} four (E) and fourteen (F) days after UUO. **(G and H)** Representative images (G) and quantification of the PT cross-sectional area (H) in kidneys from control, *Pkd1*^{Δtub}, *Pkd1*^{Δtub}; *Kif3a*^{Δtub} and *Kif3a*^{Δtub} (in red) four days after UUO. Dashed lines in images underline tubule perimeters. Scale bars: 10 μm. Each pair of linked symbols (dot and square) represents the obstructed (square) and non-obstructed (dot) kidneys of an individual female mouse. Two-way ANOVA, P-value for obstruction (grey; *P*_{obs}), genotype (brown; *P*_{gen}) and their interaction (red; *P*_{int}), followed by Tukey-Kramer test: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 or the indicated P-value. Control mice consist in *Pkd1*^{flox/flox} or *Pkd1*^{flox/flox}; *Kif3a*^{flox/flox} littermates lacking cre or rtTA transgene.

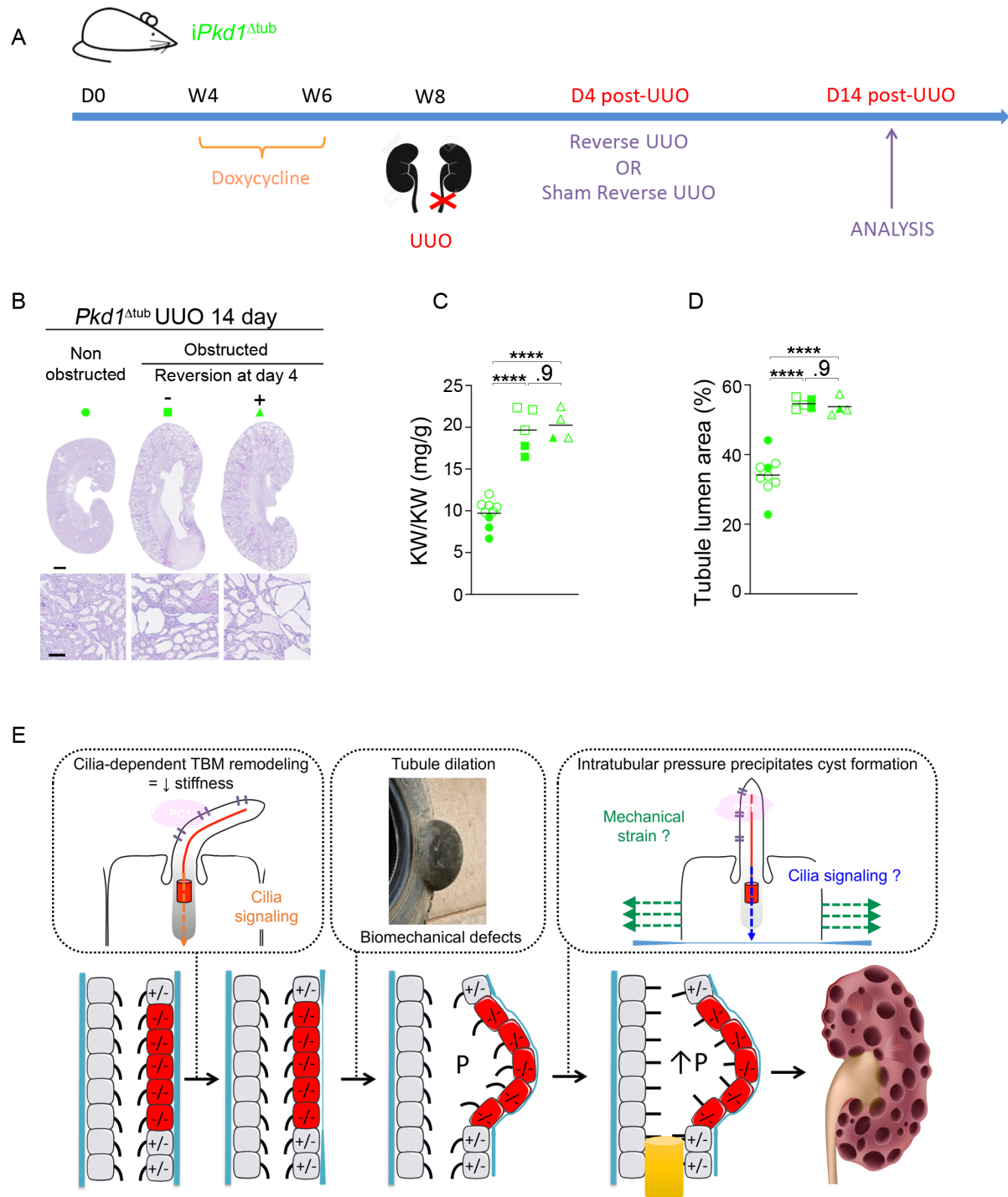


Figure 13. Transient obstruction triggers irreversible cystogenesis in *Pkd1* mutant mice. (A) Scheme of the protocol of reversible unilateral ureteral obstruction (UUO). Left kidney of 8-week-old *Pkd1* mutant mice was submitted to UUO, followed by a release of the obstruction (Reverse UUO) or a Sham operation (Sham Reverse UUO) 4 days after UUO, and mice were sacrificed 10 days after the second surgery. (B–D) Periodic-acid Schiff’s staining (B) and quantification of kidney weight to body weight ratio (KW/BW; C) and tubule dilations (D) of the kidneys from control or *Pkd1* mutant mice submitted to Reverse UUO or Sham Reverse-UUO. Scale bars: 2 mm (up) and 0.1 mm (down). Each symbol represents an individual female (closed symbol) or male (open symbol) mouse kidney. One-way ANOVA followed by Tukey-Kramer test: **** $P < 0.0001$ or the indicated P-value. (E) Proposed “tire bulge” model for cyst formation in ADPKD: Polycystin-1 loss triggers cilia dependent remodeling of basement membrane (BM). BM remodeling in turn promotes tubule distension, which is exacerbated by transmural hydrostatic pressure (P). Transient tubule obstruction increases luminal pressure, tubule dilation and tubular cell stretch, precipitating cystogenesis.