



**HAL**  
open science

## Divergent function of polycystin 1 and polycystin 2 in cell size regulation

Amandine Viau, Fruzsina Kotsis, Christopher Boehlke, Simone Braeg, Marinella Klein, Roland Nitschke, Gerd Walz, E. Wolfgang Kuehn

► **To cite this version:**

Amandine Viau, Fruzsina Kotsis, Christopher Boehlke, Simone Braeg, Marinella Klein, et al.. Divergent function of polycystin 1 and polycystin 2 in cell size regulation. *Biochemical and Biophysical Research Communications*, 2020, 521 (2), pp.290-295. 10.1016/j.bbrc.2019.10.074 . hal-03933415

**HAL Id: hal-03933415**

**<https://hal.science/hal-03933415>**

Submitted on 10 Jan 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## **Divergent function of polycystin 1 and polycystin 2 in cell size regulation.**

Amandine Viau<sup>1,2,\*</sup>, Fruzsina Kotsis<sup>1,5</sup>, Christopher Boehlke<sup>2,6</sup>, Simone Braeg<sup>1</sup>, Marinella Klein<sup>1</sup>, Roland Nitschke<sup>3,4</sup>, Gerd Walz<sup>1,2,4</sup>, E. Wolfgang Kuehn<sup>1,2,4</sup>

### **Author affiliations**

- <sup>1</sup> Renal Department, University Medical Center, Freiburg, Germany.
  - <sup>2</sup> Faculty of Medicine, University of Freiburg, Freiburg, Germany.
  - <sup>3</sup> Center for Biological Systems Analysis (ZBSA), Life Imaging Center, Albert-Ludwigs-University Freiburg, Freiburg, Germany.
  - <sup>4</sup> Center for Biological Signaling Studies (BIOSS), Albert-Ludwigs-University Freiburg, Freiburg, Germany.
  - <sup>5</sup> Institute of Genetic Epidemiology, Faculty of Medicine and Medical Center, University of Freiburg, Freiburg, Germany.
  - <sup>6</sup> Clinic for Palliative Care, Medical Center – University of Freiburg, Freiburg, Germany.
- \* Corresponding author.

### **Contact information**

Amandine Viau  
INSERM U1163 – Institut Imagine  
Laboratory of Inherited Kidney Diseases  
Team “Nephronophthisis and hypodysplasia”  
24 Boulevard du Montparnasse  
75015 Paris  
France  
phone: +33 1 42 75 43 41  
e-mail: [amandine.viau@inserm.fr](mailto:amandine.viau@inserm.fr)

**Abstract**

Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in *PKD1* or *PKD2*, the genes encoding polycystin 1 (PC1) and polycystin 2 (PC2), respectively. PC1 and PC2 localize to the primary cilium and form a protein complex, which is thought to regulate signaling events. *PKD1* mutations are associated with a stronger phenotype than *PKD2*, suggesting the existence of PC1 specific functions in renal tubular cells. However, the evidence for diverging molecular functions is scant. The bending of cilia by fluid flow induces a reduction in cell size through a mechanism that involves the kinase LKB1 but not PC2. Here, using different *in vitro* approaches, we show that contrary to PC2, PC1 regulates cell size under flow and thus phenocopies the loss of cilia. PC1 is required to couple mechanical deflection of cilia to mTOR in tubular cells. This study pinpoints divergent functions of the polycystins in renal tubular cells that may be relevant to disease severity in ADPKD.

**Keywords**

Polycystins, flow, ADPKD, cilia, mTOR

## Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most frequent monogenic disorders with a prevalence of 1:1,000 and accounts for 5-6% of end-stage renal diseases (ESRD) cases [1]. ADPKD is a late-onset multisystemic disorder characterized in the kidney by the progressive development of tubular cysts leading to massive renal enlargement. Kidney function declines and the majority of patients require kidney replacement therapy by the fifth to seventh decade of life. In addition, ADPKD patients have extra-renal manifestations, including liver and pancreatic cysts, heart disease and cerebral aneurysms [2].

Different signaling pathways are deregulated in ADPKD, including cAMP signaling, ERK and mTOR [3,4]. Pharmacologic targeting of mTOR and cAMP signaling reduces renal cyst growth in rodent models [5–7] but studies in humans have yielded heterogeneous results: mTOR inhibitors failed to alter the clinical course [8], while reducing cAMP signaling through the inhibition of the vasopressin V2 receptor modestly ameliorated kidney function decline and cystic kidney growth [9]. However, V2R inhibition has considerable side effects.

ADPKD results from mutations in *PKD1*, encoding polycystin-1 (PC1), a large orphan receptor [10] or *PKD2*, encoding polycystin-2 (PC2), a calcium-responsive cation channel of the transient receptor potential family [11]. PC1 and PC2 interact to form an oligomeric complex [12], and they localize to the primary cilium [13,14], a microtubule-based organelle that protrudes from the surface of most vertebrate cells. This localization is thought to be crucial to prevent cyst formation [14]. The precise function of PC1 and PC2 with respect to primary cilia and cyst formation remains unclear. A model where the PC1/PC2 complex functions as a ciliary mechanosensor complex, that translates cilia deflection by urinary flow into calcium flux, has recently

been refined [12,13,15,16]. Thus, the ciliary functions of PC1 and/or PC2 remain elusive and while anti-cystogenic functions are likely shared by PC1 and PC2, functional heterogeneity is likely.

The notion of divergent functions of PC1 vs. PC2 is reflected in clinical observations. Individuals with *PKD1* mutations, accounting for 85% of ADPKD cases, develop cysts earlier than patients with *PKD2*-mutations [17], have larger kidneys and faster functional decline. Kidney failure on average occurs 20 years earlier in *PKD1*-related ADPKD compared to patients with *PKD2* mutations [18]. The reason for this observation is unknown. Genetic events have been proposed: In a heterozygous state for the mutant alleles, cyst formation is the consequence of the loss of heterozygosity in a sub-population of cells [19]. A higher rate of somatic mutations has been proposed for *PKD1* which is much larger than *PKD2*. Yet, bi-allelic disruption of *Pkd1* in mice produces a more severe phenotype than *Pkd2* [20], which suggests divergences in PC1 and PC2 function in renal tubular cells.

Indeed, several observations support the idea that PC1 and PC2 can act independently. First, PC1 is highly expressed during renal development but is strongly down-regulated in the mature kidney, as opposed to PC2 [21]. Secondly, in addition to cilia, PC1 is expressed at cell junctions while PC2 localizes at the endoplasmic reticulum [22]. Thirdly, *Pkd2*, but not *Pkd1* null mice display laterality defects [23]. While this pinpoints a specific PC2 function in the embryonic node, PC1 specific functions in tubular cells remain to be established.

We previously demonstrated that primary cilia regulate mTOR signaling and cell size involving the inhibitory upstream kinase LKB1, but not PC2 [24]. Yet, recent evidence suggests a joint function of LKB1 and PC1 in cilia dependent immune

signaling [25]. This led us to investigate if PC1 functionally interacts with LKB1 and cilia in the regulation of cell size or mTOR.

## Materials and Methods

### ***Cell culture, transfections and transgenic cell lines***

Human embryonic kidney (HEK 293T) cells (ATCC Promocell; CRL-11268) are listed in the database of commonly misidentified cell lines maintained by ICLAC and NCBI Biosample and were authenticated by Interspecies Determination (Isoenzyme Analysis and STR Analysis) by the providing company. HEK 293T cells were cultured using Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (Biochrome). Plasmids were transiently transfected using calcium.

Madin-Darby canine kidney (MDCK, kind gift of Prof. Kai Simons, MPI-CBG, Dresden, Germany) cells were cultured using Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1% Penicillin-Streptomycin (Sigma). For the tetracycline-inducible knockdown of target genes, a lentivirus-based transduction system (pLVTH) was used as previously described [25,26]. The shRNA were designed using BLOCK-iT™ RNAi Designer software (Invitrogen). The shRNA targeting sequences were: *Kif3a-i* (5'-AGGCTAGAGCTGAATTAGAG-3'), *Luciferase* (5'-CGTACGCGGAATACTTCGA-3'), *Pkd1-i1* (5'-GCTTCTTTGACAAGCACAT-3'), *Pkd1-i2* (5'-CAAGGAGTTCCGCCACAAA-3'), *Pkd1-i3* (5'-GCCACAAAGTCCGCTTTGA-3'), *Pkd1-i4* (5'-CCACAAAGTCCGCTTTGAT-3') and *Pkd2-i* (5'-GGAGGAGGCAAGTTAAACT-3'). The efficiency of the knock-down was verified by qRT-PCR and/or Western blot.

*Pkd1* null MDCK cell lines were engineered with transcription activator-like effector nucleases (TALENs) as described [27].

Cells were maintained in an incubator at 5% CO<sub>2</sub> and 37°C. All cells were regularly tested for mycoplasma contamination and were mycoplasma free.

### **Quantitative real-time PCR**

Total RNAs were obtained from cells using RNeasy Mini Kit (Qiagen) and reverse transcribed using SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen) according to the manufacturer's protocol. qRT-PCR was performed on a LightCycler 480 (LC 480, Roche). *Gapdh*, *Sdha* and *Hprt* were used as the normalization controls. Each biological replicate was measured in technical duplicates. The primers used are: dog *Gapdh* forward 5'-CATGTTTGTGATGGGCGTGAACCA-3', dog *Gapdh* reverse 5'-TTTGGCTAGAGGAGCCAAGCAGTT-3', human *Gapdh* forward 5'-TGCACCACCAACTGCTTAG-3', human *Gapdh* reverse 5'-TGGATGCAGGGATGATGTT-3', dog *Hprt* forward 5'-AATGTCTTGATTGTTGAGGATAT-3', dog *Hprt* reverse 5'-ACAAAGTCAGGTTTATAGCC-3', dog *Pkd1* forward 5'-AGGACTACGAGATGGTGGAGCTCTTT-3', dog *Pkd1* reverse 5'-TTTGTGGCGGAACTCCTTGACCTT-3', mouse *Pkd1* forward 5'-AAGGTCAAGGAGTTCCGCCACAAA-3', mouse *Pkd1* reverse 5'-TGTGAAGCTTCTGAGCCTGAGCTA-3', dog *Sdha* forward 5'-TGGGTCCATCCATCGCATA-3', dog *Sdha* reverse 5'-GAAGTAGGTGCGCCCATAACC-3'.

### **Western blot**

Cells were lysed in RIPA buffer (20mM Tris pH8, 160mM NaCl, 1mM EDTA, 1mM EGTA, 1mM DTT, 0.1% SDS, 1% NP-40, 1% Na deoxycholate, 1% Triton X100) supplemented with 1mM Na<sub>3</sub>VO<sub>4</sub>, 50mM NaF and protease inhibitor cocktail tablet (Roche) using a 20G needle. After cell debris removal by centrifugation, protein content was determined using Pierce BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein were resolved on 4-15% Mini-PROTEAN TGX™ Gel (Bio-Rad)

under reducing conditions, transferred, incubated with primary and secondary antibodies and visualized on film according to standard protocols.

### ***Primary cilia immunostaining and counting***

For cilia staining, 150,000 cells/cm<sup>2</sup> were grown with or without Tetracyclin for 10 days on ibiTreat  $\mu$ -Dish<sup>35mm,high</sup> (Ibidi). Cells were fixed 10 minutes in 4% paraformaldehyde and immunostaining was performed as previously described [25]. Images were recorded using an inverted scanning confocal microscope LSM 510 Duo (Zeiss) equipped with a 63x LCI-Plan Neofluar/1.3 N.A. objective. Within the cell monolayer, five random fields of view were selected, z-stacks were recorded and 3D reconstruction was performed using ZEN Black software (Zeiss). Primary cilia (acetylated Tubulin positive) and nuclei were automatically counted using Imaris software. The mean rate of ciliated cells in a visual field was obtained by dividing cilia number by the number of counted cells in each visual field.

### ***Flow and no flow experiments***

For no flow experiments, 150,000 cells/cm<sup>2</sup> were grown with or without Tetracyclin (5 $\mu$ g/mL) for 6 days on  $\mu$ -Dish<sup>35mm,high</sup> (Ibidi) coated with Collagen IV (15 $\mu$ g/mL, BD Biosciences, 354233).

For flow experiments, 150,000 cells/cm<sup>2</sup> were seeded with or without Tetracyclin on  $\mu$ -Slide VI<sup>0.4</sup> Collagen IV coated (Ibidi) and allowed to adhere for 24 hours. The chamber was connected to a computer-controlled set up containing an air pressure pump and a two-way switching valve (Ibidi) as previously described [28], pumping 40mL of culture medium unidirectionally between two reservoirs through the flow channel at a rate of 0.6mL/min, corresponding to a shear stress of approximately 0.7 dyn/cm<sup>2</sup>. Flow was maintained over 5 days.

### ***Imaging and cell size measurement***

After 6 days of culture, single plane images were randomly recorded at ten (no flow conditions) and twenty (flow conditions) different locations using an inverted microscope Axiovert 200 (Zeiss) equipped with a 63X C-Apochromat 63X/1.2 N.A. water objective for differential interference contrast (DIC) microscopy and analyzed with the ZEN Blue software (Zeiss).

The mean cell size was calculated by counting cells manually in a defined area of 2700  $\mu\text{m}^2$  in the center of full-frame images using Imaris software. Fully visible cells were counted as one, cells at the top and right margins were counted when  $\geq 50\%$  was visible and cells at the bottom and left margins were counted when  $\geq 90\%$  was visible. The mean cell size in a visual field was obtained by dividing the area size by the number of counted cells in each visual field.

#### ***Imaging and cell height measurement***

After 6 days of culture, images were recorded using an inverted laser scanning confocal microscope LSM 510 Meta (Zeiss) equipped with a 63X C-Apochromat 63X/1.2 N.A. water objective. Within the cell monolayer, five random fields of view were selected and z-stacks were recorded using ZEN Black software (Zeiss). 3D reconstruction and cell height measurements were performed using ZEN Black software.

#### ***Plasmids, Reagents and antibodies***

The FLAG.PKD1 construct was kindly provided by Michael Caplan.

For western blot, the following antibodies were used :  $\alpha$ -TUBULIN (Sigma, T5168, 1:20,000),  $\beta$ -ACTIN (Sigma, A1978, 1:60,000), DsRed (Clontech, 632496, 1:1,000), FLAG (Sigma, F3165, 1 :3,000), GFP (B-2, Santa Cruz biotechnology, 1:3,000), HA (12CA5, Roche applied Science, 1666606, 1:1,000), PKD1 (7E12, Santa Cruz biotechnology, sc-130554, 1:200), pS6K<sup>T389</sup> (Cell signaling Technology, 9234,

1:1,000), pS6RP<sup>S235/236</sup> (Cell signaling Technology, 4858, 1:1,000), S6K (Cell signaling Technology, 9202, 1:1,000), S6RP (Cell signaling Technology, 2217, 1:1,000).

For immunofluorescence, the following antibodies were used: mouse anti-acetylated Tubulin antibody (Sigma, T6793, 1:3,000), Cy3-labelled secondary anti-mouse antibody (Jackson ImmunoResearch, 715-165-150, 1:500). Hoechst 33342 (Molecular Probes, H-1399, 1:10,000) was used for staining cell nuclei.

Rapamycin (Calbiochem, 553211) was solubilized in DMSO and used at 100 nM. DMSO was used as vehicle at 0.1%.

### ***Statistical Analysis***

Data were expressed as means  $\pm$  SD. Differences between the experimental groups were evaluated using two-tailed paired *t* test. The statistical analysis was performed using GraphPad Prism V6 software. No samples were excluded from analysis. All image analyses were performed in a blinded fashion.

## Results and Discussion

To investigate the role of PC1 in flow-dependent cell size regulation, we established inducible *Pkd1*-silenced Madin-Darby canine kidney (MDCK) cells. We designed 21 shRNA directed against common sequences of human, mouse and dog *Pkd1*. Only 4 resulted in relevant depletion of mouse *Pkd1* at both the mRNA and the protein level in human embryonic kidney (HEK 293T) cells (**Figure 1A-C**). Using these 4 shRNA sequences we generated inducible *Pkd1*-silenced (Pkd1-i) MDCK cell lines (Pkd1-i1 to i4). Tetracycline treatment resulted in efficient knock-down of dog *Pkd1* mRNA in Pkd1-i3 and Pkd1-i4 (49% and 54% decrease; **Figure 1D**). The lack of reliable antibodies did not allow us to detect endogenous canine PC1. Expression of the shRNA was documented by reporters [26]: red fluorescence indicated the repressor whereas GFP was co-expressed with the shRNA under tetracycline (**Figure 1E, F**).

To investigate if, similarly to LKB1, PC1 regulates cell size under flow, we analyzed Pkd1-i cells in a flow chamber where cells are kept for several days under permanent fluid flow, to engage cilia similar to renal tubules. After 5 days of flow, the cell size was significantly larger in *Pkd1*-depleted cells as compared to non-induced Pkd1-i cells or to control cells (Luci-i) (**Figure 2A, B**). Analysis of cross-sections excluded that differences in the x-y plane are offset by different cell heights (**Figure 2C**). The increased cell size of *Pkd1*-depleted cells recapitulated the findings in unciliated, *Kif3a*-depleted cells (**Figures 2A, B and 3B**), suggesting that this function of PC1 is linked to cilia. As described before [24], depletion of *Pkd2* did not alter flow-dependent cell size (**Figure 2B**).

Since Pkd1-i cells express residual PC1, we repeated our analyses in cells completely devoid of PC1. We employed four clonal PKD1-null renal epithelial MDCK

cell lines (*Pkd1* KO1 to KO4) produced through TALEN-based genome editing [25,27]. After 5 days of flow, the cells were significantly larger in all *Pkd1* KO cell lines when compared to wild-type parental MDCK cells (**Figure 2D**). The effect was larger than in *Pkd1*-i cells (**Figure 2B**), which may be explained by remaining PC1 protein in the latter. In the absence of flow no consistent effect on cell size was observed (**Figure 2E, F**). These findings indicate that cell size regulation by PC1 is flow specific.

Since primary cilia are the mediators of flow-dependent cell size regulation (**Figure 2 A, B**), we analyzed cilia integrity. Immunostaining for acetylated tubulin, a cilia marker, revealed that GFP-positive *Pkd1*-i MDCK cells do not lack cilia (**Figure 3A, B**). This is in accordance with the observation in humans and animal models that loss of polycystins does not affect ciliogenesis [20]. Similar to *Pkd1*-i cells, inducible depletion of *Pkd2* or *Pkd1* KO did not affect ciliogenesis (**Figure 3B, C and D**) in a way comparable to the lack of cilia observed in *Kif3a*-i MDCK cells (**Figure 3B**). These data suggest that PC1 regulates cell size under flow without impairing ciliogenesis.

mTOR is a central controller of cell mass and modulates cell size under flow [24]. We investigated whether mTORC1 is controlled by PC1 in the regulation of cell size. Immunoblots revealed that phosphorylation of the mTORC1 downstream targets S6K and S6RP was increased in *Pkd1*-i cells, but not in *Luci*-i control cells under flow (**Figure 4A**). In contrast, neither S6K nor S6RP phosphorylation were affected by PC1 in the absence of flow. Treatment of *Pkd1*-i cells with rapamycin, a specific mTORC1 inhibitor, demonstrated impaired mTORC1 activation in PC1 depleted cells after 5 days of flow, as shown by a reduction in S6K and S6RP phosphorylation (**Figure 4B**). Importantly, rapamycin rescued defective cell size control in PC1 depleted or cilia deficient cells but not control cells (**Figure 4C**). On the contrary, rapamycin treatment

had no impact on cell size in static conditions (**Figure 4D**). These results show that PC1 is required to control cell size under flow by inhibiting mTORC1.

Collectively these data reveal a specific function of PC1 in cell size regulation under flow. This function is interdependent with cilia: First, loss of *Pkd1* function and cilia ablation lead to a cell size regulation defect of the same magnitude. Second, cell size control by PC1 or primary cilia manifests only under flow, suggesting a direct or indirect involvement of PC1 in the mechanosensory response to cilia deflection. Notably, cell size regulation is common to LKB1 and PC1, which have been shown to physically interact [25] and also jointly regulate CCL2 mRNA expression through a cilia dependent mechanism [25]. However, contrary to CCL2 repression, cell size regulation by PC1 requires apical fluid flow which suggests that cell size regulation and CCL2 modulation occur through distinct ciliary pathways involving PC1 and LKB1.

Our results demonstrate a PC1 specific function in renal tubular mTOR signaling. Yet, mTOR inhibitors reduce disease severity in *Pkd1*- and *Pkd2*-mutated mouse models [5,7]. While the relative effects are difficult to compare between models using different genetic approaches, extra-tubular events may account for an effect of rapamycin in *Pkd2*-mutated mice. Indeed, recent evidence suggests that interstitial inflammation promotes cyst growth [25,29]. Therefore, anti-inflammatory effects of the immunosuppressant rapamycin may explain the effects in *Pkd2* targeted mouse models.

We find that mTOR signaling, a metabolic pathway, is regulated by PC1. Another study found that LKB1 and PC1 deficiency alters renal metabolism towards glutamine dependence [30]. Together, these observations raise the question if metabolic reprogramming in PC1 deficient renal epithelia explain disease severity in PKD1 vs. PKD2 mutated individuals and how it may be targeted therapeutically.

**Acknowledgements**

We thank the Life Imagine Center (LIC, Center for Biological systems analysis ZBSA, Freiburg, Germany) for technical assistance. Grant Support: Amandine Viau: ERA-EDTA ALTF 84-2011; Roland Nitschke: German Research Foundation (DFG) under Germany's Excellence Strategy BIOSS-EXC294, Z01; E. Wolfgang Kuehn: Else Kröner-Fresenius-Stiftung 2011\_A87 and Deutsche Forschungsgemeinschaft KFO 201, KU 1504/5-1 and SFB1140.

**References**

- [1] E. Cornec-Le Gall, A. Alam, R.D. Perrone, Autosomal dominant polycystic kidney disease, *Lancet*. 393 (2019) 919–935. doi:10.1016/S0140-6736(18)32782-X.
- [2] A.C.M. Ong, O. Devuyst, B. Knebelmann, et al., Autosomal dominant polycystic kidney disease: The changing face of clinical management, *Lancet*. 385 (2015) 1993–2002. doi:10.1016/S0140-6736(15)60907-2.
- [3] V.E. Torres, P.C. Harris, Strategies targeting cAMP signaling in the treatment of polycystic kidney disease., *J. Am. Soc. Nephrol.* 25 (2014) 18–32. doi:10.1681/ASN.2013040398.
- [4] G. Distefano, M. Boca, I. Rowe, et al., Polycystin-1 regulates extracellular signal-regulated kinase-dependent phosphorylation of tuberlin to control cell size through mTOR and its downstream effectors S6K and 4EBP1, *Mol Cell Biol.* 29 (2009) 2359–2371. [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=19255143](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19255143).
- [5] I. Zafar, K. Ravichandran, F.A. Belibi, et al., Sirolimus attenuates disease progression in an orthologous mouse model of human autosomal dominant polycystic kidney disease, *Kidney Int.* 78 (2010) 754–761. doi:10.1038/ki.2010.250.
- [6] V.H. Gattone 2nd, X. Wang, P.C. Harris, et al., Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist, *Nat. Med.* 9 (2003) 1323–1326. doi:10.1038/nm935.
- [7] J.M. Shillingford, K.B. Piontek, G.G. Germino, et al., Rapamycin ameliorates PKD resulting from conditional inactivation of Pkd1, *J Am Soc Nephrol.* 21 (2010) 489–497.

[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=20075061](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20075061).

- [8] G. Walz, K. Budde, M. Mannaa, et al., Everolimus in Patients with Autosomal Dominant Polycystic Kidney Disease, *N. Engl. J. Med.* 363 (2010) 830–840. doi:10.1056/NEJMoa1003491.
- [9] V.E. Torres, A.B. Chapman, O. Devuyst, et al., Tolvaptan in patients with autosomal dominant polycystic kidney disease. Supplementary Appendix, *N. Engl. J. Med.* (2012). doi:10.1056/NEJMoa1205511.
- [10] J. Hughes, C.J. Ward, B. Peral, et al., The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains, *Nat. Genet.* 10 (1995) 151–160. doi:10.1038/ng0695-151.
- [11] T. Mochizuki, G. Wu, T. Hayashi, et al., PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein, *Science* (80-. ). 272 (1996) 1339–1342.
- [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=8650545](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8650545).
- [12] Q. Su, F. Hu, X. Ge, et al., Structure of the human PKD1-PKD2 complex, *Science* (80-. ). 361 (2018). doi:10.1126/science.aat9819.
- [13] X. Liu, T. Vien, J. Duan, et al., Polycystin-2 is an essential ion channel subunit in the primary cilium of the renal collecting duct epithelium, *Elife.* (2018). doi:10.7554/eLife.33183.
- [14] Y. Cai, S. V. Fedeles, K. Dong, et al., Altered trafficking and stability of polycystins underlie polycystic kidney disease, *J. Clin. Invest.* 124 (2014) 5129–5144. doi:10.1172/JCI67273.
- [15] M. Delling, A.A. Indzhukulian, X. Liu, et al., Primary cilia are not calcium-

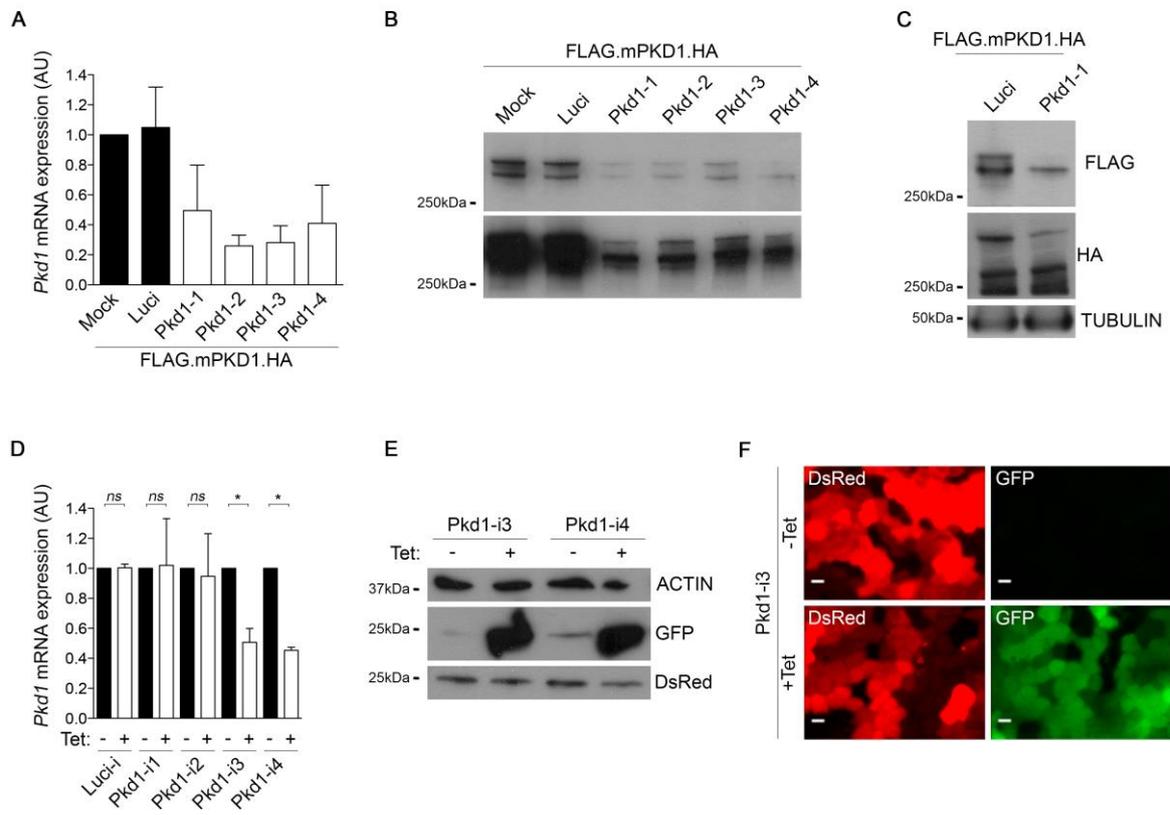
- responsive mechanosensors, *Nature*. 531 (2016) 656–660. doi:10.1038/nature17426.
- [16] T. Busch, M. Köttgen, A. Hofherr, TRPP2 ion channels: Critical regulators of organ morphogenesis in health and disease, *Cell Calcium*. (2017). doi:10.1016/j.ceca.2017.05.005.
- [17] P.C. Harris, K.T. Bae, S. Rossetti, et al., Cyst Number but Not the Rate of Cystic Growth Is Associated with the Mutated Gene in Autosomal Dominant Polycystic Kidney Disease, *J. Am. Soc. Nephrol.* 17 (2006) 3013–3019. doi:10.1681/ASN.2006080835.
- [18] E. Cornec-Le Gall, M.-P.P. Audrezet, J.-M.M.J.-M. Chen, et al., Type of PKD1 Mutation Influences Renal Outcome in ADPKD, *J. Am. Soc. Nephrol.* 24 (2013) 1006–1013. doi:10.1681/ASN.2012070650.
- [19] G. Wu, V. D'Agati, Y. Cai, et al., Somatic inactivation of Pkd2 results in polycystic kidney disease, *Cell*. 93 (1998) 177–188. [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=9568711](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9568711).
- [20] M. Ma, X. Tian, P. Igarashi, et al., Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease, *Nat Genet.* (2013). doi:10.1038/ng.2715.
- [21] V. Chauvet, F. Qian, N. Boute, et al., Expression of PKD1 and PKD2 transcripts and proteins in human embryo and during normal kidney development, *Am. J. Pathol.* (2002). doi:10.1016/S0002-9440(10)64919-X.
- [22] Y. Cai, Y. Maeda, A. Cedzich, et al., Identification and characterization of polycystin-2, the PKD2 gene product, *J. Biol. Chem.* (1999). doi:10.1074/jbc.274.40.28557.

- [23] P. Pennekamp, C. Karcher, A. Fischer, et al., The ion channel polycystin-2 is required for left-right axis determination in mice, *Curr Biol.* 12 (2002) 938–943. [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=12062060](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12062060).
- [24] C. Boehlke, F. Kotsis, V. Patel, et al., Primary cilia regulate mTORC1 activity and cell size through Lkb1, *Nat Cell Biol.* 12 (2010) 1115–1122. doi:10.1038/ncb2117.
- [25] A. Viau, F. Bienaimé, K. Lukas, et al., Cilia-localized LKB1 regulates chemokine signaling, macrophage recruitment, and tissue homeostasis in the kidney., *EMBO J.* (2018) e98615. doi:10.15252/emj.201798615.
- [26] M. Wiznerowicz, D. Trono, Conditional suppression of cellular genes: lentivirus vector-mediated drug-inducible RNA interference., *J. Virol.* 77 (2003) 8957–61. doi:10.1128/JVI.77.16.8957-8961.2003.
- [27] A. Hofherr, T. Busch, N. Huber, et al., Efficient genome editing of differentiated renal epithelial cells, 469 (2017) 303–311. doi:10.1007/s00424-016-1924-4.
- [28] F. Kotsis, R. Nitschke, M. Doerken, et al., Flow modulates centriole movements in tubular epithelial cells, *Pflugers Arch.* 456 (2008) 1025–1035. [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=18425533](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18425533).
- [29] A. Karihaloo, F. Korashy, S.C. Huen, et al., Macrophages Promote Cyst Growth in Polycystic Kidney Disease, *J. Am. Soc. Nephrol.* 22 (2011) 1809–1814. doi:10.1681/ASN.2011010084.
- [30] E.M. Flowers, J. Sudderth, L. Zacharias, et al., Lkb1 deficiency confers glutamine dependency in polycystic kidney disease, *Nat. Commun.* 9 (2018) 1–11. doi:10.1038/s41467-018-03036-y.



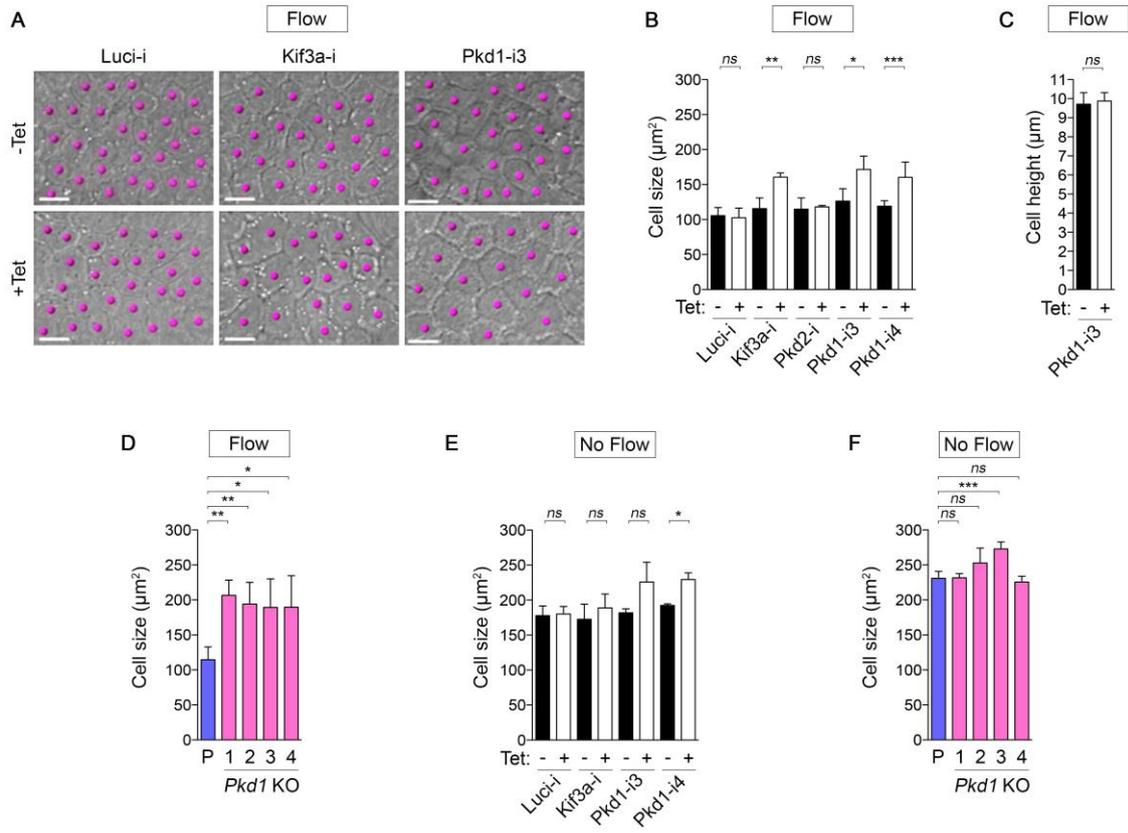
## Figure legends

Figure 1



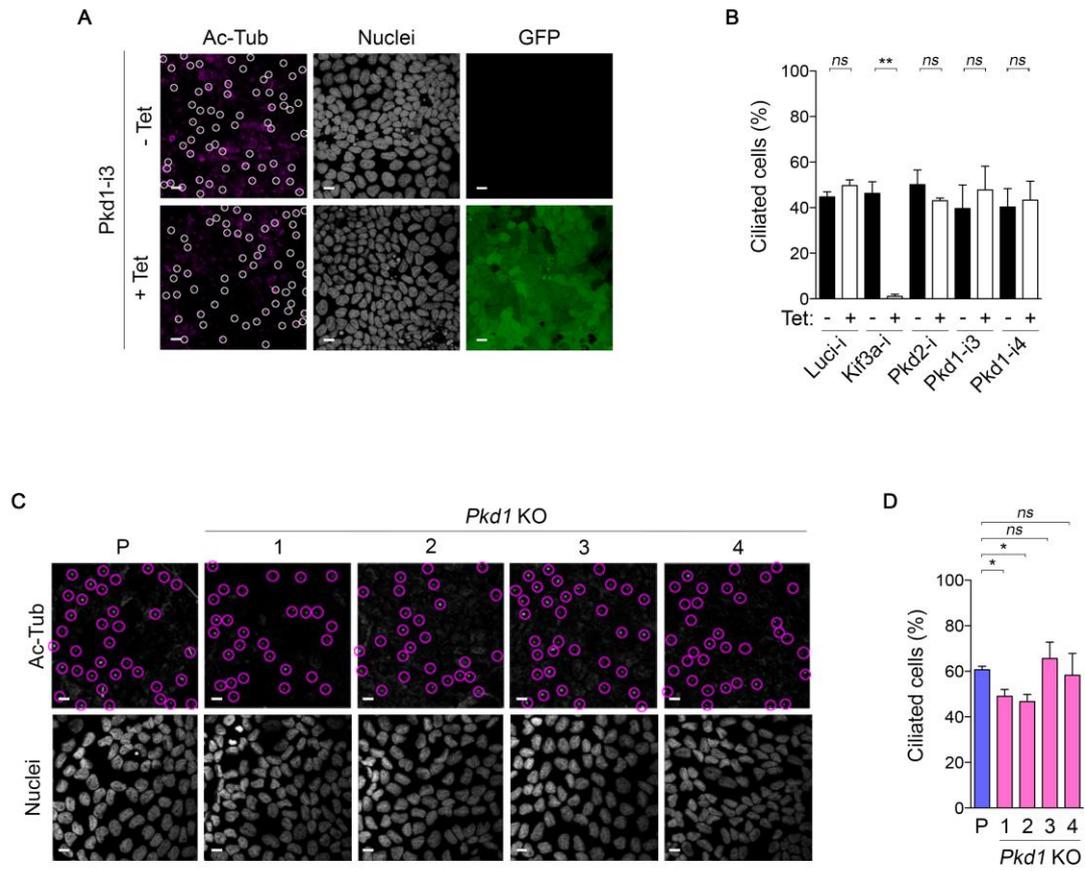
**Figure 1. Generation of *Pkd1* depleted MDCK cells.** (A) Mouse *Pkd1* mRNA expression in HEK 293T cells co-expressing a tagged mouse *Pkd1* construct (FLAG.mPKD1.HA), one of the 4 designed shRNAs targeting common sequences in dog, human and mouse *Pkd1* (Pkd1-1 to 4) or shRNA against Luciferase used as control (Luci) or no shRNA (Mock), 24 hours (24h) after transfection. ( $n=2$ ). (B) Western blot of FLAG.mPKD1.HA with anti-PKD1 in transfected HEK 293T cells at 24h (upper) and 48h (lower). (C) Western blot of FLAG.mPKD1.HA with anti-FLAG or anti-HA, in transfected HEK 293T cells (48h). (D) Canine *Pkd1* mRNA expression in MDCK cells expressing inducible shRNA against luciferase (Luci-i) or *Pkd1* (Pkd1-i1 to 4), 3 days (3d) after tetracycline induction (Tet). ( $n=3$ ). Mean  $\pm$  SD. Paired  $t$  test,  $*P<0.05$ ,  $***P<0.001$ ,  $ns$  not statistically different. (E) GFP is expressed in Pkd1-i3 and Pkd1-i4 MDCK cells (3d, +/- Tet). (F) Pkd1-i3 MDCK cells co-express DsRed with a repressor at baseline. Induction with tetracycline releases expression of shRNA and GFP (6d, +/- Tet). Scale bar: 10 $\mu$ m. (B, E) Representative of 2 independent experiments.

Figure 2



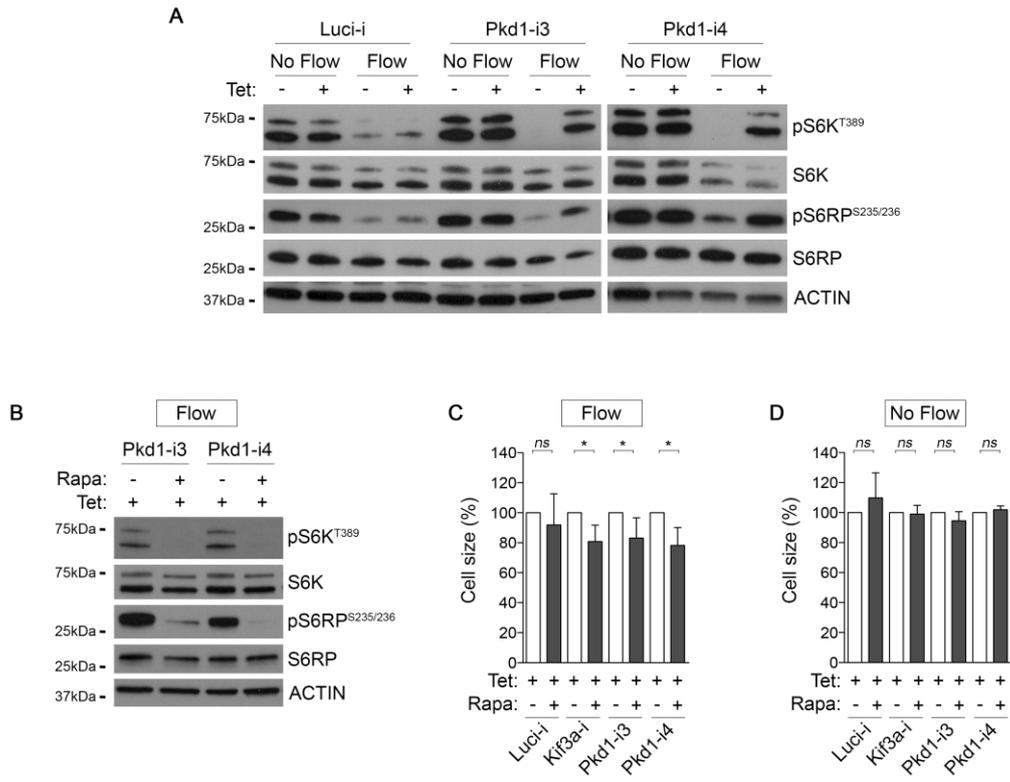
**Figure 2. PC1 but not PC2 regulates cell size under flow.** (A) Representative DIC microscopy images of MDCK cells expressing inducible shRNA against luciferase (Luci-i), *Kif3a* (Kif3a-i) or *Pkd1* (Pkd1-i3) (6d, +/- Tet, flow). One pink dot per cell. Scale bar: 10 $\mu$ m. (B) Cell size in MDCK cells expressing the indicated shRNAs (6d, +/- Tet, flow;  $n=4$ ). (C) Cell height in Pkd1-i3 MDCK cells (6d, +/- Tet, flow;  $n=3$ ). (D) Cell size in *Pkd1*-null MDCK cells (*Pkd1* KO1 to KO4) compared to wild-type parental MDCK cells (P), (6d, flow;  $n=3$ ). (E) Cell size in MDCK cells expressing the indicated shRNAs (6d, +/- Tet, no flow;  $n=3$ ). (F) Cell size in *Pkd1* KO MDCK compared to parental cells, (6d, no flow;  $n=3$ ). (B-F) Mean  $\pm$  SD. Paired  $t$  test, \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ ,  $ns$  not statistically different. Blinded quantification of 20 (B, D) or 10 (E, F) fields of view per biological sample. (C) Blinded quantification of 5 z-stacks per biological sample.

Figure 3



**Figure 3. *Pkd1* depletion does not affect cilia number.** (A) Confocal microscopy images of primary cilia (Ac-Tub), GFP and nuclei in *Pkd1*-i3 MDCK cells (10d, +/- Tet). (B) Quantification of primary cilia in MDCK cells expressing the indicated shRNAs (10d, +/- Tet). (C) Confocal microscopy images of primary cilia and nuclei in *Pkd1*-null MDCK cells (*Pkd1* KO1 to KO4) compared to wild-type parental MDCK cells (P) (10d). (D) Quantification of primary cilia in *Pkd1*-null MDCK compared to parental cells (10d). (A, C) Representative images of 3 biological replicates. Scale bar: 10 $\mu$ m. (B, D) Blinded quantification of 5 fov per biological sample. ( $n=3$ ). Mean  $\pm$  SD. Paired  $t$  test, \* $P<0.05$ , \*\* $P<0.01$ ,  $ns$  not statistically different.

Figure 4



**Figure 4. PC1 modulates flow-dependent mTORC1 regulation.** (A) Western blot of mTORC1 targets S6K and S6RP in MDCK cells expressing the indicated shRNAs. (6d, +/- Tet, +/- flow). (B) Western blot of S6K and S6RP activation in Pkd1-i MDCK cells (6d, + Tet, +/- Rapamycin (Rapa), flow). (C) Cell size in MDCK cells expressing the indicated shRNAs (6d, +Tet, +/- Rapa, flow,;  $n=3$ ). (D) Cell size in MDCK cells expressing the indicated shRNAs (6d, +Tet, +/- Rapa, no flow;  $n=3$ ). (A, B) Representative of 3 independent experiments. (C, D) Blinded quantification of 20 (C) or 10 (D) fields of view per biological sample. Mean  $\pm$  SD. Paired  $t$  test,  $*P<0.05$ ,  $ns$  not statistically different.