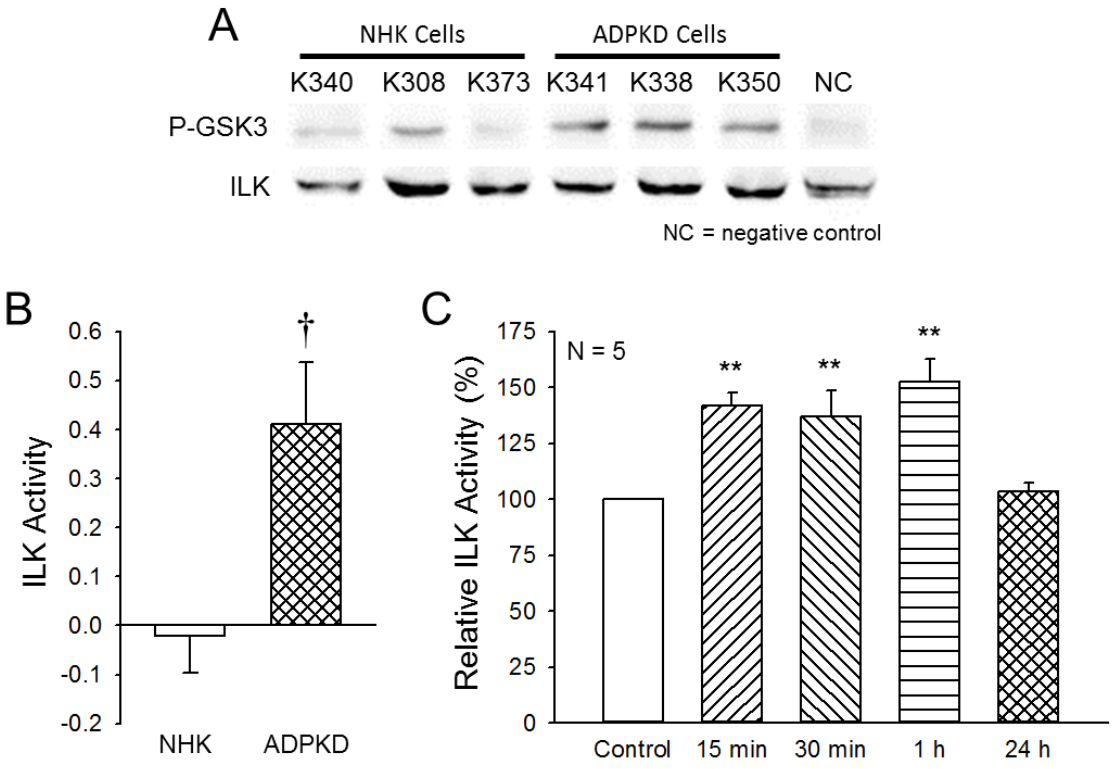
Supplemental Table 1

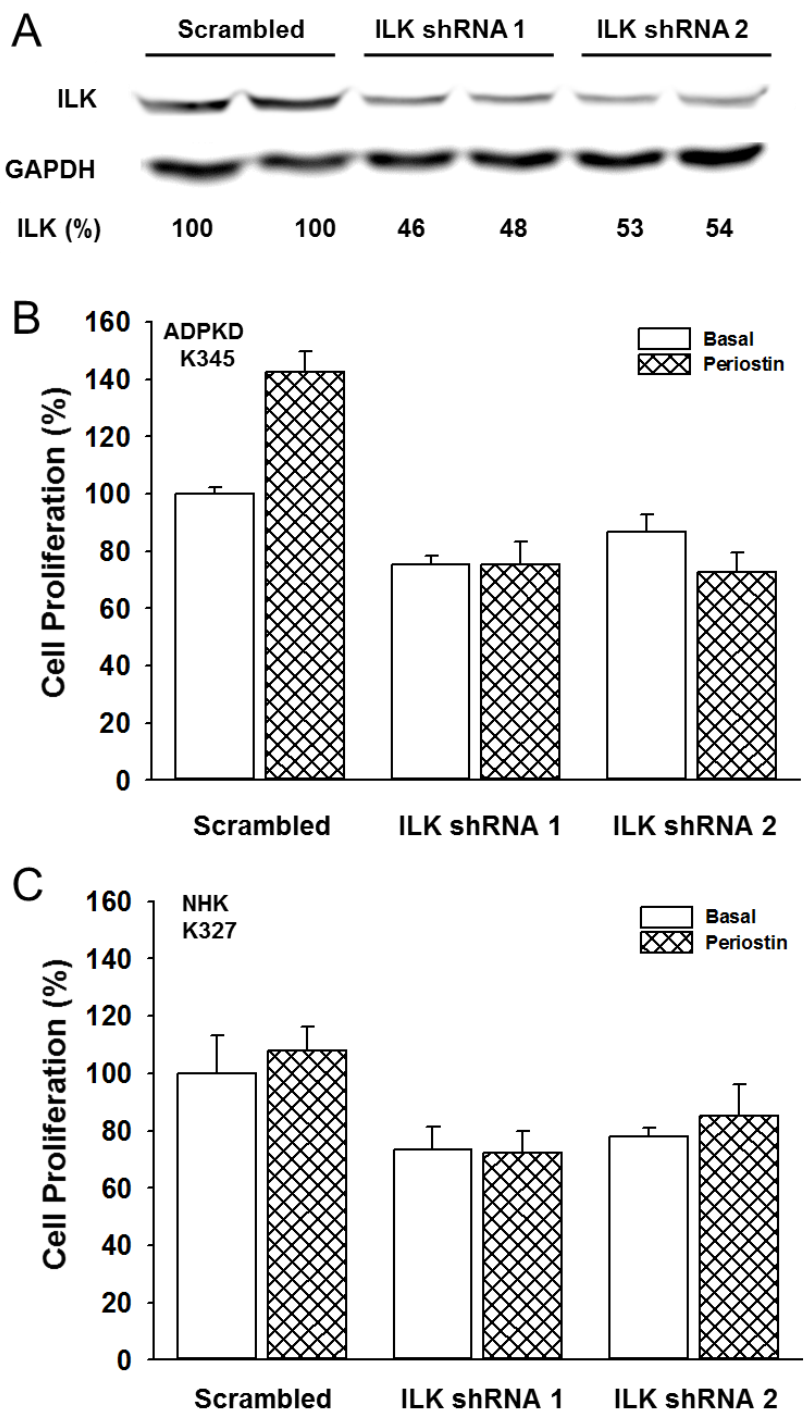
Genotype	Age	N	BW (g)	KW (g)	KW/BW (%)	Urine OsM
$Ilk^{+/+}$ CD	25 d	6	11.5 ± 0.9	0.15 ± 0.01	1.31 ± 0.07	2621 ± 116
$Ilk^{+/-}$ CD	25 d	4	13.1 ± 1.1	0.17 ± 0.02	1.32 ± 0.04	2336 ± 143
$Ilk^{-/-}$CD	25 d	5	8.7 ± 1.0	0.18 ± 0.02	2.02 ± 0.10*	655 ± 175*
$Ilk^{+/+}$ CD	10 wk	4	24.5 ± 1.8	0.33 ± 0.02	1.36 ± 0.11	3337 ± 193
$Ilk^{+/-}$CD	10 wk	3	22.6 ± 2.0	0.30 ± 0.04	1.35 ± 0.06	3066 ± 86
$Ilk^{-/-}$CD	10 wk	4	10.2 ± 1.6*	0.17 ± 0.02*	1.66 ± 0.13	535 ± 26*

Effect of collecting duct (CD)-specific knockout of ILK on body weight, kidney weight, and urine concentrating ability.Body and kidney weights and urine concentrating ability were determinedat 25 days and 10 weeksof age for wild type ILK (*$Ilk^{+/+}$ CD*), *$Ilk^{fl/+}$;Pkhd1-Cre* (*$Ilk^{+/-}$ CD*) and *$Ilk^{fl/fl}$;Pkhd1-Cre* (*$Ilk^{-/-}$ CD*) mice.To evaluate urine concentrating ability, drinking water was removed for 3 h, spot urine was collected, and urine osmolality (OsM, mosmoles/kg water) was measured using a vapor pressure osmometer.*$Ilk^{-/-}$ CD* mice had decreased body weight (BW)and lower urine osmolality(OsM, mosmoles/kg) compared to *$Ilk^{+/+}$ CD* and *$Ilk^{+/-}$ CD* mice, confirmingthat ILK ablation leads to a defect in concentrating ability of the kidneys[1]. *$Ilk^{+/-}$ CD* mice had normal BW, KW (%BW) and urine OsM.Moreover, $ILK^{+/+}$ CD and $ILK^{+/-}$CD mice (N = 1 per genotype) were aged to 51 weeks, and then BUN and KW/BW were determined. The $ILK^{+/+}$ CD mouse had a KW/BW of 1.55% and BUN of 20.7 mg/dl and the $ILK^{+/-}$CD mice had a KW/BW of 1.49% and a BUN of 17.8 mg/dl, confirming that loss of one allele of ILK does not induce renal damage. *P < 0.01, compared to age-matched*$Ilk^{+/+}$ CD*mice.



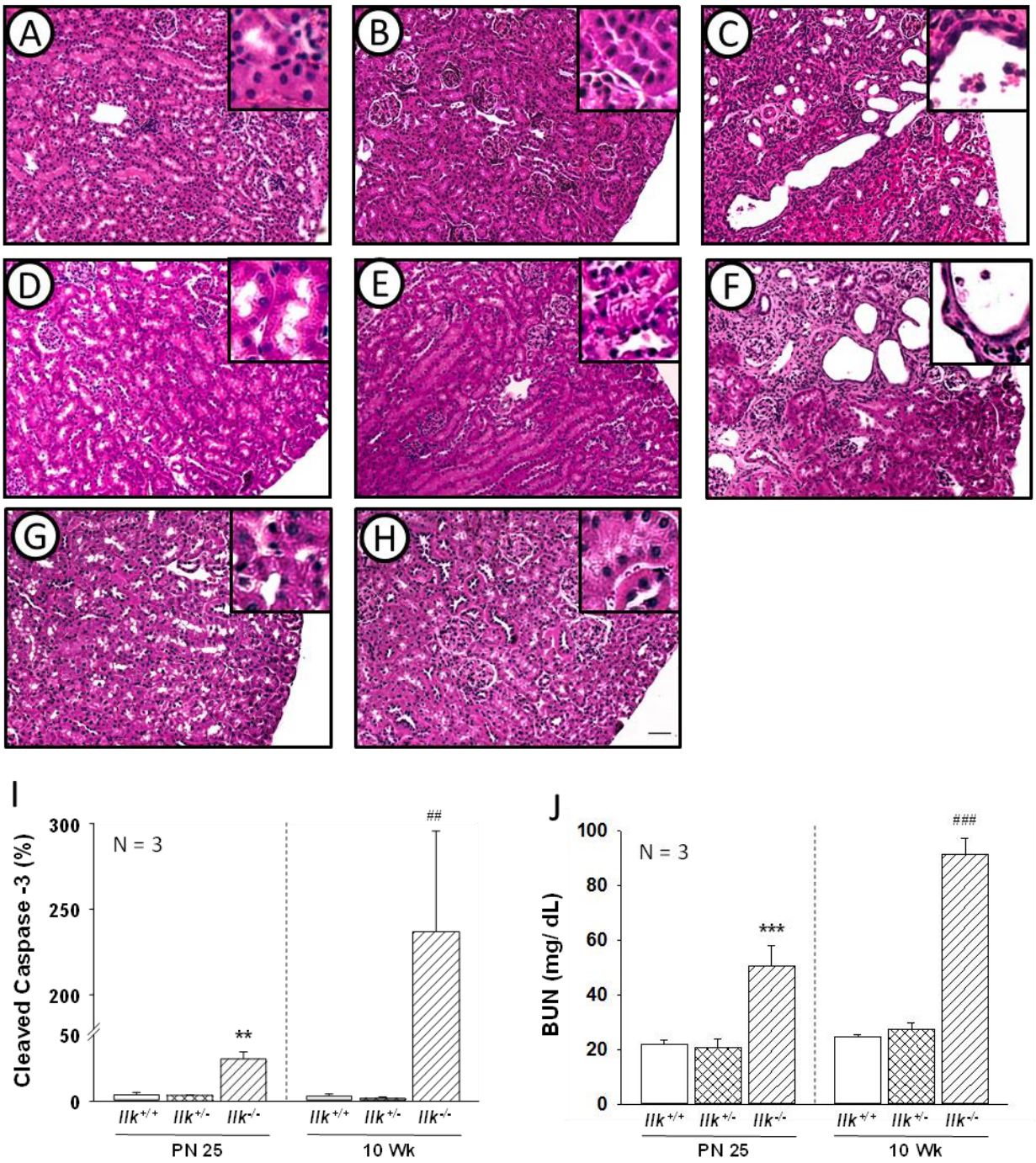
Supplemental Figure1. Effect of periostin on ILK activity in cultured ADPKD cells. (A) Primary ADPKD and normal human kidney (NHK) cells were grown in 10 cm petri dishes in DME/F12 + 1% fetal bovine serum.Numbers above the bands indicate the kidney number in the PKD biospecimen repository.Cells weregrown to confluency, and then ILK was immunoprecipitated from cell lysates using an ILK antibody attached to magnetic beads. ILK activity was determined from the phosphorylation of GSK3α/β fusion protein (P-GSK3), a substrate of ILK, in an immune complex kinase assay[2,3].The negative control (NC) represents the level P-GSK3 in a kinase assay containing ILK that was immunoprecipitated from ADPKD cells, and all other reagents, except ATP.(B) ILK activity was defined as the P-GSK3 band intensity minus the band intensity of the NC, divided by the intensity of the total ILK band.There was little basal ILK activity in NHK cells (N = 8). ILK activity appeared to beelevated in ADPKD

cells compared to NHK cells. (N = 6, †P< 0.05) (C) ADPKD cells were treated with 250 ng/ml periostin from 15 min to 24 h, and ILK activity was determined. Baseline activity (control treatment) was set to 100%. ILK activity increased as early as 15 min and remained elevated for at least 1 h.**P < 0.01, compared to control treated ADPKD cells. It is possible that other kinases, including Akt, or phosphatases were immunoprecipitated with ILK [4-6]; therefore, it remains unclear if phosphorylation of the substrates was directly due to ILK.



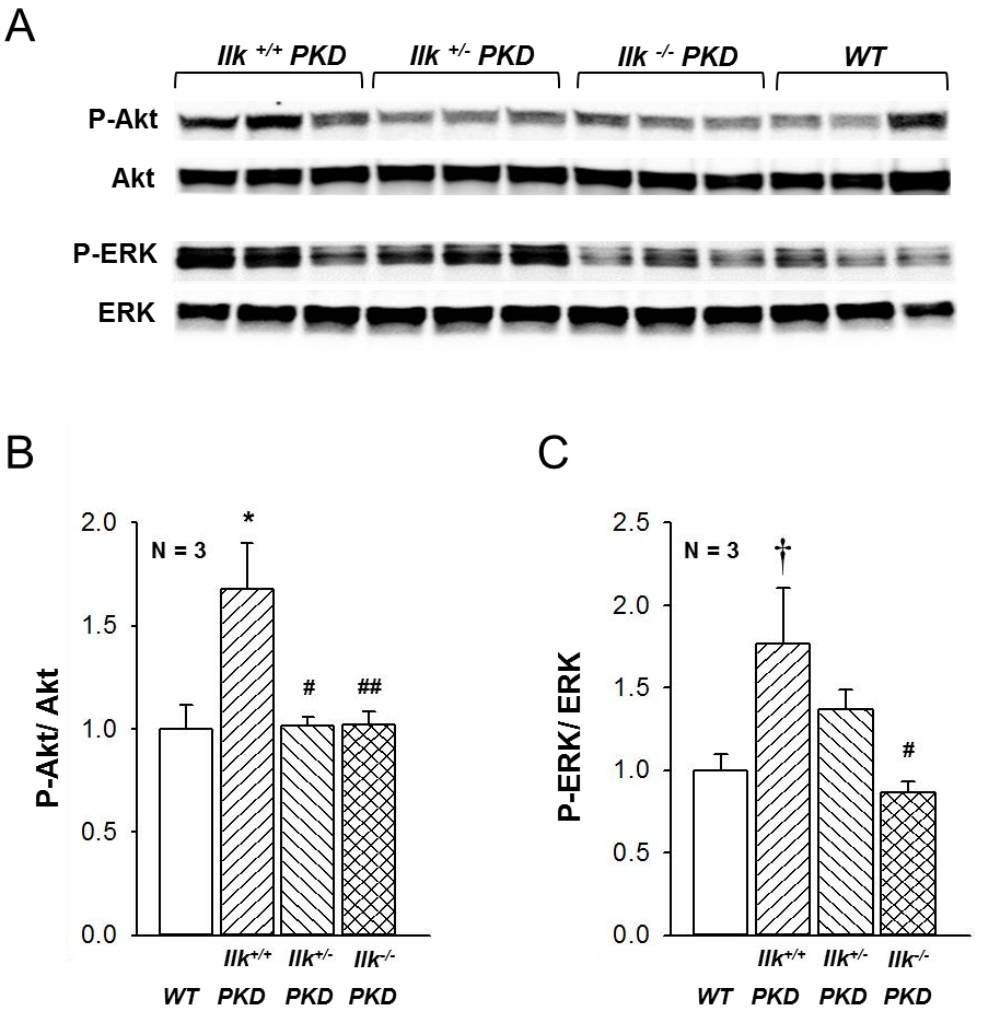
Supplemental Figure 2. Effect of lentiviral shRNA knockdown of ILK on periostin-induced proliferation.(A) Representative immunoblot of ILK in ADPKD and NHK cells after infection by lentiviral ILK shRNA (ILK shRNA 1 or ILK shRNA 2) or a non-specific (Scrambled)

sequence. Numbers below the bands indicate the percentage of ILK, relative to scrambled shRNA cells. (B) ADPKD and (C) NHK cells were infected with scrambled shRNA, ILK shRNA 1 or shRNA 2 lentivirus and plated in 24 well plates containing DME/F12 supplemented with 1% FBS.Media was reduced to 0.002% FBS for 24 h, and then 250 ng/ml periostin or control media was added to the cells.After an additional 24 h, the number of cells in each well was measured using a cell counter.Percent proliferation represents the number of cells, normalized to control-treated scrambled shRNA cells (3 wells per condition). These data confirm that ILK knockdown decreased periostin-induced proliferation of ADPKD cells, as demonstrated by MTT assay (Figure2F) and that periostin does not promote NHK cell proliferation[7].

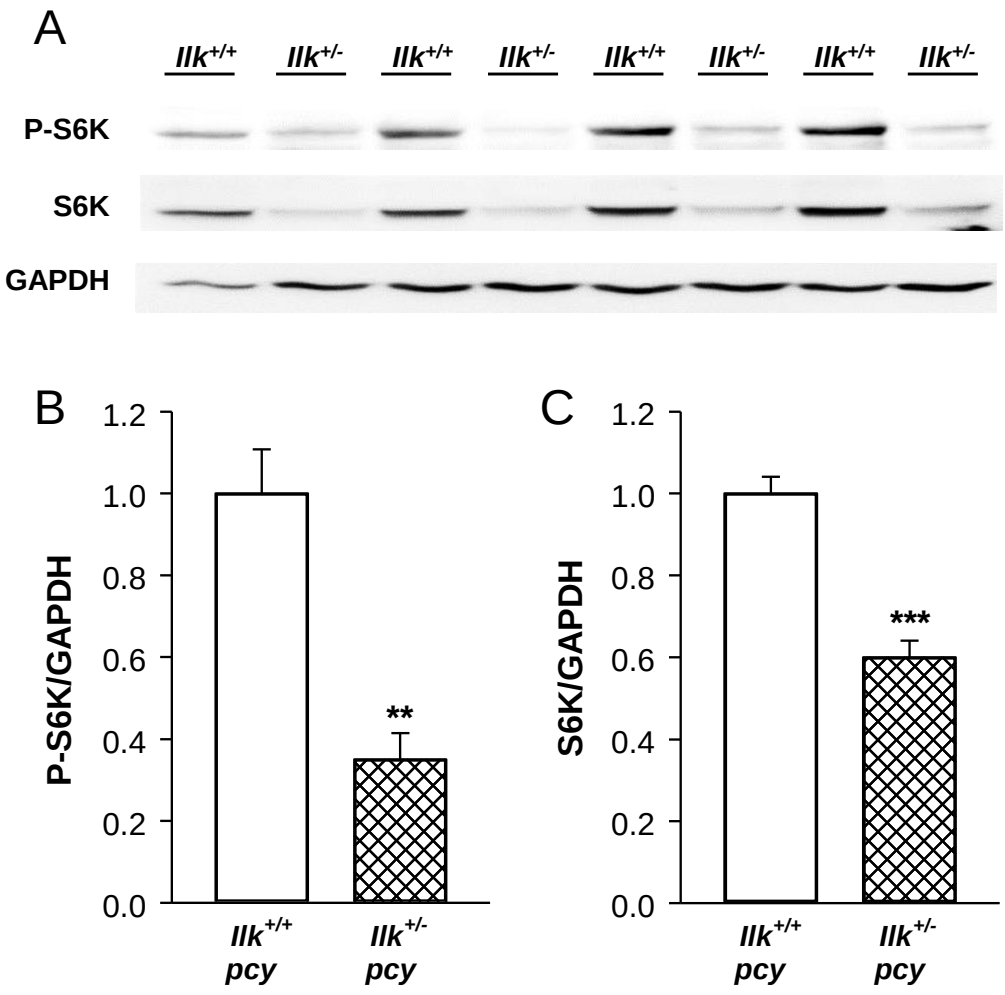


Supplemental Figure 3. Effect of collecting duct-specific ILK deletion. Kidney sections from wild type ILK (*Ilk*$^{+/+}$ *CD;*A, D, G),*Ilk*$^{fl/+}$*;Pkhd1-Cre*(*Ilk*$^{+/-}$ *CD*;B, E, H) and *Ilk*$^{fl/fl}$*;Pkhd1-Cre*(*Ilk*$^{-/-}$ *CD*;C, F) mice at 25 days (A, B, C), and 10 (D, E, F) and 51 weeks(G, H) were stained with

hematoxylin and eosin. All images are the same magnification. Scale bar = 50 μm. (I)Tissue sections were stained for cleaved caspase-3 to determine if the cellular loss of ILK causedapoptosis/anoikis[8]. The number of cleaved caspase-3 positive cells in the kidney sections were countedand represented as a percentage of non-cystic surface area (% cleaved caspase-3).(J) Blood collected from mice at postnatal day 25 (PN25) and 10 wk, were analyzed forblood urea nitrogen (BUN).



Supplemental Figure 4. Effect of ILK expression on renal P-Akt levels in PKD mice.(A) Immunoblot analysis was used to compare phosphorylated levels of renal Akt (P-Akt) and ERK (P-ERK) in *Ilk*$^{+/+}$;*Pkd1*$^{fl/fl}$;*Pkhd1-Cre*(*Ilk*$^{+/+}$ *PKD*), *Ilk*$^{fl/+}$;*Pkd1*$^{fl/fl}$;*Pkhd1-Cre*(*Ilk*$^{+/-}$ *PKD*),*Ilk*$^{fl/fl}$;*Pkd1*$^{fl/fl}$;*Pkhd1-Cre*(*Ilk*$^{-/-}$ *PKD*)andwildtype (*WT*)mice. Each lane represents a single mouse kidney lysate (N = 3 per group). Summary of the effect of CD-specific ILK expression on renal (B) P-Akt/Akt levels and (C) P-ERK/ERK levels in WT, *Ilk*$^{+/+}$ *PKD, Ilk*$^{+/-}$ *PKD* and *Ilk*$^{-/-}$ *PKD* mice.Data are means ± S.E. for kidneys from three mice per group †P= 0.05 and *P<0.01,compared to *WT*.$^{\#}$P <0.05 and$^{\#\#}$P <0.01, compared to *Ilk*$^{+/+}$ *PKD.*



Supplemental Figure 5. Effect of ILK expression on renal mTOR signaling in *pcy* kidneys. (A) Immunoblot analysis was used to compare phosphorylated S6 kinase (P-S6K) and total S6K in kidneys of 10-week old *Ilk*$^{+/+}$*;pcy/pcy* (*Ilk*$^{+/+}$), and *Ilk*$^{fl/+}$*;Pkhd1-Cre;pcy/pcy* (*Ilk*$^{+/-}$) mice. P-S6K and S6K were normalized to GAPDH, a reference protein. The bar graphs are mean ± SE for the effect of ILK expression on renal (B) P-S6K and (C) total S6K, normalized to GAPDH(N = 4 per group).Previously, total S6K and P-S6K were shown to be upregulated in *pcy* mice, relative to total protein[9]. These data show that reduced ILK expression in CDs of *pcy* mice significantly reduced P-S6K and total S6K, demonstrating that ILK is central for the aberrant mTOR activity in cystic disease. **P <0.01 and ***P <0.001, compared to *Ilk*$^{+/+}$ *pcy*.

Supplemental Methods

*Measurement of ILK kinase activity.*ILK kinase assay was performed as described [2,3] with minor modifications. Briefly, 300 µg protein was mixed with anti-rabbit IgG magnetic beads (Biomag; Polysciences, Warrington, PA) and 2 µg ILK antibody and rotated at 4° C overnight. Using a magnetic separation stand, the antibody-immune complex was isolated, washed with lysis buffer and resuspended in kinase buffer (25 mM Tris pH 7.5, 5 mM β-glycerophosphate, 0.1 mM Na_3VO_4 , 10 mM $MgCl_2$, 6 mM $MnCl_2$), 1000 µM ATP and 2.5 µg of GSK3-α/β fusion protein (CS 9237) and incubated for 1.5 hours at 30° C.The kinase reaction was stopped by adding 4× loading buffer. The beads were spun down, and antibodies to phosphorylated GSK3β (Ser 9; CS9336) or myelin basic protein (13-173; EMD Millipore) were used to determine phosphorylated levels by immunoblot analysis as described above.

References for supplemental material

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