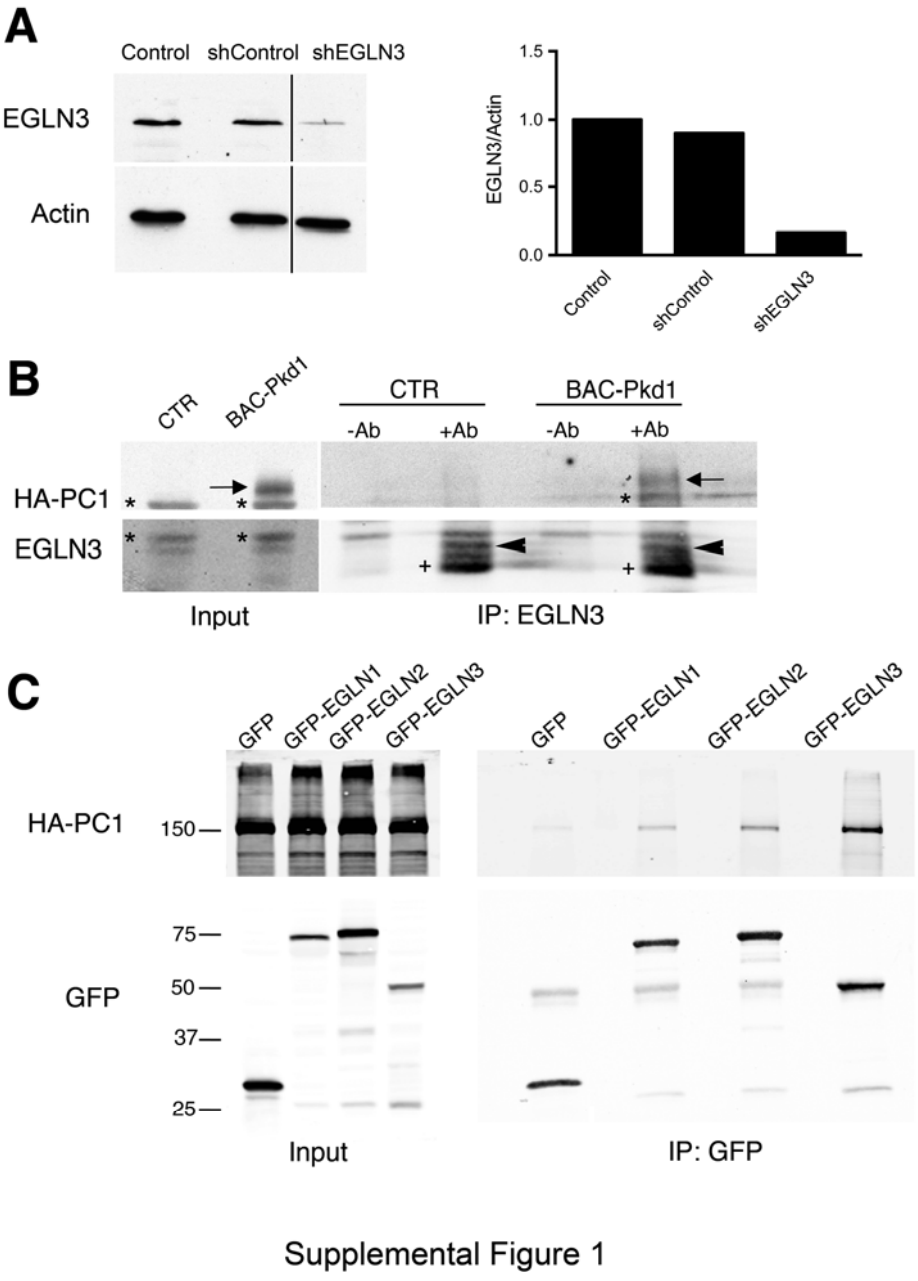
Supplemental Materials

Molecular Biology of the Cell

Padovano et al.

Supplemental Figure Legends

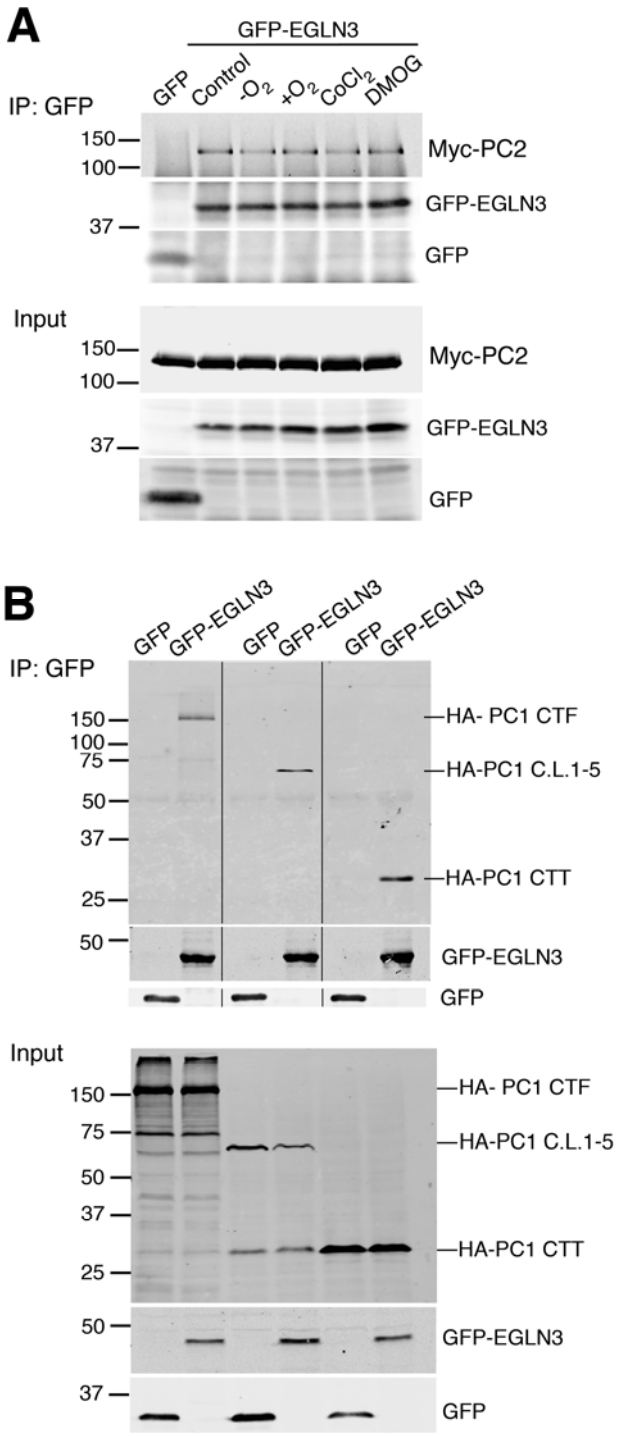


Supplemental Figure 1

Supplemental Figure 1

EGLN3 knock down and co-immunoprecipitation of EGLNs with PC1. (A) Western blot analysis of EGLN3 levels in LLC-PK_1 cells over-expressing PC1 and PC2 that were not

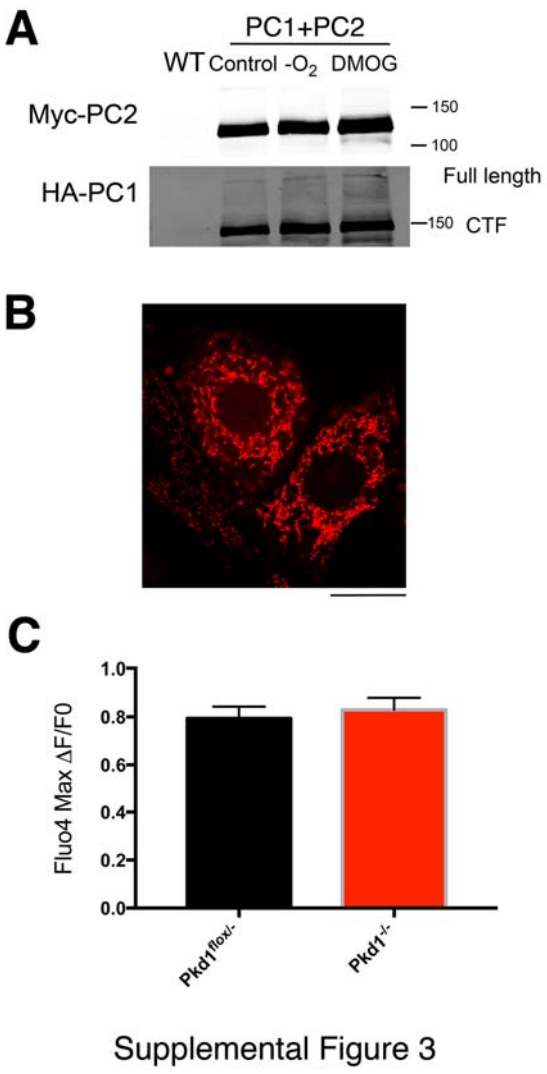
infected or were infected with retrovirus containing a scrambled shRNA (shControl) or with a retrovirus containing EGLN3 targeting shRNA (shEGLN3). Actin is detected as a loading control; the graph shows EGLN3 levels normalizedto those of actin. (B) EGLN3-PC1 co-immunoprecipitation from BAC mice. Asterisks represent non-specific bands, arrows represent specific HA-PC1 bands, arrowheads represent EGLN3 bands, and plus sign represent light chain IgG. (C) Western blot analysis of the co-immunoprecipitation of GFP tagged EGLN1, 2 and 3 with PC1.



Supplemental Figure 2

Supplemental Figure2

Polycystin-2 co-immunoprecipitates with EGLN3 (A) Co-immunoprecipitation of GFP-EGLN3 and PC2 in HEK293 cells; cells were incubated under conditions of hypoxia (-O_2) or hyperoxia (+O_2) for 2 hours or with 1mM DMOG or 200 μM $CoCl_2$for 24 hours. (B) Co-immunoprecipitation of GFP-EGLN3 and PC1 cytosolic loops 1-5 (PC1 C.L. 1-5) and the PC1 c-terminal tail (PC1-CTT). EGLN3 was co-expressed with each of the PC1 cytoplasmic domain constructs in HEK293 cells. The bottom blot depicts the level of expression of HA-tagged proteins in total lysates from the transfected cells that were used in the co-immunoprecipitation experiments. It should be noted that the band in lanes 4 and 5 that appear to co-migrate with the CTT construct (lanes 6 and 7) in our likelihood corresponds to a cleavage fragment of the C.L. 1-5 construct that coincidentally as a molecular weight similar to that of the CTT construct.

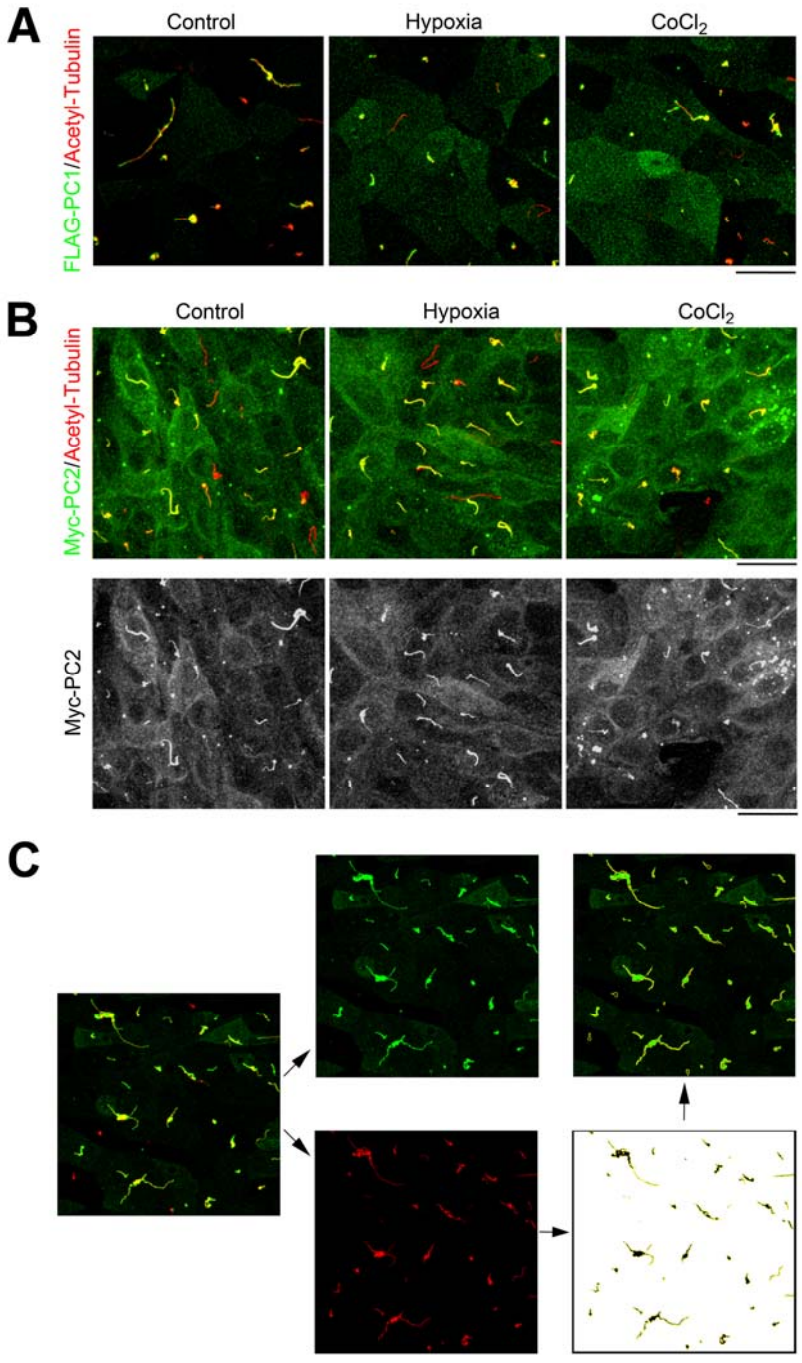


Supplemental Figure 3

Supplemental Figure3

The microsome fraction and the MAM fraction contain both of the polycystin proteins. (A) Western blot analysis of the microsome fractions isolated from LLCPK1 cells that were untransfected (WT) or transfected to over-express PC1 and PC2; prior to lysis, the cells were exposed to the indicated treatments. (B) Representative immunofluorescence image of MTS-RCaMP transfected cells. (C) Graph showing the maximum cytoplasmic Fluo4 ΔF/F0 fluorescence induced by bradykinin in $Pkd1^{flox/-}$ and $Pkd1^{-/-}$ proximal tubule

cell lines performed in the presence of extracellular calcium (n=3 experiments, 109 cells for both $Pkd1^{flox/-}$ and $Pkd1^{-/-}$ cell lines were analyzed).



Supplemental Figure 4

Supplemental Figure4

Acetylated tubulin staining was used to define the ciliary and apical population of PC1 and 2 for the purpose of image quantification. Acetylated tubulin staining was used to create a mask that defines the ciliary outline. This mask was used to measure the intensity of the cilia associate pools of PC1 and 2. Our labeling techniques detect only the surface population of PC1 (A), and the remaining surface pool of PC1 that was not associated with cilia was defined as apical PC1. Since PC2 staining was performed on permeabilized cells (B), we were unable to separately distinguish apical from intracellular staining and thus quantitate only the signal intensity associated with cilia. (C) Example of ciliary mask generation and overlay on surface PC1 staining. The image on the left was divided in its red and green component images. ImageJ was used to generate a mask corresponding to the pattern of ciliary staining as detected in the red channel using an antibody directed against acetylated tubulin. The mask was then applied to image in the green channel to quantitate fluorescence signal associated with the ciliary population of polycystin proteins. Immunofluorescence labeling was performed on LLC-PK_1 cellsuntreated or treated with hypoxia or $CoCl_2$ A maximum intensity projection of a z-stack taken with a 0.69 μm step is shown. Bars 20 μm.

Supplemental Experimental Procedures

Cell lines and antibodies

LLC-PK_1 cells and HEK293 cells were maintained as previously described (Joly *et al.*, 2006; Chapin *et al.*, 2010). Stable expression of PC1 (carrying a Flag tag appended to its N-terminus and an HA tag appended to its C-terminus) and PC2 (carrying a Myc-tag appended to its C-terminus) in LLC-PK_1 and HEK293 has been previously described (Chapin *et al.*, 2010).

The following antibodies and labeling reagents were used: anti-HA (Roche, Indianapolis, IN), anti-FLAG and anti-actin (Sigma-Aldrich, St. Louis, MO),anti-cMyc (polyclonal from Sigma-Aldrich; monoclonal from Santa Cruz Biotechnology, Santa Cruz, CA), anti-Acetylated-alpha-Tubulin (polyclonal from Cell Signaling Technology; monoclonal from Santa Cruz Biotechnology), anti-GFP (MBL, Woburn, MA), anti-OH-Proline (Abcam, Cambridge, MA). The following secondary reagents were used: anti-mouse and anti-rabbit Alexa Flour 594 and 488 (Invitrogen, Carlsbad, CA), anti-rat HRP and anti-rabbit HRP (Jackson ImmuoResearch Laboratories, West Grove, PA), anti-mouse, anti-rabbit and anti-rat 680RD and 800CW IRDyes (LI-COR, Lincoln, NE).

Immunofluorescence

LLC-PK_1 cells were grown on polycarbonate Transwell Permeable Supports (Corning, Corning, NY) and processed 4 days after reaching confluence. To stain surface PC1

protein (Chapin *et al.*, 2009), the cells were incubated for 1 h in a humidified chamber at 4°C with polyclonal anti-FLAG antibody diluted in a blocking buffer of 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) supplemented with 100 nM $CaCl_2$ and 1 mM $MgCl_2$ (PBS++). Cells were then washed with PBS++, fixed for 1 hour in 4% paraformaldehyde at 4°C, permeabilized in PBS++ with 0.3% TritonX-100 and 0.1% BSA, and blocked for 30 min in goat serum dilution buffer (GSDB; 16% goat serum, 120 mM sodium phosphate, 0.3% Triton X-100, and 450 mMNaCl)(Chapin *et al.*, 2009). They were then incubated for 1 hour in a humidified chamber with the monoclonal anti-Acetylated-alpha-Tubulin primary antibody diluted in GSDB, washed, and incubated for 1 h with the Alexa Fluor-conjugated secondary antibodies diluted in GSDB. For immunofluorescence analysis without surface labeling, cells were fixed for 20 minutes in 4% paraformaldehyde after washing with PBS++ and then permeabilized and processed as described above. Images were acquired using a 63x objective on a Zeiss LSM 780 confocal microscope and analyzed with Zen 2010 software.

EGLN3 Knock Down

To knock down EGLN3 expression in LLC-PK1 cells, we used the sequence of one of the siRNAs that was identified in the genome wide screen (GGAGCCGGCTGGGCAAATA). This sequence is directed against a portion of the EGLN3 mRNA that is conserved in pig. This sequence did not have any significant homology to other genes in the pig genome database. The forward and reverse oligos were annealed and cloned into the BglII–HindIII restriction sites of the pSUPER.retro.puro RNAi system (OligoEngine, Seattle, WA), and the resulting plasmid

was amplified. All of the plasmid sequences were verified by means of automated sequencing to exclude unwanted substitutions. Virus production was performed as previously described by co-tranfecting the generated pSUPER.retro.puro EGLN3 shRNA together with VSV-G(Schuck *et al.*, 2004). LLC-PK_1 cells overexpressing PC1 and PC2 were infected with the virus and positive cells were selected with 4 μg/ml puromycin (Invitrogen). The efficiency of knock down was tested by western blotting with antibody directed against EGLN3, and EGLN3 levels were normalized to those of actin.

Live cell mitochondrial calcium imaging

$Pkd1^{-/-}$ and $Pkd1^{flox/-}$ mouse renal cells were plated at subconfluent density on poly-L-lysine coated glass bottom microwells (Corning) for 24 hours. Cells were then transiently transfected with MTS-RCaMP1h (generous goft of Dr. A. Aperia, Karolinska Institute, Stockholm, Sweden) usingLipofectamine 2000 Reagent (Thermo Fisher Scientific) and imaged 24 hours post transfection in calcium free HEPES buffer. To measure mitochondrial calcium, cells were washed with and imaged in a calcium-free HEPES buffer at 37°C pH 7.4. The calcium-free HEPES buffer consisted of 118 mMNaCl, 5.3 mM KCL, 0.8 mM MgSO4, 14 mM Na-Hepes, 5.6 mM D-glucose and 1.0 mM EGTA. The microwell containing cells was placed on top of a Zeiss LSM 780 confocal microscope and time-dependent changes in fluorescence were measured by acquiring images using an inverted 60x magnification objective and a Rhodamine 549nM laser. Data was analyzed using Zen 2010 software and ImageJ. The following calcium agonists were applied to the imaging buffer/directly to the bath, 1uM Bradykinin Acetate Salt

(Sigma) dissolved in deionized water and 1uM Ionomycin from Streptomyces conglobatus (Sigma) dissolved in DMSO.

Data analysis

MTS-RCaMP transfected cells were selected individuallyusing the region of interest (ROI) tool in ImageJ and arbitrary fluorescence units (AFU) were measured based on mean pixel intensity. We calculated the ΔF/F0 for each ROI, which represents the change in fluorescence (ΔF) normalized to baseline fluorescence (F0).

Live cell cytosolic calcium imaging

Pkd1$^{-/-}$ and Pkd1 $^{flox/-}$ mouse renal cells were plated at subconfluent density on poly-L-lysine coated glass bottom microwells (Corning) for 24 hours. Cells were washed with a calcium-containing HEPES buffer pH 7.4 (118 mMNaCl, 5.3 mM KCL, 1.8 mM $CaCl_2$, 0.8 mM MgSO4, 14 mM Na-Hepes, 5.6 mM D-glucose, 5mM probenecid) and incubated in the same buffer containing 5μM Fluo-4 AM (ThermoFisher Scientific) for 30 minutes. The cells were imaged using an inverted 60x magnification objective mounted on a Zeiss LSM 780 confocal microscope. Changes in fluorescence were measured and analyzed using ImageJ as described for the mitochondrial calcium uptake experiments.

Generation and expression of plasmids encoding PC1 Cytosolic Loops 1-5 and the PC1 CTT

The PC1 cytosolic loops 1 (corresponding to amino acids 3087-3273), 2 (corresponding to amino acids 3336-3550), 3 (corresponding to amino acids 3593-3664), 4 (corresponding to amino acids 3907-3929), and 5 (corresponding to amino acids 3990-4018) were amplified by PCR from the mouse PC1 construct used for the generation of the stable cell lines (Chapin *et al.*, 2010). In subsequent rounds of PCR, these PCR products were connected to one another by a sequence encoding flexible linker (Ggtggaggaggttctggaggcggtggaagtggtggcggaggtagc, 3xGGGGS). An HA tag was added to the N-terminus of the protein, and the PC1 C.L. 1-5 construct thus generated was subcloned into the HindIII-XbaI sites of the pcDNA3.1/Zeo+ vector. All primer sequences are available upon request. The PC1 CTT plasmid has been previously described (Merrick *et al.*, 2012). EGLN3 was co-expressed by transient transfection with either PC C.L. 1-5 or the PC1-CTT in HEK cells and immunoprecipitation was performed as described below.

Immunoprecipitation

For PC1 immunoprecipitation (IP), HEK293 cells stably expressing PC1 and PC2 were lysed by probe sonication (3 pulses of 10 seconds at 40% power at 4^{o} C) in lysis buffer (100 mMNaCl, 5 mM EDTA, 50 mMTris pH 7.4, 0.5% NP40) plus protease inhibitor cocktail (Roche). Precleared lysates (13,000 × g, 30 min) were incubated at 4°C overnight with monoclonal-anti-HA agarose beads (Sigma). Beads were collected by centrifugation, and the pellets were washed in lysis buffer four times for 10 min with rotation at 4°C. Immunoprecipitates were eluted in SDS-PAGE loading buffer (25 mMTris-HCl [pH 6.7], 10% glycerol, 1% SDS, 50 mM DTT, bromophenol blue).

PC1 immunoprecipitation from kidneys expressing HA-tagged PC1 was performed as previously described(Wodarczyk *et al.*, 2009). For PC1-EGLN3 coIP, HEK293 cells stably expressing PC1 and PC2 were transiently transfected with the pEGFP-N1-PHD3 plasmid(Metzen *et al.*, 2003) (Addgene plasmid 21402) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. After treatments, cells were lysed and incubated at 4°C overnight with protein A conjugated agarose beads (Thermo Fisher Scientific, Rockford, IL) pre-incubated with anti-GFP polyclonal antibody (MBL). Beads were then collected and washed as described above.

Cell Surface Biotinylation

LLC-PK1 cells over-expressing PC1 and PC2 were plated at confluent density on polycarbonate Transwell Permeable Supports (Corning) and allowed to grow for 5 days. Cells were washed 3 times with ice-cold PBS++. Ice-cold biotinylation buffer (10mM Triethanolamine, 2 mM $CaCl_2$, 125 mMNaCl, pH8.9) containing 1 mg/ml biotin (EZ-Link Sulpho-NHS-SS_Biotin, Thermo Scientific) was added to only the apical side of the Transwell filter, while biotinylation buffer alone was added on the basolateral side. One well was incubated with no biotin as a control. Plates were incubated on ice at 4°C with slow rocking for 25 minutes, after which biotin solution was replaced with fresh solution and the incubation was continued for 25 more minutes. Cells were then incubated twice for 2 x 10 minutes in quenching solution (100 mM glycine in PBS++) on ice at 4°C with slow rocking. After 4 washes in PBS ++, cells were collected in lysis buffer (25 mMTris pH 7.4, 100 mMNaCl, 1 mM MgCl2, 1% Tx100, 0.1% SDS, 5 mM EDTA plus protease inhibitor cocktail) and centrifuged for 20 min at 14,000 rpm at 4°C. Supernatants were

incubated with strepavidin agarose beads (Thermo Scientific) and rotated overnight at 4°C. Beads were then washed 5 times in lysis buffer and proteins were eluted in 4x SDS-PAGE loading buffer.

Mouse strain

The generation of the Pkd1-BAC and Pkd1-HA knock-in mice has been previously described(Wodarczyk *et al.*, 2009; Fedeles *et al.*, 2011). All animals were used in accordance with scientific, humane and ethical principles and in compliance with regulations approved by the Yale University Institutional Animal Care and Use Committee.

Ciliary staining quantification

Ciliary PC1 and PC2 staining levels were quantified on immunofluorescence images from cells co-stained for Acetylated Tubulin and for Flag or Myc (Supplemental Figure 4), respectively, using ImageJ. A mask identifying the primary cilia was generated based upon the Acetylated Tubulin staining using the threshold command. Each cilium was selected using the magic wand tool and the resulting selection was applied to the Flag or Myc staining followed by the measure command to quantify total and mean pixel intensity.

Isolation of mitochondria-associated ER membranes (MAMs)

MAMs and mitochondria were isolated from the kidneys of Pkd1-BAC mice as previously described (Wieckowski *et al.*, 2009).

Mass Spectrometry

In-gel digestion

PC1 was immuno-purified from HEK 293 cells (four 100 mm petri dishes) stably transfected with Flag-PC-HA and PC2 using anti-HA conjugated agarose beads (Sigma). The immunoprecipitated protein was separated by SDS-PAGE, lightly stained with Coomassie Brilliant Blue, and the band at 150 kDa corresponding to the PC1 C-terminal fragment was excised and subjected to in-gel digestion with trypsin according to a protocol by Shevchenko(Shevchenko *et al.*, 2006). Briefly, gel pieces were cut into small cubes of 1×1 mm diameter, washed twice with 200 µl 50 % acetonitrile (ACN)/50 mM NH_4HCO_3 (AMBIC) and once with 50 % ACN/10 mM AMBIC followed by dehydration of the gel pieces with 500 µl ACN. The remaining solvent was removed in a rotary vacuum centrifuge. Dried gel pieces were rehydrated for ≥1 h on ice after covering the gel pieces with a solution of 13.3 ng/µl sequencing grade trypsin in 90 % 50 mM AMBIC/10 % ACN. Digestion proceeded for 16 h at 37 °C. Gel pieces were extracted once with 200 µl of 1:2 (by volume) 5 % formic acid (FA)/ACN and the extract was dried in a rotary vacuum centrifuge. Finally, peptides were desalted using stage tips constructed with two 1.06 mm diameter C_{18} Empore Disk membrane discs (3M St. Paul,

MN) in a 200 µl pipette tip as described(Rappsilber *et al.*, 2003). Peptides were reconstituted in 20 µl of a mixture of 3:8 (by volume) of 70 % FA/0.1 % TFA and stored at -80 °C until analysis.

Liquid chromatography and mass spectrometry

Nano liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed on a nanoAcquity UPLC (Waters) connected to aOrbitrapVelos mass spectrometer (Thermo Fisher Scientific). Details of this setup are described elsewhere(Lajoie *et al.*, 2013). In short, 4 µl of desalted digest was injected for analysis. Eluent A consisted of 0.1 % FA and eluent B was 0.1 % FA in ACN. Peptides were eluted from the column using a 90 or 200 min linear gradient program from 1 to 50 % eluent B with a brief hold at 95 % B at the end of the gradient. The mass spectrometer was operated in data-dependent acquisition mode with a top10 HCD (Higher Collisional Energy Dissociation) method. Precursor ion scans were acquired over the mass range of 298-1700 Da with a resolving power of 30,000 or 7,500 for the fragment ion scans, respectively. Methyl stearate was introduced into the ion source for lock mass calibration(Pelander *et al.*, 2011). Dynamic exclusion was programmed to collect up to two scans of peptides within 30 s and then excluding the peptide from further analysis for 60 s. Singly charged peptides were also excluded from the analysis.

Data analysis

Raw files were processed and searched with MaxQuant(Neuhauser *et al.*, 2012) software version 1.4.1.2 using the Andromeda search engine. Recalibrated MS/MS spectra were first matched against the Homo sapiens UniProt (release 2013/10) protein database with 39,670 reviewed protein sequences and protein isoforms. The NCBI reference sequence NP_038658.1 for Pkd1 from Mus musculus was appended to this database. Processing and database searching was performed with the default software settings for MaxQuant. The precursor and MS/MS mass tolerance was set to 6 ppm and 20 ppm respectively. Trypsin/P was selected as the proteolytic enzyme and up to 3 missed cleavages were allowed. Peptides with less than 7 amino acids were excluded from the analysis. The decoy mode was set to revert and the PSM, Protein, and Site false discovery rate (FDR) were at 0.01 (1 % FDR). Modified peptides required a minimum score of 40 and a minimum delta score of 17 to be reported. All searches were performed with the variable modifications oxidation(M) and carbamidomethyl(C). The initial search identified 622 proteins containing at least 1 razor peptide. These proteins were used to generate a concatenated database that was used for in-depth mining of the data for posttranslational modifications including the acrylamide modification propionamide(C), deamidation(NQ) oxidation(P), phosphorylation(STY), glutathione(C), S-nitrosylation(C), sulfinic acid(C), sulfenic acid(C) and sulfonic acid(C). Proteins matching known contaminants or the decoy database were filtered with Perseus software(Cox and Mann, 2012) version 1.4.0.20. Modified peptides were manually inspected to validate the correct site assignment of the modification. Only the highest scoring peptide was reported for each peptide.

Genome Wide siRNA screen

PC1 localizes to the apical and ciliary membrane as well as to intracellular compartments (Chapin and Caplan, 2010) and undergoes cleavages at both its N- and C-terminal domains (Qian *et al.*, 2002), however the regulation and mechanisms involved in its trafficking and C-terminal tail (CTT) cleavage are poorly understood. We designed a cell-based, high-throughput assay to simultaneously quantify the relative amount of PC1 present in each of three localization/cleavage states: PC1 at the plasma membrane, total PC1 protein in the entire cell, and nuclear localization of the cleaved PC1 C-terminal fragment. We used HEK293 cells overexpressing both a full-length cDNA construct encoding mouse PC1 that contains both an N-terminal FLAG tag and a C-terminal triple-hemagglutinin (HA) tag (Grimm *et al.*, 2003) and a cDNA encoding c-Myc-tagged PC2 (Chapin *et al.*, 2010). For siRNA screening, we used the DharmaconSmartPool human siRNA library**,** which covered 13,394 genes in triplicate, 3,262 genes in duplicate and 678 genes in singlicate. Plates were run in triplicate, with each plate including Pkd1 and Pkd2 siRNA as a positive control and RISC-free siRNA as a negative control. Cells were transfected with siRNA by reverse transfection: 10µl of 100 nM siRNA in siRNA buffer (Thermo Scientific) were added in each 384-well assay plate (Nexus Biosystem, Poway, CA) followed by 10 µl of RNAiMAX (Invitrogen) diluted 1:100 in Opti-MEM (Invitrogen). Cells were added to each well after 20 minutes and incubated at 37°C for 48 hours. The immunofluorescence-based analysis protocol involved incubation with polyclonal anti-FLAG antibody (Sigma-Aldrich) added to the extracellular media, followed by fixation, permeabilization and incubation with a monoclonal anti-HA antibody (Covance Research Products, Princeton, NJ), followed by a final incubation with anti-rabbit Alexa488 and anti-mouse Alex594, as previously described (Chapin *et*

al., 2009). This final step in the staining protocol included Hoescht 33342 (Sigma-Aldrich) to stain nuclei. Wells were then imaged using the Opera Confocal Imager (Perkin Elmer, Waltham, MA), taking a single image that included the nucleus and the plasma membrane. Images were analyzed using the Acapella software package (Perkin Elmer). For each well the total pixel intensities were calculated for the fluorescence from the surface anti-FLAG antibody, cytoplasmic anti-HA antibody and nuclear anti-HA antibody. Nuclear fluorescence signal was defined as pixels that overlapped with pixels from the Hoechst nuclear stain, while all other HA fluorescence was designated cytoplasmic signal. These quantifications were then employed in calculations of FLAG:cytoplasmic HA signal (to calculate the relative amount of polycystin-1 protein present at the plasma membrane) and nuclear:cytoplasmic HA signal (to determine what portion of the C-terminal tail had been cleaved and translocated to the nucleus). We used a sample-based normalization, using the samples themselves as *de facto* negative controls, and a robust z score for each ratio comparison to identify significant hits. The robust z score threshold for significance at the $p \leq 0.05$ level is given in the table below. Genes whose knock down negatively affected the surface localization or C-terminal tail cleavage and nuclear translocation were identified as "inhibitors", whereas genes whose knock down had a positive effect were identified as "potentiators". All replicates were inspected to ensure that the hit was repeated in each internal repeat, with replication suggesting the effects were on-target rather than non-specific. The majority of the genes that decreased overall PC1 levels when knocked down were genes involved with translation initiation and elongation. In keeping with published observations of the interaction between PC1 and 2, knocking down Pkd2 caused both a decrease in PC1

surface localization (Chapin *et al.*, 2010) and an overall decrease in the level of cytoplasmic PC1. The identification of Pkd2 in our results validated our screen design and suggested that additional findings would be relevant to PC1 trafficking. Hits selected based upon the magnitude and reproducibility of their Z-scores were re-assayed through a second round of validation screening run in duplicate using 4 un-pooled siRNAs for each gene, after which 155 were selected for further study. The Robust z score for EGLN3 was 5.38. A complete description of the nature and activities of other hits from the screen will be presented in a subsequent publication.

	Robust z Score FLAG:HA_cytosol	Robust z Score HA_Nucleus:HA_Cytosol	Robust z Score HA_Cyt
Inhibitors	<-2.66	<-2.17	<-2.07
Potentiators	>2.78	>3	>4.1

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Supplemental Table. 1. Identification of polycystin-1 proline hydroxylation sites by Mass Spectrometry

Sequence	Modified Residue	Score	PEP	Mass Error [ppm]	Charge	m/z
NSLDIFQIATPHSLGSVWK	P3165	71.6	1.789E-06	0.012	3	710.37
HIWLSIWDRPPR	P3262, P3263	69.7	4.811E-03	0.037	3	536.62
HIWLSIWDRPPR	P3263	46.8	6.854E-02	-0.765	3	531.29
VRPPHGFALFLAK	P3638, P3639	93.6	1.311E-04	-0.580	3	495.62
VRPPHGFALFLAK	P3638	58.2	2.444E-02	-1.635	2	734.92
GELYRPAWEPQDYEMVELFLR	P4110, P4114	93.5	1.202E-06	0.267	3	891.76
PAWEPQDYEMVELFLR	P4110, P4114	53.6	4.241E-03	0.534	3	685.66
VSLWPNNK	P4284	77.9	6.259E-03	-0.264	2	487.26

Underlined residues indicate proline hydroxylation. For peptides identified multiple times only the highest scoring hit is reported. Modified residue numbers correspond to the mouse polycystin 1 sequence (NP_038658.2). PEP=posterior error probability