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4 **A plasma membrane-localized polycystin-1/polycystin-2 complex**
5 **in endothelial cells elicits vasodilation**
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Abstract

Polycystin-1 (PC-1, PKD1), a receptor-like protein expressed by the *Pkd1* gene, is present in a wide variety of cell types, but its cellular location, signaling mechanisms and physiological functions are poorly understood. Here, by studying tamoxifen-inducible, endothelial cell (EC)-specific *Pkd1* knockout (*Pkd1* ecKO) mice, we show that flow activates PC-1-mediated, Ca^{2+} -dependent cation currents in ECs. EC-specific PC-1 knockout attenuates flow-mediated arterial hyperpolarization and vasodilation. PC-1-dependent vasodilation occurs over the entire functional shear stress range and via the activation of endothelial nitric oxide synthase (eNOS) and intermediate (IK)- and small (SK)-conductance Ca^{2+} -activated K^+ channels. EC-specific PC-1 knockout increases systemic blood pressure without altering kidney anatomy. PC-1 coimmunoprecipitates with polycystin-2 (PC-2, PKD2), a TRP polycystin channel, and clusters of both proteins locate in nanoscale proximity in the EC plasma membrane. Knockout of either PC-1 or PC-2 (*Pkd2* ecKO mice) abolishes surface clusters of both PC-1 and PC-2 in ECs. Single knockout of PC-1 or PC-2 or double knockout of PC-1 and PC-2 (*Pkd1/Pkd2* ecKO mice) similarly attenuates flow-mediated vasodilation. Flow stimulates non-selective cation currents in ECs that are similarly inhibited by either PC-1 or PC-2 knockout or by interference peptides corresponding to the C-terminus coiled-coil domains present in PC-1 or PC-2. In summary, we show that PC-1 regulates arterial contractility through the formation of an interdependent signaling complex with PC-2 in endothelial cells. Flow stimulates PC-1/PC-2 clusters in the EC plasma membrane, leading to eNOS, IK channel and SK channel activation, vasodilation and a reduction in blood pressure.

Introduction

Blood vessels are lined by endothelial cells, which regulate several physiological functions, including contractility, to control regional organ flow and systemic pressure. Endothelial cells can release several diffusible factors, including nitric oxide (NO), which relaxes arterial smooth muscle cells, leading to vasodilation (1). Endothelial cells also electrically couple to smooth muscle cells and directly modulate their membrane potential to modify arterial contractility (2). Several receptor agonists, substances and mechanical stimuli, such as intravascular flow, are known to act in an endothelial cell-dependent manner to regulate arterial functions. In many cases, the molecular mechanisms by which these physiological stimuli activate signaling in endothelial cells to modulate vascular contractility are unclear.

Polycystin-1 (PC-1, PKD1) is a receptor-like transmembrane protein encoded by the *Pkd1* gene (3). PC-1 is expressed in various cell types, including endothelial cells, and is predicted to form eleven transmembrane helices, an extracellular N-terminus and an intracellular C-terminus (3-8). The PC-1 N-terminus is large (>3000 amino acid residues) and contains multiple putative adhesion- and ligand-binding sites (3, 7-11). As such, PC-1 is proposed to act as a mechanical sensor and ligand receptor, although stimuli that activate PC-1 and its functional significance are unclear.

Endothelial cells also express polycystin-2 (PC-2, PKD2), a protein encoded by the *Pkd2* gene (6). PC-2 is a member of the transient receptor potential (TRP) channel family and is also termed TRP polycystin 1 (TRPP1) (12). PC-1 and PC-2 have been proposed to signal through independent and interdependent mechanisms, with much of this knowledge derived from experiments studying recombinant proteins and cultured cells (9, 13). Supporting their independence, PC-1 and PC-2 exhibit distinct developmental and expression profiles in different kidney cell types (14). PC-2 channels do not require PC-1 to traffic to primary cilia and generate currents in primary-cultured kidney collecting duct cells (15, 16). PC-1 is also proposed to act as

an atypical G protein-coupled receptor (17). In addition, C- and N-terminus-deficient PC-2 can alone form a homotetrameric ion channel with each subunit containing six transmembrane domains, when visualized using cryo-EM (18, 19). Evidence supporting PC-1 and PC-2 interdependency includes that mutations in either *Pkd1* or *Pkd2* result in autosomal dominant polycystic kidney disease (ADPKD), the most prevalent monogenic disorder in humans (20). ADPKD is typically characterized by the appearance of renal cysts, but patients can develop hypertension before any kidney dysfunction and cardiovascular disease is the leading (~50 %) cause of death in patients (21-25). Experiments studying recombinant proteins and cultured kidney cell lines have provided evidence that PC-1 and PC-2 can exist in a protein complex (11, 26-32). Several domains in PC-1 and PC-2 may physically interact, including their C-terminus coiled-coils (26, 27, 29, 30, 33). The structure of a PC-1/PC-2 heterotetrameric complex, which forms in a 1:3 stoichiometry, has also been resolved using cryo-EM (26). Despite more than two decades of research, signaling mechanisms and physiological functions of PC-1 and PC-2 and their potential dependency or independence are poorly understood, particularly in extra-renal cell types, such as endothelial cells.

Here, we generated inducible, cell-specific PC-1 knockout (*Pkd1* ecKO) mice to investigate signaling mechanisms and physiological functions of PC-1 in endothelial cells of resistance-size arteries. We also studied endothelial cell-specific PC-2 knockout (*Pkd2* ecKO) mice and produced PC-1/PC-2 double knockout (*Pkd1/Pkd2* ecKO) mice to investigate whether PC-1 acts in an independent manner or is dependent on PC-2 to respond to physiological stimuli and elicit functional responses. Our data demonstrate that PC-1 and PC-2 protein clusters form an interdependent plasma membrane complex in endothelial cells which is activated by flow to produce vasodilation and reduce blood pressure.

Results

Generation and validation of tamoxifen-inducible, endothelial cell-specific *Pkd1* knockout mice

Mice with loxP sites flanking exons 2-4 of the *Pkd1* gene (*Pkd1^{fl/fl}*) were bred with tamoxifen-inducible endothelial cell-specific Cre mice (*Cdh5(PAC)-CreERT2*) to generate *Pkd1^{fl/fl};Cdh5(PAC)-CreERT2* (*Pkd1* ecKO) mice. Genomic PCR indicated that tamoxifen (i.p.) stimulated recombination of the *Pkd1* gene in mesenteric arteries of *Pkd1* ecKO mice but did not modify the *Pkd1* gene in arteries of *Pkd1^{fl/fl}* controls (Figure 1 – figure supplement 1). Western blotting demonstrated that tamoxifen treatment reduced PC-1 protein in mesenteric arteries of *Pkd1* ecKO mice to ~66.7 % of that in *Pkd1^{fl/fl}* control mice (Figure 1A-B). This reduction in PC-1 protein in *Pkd1* ecKO mice is expected given that arterial smooth muscle cells also express PC-1 (34). In contrast, tamoxifen did not alter the expression of PC-2, small-conductance Ca^{2+} -activated K^+ (SK3) channels, intermediate-conductance Ca^{2+} -activated K^+ (IK) channels, TRP vanilloid 4 (TRPV4) channels, Piezo1 channels, endothelial NO synthase (eNOS) or G protein-coupled receptor 68 (GPR68) in arteries of *Pkd1* ecKO mice (Figure 1A-B). Immunofluorescence experiments demonstrated that PC-1 labeling was absent in endothelial cells of *Pkd1* ecKO mouse mesenteric arteries imaged en face (Figure 1C). These data indicate that PC-1 expression is abolished in endothelial cells of *Pkd1* ecKO mice.

Flow stimulates a PC-1-dependent reduction in inward current in endothelial cells

Patch-clamp electrophysiology was performed to investigate the regulation of plasma membrane currents by PC-1 in mesenteric artery endothelial cells. Currents were recorded at steady-state voltage (-60 mV) using the whole-cell configuration with physiological ionic gradients. In a static bath, endothelial cells of *Pkd1^{fl/fl}* mice generated a mean steady-state

inward current of ~ -5.5 pA/pF (Figure 1D, F). Flow stimulated an initial transient increase in inward current of ~ -1.3 pA/pF (Figure 1D-E). This transient increase in inward current was followed by a sustained reduction in inward current that reached steady-state at ~ -0.97 pA/pF in *Pkd1^{fl/fl}* cells (Figure 1D, F). During flow, the removal of bath Ca^{2+} increased mean inward current to ~ -3.7 pA/pF in *Pkd1^{fl/fl}* cells (Figure 1D, F). In a static bath, mean steady-state inward currents were similar in *Pkd1^{fl/fl}* and *Pkd1* eKO cells (Figure 1D, F). In contrast, the flow-activated transient inward current in *Pkd1* eKO cells was $\sim 15.4\%$ of that in *Pkd1^{fl/fl}* cells (Figure 1D-E). Similarly, the sustained flow-mediated reduction in inward current in *Pkd1* eKO cells was $\sim 42.2\%$ of that in *Pkd1^{fl/fl}* cells (Figure 1D, F). In the continuous presence of flow, the removal of bath Ca^{2+} also resulted in a smaller increase in inward current in *Pkd1* eKO cells than in *Pkd1^{fl/fl}* cells (Figure 1D, F). Ca^{2+} removal under flow increased inward current only ~ -0.2 pA/pF in *Pkd1* eKO cells, or $\sim 7.1\%$ of that in *Pkd1^{fl/fl}* cells (Figure 1D, F). These data demonstrate that flow stimulates a PC-1-dependent biphasic response composed of an initial transient inward current followed by a sustained Ca^{2+} -dependent reduction in inward current in endothelial cells.

Endothelial cell PC-1 contributes to flow-mediated arterial hyperpolarization

The flow-mediated, PC-1-dependent reduction in inward current in endothelial cells suggested that PC-1 may regulate arterial potential, a major determinant of contractility (35). Arterial potential was measured by impaling sharp glass microelectrodes into pressurized (80 mmHg) third-order mesenteric arteries of *Pkd1^{fl/fl}* and *Pkd1* eKO mice. In the absence of intraluminal flow, the membrane potentials of *Pkd1^{fl/fl}* and *Pkd1* eKO arteries were similar at either low (10 mmHg) or physiological (80 mmHg) pressures (Figure 1G, H). At 80 mmHg, intraluminal flow hyperpolarized *Pkd1^{fl/fl}* arteries by ~ 10 mV, but *Pkd1* eKO arteries by only ~ 2.6 mV, or $\sim 27.1\%$ of that in controls (Figure 1G, H). These data suggest that PC-1 expressed in endothelial cells contributes to flow-mediated arterial hyperpolarization.

PC-1 activates eNOS, IK channels and SK channels in endothelial cells to elicit vasodilation

The regulation of contractility by endothelial cell PC-1 was measured in pressurized (80 mmHg) third-order myogenic mesenteric arteries. Intravascular flow (15 dyn/cm^2) stimulated sustained and fully reversible dilations in mesenteric arteries of both *Pkd1^{fl/fl}* and *Pkd1* eKO mice (Figure 2A). In *Pkd1* eKO arteries, dilations to on-off flow were $\sim 34.8\%$ of those in *Pkd1^{fl/fl}* arteries (Figure 2A, B). In contrast, ACh-induced vasodilation was similar in *Pkd1^{fl/fl}* and *Pkd1* eKO arteries (Figure 2B; figure supplement 1A). To determine the functional flow range for PC-1 in endothelial cells, we measured vasoregulation to shear stresses between 3 and 35 dyn/cm^2 (36). Cumulative stepwise increases in flow caused progressive dilation, with a maximum at 27 dyn/cm^2 in *Pkd1^{fl/fl}* arteries (Figure 2C, D). Increasing shear stress above 27 dyn/cm^2 slightly attenuated maximal vasodilation (Figure 2C, D). The PC-1-sensitive component of flow-mediated dilation was calculated by subtracting responses in *Pkd1* eKO arteries from those in *Pkd1^{fl/fl}* arteries. Flow-mediated dilation in *Pkd1* eKO arteries was attenuated over the entire shear-stress range to between ~ 38.0 and 50.0% of that in *Pkd1^{fl/fl}* arteries (Figure 2C, D; Figure 2 – figure supplement 1B). Myogenic tone, depolarization (60 mM K^+)-induced constriction and passive diameter were similar in *Pkd1^{fl/fl}* and *Pkd1* eKO arteries (Figure 2 – figure supplement 1C-F). These data indicate that a broad intravascular flow range activates PC-1 in endothelial cells to induce vasodilation.

Ca^{2+} influx activates eNOS, IK channels and SK channels in endothelial cells, leading to vasodilation (1, 2). Next, we studied contributions of each of these proteins to flow-mediated, PC-1-dependent vasodilation. L-NNA, a NOS inhibitor, Tram-34, an IK channel blocker, and apamin, a SK channel inhibitor, inhibited flow-mediated dilation in pressurized (80 mmHg)

myogenic *Pkd1^{fl/fl}* arteries (Figure 2E-H). PC-1 knockout reduced the L-NNA-, Tram-34-, and apamin-sensitive components of flow-mediated dilation to ~21.6, 13.5 and 18.6 % of those in *Pkd1^{fl/fl}* arteries, respectively (Figure 2E-H). We have previously shown that in the absence of intravascular flow, L-NNA, Tram-34 and apamin alone do not alter the diameter of pressurized mesenteric arteries (6). These data indicate that flow stimulates PC-1-mediated dilation via NOS, IK channel and SK channel activation in endothelial cells.

Flow activates endothelial nitric oxide synthase (eNOS) in endothelial cells, but the involvement of PC-1 in mediating this response is unclear (37-39). Phosphorylation of eNOS at serine 1176 (p-eNOS (S1176)) leads to its activation (40, 41). Western blotting experiments indicated that intravascular flow increased p-eNOS (S1176) protein ~1.40-fold in *Pkd1^{fl/fl}* arteries, but did not alter p-eNOS in *Pkd1* eCKO arteries (Figure 2I, J). In contrast, flow did not alter total eNOS in either genotype (Figure 2I, J). These data indicate that flow stimulates PC-1 in endothelial cells, leading to eNOS, IK channel and SK channel activation, which produces vasodilation.

Endothelial cell PC-1 reduces systemic blood pressure

Radiotelemetry measurements were performed to measure blood pressure in freely-moving, conscious mice. Mean arterial pressure (MAP) was ~89.9 mmHg in *Pkd1^{fl/fl}* mice and ~109.6 mmHg in *Pkd1* eCKO mice, or 21.9% higher (Figure 3A-B). Underlying this increase in MAP were higher systolic and diastolic blood pressures in *Pkd1* eCKO mice (Figure 3 – figure supplement 1A). In contrast, heart rate and activity were similar in *Pkd1^{fl/fl}* and *Pkd1* eCKO mice, as were proximal tubule diameter and glomerular surface area measured in H&E-stained kidney sections (Figure 3C-F; Figure 3 – figure supplement 1B). These results suggest that flow stimulates PC-1 in endothelial cells, leading to vasodilation and a reduction in systemic blood pressure.

PC-1 and PC-2 coassemble and colocalize in endothelial cells

The vascular phenotype we describe here for *Pkd1* eCKO mice is similar to that of *Pkd2* eCKO mice (6). Thus, we tested the hypothesis that PC-1 and PC-2 are components of the same flow-sensitive signaling pathway in endothelial cells. PC-1 and PC-2 coimmunoprecipitated in mesenteric artery lysate, suggesting they coassemble (Figure 4A). Next, we used several different imaging techniques to measure the spatial proximity of PC-1 and PC-2 proteins in endothelial cells. Immunofluorescence energy transfer (immunoFRET) microscopy using Alexa Fluor 546 and Alexa Fluor 488-tagged secondary antibodies bound to PC-1 and PC-2 primary antibodies, respectively, generated mean N-FRET of ~24.3 % in endothelial cells of *Pkd1^{fl/fl}* mice (Figure 4B, C). In contrast, N-FRET was only ~1.3 and 0.9 % in *Pkd1* eCKO and *Pkd2* eCKO endothelial cells, respectively, when using the same labeling procedure (Figure 4C; Figure 4 - figure supplement 1A, B). Given that the Förster coefficient of the Alexa Fluor pair used is ~6.3 nm, these data suggest PC-1 and PC-2 locate in close spatial proximity. Lattice Structured Illumination super-resolution Microscopy (Lattice-SIM) was used to measure colocalization of PC-1 and PC-2 in endothelial cells of en face mesenteric arteries and in isolated mesenteric artery endothelial cells (Figure 4D-G). Endothelial cells were identified through immunolabeling of CD31, an endothelial cell-specific marker (Figure 4D, F). Analysis of these data using both Pearson's and Mander's coefficients also indicated that PC-1 and PC-2 colocalize in endothelial cells (Figure 4E, G) (42).

PC-1 and PC-2 surface clusters exhibit nanoscale colocalization and interdependency in endothelial cells

Single-Molecule Localization Microscopy (SMLM) in combination with Total Internal Reflection Fluorescence (TIRF) imaging (SMLM-TIRF) was used to measure the properties and nanoscale

proximity of PC-1 and PC-2 protein clusters in the plasma membrane of endothelial cells. Imaging was performed on cells which labeled for CD31, an endothelial-specific marker. Localization precision of the Alexa Fluor 488 and Alexa Fluor 647 fluorophores on secondary antibodies that were used for SMLM-TIRF were 29.6 ± 0.6 (n=53) and 24.6 ± 0.7 (n=45) nm, respectively, when imaged in endothelial cells (Figure 5 – figure supplement 1A, B). Discrete clusters of PC-1 and PC-2 were observed in the plasma membrane of *Pkd1^{fl/fl}* endothelial cells (Figure 5A). PC-1 and PC-2 clusters exhibited similar densities (Figure 5B). The sizes (areas) of individual PC-1 and PC-2 clusters were exponentially distributed, with means of ~ 3702.5 and 2157.1 nm^2 , respectively (Figure 5C; Figure supplement 2A, B). PC-2 clusters were smaller than PC-1 clusters (Figure 5C; Figure 5 – figure supplement 2A, B). Histograms were constructed that contained the distance between the center of each PC-1 cluster and that of its nearest PC-2 neighbor. These data were exponentially distributed (Figure 5 – figure supplement 2C). The mean PC-1 to PC-2 intercentroid distance was ~ 126.8 nm in *Pkd1^{fl/fl}* cells (Figure 5D). Approximately 27.0 % of PC-1 clusters overlapped with a PC-2 cluster and ~ 26.6 % of PC-2 clusters overlapped with a PC-1 cluster in *Pkd1^{fl/fl}* endothelial cells (Figure 5E, F; Figure 5 - figure supplement 2D). When experimental data were randomized using Costes' simulation algorithm (42), PC-1 to PC-2 and PC-2 to PC-1 overlap were only ~ 7.8 and 7.2 %, respectively in *Pkd1^{fl/fl}* cells (Figure 5E, F; figure supplement 2D). Flow did not alter PC-1 or PC-2 cluster density, PC-1 or PC-2 cluster size, the distance between PC-1 and PC-2 centroids, or their overlap (Figure 5B-F; Figure 5 – figure supplement 2D, E). In *Pkd1* eCKO cells, the densities of PC-1 and PC-2 clusters were far lower, at ~ 15.7 and 18.3 %, respectively of those in endothelial cells of *Pkd1^{fl/fl}* mice (Figure 5A, B). The mean sizes of PC-1 and PC-2 clusters in *Pkd1* eCKO endothelial cells were ~ 17.7 and 28.3 % of those, respectively, in *Pkd1^{fl/fl}* cells (Figure 5C; Figure 5 – figure supplement 2A, B). The mean PC-1 to PC-2 distance was ~ 5.79 -fold larger in *Pkd1* eCKO than *Pkd1^{fl/fl}* cells (Figure 5D; Figure 5 – figure supplement 2C). Furthermore, only ~ 1.2 % of PC-1 clusters overlapped with a PC-2 cluster in *Pkd1* eCKO cells, with similar results for PC-2 to PC-1 overlap (Figure 5E, F; figure supplement 2D). Costes' randomization of data did not alter PC-1 to PC-2 or PC-2 to PC-1 overlap in *Pkd1* eCKO cells. These data indicate that: 1) PC-1 and PC-2 surface protein clusters are colocalized in endothelial cells, 2) PC-1 or PC-2 knockout inhibits surface expression of both PC-1 and PC-2 proteins and 3) fluorescent clusters in *Pkd1* eCKO cells are likely due to non-specific labeling by secondary antibodies.

To determine whether data obtained using SMLM were specific to endothelial cells of *Pkd1^{fl/fl}* and *Pkd1* eCKO mice, we performed similar experiments using tamoxifen-inducible, endothelial cell-specific PC-2 knockout (*Pkd2* eCKO) mice and their controls (*Pkd2^{fl/fl}*). The mean densities and sizes of PC-1 and PC-2 clusters, PC-2 to PC-1 intercluster distance, PC-1 to PC-2 overlap and PC-2 to PC-1 overlap were all similar in endothelial cells of *Pkd2^{fl/fl}* mice and *Pkd1^{fl/fl}* mice (Figure 5 – figure supplement 3A-H and Figure 5 – figure supplement 4A, B). Similarly to observations in *Pkd1^{fl/fl}* cells, flow did not alter the densities, sizes, intercluster distances or overlap of PC-1 or PC-2 clusters in *Pkd2^{fl/fl}* cells (Figure 5 – figure supplement 3B, E, G, H and Figure 5 – figure supplement 4A, B). In *Pkd2* eCKO cells, PC-1 and PC-2 cluster densities were far lower, at ~ 12.7 and 11.1 % of those in *Pkd2^{fl/fl}* cells (Figure 5 – figure supplement 3A, B). PC-1 and PC-2 clusters were smaller and the mean distance from PC-2 clusters to their nearest PC-1 neighbor was far greater in *Pkd2* eCKO cells than in *Pkd2^{fl/fl}* cells (Figure 5 – figure supplement 3C-H). PC-1 to PC-2 and PC-2 to PC-1 overlap were also lower in *Pkd2* eCKO than *Pkd2^{fl/fl}* cells (Figure 5 – figure supplement 4A, B). These data demonstrate that PC-1 and PC-2 surface clusters colocalize in endothelial cells. Knockout of either PC-1 or PC-2 inhibits the surface expression of both PC-1 and PC-2 proteins, supporting their interdependency. Fluorescent clusters observed in *Pkd1* eCKO and *Pkd2* eCKO cells appear to represent non-specific secondary antibody labeling.

Flow-mediated vasodilation is similarly attenuated in arteries of *Pkd1* eKO and *Pkd1/Pkd2* eKO mice

Next, we investigated the functional interdependency of PC-1 and PC-2 in flow-mediated vasodilation. *Pkd1^{fl/fl}·Cdh5(PAC)-CreERT2* and *Pkd2^{fl/fl}·Cdh5(PAC)-CreERT2* mice were crossed to generate a *Pkd1^{fl/fl}/Pkd2^{fl/fl}·Cdh5(PAC)-CreERT2* line. Control Cre-negative *Pkd1^{fl/fl}/Pkd2^{fl/fl}* mice were generated using a similar breeding strategy. Tamoxifen-treatment reduced PC-1 and PC-2 proteins in mesenteric arteries of *Pkd1^{fl/fl}/Pkd2^{fl/fl}·Cdh5(PAC)-CreERT2* mice to ~60.9 and 66.1 %, respectively of those in *Pkd1^{fl/fl}/Pkd2^{fl/fl}* mice (Figure 6A, B, $P < 0.05$). In contrast, eNOS was similar in *Pkd1/Pkd2* eKO arteries and *Pkd1^{fl/fl}/Pkd2^{fl/fl}* arteries (Figure 6A, B). Intravascular flow stimulated vasodilation in pressurized *Pkd1^{fl/fl}/Pkd2^{fl/fl}* arteries that was similar in magnitude to that in arteries of both *Pkd1^{fl/fl}* and *Pkd2^{fl/fl}* mice (Figure 2A-C; Figure 6C, D and ref. (6)). In *Pkd1/Pkd2* eKO mouse arteries, mean flow-mediated vasodilation at 15 and 23 dyn/cm² were ~38.8 and 50.4 % of those in *Pkd1^{fl/fl}/Pkd2^{fl/fl}* arteries, respectively (Figure 6C, D). This inhibition of flow-mediated dilation is similar to that in arteries of *Pkd1* eKO and *Pkd2* eKO mice when compared to their respective controls (Figs. 2A-C, 6C, D and ref. (6)). In contrast, 60 mM K⁺-induced constriction, myogenic tone, dilations to ACh or sodium nitroprusside (SNP), a nitric oxide donor, and passive diameter were similar in *Pkd1^{fl/fl}/Pkd2^{fl/fl}* and *Pkd1/Pkd2* eKO arteries (Figure 6D; Figure 6 - figure supplement 1A-E). Collectively, these data indicate that intravascular flow stimulates vasodilation via a mechanism that involves both PC-1 and PC-2 in endothelial cells.

PC-1 knockout, PC-2 knockout and coiled-coil domain peptides similarly inhibit flow-activated non-selective cation current (I_{Cat}) in endothelial cells

Recombinant PC-1 and PC-2 can physically interact via several domains, including their C-terminal coiled-coils (27, 29, 30, 33). Next, we investigated the functional significance of the coiled-coil domains present in PC-1 and PC-2 to flow-mediated signaling. As PC-2 is a non-selective cation channel, we performed these experiments by isolating and measuring I_{Cat} in endothelial cells. I_{Cat} was measured using solutions that inhibit K⁺ channels and a bath solution that was Ca²⁺-free to prevent Ca²⁺ influx-dependent activation of channels. At a steady-state holding potential of -60 mV, flow reversibly stimulated sustained inward I_{Cat} s that were of similar amplitude in *Pkd1^{fl/fl}* and *Pkd2^{fl/fl}* endothelial cells (Figure 6E-G). In *Pkd1* eKO endothelial cells, mean flow-activated I_{Cat} was ~23.3 % of that in *Pkd1^{fl/fl}* controls (Figure 6E, G). Flow-activated I_{Cat} in *Pkd2* eKO cells was similarly smaller, at ~22.5 % of that in *Pkd2^{fl/fl}* cells (Figure 6E, G). These data indicate that PC-1 and PC-2 similarly contribute to flow-activated I_{Cat} in endothelial cells. These data also suggest that when using physiological ionic gradients, flow activates PC-1/PC-2 to induce a transient inward current which then stimulates K⁺ channels to produce the sustained reduction in inward current (Figure 1D-F and ref. 6).

Peptides were constructed that correspond to regions within the C-terminal coiled-coil domains in PC-1 (amino acids 4216-4233) and PC-2 (amino acids 874-883) that physically interact (27, 29-31). Intracellular introduction via the pipette solution of either the PC-1 or PC-2 coiled-coil domain peptide similarly reduced flow-activated I_{Cat} s to between ~30.1 and 29 % of control currents in both *Pkd1^{fl/fl}* and *Pkd2^{fl/fl}* endothelial cells (Figure 6F, G). In contrast, scrambled PC-1 and PC-2 peptides did not alter flow-activated I_{Cat} s (Figure 6G; figure 6 - figure supplement 2). Lowering bath Na⁺ concentration inhibited flow-activated I_{Cat} in all genotypes and under all conditions. These data indicate that PC-1 and PC-2 are interdependent for flow to activate I_{Cat} in endothelial cells. Data also suggest that physical coupling of PC-1 and PC-2 C-termini is necessary for flow to activate I_{Cat} .

Discussion

Here, we investigated the regulation of arterial contractility by PC-1 and the potential involvement of PC-2 in mediating responses in endothelial cells. Our data show that flow stimulates PC-1-dependent cation currents in endothelial cells that induce arterial hyperpolarization, vasodilation and a reduction in blood pressure. PC-1-dependent vasodilation occurs over the entire functional shear stress range due to eNOS, IK channel and SK channel activation. PC-1 and PC-2 proteins coassemble and their surface clusters colocalize. Knockout of either PC-1 or PC-2 abolishes surface expression of both proteins. Flow activates a PC-1- and PC-2-dependent I_{Cat} that is inhibited by peptides corresponding to the C-terminus coiled-coil domains on either polycystin protein. These data demonstrate that PC-1 regulates arterial contractility by forming an interdependent complex with PC-2 in endothelial cells. Flow stimulates plasma membrane-localized PC-1/PC-2 clusters, leading to eNOS, IK channel and SK channel activation, vasodilation and a reduction in blood pressure.

Intraluminal flow stimulates endothelial cells to induce vasodilation, but signaling mechanisms involved are poorly understood. Data here demonstrate that flow stimulates signaling mechanisms in endothelial cells that are similarly dependent on PC-1 and PC-2. When combined with the results of our earlier study, flow-mediated signaling and vasodilation are similarly attenuated in endothelial cells and arteries of *Pkd1* eCKO, *Pkd2* eCKO and *Pkd1/Pkd2* eCKO mice, further strengthening this conclusion (6). Specific results include that inducible, endothelial cell-specific knockout of either PC-1 or PC-2 similarly inhibits flow-mediated: 1) biphasic currents in endothelial cells, 2) Ca^{2+} influx-dependent cation currents in endothelial cells, 3) arterial hyperpolarization, 4) vasodilation over a broad shear stress range and 5) vasodilation that occurs via eNOS, IK channel and SK channel activation. We also show that PC-1 knockout elevates blood pressure, consistent with results obtained in *Pkd2* eCKO mice (6). In contrast, PC-1 and PC-2 do not contribute to ACh-induced vasodilation, indicating that these proteins are activated by specific stimuli and that their knockout does not cause generalized endothelial cell dysfunction. Myogenic tone, depolarization-induced constriction and passive diameter are also similar in *Pkd1* eCKO, *Pkd2* eCKO, *Pkd1/Pkd2* eCKO and control mouse arteries, illustrating that smooth muscle function is not altered in these knockout mice.

Previous studies have demonstrated that flow activates SK and inwardly rectifying K^+ (K_{ir}) channels to induce vasodilation (43-45). $\text{K}_{\text{ir}}2.1$ activation was shown to contribute to eNOS phosphorylation, but not to SK channel activation (43). The functional significance of PC-1 and PC-2 to the regulation of K_{ir} channels remains to be determined. In arterial membrane potential measurements, we did not verify cell types from which impalements were obtained, but endothelial cells and smooth muscle cells are electrically coupled, particularly in resistance-size arteries (2). Our data support the following signaling mechanism. Flow-activated PC-1/PC-2-mediated stimulation of eNOS, IK channels and SK channels produces arterial hyperpolarization, reducing the activity of voltage-dependent ($\text{Ca}_v1.2$) channels in smooth muscle cells (2). The subsequent reduction in intracellular Ca^{2+} concentration in smooth muscle cells produces vasodilation and a decrease in blood pressure (46). These data suggest that PC-1/PC-2 in endothelial cells of other resistance-size arteries may also function to induce vasodilation. Future studies should aim to investigate PC-1/PC-2 expression and function throughout the vasculature.

Virtually all vertebrate cells possess at least one primary cilia, a tiny (~0.2-0.5 μm width, 1-12 μm length) immotile organelle electrically distinct from the cell body (47-49). Cilia generate compartmentalized signaling and maintain an intracellular Ca^{2+} concentration distinct from the cell soma (47-49). A great deal of debate has taken place over whether cells sense external fluid flow through proteins located in primary cilia or the plasma membrane. Primary cilia act as flow sensors in the embryonic node and in kidney collecting duct cells (50, 51). In contrast, flow

does not activate Ca^{2+} influx in primary cilia of cultured kidney epithelial cells, kidney thick ascending tubules, embryonic node crown cells or in the kinocilia of inner ear hair cells (52). Instead, flow stimulates a cytoplasmic Ca^{2+} signal in the cell body that propagates to cilia to elevate intraciliary Ca^{2+} concentration (52). Here, flow stimulated plasma membrane cation currents, indicating that flow-sensing takes place in the cell body of endothelial cells.

Physiological functions of PC-1 and the potential involvement of PC-2 in PC-1-mediated signaling mechanisms in endothelial cells were unclear. Whether physical coupling of PC-1 and PC-2 is required for these proteins to traffic to the surface and to activate cellular signaling has also been a matter of debate. Studies that aimed to answer this question primarily used renal epithelial cells or recombinant proteins expressed in cultured cells or *Xenopus* oocytes. Ciliary localization of PC-2 is necessary for flow-sensing in perinodal crown cells and left-right symmetry in mouse embryos (50). In contrast, other studies demonstrated that a physical interaction between PC-1 and PC-2 is essential for these proteins to traffic to the cell surface and generate plasma membrane currents (26, 30-32, 53-55). It was essential to include an IgK-chain secretion sequence into recombinant PC-1 to induce its trafficking and that of associated PC-2 to the plasma membrane in HEK293 cells (55). We show that when PC-1 and PC-2 are both expressed, they readily traffic to the plasma membrane in endothelial cells. PC-1 and PC-2 protein clusters are present in the endothelial cell plasma membrane with more than one-quarter exhibiting nanoscale overlap, supporting their coassembly. Knockout of either PC-1 or PC-2 does not alter the amount of the other polycystin protein, suggesting that the expression of each polycystin is not influenced by that of the other. In contrast, PC-1 or PC-2 knockout prevents surface localization of the other polycystin protein, leading to its intracellular retention, as can be seen in SMLM and immunofluorescence imaging experiments. Flow did not alter the properties of surface PC-1 or PC-2 clusters, or their intercluster distance or overlap, suggesting that flow activates a heteromeric PC-1/PC-2 complex in the plasma membrane. The cluster sizes we report reflect those of the proteins and the antibodies used to label them. Western blotting, immunofluorescence, immunoFRET and SMLM experiments on endothelial cells and arteries of *Pkd1*^{fl/fl}, *Pkd2*^{fl/fl}, *Pkd1* eCKO and *Pkd2* eCKO mice have validated the PC-1 and PC-2 antibodies (here and ref. (6)). A small amount of fluorescence was observed in *Pkd1* eCKO and *Pkd2* eCKO cells during SMLM experiments. We use the term “non-specific labeling” as an umbrella term to refer to secondary antibodies that may be free, bound to primary antibodies or both. It is for this reason that the comparison between endothelial cells of *Pkd1*^{fl/fl}, *Pkd2*^{fl/fl}, *Pkd1* eCKO and *Pkd2* eCKO mice is useful, particularly as the same labeling protocols were performed on endothelial cells of these different mouse models. A previous study showed that PC-2 immunolabeling colocalized with α -tubulin, a ciliary marker, in endothelial cells of embryonic (E15.5) conduit vessels (56). We did not examine if PC-1 or PC-2 are present in cilia or if flow activates PC-1- or PC-2-dependent currents in the cilia of endothelial cells. Future studies should investigate these possibilities.

PC-1 and PC-2 did not generate currents in the plasma membrane of renal collecting duct cells or following heterologous expression in cell lines (15, 16, 18, 31, 32, 49). Knockout of either PC-1 or PC-2 also did not alter plasma membrane currents in inner medullary collecting duct (pIMCD) epithelial cells (15). Recently, it was demonstrated that plasma membrane PC-1/PC-2 channels are silent but can be measured when a gain-of-function mutation (F604P) is introduced into PC-2 (55, 57). We show that in the absence of flow, surface PC-1/PC-2 channels generate little current in endothelial cells (6). Rather, flow-activates plasma membrane currents in endothelial cells that are similarly attenuated by either PC-1 or PC-2 knockout or by the introduction of peptides that correspond to the C-terminus coiled-coil domains that interact on each protein (here and ref. (6)). Flow-mediated intracellular Ca^{2+} elevations and NO production were attenuated in cultured embryonic aortic endothelial cells from *Pkd1* and *Tg737*^{orp/orp} global knockout mice (58). As *Tg737*^{orp/orp} global knockout mice have shorter cilia

or no cilia, the authors proposed that PC-1 located in cilia is a flow sensor in embryonic endothelial cells (58). In contrast, more recent studies have demonstrated that flow did not elicit Ca^{2+} signaling in primary cilia and homomeric PC-2 channels did not require PC-1 in primary cilia of pIMCD epithelial cells (15). Our data support the conclusion that flow activates PC-1/PC-2-dependent cation currents in the plasma membrane of endothelial cells.

Multiple different domains in PC-1 and PC-2 physically interact. Several groups have demonstrated that PC-1 and PC-2 couple via their C-terminal coiled-coils (27, 29, 30, 33). Recombinant PC-1 and PC-2 lacking coiled-coils can also interact via N-terminal loops (59, 60). The structure of a PC-1/PC-2 heterotetramer resolved using cryo-EM indicated that a region between TM6 and TM11 of PC-1 interdigitates with PC-2 (26). PC-1 is proposed to act both as a dominant-negative subunit in PC-1/PC-2 channels and to increase Ca^{2+} permeability over that in PC-2 homotetramers (26, 57). Our data suggest that flow stimulates physical coupling of the coiled-coil domains in PC-1/PC-2 to activate current. Alternatively, the interference peptides may uncouple constitutively bound coiled coils in PC-1/PC-2 heteromers, thereby preventing current activation by flow. It is unlikely that the interference peptides dissolve PC-1/PC-2 into individual subunits as several other domains can interact and the PC-1/PC-2 heteromeric structure was resolved in the absence of the coiled-coils (26, 60). PC-1 has been proposed to act as an atypical G protein due to the presence of a G protein-binding domain located in the C-terminus (17). The PC-1 interference peptide we used does not overlap with this G protein-binding domain, which is located between amino acids 4125 and 4143. Thus, G protein signaling by PC-1 may not be involved in flow-mediated PC-1/PC-2-dependent current activation in endothelial cells, although this remains to be determined.

Our data suggest that flow activates Ca^{2+} influx through PC-1/PC-2 channels. Although homomeric PC-2 is a K^{+} - and Na^{+} -permeant channel with low Ca^{2+} permeability, heteromeric assembly of PC-2 with PC-1 increases Ca^{2+} permeability and reduces block by external Ca^{2+} , supporting this concept (15, 57). We did not determine the ionic permeability of flow-activated PC-1/PC-2-dependent currents in endothelial cells (57). Endothelial cells express a wide variety of ion channels, including several other TRPs and K^{+} channels, making the isolation of a pure PC-1/PC-2-dependent current challenging. PC-1/PC-2 may also interact with other TRP channels. For example, heteromeric channels containing PC-2 and TRPM3 exhibit higher Ca^{2+} permeability than homomeric PC-2 in primary cilia of cultured renal epithelial cells (61, 62). Thus, PC-1/PC-2 channels in endothelial cells may contain other TRP subunits.

Flow may directly or indirectly activate PC-1/PC-2 in endothelial cells. PC-1 is proposed to act as a mechanical sensor and ligand-receptor in cultured kidney epithelial cells (9-11). The PC-1 extracellular N-terminus contains several putative adhesion- and ligand-binding sites that may confer mechanosensitivity (3, 7, 8). Recent evidence indicates that the C-type lectin domain located in the PC-1 N-terminus activates recombinant PC-1/PC-2 channels, providing one possible activation mechanism for flow (55). Other mechanosensitive proteins, such as Piezo1 and GPR68, may also stimulate PC-1/PC-2-dependent currents in endothelial cells (63, 64). Investigating the mechanisms by which flow activates PC-1/PC-2-dependent currents should be a focus of future studies.

ADPKD is typically characterized by the appearance of renal cysts, but patients can develop hypertension prior to any kidney dysfunction, with cardiovascular disease the leading (~50 %) cause of death in patients (21-25). Hypertension occurs prior to loss of kidney function in more than 60 % of patients, with the average age of onset between 30 and 34 years of age (65). Our study raises the possibility that hypertension and cardiovascular disease in ADPKD patients may involve altered PC-1/PC-2 function in endothelial cells. Consistent with our data, human ADPKD patients exhibit loss of nitric oxide release and a reduction in endothelium-dependent dilation during increased blood flow (6, 66). Hypertension in humans is also associated with endothelial dysfunction and attenuated flow-mediated dilation (67). Conceivably, hypertension

may also be associated with dysfunctional PC-1/PC-2 signaling in endothelial cells. Our demonstration that PC-1/PC-2 elicits vasodilation may lead to studies identifying potential dysfunction in patients with hypertension, ADPKD and other cardiovascular diseases.

In summary, we show that PC-1 regulates arterial contractility through the formation of an interdependent plasma membrane signaling complex with PC-2 in endothelial cells. Flow stimulates PC-1/PC-2-dependent currents in endothelial cells, leading to eNOS, IK channel and SK channel activation, vasodilation and a reduction in blood pressure.

Materials and Methods

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Strain, strain background (<i>M. musculus</i>)	<i>Pkd1^{fl/fl}</i>	Baltimore PKD Core Center	PMID: 15579506 MGI: 3617325	Mice with <i>Pkd1</i> gene flanked by <i>loxP</i> regions.
Strain, strain background (<i>M. musculus</i>)	<i>Pkd2^{fl/fl}</i>	Baltimore PKD Core Center	PMID: 20862291 MGI: 4843127	Mice with <i>Pkd2</i> gene flanked by <i>loxP</i> regions.
Strain, strain background (<i>M. musculus</i>)	<i>Pkd1^{fl/fl} /Pkd2^{fl/fl}</i>	This paper		Mouse line created in-house by mating <i>Pkd1^{fl/fl}</i> with <i>Pkd2^{fl/fl}</i> . Mice with <i>Pkd1</i> and <i>Pkd2</i> genes flanked by <i>loxP</i> regions.
Strain, strain background (<i>M. musculus</i>)	<i>Cdh5(PAC)-CreERT2</i>	Cancer Research UK	RRID: MGI: 3848982	Mice with tamoxifen-inducible Cre recombinase that is expressed specifically in endothelial cells.
Strain, strain background (<i>M. musculus</i>)	<i>Pkd1^{fl/fl}:Cdh5(PAC)-CreERT2</i>	This paper		Mouse line created in-house by mating <i>Pkd1^{fl/fl}</i> with <i>Cdh5(PAC)-</i>

<i>musculus</i>)				CreERT2. Mice with inducible endothelial cell-specific deletion of PC-1.
Strain, strain background (<i>M. musculus</i>)	<i>Pkd2^{fl/fl}</i> : <i>Cdh5</i> (PAC)-CreERT2	PMID: 32364494		Mouse line created in-house by mating <i>Pkd2^{fl/fl}</i> with <i>Cdh5</i> (PAC)-CreERT2. Mice with inducible endothelial cell-specific deletion of PC-2.
Strain, strain background (<i>M. musculus</i>)	<i>Pkd1^{fl/fl}/Pkd2^{fl/fl}</i> : <i>Cdh5</i> (PAC)-CreERT2	This paper		Mouse line created in-house by mating <i>Pkd1^{fl/fl} Cdh5</i> (PAC)-CreERT2 with <i>Pkd2^{fl/fl} Cdh5</i> (PAC)-CreERT2. Mice with inducible endothelial cell-specific deletion both PC-1 and PC-2.
Antibody	Anti-PC-1 (mouse monoclonal)	Baltimore PKD Core	Mouse mAB SF4AZ E3	IF (1:100) Lattice SIM (1:100) SMLM (1:100)
Antibody	Anti-PC-2 (rabbit monoclonal)	Baltimore PKD Core	Rabbit mAB 3374 CT-14/4	IF (1:100) Lattice SIM (1:100) SMLM (1:100)
Antibody	Anti-CD31 (rat monoclonal)	Abcam	Cat. #: Ab7388 RRID: AB_305905	IF (1:100) Lattice SIM (1:100) SMLM (1:100)
Antibody	Anti-PC-1 (H-260) (rabbit polyclonal)	Santa Cruz Biotechnology	Cat. #: sc-25570, RRID: AB_2163357	N-FRET (1:100)
Antibody	Anti-PC-2 (mouse monoclonal)	Santa Cruz Biotechnology	Cat. #: sc-28331, RRID: AB_672377	N-FRET (1:100)

Antibody	Alexa 488-conjugated anti-rat IgG (donkey polyclonal)	Thermo Fisher Scientific	Cat. #: A-21208, RRID: AB_2535794	IF (1:500)
Antibody	Alexa 546-conjugated anti-mouse IgG (donkey polyclonal)	Thermo Fisher Scientific	Cat. #: A-10036, RRID: AB_2534012	IF (1:500)
Antibody	Alexa 488-conjugated anti-mouse IgG (donkey polyclonal)	Thermo Fisher Scientific	Cat. #: A-21202, RRID: AB_141607	Lattice SIM (1:500) SMLM (1:500)
Antibody	Alexa 546-conjugated anti-rabbit IgG (donkey polyclonal)	Thermo Fisher Scientific	Cat. #: A-10040, RRID: AB_2534016	Lattice SIM (1:500)
Antibody	Alexa 647-conjugated anti-rat IgG (goat polyclonal)	Thermo Fisher Scientific	Cat. #: A-21247, RRID: AB_141778	Lattice SIM (1:500)
Antibody	Alexa 555-conjugated anti-rat IgG (goat polyclonal)	Thermo Fisher Scientific	Cat. #: A-21434, RRID: AB_2535855	SMLM (1:500)
Antibody	Alexa 647-conjugated anti-rabbit IgG (donkey polyclonal)	Thermo Fisher Scientific	Cat. #: A-31573, RRID: AB_2536183	SMLM (1:500)
Antibody	Alexa Fluor 488-conjugated anti-mouse (goat polyclonal)	Thermo Fisher Scientific	Cat. #: A-11001, RRID: AB_2534069	N-FRET (1:100)

Antibody	Alexa Fluor 555-conjugated anti-rabbit (donkey polyclonal)	Thermo Fisher Scientific	Cat. #: A-31572, RRID: AB_162543	N-FRET (1:100)
Antibody	Anti-PC-1 (mouse monoclonal)	Santa Cruz Biotechnology	Cat. #: sc-130554, RRID: AB_2163355	WB (1:100)
Antibody	Anti-PC-2 (mouse monoclonal)	Santa Cruz Biotechnology	Cat. #: sc-47734, RRID: AB_672380	WB (1:100) IP (1:20)
Antibody	Anti-IK1 (D-5) (mouse monoclonal)	Santa Cruz Biotechnology	Cat. #: sc-365265, RRID: AB_10841432	WB (1:100)
Antibody	Anti-SK3 (rabbit polyclonal)	Abcam	Cat. #: ab28631, RRID: AB_775888	WB (1:100)
Antibody	Anti-eNOS [M221] (mouse monoclonal)	Abcam	Cat. #: ab76198, RRID: AB_1310183	WB (1:100)
Antibody	Anti-TRPV4 (clone 1B2.6) (mouse monoclonal)	Millipore Sigma	Cat. #: MABS466	WB (1:100)
Antibody	Anti-Actin (clone C4) (mouse monoclonal)	Millipore Sigma	Cat. #: MAB1501, RRID: AB_2223041	WB (1:1000)
Antibody	Anti-p-eNOS (rabbit polyclonal)	Cell Signaling Technology	Cat. #: 9571, RRID: AB_329837	WB (1:100)

Antibody	Anti-GPR68 (rabbit polyclonal)	NOVUS Biologicals	Cat. #: NBP2-32747	WB (1:100)
Antibody	Anti-Piezo1 (rabbit polyclonal)	Proteintech	Cat. #: 15939-1-AP, RRID: AB_2231460	WB (1:100)
Antibody	Mouse IgG control (mouse polyclonal)	Millipore Sigma	Cat. #: 12-371, RRID: AB_145840	IP (1:20)
Peptide, recombinant protein	PC-1 coiled- coil domain peptide	Genscript	FDRLNQATEDVYQL EQQL	1 μ M
Peptide, recombinant protein	PC-1 scrambled peptide	Genscript	QLNDLFQTEVAEDL QRYQ	1 μ M
Peptide, recombinant protein	PC-2 coiled- coil domain peptide	Genscript	KRREVLGRLL	1 μ M
Peptide, recombinant protein	PC-2 scrambled peptide	Genscript	VLKLLRRRGE	1 μ M
Commercial assay or kit	Pierce crosslink magnetic IP/co-IP Kit	Thermo Fisher Scientific	Cat. #: 88805	
Other	DAPI stain	Thermo Fisher Scientific	Cat. #: 3571 RRID: AB_2307445	1:1000

Animals

All animal studies were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee Health Science Center. *Pkd1^{fl/fl}* and *Pkd2^{fl/fl}* mice were obtained from the Baltimore PKD Center (Baltimore, MD). *Cdh5(PAC)-CreERT2* mice were a kind gift from Cancer Research UK (68). *Pkd1^{fl/fl}* mice were crossed with tamoxifen-inducible endothelial cell-specific Cre mice (*Cdh5(PAC)-CreERT2*, Cancer Research UK) to generate *Pkd1^{fl/fl};Cdh5(PAC)-CreERT2* mice. *Pkd2^{fl/fl};Cdh5(PAC)-CreERT2* mice were generated as previously described (6). *Pkd1^{fl/fl}* mice were crossed with *Pkd2^{fl/fl}* mice to produce *Pkd1^{fl/fl}/Pkd2^{fl/fl}* mice. *Pkd1^{fl/fl};Cdh5(PAC)-CreERT2* mice were crossed with *Pkd2^{fl/fl};Cdh5(PAC)-*

CreERT2 mice to generate *Pkd1^{fl/fl}/Pkd2^{fl/fl}:Cdh5(PAC)-CreERT2* mice. The genotypes of all mice were confirmed using PCR (Transnetyx, Memphis, TN) before use. *Pkd1^{fl/fl}*, *Pkd2^{fl/fl}* and *Pkd1^{fl/fl}/Pkd2^{fl/fl}* cre negative mice were used as controls. All mice (male, 12 weeks of age) were injected with tamoxifen (50 mg/kg, i.p.) once per day for 5 days and studied between 7 and 14 days after the last injection.

Tissue preparation and endothelial cell isolation

Mesenteric artery branches from first to fifth order were cleaned of adventitial tissue and placed into ice-cold physiological saline solution (PSS) that contained (in mM): 112 NaCl, 6 KCl, 24 NaHCO₃, 1.8 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄ and 10 glucose, gassed with 21% O₂, 5% CO₂ and 74% N₂ (pH 7.4). Endothelial cells were dissociated by introducing endothelial cell basal media (Endothelial cell GM MV2, Promocell) containing 2 mg/ml collagenase type 1 (Worthington Biochemical) into the arterial lumen and left to incubate for 30-40 minutes at 37°C. Endothelial cell isolate was placed into endothelial cell basal media containing growth supplements (Promocell) that support endothelial cell survival. Primary-cultured endothelial cells were studied within 5 days of isolation.

Genomic PCR

Genomic DNA was isolated from mesenteric arteries using a Purelink Genomic DNA kit (Thermo Fisher Scientific). PCR was then performed on an Eppendorf Gradient thermal cycler using the following protocol: 95°C for 2 min, then 35 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 30 s. Primer sequences were as follows: *Pkd1^{fl/fl}*: GTTATTTCGAGGTCGCTAGACCCTATC (forward), GTTACAGATGAGGCCCGAGGAAAG (reverse), *Pkd1* eKO: GGTACGAGAGAGAAGTGGTCTCAGGA (forward), GAGATCCCACCGCGGTTTTGCTAGAAGGCA (reverse). PCR products were separated on 1.5% agarose gels.

Western Blotting

Mesenteric artery segments comprising second- to fifth-order vessels were used for Western blotting. Arteries were transferred to an eppendorf tube containing RIPA Buffer (Sigma: R0278) and protease inhibitor cocktail (Sigma: P8340, 1:100 dilution). For experiments measuring effect of flow of eNOS phosphorylation, arteries were exposed to flow (15/dyn) for 5 mins at 37 °C and then immediately transferred to an Eppendorf tube containing RIPA buffer with protease and phosphatase inhibitor cocktail (1:100 dilution). Arteries were cut into small segments using micro scissors and mechanically broken down using a homogenizer (Argos Technologies: A0001). The lysate was centrifuged at 4°C, 10,000 rpm for 2 minutes. This process was repeated 3 times, after which the supernatant was collected. Proteins in lysate were separated on 7.5% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes. Membranes were blocked with 5% milk and incubated with one of the following primary antibodies: PC-1 (Santa Cruz), PC-2 (Santa Cruz), Piezo1 (Proteintech), GPR68 (NOVUS), SK3 (Sigma (P0608)), IK (Alomone), TRPV4 (MilliporeSigma), eNOS (Abcam), or actin (MilliporeSigma) overnight at 4°C. Membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature. Protein bands were imaged using a ChemiDoc Touch Imaging System (Bio-Rad), quantified using ImageJ software and normalized to actin.

Laser-scanning confocal microscopy

Arteries were cut open longitudinally and fixed with 4% paraformaldehyde in PBS for 1 hr. Following a wash in PBS, arteries were permeabilized with 0.2% Triton X-100 and blocked with 3% BSA + 5% serum for 1 hour at room temperature. For en face imaging experiments, arteries were incubated overnight with anti-PC-1 monoclonal primary antibody (E3 5F4A2, Baltimore

PKD Center) and anti-CD31 primary monoclonal antibody (Abcam 7388) at 4°C. Arteries were then incubated with Alexa Fluor 488 donkey anti-rat, Alexa Fluor 546 donkey anti-mouse secondary antibodies (1:500; Molecular Probes) and 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI) (1:1000; Thermo Scientific) for 1 hour at room temperature. After washing with PBS, arteries were mounted in 80% glycerol solution. DAPI, Alexa 488 and Alexa 546 were excited at 405, 488 and 561 nm with emission collected at ≤ 460 nm and ≥ 500 nm, respectively, using a Zeiss LSM 710 laser-scanning confocal microscope.

Patch-clamp electrophysiology

The conventional whole-cell configuration was used to measure steady-state currents in primary-cultured endothelial cells at a holding potential of -60 mV. Cells used label with CD31 (Figure 4E), respond to flow and produce SK and IK currents (6), consistent with endothelial cells. For experiments using physiological ionic gradients, the bath solution contained (in mM): NaCl 134, KCl 6, HEPES 10, MgCl₂ 1, CaCl₂ 2 and glucose 10 (pH 7.4). The Ca²⁺-free bath solution was the same composition as bath solution except Ca²⁺ was omitted and 1 mM EGTA was included. The pipette solution contained (in mM): K aspartate 110, KCl 30, HEPES 10, glucose 10, EGTA 1, Mg-ATP 1 and Na-GTP 0.2, with total MgCl₂ and CaCl₂ adjusted to give free concentrations of 1 mM Mg²⁺ and 200 nM Ca²⁺, respectively (pH 7.2). For experiments measuring I_{Cat}, the bath solution contained (in mM): Na aspartate 135, NaCl 5, HEPES 10, glucose 10 and MgCl₂ 1 (pH 7.4). A low Na⁺ bath solution used when measuring I_{Cat} was (in mM): NMDG-aspartate 135, NaCl 5, HEPES 10, glucose 10 and MgCl₂ 1 (pH 7.4). The pipette solution contained (in mM): Na aspartate 135, NaCl 5, HEPES 10, glucose 10, EGTA 1, Mg-ATP 1 and Na-GTP 0.2, with total Mg²⁺ and Ca²⁺ adjusted to give free concentrations of 1 mM and 200 nM, respectively (pH 7.2). PC-1 and PC-2 coiled-coil domain and corresponding scrambled peptides were custom-made (Genscript) and added to the pipette solution immediately before use. Free Mg²⁺ and Ca²⁺ were calculated using WebmaxC Standard (<https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/webmaxc/webmaxcS.htm>). The osmolality of solutions was measured using a Wescor 5500 Vapor Pressure Osmometer (Logan, UT, USA). Currents were filtered at 1 kHz and digitized at 5 kHz using an Axopatch 200B amplifier and Clampex 10.4 (Molecular Devices). Offline analysis was performed using Clampfit 10.4. The flow-activated transient inward current was measured at its peak in each cell. The steady-state inward current was the average of at least 45 seconds of contiguous data.

Pressurized artery membrane potential measurements

Membrane potential was measured by inserting sharp glass microelectrodes (50–90 MΩ) filled with 3 M KCl into the adventitial side of pressurized third- and fourth-order mesenteric arteries. Membrane potential was recorded using a WPI FD223a amplifier and digitized using a MiniDigi 1A USB interface, pClamp 9.2 software (Axon Instruments) and a personal computer. Criteria for successful intracellular impalements were a sharp negative deflection in potential on insertion, stable voltage for at least 1 min after entry, a sharp positive voltage deflection on exit from the recorded cell and a <10% change in tip resistance after impalement.

Pressurized artery myography

Experiments were performed using isolated third- and fourth-order mesenteric arteries using PSS gassed with 21% O₂/5% CO₂/74% N₂ (pH 7.4). Arterial segments 1–2 mm in length were cannulated at each end in a perfusion chamber (Living Systems Instrumentation) continuously perfused with PSS and maintained at 37°C. Intravascular pressure was altered using a Servo pump model PS-200-P (Living systems) and monitored using pressure transducers. Following development of stable myogenic tone, intraluminal flow was introduced using a P720 peristaltic pump (Instech). The intraluminal flow rate required to apply a specific amount of shear stress to

each artery was calculated using internal diameter. Arterial diameter was measured at 1 Hz using a CCD camera attached to a Nikon TS100-F microscope and the automatic edge-detection function of IonWizard software (Ionoptix). Myogenic tone was calculated as: $100 \times (1 - D_{\text{active}}/D_{\text{passive}})$ where D_{active} is active arterial diameter and D_{passive} is the diameter determined in the presence of Ca^{2+} -free PSS supplemented with 5 mM EGTA.

Telemetric blood pressure and locomotion measurements

Telemetric blood pressure recordings were performed at the University of Tennessee Health Science Center. Briefly, transmitters (PA-C10, Data Sciences International) were implanted subcutaneously into anesthetized mice, with the sensing electrode placed in the aorta via the left carotid artery. Mice were allowed to recover for 7-10 days. Blood pressure was then recorded every 20 s for 5 days prior to tamoxifen injection and again for the entire time period between 7 and 22 days after the last tamoxifen injection (50 mg/kg per day for 5 consecutive days, i.p.) using a PhysioTel Digital telemetry platform (Data Sciences International). Dataquest A.R.T. software was used to acquire and analyze data.

Kidney histology

Kidney sections were stained with H&E and examined by Probetex, Inc (San Antonio, Texas). Briefly, image analysis was performed to measure the glomerular size and tubular cross-sectional diameter. The glomerular size was measured by tracing the circumference of 25 random glomeruli and surface area was calculated using the polygonal area tool of Image-Pro 4.5 image analysis software calibrated to a stage micrometer. Tubular size was measured using the linear length tool of Image-Pro 4.5 imaging software. The tracing tool was applied at the diameter of cross-sectional profiles of 5 proximal tubules/image (total of 25/section). Glomerular and tubular images were calibrated to a stage micrometer and data was transferred to an Excel spreadsheet and statistical analysis was performed by Excel analysis pack.

Co-immunoprecipitation

Mesenteric artery segments comprising second- to fifth-order vessels were used. Arteries were transferred to an eppendorf tube containing lysis buffer and protease inhibitor cocktail (Sigma: P8340, 1 in 100 dilution). Arteries were cut into small segments using micro scissors and broken down using a mechanical homogenizer (Argos Technologies: A0001). Arterial lysate was centrifuged for 2 minutes at 10,000 rpm and at 4°C. This process was repeated 3 times, after which the supernatant was collected. Proteins were pulled down from arterial lysate using a Pierce crosslink Magnetic IP/colP kit (Thermoscientific) as per the manufacturer's instructions. Samples were incubated with PC-2 antibody (1:20, Santa Cruz) that was covalently bound to protein A/G Magnetic Beads (Pierce) overnight at 4 °C. Following washing and elution, immunoprecipitates were analyzed using Western blotting.

Immunofluorescence Resonance Energy Transfer (ImmunoFRET) imaging

Primary-cultured mesenteric artery endothelial cells were fixed with paraformaldehyde and permeabilized with 0.1% Triton X-100 for 2 min at room temperature. After blocking with 5% bovine serum albumin (BSA), the cells were treated overnight at 4°C with anti PC-1 (Rabbit polyclonal, Santa Cruz, sc-25570, 1:100 dilution, RRID:AB_2163357) and anti PC-2 (mouse monoclonal, Santa Cruz, sc-28331, 1:100 dilution, RRID:AB_672377) antibodies in PBS containing 5% BSA. After a wash, cells were incubated for 1 h at 37°C with secondary antibodies: Goat anti-Mouse Alexa Fluor 488 (ThermoFisher, A-11001) and Donkey anti-Rabbit Alexa Fluor 555 (ThermoFisher, A-31572). Coverslips were then washed and mounted on glass slides. Fluorescence images were acquired using a Zeiss 710 laser-scanning confocal microscope. Alexa 488 and Alexa 546 were excited at 488 and 543 nm and emission collected

at 505–530 and >560 nm, respectively. Images were acquired using a z-resolution of ~1 μm . Images were background-subtracted and normalized FRET (N-FRET) was calculated on a pixel-by-pixel basis for the entire image and in regions of interest (within the boundaries of the cell) using the Xia method (41) and Zeiss LSM FRET Macro tool version 2.5 as previously described (48).

Lattice Structured Illumination Microscopy (Lattice-SIM)

Arteries were cut open longitudinally and fixed with 4% paraformaldehyde in PBS for 1 hr. Following a wash in PBS, arteries were permeabilized with 0.2% Triton X-100 and blocked with 3% BSA + 5% serum for 1 hour at room temperature. Arteries were incubated overnight with anti-PC-1 monoclonal primary antibody (E3 5F4A2, Baltimore PKD Center), anti-PC-2 monoclonal antibody (3374 CT-1 414, Baltimore PKD Center) and anti-CD31 primary monoclonal antibody (Abcam 7388) at 4°C. Arteries were then incubated with Alexa Fluor 488 donkey anti-mouse, Alexa Fluor 546 donkey anti-rabbit secondary antibodies and Alexa Fluor 647 goat anti-rat secondary antibodies (1:500; Molecular Probes) for 1 hour at room temperature. After washing with PBS, arteries were mounted in 80% glycerol solution. Lattice-SIM images were acquired on a Zeiss Elyra 7 AxioObserver microscope equipped with 63x Plan-Apochromat (NA 1.46) oil immersion lens and an sCMOS camera. Lattice-SIM reconstruction was performed using the SIM processing Tool of Zeiss ZEN Black 3.0 SR software. Colocalization analysis of Lattice-SIM data was performed using Pearson's and Mander's coefficients.

Single-Molecule Localization Microscopy (SMLM)

Primary-cultured mesenteric artery endothelial cells were seeded onto 35 mm glass-bottom dishes (MaTeK Corp). Cells were fixed in 4 % paraformaldehyde, permeabilized with 0.5 % Triton X-100 phosphate-buffered saline (PBS) solution and then blocked with 5 % BSA. Cells were immunolabelled using primary antibodies to PC-1 (Baltimore PKD Center), PC-2 (Baltimore PKD Center) and CD31 (Abcam 7388) overnight at 4°C. Alexa Fluor 488 donkey anti-mouse, Alexa Fluor 555 goat anti-rat and Alexa Fluor 647 donkey anti-rabbit secondary antibodies were used for detection. An oxygen scavenging, thiol-based photo-switching imaging buffer was used (GLOX: 50% glucose, 10% PBS, 24 mg/ml glucose oxidase, 12.6 mg/ml catalase supplemented with a reducing agent (cysteamine hydrochloride-MEA) at 100 mM, pH 7.8). Cells were imaged using a super-resolution Zeiss Elyra 7 microscope using 488 nm (500mW), 561 nm (500mW) and 642 nm (500mW) lasers. A 63x Plan-Apochromat (NA 1.46) oil immersion lens and a CMOS camera were used to acquire images. The camera was run in frame-transfer mode at a rate of 100 Hz (30 ms exposure time). Fluorescence was detected using TIRF mode with emission band-pass filters of 550–650 and 660–760 nm.

Localization precision was calculated using $\sigma_{x,y}^2 = [(s^2 + q^2/12) / N] + [(8\pi s^4 b^2) / (q^2 N^2)]$ (1) $\sigma_{x,y}^2 \approx s^2 / N(2)$, where $\sigma_{x,y}$ is the localization precision of a fluorescent probe in lateral dimensions, s is the standard deviation of the point-spread function, N is the total number of photons gathered, q is the pixel size in the image space, and b is the background noise per pixel. The precision of localization is proportional to $\text{DLR}/\sqrt{N}$, where DLR is the diffraction-limited resolution of a fluorophore and N is the average number of detected photons per switching event, assuming the point-spread functions are Gaussian. PC-1 and PC-2 localization were reconstructed from 35,000-40,000 images based on fitting signals to a Gaussian function and taking into account a point spread function calculated using a standardized 40 nm bead slide (ZEN Black software, Zeiss). The first 5000 frames were excluded from the reconstruction to account for time to stabilize photo-switching of the probes. Software drift correction was applied using a model-based cross-correlation.

Statistical analysis

OriginLab and GraphPad InStat software were used for statistical analyses. Values are expressed as mean $\pm$ SEM. Student t-test was used for comparing paired and unpaired data from two populations and ANOVA with Holm-Sidak post hoc test used for multiple group comparisons. $P < 0.05$ was considered significant. Power analysis was performed to verify that the sample size gave a value > 0.8 if P was > 0.05 . Kidney histology and blood pressure experiments were all done single-blind, wherein the person performing the experiments and analysis of the results was not aware of the mouse genotype.

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References

1. J. R. Vane, The Croonian Lecture, 1993. The endothelium: maestro of the blood circulation. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **343**, 225-246 (1994).
2. C. J. Garland, C. R. Hiley, K. A. Dora, EDHF: spreading the influence of the endothelium. *British journal of pharmacology* **164**, 839-852 (2011).
3. J. Hughes *et al.*, The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. *Nat Genet* **10**, 151-160 (1995).
4. C. Boulter *et al.*, Cardiovascular, skeletal, and renal defects in mice with a targeted disruption of the Pkd1 gene. *Proc. Natl. Acad. Sci. U. S. A* **98**, 12174-12179 (2001).
5. S. Bulley *et al.*, Arterial smooth muscle cell PKD2 (TRPP1) channels regulate systemic blood pressure. *eLife* **7** (2018).
6. C. E. MacKay *et al.*, Intravascular flow stimulates PKD2 (polycystin-2) channels in endothelial cells to reduce blood pressure. *eLife* **9** (2020).
7. T. C. Burn *et al.*, Analysis of the genomic sequence for the autosomal dominant polycystic kidney disease (PKD1) gene predicts the presence of a leucine-rich repeat. The American PKD1 Consortium (APKD1 Consortium). *Hum Mol Genet* **4**, 575-582 (1995).
8. F. Qian *et al.*, Cleavage of polycystin-1 requires the receptor for egg jelly domain and is disrupted by human autosomal-dominant polycystic kidney disease 1-associated mutations. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 16981-16986 (2002).
9. E. Hardy, L. Tsiokas, Polycystins as components of large multiprotein complexes of polycystin interactors. *Cell Signal* 10.1016/j.cellsig.2020.109640, 109640 (2020).
10. J. Zhou, Polycystins and primary cilia: primers for cell cycle progression. *Annu Rev Physiol* **71**, 83-113 (2009).
11. S. M. Nauli *et al.*, Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet* **33**, 129-137 (2003).
12. S. Earley, J. E. Brayden, Transient receptor potential channels in the vasculature. *Physiol Rev* **95**, 645-690 (2015).
13. A. L. Brill, B. E. Ehrlich, Polycystin 2: A calcium channel, channel partner, and regulator of calcium homeostasis in ADPKD. *Cell Signal* **66**, 109490 (2019).
14. L. Foggensteiner *et al.*, Cellular and subcellular distribution of polycystin-2, the protein product of the PKD2 gene. *Journal of the American Society of Nephrology : JASN* **11**, 814-827 (2000).
15. X. Liu *et al.*, Polycystin-2 is an essential ion channel subunit in the primary cilium of the renal collecting duct epithelium. *eLife* **7** (2018).

- 745 16. M. Arif Pavel *et al.*, Function and regulation of TRPP2 ion channel revealed by a gain-of-
746 function mutant. *Proceedings of the National Academy of Sciences of the United States*
747 *of America* **113**, E2363-2372 (2016).
- 748 17. S. C. Parnell *et al.*, The polycystic kidney disease-1 protein, polycystin-1, binds and
749 activates heterotrimeric G-proteins in vitro. *Biochem Biophys Res Commun* **251**, 625-
750 631 (1998).
- 751 18. P. S. Shen *et al.*, The Structure of the Polycystic Kidney Disease Channel PKD2 in Lipid
752 Nanodiscs. *Cell* **167**, 763-773.e711 (2016).
- 753 19. M. Grieben *et al.*, Structure of the polycystic kidney disease TRP channel Polycystin-2
754 (PC2). *Nat Struct Mol Biol* **24**, 114-122 (2017).
- 755 20. S. Rossetti *et al.*, Comprehensive molecular diagnostics in autosomal dominant
756 polycystic kidney disease. *Journal of the American Society of Nephrology : JASN* **18**,
757 2143-2160 (2007).
- 758 21. F. A. Valero *et al.*, Ambulatory blood pressure and left ventricular mass in normotensive
759 patients with autosomal dominant polycystic kidney disease. *Journal of the American*
760 *Society of Nephrology : JASN* **10**, 1020-1026 (1999).
- 761 22. A. Martinez-Vea *et al.*, Exercise blood pressure, cardiac structure, and diastolic function
762 in young normotensive patients with polycystic kidney disease: a prehypertensive state.
763 *American journal of kidney diseases : the official journal of the National Kidney*
764 *Foundation* **44**, 216-223 (2004).
- 765 23. V. E. Torres, P. C. Harris, Y. Pirson, Autosomal dominant polycystic kidney disease.
766 *Lancet* **369**, 1287-1301 (2007).
- 767 24. P. A. Gabow, Autosomal dominant polycystic kidney disease--more than a renal
768 disease. *American journal of kidney diseases : the official journal of the National Kidney*
769 *Foundation* **16**, 403-413 (1990).
- 770 25. C. Bergmann *et al.*, Polycystic kidney disease. *Nat Rev Dis Primers* **4**, 50 (2018).
- 771 26. Q. Su *et al.*, Structure of the human PKD1-PKD2 complex. *Science* **361** (2018).
- 772 27. F. Qian *et al.*, PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nat.*
773 *Genet* **16**, 179-183 (1997).
- 774 28. L. J. Newby *et al.*, Identification, characterization, and localization of a novel kidney
775 polycystin-1-polycystin-2 complex. *The Journal of biological chemistry* **277**, 20763-20773
776 (2002).
- 777 29. J. Zhu *et al.*, Structural model of the TRPP2/PKD1 C-terminal coiled-coil complex
778 produced by a combined computational and experimental approach. *Proceedings of the*
779 *National Academy of Sciences of the United States of America* **108**, 10133-10138
780 (2011).
- 781 30. Y. Yu *et al.*, Structural and molecular basis of the assembly of the TRPP2/PKD1
782 complex. *Proceedings of the National Academy of Sciences of the United States of*
783 *America* **106**, 11558-11563 (2009).
- 784 31. K. Hanaoka *et al.*, Co-assembly of polycystin-1 and -2 produces unique cation-
785 permeable currents. *Nature* **408**, 990-994 (2000).
- 786 32. P. Delmas *et al.*, Gating of the polycystin ion channel signaling complex in neurons and
787 kidney cells. *FASEB J* **18**, 740-742 (2004).
- 788 33. L. Tsiokas, E. Kim, T. Arnould, V. P. Sukhatme, G. Walz, Homo- and heterodimeric
789 interactions between the gene products of PKD1 and PKD2. *Proc. Natl. Acad. Sci. U. S.*
790 *A* **94**, 6965-6970 (1997).
- 791 34. M. D. Griffin, V. E. Torres, J. P. Grande, R. Kumar, Vascular expression of polycystin. *J.*
792 *Am. Soc. Nephrol* **8**, 616-626 (1997).

- 793 35. M. T. Nelson, J. B. Patlak, J. F. Worley, N. B. Standen, Calcium channels, potassium
794 channels, and voltage dependence of arterial smooth muscle tone. *Am. J. Physiol* **259**,
795 C3-18 (1990).
- 796 36. P. F. Davies, Flow-mediated endothelial mechanotransduction. *Physiol Rev* **75**, 519-560
797 (1995).
- 798 37. I. Fleming, Molecular mechanisms underlying the activation of eNOS. *Pflugers Arch* **459**,
799 793-806 (2010).
- 800 38. J. L. Balligand, O. Feron, C. Dessy, eNOS activation by physical forces: from short-term
801 regulation of contraction to chronic remodeling of cardiovascular tissues. *Physiol Rev* **89**,
802 481-534 (2009).
- 803 39. V. Garcia, W. C. Sessa, Endothelial NOS: perspective and recent developments. *British*
804 *journal of pharmacology* **176**, 189-196 (2019).
- 805 40. D. Fulton *et al.*, Regulation of endothelium-derived nitric oxide production by the protein
806 kinase Akt. *Nature* **399**, 597-601 (1999).
- 807 41. S. Dimmeler *et al.*, Activation of nitric oxide synthase in endothelial cells by Akt-
808 dependent phosphorylation. *Nature* **399**, 601-605 (1999).
- 809 42. S. Bolte, F. P. Cordelieres, A guided tour into subcellular colocalization analysis in light
810 microscopy. *J Microsc* **224**, 213-232 (2006).
- 811 43. S. J. Ahn *et al.*, Inwardly rectifying K⁺ channels are major contributors to flow-induced
812 vasodilatation in resistance arteries. *The Journal of physiology* **595**, 2339-2364 (2017).
- 813 44. S. Brahler *et al.*, Genetic deficit of SK3 and IK1 channels disrupts the endothelium-
814 derived hyperpolarizing factor vasodilator pathway and causes hypertension. *Circulation*
815 **119**, 2323-2332 (2009).
- 816 45. M. S. Taylor *et al.*, Altered expression of small-conductance Ca²⁺-activated K⁺ (SK3)
817 channels modulates arterial tone and blood pressure. *Circulation research* **93**, 124-131
818 (2003).
- 819 46. J. H. Jaggar, V. A. Porter, W. J. Lederer, M. T. Nelson, Calcium sparks in smooth
820 muscle. *Am. J. Physiol* **278**, C235-C256 (2000).
- 821 47. S. J. Kleene, J. L. Van Houten, Electrical Signaling in Motile and Primary Cilia.
822 *Bioscience* **64**, 1092-1102 (2014).
- 823 48. M. Delling, P. G. DeCaen, J. F. Doerner, S. Febvay, D. E. Clapham, Primary cilia are
824 specialized calcium signalling organelles. *Nature* **504**, 311-314 (2013).
- 825 49. P. G. DeCaen, M. Delling, T. N. Vien, D. E. Clapham, Direct recording and molecular
826 identification of the calcium channel of primary cilia. *Nature* **504**, 315-318 (2013).
- 827 50. S. Yoshida *et al.*, Cilia at the node of mouse embryos sense fluid flow for left-right
828 determination via Pkd2. *Science* **338**, 226-231 (2012).
- 829 51. H. A. Praetorius, K. R. Spring, The renal cell primary cilium functions as a flow sensor.
830 *Curr Opin Nephrol Hypertens* **12**, 517-520 (2003).
- 831 52. M. Delling *et al.*, Primary cilia are not calcium-responsive mechanosensors. *Nature* **531**,
832 656-660 (2016).
- 833 53. H. C. Chapin, V. Rajendran, M. J. Caplan, Polycystin-1 surface localization is stimulated
834 by polycystin-2 and cleavage at the G protein-coupled receptor proteolytic site. *Mol Biol*
835 *Cell* **21**, 4338-4348 (2010).
- 836 54. V. G. Gainullin, K. Hopp, C. J. Ward, C. J. Hommerding, P. C. Harris, Polycystin-1
837 maturation requires polycystin-2 in a dose-dependent manner. *J. Clin. Invest* **125**, 607-
838 620 (2015).
- 839 55. K. Ha *et al.*, The heteromeric PC-1/PC-2 polycystin complex is activated by the PC-1 N-
840 terminus. *eLife* **9** (2020).
- 841 56. W. A. AbouAlaiwi *et al.*, Ciliary polycystin-2 is a mechanosensitive calcium channel
842 involved in nitric oxide signaling cascades. *Circulation research* **104**, 860-869 (2009).

57. Z. Wang *et al.*, The ion channel function of polycystin-1 in the polycystin-1/polycystin-2 complex. *EMBO Rep* 10.15252/embr.201948336, e48336 (2019).
58. S. M. Nauli *et al.*, Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1. *Circulation* **117**, 1161-1171 (2008).
59. V. Babich *et al.*, The N-terminal extracellular domain is required for polycystin-1-dependent channel activity. *The Journal of biological chemistry* **279**, 25582-25589 (2004).
60. S. Feng *et al.*, Identification and functional characterization of an N-terminal oligomerization domain for polycystin-2. *The Journal of biological chemistry* **283**, 28471-28479 (2008).
61. S. J. Kleene, N. K. Kleene, The native TRPP2-dependent channel of murine renal primary cilia. *Am J Physiol Renal Physiol* **312**, F96-F108 (2017).
62. S. J. Kleene *et al.*, The TRPP2-dependent channel of renal primary cilia also requires TRPM3. *PloS one* **14**, e0214053 (2019).
63. J. Xu *et al.*, GPR68 Senses Flow and Is Essential for Vascular Physiology. *Cell* **173**, 762-775 e716 (2018).
64. S. Wang *et al.*, Endothelial cation channel PIEZO1 controls blood pressure by mediating flow-induced ATP release. *The Journal of clinical investigation* **126**, 4527-4536 (2016).
65. A. B. Chapman, K. Stepniakowski, F. Rahbari-Oskoui, Hypertension in autosomal dominant polycystic kidney disease. *Adv Chronic Kidney Dis* **17**, 153-163 (2010).
66. A. Lorthioir *et al.*, Polycystin deficiency induces dopamine-reversible alterations in flow-mediated dilatation and vascular nitric oxide release in humans. *Kidney Int* **87**, 465-472 (2015).
67. I. Antony, G. Lerebours, A. Nitenberg, Loss of flow-dependent coronary artery dilatation in patients with hypertension. *Circulation* **91**, 1624-1628 (1995).
68. Y. Wang *et al.*, Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. *Nature* **465**, 483-486 (2010).

Figure 1. Flow stimulates PC-1-mediated, Ca^{2+} -dependent currents in mesenteric artery endothelial cells that elicit arterial hyperpolarization. (A) Representative Western blots illustrating PC-1, PC-2, SK3, IK, TRPV4, Piezo1, eNOS, GPR68 and actin proteins in mesenteric arteries of *Pkd1^{fl/fl}* and *Pkd1* eCKO mice. (B) Mean data for PC-1, PC-2, SK3, IK, TRPV4, Piezo1, eNOS, GPR68, with n=4, 6, 4, 4, 7, 8, 7, 4, respectively. * indicates $P < 0.05$. (C) En-face immunofluorescence illustrating that PC-1 (Alexa Fluor 546) is abolished in endothelial cells of *Pkd1* eCKO mice mesenteric arteries (representative of 8 arteries from *Pkd1^{fl/fl}* and 7 *Pkd1* eCKO mice, respectively). CD31 (Alexa Fluor 488) and DAPI are also shown. Scale bars = 50 μm . (D) Original recordings of steady-state current modulation by flow (10 ml/min) and effect of removing bath Ca^{2+} in endothelial cells of *Pkd1^{fl/fl}* and *Pkd1* eCKO mice voltage-clamped at -60 mV. (E) Mean data for flow-induced transient inward current density. n=15 for *Pkd1^{fl/fl}* and n=16 for *Pkd1* eCKO. * indicates $P < 0.05$ versus *Pkd1^{fl/fl}*. (F) Mean data for steady-state current density (*Pkd1^{fl/fl}*: static+ Ca^{2+} , n=15; flow+ Ca^{2+} , n=15; flow with Ca^{2+} free bath solution, n=12 and *Pkd1* eCKO: static+ Ca^{2+} , n=16; flow+ Ca^{2+} , n=16; flow with Ca^{2+} free bath, n=13). * $P < 0.05$ versus static+2 mM Ca^{2+} in the same genotype, # $P < 0.05$ vs *Pkd1^{fl/fl}* under the same condition, & $P < 0.05$ versus flow Ca^{2+} in the same genotype. (G) Original membrane potential recordings obtained using microelectrodes in pressurized (80 mmHg) mesenteric arteries of *Pkd1^{fl/fl}* and *Pkd1* eCKO mice in static or flow (15 dyn/cm²) conditions. (H) Mean data (*Pkd1^{fl/fl}*: 10 mmHg, n=8; 80 mmHg, n=14; 80 mmHg+flow, n=19; *Pkd1* eCKO: 10 mmHg, n=9; 80 mmHg, n=14; 80 mmHg+flow, n=18). * $P < 0.05$ versus static at 10 mmHg in the same genotype. # $P < 0.05$ for flow versus static at 80 mmHg in the same genotype. & indicates $P < 0.05$ versus *Pkd1^{fl/fl}* under the same condition.

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898 **Figure 1 – figure supplement 1. Genomic PCR indicating that tamoxifen stimulated Cre-**
899 **recombination in mesenteric arteries of *Pkd1^{fl/fl}*: *Cdh5*(PAC)-CreERT2 mice.**

900 Representative of four separate experiments.

901

Figure 2. Endothelial cell PC-1 stimulates vasodilation via eNOS, IK channel and SK channel activation. (A) Representative traces illustrating reversible flow-mediated dilation in pressurized (80 mmHg) mesenteric arteries of *Pkd1^{fl/fl}* and *Pkd1* ecKO mice. (B) Mean dilation to flow (15 dyn/cm²) or ACh (10 μM). *P<0.05 versus *Pkd1^{fl/fl}*. n=8 for each dataset. (C) Representative diameter changes to stepwise increases in intravascular flow in pressurized (80 mmHg) mesenteric arteries from *Pkd1^{fl/fl}* and *Pkd1* ecKO mice. (D) Mean data. The *Pkd1*-sensitive component of flow-mediated vasodilation is shown in blue. n=4 each for *Pkd1^{fl/fl}* and *Pkd1* ecKO. * P<0.05 versus *Pkd1^{fl/fl}*. (E, F, G) Regulation of flow (15 dyn/cm²)-mediated dilation by L-NNA (10 μM), apamin (300 nM) and Tram-34 (300 nM) in pressurized (80 mmHg) mesenteric arteries of *Pkd1^{fl/fl}* and *Pkd1* ecKO mice. (H) Mean data for inhibition of flow-mediated vasodilation (FMD) by L-NNA (*Pkd1^{fl/fl}* n=8, *Pkd1* ecKO n=10), Tram-34 (*Pkd1^{fl/fl}* n=5, *Pkd1* ecKO n=5) and apamin (*Pkd1^{fl/fl}* n=5, *Pkd1* ecKO n=5). Symbols illustrate # P<0.05 versus flow in the same genotype and * P<0.05 versus *Pkd1^{fl/fl}* in the same condition. (I) Original Western blots illustrating effects of flow (15 dyn/cm², 5 min, 37°C) on p-eNOS (S1176) and total eNOS proteins in *Pkd1^{fl/fl}* and *Pkd1* ecKO mesenteric arteries. (J) Mean data for flow-induced change (Δ) in proteins. *Pkd1^{fl/fl}* n=4, *Pkd1* ecKO n=6. * indicates P<0.05 versus static in the same genotype.

Figure 2 – figure supplement 1. Depolarization-induced vasoconstriction and passive diameter are similar in *Pkd1^{fl/fl}* and *Pkd1* ecKO arteries. (A) Representative traces illustrating dilations to ACh (10 μ M) and Ca^{2+} -free bath solution in pressurized (80 mmHg) *Pkd1^{fl/fl}* and *Pkd1* ecKO arteries. (B) PC-1 knockout attenuates flow-mediated dilation over a broad shear stress range in pressurized (80 mmHg) mesenteric arteries. n=4 for each shear stress value. (C) Representative traces illustrating constriction to 60 mM K^+ in pressurized (10 mmHg) arteries. (D) Mean data for 60 mM K^+ -induced constriction. n=8 for each dataset. (E) Mean myogenic tone at 80 mmHg. n=8 for each dataset. (F) Mean data for passive diameter (Ca^{2+} -free PSS) in pressurized (80 mmHg) arteries. n=8 for each.

Figure 3. *Pkd1* ecKO mice are hypertensive with normal kidney anatomy. (A) Blood pressure recordings obtained over 24 hours in a *Pkd1*^{fl/fl} and *Pkd1* ecKO mouse. (B) Mean arterial pressures (MAP) in *Pkd1*^{fl/fl} (n=5) and *Pkd1* ecKO (n=7) mice. * P<0.05 versus *Pkd1*^{fl/fl}. (C) Mean heart rate (HR). *Pkd1*^{fl/fl}, n=5, *Pkd1* ecKO, n=7. (D) Images of H&E-stained kidney cortex used for histological measurements. Scale bars = 100 µm (E) Mean proximal tubule length. n=15 proximal tubules measured in each mouse, n=3 mice. (F) Mean glomeruli surface area. n=75 glomeruli measured from each mouse, n=3 mice.

Figure 3 – figure supplement 1. Diastolic and systolic blood pressures are higher in *Pkd1* ecKO mice. (A) Mean diastolic and systolic blood pressures for *Pkd1*^{fl/fl} (n=5) and *Pkd1* ecKO (n=7) mice. *= $P < 0.05$ vs *Pkd1*^{fl/fl}. (B) Mean data for *Pkd1*^{fl/fl} (n=5) and *Pkd1* ecKO (n=7) mouse activity.

Figure 4. PC-1 and PC-2 coassemble and colocalize in endothelial cells. (A)

Representative Western blots illustrating the immunoblot (IB) detection of both PC-1 and PC-2 in PC-2 immunoprecipitate (IP) (n=5). (B) PC-1 (Alexa546) and PC-2 (Alexa488) antibody labeling generates FRET in mesenteric artery endothelial cells of *Pkd1^{fl/fl}* mice that is abolished in endothelial cells of *Pkd1* ecKO and *Pkd2* ecKO mice. Scale bar = 10 μ m. (C) Mean data for immunoFRET experiments (*Pkd1^{fl/fl}* n=12, *Pkd1* ecKO n=10, *Pkd2* ecKO n=10). * P<0.05 versus *Pkd1^{fl/fl}*. (D) Lattice SIM images of PC-1, PC-2 and CD31 immunofluorescence in the same endothelial cells of an en face mesenteric artery. The merged image is also shown with yellow pixels illustrating colocalization of PC-1 and PC-2. Scale bars = 10 μ m. (E) Mean data for PC-1 and PC-2 colocalization using both Pearson's and Mander's correlation coefficients. n=25 images, 12 arteries and 6 mice for each dataset. (F) Lattice SIM image of PC-1 and PC-2 immunofluorescence in a mesenteric artery endothelial cell. Yellow pixels illustrate PC-1 to PC-2 colocalization. Scale bar = 10 μ m. (G) Mean data for PC-1 and PC-2 colocalization when using Pearson's and Mander's correlation coefficients. n=13, 4 mice for each dataset.

Figure 4 - figure supplement 1. PC-1 or PC-2 knockout abolishes immunoFRET in endothelial cells. PC-1 (Alexa546) and PC-2 (Alexa488) antibody labeling and FRET in mesenteric artery endothelial cells of *Pkd1* ecKO (panel A) and *Pkd2* ecKO (panel B) mice. Scale bars = 10 μ m.

Figure 5. Plasma membrane PC-1 and PC-2 clusters colocalize in endothelial cells. (A)

TIRF-SMLM images of PC-1 and PC-2 surface clusters in a *Pkd1^{fl/fl}* and *Pkd1* eCKO endothelial cell. Scale bars = 5 μ m. (B) Mean data for PC-1 and PC-2 cluster density measured in *Pkd1^{fl/fl}* and *Pkd1* eCKO endothelial cells under static and flow (10 ml/min) conditions. n=8 for *Pkd1^{fl/fl}* (static), n=10 for *Pkd1* eCKO (static) and n=10 for *Pkd1^{fl/fl}* (flow). * P<0.05 versus *Pkd1^{fl/fl}* (static). (C) Mean data for PC-1 and PC-2 cluster sizes measured in *Pkd1^{fl/fl}* and *Pkd1* eCKO endothelial cells under static and flow (10 ml/min) conditions. n=8 for *Pkd1^{fl/fl}* (static), n=10 for *Pkd1* eCKO (static) and n=10 for *Pkd1^{fl/fl}* (flow). * P<0.05 versus respective floxed control in static, # P<0.05 versus PC-1 cluster size in the same genotype under the same condition. (D) Mean data for PC-1 to PC-2 nearest-neighbor analysis. n=8 for *Pkd1^{fl/fl}* (static), n=10 for *Pkd1* eCKO (static) and n=10 for *Pkd1^{fl/fl}* (flow). * P<0.05 versus *Pkd1^{fl/fl}* static and *Pkd1^{fl/fl}* flow. (E) TIRF-SMLM images of PC-1 and PC-2 clusters, overlap of PC-1 and PC-2 data and overlap of PC-1 and PC-2 data following Coste's randomization (Rand) simulation in *Pkd1^{fl/fl}* and *Pkd1* eCKO cells. Scale bars = 5 μ m. (F) Mean experimental and Costes' randomized (Rand) data for PC-1 to PC-2 overlap in *Pkd1^{fl/fl}* cells in static and flow and *Pkd1* eCKO cells in static. n=8 for *Pkd1^{fl/fl}* (static), n=10 for *Pkd1* eCKO (static) and n=10 for *Pkd1^{fl/fl}* (flow). * P<0.05 versus respective floxed control in static condition, # P<0.05 versus *Pkd1^{fl/fl}* static.

Figure 5 – figure supplement 1. Localization precision of fluorophores used in SMLM experiments. Mean data illustrating the localization precision (FWHM) of Alexa Fluor 488 (panel A)- and Alexa Fluor 647 (panel B)-tagged secondary antibodies in endothelial cells under all conditions when imaged using SMLM. (A) Experimental numbers for Alexa Fluor 488 in *Pkd1^{fl/fl}* (static), *Pkd2^{fl/fl}* (static), *Pkd1^{fl/fl}* (flow), *Pkd2^{fl/fl}* (flow), *Pkd1* ecKO (static), *Pkd2* ecKO (static) are 8, 10, 10, 10, 10 and 9, respectively. (B) Experimental numbers for Alexa Fluor 647 in *Pkd1^{fl/fl}* (static), *Pkd2^{fl/fl}* (static), *Pkd1^{fl/fl}* (flow), *Pkd2^{fl/fl}* (flow), *Pkd1* ecKO (static), *Pkd2* ecKO (static) are 8, 10, 10, 10, 10 and 9, respectively. Numbers provided in the results are the combined means of all data collected for Alexa Fluor 488 and Alexa Fluor 647 in all genotypes in static and flow.

Figure 5 – figure supplement 2. Properties of PC-1 and PC-2 clusters and their spatial proximity and overlap in *Pkd1^{fl/fl}* and *Pkd1* ecKO endothelial cells. (A) Histogram illustrating PC-1 cluster size distribution in a *Pkd1^{fl/fl}* (static) and *Pkd1* ecKO (static) endothelial cell. (B) Histogram illustrating PC-2 cluster sizes in a *Pkd1^{fl/fl}* (static) and *Pkd1* ecKO (static) endothelial cell. (C) Histograms illustrating the distance from each PC-1 cluster to its nearest PC-2 neighbor in a *Pkd1^{fl/fl}* and *Pkd1* ecKO endothelial cell. (D) Mean experimental and randomized data for PC-2 to PC-1 overlap in *Pkd1^{fl/fl}* cells in static and flow and *Pkd1* ecKO cells in static. n=10 for *Pkd2^{fl/fl}* (static), n=9 for *Pkd2* ecKO (static) and n=10 for *Pkd2^{fl/fl}* (flow). * P<0.05 versus respective floxed control in static condition, # P<0.05 versus *Pkd1^{fl/fl}* static. (E) Histogram illustrating the distance from each PC-1 cluster to its nearest PC-2 neighbor in a *Pkd1^{fl/fl}* endothelial cell under flow (10 ml/min).

Figure 5 – figure supplement 3. Properties of PC-1 and PC-2 surface clusters in endothelial cells of *Pkd2^{fl/fl}* and *Pkd2* ecKO mice. (A) TIRF-SMLM images of PC-1 and PC-2 surface clusters in a *Pkd2^{fl/fl}* and *Pkd2* ecKO endothelial cell. Scale bars = 5 μ m. (B) Mean data for PC-1 and PC-2 cluster density measured in *Pkd2^{fl/fl}* and *Pkd2* ecKO endothelial cells under static and flow conditions. Experimental numbers for *Pkd2^{fl/fl}* static, *Pkd2* ecKO static and *Pkd2^{fl/fl}* flow are 10, 9 and 10 for PC-1 and PC-2. * $P < 0.05$ versus *Pkd2^{fl/fl}* (static). (C) Histogram of individual PC-2 cluster sizes in a *Pkd2^{fl/fl}* (static) and *Pkd2* ecKO (static) endothelial cell. (D) Histogram of individual PC-1 cluster sizes in a *Pkd2^{fl/fl}* (static) and *Pkd2* ecKO (static) endothelial cell. (E) Mean data for PC-1 and PC-2 cluster sizes measured in *Pkd2^{fl/fl}* and *Pkd2* ecKO endothelial cells under static and flow (10 ml/min) conditions. Experimental numbers for *Pkd2^{fl/fl}* static, *Pkd2* ecKO static and *Pkd2^{fl/fl}* flow are 10, 9 and 10 for PC-1 and PC-2. * $P < 0.05$ versus same protein in *Pkd2^{fl/fl}* static, # $P < 0.05$ versus PC-1 in same condition. (F) Histogram illustrating the distance from each PC-2 cluster to its nearest PC-1 neighbor in a *Pkd2^{fl/fl}* and *Pkd2* ecKO endothelial cell. (G) Mean data for distance from each PC-2 cluster to its nearest PC-1 neighbor. n=10 for *Pkd2^{fl/fl}* static, n=9 for *Pkd2* ecKO static, n=10 for *Pkd2^{fl/fl}* flow. * $P < 0.05$ versus *Pkd2^{fl/fl}* static and *Pkd2^{fl/fl}* flow. (H) Histogram illustrating the distance from each PC-2 cluster to the nearest PC-1 neighbor in a *Pkd2^{fl/fl}* endothelial cell under flow (10 ml/min).

Figure 5 – figure supplement 4. Analysis of PC-1 and PC-2 cluster overlap in endothelial cells of *Pkd2^{fl/fl}* and *Pkd2* ecKO mice. (A) TIRF-SMLM images of PC-1 and PC-2 clusters, overlap of PC-1 and PC-2 data and overlap of PC-1 and PC-2 data following Costes' randomization simulation. Scale bars = 5 μ m. (B) Mean experimental and randomized overlap data for PC-1 to PC-2 and PC-2 to PC-1 in *Pkd2^{fl/fl}* and *Pkd2* ecKO cells. n=10 for *Pkd2^{fl/fl}* static, n=9 for *Pkd2* ecKO static, n=10 for *Pkd2^{fl/fl}* flow. * P<0.05 versus flox under the same condition, # P<0.05 versus *Pkd2^{fl/fl}* static.

Figure 6. PC-1 and PC-2 are interdependent for flow-mediated vasodilation and I_{Cat} activation in mesenteric artery endothelial cells. (A) Representative Western blots of PC-1, PC-2 and eNOS in mesenteric arteries of *Pkd1^{fl/fl}/Pkd2^{fl/fl}* and *Pkd1/Pkd2* ecKO mice. (B) Mean data. * $P < 0.05$ versus *Pkd1^{fl/fl}/Pkd2^{fl/fl}*. $n=5$ for each dataset. (C) Representative traces illustrating flow-mediated dilation by 15 and 23 dyn/cm² shear stress in pressurized (80 mmHg) mesenteric arteries of *Pkd1^{fl/fl}/Pkd2^{fl/fl}* and *Pkd1/Pkd2* ecKO mice. (D) Mean dilation to flow (15 and 23 dyn/cm²) or ACh (10 μ M). $n=6$ each for 15 dyn/cm², 23 dyn/cm² and ACh. * $P < 0.05$ versus *Pkd1^{fl/fl}/Pkd2^{fl/fl}*. # $P < 0.05$ versus 15 dyn/cm² in the same genotype. (E) Original recordings illustrating that flow (10 ml/min) activates I_{Cat} in *Pkd1^{fl/fl}* and *Pkd2^{fl/fl}* endothelial cells that are similarly attenuated in PC-1 ecKO and PC-2 ecKO endothelial cells. (F) Intracellular introduction via the patch pipette of either a PC-1 or PC-2 C-terminus coiled-coil domain peptide reduces flow-induced I_{Cat} in *Pkd1^{fl/fl}* endothelial cells. Traces shown in panels E and F are recorded from different endothelial cells. (G) Mean data. PC-1 pep and PC-2 pep indicate peptides corresponding to the coiled-coil domains in PC-1 and PC-2, respectively. *Pkd1^{fl/fl}* $n=7$, *Pkd2^{fl/fl}* $n=8$, *Pkd1^{fl/fl}* + scrambled (scram) PC-1 pep $n=6$, *Pkd1^{fl/fl}* + scram PC-2 pep $n=7$, *Pkd1* ecKO $n=8$, *Pkd2* ecKO $n=9$, *Pkd1^{fl/fl}*+PC-1 peptide $n=6$, *Pkd2^{fl/fl}*+PC-1 peptide $n=7$, *Pkd1^{fl/fl}*+PC-2 peptide $n=6$ and *Pkd2^{fl/fl}*+PC-2 peptide $n=7$. * $P < 0.05$ versus no peptide in same genotype. # $P < 0.05$ versus respective scrambled peptide in same genotype.

Figure 6 – figure supplement 1. Smooth muscle-specific vasoconstriction and vasodilation and passive diameter are unaltered in *Pkd1/Pkd2* ecKO mouse arteries. (A) Representative traces illustrating 60 mM-induced K⁺ constriction in a pressurized (10 mmHg) *Pkd1^{fl/fl}/Pkd2^{fl/fl}* and *Pkd1/Pkd2* ecKO artery. (B) Mean data for 60 mM K⁺-induced constriction. n=6 for each dataset. (C) Mean myogenic tone in pressurized (80 mmHg) mesenteric arteries from *Pkd1^{fl/fl}/Pkd2^{fl/fl}* and *Pkd1/Pkd2* ecKO mice. n=6 for each dataset. (D) Mean data for SNP (10μM)-induced dilation in pressurized (10mmHg) *Pkd1^{fl/fl}/Pkd2^{fl/fl}* and *Pkd1/Pkd2* ecKO arteries. n=6 for each dataset. (E) Mean data for passive diameter in pressurized (80 mmHg) arteries. n=6 for each dataset.

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Figure 6 – figure supplement 2. Scrambled PC-1 and PC-2 peptides do not alter flow-activated I_{Cat} in endothelial cells. Original recordings illustrating that intracellular introduction via the patch pipette of either a PC-1 scrambled (scram) peptide or a PC-2 scrambled peptide does not alter flow (10 ml/min)-activated I_{Cat} in *Pkd1^{fl/fl}* endothelial cells. Each trace is recorded from a different endothelial cell.

A

B

C

D

E

F

A

B

A

B

C

D

E

F

G

A

B

A

B

C

D

E

F

A

B

Pkd1^{fl/fl} (static) *Pkd2^{fl/fl}* (static) *Pkd1^{fl/fl}* (flow)
 Pkd2^{fl/fl} (flow) *Pkd1* ecKO (static) *Pkd2* ecKO (static)

A

B

A

B

C

D

E

F

G

A

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