Figures and figure supplements

Arterial smooth muscle cell PKD2 (TRPP1) channels regulate systemic blood pressure

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Figure 1. Activation of Cre recombinase abolishes PKD2 in arterial myocytes of \( Pkd2^{fl/fl} \):myh11-cre/ERT2 mice. (A) RT-PCR showing the absence of PKD2 transcript in isolated myocytes from tamoxifen-treated \( Pkd2^{fl/fl} \):myh11-cre/ERT2 mice. (B) Western blots illustrating the effect of tamoxifen-treatment in \( Pkd2^{fl/fl} \) and \( Pkd2^{fl/fl} \):myh11-cre/ERT2 mice on PKD2, CaV1.2L (full-length CaV1.2) and CaV1.2S (short CaV1.2) proteins in mesenteric and hindlimb arteries. (C) Mean data for proteins in mesenteric arteries of tamoxifen-treated \( Pkd2^{fl/fl} \):myh11-cre/ERT2 mice when compared to those in tamoxifen-treated \( Pkd2^{fl/fl} \) mice. \( n = 4–7 \). * indicates \( p<0.05 \) versus \( Pkd2^{fl/fl} \). (D) En-face immunofluorescence imaging illustrating that PKD2 protein (red, Alexa Fluor 555) is abolished in myocytes of mesenteric and hindlimb arteries in tamoxifen-treated \( Pkd2^{fl/fl} \):myh11-cre/ERT2 mice (representative of 6 mesenteric and six hindlimb arteries). In contrast, PKD2 protein in endothelial cells is unaltered. Nuclear staining (DAPI) is also shown. Scale bars = 20 \( \mu m \). (E) Confocal and DIC images illustrating that PKD2 protein (Alexa Fluor 555) is abolished in isolated mesenteric artery myocytes of tamoxifen-treated \( Pkd2^{fl/fl} \):myh11-cre/ERT2 mice (representative data from 5 \( Pkd2^{fl/fl} \) and 5 \( Pkd2^{fl/fl} \):myh11-cre/ERT2 mice). Scale bars = 10 \( \mu m \). DOI: https://doi.org/10.7554/eLife.42628.002
**Figure 1—figure supplement 1.** Genotyping of mouse lines. Ethidium bromide gel illustrating PCR products in vasculature of C57BL/6J (WT) mice and tamoxifen-treated Pkd2^{fl/fl} and Pkd2^{fl/fl}:myh11cre/ERT2 mice. DOI: https://doi.org/10.7554/eLife.42628.003
Figure 1—figure supplement 2. PKD2 protein is lower in aorta and mesenteric and hindlimb arteries from tamoxifen-treated Pkd2<sup>fl/fl</sup>:myh11-cre/ERT2 mice. (A) Western blots illustrating PKD2 protein was lower in mesenteric arteries of tamoxifen-treated Pkd2<sup>fl/fl</sup>:myh11-cre/ERT2 mice, whereas other proteins were similar. (B) Mean data for proteins in hindlimb arteries of Pkd2 smKO mice (n = 4–6). (C) Western blots of proteins in aorta. Cav1.2L, full-length Cav1.2; Cav1.2S, short Cav1.2. (D) Mean data from aorta (n = 4). * indicates p<0.05 versus Pkd2<sup>fl/fl</sup>. DOI: https://doi.org/10.7554/eLife.42628.004
Figure 1—figure supplement 3. Several proteins that regulate arterial contractility are unchanged in tamoxifen-treated Pkd2\textsuperscript{fl/fl}:myh11-cre/ERT2 mice. (A) Western blots illustrating Angiotensin II type one receptor (AT1R), Piezo1, α1-adrenergic receptor A (α1A), α1-adrenergic receptor B (α1B), α1-adrenergic receptor D (α1D) and G protein-coupled receptor 68 (GPR68) protein levels in mesenteric and hindlimb arteries of Pkd2\textsuperscript{fl/fl} and Pkd2\textsuperscript{fl/fl}:myh11-cre/ERT2 mice. (B) Mean data from mesenteric and hindlimb arteries (n = 4 per group).
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Figure 2. *Pkd2* smKO mice are hypotensive with normal cardiac function and renal histology. (A) Original telemetric blood pressure recordings from *Pkd2* smKO and *Pkd2*fl/fl mice. (B) Mean systolic and diastolic blood pressures in *Pkd2*fl/fl (n = 11) and *Pkd2* smKO (n = 12) mice. * indicates p<0.05 versus *Pkd2*fl/fl. (C) Mean arterial blood pressures (MAP) in *Pkd2*fl/fl (n = 11) and *Pkd2* smKO (n = 12) mice during day and night (gray) cycles. ZT: Zeitgeber Time. * indicates p<0.05 versus *Pkd2*fl/fl for all data points. (D) Mean echocardiography data. Cardiac output (CO), fractional shortening (FS), ejection fraction (EF) and heart rate (HR). (*Pkd2*fl/fl, n = 5; *Pkd2* smKO mice, n = 4). (E) Representative images of H and E stained kidney cortex used for histological assessment (n = 3 mice used for each group).

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Figure 2—figure supplement 1. Lower blood pressure is sustained in Pkd2 smKO mice. (A) Mean arterial blood pressure (MAP) in Pkd2 smKO and Pkd2fl/fl mice (n = 6 per group). * indicates p<0.05 versus Pkd2fl/fl. (B) Mean data of locomotor activity, n = 6 per group.
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Figure 3. Pressure-induced vasoconstriction is attenuated in Pkd2 smKO mouse hindlimb arteries. (A) Representative traces illustrating diameter responses to intravascular pressure in gastrocnemius arteries of Pkd2^{fl/fl} and Pkd2 smKO mice. (B) Mean data for myogenic tone in gastrocnemius arteries (Pkd2^{fl/fl}, n = 5; Pkd2 smKO, n = 6). * indicates p<0.05 versus Pkd2^{fl/fl}. (C) Representative traces illustrating hindlimb perfusion pressure in response to increasing flow. (D) Mean data for hindlimb perfusion pressure (Pkd2^{fl/fl}, n = 6; Pkd2 smKO, n = 4). * indicates p<0.05 versus Pkd2^{fl/fl}.

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Figure 3—figure supplement 1. Myocyte PKD2 knockout does not alter phenylephrine or angiotensin II-induced vasoconstriction in hindlimb arteries. (A) Mean passive diameter at 80 mmHg of first-order gastrocnemius arteries (G) and third-, fourth- and fifth-order mesenteric arteries (M) (Pkd2<sup>fl/fl</sup>: G, n = 5; M<sup>3<sup>rd</sup></sup>n = 4; M<sup>4<sup>th</sup></sup>n = 5; M<sup>5<sup>th</sup></sup>n = 5 and Pkd2 smKO: G, n = 5; M3<sup>rd</sup>n = 7; M4<sup>th</sup>n = 4; M5<sup>th</sup>n = 5). (B) Mean data for 60 mM K<sup>+</sup>-induced constriction in pressurized (100 mmHg) gastrocnemius arteries from Pkd2<sup>fl/fl</sup> (n = 4) and Pkd2 smKO (n = 4) mice. * indicates p<0.05 versus Pkd2<sup>fl/fl</sup>. (C) Mean data of phenylephrine-induced constriction in pressurized gastrocnemius arteries (Pkd2<sup>fl/fl</sup> n = 4, Pkd2 smKO n = 5). (D) Mean data of angiotensin II-induced constriction in gastrocnemius arteries pressurized to 100 mmHg (Pkd2<sup>fl/fl</sup>, n = 5 and Pkd2 smKO, n = 5–6). (E) Mean data of phenylephrine-induced pressure responses in intact hindlimb (Pkd2<sup>fl/fl</sup>, n = 11–13 and Pkd2 smKO, n = 8–9).

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Figure 4. PKD2 channels contribute to pressure-induced hindlimb artery depolarization and swelling-activated Na+ currents in hindlimb artery myocytes. (A) Representative traces of microelectrode impalements under indicated conditions illustrating that pressure-induced depolarization is attenuated in gastrocnemius arteries of Pkd2 smKO mice. Phenylephrine (PE) = 1 μM. Scale bars: Y = 10 mV, X = 20 s. (B) Mean data for membrane potential recordings in pressurized hindlimb arteries in the absence or presence of PE (Pkd2fl/fl: 10 mmHg, n = 11; 100 mmHg, n = 10; 100 mmHg + PE, n = 13 and Pkd2 smKO: 10 mmHg, n = 11; 100 mmHg, n = 10; 100 mmHg + PE, n = 14). * indicates p<0.05 versus 10 mmHg in Pkd2fl/fl. # indicates p<0.05 versus 100 mmHg in the same genotype. (C) Representative ICats recorded between −100 and +100 mV in isotonic (300 mOsm), hypotonic (250 mOsm) and hypotonic bath solution with Gd3+ (100 μM) in the same Pkd2fl/fl and Pkd2 smKO mouse hindlimb artery myocytes. (D) Representative I-V relationships of Gd3+ -sensitive ICats activated by hypotonic solution in Pkd2fl/fl and Pkd2 smKO hindlimb myocytes. (E) Mean data for Gd3+ -sensitive ICats activated by hypotonic solution in Pkd2fl/fl and Pkd2 smKO hindlimb myocytes (n = 5 for each). * indicates p<0.05 versus 250 mOsm, # p<0.05 versus Pkd2fl/fl. (F) Representative I-V relationships between −100 and +100 mV in isotonic (300 mOsm), hypotonic (250 mOsm) and hypotonic bath solution with low (40 mM) Na+ in the same Pkd2fl/fl mouse hindlimb artery myocyte.

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Figure 4—figure supplement 1. PKD2 knockout does not alter phenylephrine (PE)-activated ICa in isolated hindlimb artery myocytes. (A) Representative I-V relationships recorded between $-100$ and $+100$ mV in the same hindlimb artery myocytes of Pkd2$^{fl/fl}$ or Pkd2 smKO mice in control and PE (10 μM). (B) Mean data for current density at $-100$ and $+100$ mV (Pkd2$^{fl/fl}$, n = 6 and Pkd2 smKO, n = 6). * indicates p<0.05 versus control in the same genotype.

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Figure 5. Pressure-induced vasoconstriction is unaltered, whereas phenylephrine-induced vasoconstriction is attenuated, in mesenteric arteries of Pkd2 smKO mice. (A) Mean vasoconstriction over a range of pressures in resistance-size mesenteric arteries from Pkd2fl/fl (n = 7) and Pkd2 smKO (n = 9) mice. (B) Original recordings of concentration-dependent, phenylephrine (PE)-induced contraction in mesenteric artery rings. (C) Mean PE-induced contraction (Pkd2fl/fl, n = 5, Pkd2 smKO, n = 6; *p<0.05 versus Pkd2fl/fl). (D) Representative phenylephrine-induced vasoconstriction in pressurized (80 mmHg) fifth-order mesenteric arteries. (E) Mean PE-induced vasoconstriction in pressurized (80 mmHg) fourth-and fifth-order mesenteric arteries (Pkd2fl/fl, n = 6, Pkd2 smKO, n = 6; *p<0.05 versus Pkd2fl/fl at the same PE concentration).

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Figure 5—figure supplement 1. Myocyte PKD2 knockout attenuates phenylephrine-induced vasoconstriction, but does not alter pressure or angiotensin II-induced vasoconstriction in hindlimb arteries. (A) Mean myogenic tone at 80 mmHg illustrating that myogenic tone is similar in third-, fourth- and fifth-order mesenteric arteries and unaltered by PKD2 knockout (Pkd2fl/fl: 3rd n = 4; 4th n = 5; 5th n = 4 and Pkd2 smKO: 3rd n = 7; 4th n = 4; 5th n = 4). (B) Mean data for 60 mM K+-induced constriction in first- and second order mesenteric artery rings (Pkd2fl/fl n = 5; Pkd2 smKO n = 6). (C) Mean data for phenylephrine-induced vasoconstriction in pressurized, endothelium-denuded 4th order mesenteric arteries (Pkd2fl/fl, n = 3 and Pkd2 smKO, n = 3). * indicates p<0.05 versus Pkd2fl/fl. (D) Mean myogenic tone at 80 mmHg in endothelium-denuded 4th order mesenteric arteries (Pkd2fl/fl, n = 3 and Pkd2 smKO, n = 3). (E) Mean data for angiotensin II-induced vasoconstriction in mesenteric arteries (Pkd2fl/fl, n = 11–12 and Pkd2 smKO, n = 10–11).

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Figure 6. PKD2 channels contribute to phenylephrine-induced mesenteric artery depolarization and INa in mesenteric artery myocytes. (A) Representative traces of microelectrode impalements illustrating that phenylephrine (PE, 1 μM)-induced depolarization is attenuated in mesenteric arteries of Pkd2 smKO mice. Scale bars: Y = 10 mV, X = 20 s. (B) Mean membrane potential recordings in pressurized (10 and 80 mmHg) mesenteric arteries and in PE at 80 mmHg (Pkd2fl/fl: 10 mmHg, n = 13; 80 mmHg, n = 9; 80 mmHg + PE, n = 15. Pkd2 smKO: 10 mmHg, n = 11; 80 mmHg, n = 12; 80 mmHg + PE, n = 12). *p<0.05 versus 10 mmHg in the same genotype. # p<0.05 versus 80 mmHg in the same genotype. (C) Original current recordings obtained between /C0 and +100 mV in the same Pkd2fl/fl and Pkd2 smKO myocytes in control, PE (10 μM), low Na+ (40 mM)+PE and low Na+ (40 mM)+PE + Gd³⁺ (100 μM). (D) Mean paired data (Pkd2fl/fl, n = 5; Pkd2 smKO, n = 5, *p<0.05 versus control in the same genotype).

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Figure 6—figure supplement 1. PKD2 knockout does not alter swelling-activated Icat in isolated mesenteric artery myocytes. (A) Representative I-V relationships from the same isolated mesenteric artery myocytes of Pkd2^{fl/fl} or Pkd2 smKO mice in isosmotic (300 mOsm), hyposmotic (250 mOsm) and hyposmotic (250 mOsm) + Gd^{3+} (100 μM) solutions. (B) Mean data for hyposmotic activated Gd^{3+} (100 μM)-sensitive cationic current density at -100 and +100 mV (Pkd2^{fl/fl}, n = 6 and Pkd2 smKO, n = 6).

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Figure 7. Angiotensin II-induced hypertension is attenuated in Pkd2 smKO mice. (A) Telemetric blood pressure time course showing the development of angiotensin II-induced hypertension in Pkd2^fl/fl (n = 6) and Pkd2 smKO mice (n = 9). Osmotic minipumps containing either saline or angiotensin II were implanted one day prior to day 0. * indicates p<0.05 versus Pkd2^fl/fl in the same condition. (B) Western blots illustrating total PKD2 protein in mesenteric and hindlimb arteries of saline-and angiotensin II-treated control mice. (C) Mean total PKD2 protein in mesenteric and hindlimb arteries of angiotensin II-treated mice compared to saline control (n = 8 for each group). * indicates p<0.05 versus saline in the same arterial preparation. (D) Western blots showing surface and intracellular PKD2 protein in arteries of saline-and angiotensin II-treated mice. (E) Mean surface PKD2 protein in mesenteric and hindlimb arteries of angiotensin II-treated mice compared to saline control (n = 8 for each group). * indicates p<0.05 versus saline in the same arterial preparation. (F) Mean data illustrating the percentage of total PKD2 located at the surface in mesenteric and hindlimb arteries of saline-and angiotensin II-treated mice (n = 4 for each group).

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Figure 8. Arterial myocyte PKD2 knockout attenuates vasoconstriction and arterial wall remodeling during hypertension. (A) Mean phenylephrine-induced vasoconstriction in pressurized (80 mmHg) mesenteric arteries from angiotensin II-treated mice (Pkd2<sup>fl/fl</sup>, n = 7–8; Pkd2 smKO, n = 8).

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