SUPPLEMENTAL METHODS

Electrophysiology

Isolated left ventricular cardiomyocytes were transferred to a temperature-controlled chamber (Warner Instruments, Hamden, CT, USA) mounted on a Leica DMi3000B inverted microscope (Leica Microsystems, Buffal Grove, IL, USA), and were continuously perfused (2 mL/min) with a bicarbonate-containing Tyrode solution having the following composition (in mmol/L): NaCl 124, NaHCO₃ 25, KCl 4, CaCl₂ 1.2, MgCl₂ 1, HEPES 10, glucose 10, with pH=7.4. The pH of bicarbonate-containing Tyrode’s solution was regularly checked (every 2-hr) and carefully adjusted using a pH meter (VWR sympHony SB70P; VWR, Radnor, PA, USA). The osmolality of all applied solutions was also regularly checked and carefully adjusted to 295-300 mOsm/L using a vapor pressure osmometer (Vapro 5520; Wescor Inc., Logan, UT, USA). Action potentials (APs) and K⁺ currents were recorded in whole-cell configuration of patch-clamp technique. Electrodes were fabricated from borosilicate glass (World Precision Instruments, Sarasota, FL, USA) having tip resistances of 2–2.5 MΩ when filled with internal solution containing (in mmol/L): K-Aspartate 100, KCl 20, NaCl 8, Mg-ATP 5, EGTA 10, CaCl₂ 4.1, HEPES 10, cAMP 0.002, phosphocreatine-K₂ 10, and calmodulin 0.0001, with pH=7.2 (free [Ca²⁺]=100 nmol/L, calculated using the WEBMAXC Extended version of the MaxChelator software, https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/webmaxc/webmaxcE.htm). The electrodes were connected to the input of an Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA). Outputs from the amplifier were digitized at 50 kHz using Digidata 1440A A/D card (Molecular Devices, San Jose, CA, USA) under software control (pClamp 10). The series resistance was typically 3–5 MΩ and it was compensated by 90%. Experiments were discarded when the series resistance was high or increased by >10%. Reported voltages are corrected to the liquid junction potentials. All experiments were conducted at 37±0.1°C.

APs were evoked by 2-ms-long supra-threshold depolarizing pulses delivered via the patch pipette at various pacing frequencies from 1 Hz to 10 Hz. Fifty consecutive APs were recorded and analyzed to examine the average behavior at each pacing frequency. Short-term variability of APD₉₀ was calculated according to the following formula: STV = Σ [(APDᵢ₊₁ – APDᵢ)] / [(nbeats -1) * √2], where APDᵢ and APDᵢ₊₁ indicate the durations of the iᵗʰ and (i+1)ᵗʰ APs, and nbeats denotes the number of consecutive beats analyzed.²⁴,⁵⁹ Changes in STV are presented as Poincaré plots, where 50 consecutive APD₉₀ values are plotted, each against the duration of the previous APD₉₀. Experiments were performed when [Ca²⁺]ᵢ was buffered to 100 nmol/L and also when EGTA and CaCl₂ were both omitted from the pipette solution listed above to preserve physiological [Ca²⁺]ᵢ transient and contraction.

Whole-cell K⁺ currents were recorded using 10 mmol/L EGTA (free [Ca²⁺]=100 nmol/L) in the pipette solution, and in the presence of Na⁺ and Ca²⁺ current inhibitors (10 µmol/L tetrodotoxin for I₅Na, and 10 µmol/L nifedipine for I₅Ca) in the bath solution. Different K⁺ current components were separated using appropriate voltage protocols, selective ionic current inhibitors (5 mmol/L 4-aminopyridine for Iₒ and I₅K,slow, 1 µmol/L E-4031 for I₅Kr, 300 µmol/L BaCl₂ for I₅K₁) and biexponential fitting (R²>0.9 in each case) to the decay of the voltage-gated outward K⁺ currents (Iᵥ) (Fig. S1) as previously described.²³,²⁸,³³ Voltage-gated K⁺ currents (I₅K₁) were elicited using a 4.5 s-long test pulses between -40 and +60 mV in 10 mV steps from a holding potential of -80 mV with an interpulse interval of 5.5 s. For Ba²⁺-sensitive I₅K₁ traces, steady-state current–voltage relationships were obtained by plotting the magnitude of I₅K₁ at the end of a 500-ms test pulse as a function of the test depolarization, arising from the holding potential of ~80 mV. Ionic currents were normalized to cell capacitance (i.e., current density), determined in each cell using short (10 ms) hyperpolarizing pulses from ~10 mV to ~20 mV.

The impact of acute PKD inhibition was tested following 10 min cell treatment with the membrane-permeable, selective PKD inhibitor CRT0066101 (1 µmol/L) in self-controlled experiments.³⁴ Diastolic arrhythmogenic activities were elicited by cessation of 1 min burst pacing (10 Hz), and membrane potential was recorded for an additional 3 min. Delayed afterdepolarizations (DADs) were defined as an increase in resting membrane potential exceeding 1 mV in amplitude within 0.5 s. Spontaneous APs (sAPs) were defined as depolarizations exceeding 0 mV (and having a fast upstroke.
phase). The term of complex sAP was used when an early afterdepolarization (EAD) superimposed on sAP repolarization. EAD was defined as the inflexion of membrane potential change during repolarization, exceeding 3 mV in amplitude. DAD parameters were assessed only in those events where no AP occurred subsequently. DAD amplitude data were fitted to a log-normal distribution curve (y = \( y_0 + A / (\sqrt{2\pi} \cdot \omega) \ast e^{-\ln[x/x_c]^2/2\omega^2} \)), where \( y_0 \) is the offset, \( x_c \) is the center, \( \omega \) is the log standard deviation and \( A \) is the area).

Data processing, analysis, and plotting have been performed using Clampfit 10 (Molecular Devices, San Jose, CA, USA), Excel for Office 365 (Microsoft, Redmond, WA, USA), GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) and Origin 2016 (OriginLab, Northampton, MA, USA) software.

Reagents
Chemicals and reagents were purchased from MilliporeSigma (St. Louis, MO, USA), if not specified otherwise. E-4031 was from Tocris (Bristol, UK).

Statistical analysis
Pooled data are presented as Mean±SEM. The number of biological and technical replicates in each experimental group is reported in the figures and figure legends. Normality of the data was assessed by Shapiro-Wilk test and the equality of group variance was tested using Brown-Forsythe test. Because multiple cells may come from one animal, we performed hierarchical statistical analysis taking into account inter-subject variability. Statistical significance of differences for continuous variables was tested by a linear-mixed model using nested ANOVA followed by a post hoc Tukey test, when applicable. Categorical outcomes were evaluated using Fisher’s exact test. GraphPad Prism 9 (San Diego, CA, USA) software was used for data analysis. Blinded data acquisition and analysis have been performed for all in vivo experiments (echocardiography). Animals were grouped with no blinding but randomized in cellular experiments. Fully blinded analysis was not performed in cellular studies because the same person carried out the experiments and analysis. Both male and female animals were used. Group sizes were determined by an a priori power analysis for a two-tailed Student’s t-test with an \( \alpha \) of 0.05 and power of 0.8, in order to detect a 20% difference signal at the endpoint. Origin 2016 (OriginLab, Northampton, MA, USA) software was used for plotting the data.
|                     | WT+Sham | PKD1 cKO+Sham | WT+TAC | PKD1 cKO+TAC |
|---------------------|---------|---------------|--------|--------------|
| **Sex**             | Male    | Female        | Male   | Female       | Male | Female |
| N (animals)         | 4       | 4             | 4      | 3            | 8    | 8      |
| EF pre-op (%)       | 53.1±5.0| 52.1±4.6<sup>NS</sup>| 53.5±2.1| 48.9±1.1<sup>NS</sup>| 56.5±3.5| 59.1±4.7<sup>NS</sup>| 54.5±2.5| 55.4±5.4<sup>NS</sup>|
| EF 7 wks post-op (%)| 53.3±4.6| 55.5±6.3<sup>NS</sup>| 48.9±1.9| 52.5±3.4<sup>NS</sup>| 26.6±2.9| 27.4±3.0<sup>NS</sup>| 22.9±3.8| 26.6±4.0<sup>NS</sup>|
| Heart weight (HW, g)| 0.25±0.01| 0.21±0.01<sup>P<0.02</sup>| 0.25±0.02| 0.22±0.02<sup>NS</sup>| 0.50±0.02| 0.46±0.02<sup>NS</sup>| 0.41±0.05| 0.31±0.04<sup>NS</sup>|
| Body weight (BW, g) | 32.7±1.2| 25.2±1.6<sup>P<0.01</sup>| 30.9±1.0| 28.2±0.5<sup>NS</sup>| 29.1±0.6| 25.5±0.7<sup>P<0.01</sup>| 28.5±1.0| 23.6±1.2<sup>P<0.01</sup>|
| HW / BW (%)         | 0.78±0.01| 0.83±0.04<sup>NS</sup>| 0.79±0.04| 0.77±0.08<sup>NS</sup>| 1.74±0.07| 1.79±0.06<sup>NS</sup>| 1.43±0.18| 1.32±0.08<sup>NS</sup>|
| n (cells)           | 35      | 22            | 26     | 33           | 30   | 35     |
| C<sub>m</sub> (pF)   | 146.7±2.0| 146.2±2.4<sup>NS</sup>| 144.4±1.8| 146.2±1.6<sup>NS</sup>| 268.0±9.2| 251.7±7.3<sup>NS</sup>| 219.0±6.0| 220.5±8.0<sup>NS</sup>|
| I<sub>K1</sub> @ -140 mV (pA/pF) | -26.5±0.9| -26.7±1.1<sup>NS</sup>| -27.2±0.8| -27.8±0.9<sup>NS</sup>| -14.2±0.6| -14.0±0.7<sup>NS</sup>| -19.2±0.8| -18.8±0.7<sup>NS</sup>|
| I<sub>to</sub> @ +60 mV (pA/pF) | 19.3±3.6| 17.0±1.2<sup>NS</sup>| 25.8±2.0| 21.5±2.8<sup>NS</sup>| 9.6±1.7| 9.0±1.3<sup>NS</sup>| 16.9±2.1| 14.2±1.2<sup>NS</sup>|
| I<sub>K,slow</sub> @ +60 mV (pA/pF) | 9.7±0.7| 8.2±1.0<sup>NS</sup>| 9.9±1.3| 8.3±0.9<sup>NS</sup>| 5.9±0.9| 4.9±0.8<sup>NS</sup>| 5.1±0.4| 4.9±0.5<sup>NS</sup>|
| I<sub>sus</sub> @ +60 mV (pA/pF) | 7.4±0.2| 7.7±0.4<sup>NS</sup>| 7.8±0.6| 8.3±0.4<sup>NS</sup>| 5.8±0.3| 5.3±0.5<sup>NS</sup>| 6.7±0.4| 7.2±0.6<sup>NS</sup>|
| I<sub>Kr</sub> @ +60 mV (pA/pF) | 0.47±0.04| 0.45±0.02<sup>NS</sup>| 0.48±0.06| 0.46±0.04<sup>NS</sup>| 0.31±0.03| 0.28±0.03<sup>NS</sup>| 0.39±0.04| 0.43±0.06<sup>NS</sup>|
| APD<sub>90</sub> @ 1 Hz (ms) | 40.2±1.7| 43.3±3.5<sup>NS</sup>| 31.3±2.2| 34.3±1.1<sup>NS</sup>| 71.1±5.9| 67.5±6.5<sup>NS</sup>| 59.1±3.9| 53.0±6.1<sup>NS</sup>|

Table S1. Sex-differences in morphometric data, contractility and electrophysiological parameters in mice.

Male-female comparisons were made in each experimental group. Male-female differences were determined in each group using unpaired Student’s t-test and P values are reported after each value in females (NS, not significant). Parameters having significant difference from WT+Sham are shown in red for PKD1 cKO+Sham and in blue for WT+TAC. Magenta color denotes significant difference between PKD1 cKO+TAC and WT+TAC. ANOVA with Tukey post hoc test.

(WT, wild-type; PKD1 cKO, protein kinase D1 cardiomyocyte-specific knockout; TAC, transverse aortic constriction; EF, ejection fraction; C<sub>m</sub>, membrane capacitance; I<sub>K1</sub>, inward rectifier K<sup>+</sup> current; I<sub>to</sub>, transient outward K<sup>+</sup> current; I<sub>K,slow</sub>, slowly inactivating K<sup>+</sup> current; I<sub>sus</sub>, sustained current; I<sub>Kr</sub>, rapid delayed rectifier K<sup>+</sup> current; APD<sub>90</sub>, action potential duration at 90% of repolarization.)
| Gene      | Protein | \( I_\text{K} \) | Forward primer (5' to 3') | Reverse primer (5' to 3') |
|-----------|---------|-----------------|--------------------------|--------------------------|
| Gapdh     | GAPDH   | N/A             | AACAGCAACTCCCACTCTTC     | CCTGTGCTGTAGGGTTATT      |
| S18       | 18S rRNA| N/A             | AAAAGACAGACATCGACCTCACC  | GTACTCCCCATCCTTAGGATCC   |
| Prkd1     | PKD1    | N/A             | GCAGTGGAATGAGGAGGAAG     | GGCTCACAGGAGACAGTAAG     |
| Prkd2     | PKD2    | N/A             | GGTATCTGGGTGGATGGAGAAG   | CACCAGGATCGGGATGAAG      |
| Prkd3     | PKD3    | N/A             | GCAATTCAAGGGCGAGTAACC    | GGCAGGGAAGACAGATTTAT     |
| Camk2d    | CaMKIIδ | N/A             | GTGACACCTGAGCCCAAGA      | CATCATGGAGGCCAAGTATTAG   |
| Camk2f    | CaMKIIγ | N/A             | AAAGCTGGAGCCCTACGATTTCC  | GCGCTTTGACAGGTTATG       |
| Nppa      | ANF     | N/A             | CAGGCATATTGGAGCAATC      | GGGCATGACCTCATCTTCTAC    |
| Myh7      | \( \beta \)-MHC | N/A | AGATGGCTGGTGGAGATAG     | TTTGCGCTTGGATGATATG      |
| Hprt      | HPRT    | N/A             | CTGATTATGGACAGGACTGAAGA  | AATCCAGGACGGTACGAAA     |
| Kcnd2     | \( \text{Kv}4.2 \) | I\(_{\text{to, fast}}\) | CGCCACATCCTCAACTTCTA    | CGGTCCCTGTACTCTCTACA     |
| Kcnd3     | \( \text{Kv}4.3 \) | I\(_{\text{to, fast}}\) | GTGCGCATCAGCCCTATTA     | GAAGATCCTGAAGACAGGAAG    |
| Kcnip2    | \( \text{KChIP2} \) | I\(_{\text{to, fast}}\) | TGATGACTGAACTGGGCTTTCC | GTAGGTGTTACTGCCCACATCAT |
| Kcna4     | \( \text{Kv}1.4 \) | I\(_{\text{to, slow}}\) | ATCGTGAGAGACGTGTGATTG   | GCCAGAATGATGAAGTAAAGG    |
| Kcna5     | \( \text{Kv}1.5 \) | I\(_{\text{K, slow}}\) | TGAGGATGAGGCGAGGAGAAG   | CGCAAACCCGAGATTTATG      |
| Kcnb1     | \( \text{Kv}2.1 \) | I\(_{\text{K, slow}}\) | GGCTTTGATACAGATCCTTCTAG | GCCACTTGCTTGATGATGAT     |
| Kcnk2     | TREK-1   | I\(_{\text{sus}}\) | CTGTTTGGCTGTGTCTCTCTT    | CTGCCACGTAGTCTGATCAATC   |
| Kcnk3     | TASK-1   | I\(_{\text{sus}}\) | GACATTCTTTCCAGGCTATT    | GAAGCTGAAAGCGCACAATCT    |
| Kcnj2     | \( \text{Kv}2.1 \) | I\(_{\text{K1}}\) | GGTACCTGGCAGACATCTTAC   | GAGCAGGGCTATCAAAAA      |
| Kcnj12    | \( \text{Kv}2.2 \) | I\(_{\text{K1}}\) | AAGGGCCCTAGACCCATCTT    | CTCAAGTACGTCTGTCACAGG    |
| Kcnh2a    | hERG1a   | I\(_{\text{Kr}}\) | CCCTCCTAAAGGACAGATAG    | GCAATCGACAGATGGAAGAAG    |
| Kcnh2b    | hERG1b   | I\(_{\text{Kr}}\) | GCTTACTGGCCCTACTCTCAC   | CTTGCCAGGGCGCATATAG     |

**Table S2. Sequences of the specific primers used in RT-qPCR.**

Examined genes, encoded proteins and related \( K^+ \) currents (\( I_\text{K} \), if applicable) with the corresponding forward and reverse primers are listed.
Figure S1. Major voltage-dependent K⁺ currents in murine ventricular myocytes.

A, Representative voltage-gated outward K⁺ current (Iₖᵥ) traces in control and following 4-aminopyridine (4-AP, 5 mmol/L) treatment in mouse ventricular cardiomyocytes. 4-AP inhibits both the fast, transient outward K⁺ current (Iₖₒ) and slowly inactivating K⁺ current (Iₖ,slow), but not the sustained K⁺ current (Iₖₘₜ). Early phase of Iₖᵥ representing Iₖₒ is enlarged in inset. Iₖₒ and Iₖ,slow were separated by biexponential fitting (R²>0.9 in each case) to the decay of Iₖᵥ. Sustained K⁺ current (Iₖₘₜ) was measured at the end of the 4.5s-long test voltage pulse. B, Representative inward rectifier K⁺ current (Iₖ₁) traces in control and following Ba²⁺ (300 μmol/L) treatment. Iₖ₁ magnitude was measured at the end of a 500 ms test pulse. C, Representative rapid delayed rectifier K⁺ current (Iₖᵣ) traces in control and following E-4031 (1 μmol/L) treatment. Iₖᵣ tail current is enlarged in inset. The tail current was completely inhibited by E-4031 treatment. Whole-cell K⁺ currents were recorded using 10 mmol/L EGTA (free [Ca²⁺]=100 nmol/L) in the pipette solution, and in the presence of Na⁺ and Ca²⁺ channel inhibitors (10 μmol/L tetrodotoxin for voltage-gated Na⁺ channel, and 10 μmol/L nifedipine for L-type Ca²⁺ channel) in the external Tyrode’s solution. Voltage protocols are shown above (CL, cycle length).
Figure S2. Rapid delayed rectifier K⁺ current in murine ventricular myocytes. 
A, Representative rapid delayed rectifier K⁺ current (I_{Kr}) tails. B, I_{Kr} tail density and deactivation time constant. (Number of animals, WT+Sham: 8, PKD1 cKO+Sham: 5, WT+TAC: 6, and PKD1 cKO+TAC: 5. The number of cells (n) in each experimental group is listed in the figure.) Nested ANOVA with Tukey post hoc test. *P<0.05 versus WT+Sham.
Figure S3. Effect of acute PKD inhibition on K\textsuperscript{+} currents in TAC-induced HF.

A, Representative voltage-gated outward K\textsuperscript{+} current (I_{Kv}) traces in wild-type (WT) ventricular cardiomyocytes 8-wk post-TAC in control and following 10-min treatment with the selective PKD inhibitor, CRT0066101 (CRT, 1 \mu mol/L). Early phase of I_{Kv} is enlarged in inset. B, Peak I_{Kv} density. C, Transient outward K\textsuperscript{+} current (I_{to}) density. D, Slowly inactivating K\textsuperscript{+} current (I_{K,slow}) density. E, Sustained K\textsuperscript{+} current (I_{sus}) density. F, I_{to} inactivation time constants (\tau_{fast} and \tau_{slow}). G, I_{to} recovery from inactivation. Rapid recovery shown in inset. H, Representative inward rectifier K\textsuperscript{+} current (I_{K1}) traces. I, I_{K1} density-voltage relationship. Outward I_{K1} is enlarged in inset. J, Representative rapid delayed rectifier K\textsuperscript{+} current (I_{Kr}) tails. K, I_{Kr} tail density and deactivation time constant. (n=6 cells/3 animals in each experimental group). Paired, two-tailed Student’s t-test. *P<0.05, **P<0.01.
Figure S4. Acute PKD inhibition enhances the transient outward K⁺ current (Iₒ).
Acute inhibition of PKD using the selective inhibitor CRT0066101 (CRT, 1 μmol/L, 10 min) increased the
density of the transient outward K⁺ current (Iₒ) in wild-type (WT) sham (n=10 cells/4 animals) and TAC
(n=6 cells/3 animals) ventricular cardiomyocytes. Iₒ density at +60 mV is shown in paired experiments
before and after CRT treatment. Paired, two-tailed Student’s t-test.
Figure S5. Expression of K⁺ channels in WT and PKD1 cKO mouse hearts. 
Expression of hypertrophic genes (atrial natriuretic peptide, Nppa; β-myosin heavy chain, Myh7) and K⁺ channel subunits in wild-type (WT) and PKD1 cardiac-specific knockout (cKO) ventricular cardiomyocytes 8-wk after TAC or sham. N=3 hearts in each experimental group, and 3 technical replicates have been performed and averaged for each heart. ANOVA with Bonferroni post hoc test. *P<0.05, **P<0.01, ***P<0.001 vs. WT Sham.
Figure S6. Delayed afterdepolarizations (DADs) in TAC-induced HF.

A. Arrhythmogenic diastolic activities were elicited using a tachypacing protocol (left). Representative records in isolated left ventricular WT cardiomyocytes 8-wk post-TAC under physiological conditions (without using exogenous Ca^{2+} buffers, middle) and in the presence of 10 mmol/L EGTA in the pipette solution ([Ca^{2+}]_{i}=100 nM, right). B. Correlation between DAD frequency and DAD amplitude in wild-type (WT) and PKD1 cKO ventricular cardiomyocytes 8-wk after TAC (or sham). DADs were measured following cessation of 1-min tachypacing at 10 Hz frequency. DAD amplitude data were fitted to a log-normal distribution curve (R^2>0.94 in all cases). C. Percentage of total DADs having amplitude of >5 mV and >10 mV. Fisher’s exact test. **P<0.001 versus WT+Sham; ###P<0.001 versus WT+TAC. D, Correlation between DAD amplitude and membrane capacitance in each experimental group measured. Solid line represents the linear fit to the data (R^2=0.99).