SUPPLEMENTAL MATERIALS

Online Materials and Methods

IVF patients

Five IVF-patients with confirmed 7q36 DPP6-associated haplotype were studied. Baseline clinical data and ECG-characteristics including ventricular extrasystole (VES)-coupling intervals were obtained for all. One patient underwent invasive electrophysiological study, including pace-mapping and radiofrequency-ablation at the presumed site of VES-origin, along with long-term follow-up.

Human cardiac tissue samples

Normal human cardiac tissues were obtained from 15 non-diseased hearts of organ donors (6 females aged 53±8 years and 9 males aged 49±12 years) whose hearts were explanted to obtain pulmonary and aortic valves for transplant surgery (University of Szeged). All experimental protocols were approved by the Albert Szent-Gyorgyi Medical University Ethical Review Board (No. 51-57/1997 OEJ) and conformed to the principles of the *Declaration of Helsinki* of the World Medical Association. Hearts were stored in cardioplegic solution at 4°C and PF false tendons, left ventricular (LV) epicardium (Epi), LV midmyocardium (Mid) and LV endocardium (Endo) were dissected and quickly frozen in liquid nitrogen.

RNA extraction and mRNA quantification

Total mRNA was extracted from non-diseased human (N=8) cardiac PF, LV Epi, LV Mid and LV Endo with TRIzol™. The frozen-tissue samples were pulverized and subjected to homogenization in TRIzol Reagent (Invitrogen), chloroform extraction and isopropanol precipitation. Total mRNA from cultured VM cells infected with Adv-GFP-CTL, Adv-GFP-DPP6, Scr or DPP6 KD adenoviruses was also extracted with TRIzol Reagent (Invitrogen), followed by similar steps. Genomic DNA was eliminated by incubation in DNase I (0.1 U/μL, 37°C) for 30 minutes, followed by phenol-chloroform acid extraction and gel verification. RNA was quantified spectrophotometrically at 260-nm wavelength and integrity was confirmed on a denaturing agarose gel. RNA-samples were stored in RNAse-free distilled H_2O at -80°C. First-strand cDNA was synthesized by RT with 1 μg of RNA, random primers and MMLV reverse transcriptase (High Capacity cDNA Archive Kit, Applied Biosystems). DNA contamination was excluded by RT-negative PCR. Real-time PCR was conducted with a Stratagene Mx3000P qPCR detection system with SYBR green quantitative assay. 18S rRNA was used as the internal standard. Primers for real-time PCR reactions are listed in Online Table I. PCR products were verified with dissociation curves. mRNA was quantified with comparative threshold-cycle quantification and ΔΔCt methods. Data are expressed as $2^{-\Delta Ct}*10^3$.

Immunoblotting

Membrane-proteins from human (n=7) or canine (n=4, adult male mongrel dogs, 25~30 kg) paired cardiac VM and PF tissues were extracted with lysis buffer containing 25-mmol/L Tris-HCl (pH 7.34), 5-mmol/L EDTA, 5-mmol/L EGTA, 150-mmol/L NaCl, 20-mmol/L NaF, 0.2-mmol/L Na_3VO_4, 20-mmol/L glycerol-2-phosphate, 0.1-mmol/L AEBSF, 1-μmol/L microcystin, 25-μg/ml leupeptin, 10-μg/ml aprotinine, and 1-μg/ml pepstatin, followed by

homogenization and centrifugation at 3000 rpm, 4°C for 10 minutes. The supernatant was then collected and further centrifuged at 48,000 rpm, 4°C for 1 hour. The precipitates containing enriched crude membrane proteins were resuspended in lysis buffer supplemented with 1% Triton X-100, and were kept at -80°C. Protein concentration was determined with the Thermo Scientific Pierce BCA™ protein assay kit. 20 μg of membrane protein samples were denatured with Laemmli sample buffer at 100°C and separated on a 10% SDS-PAGE gel, followed by electrophoretic transfer to polyvinylidene fluoride (PVDF) membranes (Immobilon™, Millipore) in 25-mmol/L Tris-base, 192-mmol/L glycine and 20%-ethanol at 0.3A for 1 hour. Membranes were blocked in PBS-T (NaCl 136.8-mmol/L, KCl 2.7-mmol/L, Na_2HPO_4 4.2-mmol/L, KH_2PO_4 1.8-mmol/L, pH 7.34, 0.1% Tween) with 5% non-fat dry milk for 1 hour and incubated respectively with the following primary antibodies overnight at 4°C: goat anti-DPP6, 1:500, R&D Systems; mouse anti-DPP6, 1:500, R&D Systems; rabbit anti-Kv4.3, 1:1000, Alomone; mouse anti-KChIP2, 1:1000, Neuromab; mouse anti-NCS-1, 1:1000, BD Bioscience; mouse anti-GAPDH (to control for protein loading), 1:5000, Fitzgerald. After washing and re-blocking, membranes were incubated with horseradish peroxidase-conjugated donkey anti-goat or donkey anti-mouse secondary antibodies (1:10,000, Jackson Immunolabs). Protein-bands were detected with Western-Lightening Chemiluminescence Reagent Plus (Perkin-Elmer Life Sciences) and quantified with Quantity-One software (Biorad). All expression data are provided relative to GAPDH for the same samples on the same gels.

Native cell isolation and culture

Because of the very limited availability of human cardiac tissue for study, experiments with native cardiomyocytes were performed on tissues obtained from dog hearts, which have relative VM and PF-cell (PC) I_{to}-properties similar to human.[1,2] Animal-care procedures followed National Institutes of Health guidelines and were approved by the animal research ethics committee of the Montreal Heart Institute. Adult male mongrel dogs (19-30 kg, n=19) were anesthetized with pentobarbital (30 mg/kg IV) under artificial ventilation. Hearts were excised under left lateral thoracotomy and immersed in room-temperature oxygenated Tyrode's solution. All subsequent procedures were performed at room temperature unless otherwise specified. After excision of PF false tendons, the transmural free wall (~30 × 50 mm) of the anterior left ventricle was dissected and the perfusing artery was cannulated, followed by perfusion with Tyrode's solution containing collagenase (120 U mL^{-1}, Worthington, type II) at 37°C. Epicardial (Epi) or mid-myocardial ventricular cardiomyocytes (VMs) from the left ventricular (LV) free wall were isolated as previously described.[3] Epi cells were used for most functional studies, with midmyocardial cells used only for KChIP2-knockdown experiments. In selected studies, right ventricular (RV) cardiomyocytes were isolated from the RV free-wall. After isolation, cells were either put in storage solution for study on the same day or kept in culture medium and centrifuged at 500 rpm for 2 minutes. Cell-pellets were resuspended in culture-medium containing Medium-199 (Invitrogen, with Earle's salts, L-glutamine and 2.2 g/L sodium bicarbonate, Na-penicillin G (100-U/mL) and streptomycin sulfate (100-μg/L), supplemented with Insulin-Transferrin-Selenium-X (Invitrogen, containing 0.01-mg/mL insulin, 5.5-μg/mL transferrin, 6.7-ng/mL sodium selenite and 2-μg/mL ethanolamine).

PF false tendons were excised from both ventricles into Dulbecco's modified eagle medium (DMEM, Invitrogen) and were first digested by elastase (1.5 U/mL, Type I) for 10 minutes, followed by collagenase (1,200 U/mL, Worthington Type II) digestion with 0.1% BSA (bovine serum albumin) for 50±5 minutes at 37°C in a water bath. During digestion, the fibers were

gently agitated by bubbling with 100%-O_2. After digestion, PFs were transferred into a high-[K^+] storage solution and individual PF-cells (PCs) were dispersed by gentle pipetting for 10 minutes. The solution containing single PCs was either used for study on the same day or filtered and centrifuged at 500 rpm for 2 minutes. The PC pellet was resuspended in fresh KB solution supplemented with 1-mmol/L $CaCl_2$. After 10-minute incubation, PCs were precipitated by 2-minute centrifugation at 500 rpm and were resuspended in PC culture medium that contained DMEM, Na-penicillin G and streptomycin sulfate supplemented with Insulin-Transferrin-Selenium-X (ITX).

Matched PCs and VMs derived from each heart were plated on laminin (20-μg/mL) pre-coated circular (12-mm diameter) glass coverslips in 24-well cell culture plate. VMs were plated at ~1×10^4 cells/cm^2. Because of the very small number of PCs from one false tendon, all isolated PCs from all false tendons in each dog were plated for each experiment. Cells were incubated at 37°C in a humidified, 5% CO_2-enriched environment. After 4-hour preincubation, any dead or unattached cells were washed off with fresh media to leave a homogeneous layer of rod-shaped cells attached to the coverslips or Petri dishes. Attached PCs and VMs were immediately subjected to adenovirus infection for 2 hours and were incubated for 48 hours in fresh culture medium. After culture, PCs and VMs were washed twice with KB solution and stored at 4°C for electrophysiological study.

Recombinant adenovirus vector construction

DPP6 over-expression

Full-length cDNA of human DPP6 was generated from a cDNA clone (RC216875, OriGene Technologies Inc) by PCR with specific primers containing the respective restriction sites. Human *DPP6* isoform 2 (NM_001936) was used since this is the most highly expressed isoform in human heart.[4] A bicistronic construct encoding triple FLAG-tagged DPP6 and/or green fluorescent protein (GFP) under control of the CMV promoter, was generated by inserting the cDNA into pShuttle-IRES-hrGFP-1 vector (Stratagene). The adenoviral vector containing DPP6 cDNA will be designated Adv-GFP-DPP6, and the control adenoviral vector containing only GFP as Adv-GFP-CTL.

DPP6 knock-down

To attenuate DPP6 expression in canine cardiomyocytes, an E1-E3-deleted adenoviral vector, over-expressing a micro-RNA embedded shRNA (shRNAmir) targeted to the canine DPP6 mRNA (GeneBank ID: XM_532774) was developed. First, we created an adenoviral shuttle plasmid that carries a CMV promoter-driven GFP expression cassette and the microRNA-context sequence in the 3`untranslated region of GFP with unique restriction sites for cloning of shRNAmirs as follows. The turbo GFP open reading frame was PCR-amplified from pGIPZ (Openbiosystems) with 5` GGTAGTCGACCACCGACTCTACTAGAGGAT sense and 5` TGCGGCCGCGGCCGCTACTTGTACATTAT antisense primers and the PCR product was cloned in pAdTrack-CMV (a gift of Bert Vogelstein, Addgene plasmid #16405) at SalI – NotI sites, hence generating the AdS-GFP plasmid. Two XbaI fragments of AdS-GFP between positions 1612 and 3298 were deleted from AdS-GFP, using the dam-, dcm- E. coli strain ER2925 (New England Biolabs), resulting in AdS-GFP-ΔXbaI. Finally, the microRNA-context sequence was PCR amplified from pGIPZ with 5` TAGCGGCCGCTTGTTTGAATGA GGCTTCAG sense and 5` TGCAAGCTTCGCATTAGTCTTCCAATTGAA antisense primers, and the PCR product was cloned in AdS-GFP- ΔXbaI between NotI – HindIII sites, by this constructing the AdS-empty plasmid. The DPP6-targeted shRNAmir sequence was cloned in

AdS-empty following previously published protocols.[5] Briefly, template for the DPP6-specific shRNA sequence was designed by the web-based 'shRNA retriever' tool available on the Sachidanandam Laboratory homepage (http://katahdin.cshl.org/, Cold Spring Harbor Laboratory). The 97 bp long synthetic oligonucleotides for the DPP6-targeted and the scrambled shRNAs (DPP6: 5` TGCTGTTGACAGTGAGCGCCGACAGTTTCTGAAACTGTTGTAGTGAAGC CACAGATGTA*CAACAGTTTCAGAAACTGTCGT*TGCCTACTGCCTCGGA, scrambled: 5` TGCTGTTGACAGTGAGCGAACGTAAGCAAAGCGGTGATCATAGTGAAGCCACAGAT GTA*TGATCACCGCTTTGCTTACGTC*TGCCTACTGCCTCGGA, the 23-bp long mature siRNA sequences are italicized) were PCR amplified with 5` CAGAAGGCTCGAGAAGGTATATTGC TGTTGACAGTGAGCG sense and 5` CTAAAGTAGCCCCTTGAATTCCGAGGCAGTA GGCA antisense primers and the PCR products were cloned in AdS-empty at XhoI and EcoRI sites.

KChIP2 knock-down

For KChIP2 knock-down, the double hairpin method was used. In these constructs, the length of the microRNA context sequence is minimized so that double or multiple units of the same shRNA can be cloned in tandem to improve knock-down efficacy.[6] The shRNA retriever tool was used to design the synthetic template for the KChIP2-specific shRNA (5` TGCTGTTGACA GTGAGCGCTGACATGATGGGCAAGTATACTAGTGAAGCCACAGATGTAGTATACTTG CCCATCATGTCATTGCCTACTGCCTCGGA). For the scrambled hairpin the same template was used as in the DPP6 experiments. The 97 bp long synthetic oligonucleotide templates were PCR amplified with the 5` GCGCGGCCGCATGGATCCGATCCAAGAAGGTATATTGCTGT TGACAGTGAGCG sense and 5` CTAAGCTTGCAGATCTATCGTAGCCCTTGAATTCCG AGGCAGTAGGCA antisense primers. Since the sense primer carries NotI and BamHI and the antisense primer carries BglII and HindIII sites, the PCR product could be cut by NotI-BglII and BamHI-HindIII in parallel reactions. The NotI-BglII and BamHI-HindIII cut PCR fragments were cloned in AdS-GFP-ΔXbaI at NotI-HindIII sites by three-way ligation, resulting in a CMV-GFP-double-hairpin construct. Integrity of all plasmid constructs was verified by sequencing.

Virus production

Recombinant adenoviral genomes and initial virus cultures were generated by employing the Adeasy system, according to previously published protocols.[7] Recombinant adenoviruses were amplified in Hek293T/17 cells (ATCC) and were purified with the Adenovirus Standard Purification ViraKit™ (Virapur LLC). Functional titers of the final virus preparations were determined by infecting Hek293T/17 cells with limiting dilutions of the virus.

CHO cell culture and transfection

Chinese hamster ovary (CHO) cells were cultured in F12 medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone®, Thermo Scientific) and 100 units/mL penicillin, 100 g/mL streptomycin (Invitrogen) at 37°C with 5%-CO_2. One day before transfection, 1×10^5 cells/well were seeded in a 24-well plate that contained sterile glass coverslips (12-mm diameter) for electrophysiological or immunocytochemical studies. For co-immunoprecipitation studies, 1×10^5 cells/well were seeded in seven 100-mm petri dishes (one for each co-transfection group). Transfection was performed with Lipofectamine™2000 (Invitrogen), with 0.1 μg (for 24-well) or 3 μg (for 100-mm petri dish) plasmid DNA encoding Kv4.3 or combinations of Kv4.3 with 0.35 μg (24-well) or 10.5 μg (100-mm petri dish) KChIP2b, DPP6 or NCS-1. Bicistronic vectors

carrying DsRed, CFP and GFP were used. In parallel, 3 μg of plasmid DNA encoding DsRed, 10.5 μg of plasmid encoding CFP only and 10.5 μg of plasmid encoding GFP alone was co-transfected into CHO cells in a 100-mm petri dish as a control for transfection efficiency and for co-immunoprecipitation studies. Fluorescent cells were used for patch-clamp experiments within 1-2 days of transfection. For the 100-mm petri dishes, after 2-day transfection and before immunoprecipitation, comparable transfection efficiency was determined by observation of DsRed, CFP and GFP fluorescence in each group. Cells were then washed with phosphate-buffered saline (PBS) before protein extraction and immunoprecipitation experiments.

The plasmids used were human Kv4.3 (provided by Dr. Gordon Tomaselli, Johns Hopkins University, GenBank #NM_172198) subcloned in pIRES-DsRed2, human KChIP2b (provided by Dr. Michael Morales and Dr. Harold Strauss, SUNY, Buffalo, NY, GenBank #NM_173192) subcloned in pIRES-CFP, human NCS-1 (purchased from Openbiosystem, USA. GenBank #NM_014286) subcloned into pIRES-CFP, and human DPP6 (GenBank #NM_001936) in pIRES-GFP. Plasmids containing only GFP, CFP or DsRed2, i.e., pIRES-GFP, pIRES-CFP or pIRES-DsRed2, were also used when necessary.

Immunoprecipitation studies

Proteins from CHO cells after 2 day-transfections were extracted with lysis buffer containing 25-mmol/L Tris-HCl (pH 7.34), 5-mmol/L EDTA, 5-mmol/L EGTA, 150-mmol/L NaCl, 20-mmol/L NaF, 0.2-mmol/L Na_3VO_4, 20-mmol/L glycerol-2-phosphate, 0.1-mmol/L AEBSF, 1-μmol/L microcystin, 25-μg/mL leupeptin, 10-μg/mL aprotinine, 1-μg/mL pepstatin, and 1% Triton X-100 followed by homogenization. After centrifugation at 3000 rpm and 4°C for 10 minutes, the supernatant was fast-frozen and stored at -80°C. Protein concentration was determined with the Thermo Scientific Pierce BCA™ protein assay kit.

Immunoprecipitation was performed with a monoclonal anti-Kv4.3 antibody (Neuromab). Dynabeads® M-280/sheep anti-mouse IgG (100 μL for each sample) were washed with PBS and preincubated with 1%-BSA for 1 hour at RT to minimize nonspecific binding. Mouse anti-Kv4.3 antibodies (2.5 μg) were incubated overnight at 4°C with 100-μL Dynabeads per sample with gentle rotation. The anti-mouse IgG-coated beads were then washed 5 times with PBS by magnetic-precipitation/resuspension, and were incubated overnight with 100 μg of total protein extracts from CHO cells expressing Kv4.3, Kv4.3+KChIP2, Kv4.3+KChIP2+DPP6, Kv4.3+DPP6, Kv4.3+NCS-1, Kv4.3+NCS-1+DPP6 or DsRed, at 4°C with gentle rotation. The supernatants were then collected. The bead-antibody-target protein complexes were washed 5 times with PBS followed by magnetic-precipitation/resuspension. The bound Kv4.3 protein-complexes were eluted from the beads and denatured by adding 50 μL of SDS sample buffer and heated at 100°C. Supernatants (40 μL) from each immunoprecipitation-reaction were denatured by adding 10 μL of 5×sample buffer and heated to 100°C for 5 minutes.

The eluted proteins and the supernatants were separated on 10% SDS-PAGE gel, electrophoretically transferred to polyvinylidene fluoride (PVDF) membranes (Immobilon™, Millipore) in 25-mmol/L Tris-base, 192-mmol/L glycine and 20%-ethanol at 0.3 A for 1 hour. Membranes were blocked in PBS-T (NaCl 136.8-mmol/L, KCl 2.7-mmol/L, Na_2HPO_4 4.2-mmol/L, KH_2PO_4 1.8-mmol/L, pH 7.34, 0.1% Tween) with 5% non-fat dry milk for 1 hour, and incubated overnight at 4°C with one of the following primary antibodies: goat anti-hDPP6, 1:1000, R&D Systems; mouse anti-Kv4.3, 1:1000, Neuromab; mouse anti-KChIP2, 1:2000, Neuromab; mouse anti-NCS-1, 1:1000, BD Bioscience. After washing and re-blocking, membranes were incubated with horseradish peroxidase-conjugated donkey anti-goat or

anti-mouse secondary antibodies (1:10,000, Jackson Immunolabs). Protein-bands were detected with Western-Lightening Chemiluminescence Reagent Plus (Perkin-Elmer Life Sciences).

Confocal microspcopy

Two-days after transfection, CHO cells were washed once with PBS, then fixed with 2%-paraformaldehyde (20 minutes, BioShop) and washed 3 times (5 minutes each) with PBS. Cells were blocked and permeablized with 2% normal donkey serum (NDS, Jackson) and 0.2% Triton X-100 (BioShop) for 1 hour. Cells were then incubated overnight at 4°C with primary antibody against Kv4.3 (mouse anti-Kv4.3, 1:200, Neuromab) in PBS containing 1%-NDS and 0.05%-Triton, followed by 3 washes and secondary antibody (donkey-anti-mouse Alexa-488, Jackson, 1:800) and WGA (wheat germ agglutinin, Alexa Fluor 647, Life technologies, 1:200) incubation at room temperature for 1 hour. Confocal microscopy was performed with a Zeiss LSM-710 system. Control experiments omitting primary antibodies revealed absent or very low-level background staining. Images were deconvolved with Huygens Professional software (Scientific Volume Imaging) using measured point spread functions (PSFs). Measured PSFs were acquired with the same parameters as the images of interest. Total, intracellular and plasma membrane Kv4.3 fluorescence were analyzed using Zeiss LSM 710 software. For each cell analyzed, the Kv4.3 fluorescence densities were determined as the sum of the pixels within cell membrane or intracellular or the whole cell regions normalized to the corresponding region areas. Measurements were repeated in 5 Z-stacks for each cell.

Electrophysiology

Whole-cell patch-clamp technique (voltage-clamp mode) was applied for I_{to} recording at 36±0.5ºC (for native cells) or at 22±0.5ºC (for CHO cells). Borosilicate-glass electrodes had tip-resistances between 1.5 and 3.0 MΩ when filled. Cell-capacitance and series resistance were compensated by ~80% to 90%. Leakage compensation was not used. Cell capacitances were not different among groups. Mean±SEM of cell capacitances are shown in online Table II. I_{to} was defined as a rapidly-activating and inactivating outward current, whether in native cells or heterologous systems, and its amplitude measured from peak outward current to quasi steady-state current at the end of the depolarizing pulse. Currents are expressed in terms of density.

The standard Tyrode solution contained (mmol/L) NaCl 136, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1, NaH_2PO_4 0.33, HEPES 5 and dextrose 10 (pH 7.35 with NaOH). The high-K^+ storage solution contained (mmol/L) KCl 20, KH_2PO_4 10, dextrose 10, mannitol 40, L-glutamic acid 70, β-OH-butyric acid 10, taurine 20, EGTA 10 and 0.1% BSA (pH 7.3 with KOH). The standard pipette solution used in most experiments contained (mmol/L) K-aspartate 110, KCl 20, $MgCl_2$ 1, MgATP 5, GTP 0.1, HEPES 10, Na-phosphocreatine 5, EGTA 5 with pH adjusted to 7.3 with KOH.

For I_{to}-recording in native cells, atropine (1-μmol/L) and $CdCl_2$ (200-μmol/L) were added to external solutions to eliminate muscarinic K^+-currents and to block Ca^{2+}-currents. Na^+-current contamination was avoided by using a holding potential (HP) of -50 mV or by substitution of equimolar Tris HCl for NaCl. For I_{to}-recordings in CHO cells, standard Tyrode solution was used as external solution. Tetraethylammonium (TEA; Sigma-Aldrich) was prepared in a 0.3-mol/L stock solution. For 100-mmol/L TEA, an equal molar concentration of NaCl was removed to maintain extracellular osmolarity.

The resting membrane potentials were similar in the absence and presence of the control virus in 48-hour cultured PC or VM cells; e.g., in PC, -44±6 mV and -45±5 mV, *P*=NS; in

VM, -64±5 mV and -60±8 mV, *P*=NS. We also analyzed the input resistances of 48-hour cultured cells by applying a 10-mV hyperpolarizing voltage-clamp step from a holding potential of -80 mV. The input resistances were not different in 48-hour cultured PC or VM cells with versus without the control virus. In PC, input resistances averaged 62±28 MΩ without (N=5) and 105±33 MΩ with (n=8) the control virus, *P*=NS. In VM, input resistances averaged 9.8±1.1 MΩ without (n=6) and 14.8±3.8 MΩ with (n=6) the control virus, *P*=NS.

Data acquisition and analysis

Clampfit 9.0 (Axon) and GraphPad Prism 5.0 were used for data-analysis; curve-fitting was performed with nonlinear least-square algorithms. Real-time PCR results were analyzed with MXPro software from Stratagene. Western blot results were analyzed with Quantity one from Bio-Rad. Statistical comparisons were performed with paired or unpaired Student *t*-tests if only 2 group means were compared. When multiple groups were studied simultaneously, group comparisons were performed with ANOVA. If significant differences were indicated by analysis of variance, posthoc *t*-tests with Bonferroni's correction were used to evaluate differences between individual mean values. A two-tailed $P<0.05$ indicated statistical significance; group data are expressed as mean±SEM.

PF action potential model

A previously-described model of the electrophysiology of the PF-cell was employed.[8] To reproduce behavior at the physiological-temperature of canine-cell recordings, the kinetics of T-type Ca^{2+}-current were accelerated with a Q-factor of 3. The I_{to}-representation was reformulated to incorporate rapidly and slowly inactivating and recovering components of similar amplitude. Current-density was set to reproduce a peak current of +10 pA/pF at +30 mV.[9]

References

1. Han W, Wang ZG, Nattel S. A comparison of transient outward currents in canine cardiac Purkinje cells and ventricular myocytes. *Am J Physiol Heart Circ Physiol*. 2000;279:H466-H474.
2. Han W, Zhang L, Schram G, Nattel S. Properties of potassium currents in Purkinje cells of failing human hearts. *Am J Physiol Heart Circ Physiol*. 2002;283:H2495-H2503.
3. Xiao, L., Zhang, L., Han, W., Wang, Z., and Nattel, S. 2006. Sex-based transmural differences in cardiac repolarization and ionic-current properties in canine left ventricles. *Am J Physiol Heart Circ Physiol.* 291:H570-H580.
4. Alders M, Koopmann TT, Christiaans I, Postema PG, Beekman L, Tanck MW, Zeppenfeld K, Loh P, Koch KT, Demolombe S, Mannens MM, Bezzina CR, Wilde AA. Haplotype-sharing analysis implicates chromosome 7q36 harboring DPP6 in familial idiopathic ventricular fibrillation. *Am J Hum Genet*. 2009;84:468-476.
5. Paddison PJ, Cleary M, Silva JM, Chang K, Sheth N, Sachidanandam R, Hannon GJ. Cloning of short hairpin RNAs for gene knockdown in mammalian cells. *Nat Methods.* 2004;1:163-167.
6. Sun D, Melegari M, Sridhar S, Rogler CE, Zhu L. Multi-miRNA hairpin method that improves gene knockdown efficiency and provides linked multi-gene knockdown. *Biotechniques.* 2006;41:59-63.

7. Luo J, Deng ZL, Luo X, Tang N, Song WX, Chen J, Sharff KA, Luu HH, Haydon RC, Kinzler KW, Vogelstein B, He TC. A protocol for rapid generation of recombinant adenoviruses using the AdEasy system. *Nat Protoc* 2007; 2:1236-1247.
8. Sampson KJ, Iyer V, Marks AR, Kass RS. A computational model of Purkinje fibre single cell electrophysiology: implications for the long QT syndrome. *J Physiol.* 2010;588(Pt 14):2643-2655.
9. Jeck C, Pinto J, Boyden P. Transient outward currents in subendocardial Purkinje myocytes surviving in the infarcted heart. *Circulation*. 1995;92:465-473.

Online Tables

Online Table I. Primers used for real-time RT-PCR

Primers	Sequences	GeneBank#
hKv4.3	F: TGTTTCCAACTTTAGCCGGATT R: TTTGTGCCCTGCGTTTATCA	NM_004980
hKv3.4	F: TGCCTGCGCCGATGGTAGTG R: ATGCCATGTCGTGCCCAGCC	NM_004978
hKv1.4	F: AGCCATTGCGGGTGTCTTAA R: CGTTAGCTGTGTCTGTTCCTCATT	NM_002233
hKChIP2	F: ATGCTTGACATCATGAAGTCCAT R: TTGACAAGACTCAATGAATTCCT	AF199598
hNCS-1	F: GGCAACGATTACCGAGAAGGAGG R: TGGAAGCCTGCCGCATCCAG	NM_001128826
hDPP6	QIAGEN QuantiTect® Primer assay Cat.No. QT00080598	NM_001936, NM_130797, NM_001039350
hKCNE1	F: AACGACCCATTCAACGTCTACA R: CCGGGCCTGGACATAGG	NM_000219
hKCNE2	F: CAAAGTTGATGCTGAGAACTTCTACTATG R: GATTTCACAGTGCTCACCAGGAT	NM_172201
hKCNE3	F: ACCTGGCCGTGATGACAACT R: TCACTACGCTTGTCCACTTTGC	NM_005472
DPP6 (dog)	F: GCCATCCGTGTGGTCTCAAC R: GGTAGATGGAGCCGGTGTAG	XM_532774

Online Table II. Cell capacitances

	Day 0	**Day 2**	**Adv-GFP**	**Adv-GFP-DPP6**	**Adv-GFP-SCr**	**Adv-GFP-DPP6 KD**
PC	117±15 pF (N=7)	112±11 pF (N=11)	154±12 pF (N=9)	188±24 pF (N=8)	96±13 pF (N=11)	93±8 pF (N=14)
VM	160±17 pF (N=5)	153±6 pF (N=6)	201±19 pF (N=15)	192±12 pF (N=15)	138±13 pF (N=11)	145±12 pF (N=8)
RV	139±9 pF (N=5)	-	-	-	-	-
	Kv4.3	**Kv4.3 +KChIP2**	**Kv4.3+ KChIP2+ DPP6**	**Kv4.3 +DPP6**	**Kv4.3 +NCS-1**	**Kv4.3+ NCS-1+ DPP6**
CHO cells	28.0±4.2 pF (N=21)	29.2±6.4 pF (N=21)	32.8±4.6 pF (N=19)	25.6±3.9 pF (N=12)	32.0±3.4 pF (N=17)	33.4±5.6 pF (N=18)

Online Table III. General ECG characteristics and properties of spontaneous VF episodes in DPP-related IVF patients

ID	Age	Gender	ECG	PR	QRS	QTc	HR	VES morph.	VES axis	VES CI
A	24	M	ECG	160	90	450	110	LBBB	Left	220
B	16	M	ECG	160	80	390	75	LBBB	Left	270
C	50	M	ECG	170	100	390	100	LBBB	Left	280
D	35	M	ECG	160	105	370	100	LBBB	Left	200
E	21	M	ECG	190	90	420	90	LBBB	Left	200

M, male; ECG, electrocardiogram; HR, heart rate; VES, ventricular extrasystole; morph., morphology; LBBB, left bundle branch block; NA, not applicable; CI, coupling interval. Data are in milliseconds. Patient E corresponds to the patient of Figure 1.

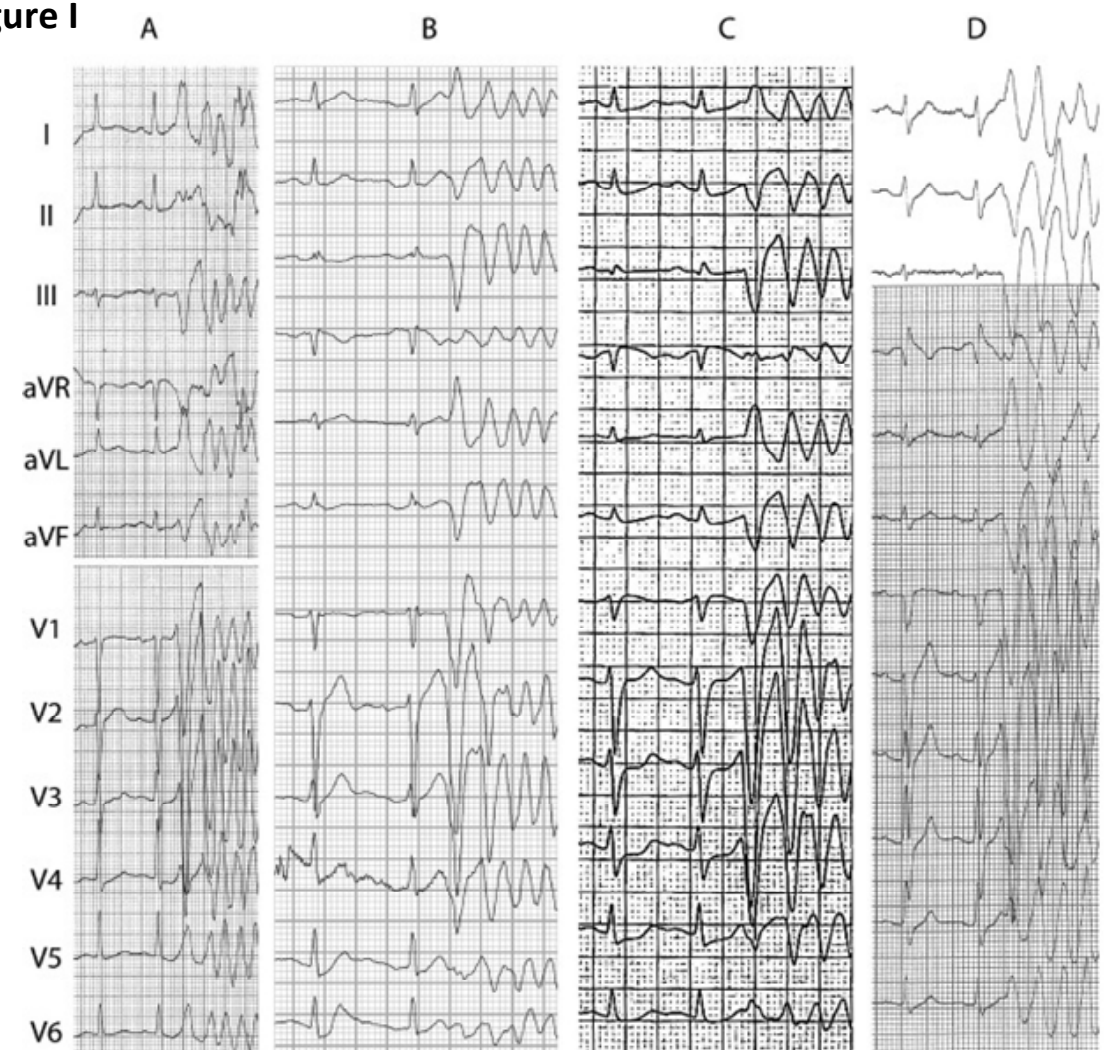
Online Table IV. Inactivation voltage-dependence in CHO cells expressing Kv4.3 along with various subunits

	Kv4.3 alone	**Kv4.3 +KChIP2**	**Kv4.3 +KChIP2+DPP6**	**Kv4.3 +NCS1**	**Kv4.3 +NCS1+DPP6**	**Kv4.3 +DPP6**
$V_{1/2}$ (mV)	-40.9±1.3	-42.3±1.9	-43.4±3.8	-38.3±3.7	-45.3±2.2	-53.4±2.4**
SLOPE (mV)	-8.4±0.7	-5.0±0.3	-6.1±0.5	-6.4±0.3	-8.2±0.8	-10.4±4.2
N	11	9	6	6	8	5

$V_{1/2}$, half-maximal voltage of inactivation. Slope, slope factor for inactivation. N, number of cells studied.

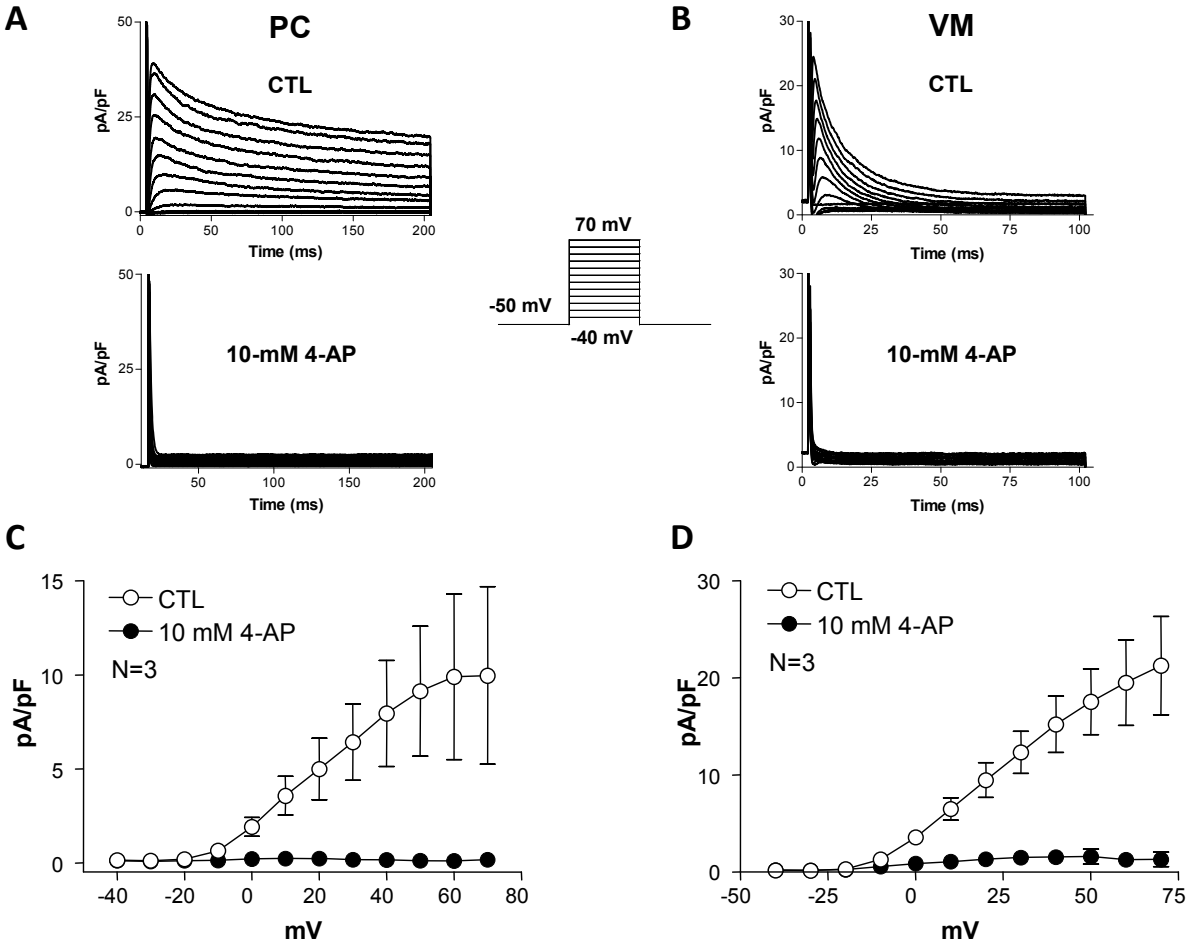
**$P<0.01$ versus Kv4.3.

Online Figure I

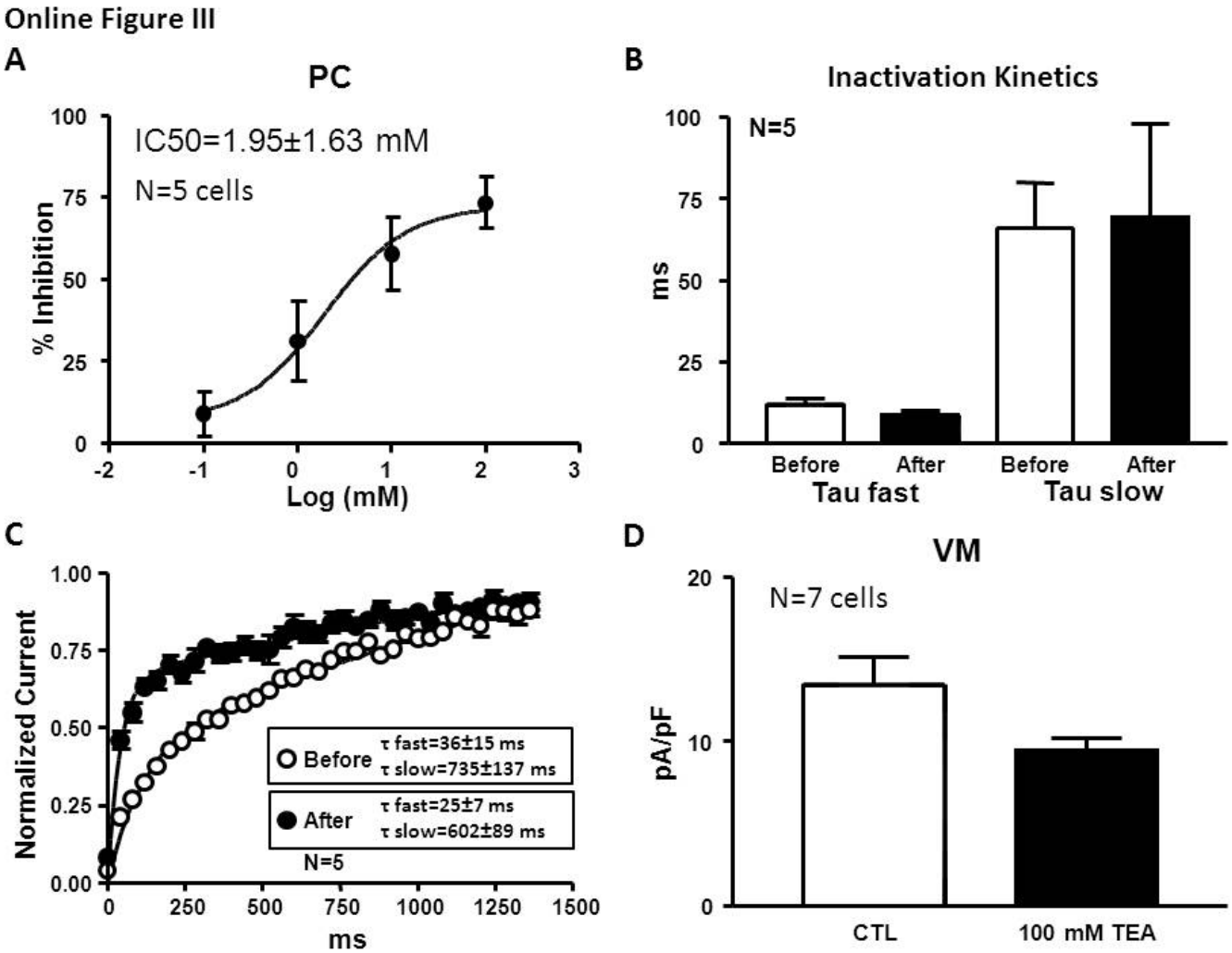


Online Figure I. Twelve-lead ECG recordings from 4 male IVF patients (Patients A, B, C and D in Online Table II) who are *DPP6* risk haplotype carriers and have been resuscitated from ventricular fibrillation.

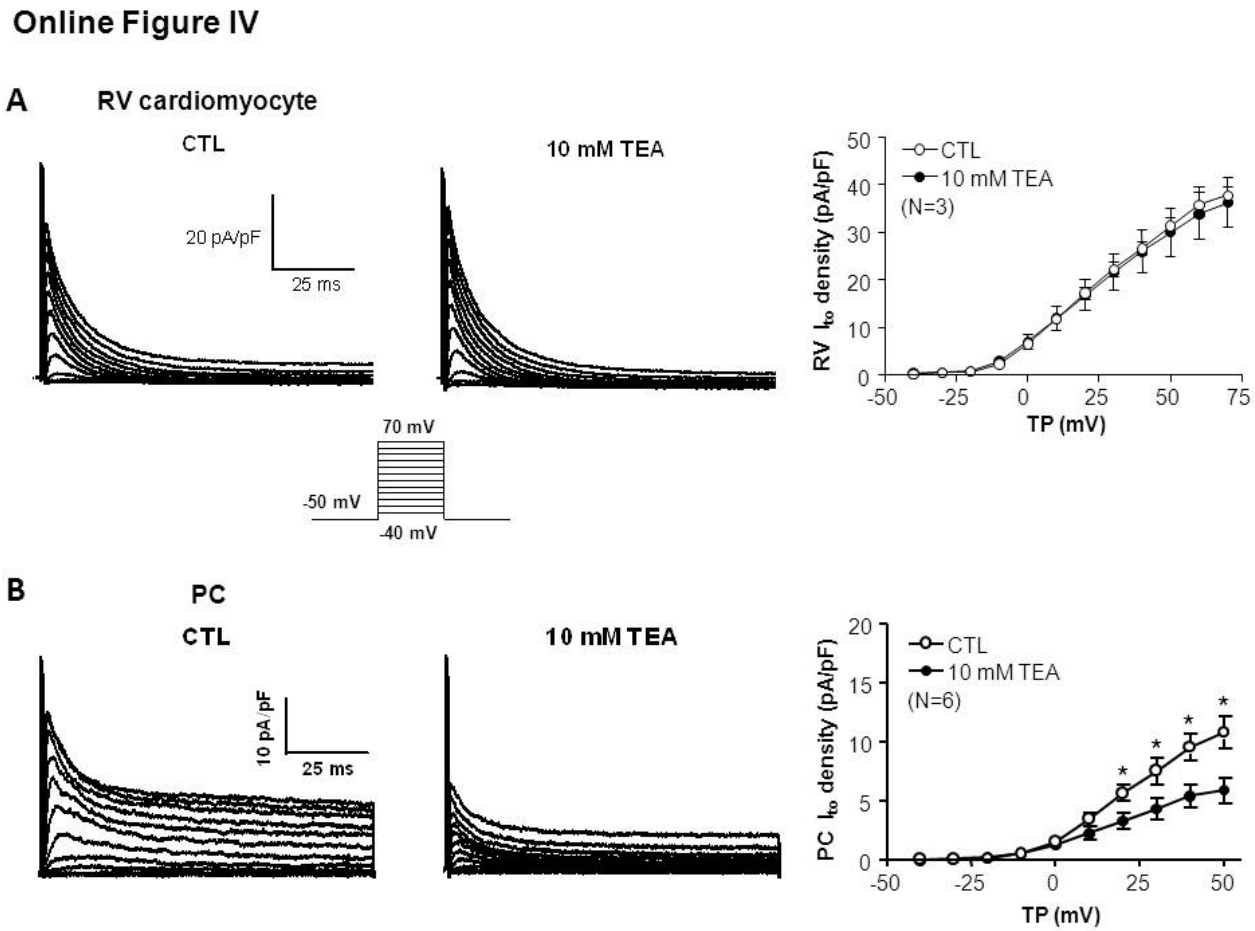
Online Figure II



Online Figure II. Effects of 10-mmol/L 4-aminopyridine (4-AP) on PC or VM I_{to}. **A and B**, representative recordings of PC (**A**) and VM (**B**) I_{to} before (top) and after (bottom) 5-minute perfusion with 10-mmol/L 4-AP. Currents were obtained with 200-ms (PC) or 100-ms (VM) depolarizations to between -40 and +70 mV, from a holding potential of -50 mV (at 0.1 Hz). **C and D**, mean±SEM current density-voltage relations from PC (**C**) and VM (**D**).

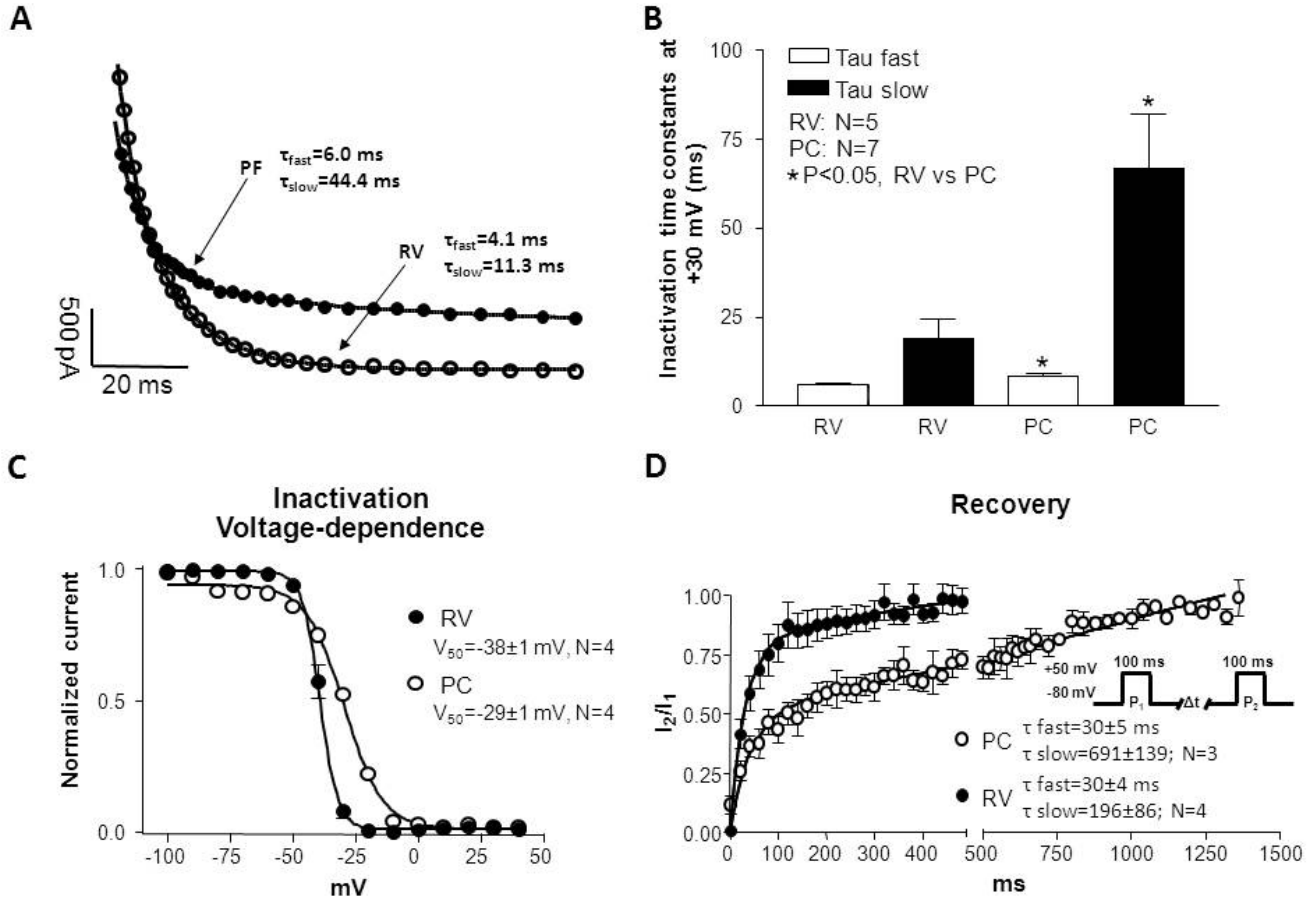


Online Figure III. A, TEA dose-response relation for PC I_{to} inhibition at +50 mV. Curve represents best-fit sigmoidal dose-response relation. **B,** I_{to} inactivation time-constants at +50 mV before and after 100-mmol/L TEA. **C**, Time course of I_{to} recovery from inactivation in fresh PCs before and after 10-mmol/L TEA. Values are normalized currents during the test pulse (P_2) as a function of I_1-I_2 interval, obtained with 100-ms P_2 test-pulses from -80 to +50 mV at 0.1 Hz. Best-fit biexponential functions are shown. **D,** I_{to} densities at +50 mV in fresh LV cells before (CTL) and after 100-mmol/L TEA perfusion. Values are mean±SEM.



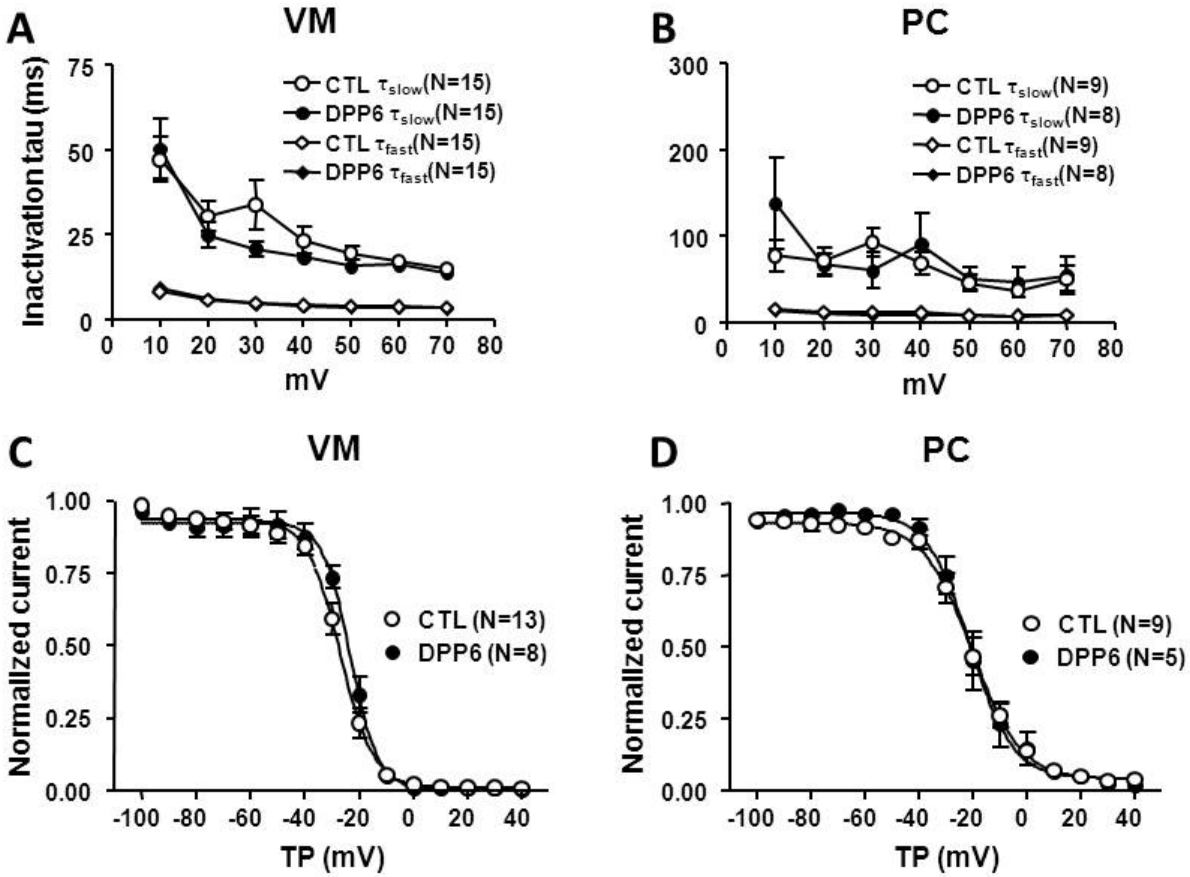
Online Figure IV. RV and PC I_{to} and their responses to 10 mmol/L TEA. **A**, typical recordings of fresh RV-cell I_{to} before (left) and after (middle) 5-minute perfusion with 10-mmol/L TEA. Currents were obtained with 100-ms pulses to voltages from -40 to 70 mV, from a holding potential of -50 mV. Right panel in **A** shows mean±SEM current density-voltage relations of RV I_{to} before and after 10-mM TEA. **B**, representative I_{to} recordings from freshly isolated PCs before (left) and after (middle) 10 mmol/L TEA. Right panel in B shows mean±SEM current density-voltage relations of PC I_{to} before and after 10-mmol/L TEA from freshly-isolated PCs (same data as in Figure 4E).

Online Figure V



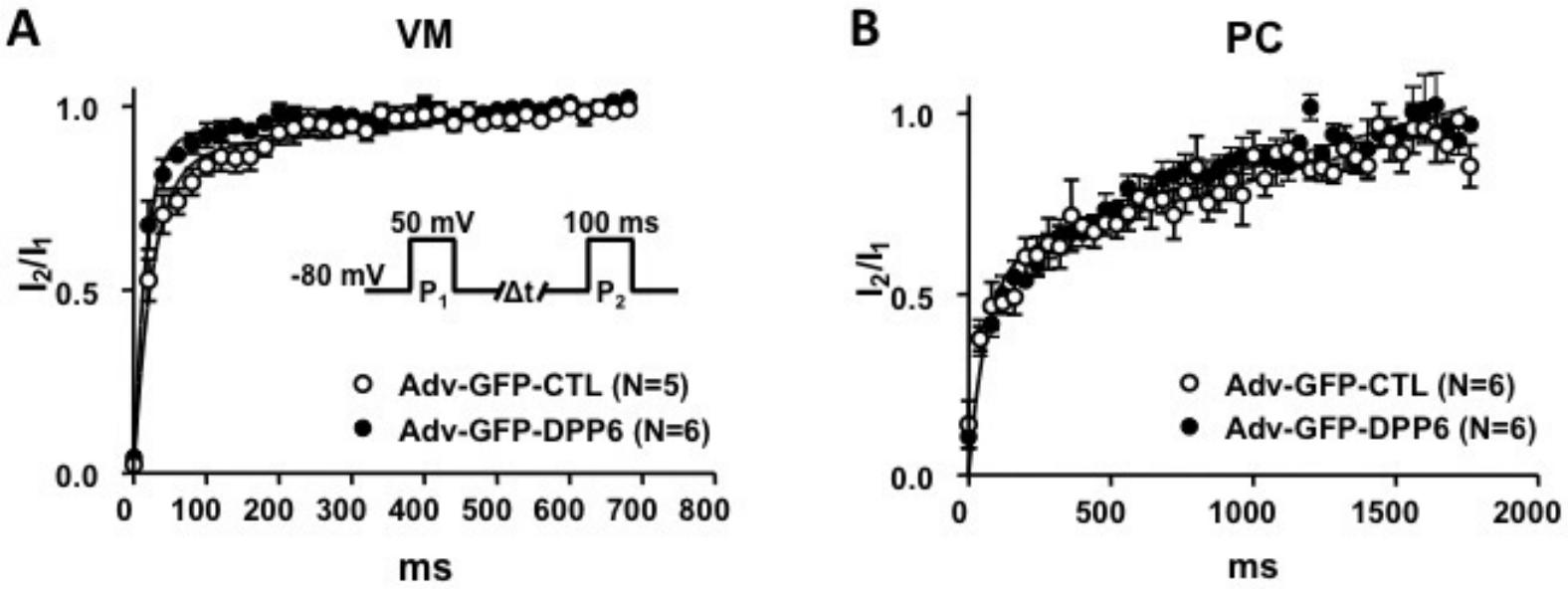
Online Figure V. RV vs PC I_{to} inactivation kinetics/voltage-dependence. **A**, Time-dependent inactivation of RV and PC I_{to}. Currents during 100-ms depolarizations to +30 mV, with best-fit biexponentials. **B**, I_{to} inactivation constants (τfast and τslow), obtained as shown in A. **P*<0.05 RV versus PC. **C**, voltage-dependence of I_{to} inactivation. Values are mean±SEM normalized currents; curves are best-fit Boltzmann relations. Data obtained with 200-ms test pulses to +50 mV preceded by 1-s conditioning pulses with a holding potential of -80 mV. **D**, I_{to} recovery from inactivation. Normalized currents as a function of I_1-I_2 interval at 0.1 Hz. Best-fit biexponentials are shown. Values are mean±SEM.

Online Figure VI



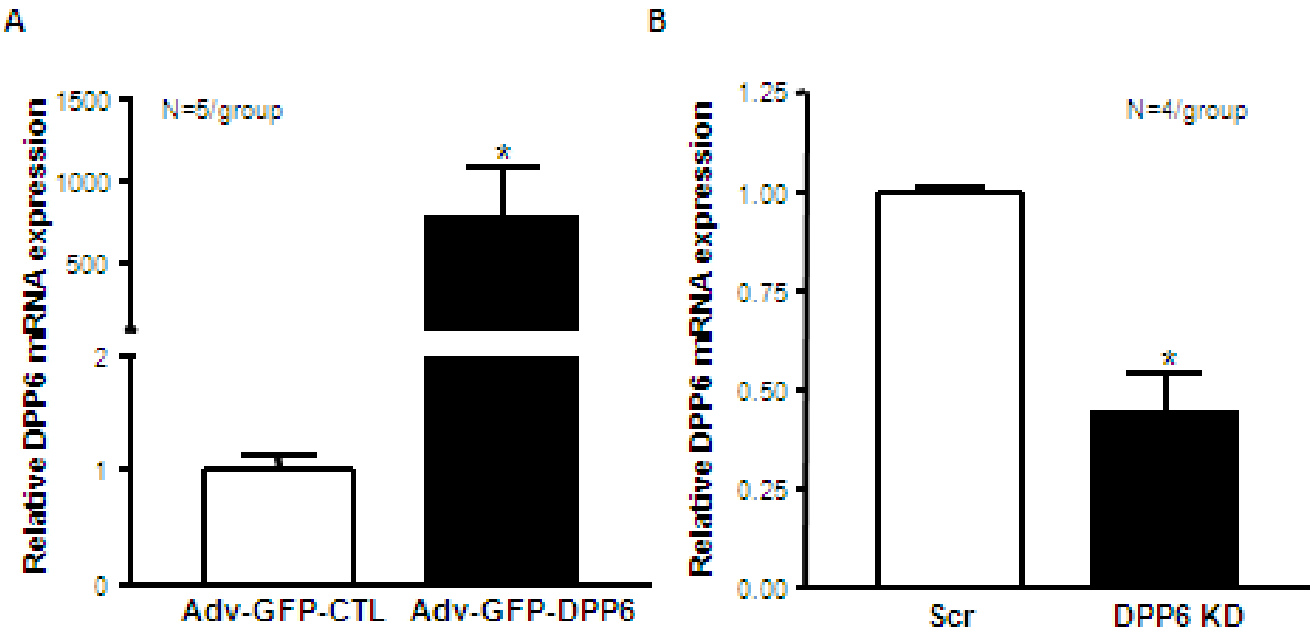
Online Figure VI. **A&B.** Voltage dependence of VM (A) and PC (B) I_{to} inactivation time constants at +10-70 mV after 48-hour Adv-GFP-CTL (CTL) or Adv-GFP-DPP6 (DPP6) infection. **C&D**, voltage-dependence of VM (C) and PC (D) I_{to} inactivation, studied with a 200-ms test pulse from -80 mV to +50 mV, preceded by 1-s conditioning pulses. Best-fit Boltzmann relations are shown. Values are mean±SEM.

Online Figure VII



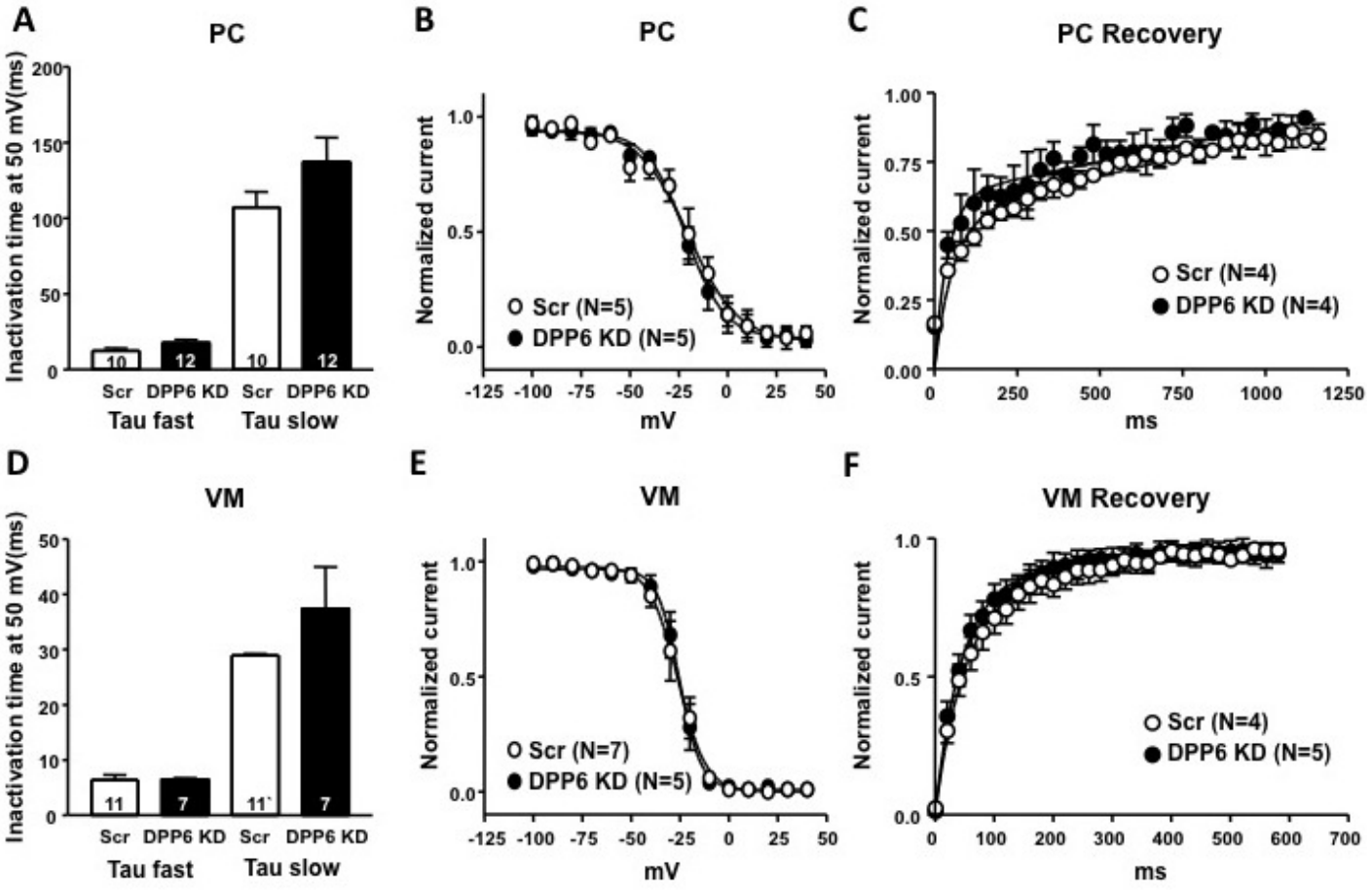
Online Figure VII. Time course of VM (A) and PC (B) I_{to} recovery from inactivation after 48-hour of Adv-GFP-CTL or Adv-GFP-DPP6 infection. Values are mean±S.E.M. of normalized currents as a function of I_1-I_2 interval, obtained with the protocol shown in panel A at 0.1 Hz. Best-fit biexponential functions are shown.

Online Figure VIII

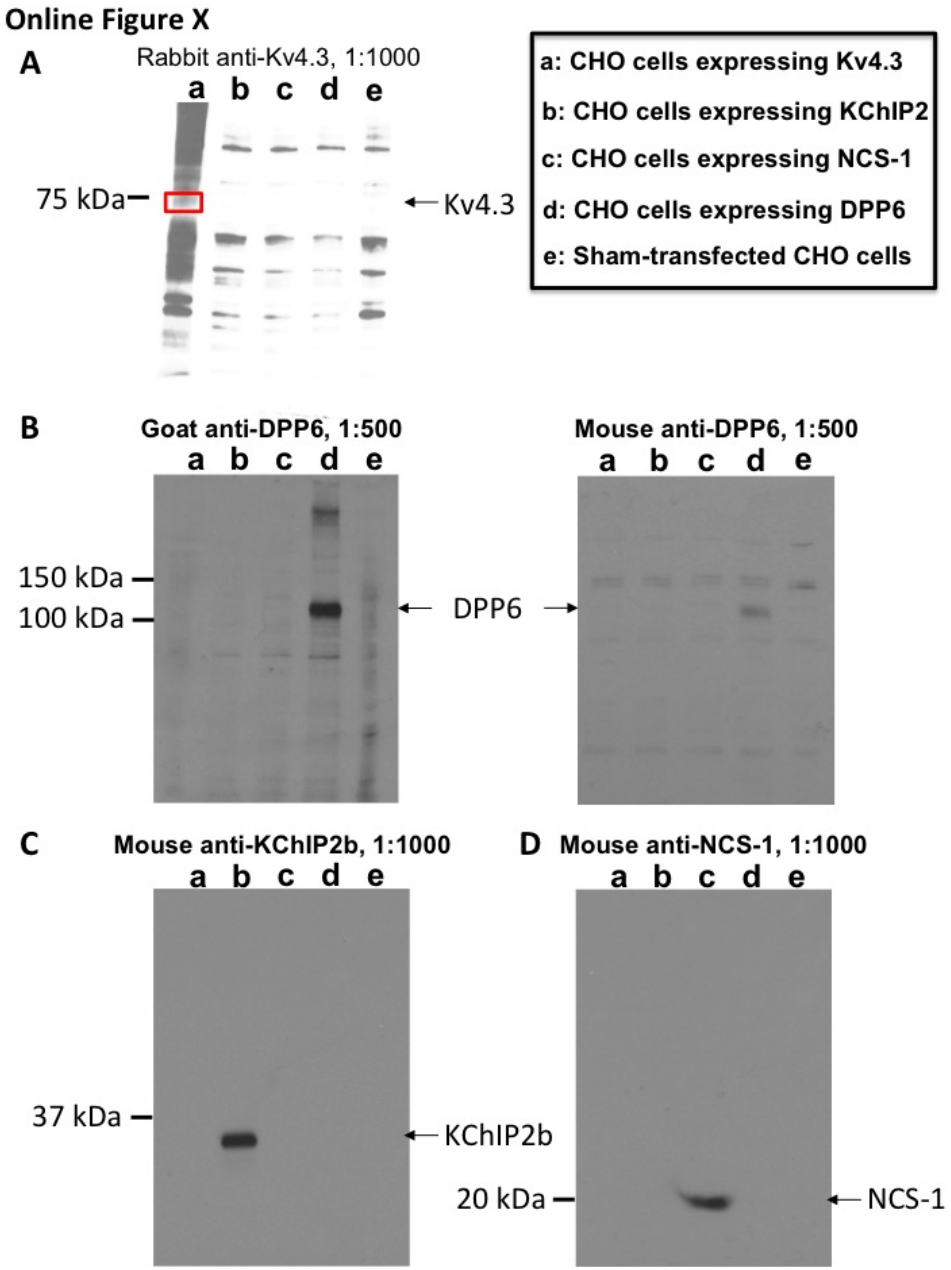


Online Figure VIII. A. Mean±S.E.M DPP6 mRNA expression in dog VM cells infected with Adv-GFP-CTL or Adv-GFP-DPP6. N=5/group, **P<0.05*, versus Adv-GFP-CTL. B. Mean±S.E.M DPP6 mRNA expression in dog VM cells infected with Scr or DPP6-KD. N=4/group, **P<0.05*, versus Scr.

Online Figure IX

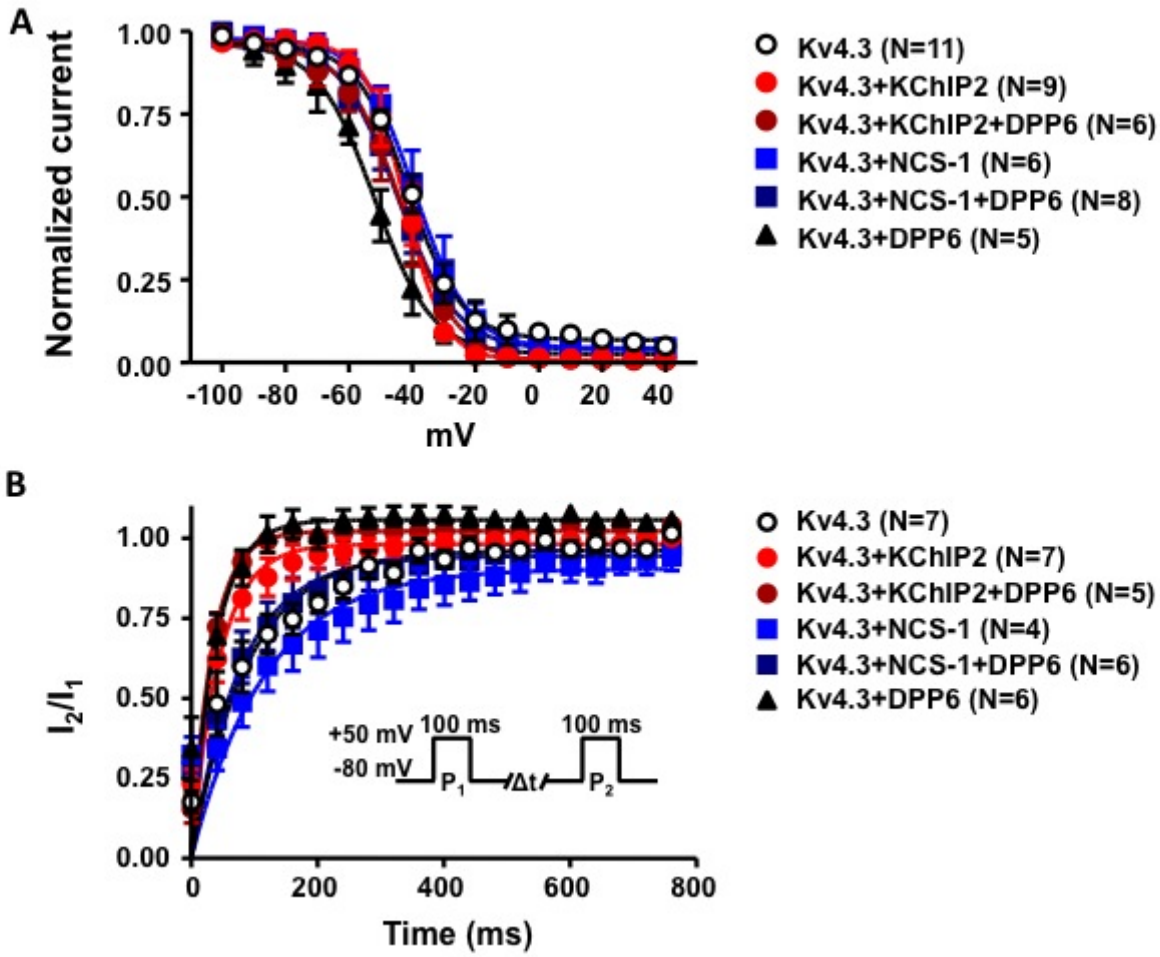


Online Figure IX. A&D, I_{to} inactivation constants at +50 mV in Scr or DPP6 KD adenoviral infected PCs (**A**) and VM cells (**D**). Data were obtained with biexponential fitting of currents recorded as shown in Figure 6. **B&E**, voltage-dependence of PC (**B**) and VM (**E**) I_{to} inactivation. Data were obtained with a 200-ms test pulse from -80 mV to +50 mV preceded by 1-s conditioning pulses. Currents were normalized to maximum; curves are best-fit Boltzmann relations. **C&F**, PC (**C**) and VM (**F**) I_{to} recovery from inactivation. Normalized currents as a function of I_1-I_2 interval (obtained with protocol in Online Figure VIIA); best-fit biexponential functions are shown. Values are mean±SEM.

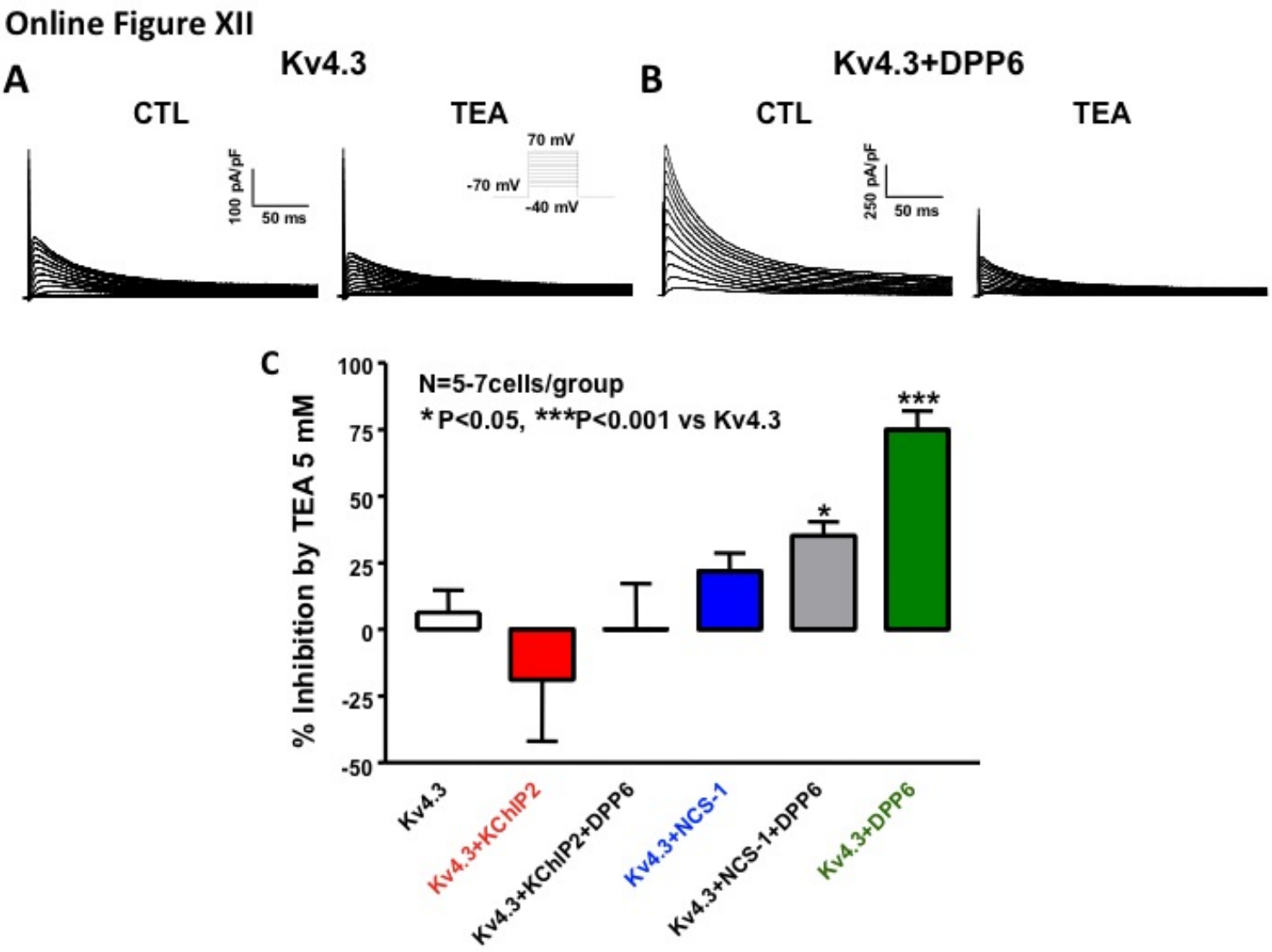


Online Figure X. Immunoblots of CHO-cell membranes incubated with primary antibodies against Kv4.3, DPP6, KChIP2 or NCS-1. Lane a-e were total protein samples from CHO cells overexpressing Kv4.3 (a), KChIP2 (b), NCS-1 (c), DPP6 (d) or non-transfected CHO cells (e). The positions of the bands quantified in our Western blot experiments are shown by arrows.

Online Figure XI

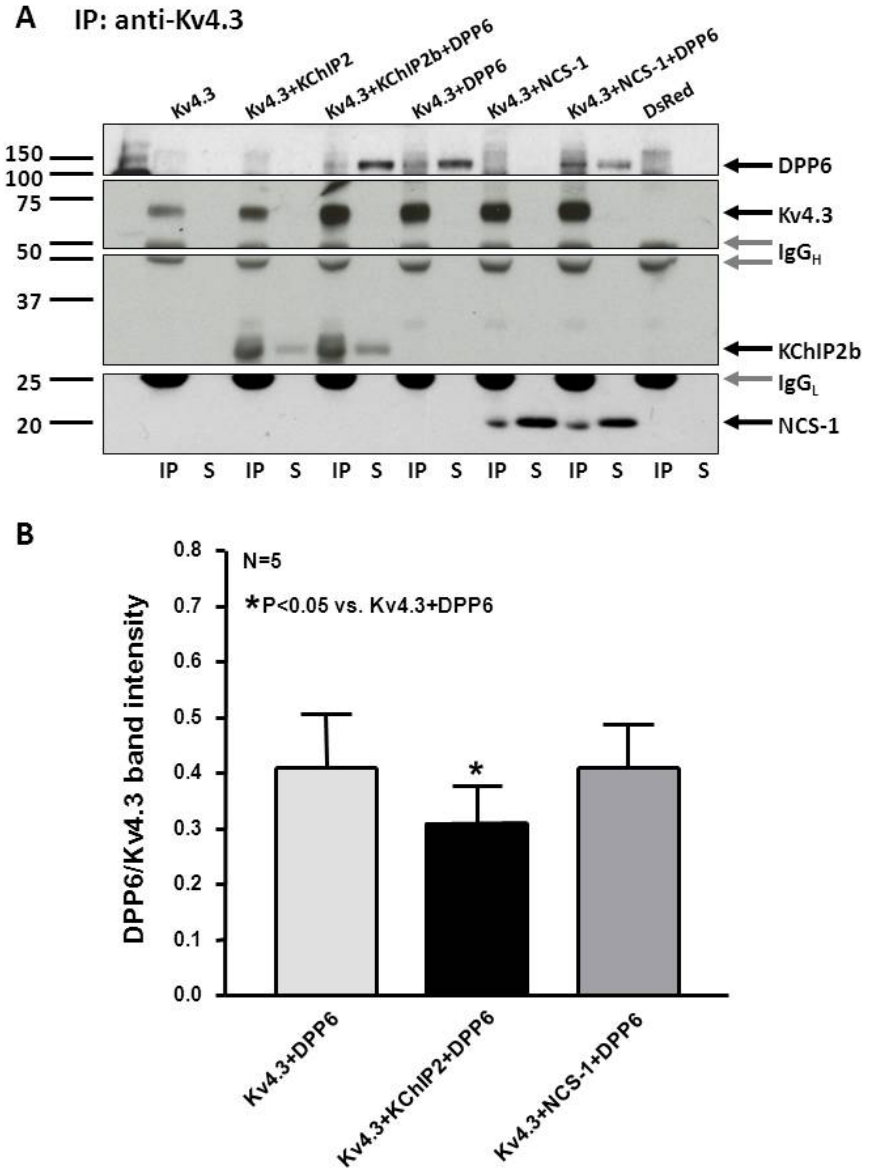


Online Figure XI. **A**, Inactivation voltage-dependence of currents in CHO cells transfected with Kv4.3, Kv4.3+KChIP2, Kv4.3+KChIP2+DPP6, Kv4.3+NCS-1, Kv4.3+NCS-1+DPP6, or Kv4.3+DPP6. Data were obtained with a 200-ms test-pulses to +50 mV preceded by 1-s conditioning pulses. HP was -80 mV. Best-fit Boltzmann relations are shown. **B**, time-dependent recovery from inactivation, studied with paired-pulse protocol (inset) at 0.1 Hz. Best-fit monoexponential functions are shown. N=numbers of cells studied. Values are mean±SEM.



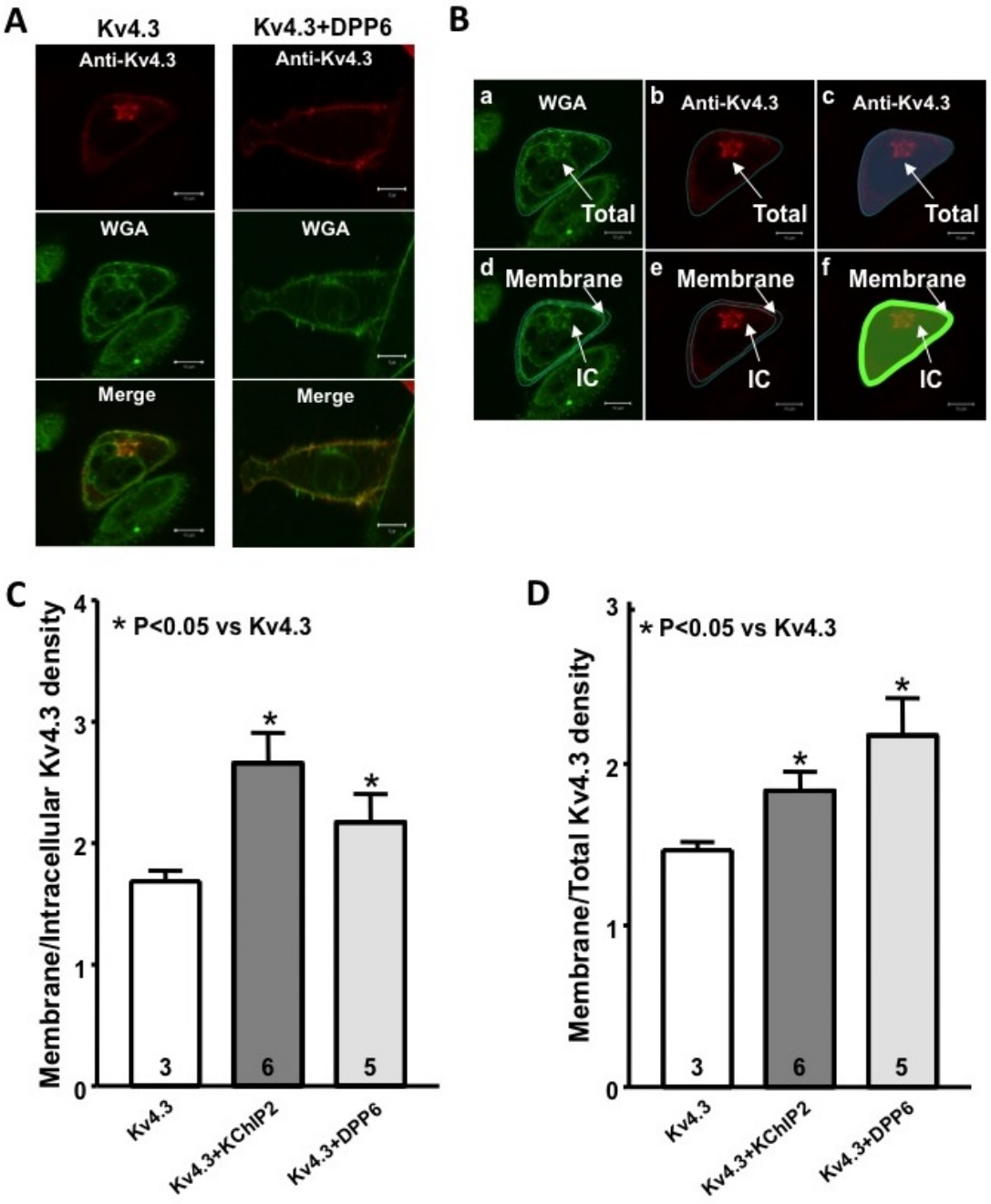
Online Figure XII. Currents recorded before and after 5-mmol/L TEA in CHO cells transiently transfected with Kv4.3 (**A**) or Kv4.3+DPP6 (**B**). Currents were recorded with 250-ms depolarizations from a HP of -70 mV at 0.1 Hz. **C**, mean±SEM percentage (%) inhibition by 5-mmol/L TEA at +30 mV. * *P<0.05* versus Kv4.3-alone.

Online Figure XIII



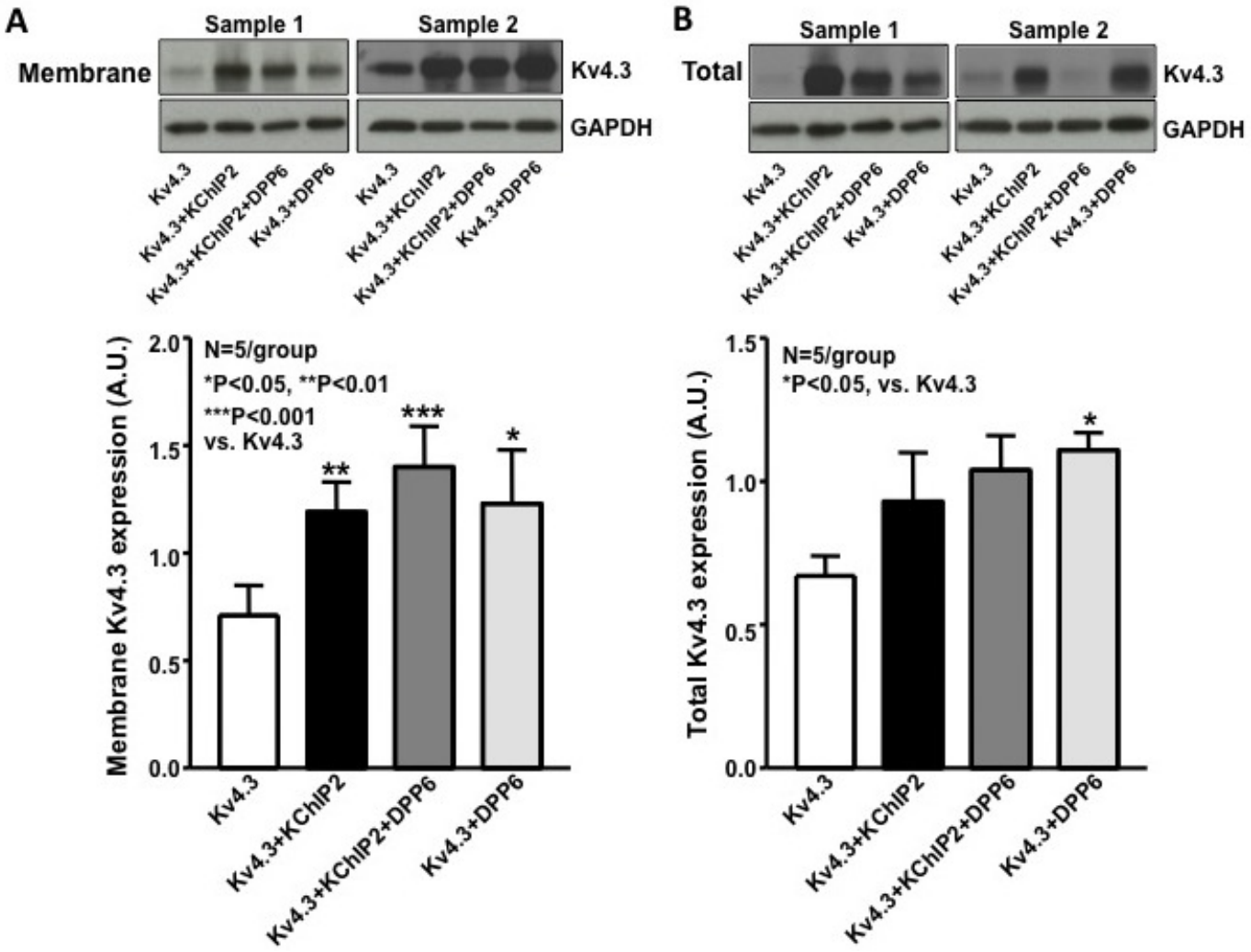
Online Figure XIII. Interactions between DPP6 and Kv4.3 in the absence or presence of KChIP2 or NCS-1 when coexpressed in CHO cells. **A,** Coimmunoprecipitation of Kv4.3, KChIP2, DPP6, and NCS-1 from total protein extracts of CHO cells expressing Kv4.3 alone, Kv4.3+KChIP2, Kv4.3+KChIP2+DPP6, Kv4.3+DPP6, Kv4.3+NCS-1, Kv4.3+NCS-1+DPP6, or DsRed. Proteins were immunoprecipitated with monoclonal anti-Kv4.3 (IP: anti-Kv4.3) and the immunoprecipitates were probed with anti-DPP6, anti-Kv4.3, anti-KChIP2 and anti-NCS-1 antibodies. IP, immunoprecipitate; S, supernatant. **B,** Mean±SEM DPP6 to Kv4.3 band-intensity ratios from Kv4.3+KChIP2+DPP6, Kv4.3+DPP6 and Kv4.3+NCS-1+DPP6 immunoprecipitates. **P*<0.05 versus Kv4.3+DPP6.

Online Figure XIV



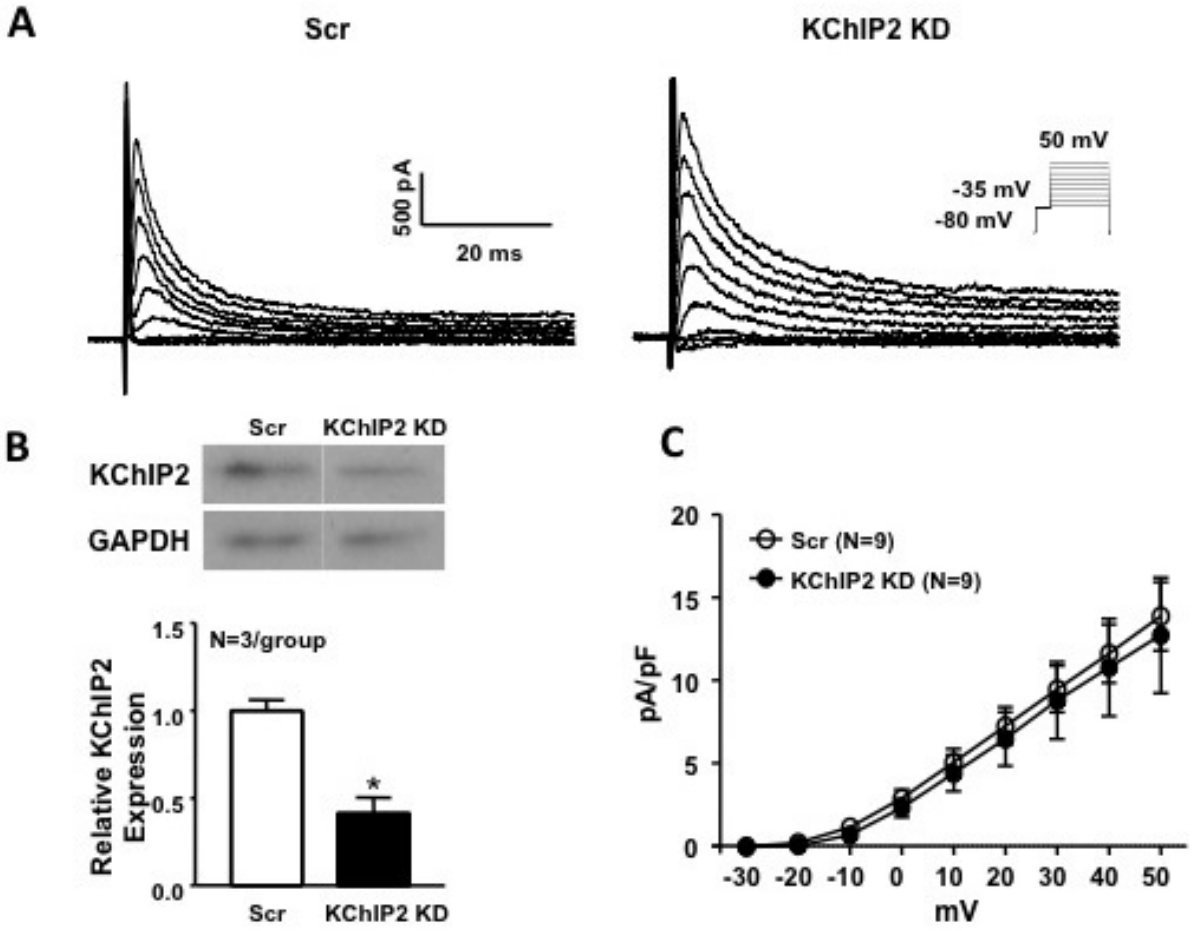
Online Figure XIV. **A**, Immunofluorescent images of CHO cells expressing Kv4.3 or Kv4.3+DPP6. Red=Kv4.3, green=wheat germ agglutinin (WGA) membrane marker. **B**, Total, membrane and intracellular (IC) Kv4.3 fluorescence calculation in one cell. **a**, WGA-staining. **b**, Anti-Kv4.3-staining. **c**, image in **b:** total Kv4.3-fluorescence was obtained from area within blue translucent mask. **d**, inner/outer cell-membrane borders outlined in blue. **e**, intracellular (IC) and membrane-zones indicated. **f**, membrane Kv4.3-fluorescence in region demarcated by bright green line; IC Kv4.3-fluorescence in area covered by translucent green mask. Horizontal scale=10 µm. **C**, **D**, mean±SEM ratios of Kv4.3 membrane/intracellular (**C**) or membrane/total (**D**) density in CHO cells expressing Kv4.3 only, Kv4.3+KChIP2, or Kv4.3+DPP6.*$P<0.05$ versus Kv4.3.

Online Figure XV



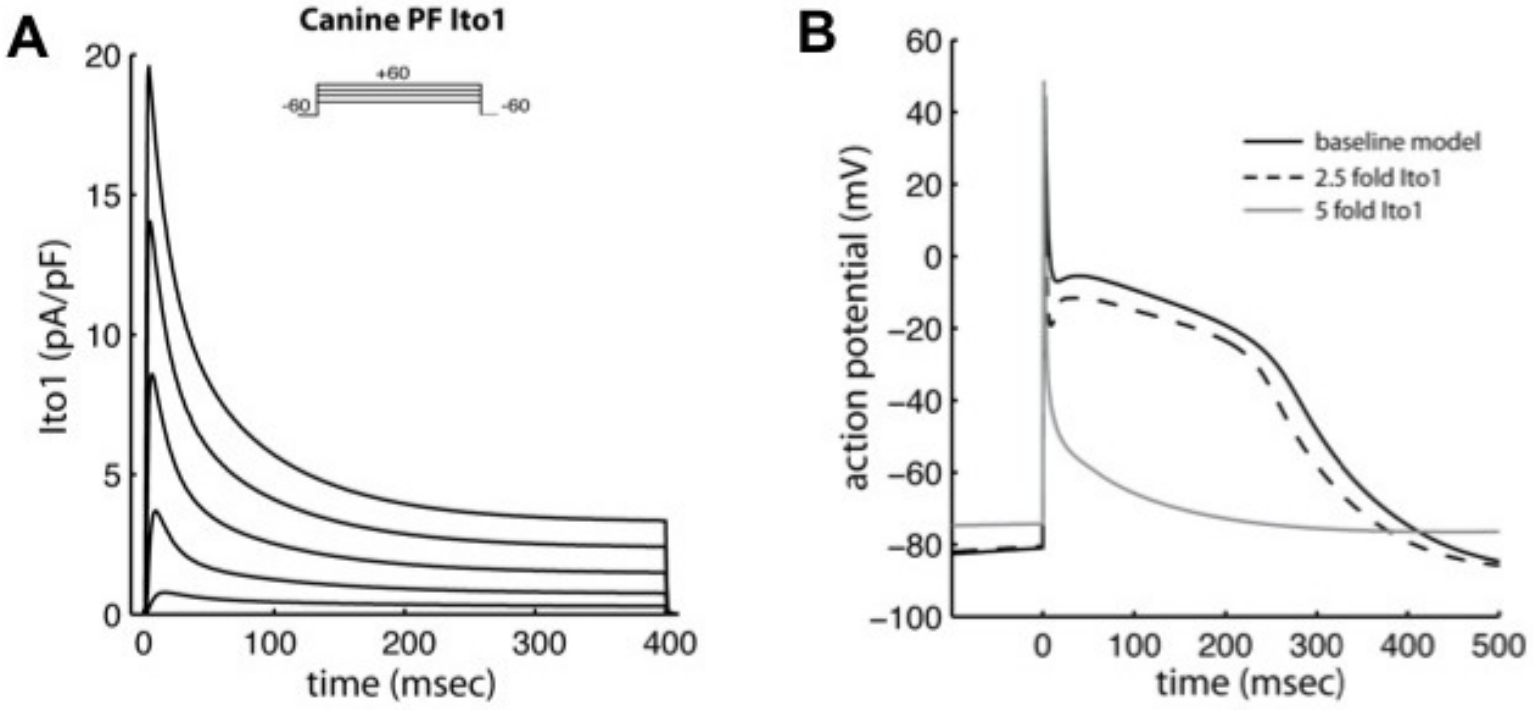
Online Figure XV. **Top:** Immunoblots for Kv4.3 and GAPDH from membrane (**A**) or total protein (**B**) fractions in CHO cells expressing Kv4.3, Kv4.3+KChIP2, Kv4.3+KChIP2+DPP6, or Kv4.3+DPP6. Bottom: corresponding mean±SEM Kv4.3 band-intensities after normalization to GAPDH. N=5/group. **P*<0.05, ***P*<0.01, ****P*<0.001, versus Kv4.3 channels.

Online Figure XVI



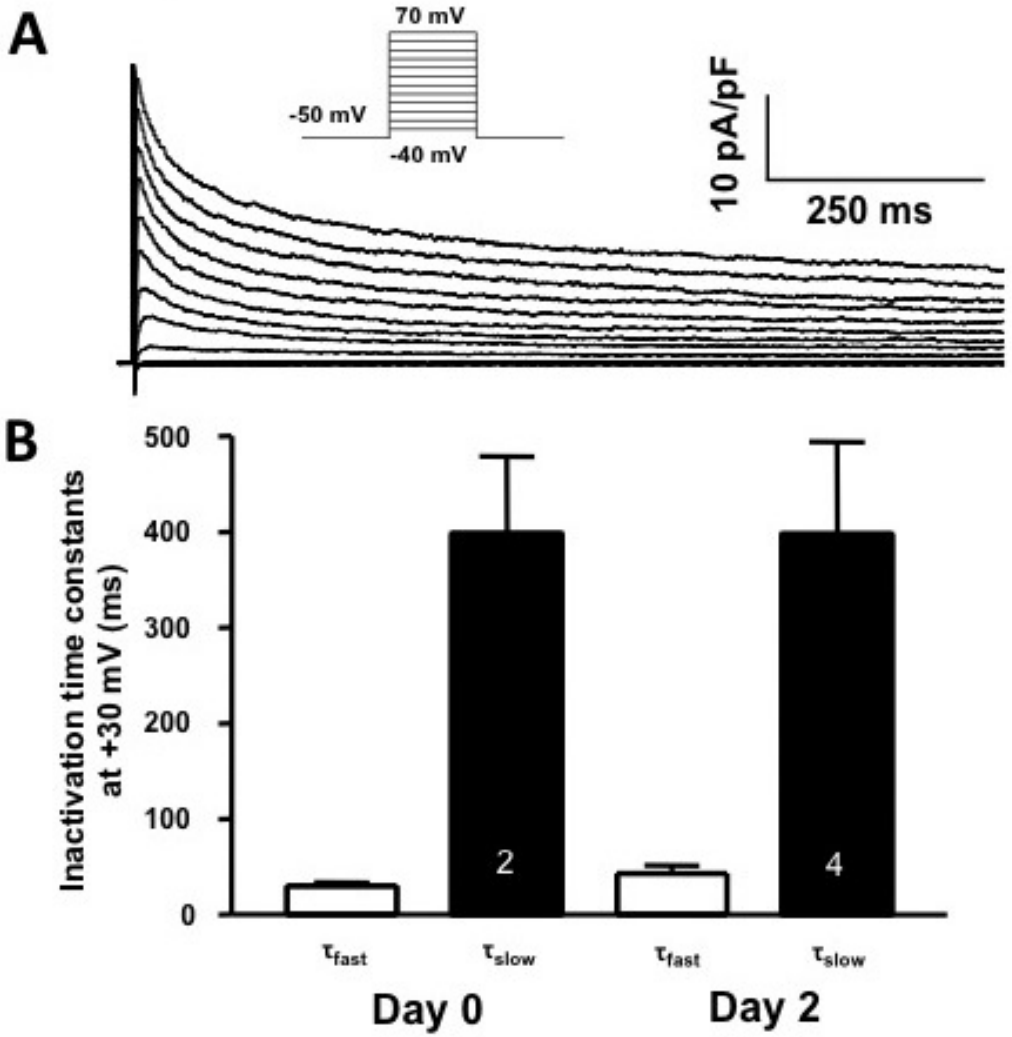
Online Figure XVI. A, I_{to} recordings from VMs infected with adenoviruses containing scrambled (Scr) or KChIP2 knockdown (KD) constructs. Currents were obtained with 100-ms depolarizations from -80 mV, preceded by a brief (5-ms) pre-pulse to -35 mV to inactivate I_{Na}. **B**, Top, Western blots in VMs infected with SCr or KChIP2 KD adenovirus; Bottom, mean±SEM KChIP2 bands normalized to GAPDH. *P<0.05 vs KChIP2 KD. **C**, mean±SEM I_{to} density-voltage relations.

Online Figure XVII



Online Figure XVII. Simulated DPP6-overexpression effects in a mathematical model of the PF action potential. **A**, a family of model-derived currents upon depolarization to various potentials reproduces the essential features of canine-PF I_{to} K^+-current. **B**, action potentials at 1 Hz for the PF model with I_{to} at normal density (solid black line); 2.5×current-density (dashed line), and 5×current-density (gray line) show progressive deepening of the phase 1 notch, leading to very rapid repolarization from phase 1.

Online Figure XVIII



Online Figure XVIII. **A**, Representative recordings of PC I_{to} after 2-day culture, obtained with 1-s depolarizations to voltages between -40 and 70 mV, from a holding potential -50 mV. **B**, mean±SEM I_{to} inactivation constants (τ_{fast} and τ_{slow}) from Day 0 and Day 2 cultured Purkinje cells. Numbers in columns indicate number of cells.

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Unique Cardiac Purkinje Fiber Transient Outward Current β-Subunit Composition: A Potential Molecular Link to Idiopathic Ventricular Fibrillation

Ling Xiao, Tamara T. Koopmann, Balázs Ördög, Pieter G. Postema, Arie O. Verkerk, Vivek Iyer, Kevin J. Sampson, Gerard J.J. Boink, Maya A. Mamarbachi, Andras Varro, Luc Jordaens, Jan Res, Robert S. Kass, Arthur A. Wilde, C.R. Bezzina and Stanley Nattel

Circ Res. 2013;112:1310-1322; originally published online March 26, 2013;
doi: 10.1161/CIRCRESAHA.112.300227
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571