SUPPLEMENTAL DATA

A. Detailed methods

1. Patch clamp recordings from myocytes

All recordings were done using ruptured-patch whole cell dialysis mode of the patch clamp method, at 34±1°C, with myocytes superfused with normal Tyrode's solution (in mM: NaCl 146, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, dextrose 10, pH 7.4) and dialyzed with pipette solution (in mM: K-aspartate 125, KCl 20, EGTA 10, ATP 10, HEPES 10, pH 7.3). Membrane voltage was recorded in the current-clamp mode, while membrane currents were recorded in the voltage-clamp mode, of Axopatch 200B amplifiers under the control of Clampex (pClamp10 suite) via DigiData 1440A interface .

To make sure that the action potentials and currents were recorded under as uniform conditions as possible among the myocytes (especially important for the L-type Ca and slow delayed rectifier currents, I_{CaL} and I_{Ks}, that tended to 'run down' during whole cell dialysis), we adhered to the following sequence of protocols: (1) 0 - 4 min after patch rupture, adjusting series resistance compensation and recording cell capacitance, (2) 5 - 10 min, recording action potentials, (3) 10 -20 min, recording I_{K1}, I_{CaL}, and I_{to}, (4) 20 - 25 min, recording I_{Kr} and I_{Ks}.

2. Action potential analysis

We recorded resting membrane potential (RMP) and action potentials elicited by a train of 0.2-ms supra-threshold depolarizing current pulses (n = 60) at a cycle length of 1 s. Action potential durations were measured as the time between the upstroke and when membrane potential was repolarized to -60 mV ($APD_{-60\ mV}$). In 12 of 39 PVC myocytes, the action potentials lasted longer than 1 s. In these cases, APD was defined as 1 s. The AP plateau voltage was quantified as the membrane potential 50 ms after the upstroke. In PVC myocytes that had prominent 'spike-and-dome' AP morphology, the most positive voltage reached during the 'dome' was defined as plateau voltage. Beat-to-beat variations in APD were quantified by

standard deviations of successive differences (SDSD) in APD, calculated as: $[\Sigma(APD_{n+1} - APD_n)^2/N]^{0.5}$, where APD_{n+1} and APD_n were successive APD values, 'N' was 'number of consecutive APs -1' ($N \geq 30$).

3. Membrane current analysis

For quantification purpose, individual current components were separated based on the voltage clamp protocols and methods of data analysis. Current density was calculated by dividing current amplitude by cell capacitance (pA/pF).

a. *Transient outward current (I_{to})*:

To separate I_{to} from overlapping Na current (I_{Na}) and I_{CaL}, we recorded I_{to} at +50 mV. I_{Na} and I_{CaL} were small because +50 mV was close to their reversal potentials. To quantify I_{to} peak amplitude (Fig. 2A), we further isolated I_{to} from overlapping I_{Ks} at +50 mV by subtracting current traces recorded after a 2-s prepulse to -30 mV (I_{to} totally inactivated with little I_{Ks} activation, Fig. 5A) from that after a 2-s prepulse to -100 mV (I_{to} fully available, Fig. 2B). To quantify voltage-dependence of I_{to} inactivation (voltage clamp protocol diagrammed in Fig. 2B): from V_h -80 mV, 2-s conditioning pulses to V_c -100 to -30 mV in 5 mV steps were followed by a test pulse to +50 mV to record current from available I_{to} channels. In each myocyte, the peak amplitudes of test pulse currents were normalized by that after V_c -100 mV (fraction available) and its relationship to V_c was fit with a Boltzmann function: fraction available = $1/(1+\exp[(V_c-V_{0.5})/k])$, to estimate the half-maximum inactivation voltage '$V_{0.5}$' and slope factor 'k'. To quantify the rate of I_{to} recovery from inactivation (restitution, voltage clamp protocol diagrammed in Fig. 2C): from V_h -80 mV, double pulses to +50 mV for 500 ms with varying interpulse interval (ΔT) were applied once every 15 s. The peak current amplitude during the second pulse (P2) was divided by that during the first pulse (P1) as an estimate of the fraction of I_{to} channels recovered from inactivation (fraction recovered) during the interpulse interval. Its relationship to ΔT was fit with a double

exponential function: fraction recovered = $A_f(1-\exp[-\Delta T/\tau_f])+ A_s(1-\exp[-\Delta T/\tau_s])+A_{ss}$, to estimate the fractions of fast and slow components of I_{to} restitution (A_f and A_s), as well as time constants of the 2 components (τ_f and τ_s). A_{ss} is the fraction of available I_{to} channels at the end of the first pulse.

b. Inward rectifier (I_{K1}) and L-type Ca (I_{CaL}) currents:

The same voltage clamp protocol was used to quantify I_{K1} and I_{CaL} (diagrammed in Fig. 3A): from V_h -40 mV, 500-ms test pulses to V_t +60 to -130 mV in 10 mV steps were applied once every 10 s. I_{K1} was quantified by current amplitudes at the end of the test pulses in the -40 to -130 mV range. I_{CaL} was measured as the difference between the initial peak current and current level at the end of the 500-ms pulse in the -30 to +60 mV range.

c. *Rapid and slow delayed rectifier (I_{Kr} and I_{Ks}) currents*:

To separate I_{Kr} and I_{Ks} from overlapping I_{to} and I_{CaL}, we quantified their tail currents after 5-s depolarization pulses when I_{to} and I_{CaL} were inactivated. I_{Kr} and I_{Ks} were further separated from each other based on their different voltage ranges of activation. The validation experiment is shown in Fig. 5B. The upper panel of Fig. 5B depicts 3 sets of tail currents recorded from the same CON myocyte under the control conditions, after applying 1 uM dofetilide (DOF), and the difference currents (subtracting currents in DOF from those under the control conditions). Currents were elicited by the diagrammed voltage clamp protocol: from V_h -50 mV, 5-s test pulses to -40 to +60 mV in 10 mV steps were applied once every 15 s to activate available I_{Kr} and I_{Ks} channels. Tail currents recorded upon repolarization to -50 mV (gray shading) reflected currents through available I_{Kr} and I_{Ks} channels. The lower panel of Fig. 5B illustrates tail I-Vs from the same data set fit with Boltzmann functions. Currents in DOF represented I_{Ks}. The Boltzmann fit generated $V_{0.5}$ of 24 mV and k of 15 mV. Difference currents (DOF-sensitive)

represented I_{Kr}. The Boltzmann fit generated $V_{0.5}$ of -6 mV and k of 6.5 mV. Control currents represented the sum of I_{Kr} and I_{Ks}. The data points are superimposed on a curve summed from the I_{Kr} and I_{Ks} Boltzmann fits. The same observations were obtained in 3 other CON myocytes. Based on this analysis, we quantified I_{Kr} as tail currents at -50 mV after 5-s pulse to 0 mV (before significant activation of I_{Ks}), and I_{Ks} as tail currents at 0 mV (when I_{Kr} activation approached its plateau and thus would not produce tail current) after 5-s pulse to +50 mV. These measurements are indicated by dashed lines with arrows in the right panel of Fig. 5B.

4. Correlating APD and key current densities in individual myocytes

This was done by 2 methods: linear regression and Pearson correlation coefficient. The Pearson correlation coefficient tells us whether 2 parameters (current density and APD) change in the same direction (e.g. increase in current and prolongation in APD), in the opposite directions, or unrelated (+1 to -1, with '0' if unrelated). Neither linear regression nor Pearson correlation coefficient was ideal. For example, the relationship between APD and any current density is likely to be non-linear. However, consistency in results from these two analyses validates each other. This is the case for our data analysis (Fig. 8A vs 8B)

5. Immunoblot experiments

a. Preparation of whole tissue lysates (WTLs):

Tissue chunks were taken from left ventricular free wall (adjacent to the anterior wall where myocytes were isolated for patch clamp and $[Ca]_i$ experiments) and snap frozen in liquid nitrogen. Tissue chunks were kept in -80^{o}C freezer until experiments. To prepare WTLs, frozen tissue chunks were pulverized in 10 vol of lysis buffer (in mM: NaCl 145, $MgCl_2$ 0.1, HEPES 15, EGTA 10, pH 7, Triton X-100 0.5, with protease inhibitor cocktail), and solubilized for 30 min on ice. This crude lysate was homogenized by tip sonicator (2 of 15-s bursts), and then centrifuged

to pellet nuclei and debris. The supernatants were saved as WTLs, and the protein concentrations were quantified using bicinchoninic acid (BCA) kit (Pierce).

b. Sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis and immunoblotting:

WTLs were loaded onto denaturing (SDS)/reducing (mercaptoethanol) polyacrylamide gels. After electrophoresis, the proteins were blotted onto PVDF membranes and the membranes were probed with suitable antibodies (see below). Immunoreactivity was visualized using an ECL detection kit (Amersham). Remaining proteins in the gels were stained with Coomassie blue to check loading variations. Densitometry was performed using FluoChem E and associated software (ProteinSimple).

6. $[Ca]_i$ monitoring

a. *Setup for $[Ca]_i$ monitoring*:

The setup consists of an Olympus IX70 microscope equipped with a Polycrome V (Till Photonics, Gräfelfing, Germany) as light source, a Luca S digital camera (Andor Technology, Belfast, UK), and an automatic perfusion system (AutoMate Scientific, Berkeley, CA). The imaging and perfusion systems were controlled by the Live Acquisition Software from Till Photonics. Fluo-4 was excited by 490/10 nm light via a 505LP dichroic mirror, and emission was measured at wavelength of 535/50 nm. The objective was an Olympus 20X 0.80 NA Oil and the acquisition rate was set at 50 Hz. The field electrical stimulation was delivered by platinum wires positioned near the cells. Electrical pulses of 0.5-ms duration were generated by a Grass S48 stimulator; the voltage applied was set at stimulation threshold for each cell.

b. *Data acquisition and analysis*:

Ca sensitive dye Fluo-4 AM (Life Technologies) was dissolved in DMSO along with pluronic F-127 (20%) and diluted in imaging solution (IS, in mM: 130 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10

HEPES, 10 glucose, pH 7.4) to reach a final concentration of 5.5 μM. Myocytes were plated on Matrigel-coated coverslips and loaded with Fluo-4 AM for 15 min at 37°C. Cells were then washed twice with IS and placed on the stage of epifluorescence microscope described above. Recordings were made at room temperature, with myocytes superfused with IS and field stimulated at cycle length 2 s. Fluorescence signals were background-subtracted and normalized by initial baseline fluorescence ($\Delta F/F_o$). Each myocyte experienced the same sequence of protocols: (1) $\Delta F/F_o$ transients evoked by field stimulations in 2 mM [Ca] bath solution (reflecting Ca^{2+}-induced Ca^{2+} release from SR), (2) tetracaine-induced decrease in $\Delta F/F_o$ (reflecting blockade of SR Ca leak with continued Ca uptake by SR Ca pump, while plasma membrane Na/Ca exchanger suppressed by Ca- and Na-free bath solution, in mM: 130 LiCl, 4 KCl, 1 $MgCl_2$, 2 EGTA, 10 HEPES, 10 glucose, pH 7.4), and (3) caffeine (20 mM)-induced $\Delta F/F_o$ spike (reflecting emptying of SR Ca store).

7. Immunocytochemistry and confocal microscopy

Myocytes attached to poly-L-lysine coated coverslips were fixed by 2% paraformaldehyde (room temperature, 10 min) and stored in non-permeabilizing blocking buffer (PBS supplemented with 5% fetal calf serum and 0.1% NaN_3) at 4^oC until experiments. Myocytes were permeabilized by 0.2% saponin (room temperature, 2 hr). Cells were incubated with primary antibodies, followed by Alexa-conjugated secondary antibodies. After antibody incubation, cell membrane and t-tubules were stained with Alexa-conjugated wheat germ agglutinin (WGA) and nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). Coverslips were mounted on slides with anti-fade agent. Fluorescence images were obtained with a Zeiss 710 confocal microscope using the following laser lines/emission filters (in nm): DAPI (405/BP 420–480), Alexa488 (488/BP 493–530), Alexa568 (561/BP573-630), and Alexa647 (633/LP657). Image analysis was done using NIH ImageJ. To quantify signal colocalization

between dyad proteins (Cav1.2, Junctophilin-2 and RyR2) and WGA, a line was drawn along the longitudinal axis of a myocyte where a clear array of WGA signals (marking t-tubule locations) was seen (Fig. 7A). The immunofluorescence signals of dyad proteins along this line were measured and background-subtracted. The degree of colocalization between immunofluorescence signals and WGA signals was quantified by Pearson correlation coefficient (Fig. 7B).

To count the number of $LAMP1^+$ vesicles per myocyte (Fig. 7D and 7E), the z-stack images of the whole myocyte was examined and the z-slice containing the most abundant $LAMP1^+$ vesicles was chosen for ImageJ quantification of vesicle number. Most lysosomes clustered to the peri-nuclear and inter-nuclear regions of cardiac myocytes. Given the spatial resolution along the z-axis of $\geq$ 2 μm (561 nm laser excitation), we estimate that $\geq$ 90% of the lysosomes in each myocyte were counted by our method.

8. Antibodies

The following primary antibodies were used in immunoblot and/or immunocytochemistry experiments: mAbs against Cav1.2, Kv4.3, and KChIP2 (NeuroMabs), rabbit pAbs against Kir2.1, Kir2.2, KCNQ1 and ERG1 (Alomone), mAb against KCNE1 (AbNova), mAb against RyR2 and goat pAb against junctophilin-2 (AbCam), and a rabbit pAb against KCNE2 (made by ourselves).

B. Numerical values of quantified parameters

Table S1 Summary of patch clamp data						
		CON	n	PVC	n	p value
Cell size	Cell capacitance (pF)	189±5	51	225±6	50	<0.001
and morphology	Length : width	5.7±0.3	11	7.7±0.6	12	< 0.05
Membrane voltage	RMP (mV)	-90.5±0.5	49	-91.2±0.5	43	>0.05
	$APD_{-60\ mV}$ (ms)	455±20	44	649±47	39	<0.01
	Plateau (mV)	28.8±1.5	44	26.8±2.2	26	>0.05
	SDSD in APD (ms)	26.5±1.4	38	63.0±12.7	28	<0.05
I_{to} peak amplitude at +50 mV (pA/pF)		18.6±1.0	44	14.1±1.1	42	<0.01
I_{to} inactivation	$V_{0.5}$ (mV)	-68.6±0.9	40	-63.6±3.5	40	>0.05
	k (mV)	3.9±0.2	40	4.4±0.4	40	>0.05
I_{to} restitution at -80 mV	%Fast	38±4	30	42±3	27	>0.05
	τ_{Fast} (ms)	324±39	30	215±24	27	>0.05
	τ_{slow} (ms)	1502±354	30	1125±152	27	>0.05
I_{K1} amplitude (pA/pF)	Outward at -70 mV	2.63±0.19	45	1.67±0.09	39	<0.001
	Inward at -130 mV	-24.8±2.0	40	-13.7±1.7	33	<0.001
I_{CaL} peak amplitude at 0 mV (pA/pF)		-4.79±0.28	48	-3.34±0.25	42	<0.001
I_{Kr} tail current amplitude (pA/pF)		0.31±0.02	26	0.27±0.04	28	>0.05
I_{Ks} tail current amplitude (pA/pF)		0.46±0.04	24	0.56±0.05	27	>0.05

Voltage clamp protocols and methods of data analysis for individual currents are described in *Detailed Methods*. 'n': number of myocytes examined (from 4 control and 5 PVC hearts).

Statistical analysis: t-test between CON and PVC myocytes.

Table S2 Summary of immunoblot data				
	CON	n	PVC	n
Kv4.3	1.00±0.08	4	0.57±0.08	4
KChIP2	1.00±0.10	4	0.52±0.15	4
Kir2.1	1.00±0.16	4	0.92±0.11	4
Kir2.2	1.00±0.20	4	0.49±0.09	4
Cav1.2	1.00±0.10	5	0.60±0.15	4
KCNQ1	1.00±0.16	4	0.81±0.35	4
KCNE1	1.00±0.01	4	0.94±0.15	4
ERG1	1.00±0.30	4	0.43±0.12	4
KCNE2	1.00±0.23	4	0.74±0.12	4

Experimental procedures are provided in *Detailed Methods*. Immunoblot band intensities were background subtracted, each divided by Coomassie blue intensity of the same lane, and normalized by the mean of CON samples. 'n': number of hearts examined.

Table S3 Summary of Fluo-4 $\Delta F/F_o$ data					
	CON	n	PVC	n	p value
$\Delta F/F_o$ transients induced by field stimulation in 2 mM $[Ca]_o$	0.61±0.03	42	0.41±0.02	60	< 0.0001
Tetracaine-induced decrease in $\Delta F/F_o$ in Ca_o-, Na_o-free solution	-0.142±0.009	42	-0.132±0.007	60	>0.05
$\Delta F/F_o$ spike induced by caffeine in Ca_o-, Na_o-free solution	1.12±0.05	42	1.13±0.05	60	>0.05

Procedures of data acquisition and analysis are provided in *Detailed Methods*. 'n': number of myocytes examined (from 4 control and 5 PVC hearts). Statistical analysis was done by t-test.