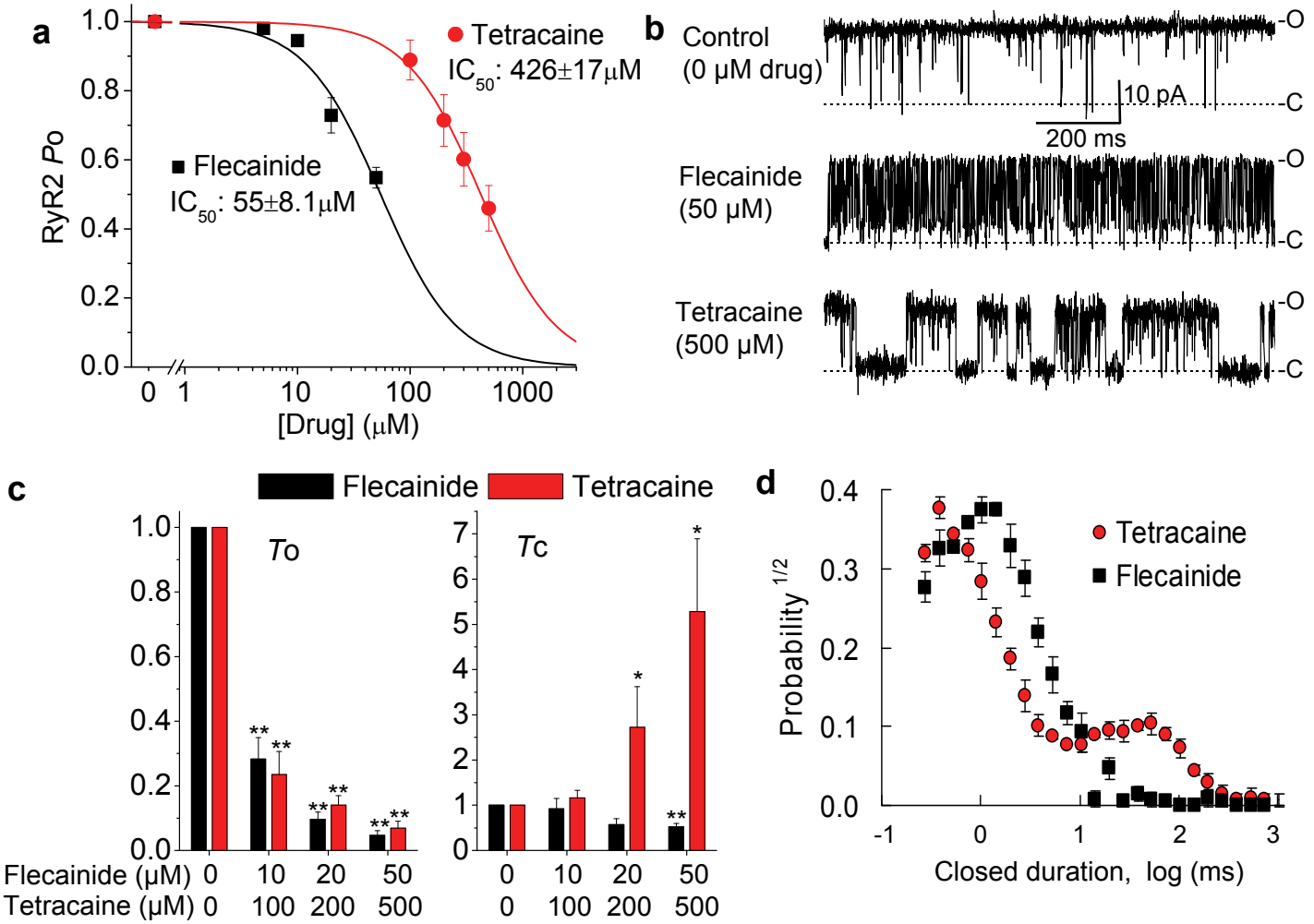


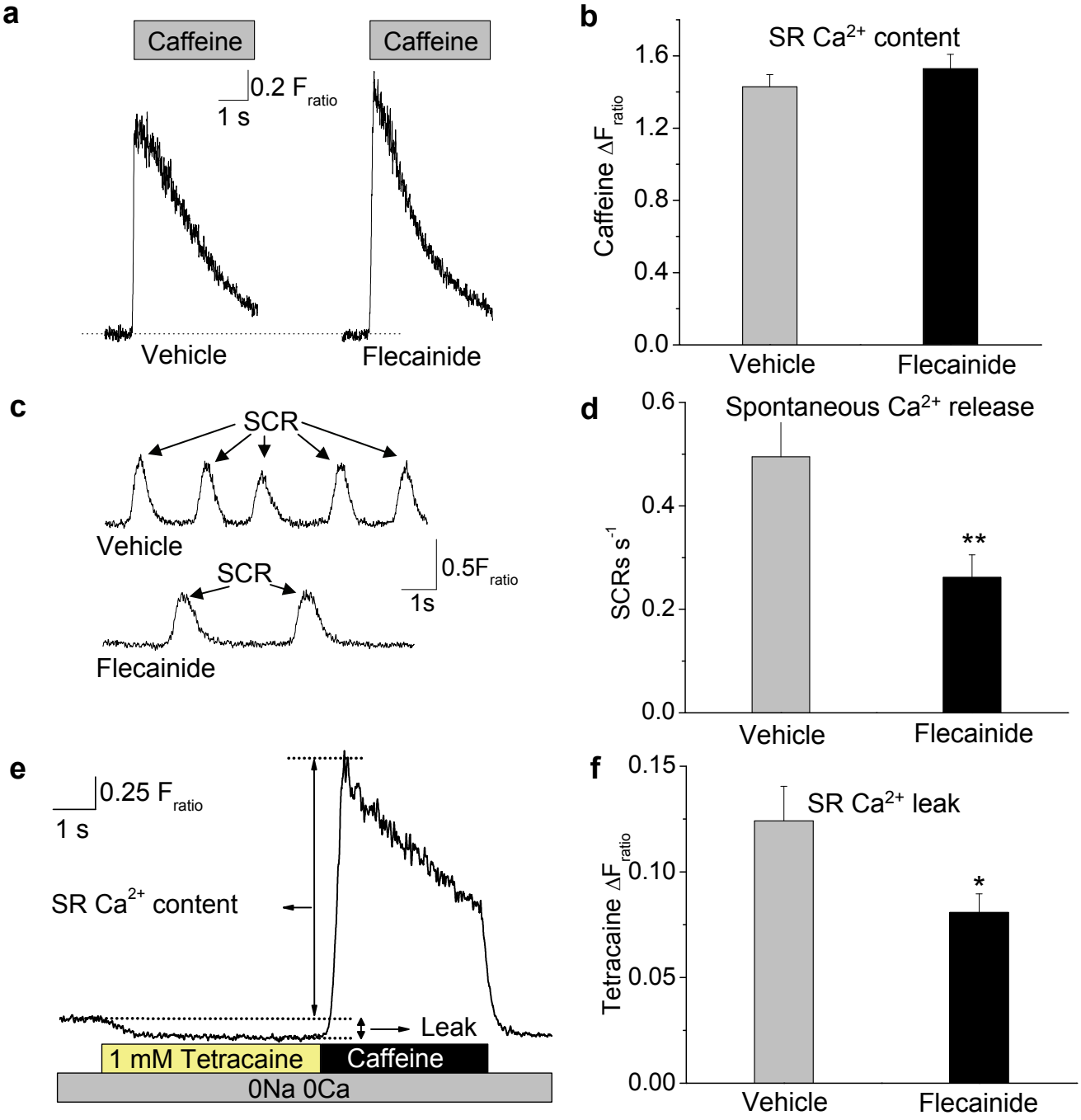
Supplementary Fig. 1 Comparison of RyR2 inhibition by flecainide and tetracaine. Compared to tetracaine, flecainide is a much more potent inhibitor of RyR2 channels with a different mechanism of action.

(a) Concentration-response relationship of relative mean open probability (*Po*).

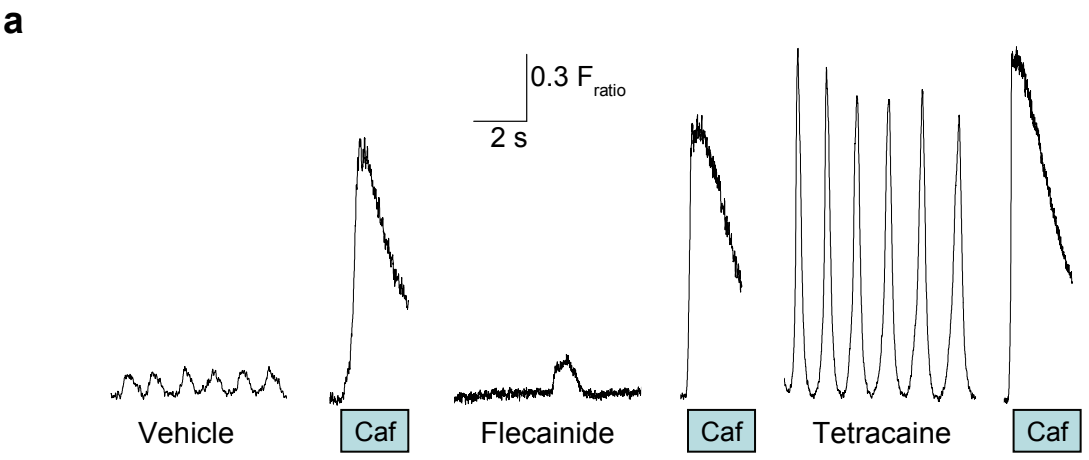
(b) Representative examples of the activity of single sheep RyR2 incorporated in lipid bilayers at the IC_{50} of each drug. C = closed, O = open.

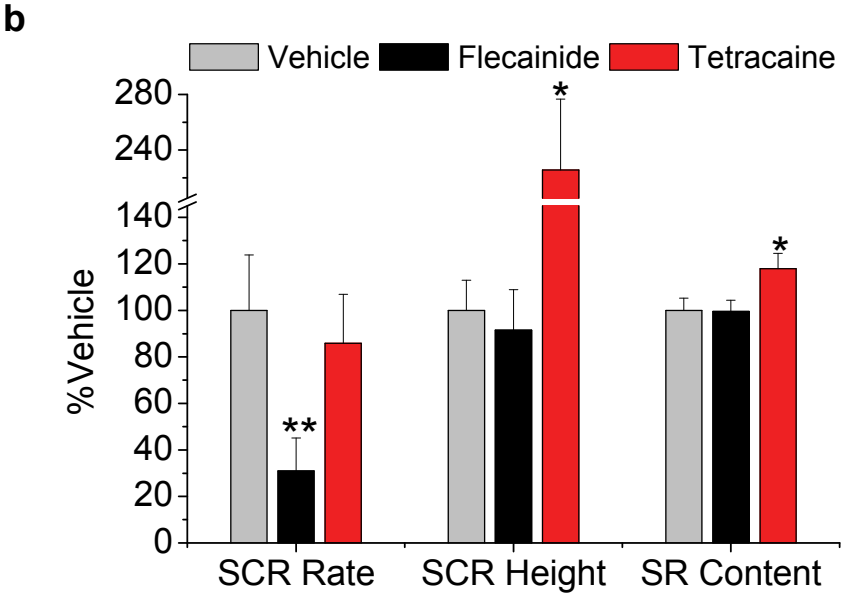
(c) Concentration-response relationship of relative mean open times (*To*) and mean closed times (*Tc*). Flecainide and tetracaine were compared at concentrations that produced comparable inhibition of *Po* in **(a)**. Note that tetracaine drastically increases mean *Tc*, whereas flecainide has the opposite effect. Data are expressed relative to values at 0 μM drug, with absolute *Po* = 0.86±0.02, *To* = 45±25 ms and *Tc* = 2.4±0.8 ms (flecainide) and *Po* = 0.99±0.007, *To* = 170±52 ms and *Tc* = 2.4±0.6 ms (tetracaine). N=3-8 per concentration for flecainide and N=4-6 for tetracaine, *P<0.05, **P<0.01 compared to 0 μM drug. RyR2 channels were activated by 0.1 mM Ca^{2+} on the *cis* (cytosolic) side (**Supplementary Methods**).

(d) Comparison of RyR2 channel closed dwell-time disctributions at IC_{50} of each drug (N=3). Dwell-times are grouped into logarithmically spaced bins (**Supplementary Methods**). Hence, individual exponential decay components of the distributions appear as peaks centered at times coinciding with their decay constants (τ). Flecainide blocks RyR2 by causing brief subconductance states with τ = 2ms, whereas tetracaine causes short- and long-lived channel closings (τ =0.5 & 50 ms).



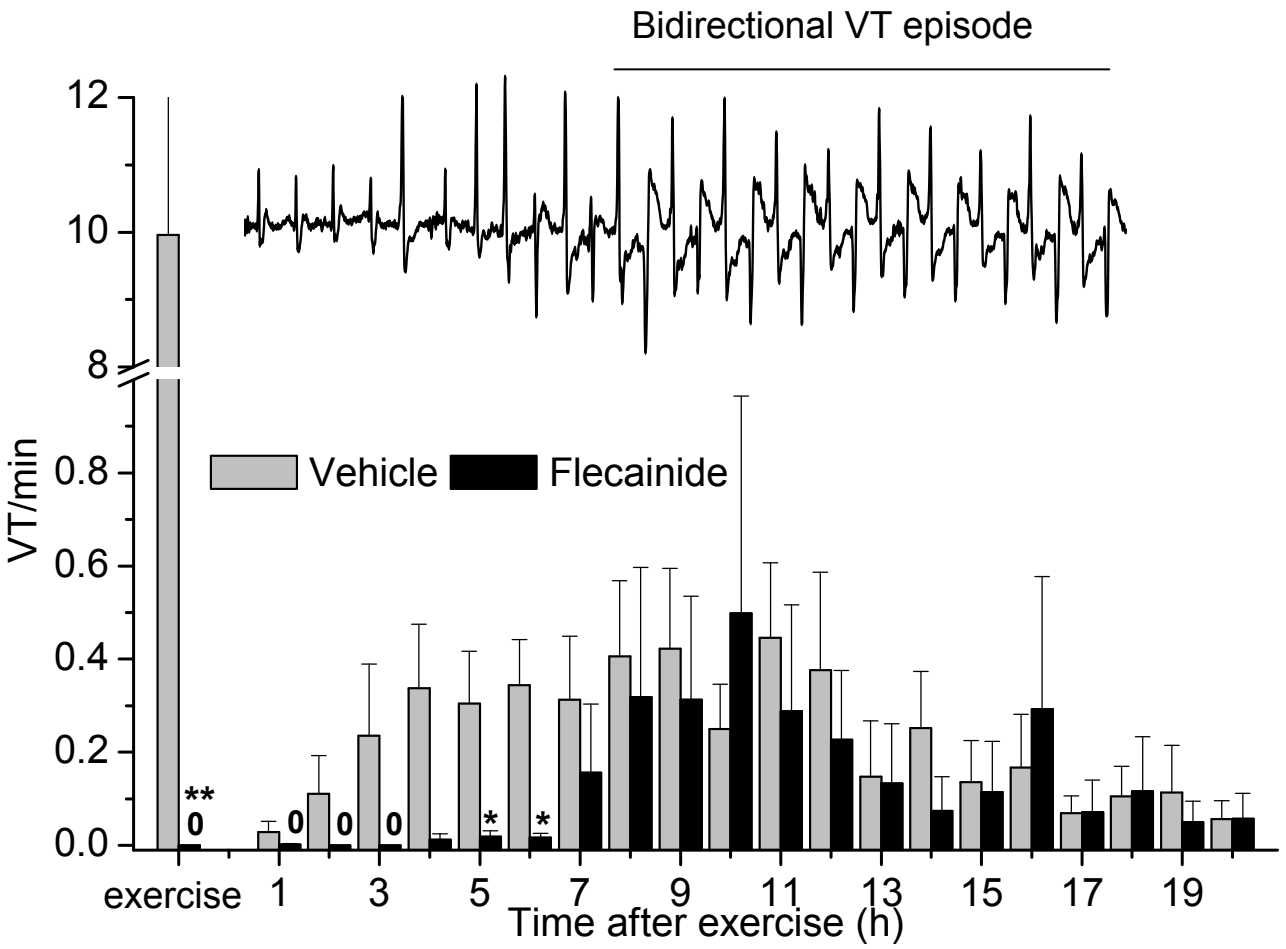
Supplementary Fig. 2 Effect of flecainide (6 μM) on SR Ca^{2+} content, spontaneous SR Ca^{2+} release and SR Ca^{2+} leak in Casq2$^{-/-}$ myocytes stimulated with isoproterenol (1μM). All data are mean and s.e.m., with 3-4 independent myocyte preparations for each experiment.
(a,b) SR Ca^{2+} content was estimated by the height of the Ca^{2+} transient induced by rapid caffeine (10 mM) application. Flecainide had no significant effect on average SR Ca^{2+} content. N=45 (vehicle) and 44 (flecainide) myocytes. **(c,d)** Rate of spontaneous Ca^{2+} releases after removal of Na^+ and Ca^{2+} from the extracellular solution, which prevents trans-sarcolemmal Ca^{2+} flux. N=55 myocytes per group, **p<0.01, *p<0.05 compared to vehicle. Flecainide decreased spontaneous Ca^{2+} releases also in *Casq2*$^{+/+}$ (wild-type) myocytes (vehicle: 0.34±0.11 SCR s^{-1}, N=25, flecainide 0.10±0.03 SCR s^{-1}, N=31, p=0.023).
(e,f) Tetracaine application was used to estimate SR Ca^{2+} leak as illustrated in **(e).** Removal of Na^+ and Ca^{2+} from the extracellular solution prevents trans-sarcolemmal Ca^{2+} flux. The drop in the fluorescent signal after application of the RyR2 blocker tetracaine (1 mM) was used to estimate diastolic SR Ca^{2+} leak. N=55 myocytes per group, *p<0.05 compared to vehicle.



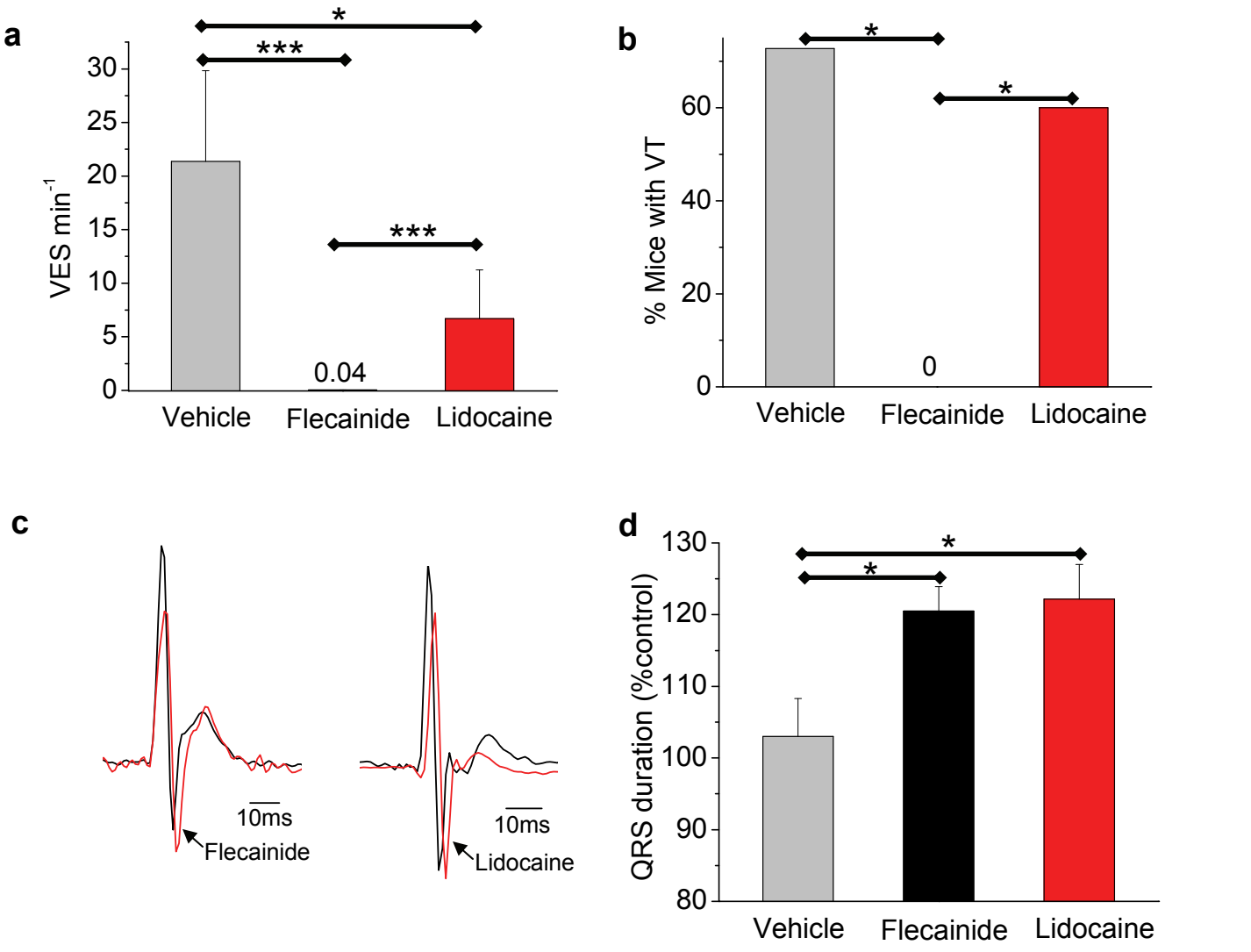


Supplementary Fig. 3 Effect of prolonged exposure to flecainide or tetracaine on spontaneous SR Ca^{2+} release (SCR) and SR Ca^{2+} content of quiescent Casq2$^{-/-}$ myocytes exposed to isoproterenol (1μM). **(a)** Representative examples of SCRs and caffeine-induced Ca^{2+} transients (Caf). The height of the caffeine transient was used to estimate SR Ca^{2+} content. **(b)** Comparison of means. All data were expressed relative to mean values obtained in vehicle-treated myocytes and averaged for each group. Myocytes were incubated for ~30 min in Tyrode's solution containing either vehicle, flecainide (6 μM) or tetracaine (50 μM). Acute application of 50 μM tetracaine had previously been reported to suppress spontaneous SR Ca^{2+} release in ventricular myocytes (Venetucci et al 2006). Experimental protocols as described in **Supplementary Methods.** Note that in contrast to flecainide, chronic application of tetracaine failed to suppress SCRs, and increased SCR height and SR Ca^{2+} content. All data are mean and s.e.m., with 3-4 independent myocyte preparations for each experiment. Vehicle N=45 myocytes, flecainide N=47 myocytes, tetracaine N=48 myocytes, **p<0.01 by Mann-Whitney test and *p<0.05 by *Student's t*-test.

Venetucci, L.A., Trafford, A.W., Diaz, M.E., O'Neill, S.C. & Eisner, D.A. Reducing ryanodine receptor open probability as a means to abolish spontaneous Ca^{2+} release and increase Ca^{2+} transient amplitude in adult ventricular myocytes. *Circ Res* **98**, 1299-1305 (2006).



Supplementary Fig. 4 Flecainide treatment prevents ventricular tachycardia (VT) during and after treadmill exercise in conscious *Casq2*$^{-/-}$ mice.
Exercise challenge test and telemetric ECGs recordings were carried out as described in **Supplementary Methods**. The inset illustrates a typical bidirectional VT episode. Note that Casq2$^{-/-}$ mice exhibit VT episodes even during normal activity, albeit at a lower rate than during exercise challenge. Flecainide (20 mg kg^{-1}) or vehicle were injected intraperitoneally (i.p.) 15-30 minutes before exercise. Flecainide completely prevented ventricular tachycardia during exercise and for 3 hrs after exercise, and significantly suppressed VT up to 6 hrs after administration. Note that there was no rebound increase of ventricular tachycardia. VT incidence per minute was averaged for the full duration of the treadmill exercise test (10-15 min per mouse) and for each hour after the exercise. Data are mean and s.e.m., N=8 mice, **p<0.01, *p<0.05 compared to vehicle. A single administration of flecainide (20 mg kg^{-1} i.p.) resulted in serum concentrations of 2.5±0.2 μM (1.2±0.08 mg l^{-1}, n=3 mice) one hour after injection, and of 0.02±0.006 μM (0.009±0.003 mg l^{-1}) 14 hrs after injection. Serum concentrations of 0.4 -1 mg l^{-1} are considered within the therapeutic range of flecainide in humans. Heart tissue concentration were 33±0.8 μM (16±0.4 mg kg^{-1} heart tissue), n=3 mice) one hour after injection, and of 0.23±0.11 μM (0.11±0.05 mg kg^{-1} heart tissue) 14 hrs after injection.



Supplementary Fig. 5 Comparison of lidocaine and flecainide treatment in conscious *Casq2*$^{-/-}$ mice. Lidocaine, a Na^+ channel blocker that does not inhibit RyR2 channels, was significantly less effective in suppressing exercise-induced ventricular extrasystoles (VES) than flecainide **(a)**, and did not prevent exercise-induced VT **(b)**, even though QRS duration, which indicates Na^+ channel block (Vaughan Williams 1989), was increased to a similar extent by both drugs **(c,d)**. Exercise challenge test and telemetric ECGs recordings were carried out as described in **Supplementary Methods**. To obtain VES and VT incidence, the full duration of the treadmill exercise test (10-15 min per mouse) was analyzed. Panel **(c)** shows representative QRS complexes before and treadmill exercise. Note the wider QRS complex in presence of flecainide and lidocaine, respectively. For each mouse, QRS duration was measured as described in Casimiro et. al after the completion of the treadmill exercise test. QRS duration was expressed as % of the QRS duration before drug/vehicle injection (control). Data are mean and s.e.m., vehicle N=11 mice, flecainide N=8 mice, lidocaine N=5 mice, ***p<0.001, *p<0.05 by Mann-Whitney test for **(a)**, Fisher-exact test for **(b)**, and *Student's t*-test for **(d).**

Vaughan Williams, E.M. Relevance of cellular to clinical electrophysiology in interpreting antiarrhythmic drug action. *Am J Cardiol* **64**, 5J-9J (1989).

Casimiro, M.C., *et al.* Targeted disruption of the Kcnq1 gene produces a mouse model of Jervell and Lange-Nielsen Syndrome. *Proc Natl Acad Sci U S A* 98, 2526-2531 (2001).

Flecainide Prevents Catecholaminergic Polymorphic Ventricular Tachycardia in Mice and Humans

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Supplementary Methods

Human Studies

Both CPVT patients described in our report were refractory to standard drug therapy and awaiting surgical stellate ganglionectomy, an investigational procedure currently being evaluated for effectiveness in CPVT. Given this therapeutic dilemma, the treating physician (AAMW) elected a therapeutic trial of flecainide, which is an approved anti-arrhythmic drug. Given the lack of clinical data in CPVT, the standard dose for treating atrial fibrillation was used. The patient or the parents provided informed consent, and the study was reviewed and approved by the institutional medical ethical review board of the Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands. Treadmill exercise testing and ICD interrogations were performed as part of routine clinical care. Arrhythmia burden during exercise testing was quantified as previously described.[1]

Animal studies

All studies were approved by the institutional animal care and use committees at Vanderbilt University, and performed in accordance with NIH guidelines. Adult *Casq2*$^{-/-}$ mice (3-5 months old) were used for all experiments[2]. *Casq2*$^{-/-}$ mice consistently develop VT during exercise or after catecholamine challenge.

Single RyR2 Ca^{2+} release channel measurements

SR vesicles containing ryanodine receptor Ca^{2+} release channels were obtained from sheep hearts and were reconstituted into artificial lipid bilayers as previously described [3]. Lipid bilayers were formed from phosphatidylethanolamine and phosphatidylcholine (8:2 wt/wt, Avanti Polar Lipids, Alabaster, AL) in n-decane, (50 mg ml^{-1}, ICN Biomedicals) across an aperture of 150–250 μm diameter in a Delrin cup which separated two baths (*cis* and *trans*). During SR-vesicle incorporation, the *cis* (cytoplasmic) bath contained (in mM) 250 Cs^+ (230 $CsCH_3O_3S$, 20 CsCl), 1.0 $CaCl_2$ and 500 mannitol, while the *trans* (luminal) solution contained 50 Cs^+ (30 $CsCH_3O_3S$, 20 $CsCl_2$) and 1.0 $CaCl_2$. After detection of channels in the bilayer the compositions of the *cis* and *trans* solutions were changed as follows. The [$CsCH_3O_3S$] in the *trans* solution was increased to 230 mM (*i.e*. establishing 250 mM Cs^+ in both *cis* and *trans* baths) by means of aliquot addition of 4 M stock. The *cis* solution was exchanged for one containing 250 mM Cs^+, 2 mM ATP, 0.1 M free Ca^{2+} and the desired flecainide or tetracaine concentration. Rapid solution exchange was performed using a tube (300 m ID) located within 100 m of the bilayer. Flow rates of ~1 l per second produced total solution exchange at the bilayer in <1 s.[4] Free [Ca^{2+}] of 0.1 M was achieved by a combination of 4.5 mM BAPTA and 1 mM $CaCl_2$. Mannitol was obtained from Ajax chemicals and $CaCl_2$ from BDH Chemicals. Solutions were pH-buffered with 10 mM N-tris [Hydroxymethyl]methyl-2-aminoethanesulfonic acid (TES, ICN Biomedicals) and solutions were titrated to pH 7.4 using CsOH (optical grade, ICN Biomedicals) and were redox buffered with 5 mM glutathione (ICN Biomedicals). Flecainide (acetate salt), tetracaine (hydrochloride) and caesium salts were obtained from Sigma Corporation.

Bilayer potential was controlled using an Axopatch 200B amplifier (Axon Instruments). Electrical potentials are expressed using standard physiological convention (*i.e*., cytoplasmic side relative to the luminal side at virtual ground). Single channel recordings were obtained using bilayer potential difference of +40 mV. The current signal was digitized at 5 kHz and low-pass filtered at 1 kHz with a Gaussian digital filter. Open probability (P_o) as well as open and closed durations was measured by the 50% threshold detection method. Analysis was carried out using Channel2 software (P.W. Gage and M. Smith, Australian National University, Canberra). Dwell-time distributions were compiled from

single channel records and displayed using log-binned histograms with 7 bins per decade.[5] This method transforms individual exponential decay components to peaked distributions which are centered on time values that correspond to their decay constants.

Treadmill exercise testing in mice

Exercise testing in conscious mice was carried out as previously described.[2] Briefly, mice were initially anesthetized (pentobarbital, 70 mg g^{-1}) and an electrocardiogram (ECG) transmitter (Data Sciences International, St. Paul, MN) was implanted into the abdominal cavity with subcutaneous electrodes in lead 2 configuration. Animals were allowed to recover for at least 72 h after surgery before participating in the treadmill exercise studies. Flecainide (20 mg kg^{-1}), lidocaine (20 mg kg^{-1}) or vehicle (70%ethanol) was injected intraperitoneally 15-30 minutes before exercise. Mice were placed individually into a special chamber of the motorized rodent treadmill (Exer-6M, Columbus Instruments, Columbus, OH) and exercised until they exhibited signs of exhaustion, as described previously.[2] Exhaustion was defined as the mouse spending more than 50% of the time or more than 15 s consecutively on the shock grid. For each mouse, treadmill testing was performed two times (drug and placebo) at an interval of >72 h. High-quality ECGs were recorded from 15 min before exercise until 20 h after exercise. An analysis program (Dataquest A.R.T. version 2.3, Data Sciences International) was used to review the ECG records during the exercise test and quantify ventricular extrasystoles (VEs) and runs of ventricular tachycardia (>3 consecutive ventricular beats). To analyze arrhythmia incidence during the 20 h recording period, we utilized the multi-purpose MultiJobECG analysis and visualization system (multijobECG@shaw.ca), which applies multi-scale, hierarchical, contextual signal processing (CSP) to high volumes of raw ECG data.[6] Using the MultiJobECG's episode learning library, we defined different spectral classes of ECG episodes (sinus rhythm, VEs, run of ventricular tachycardia); these episodes were then automatically located and displayed on the user-interface for confirmation by an experienced reviewer. Once confirmed, ventricular arrhythmias were categorized, counted and quantified as incidence rate per minute. ECG analysis was performed blinded to the treatment group.

ECG recordings in anesthetized, isoproterenol- treated mice

The effects of flecainide on isoproterenol-induced ventricular arrhythmia were studied in anesthetized *Casq2*$^{-/-}$ mice as previously described.[2] Briefly, mice were anesthetized with isoflurane vapor titrated to maintain the lightest anesthesia as possible. On average, 1.0% vol/vol isoflurane vapor was sufficient to maintain adequate anesthesia. Loss of toe pinch reflex and respiration rate were used to monitor levels of anesthesia. Average respiration rate was not different between placebo and flecainide administration. Baseline ECG was recorded for 5 minutes, followed by an additional 20 minutes after administration of isoproterenol (1.5 mg kg^{-1} i.p.). Flecainide (20 mg kg^{-1} i.p.) or vehicle (70% ethanol) was administered 15 minutes before administration of isoproterenol. Isoproterenol challenge was performed two times in the same mouse at an interval of >72 h, with half the animals receiving first placebo, then flecainide, and the other half receiving first flecainide, then placebo. ECG analysis was performed by an experienced cardiologist blinded to the treatment group.

Myocyte isolation and Ca^{2+} indicator loading

Single ventricular myocytes were isolated by a modified collagenase/protease method as previously described.[7] Ventricular myocytes were incubated with 2 μM fura-2 acetoxymethyl ester (Fura-2 AM) for 8 minutes at room temperature to load the indicator into the cytosol. Myocytes were washed two times for 10 minutes with 1.2 mM Ca^{2+} Tyrode's solution containing 250 μM probenecid to retain the indicator in the cytosol. An additional 30 minutes were allowed for de-esterfication of the indicator before imaging the cells. All the experiments were performed in 2 mM Ca^{2+} Tyrode's solution containing (in mM): NaCl 134, KCl 5.4, $MgCl_2$ 1, glucose 10, and HEPES 10, pH adjusted to 7.4 with NaOH. All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise specified.

Ca^{2+} fluorescence measurements

Ca^{2+} transients were measured using a dual beam excitation fluorescence photometry set-up (IonOptix, Milton, MA) as described previously.[2] Briefly, fura-2 loaded ventricular myocytes were incubated with 6 μM flecainide or vehicle (70% ethanol) for 10 minutes. In an additional set of experiments,

myocytes were incubated for 30 min with flecainide (6 μM), tetracaine (50 μM) or vehicle. After the cells attached to the bottom of the laminin coated chamber, they were superfused with Tyrode's solution containing 2 mM Ca^{2+}, 6 μM flecainide and 1 μM isoproterenol. SR Ca^{2+} content was estimated by measuring the amplitude of a Ca^{2+} transient induced by rapid application of 10 mM caffeine.[8] All experiments were conducted at room temperature (~23 ºC).

The emission at excitation wavelengths of 360 and 380 nm (background subtracted) was used to monitor the fluorescence signals of Ca^{2+}-bound and Ca^{2+}-free Fura-2. Intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ is proportional to the fluorescence ratio at 360 nm and 380 nm excitation.[9] Fura-2 AM compartmentalizes into intracellular organelles,[10] and therefore calculating $[Ca^{2+}]_i$ from Fura-2 fluorescence ratios may not be accurate in intact cells. Thus, $[Ca^{2+}]_i$ measurements are reported as fluorescence ratios (F_{ratio}). Data were analyzed using a specialized analysis software package (IonWizard™, IonOptix).

Analysis of spontaneous SR Ca^{2+} releases and triggered beats

A spontaneous Ca^{2+} release event was defined as a spontaneous increase of 0.07 F_{ratio} (two times the average background noise) or more from the diastolic F_{ratio} as previously described.[11] Spontaneous Ca^{2+} releases and triggered beats were recorded while myocytes were exposed to 2 mM Ca^{2+} and1 μM isoproterenol Tyrode's solution additionally containing 6 μM flecainide, 50 μM tetracaine, or 70% ethanol respectively. To eliminate the effect of trans-sarcolemmal Ca^{2+} or Na^+ fluxes,[11] spontaneous Ca^{2+} releases were recorded in a subset of myocytes bathed for 30s in 0 mM Na^+ and Ca^{2+} Tyrode's solution containing either 6 μM flecainide or vehicle and 1 μM isoproterenol. The 0 mM Na^+ and Ca^{2+} Tyrode's solution contained (in mM): LiCl 134, KCl 5.4, $MgCl_2$ 1, EGTA 1, glucose 10, and HEPES 10, pH adjusted to 7.4 with LiOH.

Measurement of diastolic SR Ca^{2+} leak

Leak was measured as previously described.[2,11] Briefly, quiescent Fura-2 loaded cardiac myocytes were bathed in 0 mM Na^+ and Ca^{2+} Tyrode's solution containing 6 μM flecainide (or vehicle) and 1 μM isoproterenol. After spontaneous Ca^{2+} release frequency reached a steady-state, the external solution was quickly changed to 0 mM Na^+ and Ca^{2+} Tyrode's solution containing 1 mM tetracaine. Tetracaine blocks RyR2 channels and as a result Ca^{2+} shifts from the cytosol into the SR. The tetracaine-induced decline in diastolic Fura-2 fluorescence ratio was used as an estimate of SR Ca^{2+} leak,[2,11] which is insensitive to changes in SR Ca^{2+} uptake.[12] The amplitude of the caffeine-induced Ca^{2+} transient was used as an estimate of total $[Ca^{2+}]_i$, which included the Ca^{2+} leak.

Analysis of [flecainide] in mouse plasma and heart tissue

The concentration of flecainide in plasma and whole heart tissue was measured by reversed phase high performance liquid chromatography with positive electrospray ionization mass spectrometric detection HPLC-MS as previously described[13] with slight modifications. Briefly, 50 l of 20 M verapamil was added to the samples as an internal standard, the tissue homogenized in phosphate buffered saline, protein precipitated with 2 volumes acetonitrile:methanol (85:15), and acidified supernatant loaded on Waters Oasis MCX cartridges. After washing with acidic phosphate buffer and 60% methanol, the drugs were eluted with acetonitrile containing 0.1% triethylamine and analyzed by MS using selective reaction monitoring of the *m/z* 415 ◊ 301 transition for flecainide and the *m/z* 455 ◊ 165 transition for verapamil. The HPLC gradient went from 95% Solvent A (water with 0.1% acetic acid to 90%) initially to 90% Solvent B (acetonitrile) over 5 minutes using a C18 column (Magic Bullet 3A, Michrom BioResources, Auburn, CA). Under these conditions flecainide eluted at 2.7 min and verapamil at 3.0 min. Tissue concentration was calculated as the ratio of peak heights for the sample relative to flecainide calibration standards and normalized to volume of plasma or tissue (1 mg heart tissue assumed to be 1 l.)

Statistical analysis

Unless otherwise stated, data are presented as mean ± s.e.m.. All statistical tests were two-tailed and conducted with SPSS, version 12.0 or 16.0 (SPSS Inc, Chicago, IL). For normally distributed data, differences between groups were assessed using a one-way analysis of variance (ANOVA). If

statistically significant differences were found, individual groups were compared with *Student's t*-test. For non-normally distributed data such as VT incidence rates, Wilcoxon signed ranks test (for paired comparisons) or Mann-Whitney test (for unpaired comparisons) were used. The level of significance is stated in the text.

References

1. Wilde, A.A.*, et al.* Left cardiac sympathetic denervation for catecholaminergic polymorphic ventricular tachycardia. *N Engl J Med* **358**, 2024-2029 (2008).
2. Knollmann, B.C.*, et al.* Casq2 deletion causes sarcoplasmic reticulum volume increase, premature Ca2+ release, and catecholaminergic polymorphic ventricular tachycardia. *J Clin Invest* **116**, 2510-2520 (2006).
3. Laver, D.R.*, et al.* Cytoplasmic Ca2+ inhibits the ryanodine receptor from cardiac muscle. *J Membr Biol* **147**, 7-22 (1995).
4. Laver, D.R. & Curtis, B.A. Response of ryanodine receptor channels to Ca2+ steps produced by rapid solution exchange. *Biophys J* **71**, 732-741 (1996).
5. Sigworth, F.J. & Sine, S.M. Data transformations for improved display and fitting of single-channel dwell time histograms. *Biophys J* **52**, 1047-1054 (1987).
6. Guo, J.*, et al.* Decrease in density of INa is in the common final pathway to heart block in murine hearts overexpressing calcineurin. *Am J Physiol Heart Circ Physiol* **291**, H2669-2679 (2006).
7. Knollmann, B.C.*, et al.* Familial hypertrophic cardiomyopathy-linked mutant troponin T causes stress-induced ventricular tachycardia and Ca2+-dependent action potential remodeling. *Circ Res* **92**, 428-436 (2003).
8. Bers, D.M. Sarcoplasmic reticulum. . in *In Excitation and Contraction Coupling and Cardiac Contractile Force* 161-202 (Kluwer Academic Publishers; 2001, Dordrecht, The Netherlands/Boston, Massachusetts, USA/London, United Kingdom).
9. Grynkiewicz, G., Poenie, M. & Tsien, R.Y. A new generation of Ca2+ indicators with greatly improved fluorescence properties. *J Biol Chem* **260**, 3440-3450 (1985).
10. Williford, D.J., Sharma, V.K., Korth, M. & Sheu, S.S. Spatial heterogeneity of intracellular Ca2+ concentration in nonbeating guinea pig ventricular myocytes. *Circ Res* **66**, 241-248 (1990).
11. Chopra, N., Laver, D., Davies, S.S. & Knollmann, B.C. Amitriptyline Activates Cardiac Ryanodine Channels And Causes Spontaneous Sarcoplasmic Reticulum Calcium Release. *Mol Pharmacol* (2008).
12. Curran, J., Hinton, M.J., Rios, E., Bers, D.M. & Shannon, T.R. Beta-adrenergic enhancement of sarcoplasmic reticulum calcium leak in cardiac myocytes is mediated by calcium/calmodulin-dependent protein kinase. *Circ Res* **100**, 391-398 (2007).
13. Kristoffersen, L.*, et al.* Simultaneous determination of 6 beta-blockers, 3 calcium-channel antagonists, 4 angiotensin-II antagonists and 1 antiarrhythmic drug in post-mortem whole blood by automated solid phase extraction and liquid chromatography mass spectrometry. Method development and robustness testing by experimental design. *J Chromatogr B Analyt Technol Biomed Life Sci* **850**, 147-160 (2007).