Supplementary Methods:

Adult Murine Cardiac Myocyte Dissociation: Isolation of cardiac cardiomyocytes was as in with slight modifications[1]. Nine to 11 week-old male BALB/c mice were anesthetized with pentobarbital. After mechanical ventilation, the aorta was cannulated *in situ* and the heart rapidly excised and retrogradely perfused at 2-3 ml/min (~80-100 mmHg) for 5 min with Tyrode solution (137 mM NaCl, 5.4 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose), followed by 1 min with a 'low-calcium' medium (100 mM NaCl, 10 mM KCl, 1.2 mM KH_2PO_4, 5 mM MgSO4, 20 mM glucose, 50 mM taurine, 10 mM HEPES) supplemented with 0.13 mM $CaCl_2$ and 2.1 mM EGTA. Enzymatic digestion was started by 15 min perfusion with 'low-calcium' medium supplemented with 1% BSA, 0.2 mM $CaCl_2$, collagenase (type IV, 22 U/ml; type II, 7U/ml; Worthington) and pronase (100 µg/ml; Serva). Perfusion solutions were oxygenated by bubbling with 95%/5% O_2/N_2. The left ventricle was then removed, cut into pieces (~3x3 mm) and incubated at 37°C for 5 min in the enzyme solution with gentle stirring. Cardiac myocytes were finally mechanically dissociated by gentle pipetting tissue pieces. Cells were centrifuged at 500 r.p.m. for 1 min and the pellet was washed twice in 'low-calcium' medium supplemented with 0.2 mM $CaCl_2$ ('wash'). Cell pellet was finally suspended in 'wash' and kept at room temperature until use. All solutions were kept at 37ºC with pH 7.3.

HEK Cell culture: Cells were grown in T25 flasks in Minimum Essential Medium, 10% horse serum, non-essential amino acids, sodium pyruvate and penicillin-streptomycin (Invitrogen, Carlsbad, CA). Cells were grown to about 75% confluency. One day prior to the experiments, flasks were transfected with SCN5A encoding hH1 isoforms c1 or c3, and with green fluorescent

protein (GFP) pEGFP-C1 (Clontech, Palo Alto, CA), using Lipofectamine 2000 reagent and OPTI-MEM I reduced serum medium (Invitrogen, Carlsbad, CA). On the morning of experiments, cells were trypsinized and resuspended in Ringers solution and plated onto the recording chamber 15 minutes prior to the initiation of experiments.

Recording Solutions:

Whole cell electrophysiology: The intracellular solution contained (in mM): 125 $CH_3SO_3^-$, 35 Cl^-, 145 Cs^+, 5 Na^+, 5 Mg^{2+}, 5 HEPES, 2 EGTA. pH was adjusted to 7.0 with CsOH. The extracellular solution contained (in mM): 159 Cl^-, 139 Cs^+, 15 Na^+, 4.7 K^+, 2.5 Ca^{2+}, 10 HEPES, and 5.5 glucose. pH was adjusted to 7.4 with NaOH. The predicted whole cell liquid junction potential of 7.4 mV was subtracted during whole cell analysis.

Cell-attached patches: For patch experiments on HEK cells, bath solution was *high-K* for macroscopic recordings and *high-Cs* for single channel recordings. *High-K* solution contained (in mM): 149 K^+, 2.5 Ca^{2+}, 5 HEPES, 5.5 glucose. *High-Cs* solution contained (in mM): 139 Cs^+, 15 Na^+, 4.7 K^+, 2.5 Ca^{2+}, 10 HEPES, 5.5 glucose. The cardiac myocyte bath solution was the "low calcium" solution 100 mM NaCl, 10 mM KCl, 1.2 mM KH_2PO_4, 5 mM MgSO4, 20 mM glucose, 50 mM taurine, 10 mM HEPES. Pipette solution was (in mM) 149 Na^+, 4.7 K^+, 2.5 Ca^{++}, 5 HEPES. $GdCl_3$ (10μM) was added to the pipette solution on the day of experiments, except when ranolazine, QX-314 and pH 5.0 were used in the pipette. This concentration of $GdCl_3$ was previously established to provide sufficient blockade of the endogenous stretch channels, without a significant effect on mechanosensitivity of $Na_V1.5$ channels[2]. For pH 5.0 experiments adjustments were made with NaOH. Osmolality for all solutions was 290 mmol/kg.

Mechanical Activation of Voltage-clamped Currents:

Whole cell electrophysiology with bath flow: After a seal and whole cell access were obtained, control currents were recorded in ranolazine-free solution until a stable baseline was established. Next, an elliptical 0.7 mL bath was flushed with ranolazine-free solution at a rate of 10 mL/min for the duration of the 90 s recording to obtain flow data as a paired control. Then, extracellular solution plus ranolazine (premixed to 50 μM) was washed into the chamber at the conclusion of the flow record. Previous work has shown that mechanical activation of $Na_V1.5$ is reversible after minutes[3]. Therefore, ranolazine solution was allowed to incubate on cells for 10 min before the ranolazine data was recorded. Finally, the bath was flushed with ranolazine solution at 10 mL/min to determine the effect of ranolazine on mechanosensitvity of $Na_V1.5$ by flow. In alternate experiments, ranolazine data were collected first before paired controls 10 min later.

Cell-attached patch with direct stretch by pipette pressure: Due to the mechanical remodeling of patches with prolonged stretch pulses[2] we used a rapid pressure clamp (rise time to -30 mmHg in 5 msec) stretch steps were limited to 100 msec during activation/inactivation steps only. Seal history is important for such experiments[2], therefore we recorded the pressures required for patch formation. Patches were typically formed at <5 mmHg. Patches with seals that were formed at >10 mmHg were discarded. Pressure was stepped to the desired level for a few seconds prior to protocol execution, and then stepped back to 0 mmHg between successive datasets.

For experimental systems that aim to combine voltage- and mechano-clamp, application of pressure to a voltage-clamped membrane patch is the most reproducible stimulus available[4]. Determination of the actual stimulus at the patch is complicated since the geometry is not

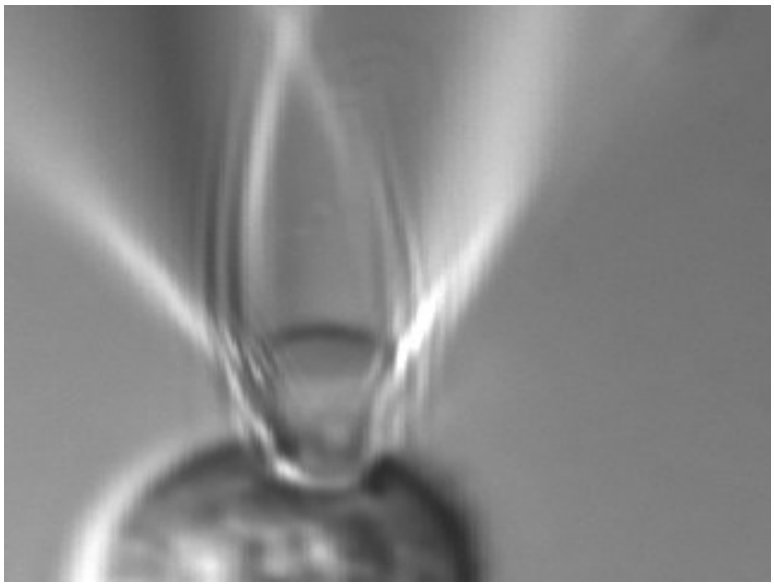
spherical, and the patch anatomy is inhomogeneous and changes in time[4]. Therefore, we focused on differential effects within the same patch[5].

Data Analysis:

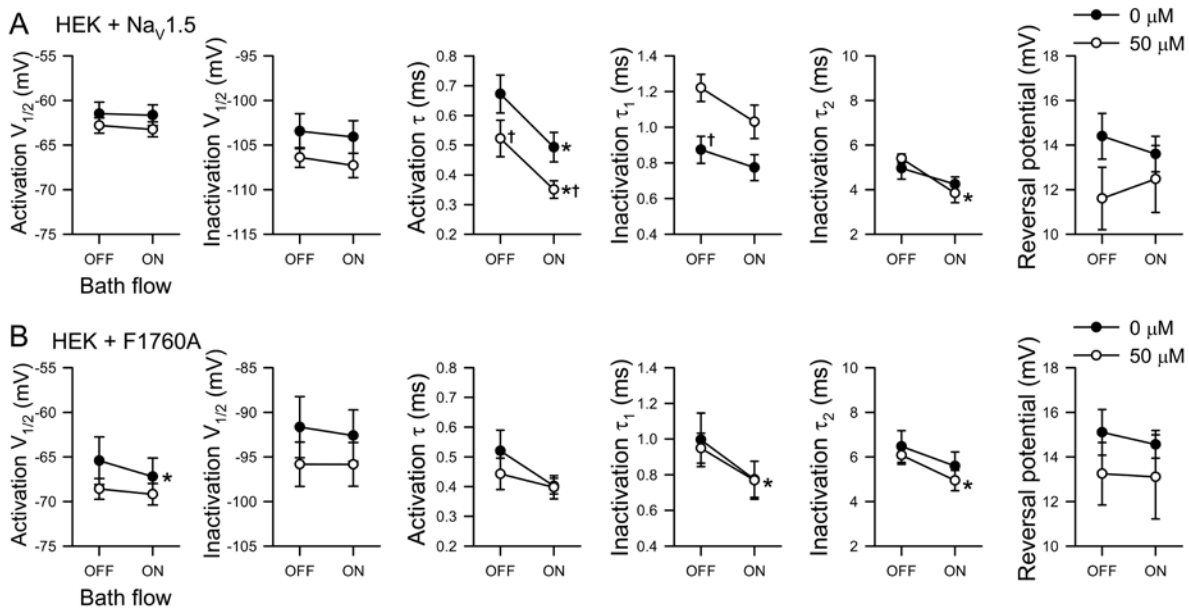
Whole cell electrophysiology: Peak currents during the first series of test pulses determined the voltage dependence of activation, whereas peak currents at test pulse 2 versus the voltages of test pulse 1 determined the voltage dependence of inactivation. Peak currents normalized to the equation $I_{norm}=100(I_V)/I_{peak}$ were fit with a sigmoid 3-parameter curve: $y=1/(1+exp^{((x_0-x)/b)})$, where x_0 is $V_{1/2}$, the voltage of half-activation or half-inactivation. Currents activated over the first 50 ms were fit with a 3-term weighted exponential equation: $f(t)=K_0(\sum f_i exp^{(-t/\tau_i)})$, in which three time constants (τ_i), represent one activation and two inactivation states of $Na_V1.5$. Peak currents are expressed as a fraction of cell capacitance (pA/pF).

Cell-attached patches: Voltage dependence of activation and steady-state inactivation were fit using a two state Boltzmann model ($I=A/[1+exp((V_{1/2}-V)/dV)]$) where normalized peak currents (I) were plotted against applied voltage. Bath exchange time response was fit using an exponential decay function ($I=I_o+Aexp^{(t/\tau)}$), where peak currents from successive steps were plotted against time (t). Two dose-response functions were used. A single dose-response function $y=A_1+(A_2-A_1)/(1+10\text{^}(log(x_0)-x)h)$, where x was ranolazine concentration, A were constants, x_0 was IC_{50} and h was the hill slope. Double dose-response function $y=A_1+(A_2-A_1)[p/(1+10\text{^}(x_{0,1}-x)*h_1)+(1-p)/(1+10\text{^}(x_{0,2}-x)*h_2)]$ was used to obtain two IC_{50} values ($x_{0,1}$ and $x_{0,2}$) and two hill slopes (h_1 and h_2).

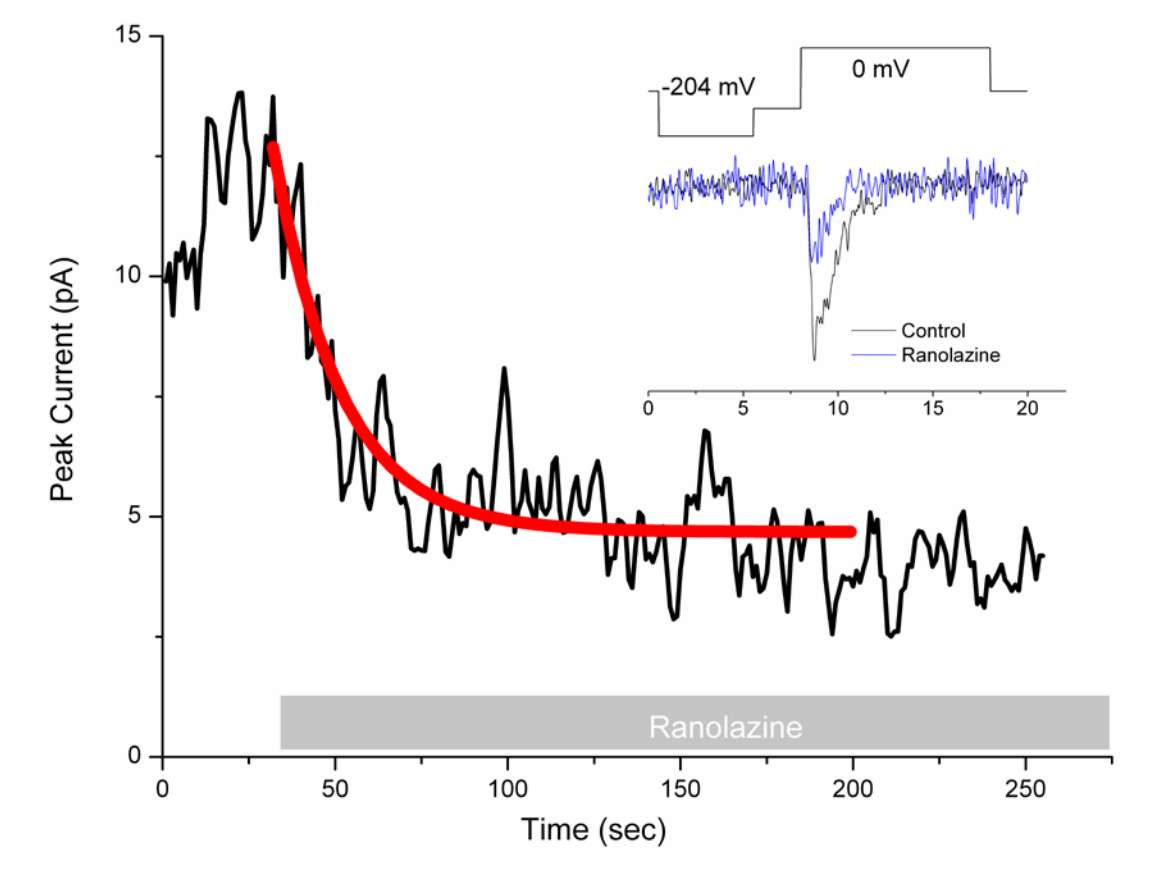
Single channels: First, acquired traces were chopped into segments of desired length. Typical baseline and open channel amplitudes were input into a two-state kinetic model. Log-likelihood minimization was used to idealize the single channel events[6].



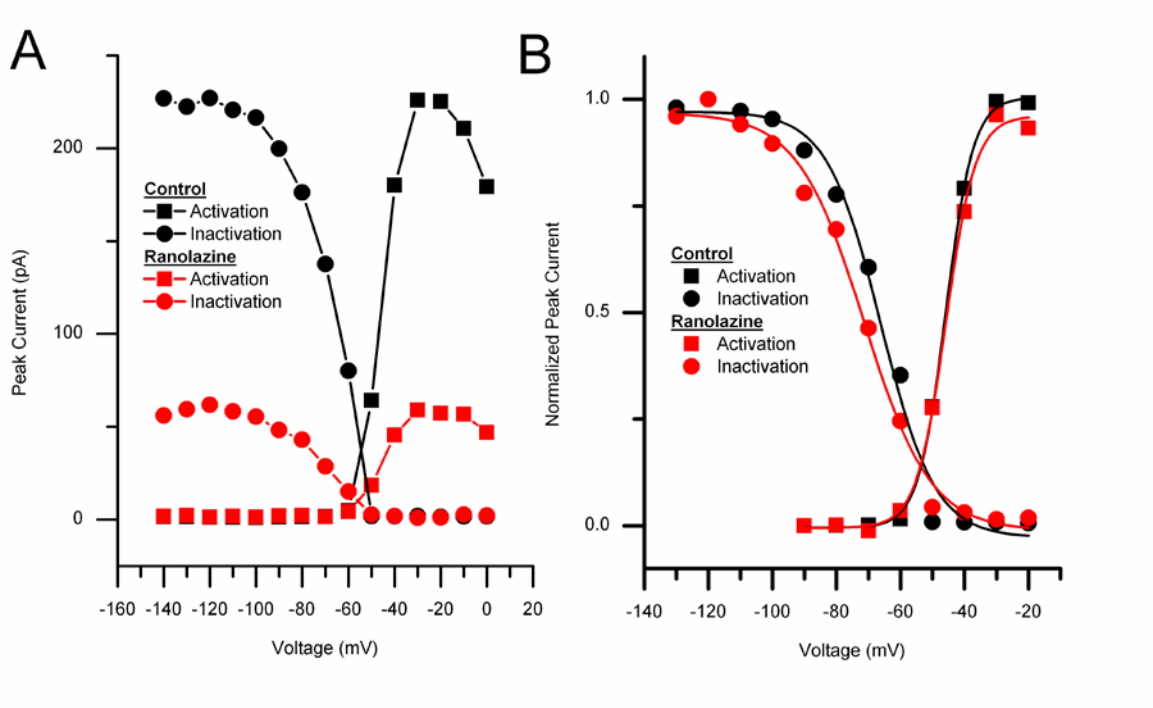
Supplementary Video 1. Negative pressure stretches the patch. Transfected cell was patch-clamped and suction-induced changes were imaged with Olympus IX70 using DIC optics through 60x (NA 0.90) long-working distance objective and recorded as time-sequence images (10 fps) using a CCD camera (Hamamatsu Photonics, Japan). Suction was applied through the side-port of the patch electrode holder. Pressure was stepped in 100 msec intervals from 0 mmHg to -30 mmHg.



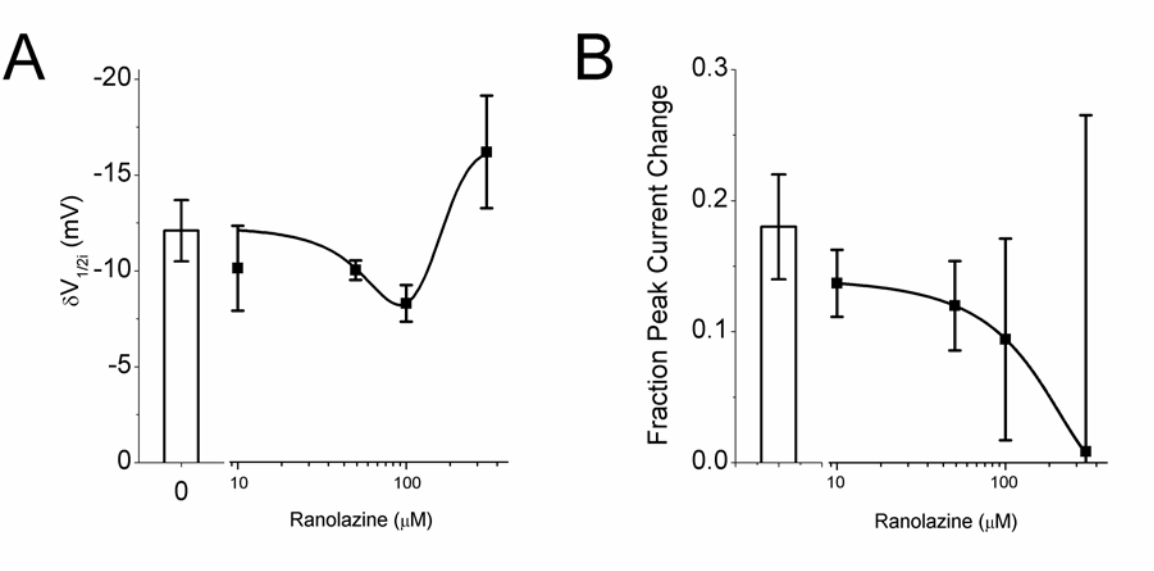
Supplementary Figure 1. Effects of flow of solution with or without ranolazine on the voltage dependence and kinetics of Na^+ channels in HEK293 cells transfected with *SCN5A* F1760 (wild-type $Na_V1.5$) or F1760A. *A-B*, Effects of bath flow at a rate of 0 (*OFF*) or 10 mL/min (*ON*) of solution containing 0 (filled circles) or 50 μM ranolazine (empty circles) on the voltage dependent and kinetic properties of Na^+ channels in HEK293 cells transfected with $Na_V1.5$ F1760 (*A*) or F1760A (*B*). *Columns,* from *left* to *right*, $V_{1/2}$ of steady-state activation, $V_{1/2}$ of steady-state inactivation, time constant (τ) of activation, fast time constant of inactivation ($τ_1$), and slow time constant of inactivation ($τ_2$). (n=6-9; **P*<0.05 compared to 0 mL/min bath flow, †*P*<0.05 compared to 0 μM ranolazine, and *P*>0.05 interaction between bath flow and ranolazine blockade by two-way repeated measures ANOVA with Bonferroni multiple comparisons posttest).



Supplementary Figure 2. Ranolazine can be applied to cell-attached patches using bath exchange. Cell-attached patches were voltage-clamped and stepped at 1Hz to 0 mV from HP=-120 mV and using a -204 mV pre-pulse to recover from inactivation. Peak currents were monitored before and after application of Ranolazine to the bath. In this example, 300 μM Ranolazine was applied to the bath at 30 seconds and peak current reduced by ~7.5 pA within seconds. When fit to an exponential decay function (solid green line) (t from 0 to 200 sec), τ was 19.4±1.6 sec. Average time constant (τ) was 20.7±5.5 sec (n=5). Bath exchange with control solution did not produce such changes.



Supplementary Figure 3. A typical cell-attached patch with and without ranolazine (100 μM). Peak current versus voltage for activation (squares) and steady-state inactivation (circles) were obtained at rest (0 mmHg) without ranolazine (black traces) and with 100 μM ranolazine (red traces). *A*, peak current decreased from 228 pA for control to 59 pA for ranolazine. *B*, voltage dependence of activation did not change from $V_{1/2a}$ of -46 mV for control to -46 mV for ranolazine, while voltage dependence of steady-state inactivation $V_{1/2i}$ was hyperpolarized from -66 mV to -71 mV. Slopes of activation (dV_a) changed from 4.3 mV for control to 4.6 mV for ranolazine and inactivation (dV_i) from 8.0 mV for control to 10.0 mV for ranolazine.



Supplementary Figure 4. Scatter plots (n≥3 per data point) showing ranolazine concentration versus shift in the voltage-dependence of inactivation ($\Delta V_{1/2i}$) (*A*), and fractional change in peak current (I_{30}/I_0) (*B*). Solid lines are data fits using single dose-response functions (*B*), and biphasic dose-response (*A*).

A) The shift of voltage-dependence of inactivation ($\Delta V_{1/2i}$) in response to ranolazine was biphasic. At the smaller concentrations of ranolazine (10 – 100 μM), we observed a decrease in $\Delta V_{1/2i}$ of similar amplitude to $\Delta V_{1/2a}$. However, at the highest concentration tested (300 μM), the shift in $V_{1/2i}$ exceeded that for the controls. This behavior was fit using a biphasic dose-response function, with IC_{50}s of $x_{0,1}$=60.0 and $x_{0,2}$=120.0 μM and hill slopes of h_1=0.02 and h_2=-0.01. The two IC_{50} values match the mechanically sensitive changes of $V_{1/2a}$ ($x_{0,1}$) above and previously published IC_{50}=135 μM for peak current inhibition ($x_{0,2}$)[7]. A possible explanation is that, the biphasic effect is due to the fact that ranolazine stabilizes inactivation independently of pressure, and at high concentrations these effects overcome those of mechano-inhibition (see Supplementary Figure 3 above).

B) Analysis of peak current decrease with pressure was complicated by the very small residual current at high ranolazine concentrations and the variability of response. Peak current concentration-response was fit to a dose-response function, yielding IC_{50} of 123.1 μM and a hill slope of h=-0.005.

REFERENCES

1. Hodgson DM, Zingman LV, Kane GC, Perez-Terzic C, Bienengraeber M, Ozcan C, Gumina RJ, Pucar D, O'Coclain F, Mann DL, Alekseev AE, Terzic A. Cellular remodeling in heart failure disrupts k(atp) channel-dependent stress tolerance. *Embo J.* 2003;22:1732-1742
2. Morris CE, Juranka PF, Lin W, Morris TJ, Laitko U. Studying the mechanosensitivity of voltage-gated channels using oocyte patches. *Methods Mol. Biol.* 2006;322:315-329
3. Saito YA, Strege PR, Tester DJ, Locke GR, 3rd, Talley NJ, Bernard CE, Rae JL, Makielski JC, Ackerman MJ, Farrugia G. Sodium channel mutation in irritable bowel syndrome: Evidence for an ion channelopathy. *Am J Physiol Gastrointest Liver Physiol*. 2009;296:G211-218
4. Suchyna TM, Markin VS, Sachs F. Biophysics and structure of the patch and the gigaseal. *Biophysical journal*. 2009;97:738-747
5. Opsahl LR, Webb WW. Lipid-glass adhesion in giga-sealed patch-clamped membranes. *Biophysical Journal*. 1994;66:75-79
6. Qin F, Auerbach A, Sachs F. Maximum likelihood estimation of aggregated markov processes. *Proc Biol Sci*. 1997;264:375-383
7. Fredj S, Sampson KJ, Liu H, Kass RS. Molecular basis of ranolazine block of lqt-3 mutant sodium channels: Evidence for site of action. *British Journal of Pharmacology*. 2006;148:16-24