SI Methods

Electrophysiological Characterization and Analysis.

Ca^{2+} currents and action potentials were recorded with the ruptured whole-cell patch-clamp technique in voltage clamp or current clamp mode respectively. For Ca^{2+} current recordings the patch pipettes (borosilicate glass, Harvard apparatus LTD) had resistance of 1.5 – 3 MΩ when filled with (mM): 145 Cs-aspartate, 2 $MgCl_2$, 10 HEPES, 0.1 Cs-EGTA, 2 Mg-ATP and 0.2 Fluo-4 pentapotassium salt to record Ca^{2+} transients in AP-clamp mode (pH 7.4 with CsOH). The extracellular bath solution contained (mM): 10 $CaCl_2$, 145 tetraethylammoniumchloride, 10 HEPES (pH 7.4 with TEA). Recordings were made with an Axopatch 200A amplifier (Axon Instruments Inc, Foster City, CA). Data acquisition and command potentials were controlled by pClamp software (version 8.0 Axon Instruments).

For current clamp experiments the patch pipette (borosilicate glass, Harvard apparatus LTD) had resistance of 2 - 3.5 MΩ when filled with (mM): 120 potassium aspartate; 8 KCl; 7 NaCl; 1 $MgCl_2$; 10 Hepes; 5 Mg-ATP; 0.3 Na-GTP; and 0.2 Fluo-4, pentapotassium salt (pH 7.2 with KOH). The bath solution was a modified Ringer solution containing (mM): 145 $NaCl_2$; 5 KCl; 1 $MgCl_2$; 10 Hepes; 2 $CaCl_2$ (pH = 7.4 with NaOH). Liquid-junction potentials were corrected before the recording started. APs were triggered at 0.5 Hz by 2ms long square pulses at amplitudes above the threshold, and the 20^{th} AP was selected for analysis. Recordings were made with an Axoclamp 2B amplifier (Axon Instruments Inc, Foster City, CA) controlled by pClamp software (version 10.0 Axon Instruments). The duration of the AP was measured at AP/2.

The current-voltage dependence was fitted according to:

$$I = G_{\max} \cdot (V - V_{rev}) / 1(1 + \exp(-(V - V_{1/2}) / k)) \quad (1)$$

where, G_{max} is the maximum conductance of the L-type Ca^{2+} channels, V_{rev} is the extrapolated reversal potential of the Ca^{2+} current, $V_{1/2}$ is the potential for half maximal conductance and k is the slope.

The voltage dependence of the Ca^{2+} conductance was fitted according to a Boltzmann distribution:

$$G = G_{\max} / (1 + \exp(-(V - V_{1/2}) / k)) \quad (2)$$

The voltage dependence of the steady-state inactivation curves was fitted with a modified Boltzmann equation:

$$G = (1 - G_{\max}) / (1 + \exp((V - V_{1/2}) / k)) + G_{\max} \quad (3)$$

The time dependence of recovery from inactivation was fitted with a single exponential rise to maximum function:

$$A = A_{\max} \cdot (1 - \exp(-\tau \cdot t)) \quad (4)$$

where A_{max} is the maximum recovery amplitude and τ is the recovery rate.

The kinetic properties of the Ca^{2+} currents were determined by fitting the entire duration of the maximum sweep with a triple exponential function:

$$I = A_{fast} \cdot (\exp(-t / \tau_{fast})) + A_{slow} \cdot (\exp(-t / \tau_{slow})) + A_{inactivation} \cdot (\exp(-t / \tau_{inactivation})) + C \quad (5)$$

where I is the current, A_{fast}, A_{slow}, and $A_{inactivation}$ are the individual current amplitudes, while τ_{fast}, τ_{slow}, and $\tau_{inactivation}$ are their specific time constants.

Simulating Experimentally Determined Effects of $\alpha_2\delta$-1 siRNA Depletion with a Computer Model for Cardiac Ventricular Myocytes.

Here we simulated the effects of $\alpha_2\delta$-1 siRNA treatment on cardiac function by introducing the relative changes of the affected current parameters into the model as described below. The alternative approach of introducing the absolute mean values from our experiments was also tested and essentially produced the same effects on the action potential and Ca^{2+} transients. However, due to the generally slow current kinetics of the experimentally determined currents, the model became instable during longer simulations. Therefore the more conservative approach of simulating the relative changes is the method of choice.

The difference in the mean value of half-maximal activation potential calculated for control and $\alpha_2\delta$-1 siRNA conditions were directly inserted into the equation (where *d* is the voltage dependence of activation):

$$\bar{d} = \frac{1}{1+e^{-\frac{v+14.5}{6}}},\ \bar{d}_{(m)} = \frac{1}{1+e^{-\frac{v+5.6}{6}}} \tag{6}$$

Effects of $\alpha_2\delta$-1 depletion on the time constants of inactivation were simulated by introducing the relative changes into the equation:

$$\tau_f = \left(\frac{1}{\left(0.0197\cdot e^{-(0.0337\cdot(v+14.5))^2}\right)+0.02}\right),\ \tau_{f(m)} = \left(\frac{1}{\left(0.0197\cdot e^{-(0.0337\cdot(v+14.5))^2}\right)+0.02}\right)/0.5025 \tag{7}$$

The computer model (1) describes the activation of Ca^{2+} currents in normal cardiac myocytes with a single time constant. However, in our experimental system the Ca^{2+} currents were best described by two activation components. Upon depletion of $\alpha_2\delta$-1 the

time constants remain unchanged while the ratio of slow to fast components changed from 20:80 to 46:54, respectively. In order to convert these changes to a corresponding change of the single component in the computer model, the following calculation was performed:

$$\tau_{average} = \tau_{fast} \cdot AFC + \tau_{slow} \cdot ASC \quad (8)$$

where:

$\tau_{average}$ = average time constant

τ_{fast}, τ_{slow} = time constant of fast and slow components

AFC = the contribution of the fast component (A_{fast}) to the total current

ASC = the contribution of the slow component (A_{slow}) to the total current

The following mean values for τ_{average} were obtained for the control and $\alpha_2\delta$-1 siRNA conditions by calculating τ_{average} for each recording and then forming the mean:

Table 1. Kinetic properties of activation.

Experimental condition	τ_{fast}	τ_{slow}	*AFC*	*ASC*	$\tau_{average}$
control-siRNA	3.3 ± 0.3	14.5 ± 2.2	80	20	5.3 ± 0.6
$\alpha_2\delta$-1-siRNA	4.3 ±0.5	16.1 ±1.5	54	46	9.1 ± 0.7

According to this calculation the observed kinetic changes correspond to an increase of $\tau_{average}$ by 40.63 % in the $\alpha_2\delta$-1 siRNA condition. Therefore the term for the time constant of activation in the mode for $\alpha_2\delta$-1 siRNA was divided by 0.4063.

$$\tau_d = \left(\frac{\bar{d}\left(1 - e^{-\frac{v+14.5}{6}}\right)}{0.035 \cdot (v+14.5)} \right), \quad \tau_{d(m)} = \left(\frac{\bar{d}_{(m)}\left(1 - e^{-\frac{v+14.5}{6}}\right)}{0.035 \cdot (v+14.5)} \right) / 0.4063 \qquad (9)$$

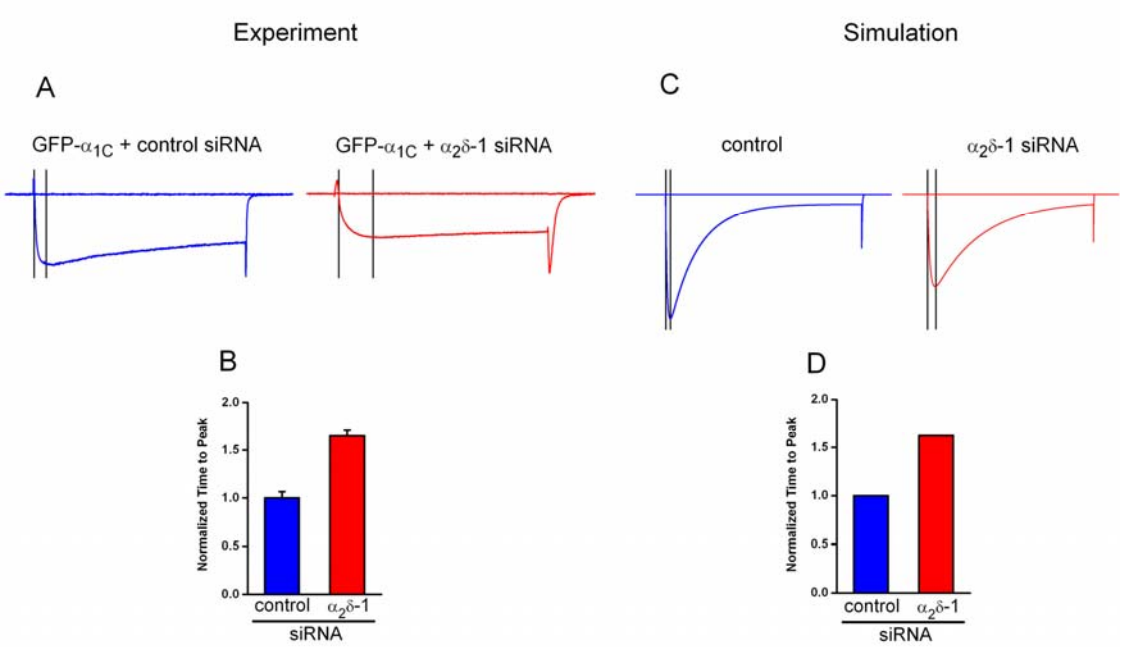
Figure 1 (below) shows that modifying the term for the single activation time constant according to this procedure appropriately describes the behavior of the experimental data, although a single component fit of activation would not provide the mechanistic explanation of the $\alpha_2\delta$-1 effects. The modeled double exponential fit (red line) was calculated using the equation:

$$I = A_{total} \cdot \left(\exp\left(-t / \tau_{average}\right)\right) + A_{inactivation} \cdot \left(\exp\left(-t / \tau_{inactivation}\right)\right) + C \qquad (10)$$

where: $A_{total} = A_{slow} + A_{fast}$, while $\tau_{average}$ was determined using the equation (8) and using the time constants and amplitude contribution determined for this example recording.

SI Fig. 8. Comparison of the experimental data (open circles), the fit of the data with a triple exponential function (blue line), and the modeled double exponential fit (red line).

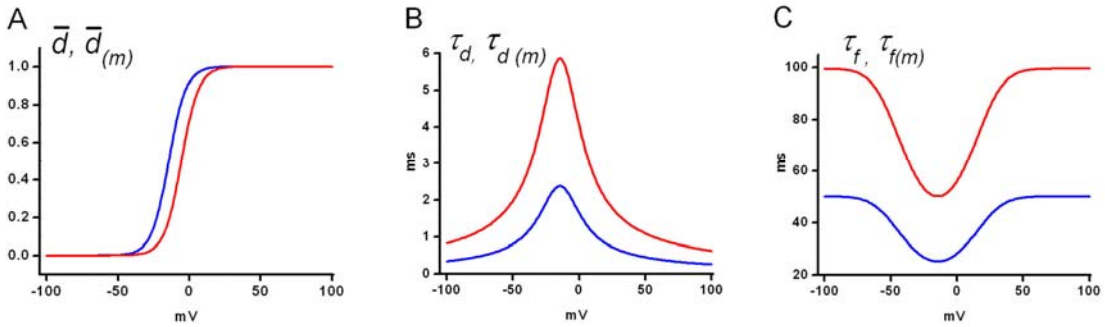
Figure 2 (below) compares the experimentally recorded Ca^{2+} currents with the modeled Ca^{2+} currents running the computer model (1) under voltage clamp conditions. The currents recorded in dysgenic mouse myotubes expressing rabbit GFP-α_{1C} and $\alpha_2\delta$-1 siRNA showed a 65% increase of time-to-peak compared to the currents in myotubes transfected with GFP-α_{1C} and control siRNA ($p<0.001$).



SI Fig. 9. Comparison of experimental and simulated effects of $\alpha_2\delta$-1 siRNA depletion on current kinetics (time-to-peak). (*A*) Maximum sweep for the representative currents recorded from control and $\alpha_2\delta$-1 depleted myotubes. Vertical lines indicate the time interval of time-to-peak. (*B*) By depleting the $\alpha_2\delta$-1 subunit the time-to-peak was increased by 65%. (*C*) Maximum sweeps of the modeled Ca^{2+} currents. (*D*) Modifying the time constants of activation and inactivation resulted in a similar increase of time-to-peak.

Although the overall kinetics of the currents in the rabbit ventricular myocyte model differed, modifying the time constants of activation and inactivation in the computer model resulted in a similar 62% increase of time-to-peak indicating that the modifications made in the model closely resemble the effects of $\alpha_2\delta$-1 depletion observed in the experiments.

Figure 3 (below) demonstrates the modified Ca^{2+} current components .



SI Fig. 10. Parameter for the Ca^{2+} current I_{CaL}. The graphs show the modeled voltage dependence of activation, and the time constants of activation and inactivation for the control- (blue) and $\alpha_2\delta$-1 siRNA (red; *m*) condition. (*A*) Steady-state activation ($\bar{d}, \bar{d}_{(m)}$). (*B*) Activation time constants. (*C*) Inactivation time constants.

The cumulative effects of the altered voltage-dependence and current kinetics caused a decrease in current density. In order to recapitulate the experimentally determined values an additional reduction of current density in the model was necessary:

$$I_{CaL(m)} = I_{CaL} \cdot 0.83 \qquad (11)$$

1. Shannon TR, Wang F, Puglisi J, Weber C, & Bers DM (2004) *Biophys J* **87,** 3351-3371.