Supplemental material

Morales et al., https://doi.org/10.1085/jgp.201812236

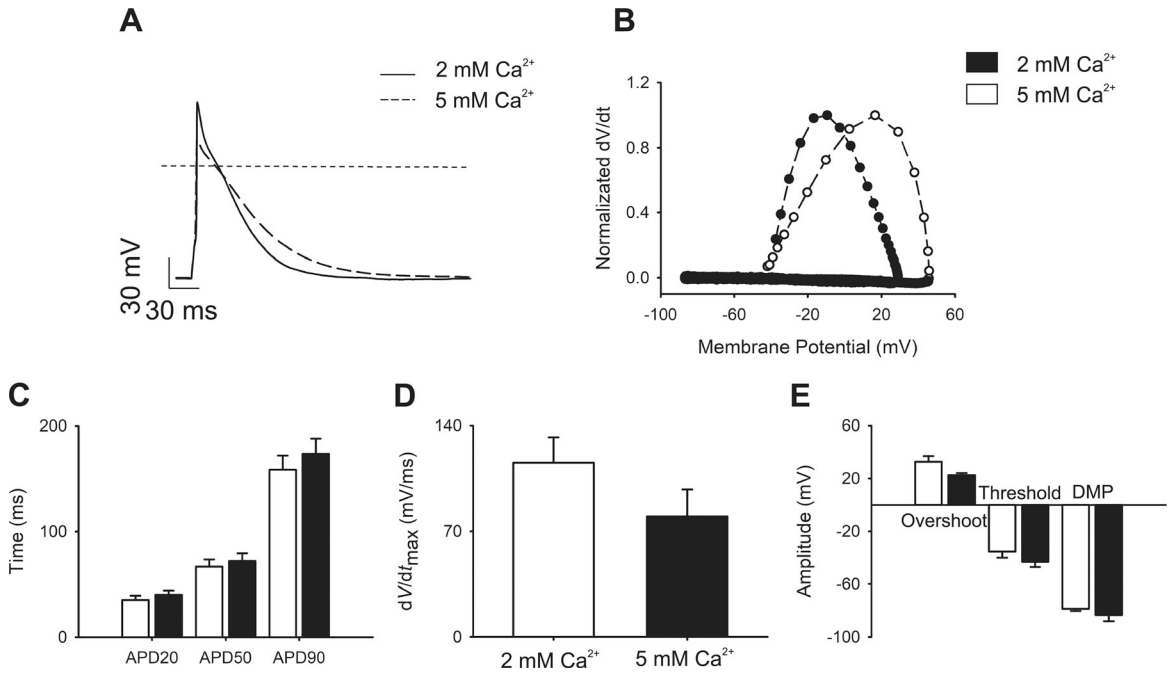

Figure S1. **APs from cardiomyocytes with high external calcium. (A)** Representative AP waveforms from a cardiomyocyte in the presence of 2 mM (solid line) or 5 mM (dashed line) external Ca^{2+}. APs were elicited with short (2–5 ms) depolarizing current injections (100–200 pA) at a 1-Hz frequency. The horizontal line represents zero level. **(B)** Phase plot of the normalized first derivative of membrane potential (dV/dt) against membrane potential (V_m) for the APs shown in A. Controls are represented by empty symbols, and isoproterenol-treated cardiomyocytes are represented by filled symbols. **(C)** Bar graph (mean ± SEM) of overshoot, threshold potentials, and mean resting membrane potentials. **(D)** Bar graph (mean ± SEM) of maximum rate of potential change (dV/dt_{max}). **(E)** Bar graph (mean ± SEM) of APD estimated at 20% (APD_{20}), 50% (APD_{50}), and 90% (APD_{90}) repolarization. For each bar graph, empty bars represent cardiomyocytes at 2 mM external Ca^{2+} and hatched bars, cardiomyocytes at 5 mM Ca^{2+} (n = 10).

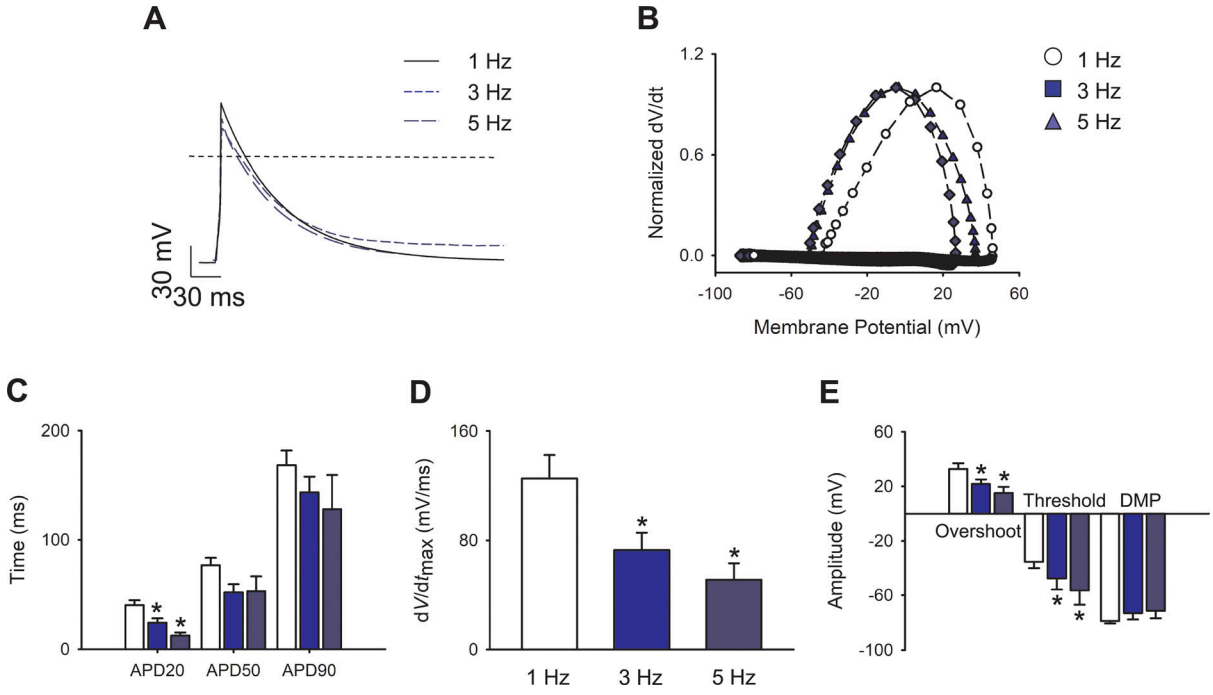

Figure S2. **APs from cardiomyocytes with different stimulation frequency. (A)** Representative AP waveforms from a cardiomyocyte stimulated at a frequency of 1 Hz (solid line), 3 Hz (blue dashed line), or 5 Hz (gray dashed line). APs were elicited with short (2–5 ms) depolarizing current injections (100–200 pA) at a 1-Hz frequency. The horizontal line represents zero level. **(B)** Phase plot of the normalized first derivative of membrane potential (dV/dt) against membrane potential (V_m) for the APs shown in A. Cardiomyocytes stimulated at 1 Hz are shown with empty symbols, at 3 Hz with blue symbols, and at 5 Hz with gray symbols. **(C)** Bar graph (mean ± SEM) of APD estimated at 20% (APD_{20}), 50% (APD_{50}), and 90% (APD_{90}) repolarization. **(D)** Bar graph (mean ± SEM) of maximum rate of potential change (dV/dt_{max}). **(E)** Bar graph (mean ± SEM) of overshoot, threshold potentials, and mean resting membrane potentials. For each bar graph, empty bars represent cardiomyocytes at 1-Hz frequency stimulation; blue bars represent cardiomyocytes at 3-Hz frequency stimulation; and gray bars represent cardiomyocytes at 5-Hz frequency stimulation (n = 10; *, P < 0.01, with respect to 2 mM Ca^{2+}).

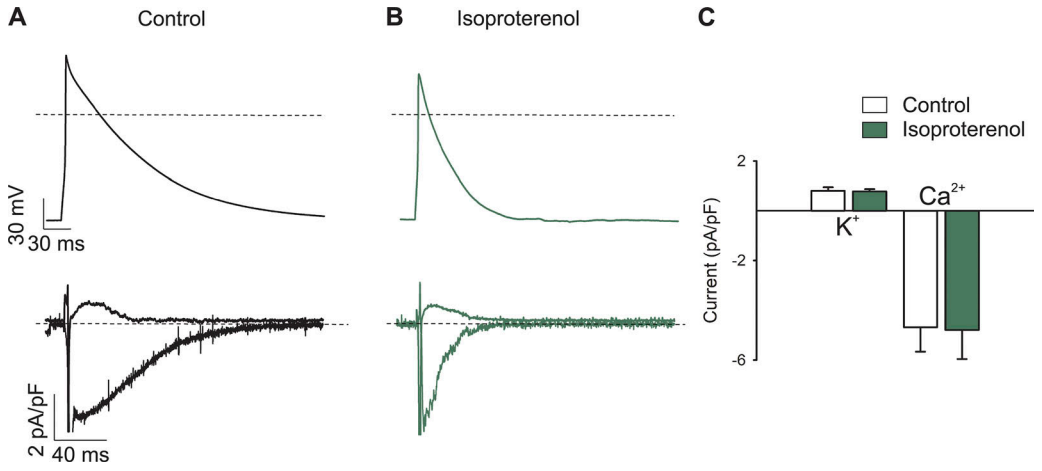

Figure S3. **Calcium-dependent potassium currents and L-type calcium currents during an AP. (A and B)** Upper panels depict representative AP waveforms from a control cardiomyocyte (A) or one treated with 100 nM isoproterenol (B). Bottom panels represent the respective calcium-dependent potassium currents (outward currents) and the L-type calcium currents. The horizontal line represents zero level. **(C)** Bar graph (mean ± SEM) of maximal current normalized by cell capacitance (pA/pF). Empty bars represent control cardiomyocytes and hatched bars represent cardiomyocytes treated with 100 nM isoproterenol (n = 4).

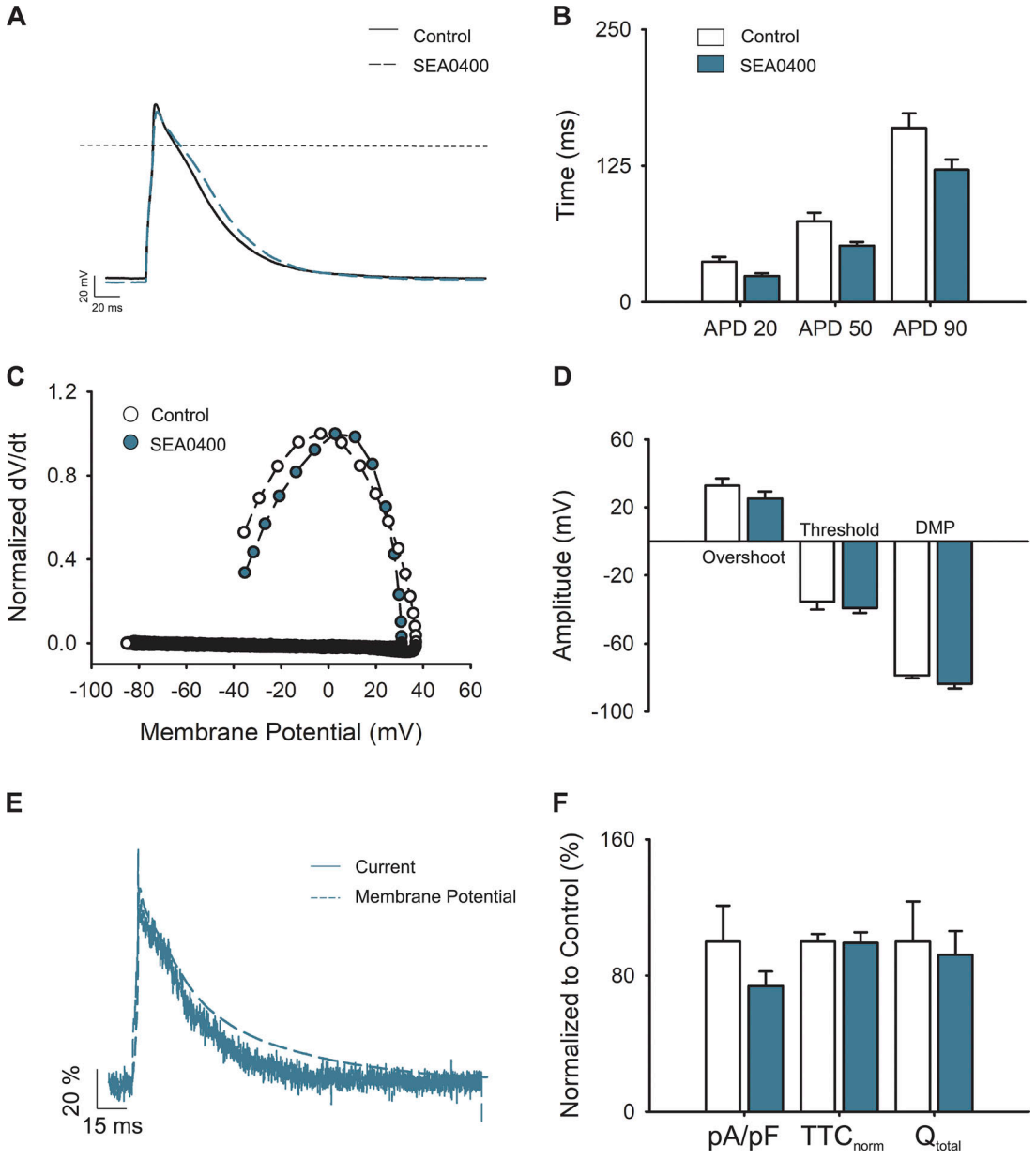

Figure S4. **Role of NCX on APs and L-type calcium currents in newborn rat cardiomyocytes. (A)** Representative AP waveforms from a cardiomyocyte control (solid line) or with an inhibitor of NCX (1 µM SEA0400; dashed light blue line). APs were elicited with short (2–5 ms) depolarizing current injections (100–200 pA) at a 1-Hz frequency. **(B)** Bar graph (mean ± SEM) of APD estimated at 20% (APD_{20}), 50% (APD_{50}), and 90% (APD_{90}) repolarization. **(C)** Phase plot of the normalized first derivative of membrane potential (dV/dt) against membrane potential (V_m) for the APs shown in A. Cardiomyocyte controls are shown with empty symbols and cardiomyocytes treated with 1 µM SEA0400 with light blue symbols. **(D)** Bar graph (mean ± SEM) of overshoot, threshold potentials, and mean DMPs. **(E)** Representative nifedipine-sensitive current (solid line) elicited by the AP (dashed line) prerecorded from the same cardiomyocyte treated with 1 µM SEA0400. **(F)** Bar graph (mean ± SEM) of maximal current normalized by cell capacitance (pA/pF), total time of nifedipine-sensitive current normalized by its APD (TTC_{norm}), and the total current integral normalized by cell capacitance (Q_{total}). Data are presented as percentages with respect to cardiomyocyte controls. For each bar graph, empty bars represent cardiomyocyte controls and light blue bars represent cardiomyocytes treated with 1 µM SEA0400 (n = 5; *, P < 0.01 with respect to control).

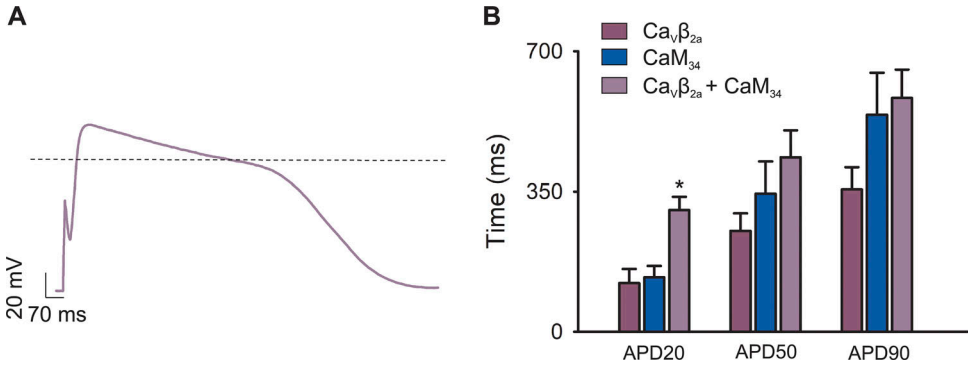

Figure S5. **Complementary role of VDI and CDI on APs. (A)** Representative AP waveform from cardiomyocytes overexpressing $Ca_V\beta_{2a}$ and CaM_{34}. APs were elicited with short (2–5 ms) depolarizing current injections (100–200 pA) at a 1-Hz frequency. **(B)** Bar graph (mean ± SEM) of APD estimated at 20% (APD_{20}), 50% (APD_{50}), and 90% (APD_{90}) repolarization for cardiomyocytes overexpressing $Ca_V\beta_{2a}$ (light red bars), CaM_{34} (blue bars), or both (light purple bars). n = 7; *, $P < 0.01$, one-way ANOVA.