Supplemental Methods.

**Cell population analysis:**

For the experiments described in Fig. 1, viral transduction efficiency was approximately 50% and transduced and untransduced cells could not be readily determined in our myocyte $Ca^{2+}$ measurement system. Consequently, contributions of non-expressing AVM to the aggregate cell population would likely result in an underestimation of the contribution of PLCε expression to Iso and cpTOME responsiveness. Population analysis of PLCε$^{-/-}$ myocytes transduced with wild type PLCε shows that a significant fraction of the cells had responses to Iso (Supplemental Fig. 1A top panel) or cpTOME (Supplemental Fig. 1B top panel) that were greater than YFP transduced cells. However, Iso- and cpTOME-treated cells transduced with each of the mutant PLCε viruses showed very few cells with responses greater than YFP transduced cells (Supplemental Fig. 1A and B bottom panels). These results further indicate that PLCε-Rap-GEF activity, Rap-binding to the RA2 domain and PLC hydrolytic activity are all required for PLC dependent effects on CICR.

**Supplemental Figure Legends**

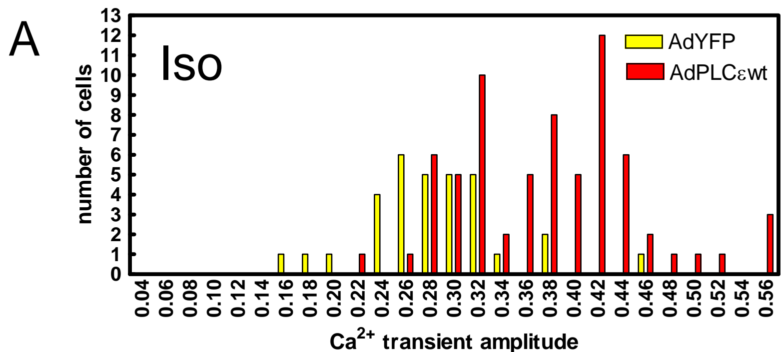
**Supplemental Figure 1.** A) (*upper panel*) Distribution of $Ca^{2+}$ transient amplitudes for all PLCε$^{-/-}$ cells transfected with either YFP or wtPLCε expressing adenoviruses and treated with isoproterenol. (*lower panel*) Distribution of $Ca^{2+}$ transient amplitudes in all PLCε$^{-/-}$ cells transfected with either YFP or various mutant PLCε expressing adenoviruses as indicated and treated with isoproterenol. B) Same as A except stimulated with cpTOME.

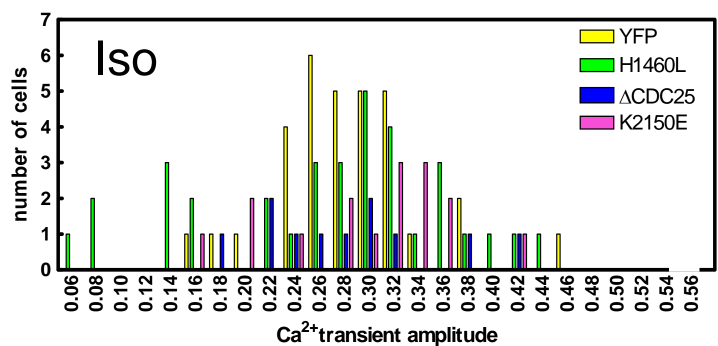
**Supplemental Figure 2.** Representative fluorescence (*left*) and phase (*right*) micrographs of Cy3-labeled siRNA transfected myocytes (*upper*) and mock transfected (*lower*) myocytes.

**Supplemental Figure 3.** Representative L-type $Ca^{2+}$ currents (*lower black traces*) and intracellular $Ca^{2+}$ transients (*upper red traces*) in myocytes from PLCε$^{+/+}$ (A and B) and PLCε$^{-/-}$ mice in both the absence (A and C) and presence (B and D) of 1μM isoproterenol. $Ca^{2+}$ currents and transients were elicited by 200 ms depolarizations to test potentials to -50 mV, -20 mV, 0 mV, 30 mV, and 50 mV. The kinetics of $Ca^{2+}$ current inactivation for test potentials to 0 mV was fitted by a single exponential function (*green solid lines*). The time constant of inactivation was not significantly different ($p>0.3$) between PLCε$^{+/+}$ ($\tau_{inact} = 37.5 \pm 1.9$ ms) and PLCε$^{-/-}$ ($\tau_{inact} = 34.9 \pm 1.9$ ms) myocytes under basal conditions. However, in the presence of 1μM isoproterenol, the time constant of inactivation was significantly higher in PLCε$^{-/-}$ myocytes compared to PLCε$^{+/+}$ (PLCε$^{+/+}$: $\tau_{inact} = 19.0 \pm 1.2$ ms; PLCε$^{-/-}$: $\tau_{inact} = 24.6 \pm 1.3$ ms, $p<0.01$). The scale bars shown in D apply to all panels.

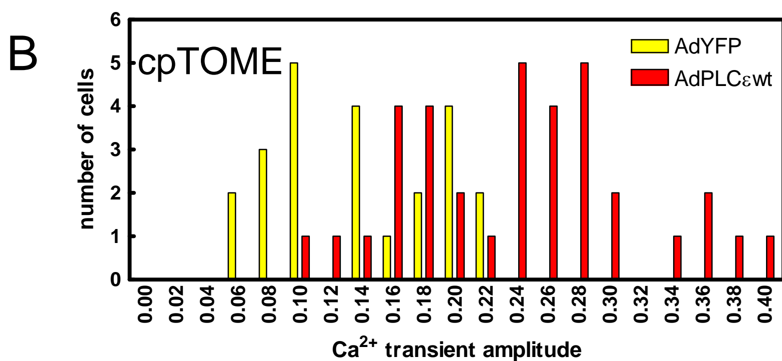
**Supplemental Figure 4.** Diagram depicting the Epac/PLCε pathway.

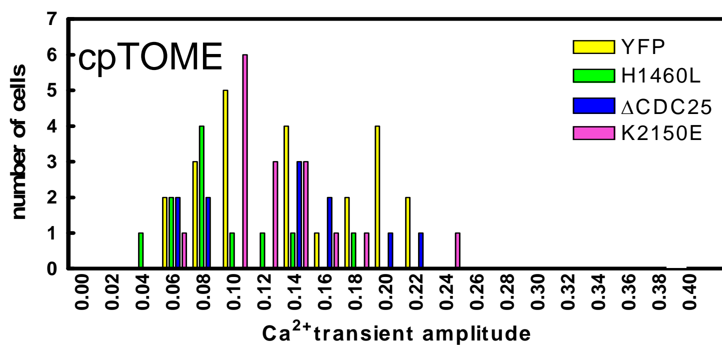
A



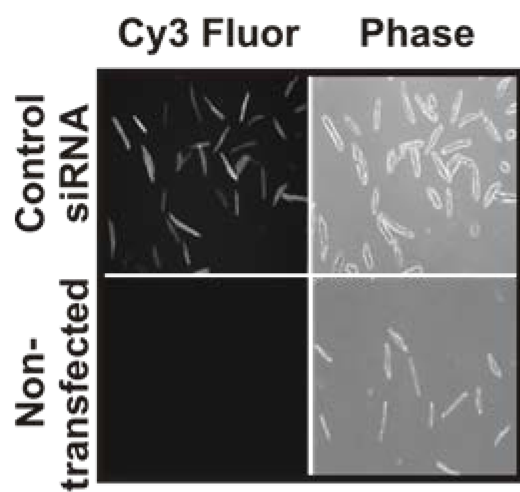




B

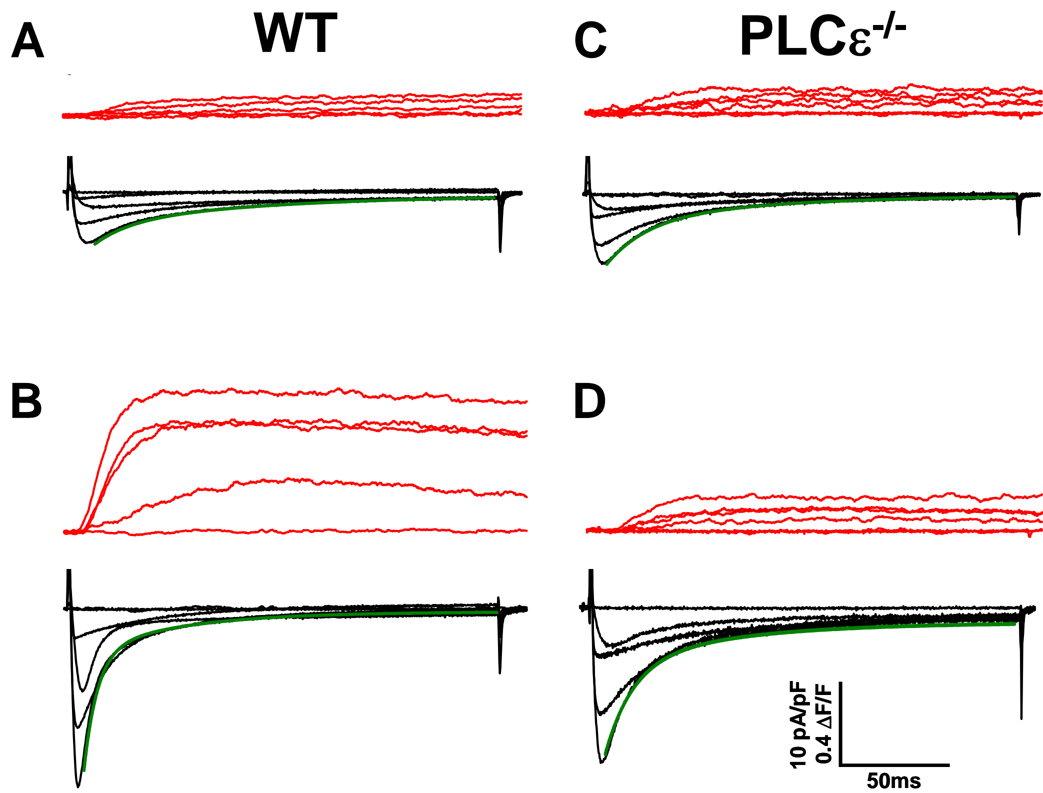


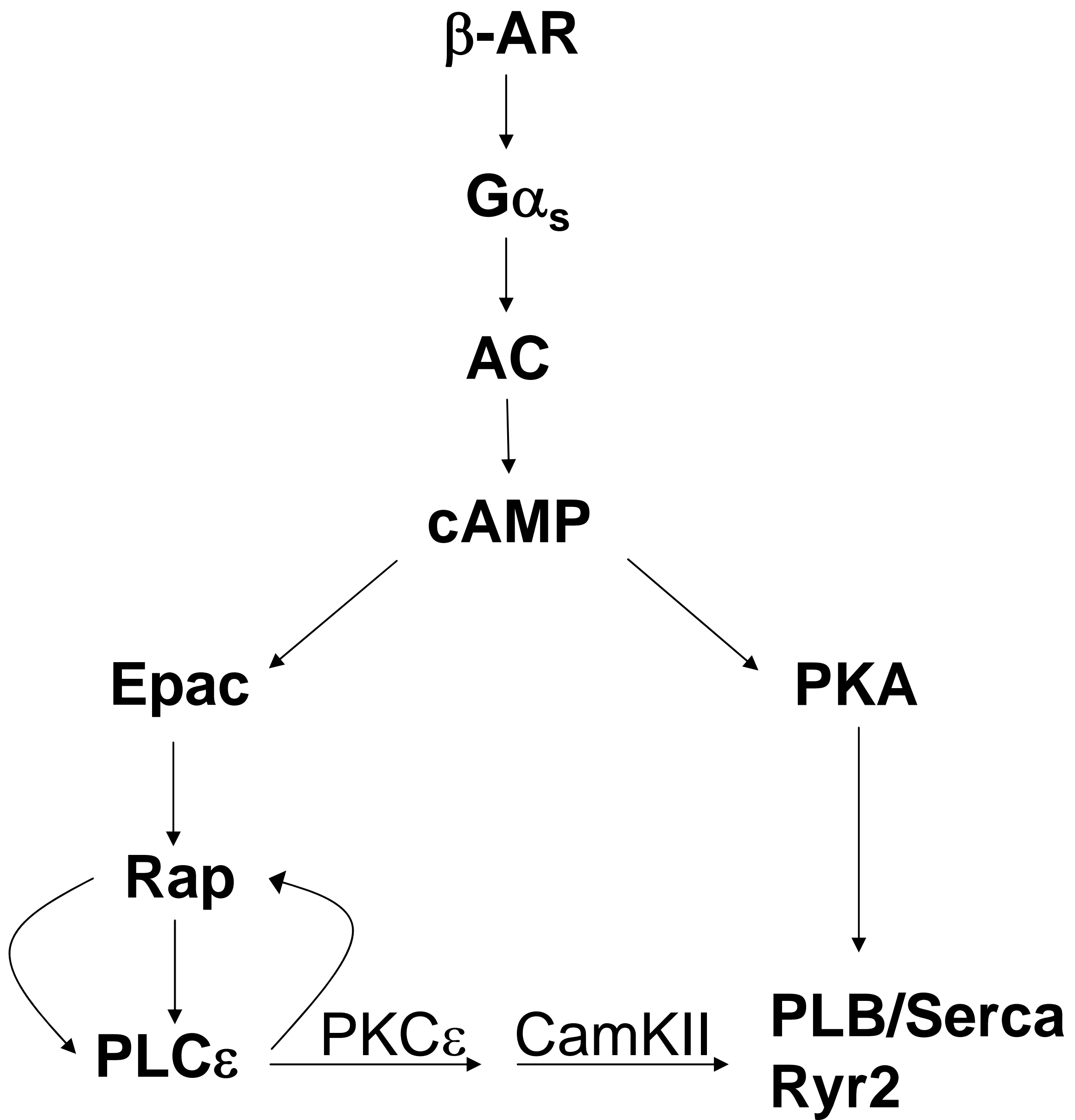

Supplemental Fig. 1




Supplemental
Figure 2.




Supplemental Fig. 3




Supplemental Fig. 4