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Supplementary Materials for

Ca^{2+}-Dependent Phosphorylation of Ca^{2+} Cycling Proteins Generates Robust Rhythmic Local Ca^{2+} Releases in Cardiac Pacemaker Cells

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The PDF file includes:

Figure S1

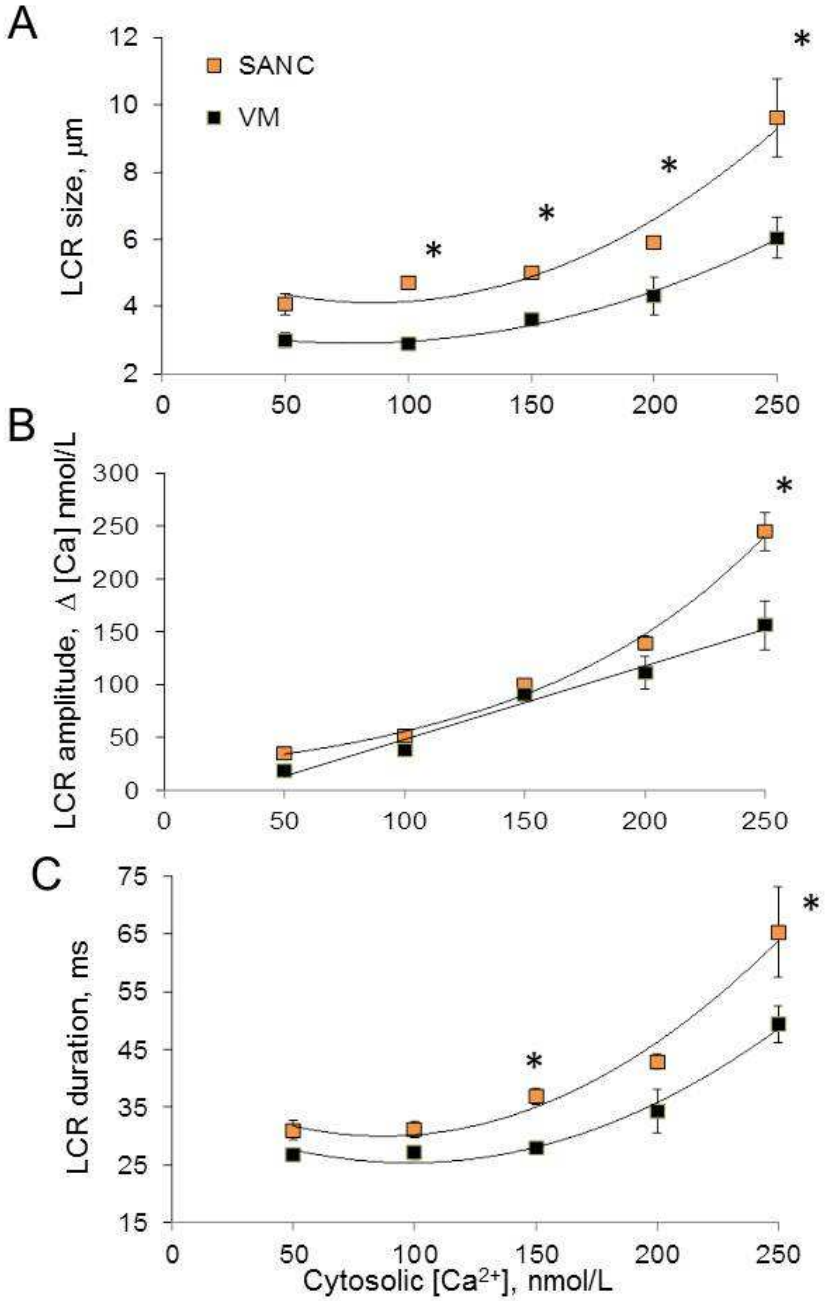


Figure S1: Local Ca^{2+} release characteristics are Ca^{2+}-dependent and differ in sinoatrial node cells and ventricular myocytes. Spatiotemporal characteristics of local Ca^{2+} releases (LCRs) in permeabilized sinoatrial node cells (yellow squares) and sparks in permeabilized ventricular myocytes (black squares) at different free cytosolic Ca^{2+} concentrations. (A) LCR size; (B) and (C) amplitude and duration of spontaneous LCRs, respectively. Number of sinoatrial node cells was as follows: n=16 at 50 nmol/L; n=20 at 100 nmol/L; n= 60 at 150 nmol/L; n=54 at 200 nmol/L; n=9 at 250 nmol/L. Number of ventricular myocytes was as follows: n=5 at 50 nmol/l; n=5 at 100 nmol/l; n=11 at 150 nmol/L; n=9 at 200 nmol/L; n=12 at 250 nmol/L free cytosolic Ca^{2+}. * P< 0.05.

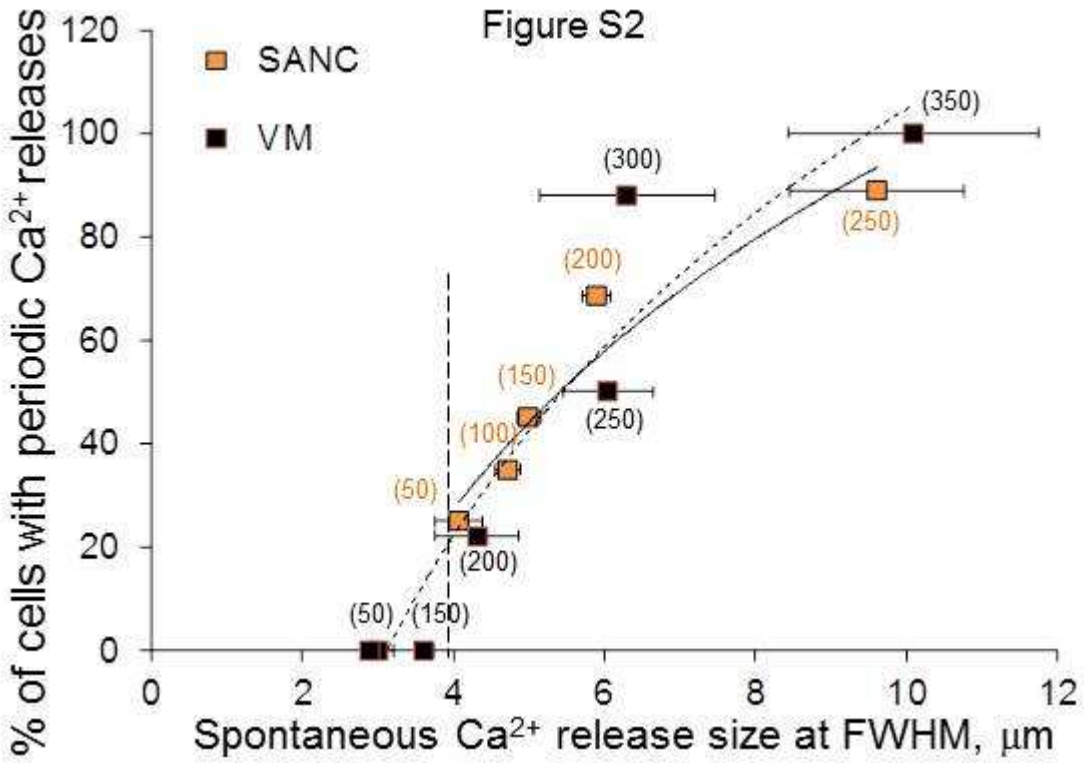


Figure S2: The size of spontaneous local Ca^{2+} releases delineates their periodicity. Correlation between spontaneous Ca^{2+} release size and percentage of cells that generated periodic spontaneous Ca^{2+} releases in sinoatrial node cells: solid line, Y = 75.46 ln(x) - 77.33; R^2= 0.93; in ventricular myocytes: dashed line, Y = 89.89 ln(x) - 102.29; R^2= 0.91. Dashed vertical line specifies the size of spontaneous Ca^{2+} releases that produced a shift from stochastic to periodic spontaneous Ca^{2+} releases. Concentrations of intracellular free Ca^{2+} (nmol/L) are shown near each data point in parentheses. Number of sinoatrial node cells was as follows: n=16 at 50 nmol/L; n=20 at 100 nmol/L; n= 60 at 150 nmol/L; n=54 at 200 nmol/L; n=9 at 250 nmol/L. Number of ventricular myocytes was as follows: n=7 at 50 nmol/l; n=10 at 100 nmol/l; n=15 at 150 nmol/L; n=9 at 200 nmol/L; n=12 at 250 nmol/L; n=9 at 300 nmol/L and n=3 at 350 nmol/L free cytosolic Ca^{2+}.

Figure S3

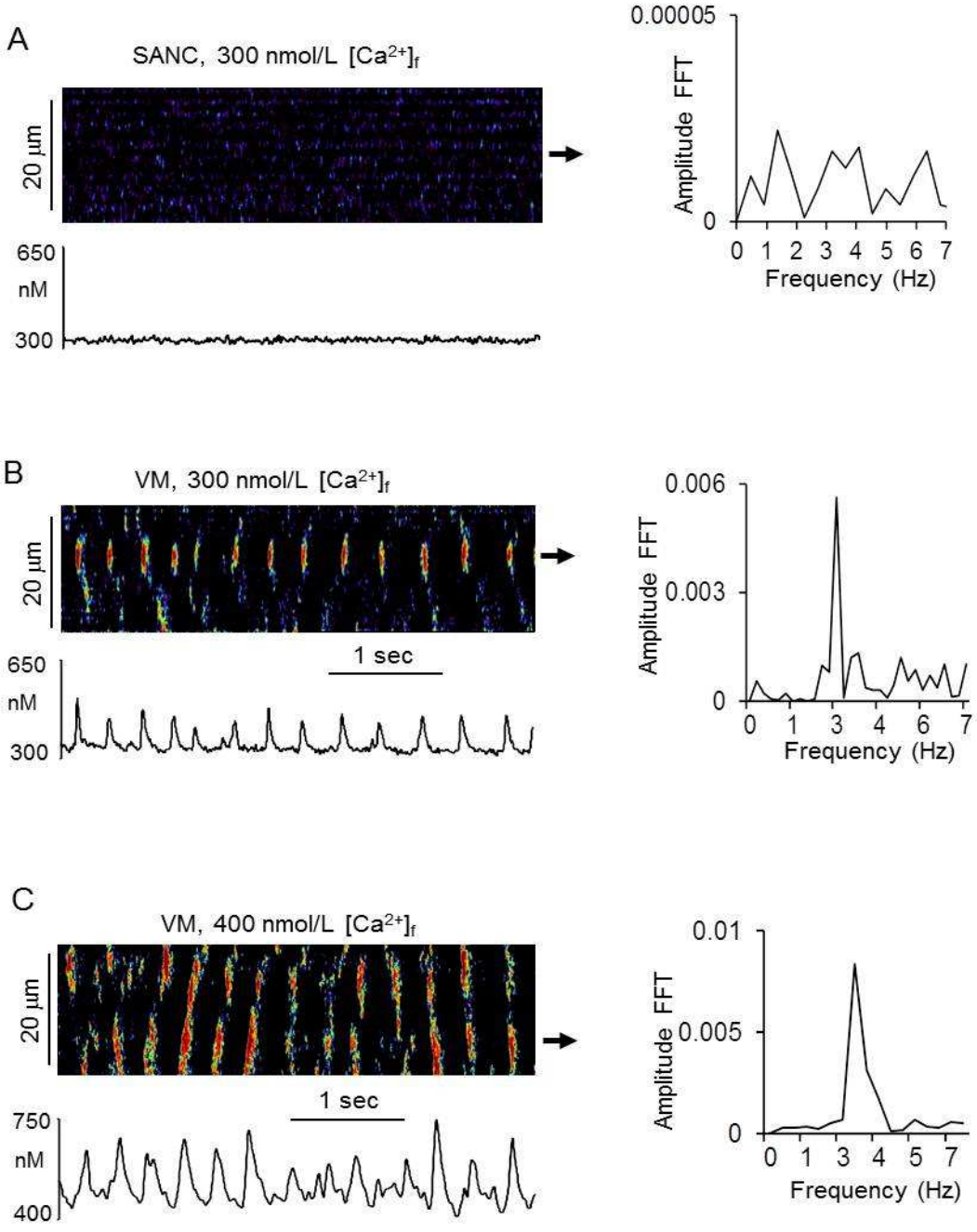
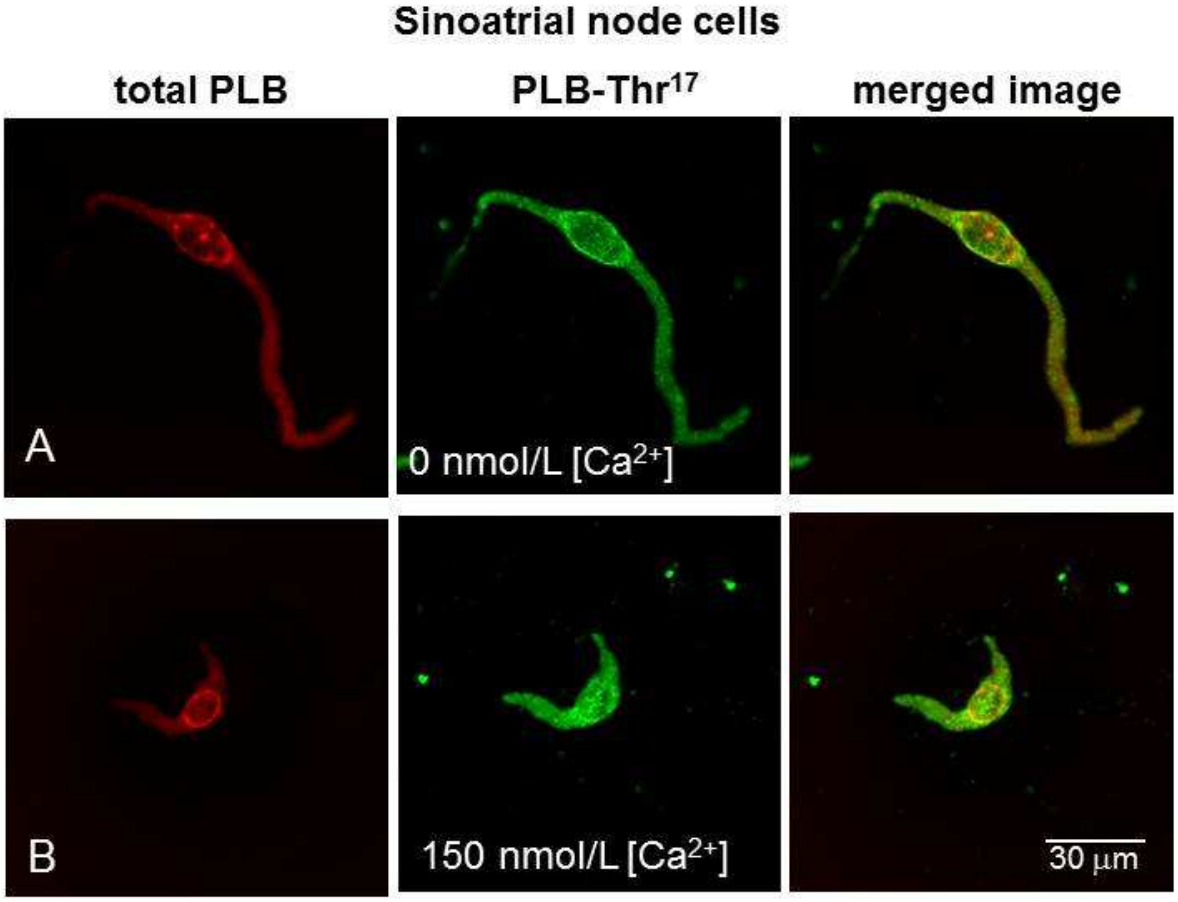


Figure S3: High cytosolic Ca^{2+} concentrations eliminate local Ca^{2+} releases in sinoatrial node cells. (A) Left: Representative confocal line-scan images of sinoatrial node cells bathed at 300 nmol/L (n=3 cells) and (right) FFT of the Ca^{2} waveform obtained along a line indicated by arrow in the image to the left. (B and C) Left: Representative confocal line-scan images of ventricular myocytes bathed at 300 nmol/L (n=9 cells) and 400 nmol/L (n=3 cells) cytosolic free Ca^{2+}, respectively. Right, FFT of Ca^{2} waveforms obtained along lines indicated by arrows in images to the left.

Figure S4


Sinoatrial node cells
total PLB
PLB-Thr17
merged image
A
0 nmol/L [Ca2+]
B
150 nmol/L [Ca2+]
30 μm

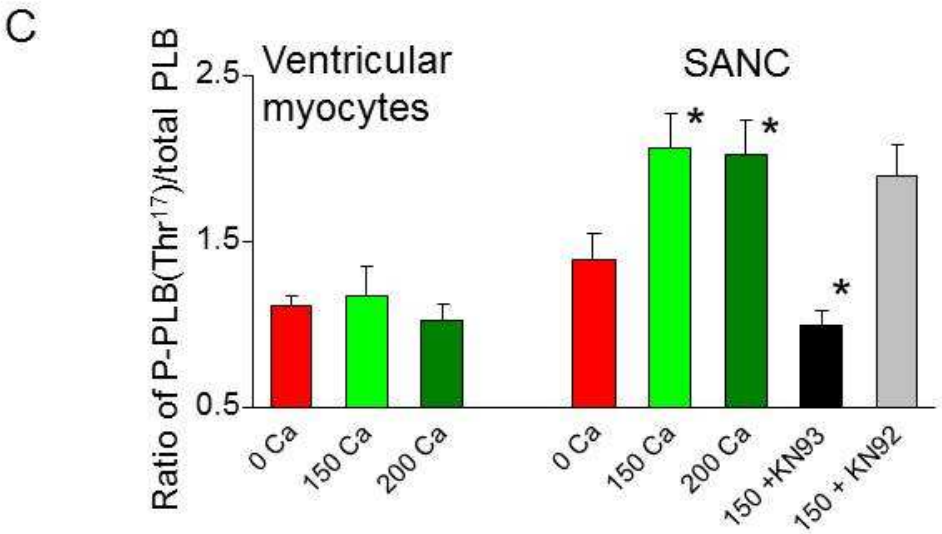

C
Ratio of P-PLB(Thr17)/total PLB
2.5
1.5
0.5
Ventricular myocytes
SANC
*
*
*
0 Ca
150 Ca
200 Ca
0 Ca
150 Ca
200 Ca
150 +KN93
150 + KN92

Figure S4: Increased cytosolic Ca^{2+} concentrations increase phosphorylation of PLB at Thr^{17} in sinoatrial node cells, but not in ventricular myocytes. (A) Representative confocal images of permeabilized sinoatrial node cells at 0 nmol/L (n=49 cells) and (B) 200 nmol/L (n=66 cells) cytosolic free Ca^{2+} cells were labeled for total PLB (red) and PLB phosphorylated at Thr^{17} (green). (C) Relative changes of phosphorylation of PLB at Thr^{17} normalized to total PLB in permeabilized sinoatrial node cells or ventricular myocytes at different cytosolic free Ca^{2+} concentrations. Number of immunostained sinoatrial node cells in (C) was as follows: n= 49 cells at 0 nmol/L; n=64 cells at 150 nmol/L; n=66 cells at 200 nmol/L cytosolic free Ca^{2+}; 68 cells were treated with KN-93 and 60 cells with KN-92 at 150 nmol/L cytosolic free Ca^{2+}. Number of immunostained ventricular myocytes in (C) was as follows: n=80 cells at 0 nmol/L; n=74 cells at 150 nmol/L and n=81 cells at 200 nmol/L cytosolic free Ca^{2+}. * $P<0.05$

Figure S5

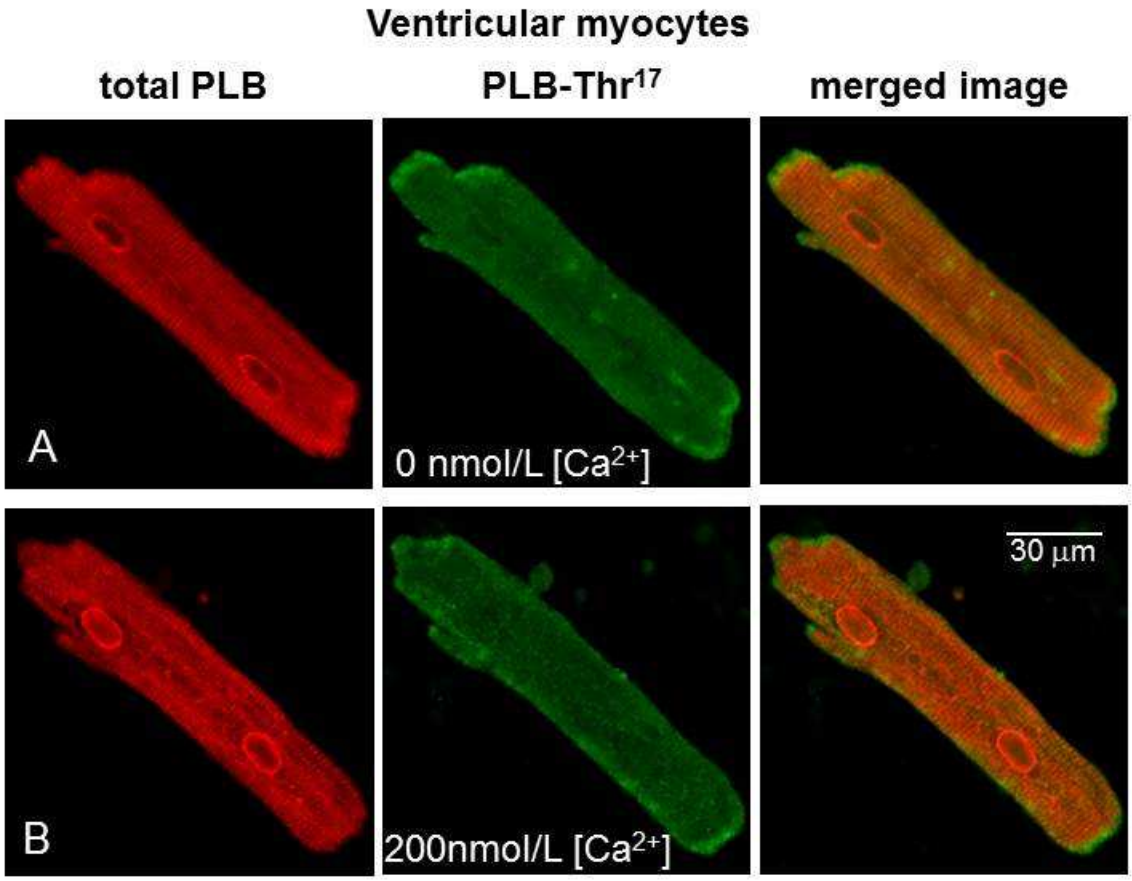


Figure S5: Increased cytosolic Ca^{2+} concentrations do not change the phosphorylation of PLB at Thr^{17} in ventricular myocytes. Representative confocal images of permeabilized VM at 0 nmol/L (A) and 200 nmol/L (B) cytosolic free Ca^{2+}. Cells were labeled for total PLB (red) and PLB phosphorylated at Thr^{17} (green). Number of immunostained ventricular myocytes was as follows: n=80 at 0 nmol/L; n=81 at 200 nmol/L cytosolic free Ca^{2+}.

Figure S6

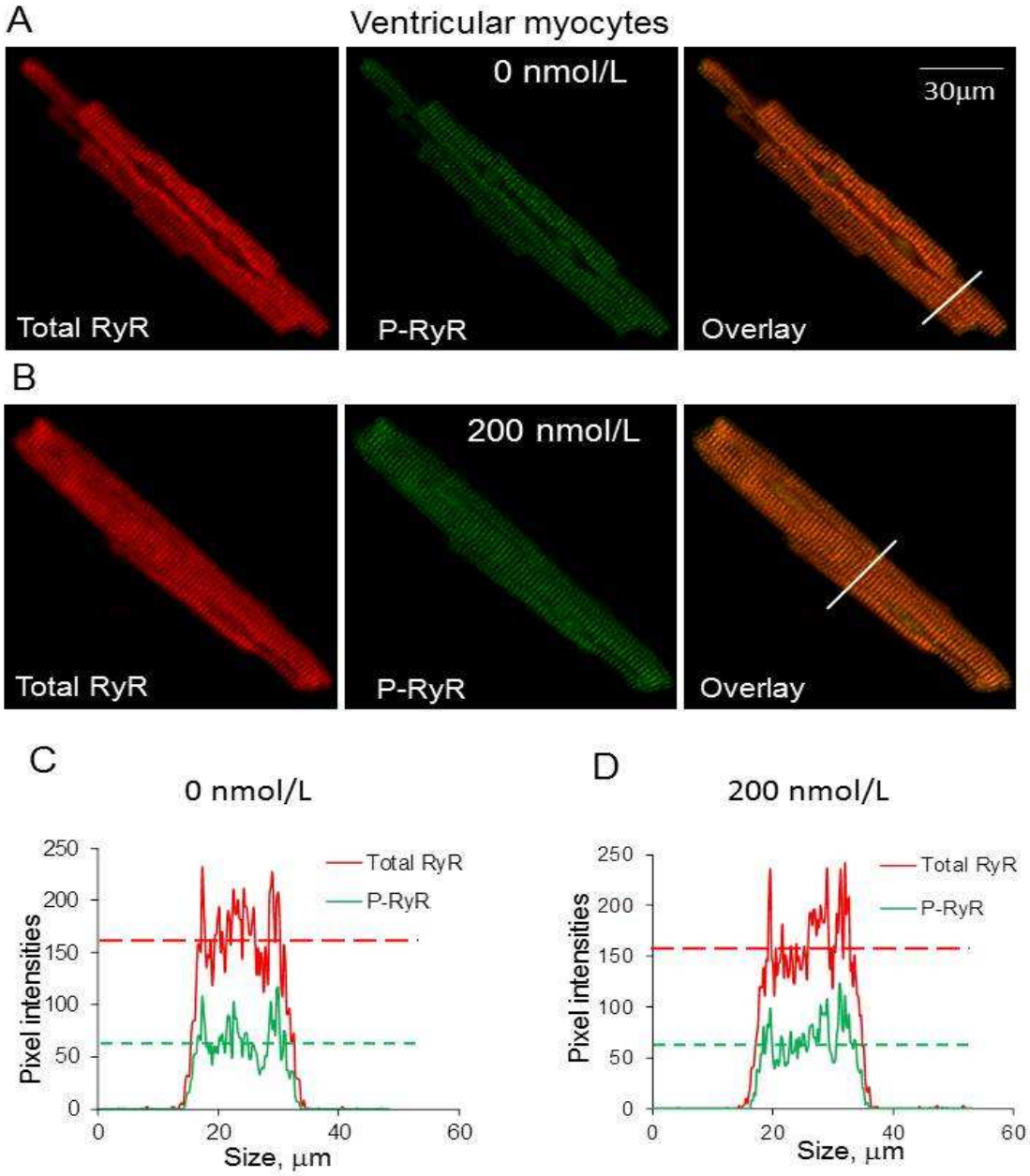


Fig. S6: Phosphorylated RyR immunolabeling in ventricular myocytes is not Ca^{2+}-dependent. Representative confocal images of VM immunolabeled for total RyR (red) and RyR phosphorylated at Ser^{2809} (green) at 0 nmol/L (A) and 200 nmol/L (B) cytosolic free Ca^{2+}. (C) and (D) A graph of the pixel-by-pixel fluorescence intensities of total and phosphorylated RyR labeling along an arbitrary line, indicated by a white line in (A) and (B) of VM at 0 nmol/L (A) and 200 nmol/L (B) cytosolic free Ca^{2+}. The dashed lines in (C) and (D) show the average (over the entire cell) pixel intensity for total RyR (red) and phosphorylated RyR (green). Number of immunostained ventricular myocytes was as follows: n=68 at 0 nmol/L and n=53 at 200 nmol/L cytosolic free Ca^{2+}.

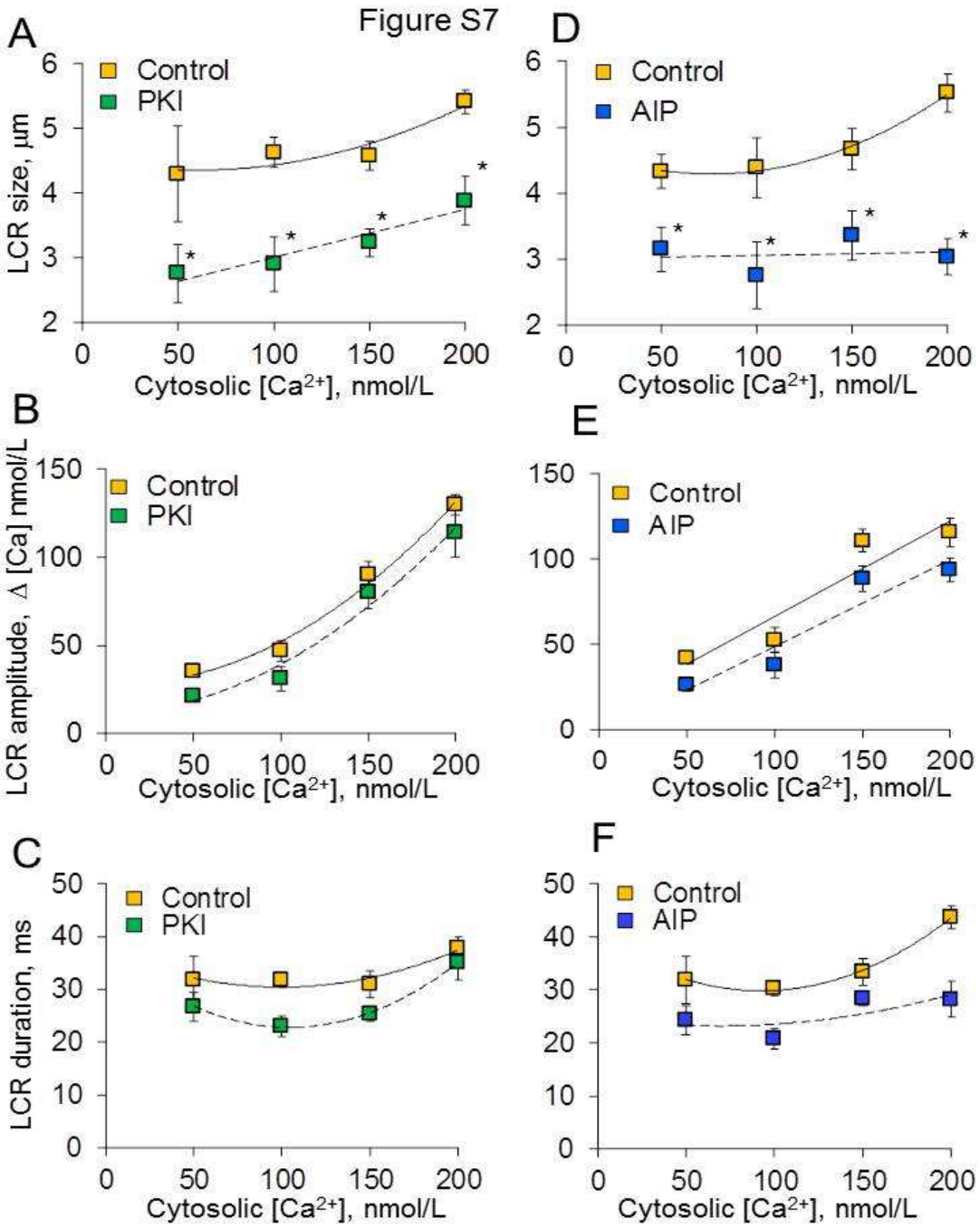


Fig. S7: Effects of PKI and AIP on the characteristics of local Ca^{2+} releases in permeabilized sinoatrial node cells. Spatiotemporal characteristics of local Ca^{2+} releases (LCR) before (yellow squares) and after treatment with PKI (A-C, green squares) or AIP (D-F, blue squares). (A, D) Spatial size of local Ca^{2+} releases. (B, E) Amplitude of spontaneous Ca^{2} releases. (C, E) Duration of local Ca^{2+} releases. Number of sinoatrial node cells treated with PKI at different cytosolic Ca^{2+} was as follows: n=4 at 50 nmol/L; n=4 at 100 nmol/l; n=12 at 150 nmol/L; n=8 at 200 nmol/L. Number of sinoatrial node cells treated with AIP at different cytosolic Ca^{2+} was as follows: n=4 at 50 nmol/L; n=3 at 100 nmol/l; n=6 at 150 nmol/L; n=8 at 200 nmol/L. * P< 0.05.