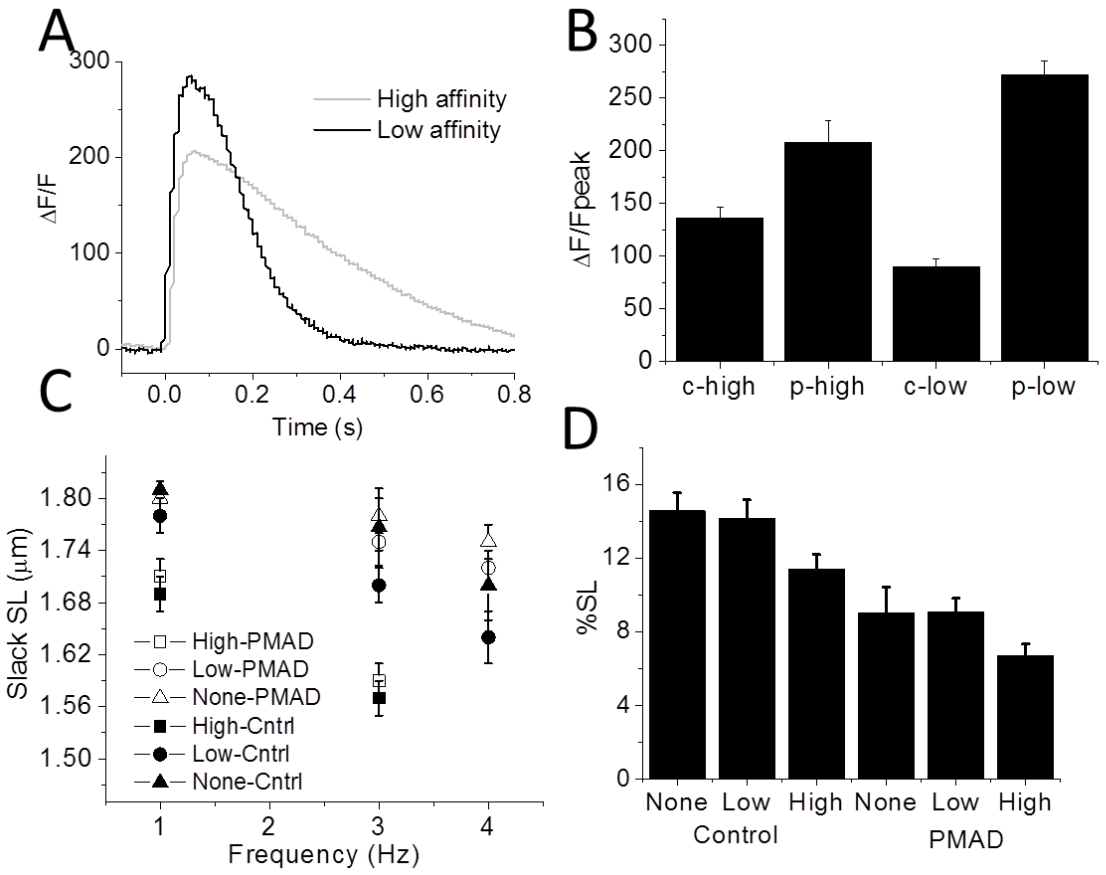
SUPPLEMENTAL MATERIAL

In vivo Post-cardiac arrest myocardial dysfunction is supported by CaMKII-mediated calcium long-term potentiation and mitigated by Alda-1, an Agonist of Aldehyde Dehydrogenase Type 2

Running Title: Post-cardiac arrest myocardial dysfunction

Christopher Woods, MD, PhD;[1*†] Ching Shang, PhD;[2*] Fouad Taghavi, MBChB, MRCS;[3*] Peter Downey, MD;[4] Adrian Zalewski, BSc;[4] Gabriel R. Rubio, BSc;[2] Jing Liu, MD;[2] Julian R. Homburger, BSc;[2] Zachary Grunwald, BA;[2] Wei Qi, PhD;[4] Christian Bollensdorff, PhD;[5] Porama Thanaporn, MD;[2] Ayyaz Ali, MBChB MRCS;[3] Kirk Riemer, PhD;[6] Peter Kohl, MD,PhD;[7] Daria Mochly-Rosen, PhD;[8] Edward Gerstenfeld, MD;[9] Stephen Large, MS, FRCS FRCP;[3] Ziad Ali, MD, DPhil;[4] Euan Ashley, MRCP, DPhil[2†]

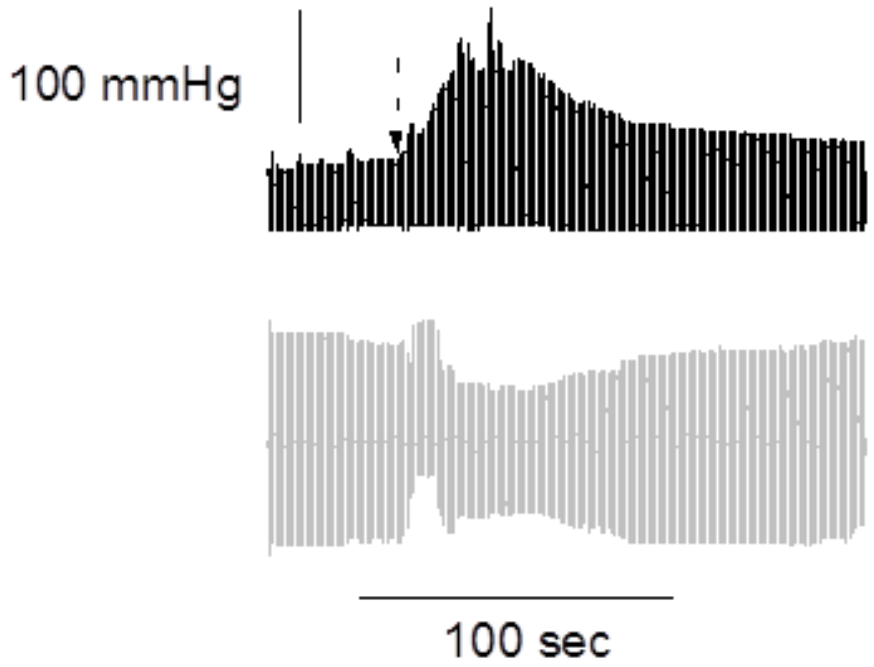
Supplemental Figure 1



Supplemental Figure 1: **High vs Low affinity calcium indicators in PMAD calcium measurements. A.** Two superimposed CICR transients from the same PMAD cell population loaded with either with Fluo-5f (low affinity, black) or with Fluo-4 (high affinity, gray) are shown demonstrating the significant filtering effect of the higher affinity dye both in terms of amplitude and time course. On average, decay phase was significantly prolonged to 533 ± 16 ms with Fluo-4 (data not shown), ~100 ms longer than Fluo-5f (see text). **B.** Calcium transient ΔF/Fpeak for both control (C) and PMAD (P) cells measured using the high and low affinity Fluo derivatives.. The difference between control and PMAD was reduced using the high affinity dye ($p=0.02$, $n=32$ from 2 rats for high affinity PMAD and control). **C.** The resting diastolic length

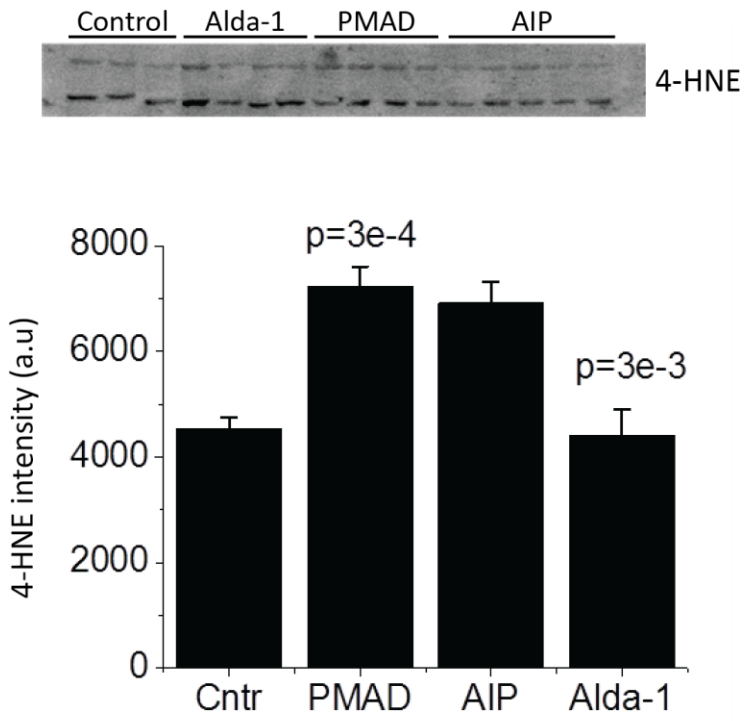
measured just prior to the next stimulus in a pacing train (y axis) is plotted against pacing frequency (x-axis) in cells load without dye (triangles), with low affinity dye (circles) or high affinity dye (squares) in control (open) and PMAD cells (filled). As can be seen, high affinity dye significantly prevents the cell from returning to resting length at all frequencies tested, a process that results from the dominant buffering feature of the dye. **D.** %SL in unloaded cells from control and PMAD cells loaded with high or low affinity calcium dye at steady-state1 Hz pacing. The high affinity dye reduces the %SL in control cells almost as much as cardiac death does alone, while the low affinity dye and 1 Hz pacing does not appreciably alter contractile performance. For all experiments above, n=32 for cells from 2 rats for high affinity experiments in both PMAD and control. Low affinity numbers are as specified in text.

Supplemental Figure 2



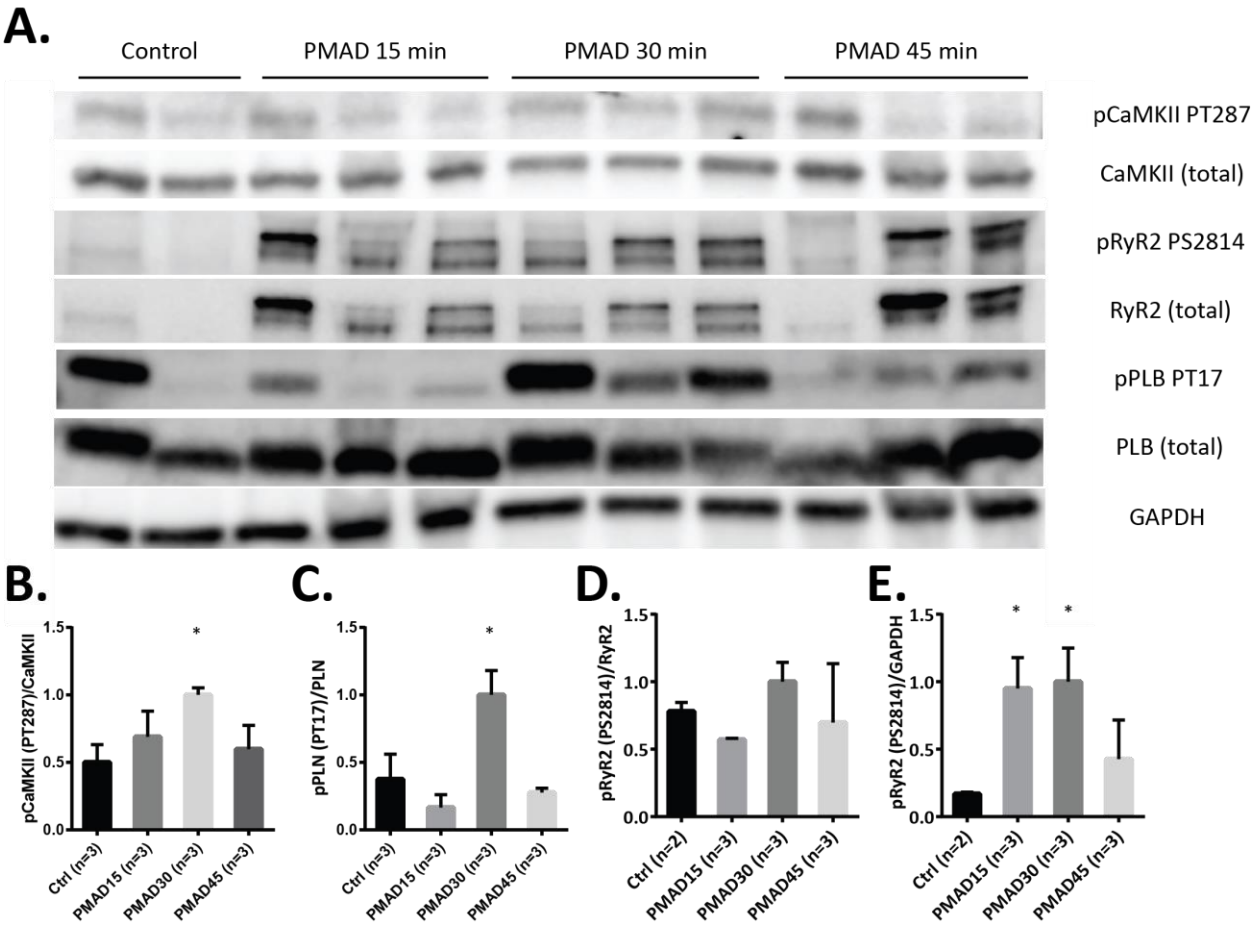
Supplemental Figure 2: **LV Pressure and heart rate tracings during brain stem herniation. A.** Example LV pressure trace contour (black) and heart rate contour (gray) at baseline and after intracranial fogarty balloon inflation (dotted arrow) demonstrating hypertensive response and gradual return to near normal LV pressures.

Supplemental Figure 3



Supplemental Figure 3. Western blot and quantitative assessment of 4-HNE adducts. Example Western blot against 4-HNE adducts for control (Cntr), PMAD in the presence of Alda-1 (Alda-1), PMAD, PMAD in the presence of AIP (AIP). F statistic=13. P value compared to control.

Supplemental Figure 4



Supplemental Figure 4. Temporal changes of CaMKII-dependent phosphorylation after cardiac arrest and ECMO reperfusion. A. Example Western blots showing CaMKII-dependent phosphorylation and protein level of CaMKII, phospholamban and RyR2 in Control group, PMAD-15 min (15 minutes of cardiac arrest and 45 minutes of reperfusion), PMAD-30 min (30 minutes of cardiac arrest and 30 minutes of reperfusion), and PMAD-45 min (45 minutes of cardiac arrest and 15 minutes of reperfusion). **B-E.** Quantitative summary of Western blot analysis. CaMKII-dependent phosphorylation of CaMKII at T287 (PT287/CaMKII, in **B**) and phosphorylation of PLN at T17 (PT17/PLN, in **C**) appeared to be variable with the duration of

cardiac arrest and reperfusion, but not consistent with the time passed since the onset of cardiac arrest (60 minutes in all PMAD samples). **D**. CaMKII-dependent RyR2 phosphorylation at S2814 (PS2814/RyR2) did not vary considerably with the length of cardiac arrest and reperfusion. However, RyR2 protein level surged in all PMAD groups therefore overall pRyR2 PS2814 in PMAD was also shown to increase dramatically compared with the Control group (PS2814/GAPDH in **E**). Graph data represent the Mean ± SEM of each group; *, $p<0.05$. P values were reported by ordinary one-way ANOVA with multiple comparisons and shown as comparison between the designated group and the Control group.