Electronic Supplementary Material

THE REDUCED MYOFILAMENT RESPONSIVENESS TO CALCIUM CONTRIBUTES TO THE NEGATIVE FORCE-FREQUENCY RELATIONSHIP IN RAT CARDIOMYOCYTES: ROLE OF REACTIVE OXYGEN SPECIES AND P-38 MAP KINASE

María Sofía Espejo, Ignacio Aiello, Marisa Sepúlveda, Martín G. Vila Petroff, Ernesto A. Aiello[#], Verónica C. De Giusti[#].

Centro de Investigaciones Cardiovasculares "Dr. Horacio E. Cingolani", Facultad de Ciencias Médicas, Universidad Nacional de La Plata-CONICET, La Plata, Argentina.

Running title: p38-K contributes to the negative staircase of rat myocardium

[#]**Address for correspondence:**

Dr. V. C. De Giusti or Dr. E. A. Aiello

Centro de Investigaciones Cardiovasculares

"Horacio E. Cingolani"

Facultad de Ciencias Médicas, UNLP-CONICET.

Calle 60 y 120, 1900, La Plata. Argentina

Email: vdegiusti@med.unlp.edu.ar; aaiello@ciclaplata.org.ar

Materials and Methods

Intracellular ROS in isolated myocytes

Intracellular ROS production was measured on a Zeiss 410 microscope using rat ventricular myocytes loaded with 5 μmol/L 5-(6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H2DCF DA, Molecular Probes) for 30 min at 37 °C. CMH2DCF DA (DCF) was excited at 488 nm and the emitted fluorescence was recorded at 510–560 nm. As DCF can produce artefactual signal amplification upon continuous light exposure, cells were imaged at extremely low intensity and images were taken every 1 second for the first min of stimulation. Nevertheless, in order to avoid misinterpretation of the recordings, the slight spontaneous increase in the fluorescence signals were fitted with regression lines before and after the change in the frequency of stimulation and the ratio between both slopes (0.5 and 2 Hz) were used to express the data. The control condition was measured at 0.5 Hz all the time, and we also expressed the result as the ratio between the second slope at 0.5 Hz vs the first one.

Figure Legends

Figure S1: *Effect of the drugs in control conditions (HCO_3^- buffered solution) on the percentage of sarcomere length shortening.* Average data of sarcomere length shortening at 0.5 Hz in the absence and presence of the following drugs: MPG (4 rats, n=6), Apo (3 rats, n=6), 5-HD (3 rats, n=7), SB-202190 (3 rats, n=9) and KN-93 (3 rats, n=5). * indicates $p<0.05$ vs control.

Figure S2: *Effect of the FFR on the basal sarcomere length*. Average data of change in basal sarcomere length expressed as the ratio of 2 Hz/0.5 Hz. There is no difference between control condition (3 rats, n=15) and during the different treatments: 5-HD (3 rats, n=6), Apo (3 rats, n=10), MPG (3 rats, n=10), KN-93 (3 rats, n=7), SB-202190 (3 rats, n=7).

Figure S3: *The increase in the frequency of stimulation increases ROS production.* Average slopes of DCF fluorescence during the increase in stimulation frequency from 0.5 to 2 Hz (**A, upper panel**) or when the cells were continuously stimulated at 0.5Hz (**A, bottom panel**). The fitting lines and their respective R^2 are shown. **B, left side** shows the normalized slopes (2 Hz slope/0.5 Hz slope in FFR situation, and the last 0.5 Hz slope/first 0.5 Hz slope in control condition, *see Methods*) of DCF fluorescence (5 rats, n=8 per each situation). * indicates $P<0.05$ vs. control. On **B, right side** representative fluorescence images of the same myocyte exposed at 0.5 Hz and 2 Hz are shown.

Figure S4: *Effect of H_2O_2 on sarcomere length shortening and calcium transients in myocytes loaded with Fura-2.* **A** shows representative twitches of sarcomere shortening and calcium transient in the same cell and at the same time at 0.5 Hz in control conditions **(left side)** and in the presence of 100 μM H_2O_2 **(right side)**. **B** illustrates the average data of the previous situations, showing that H_2O_2 (3 rats, n=8) decreases the amplitude of calcium transient without affecting the diastolic calcium. **C** shows average data of percentage sarcomere length shortening (%SLS) and **D** shows the ratio between the delta of sarcomere length shortening and the delta of the amplitude of calcium transient (ΔSLS/ΔCa) (3 rats, n=8). The data suggest that the decrease in cell contractility in the presence of H_2O_2 is due to both, a decrease in the amplitude of the

calcium transient and a decrease in myofilament calcium sensitivity. * indicates $p<0.05$ vs 0.5 Hz.

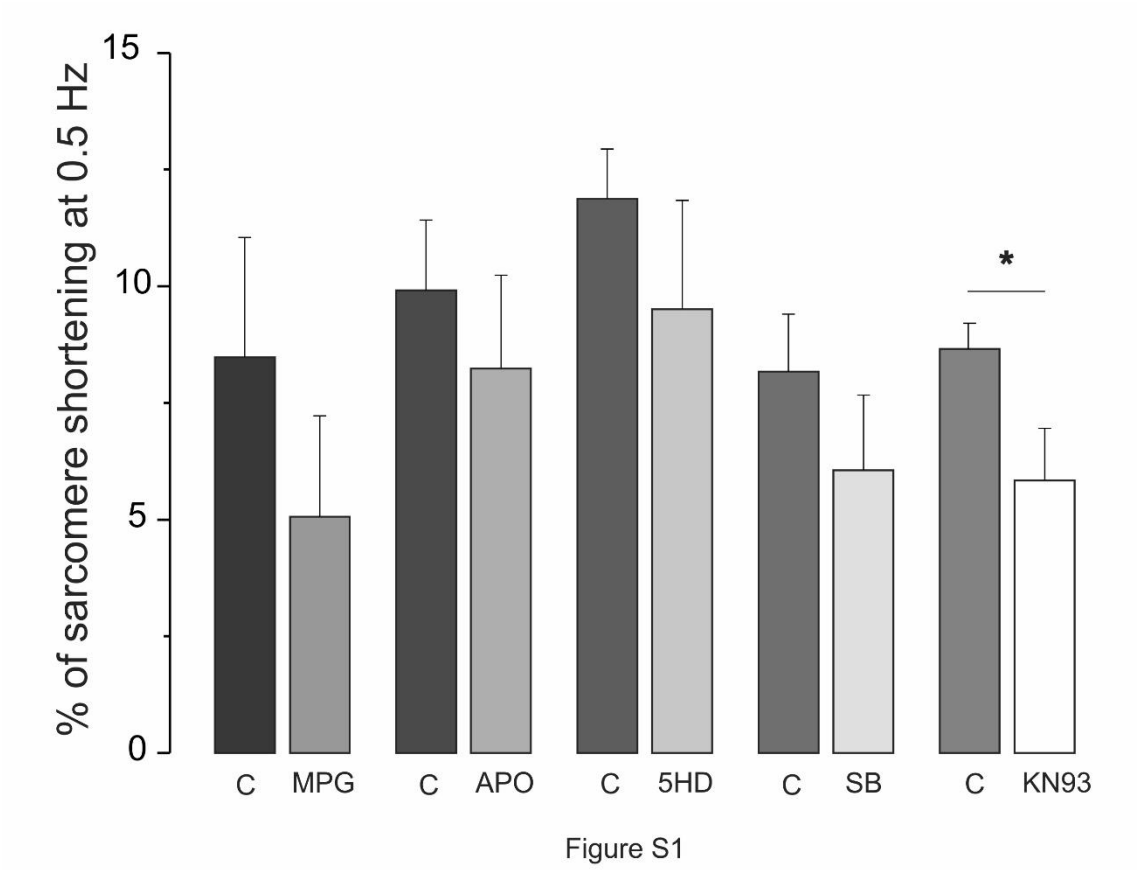

Figure S1

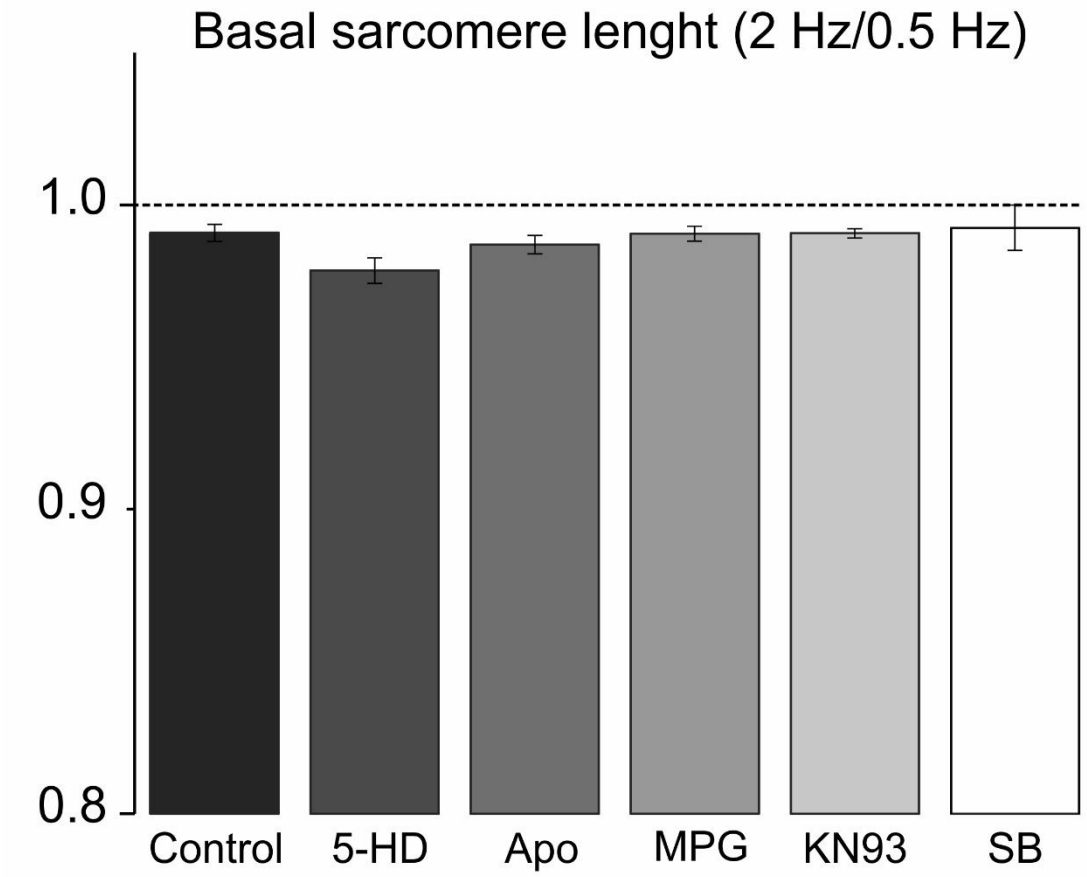
Basal sarcomere lenght (2 Hz/0.5 Hz)
1.0
0.9
0.8
Control
5-HD
Apo
MPG
KN93
SB

Figure S2

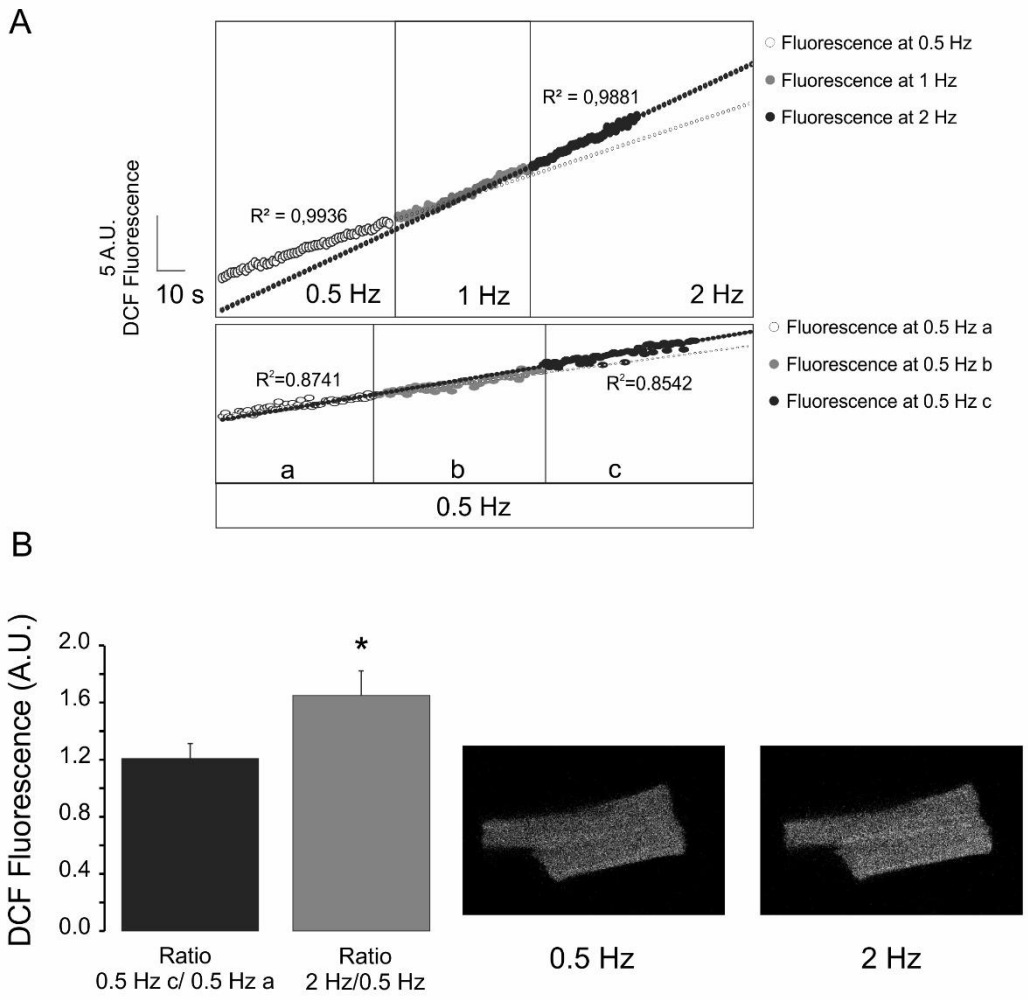
A
Fluorescence at 0.5 Hz
Fluorescence at 1 Hz
Fluorescence at 2 Hz
R² = 0,9881
R² = 0,9936
5 A.U.
DCF Fluorescence
10 s
0.5 Hz
1 Hz
2 Hz
Fluorescence at 0.5 Hz a
Fluorescence at 0.5 Hz b
Fluorescence at 0.5 Hz c
R²=0.8741
R²=0.8542
a
b
c
0.5 Hz
B
DCF Fluorescence (A.U.)
2.0
1.6
1.2
0.8
0.4
0.0
*
Ratio
0.5 Hz c/ 0.5 Hz a
Ratio
2 Hz/0.5 Hz
0.5 Hz
2 Hz

Figure S3

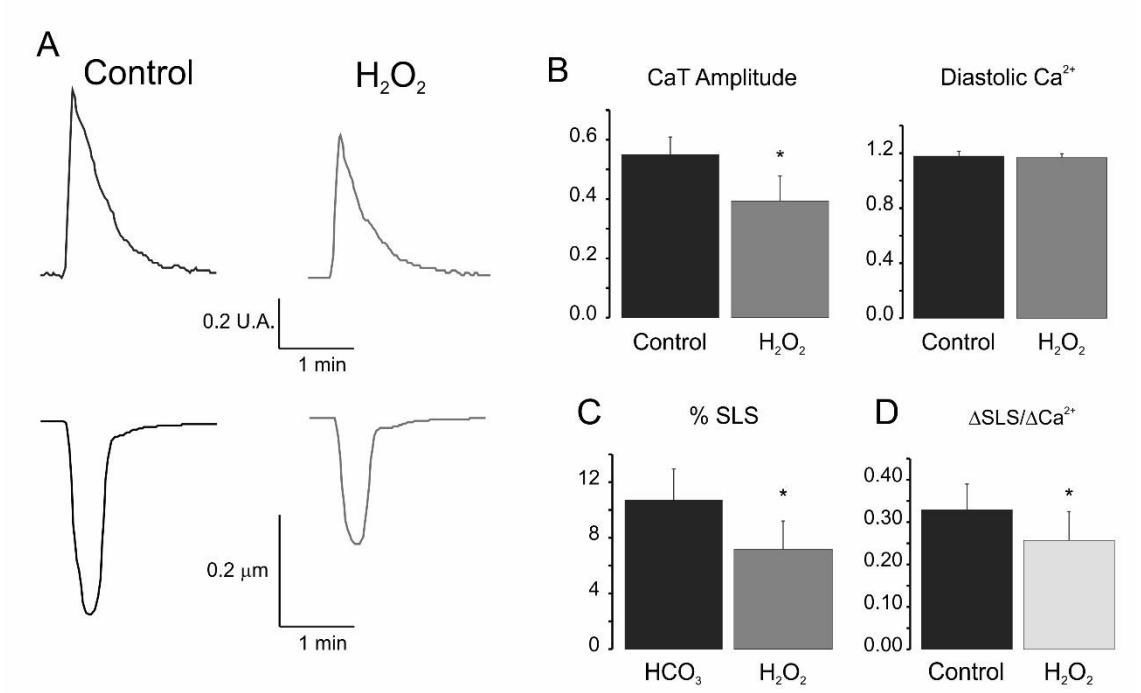
A
Control
H2O2
0.2 U.A.
1 min
0.2 μm
1 min
B
CaT Amplitude
Diastolic Ca2+
0.6
0.4
0.2
0.0
1.2
0.8
0.4
0.0
*
Control
H2O2
Control
H2O2
C
% SLS
D
ΔSLS/ΔCa2+
12
8
4
0
0.40
0.30
0.20
0.10
0.00
*
*
HCO3
H2O2
Control
H2O2

Figure S4