Supplemental Material For:

TRPA1 activation within the cardiac myocyte limits ischemia-reperfusion injury in rodents

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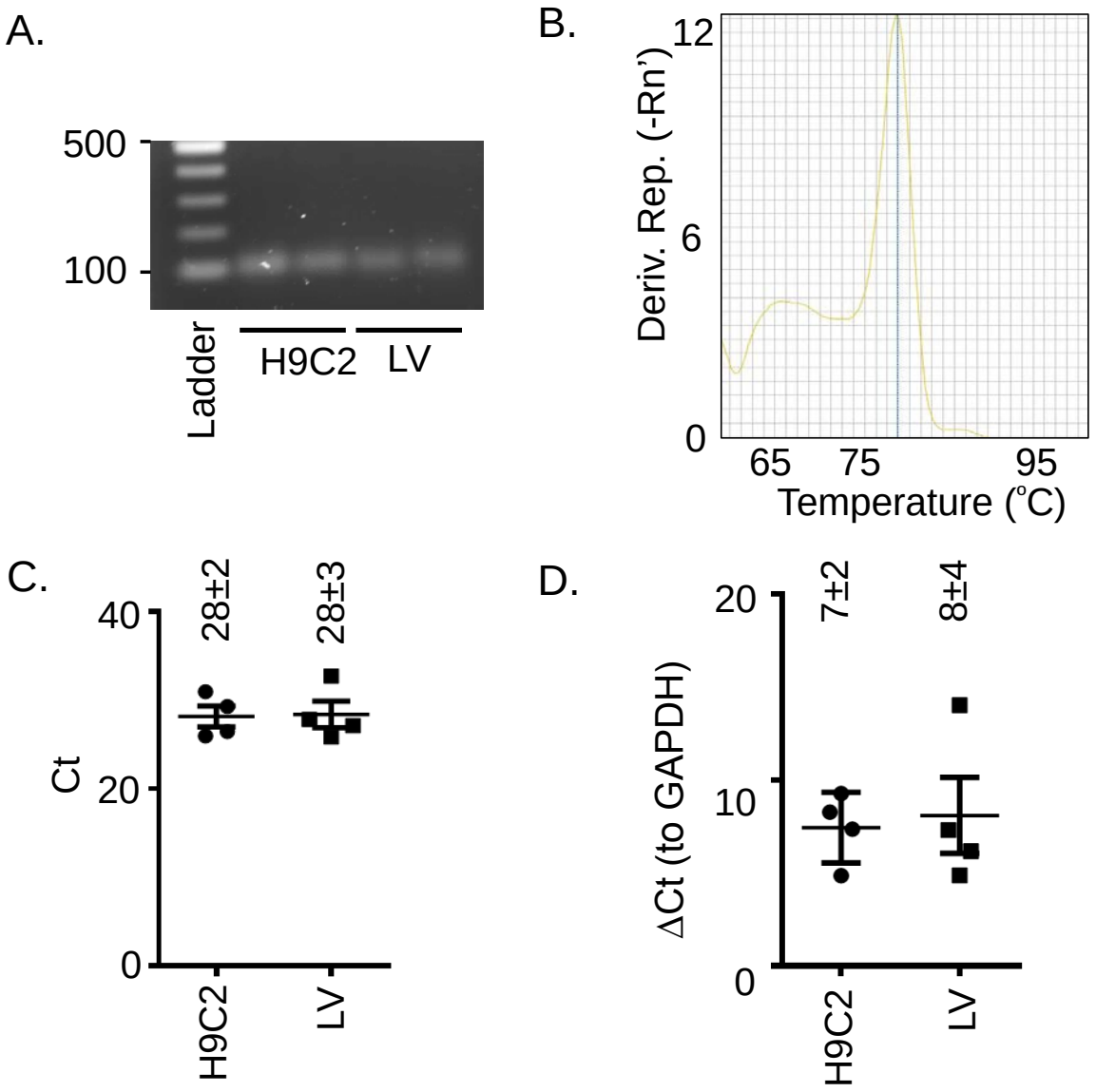
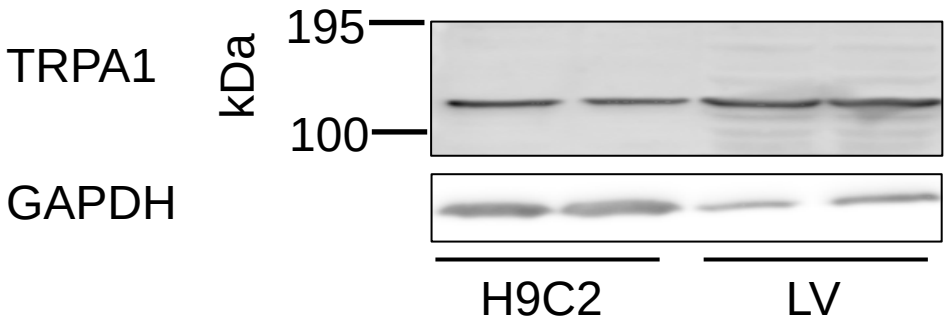
Supplemental Digital Content 1.



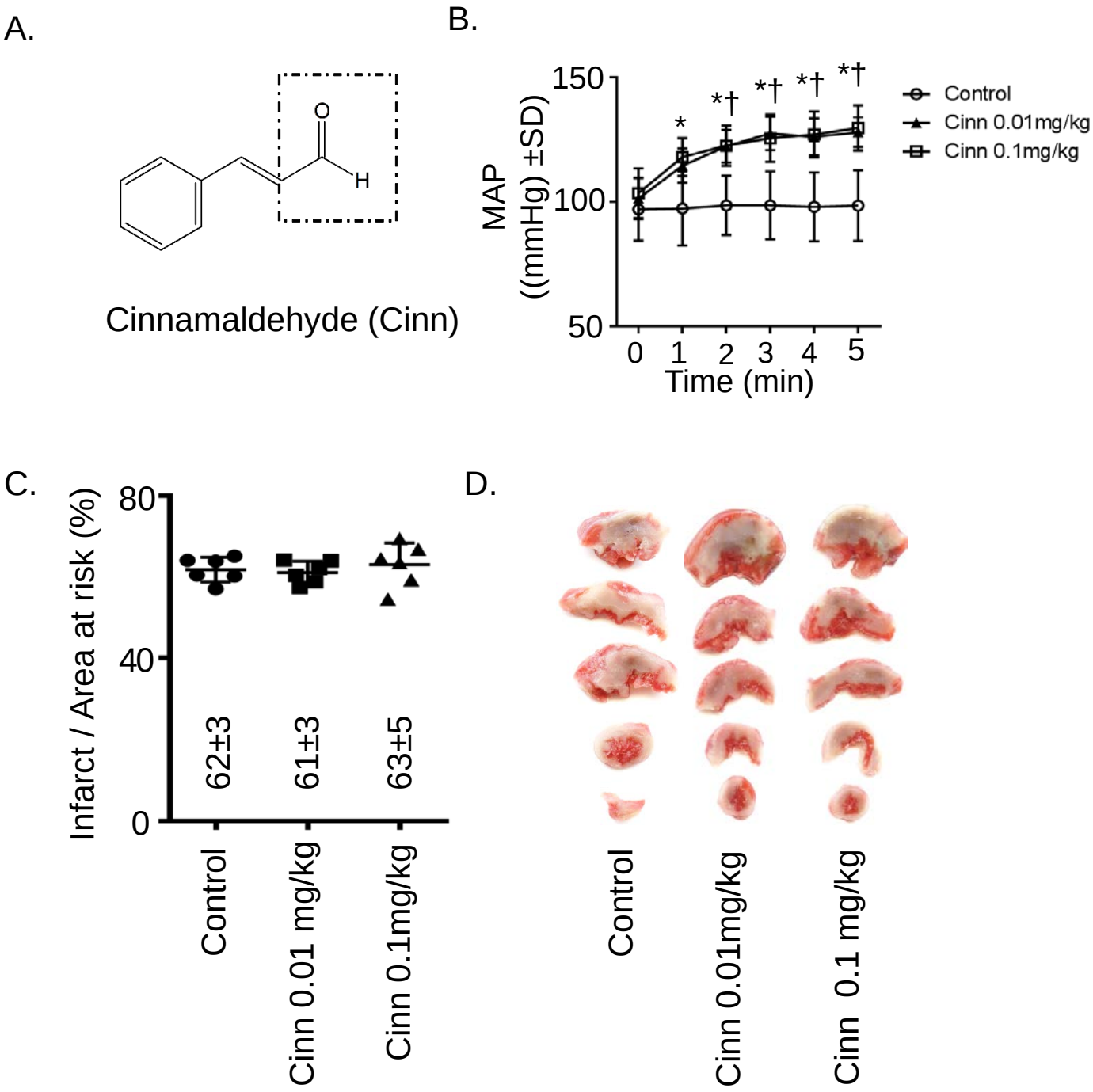
Supplemental Digital Content 1. qPCR validation. **A.** Representative TRPA1 DNA gel for qPCR experiments. **B.** Representative TRPA1 melt curve for qPCR experiments. **C.** qPCR of heart tissue and H9C2 cells with 4 biological replicates performed in triplicate. **D.** Delta Ct relative to GAPDH for heart chambers and cells.

Supplemental Digital Content 2.



Supplemental Digital Content 2. Western blot validation. Western blot of total left ventricle heart homogenate and H9C2 cells representative of 3 biological replicates.

Supplemental Digital Content 3.



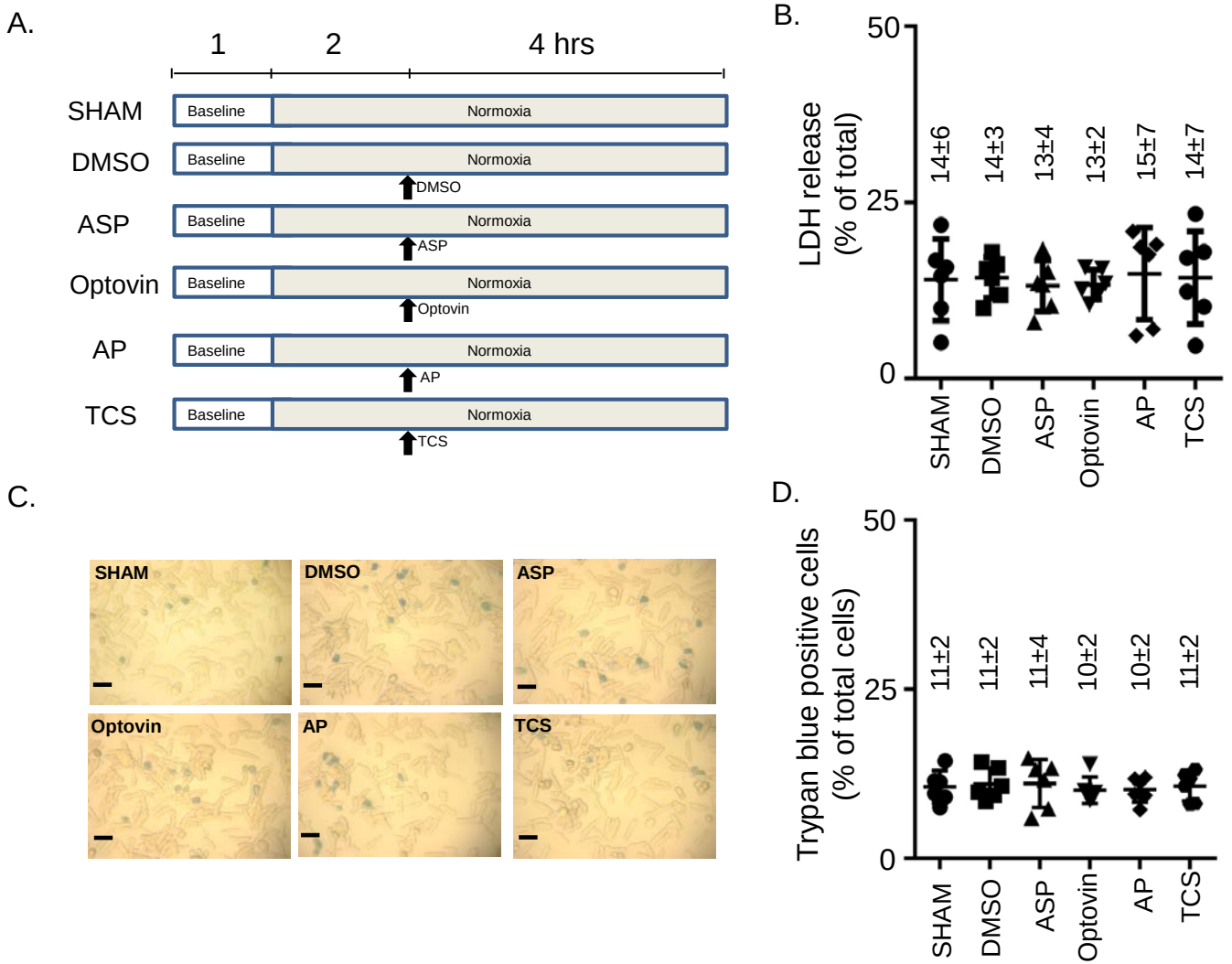
Supplemental Digital Content 3. Cinnamaldehyde does not reduce myocardial infarct size. A. Chemical structure of cinnamaldehyde (Cinn). The aldehyde is highlighted in a dashed box. **B.** Five minutes of mean blood pressure (MAP) changes after cinnamaldehyde administrated. **C.** Infarct size per area at risk percentage. Rats were given cinnamaldehyde at a dose of 0.01 and 0.1 mg/kg, 5 minutes prior to 30 minutes of left anterior descending coronary artery ligation to cause ischemia followed by 2 hours of reperfusion. Data presented as mean ± SD (n=6). *P<0.05 vs. control; †P<0.05 vs. Baseline. **D.** Representative images of left ventricle area at risk for each group.

Supplemental Digital Content 4.

Groups	n	AAR/LV%	Baseline			15 min ischemia			2 hr reperfusion		
			HR	MAP	RPP	HR	MAP	RPP	HR	MAP	RPP
Control	6	39±3	414±20	103±25	48±12	408±20	101±18	46±9	374±25†	79±9	37±6
Cinn 0.01mg/kg	6	42±5	455±28	106±11	54±5	459±38	110±20	56±10	427±32	81±20	43±11
Cinn 0.1mg/kg	6	39±6	442±37	107±13	54±9	444±34	109±26	56±17	389±44	85±14	43±6

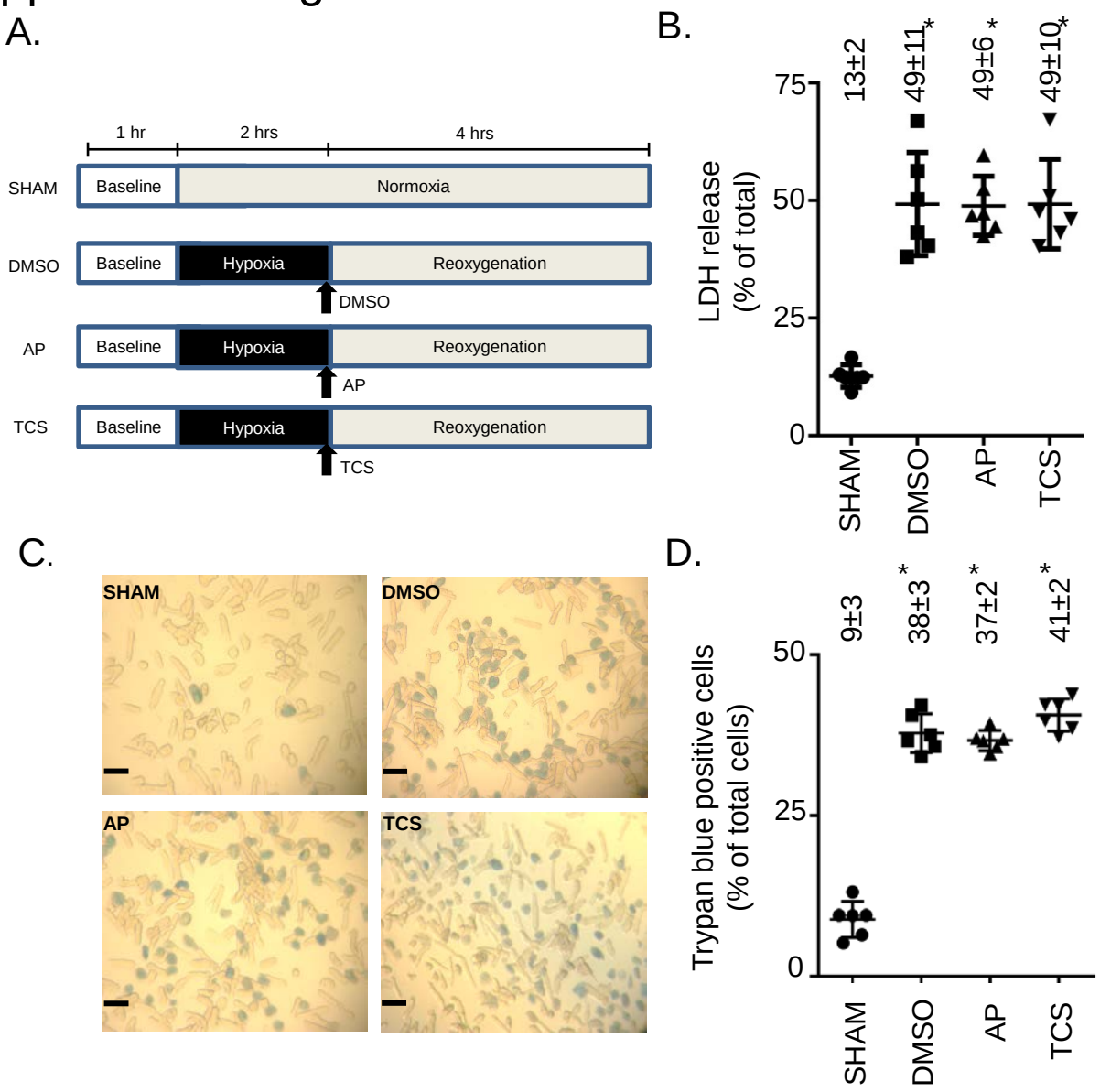
Supplemental Digital Content 4. Number of animals used, area at risk per left ventricle %, and hemodynamic values measured. Described are the groups, number of animals per group (n), area at risk per left ventricle percent (AAR/LV), and hemodynamics acquired for the *in vivo* studies. Heart rate (HR), mean blood pressure (MAP), and rate pressure product (RPP) defined as the product of heart rate and systolic blood pressure, were assessed at baseline, during ischemia, and at 2 hours of reperfusion. Data presented as mean ± SD (n=6). No significant differences were found between groups. Cinn = cinnamaldehyde †P<0.05 vs. Baseline.

Supplemental Digital Content 5.



Supplemental Digital Content 5. Inhibition of TRPA1 at reperfusion in isolated adult cardiomyocytes dose not reduce cell death. A. Experimental protocol for cardiac myocyte hypoxia-reoxygenation studies. The two TRPA1 inhibitors (AP and TCS) were given immediately after hypoxia. **B.** Percentage of LDH for each experimental group (n=6 biological replicates from two cardiomyocyte preparations). **C.** Representative images of trypan blue positive and negative cardiac myocytes for each group (black bar is 50mm). **D.** Percentage of dead cells for each experimental group (n=6 biological replicates from two cardiomyocyte preparations). Data points represent individual biological results for each experiment in addition to values presented as mean ± SD, *P<0.05 vs. SHAM.

Supplemental Digital Content 6.



Supplemental Digital Content 6. Effects of treatments in isolated adult cardiomyocytes subject to normoxia. A. Experimental protocol for cardiac myocyte hypoxia-reoxygenation studies. DMSO, the TRPA1 activators (ASP and optovin) and inhibitors (AP and TCS) were given at the same time point shown in Figure 4 and Supplemental Digital Content 5. **B.** Percentage of LDH for each experimental group (n=6 biological replicates from two cardiomyocyte preparations). **C.** Representative images of trypan blue positive and negative cardiac myocytes for each group (black bar is 50mm). **D.** Percentage of dead cells for each experimental group (n=6 biological replicates from two cardiomyocyte preparations). Data points represent individual biological results for each experiment in addition to values presented as mean ± SD. No significant differences were found between groups.