Online Data Supplement

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HDAC inhibition blunts ischemia/reperfusion injury by inducing cardiomyocyte autophagy

Min Xie, MD, PhD[a], Yongli Kong, MD[a], Wei Tan, MD[a], Herman May, BS[a],

Pavan K. Battiprolu, PhD[a], Zully Pedrozo, PhD[a], Zhao Wang, PhD[a], Cyndi Morales, BS[a],

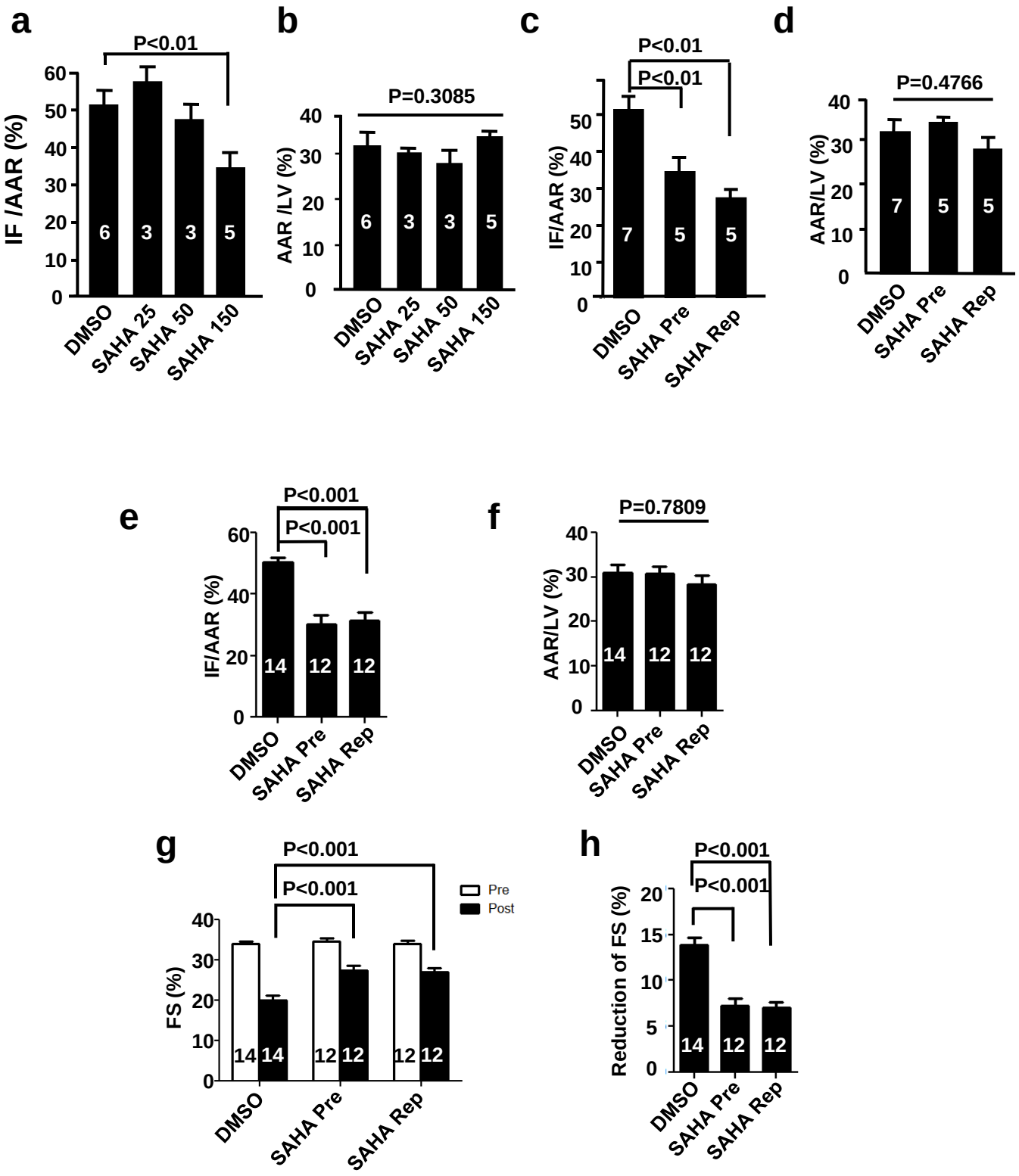
Xiang Luo, MD[a], Geoffrey Cho, MD[a], Nan Jiang, MS[a], Michael E. Jessen, MD[b],

John J. Warner, MD[a], Thomas G. Gillette, PhD[a],

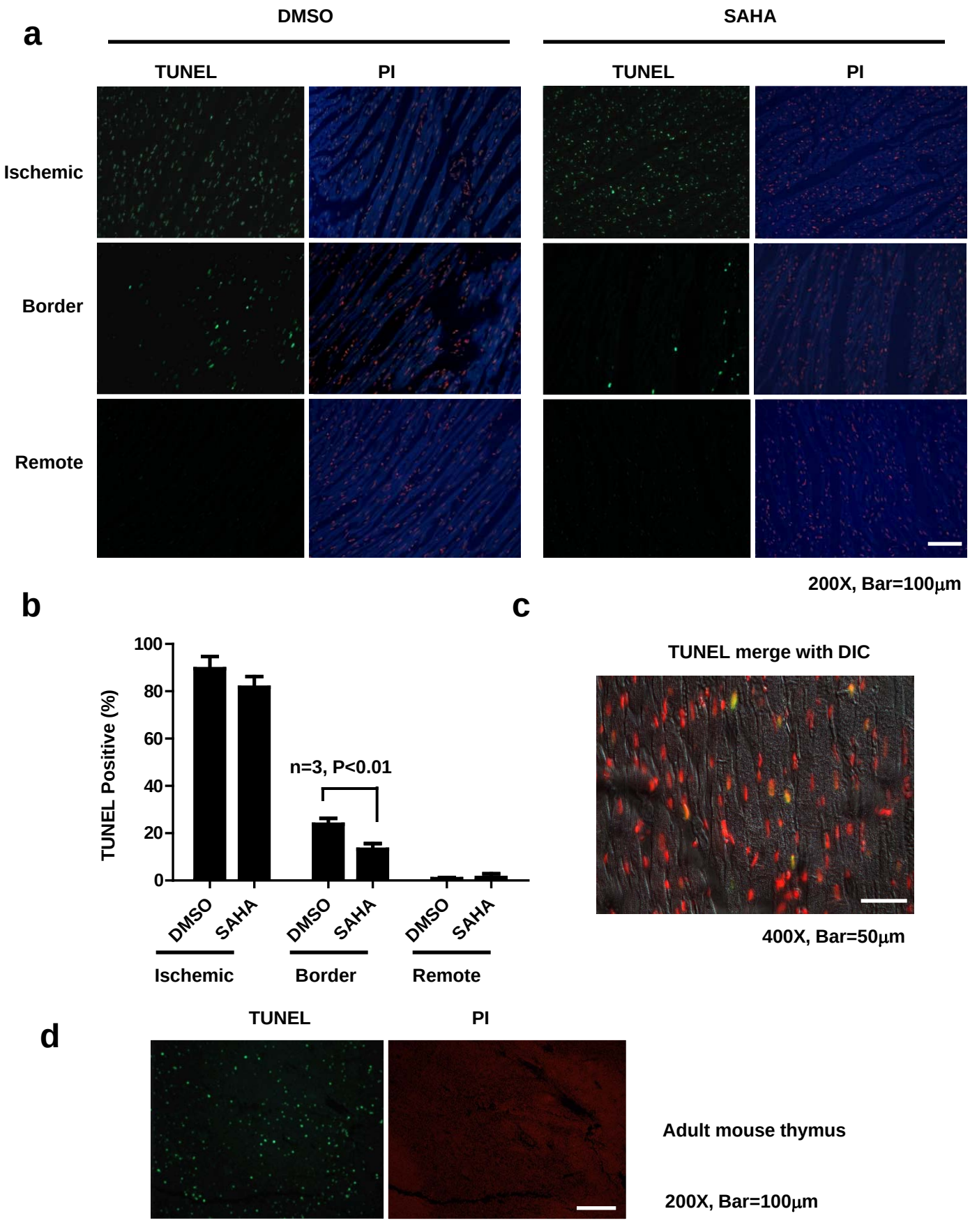
Aslan T. Turer, MD[a], and Joseph A. Hill, MD, PhD[a,c]

Departments of [a]Internal Medicine (Cardiology), [b]Cardiovascular and Thoracic Surgery, and [c]Molecular Biology, University of Texas Southwestern Medical Center, Dallas, Texas

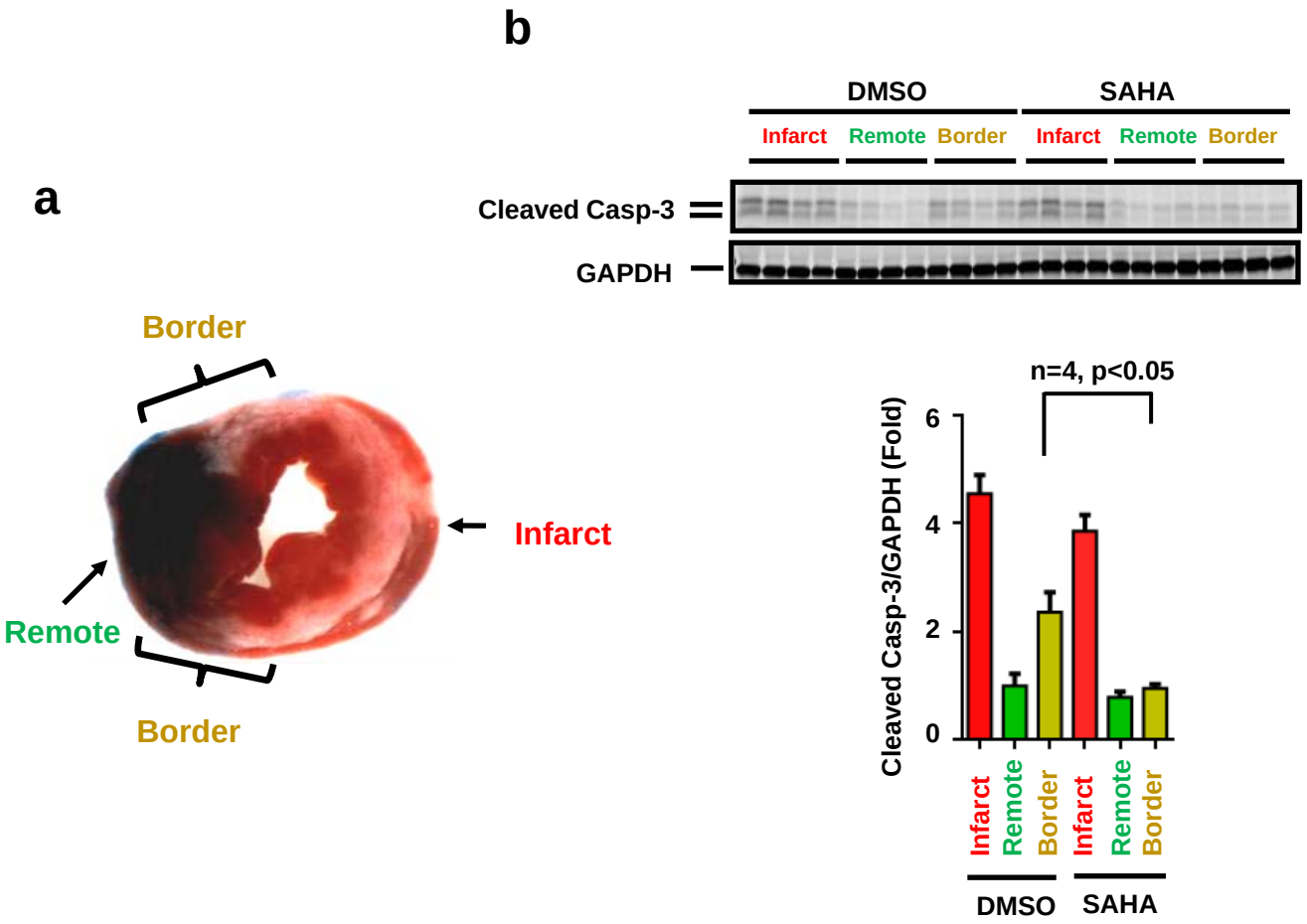
Address correspondence to:
Joseph A. Hill, MD, PhD
Division of Cardiology
University of Texas Southwestern Medical Center
NB11.200
6000 Harry Hines Boulevard
Dallas, TX 75390-8573, USA
joseph.hill@utsouthwestern.edu



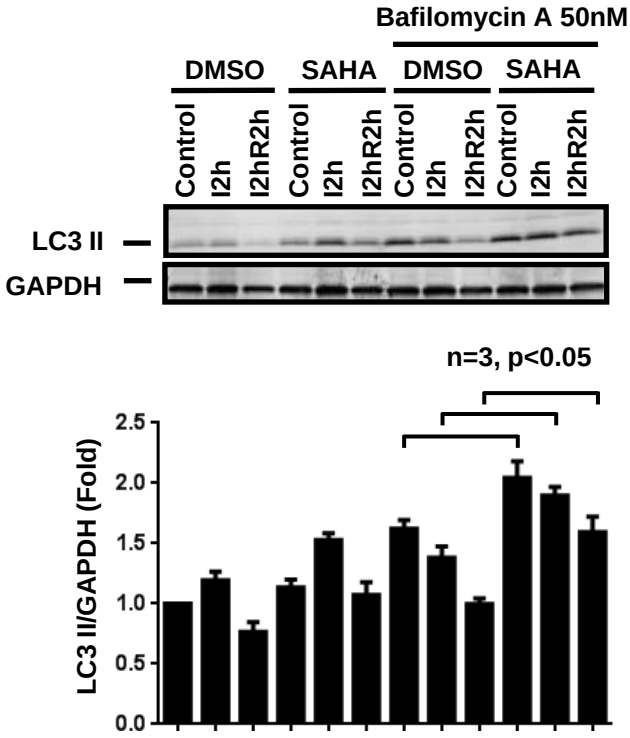
Supplemental Figure 1. SAHA reduces infarct size in rabbit I/R model



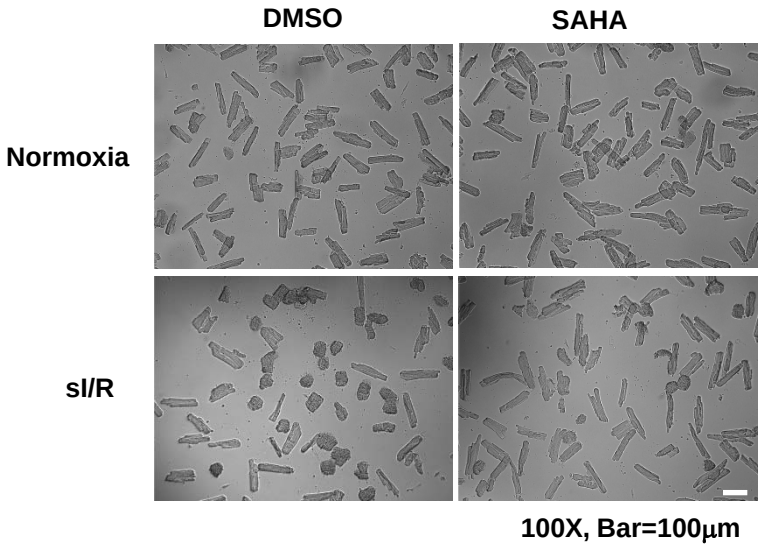
Supplemental Figure 2. SAHA decreases apoptosis by TUNEL.

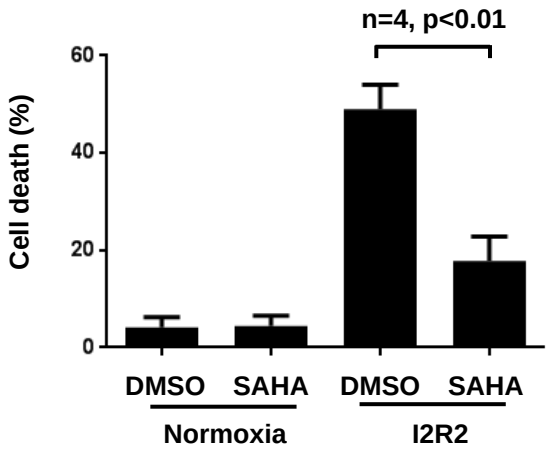


Supplemental Figure 3. SAHA decreased apoptosis in the infarct border zone as measured by cleaved caspase-3.

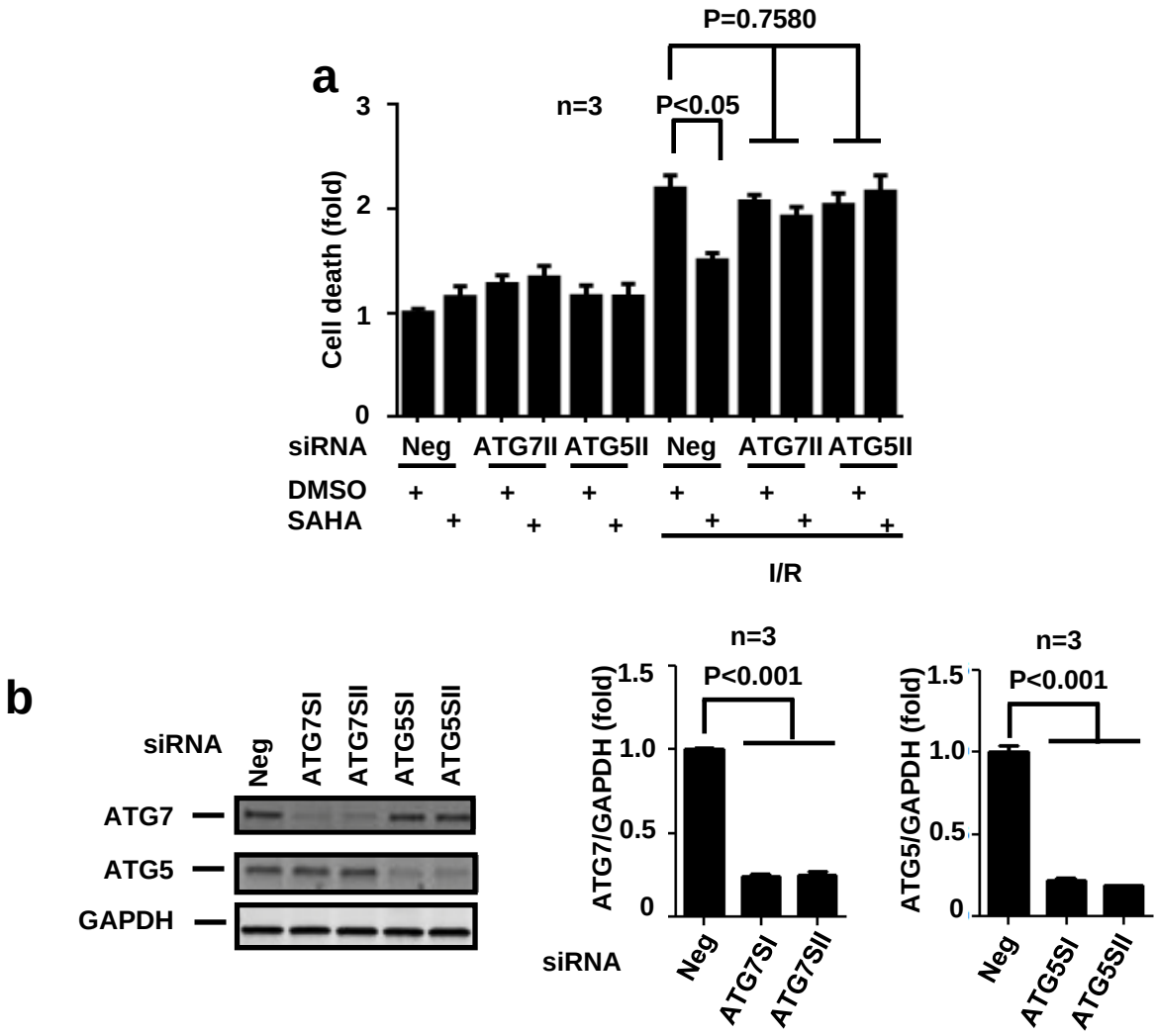


Supplemental Figure 4. SAHA induces autophagic flux in adult rat ventricular myocytes subjected to simulated IR

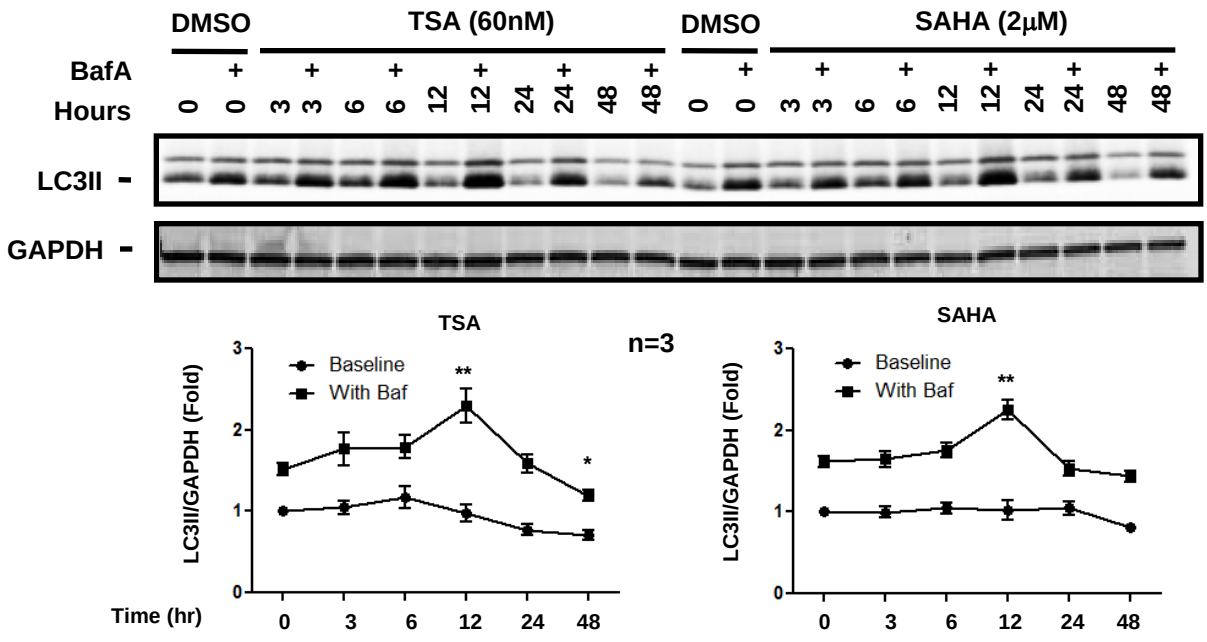




Supplemental Figure 5. SAHA protects ARVMs from cell death after simulated IR



Supplemental Figure 6. SAHA's cardioprotective effects are dependent on autophagic flux as tested using a second, sequence-independent set of ATG7 and ATG5 siRNAs.



Supplemental Figure 7. TSA and SAHA induce autophagic flux at early time points, and prolonged TSA treatment reduces autophagic flux in serum-free conditions.

	Study Type	n	Shapiro-Wilk normality	Test used	Significance
Figure 1bcd	Mouse	11	Passed	Parametric Unpaired t test	p<0.05 DMSO vs TSA
Figure 1e	Mouse	10	Passed	Parametric Repeated measure ANOVA	p<0.05 DMSO vs TSA at all four time points
Figure 1ghi	Mouse	6-7	SAHA 30 passed, DMSO, SAHA50 n too small	Non-parametric Anova Kruskal-Wallis test	IF/AAR, FS p<0.05 DMSO vs SAHA50
Figure 3bcdfg	Rabbit	7	All group passed	Parametric ANOVA with post Tukey's multiple comparisons test	IF/AAR, IF/LV and FS p<0.05 DMSO vs SAHA pre and SAHA reper
Figure5a	Molecular	4	n too small	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 SAHA border vs DMSO border
Figure5d	Molecular	3-5	n too small	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 Autophagosome Autolysosome DMSO vs SAHA reper and SAHA pre
Figure6a	Molecular	3 (triplicate 9)	All group passed	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO vs SAHA
Figure6b	Molecular	3	n too small	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO vs SAHA
Figure6c	Molecular	3	n too little	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO vs SAHA
Figure6d	Molecular	3 (triplicate 9)	All group passed	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO vs SAHA
Figure6e	Molecular	4	n too small	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 Control vs ATG7 and ATG5
Figure7a	Molecular	3	n too small	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO vs SAHA
Figure7b	Molecular	3 (triplicate 9)	All group passed	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 hypo DMSO neg vs hypo SAHA neg
Supp Figure 1ab	Rabbit	3-6	n too small	Non-Parametric Mann Whitney t test between DMSO and SAHA 150	p<0.05 DMSO vs SAHA150
Supp Figure 1cd	Rabbit	5-7	n too small	Non-Parametric Kruskal-Wallis test with Dunn's multiple comparisons test	p<0.05 DMSO vs SAHA reper and SAHA pre
Supp Figure 1efgh	Rabbit	12-14	All group passed	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO vs SAHA reper and SAHA pre
Supp Figure 2	Molecular	3	n too small	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO border vs SAHA border
Supp Figure 3	Molecular	3	n too small	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO border vs SAHA border
Supp Figure 4	Molecular	3	n too small	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO vs SAHA across neg, I2 and I2R2
Supp Figure 5	Molecular	4 (triplicate 12)	All group passed	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO vs SAHA after I2R2
Supp Figure 6a	Molecular	3 (triplicate 9)	All group passed	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 DMSO vs SAHA
Supp Figure 6b	Molecular	3	n too small	Parametric ANOVA with post Tukey's multiple comparisons test	p<0.05 Neg vs ATG7 and ATG5
Supp Figure 7	Molecular	3	n too small	t test between each time point to time 0h	p<0.05 TSA time 12h and 48h vs time 0h,SAHA 12h vs 0h

Supplemental Table 1. Experiment paradigm and statistical methods used

	Study Type	n	Shapiro-Wilk normality	Non-parametric Test used	Significance (exact p value)
Figure 1b	Mouse (TSA infarct size)	11	Passed	Mann Whitney test	IF/AAR, DMSO vs TSA, p=0.0128
Figure 1e	Mouse (TSA FS)	10	Passed	Friedman test, with Dunn's post test of two groups	FS, DMSO vs TSA, Day1 p=0.0394, Day3 p=0.0052, Day7 p=0.0001, Day14 p=0.024
Figure 1g	Mouse (SAHA infarct size)	6-7	SAHA 30 passed, DMSO, SAHA50 n too small	Anova Kruskal-Wallis test, with Dunn's post test of two groups	IF/AAR, DMSO vs SAHA50, p=0.0179
Figure 1i	mouse (SAHA FS)	6-7	SAHA 30 passed, DMSO, SAHA50 n too small	Anova Kruskal-Wallis test, with Dunn's post test of two groups	Post IR FS, DMSO vs SAHA Pre, p=0.0381
Figure 3b	Rabbit (SAHA infarct size)	7	All group passed	Anova Kruskal-Wallis test, with Dunn's post test of two groups	IF/AAR, DMSO vs SAHA Pre, p=0.0015, DMSO vs SAHA Reper, p=0.0423
Figure 3f	Rabbit (SAHA FS)	7	All group passed	Anova Kruskal-Wallis test, with Dunn's post test of two groups	Post IR FS, DMSO vs SAHA Pre, p=0.0083, DMSO vs SAHA Reper, p=0.0025

Supplemental Table 2. Non-parametric statistical analyses of all animal studies

Supplemental Figure Legends

Supplemental Figure 1. SAHA reduces infarct size in rabbit I/R model. a. SAHA pretreatment reduced infarct size normalized to area at risk (IF/AAR) as determined by TTC staining (n=3-6, $p<0.05$). All rabbits were subjected to I/R (30 min/24 hour). **b.** There were no significant differences in AAR among groups. **c.** In a non-randomized cohort, both SAHA pretreatment and reperfusion-only treatment reduced IF/AAR (n=5-7, $p<0.01$). **d.** There were no significant differences in AAR among groups. **e**. Data from both randomized and non-randomized cohorts are combined. SAHA reduced IF/AAR significantly (n=12-14, $p<0.001$). **f.** There were no significant differences in AAR among groups. **g.** Data from both randomized and non-randomized cohorts are combined. SAHA partially preserved systolic function quantified as %FS (n=12-14, $p<0.001$). **h**. Declines in contractile performance after I/R, measured as %FS, were significantly blunted in the SAHA treatment group (n=12-14, $p<0.001$).

Supplemental Figure 2. SAHA decreases apoptosis by TUNEL. a. TUNEL staining of three tissue zones: infarct zone, border zone, and remote zone. Green, TUNEL-positive nuclei, Red, PI staining of nuclei. Rabbits were subjected to I/R (30 min/2 hour). **b.** Quantification of TUNEL-positive nuclei. SAHA treatment significantly reduced TUNEL-positive nuclei in the I/R border zone (n=3, $p<0.01$). **c.** Merged image of DIC and TUNEL staining reveals that TUNEL-positive cells are predominantly within striated myocytes. **d.** Adult mouse thymus was used as a positive control for TUNEL staining.

Supplemental Figure 3. SAHA decreased apoptosis in the infarct border zone as measured by cleaved caspase-3. a. Rabbit myocardium samples were harvested as depicted. Red: infact zone, Green: remote zone, Yellow: border zone. **b. Upper:** Immunoblot analysis of cleaved caspase 3. **Lower:** Quantification of cleaved caspase 3 revealed that levels were significantly lower in the SAHA-treated group (n=4, $p<0.05$).

Supplemental Figure 4. SAHA induces autophagic flux in adult rat ventricular myocytes subjected to simulated IR. Upper: Western blots. **Lower:** mean data. SAHA pretreatment induced autophagic flux in ARVMs at baseline and after simulated ischemia 2hr (I2h). Furthermore, SAHA treatment exclusively at reperfusion induced autophagic flux after simulated ischemia (2hr) plus reperfusion (2hr) [I2R2].

Supplemental Figure 5. . SAHA protects ARVMs from cell death after simulated IR. SAHA treatment (2μM) at reperfusion reduced cell death around 60% after simulated I/R (sI/R; 2hour/2 hour) (n=4, $p<0.05$).

Supplemental Figure 6. . SAHA's cardioprotective effects are dependent on autophagic flux as tested using a second, sequence-independent set of ATG7 and ATG5 siRNAs. a. LDH cell death assays were conducted in the settings of ATG7 or ATG5 knockdown using sequence-independent siRNA constructs (ATG7II and ATG5II). Suppression of either ATG7 or ATG5 abolished SAHA's cardioprotective effects after sI/R (5 hour/1.5 hour) (n=3, $p<0.05$). **b.** RNAi knockdown of ATG7 or ATG5 using ATG7II and ATG5II siRNA. This independent set of siRNAs suppressed ATG7 and ATG5 levels as efficiently as the original set of constructs. **Left:** Western blots. **Right:** mean data.

Supplemental Figure 7. TSA and SAHA induce autophagic flux at early time points, and prolonged TSA treatment reduces autophagic flux in serum-free conditions. Upper: Autophagic flux measurement by LC3 II Western blot in the presence/absence of Bafilomycin A (Bfa) in NRVM treated with TSA. **Lower:** Quantification (n=3). **, $p<0.01$; *, $p<0.05$ compared with DMSO control.

Supplemental Tables

Supplemental Table 1. Experiment paradigm and statistical methods used. Methods employed in each figure are listed. In the animal studies, if the data did not pass criteria for normality, non-parametric analysis was performed. In the molecular studies, parametric analyses were performed throughout. The cell death assay data fulfilled normality. Statistical significance is reported if $p<0.05$.

Supplemental Table 2. Non-parametric statistical analyses of all animal studies. As the animal sample sizes are low, and in an effort to maximize stringency, we also conducted non-parametric analyses in all animal studies. In each case, these analyses confirmed the statistical significance derived from parametric analyses.