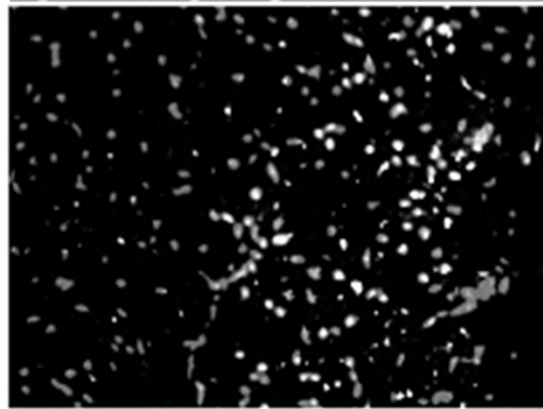
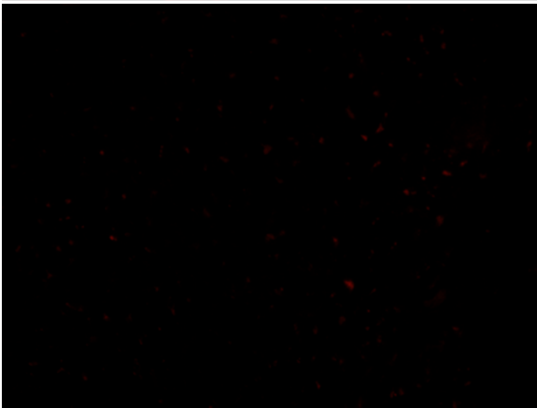
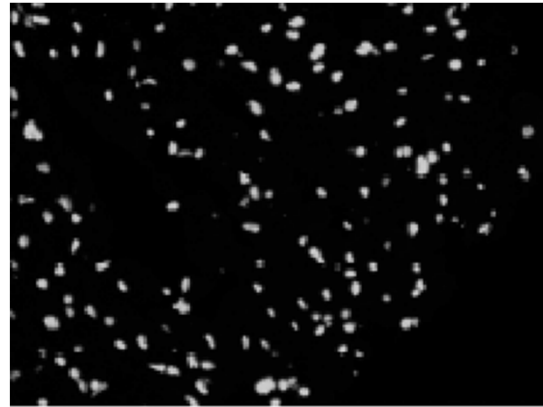
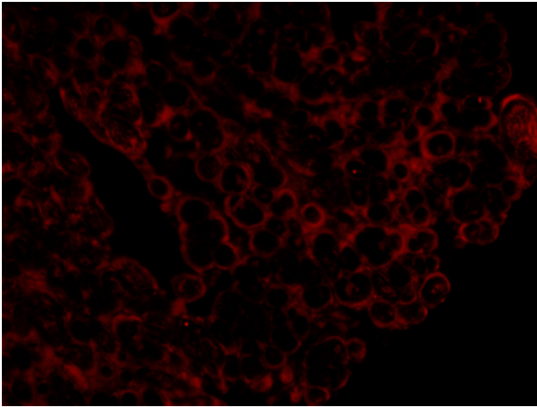


Cardiac Findings	Infarcted	Non-Infarcted
Patient#	11	11
Male/Female	6/5	5/6
Mean Age	66	62
RISK FACTORS		
Hyperlipidemia	8 (73%)	1 (36%)
Hypertension	7 (63%)	5 (45%)
Obesity	5 (45%)	5 (45%)

Supplementary Table 1. Autopsy Study Population. Table of patient characteristics for human study in Figure 1.

anti-CD47

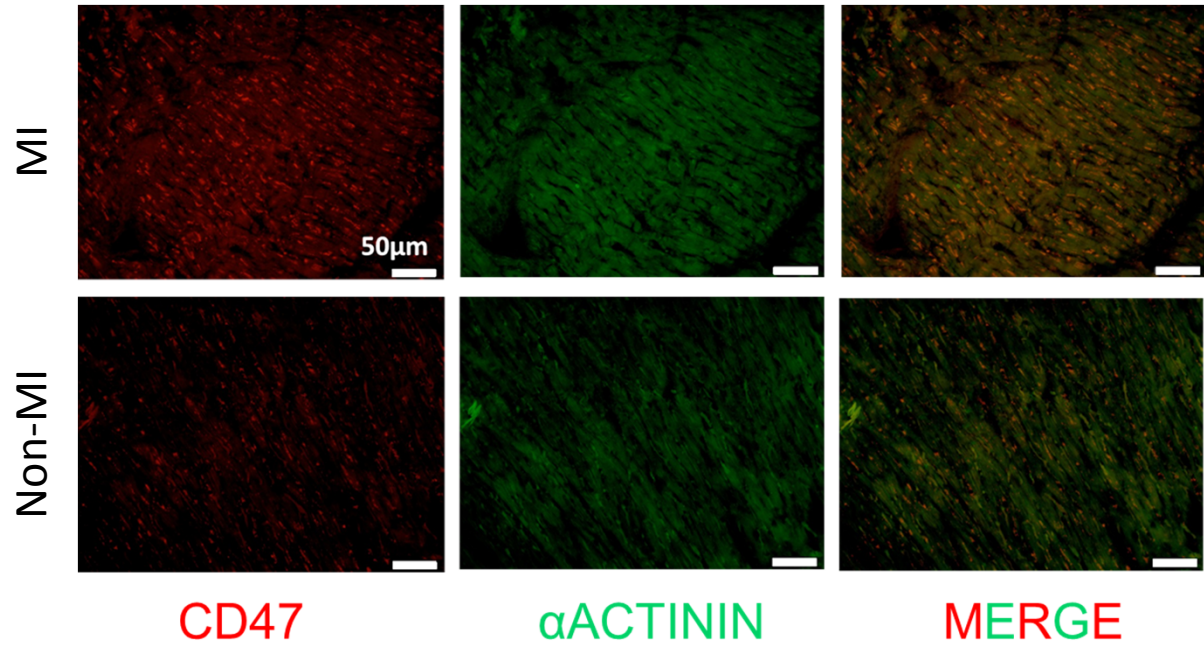
nuclei



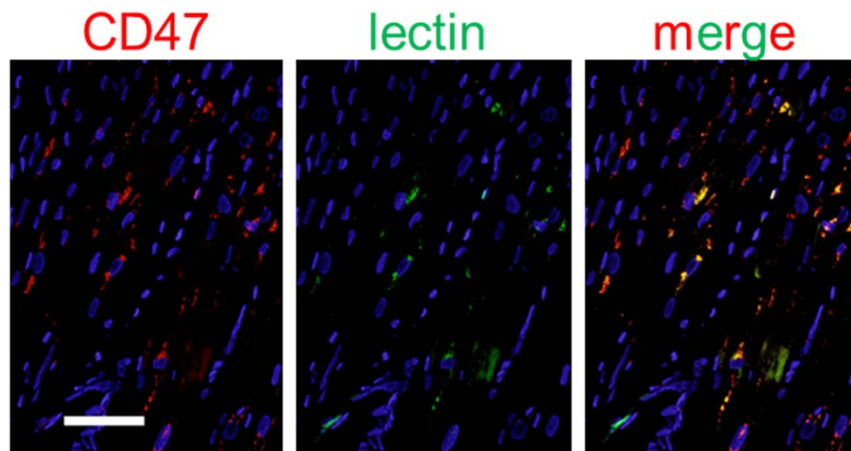
isotype control

nuclei

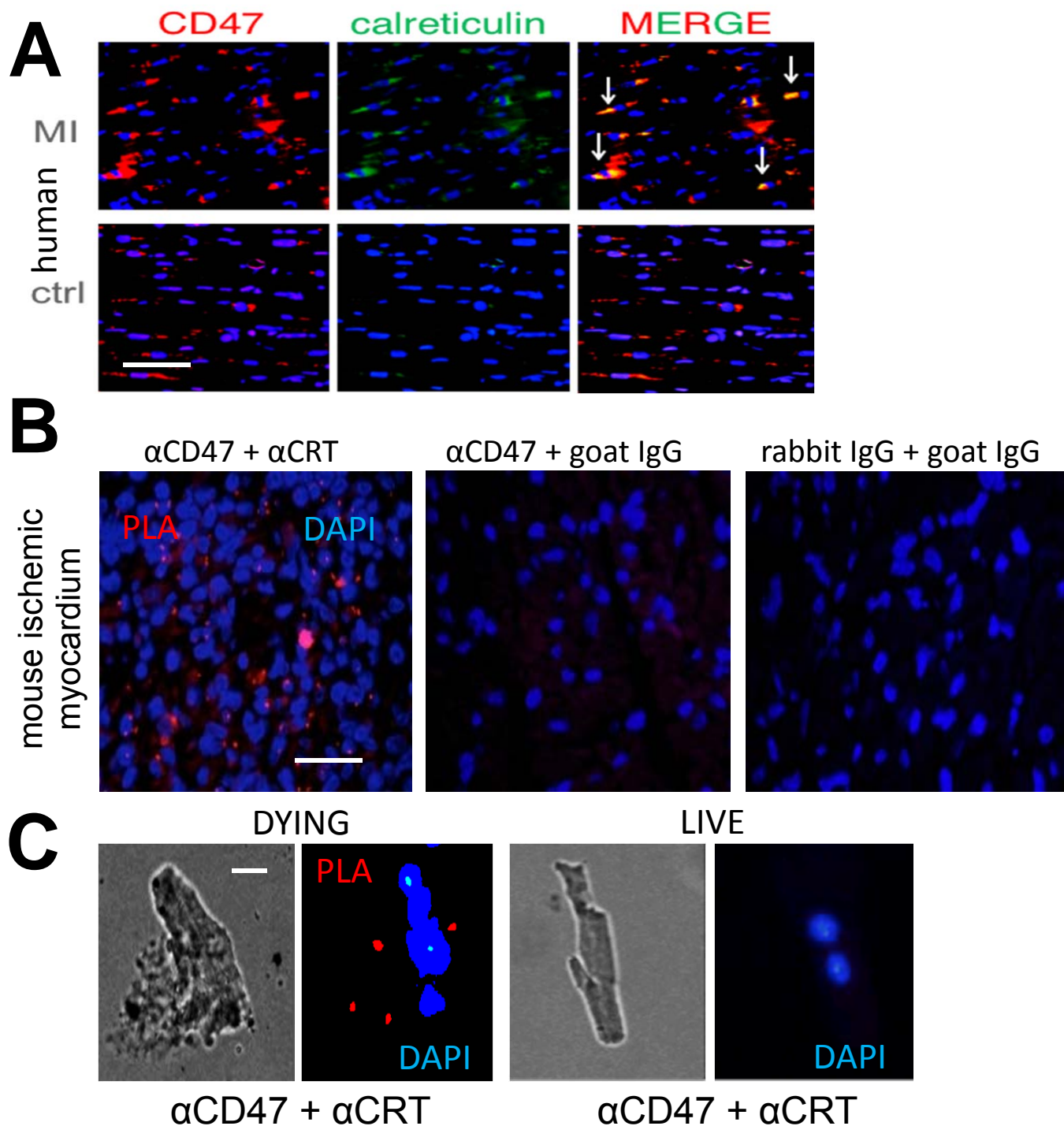
Supplemental Figure 1. CD47 staining of human cardiac tissue. Primary Antibody: Rabbit pAB-CD47 (Abcam ab175388) (1:100). Secondary antibody: Alexa Fluor 594 Donkey α -Rabbit IgG(H+L) (1:100). Nuclei stains are DAPI.



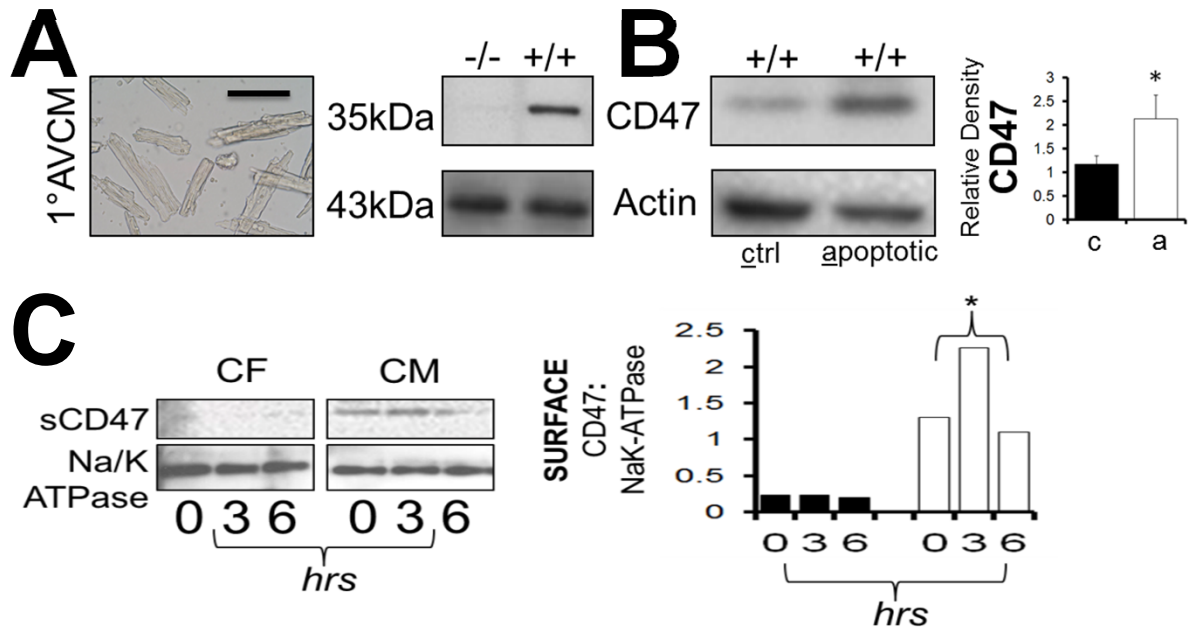
Supplemental Figure 2. CD47 staining of human myocardial samples. Autopsy heart immunohistochemistry from myocardial infarction individuals (top panel) versus non-MI (bottom panel) were stained with antibodies against CD47 (red) and cardiomyocyte-marker alpha-actinin (green). To the right is the merge of red and green channels. Scale bar is 50 μ m.



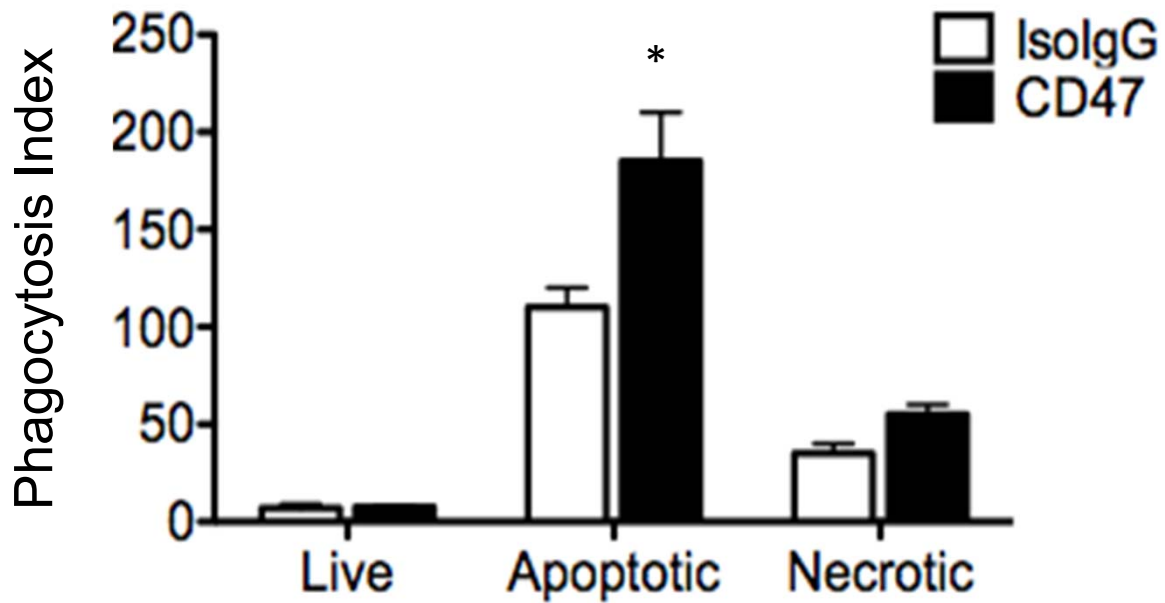
Supplementary Figure 3. Cardiac CD47 co-localization with endothelial lectin from human patients post myocardial infarction. Representative images are shown. Scale Bar is 100 μm . CD47 antibody is Abcam ab175388. Isolectin antibody from Thermo Fisher Scientific I21411. DAPI nuclei are blue.



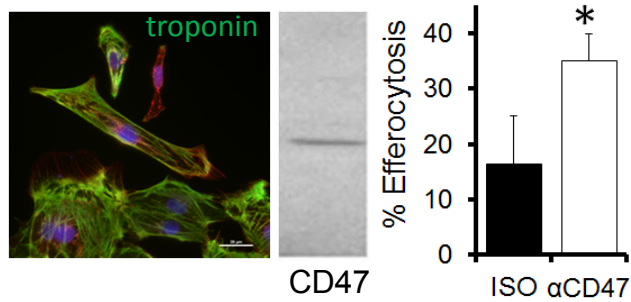
Supplemental Figure 4. CD47 and calreticulin staining on human and mouse hearts and cells. (A) Top panel images depict immunohistochemistry for indicated markers from myocardial infarction (MI) and non-MI (ctrl) human hearts. Scale bar is 50 μ m. (B) Proximity Ligation Assay (PLA) for CD47 and Calreticulin colocalization in ischemic mouse heart and on Scale bar is 50 μ m. (C) primary adult, differentiated mouse cardiomyocytes. For B: Paraffin embedded mouse heart was stained for CD47 (abcam ab175388 rabbit polyclonal to CD47) and calreticulin (Santa Cruz SC6468 goat polyclonal to caveolin-3) for PLA. Calreticulin antibody (goat) and CD47 antibody (rabbit). RIgG, rabbit isotype IgG. GIgG, goat isotype IgG. For C: Tissue cultures were treated with α CD47 and α Calreticulin for PLA. In PLA, red dots on images indicate that the two proteins are close enough for the probe to amplify and emit a red signal. Anti-calreticulin (CRT) is from Goat and anti-CD47 from rabbit. Scale bar is 15 μ m.



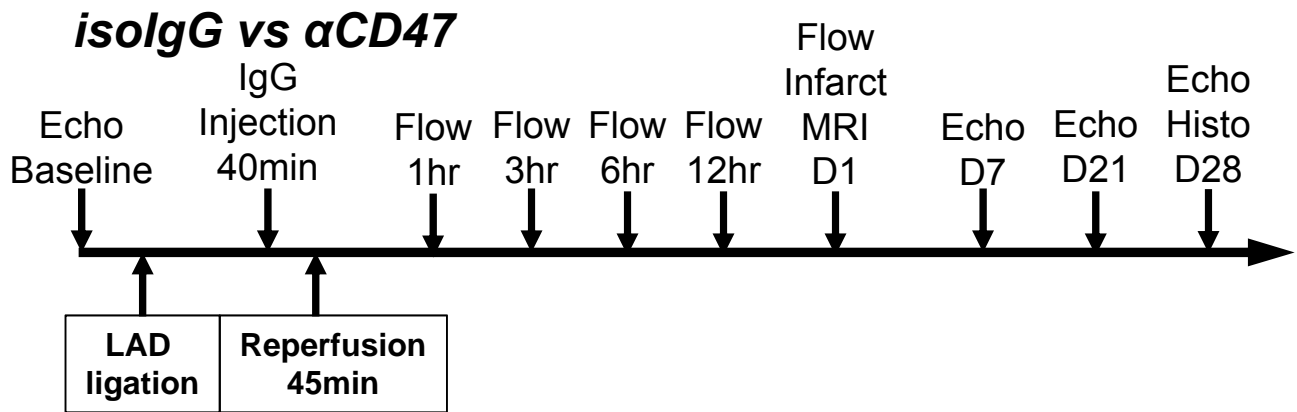
Supplemental Figure 5. CD47 surface protein is induced in primary adult cardiomyocytes (CMs) during apoptosis. (A) Primary adult ventricular cardiomyocytes (AVCMs) from mice were isolated (image; scale bar = 60 μ m) and extracts from $Cd47^{-/-}$ versus $Cd47^{+/+}$ cell lysates were subjected to Western blot for CD47. (B) AVCMs were cultured in staurosporine (STS) at 1 μ M to induce apoptosis and representative image is of Western blot performed from lysates taken 3 hours post apoptotic stimuli. Bar graph scale is quantitation of ctrl (c) versus apoptotic (a) bands by densitometry. * indicates $p = 0.04$ relative to control. (C) Western Blot shows kinetics of surface (biotinylated) CD47 expression in adult mouse primary cardiac fibroblasts (CFs) versus AVCMs after indicated times post apoptotic stimulus. Surface CD47 was quantified by densitometry and normalized to surface Na-K ATPase. * indicates $p = 0.04$ relative to control time points.



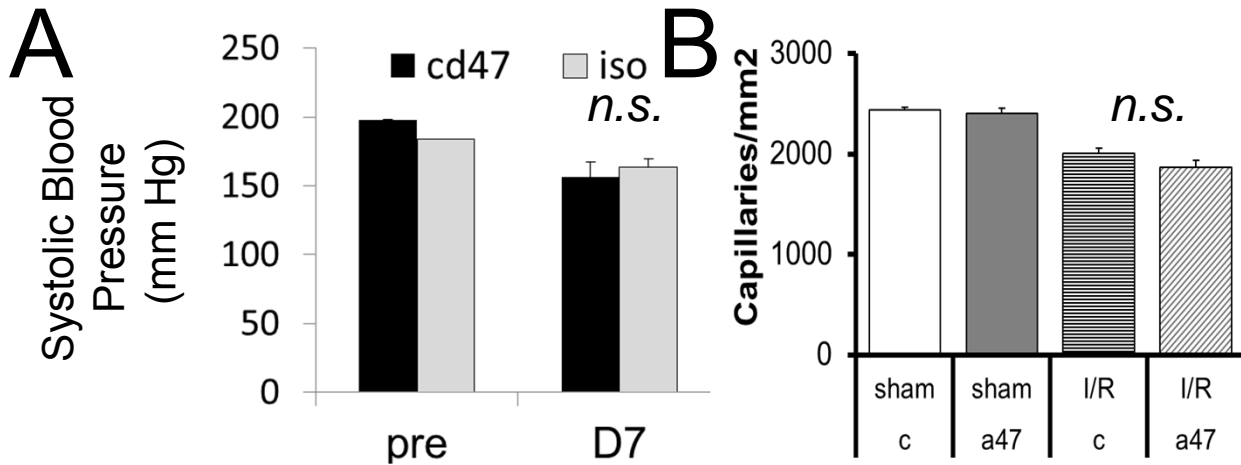
Supplemental Figure 6. Comparative analysis of phagocytosis enhancements by CD47 blockade. H9C2 CMs cells were treated induced to apoptosis by staurosporine or were subjected to multiple freeze/thaw cycles to induce necrosis. Green fluorescently labeled apoptotic, necrotic, and live H9C2 cells were fed to primary peritoneal macrophages for 90mins followed by observing under a fluorescent microscope for efferocytosis quantification. Phagocytosis Index was calculated as number of CM-bodies taken up by macrophages, divided by total macrophage number. P = 0.04.



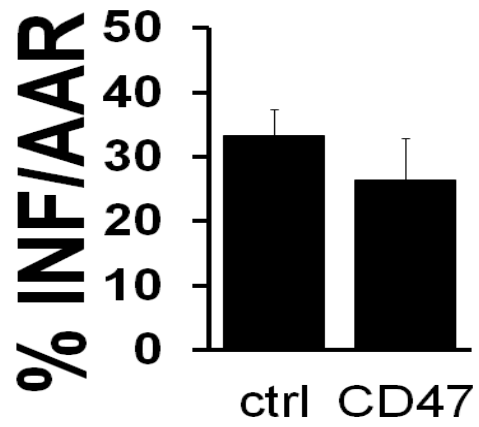
Supplemental Figure 7. CD47 is expressed in human iPSCMs and its blockade enhances efferocytosis. Western Blot of CD47 from iPSCMs (micrograph; cardiac troponin TNNT2 is green and ACTN2 actinin is red). iPSCMs were fluorescently labelled and treated with isotype (ISO) control versus anti-CD47 and added to human monocyte-derived macrophages to quantify efferocytosis. P = 0.03.



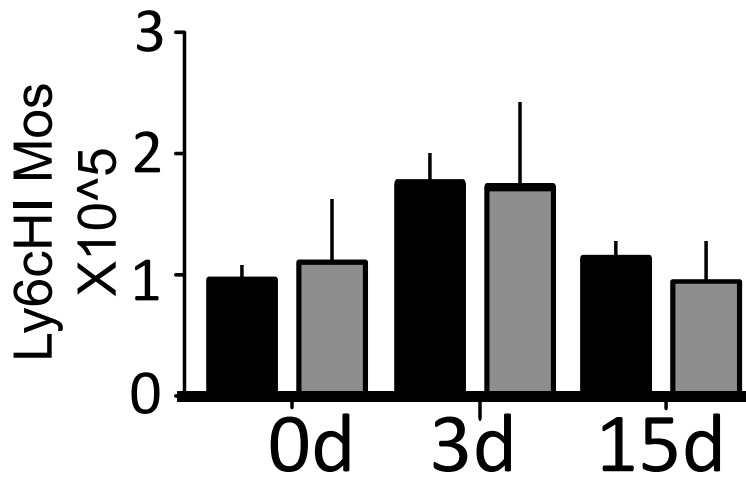
Supplemental Figure 8. Scheme for in vivo experiments. Before and after ischemia reperfusion, hearts of control (*isIgG* injected) vs experimental (α CD47 *igG* injected) mice were assessed by Echocardiography, Histology, and MRI for cardiac function and repair, and Flow cytometry for efferocytosis and inflammatory cells.



Supplemental Figure 9. Acute CD47 blockade does not significantly alter systolic blood pressure nor markers of angiogenesis. (A) Systolic blood pressure was measured on conscious mice using CODA tail-cuff system, pre-surgery and also on day/D7 after ischemia/reperfusion on both CD47 and isotype/IgG injection groups. No significant (n.s). change was detected between the two groups. (B) Angiogenesis was assessed by lectin antibody staining in the border zone +/- aCD47 treatment, 28 days post I/R.



Supplemental Figure 10. Infarct sizes post ADENO-CD47 treatment vs. control. Shown are infarcts per area at risk (AAR).



Supplemental Figure 11. Cellular monocyte inflammation levels in blood post I/R +/- anti-CD47 treatment. Flow cytometric analyses of myeloid phagocytes at indicated times post I/R from circulating blood.