SUPPLEMENTAL MATERIAL

Supplemental Methods

Surgical Procedures

All animals were loaded with oral 400mg twice daily of amiodarone over 5d prior to inducing myocardial ischemia to reduce the incidence of fatal arrhythmias post-balloon occlusion. Animals were sedated with 2mg/kg Telazol® (tiletamine/zolazepam, 1:1) IM prior to central line placement and 10mg/kg ketamine IV prior to all cardiac catheterization and imaging procedures. Additionally, animals received 1g cefazolin IV for pre-procedural antibiotic prophylaxis. General anesthesia was maintained with 1-2% inhaled isofluorane. Post-procedural pain was treated with 0.03mg/kg buprenorphine IV and/or a 25mcg/h fentanyl patch transdermally. In order to facilitate blood collection and administration of experimental agents and medications, a tunneled silastic central venous catheter was placed into the external jugular vein in a sterile fashion 2-5d prior to induction of myocardial ischemia. Lines were cleaned with alcohol and flushed daily with 5mL of heparinized saline (100 units/mL).

During the induction of myocardial ischemia, arterial blood pressure, heart rate and electrocardiogram (ECG) tracing, oxygen saturation, and end-tidal carbon dioxide were continuously monitored and recorded every 15-20min. Core body temperature was maintained at 37-38°C with a Bair hugger warming blanket (Arizant Healthcare Inc., St. Paul, MN). A continuous IV infusion of amiodarone 1mg/min was maintained throughout the procedure to prevent arrhythmia. The right femoral artery was accessed percutaneously and cannulated with a 6Fr or 7Fr introducer sheath. A 6Fr 100cm H-STICK guiding catheter (Cordis Corp., Bridgewater, NJ) was inserted and advanced into the right and left main coronary artery under fluoroscopic imaging to perform selective right and left coronary angiography. Following systemic heparinization (10 u/kg), an Apex PTCA 8x2.5mm Balloon Angioplasty Catheter (Boston Scientific Corp., Natick, MA) was then advanced into the mid-segment of the LAD and inflated to 4-6 mmHg, typically distal to the second diagonal branch. Evidence of occlusion (TIMI 0 flow) was confirmed by angiography and ST elevations in anterior leads of the ECG

tracing (Figure 1 in the Data Supplement). Complete reperfusion (TIMI 3 flow) was confirmed by angiography. Ventricular ectopy was treated with Lidocaine 10-20mg IV boluses as needed. The model carried a mortality rate of 16.7% due to intractable fatal ventricular arrhythmia within the ischemic period. Whole blood collection for complete blood counts (CBCs) and serum analysis of cardiac markers, metabolic parameters, and presence of experimental compounds were performed at baseline, 8h, 16h, 24h, and then once daily for 5d reperfusion in both the 5d and 21d treatment groups.

At the termination of the experiment, sharp tissue biopsies of lung, liver, spleen, and kidney were collected and stored in formalin for histopathologic analysis. Cardiac harvest was then performed following median sternotomy, aortic cannulation and cross clamp, followed by infusion of cold cardioplegia.

Area at Risk and Infarct Size Determination

Following cardioplegic arrest and prior to cardiac harvest, the LAD was ligated with a 2-0 silk tie at the precise point of balloon occlusion. Evans blue dye (4%) was infused through the aortic cannula, and thus the unstained region of the left ventricle (LV) defined the area at risk (AAR). Hearts were then explanted and sliced into 4-5 sections of equal thickness starting from the apex. Sections were weighed and then incubated in 1% triphenyltetrazolium chloride (TTC) for 37^0C for 15 min, which stains viable tissue in red and infracted tissue in white (Figure 2 in the Data Supplement). Sections were then photographed on both sides using a Nikon Coolpix 8800 digital camera (Nikon Inc., Melville, NY). Infarct size was then determined by computerized planimetry using Adobe Photoshop Software, Version 7 (Adobe Systems Inc., San Jose, CA) as previously described.[1] Infarct size was calculated as a percentage of the AAR as well as the total area of the LV.

Production and Labeling of m21G6

Production of m21G6 antibody was as previously described.[1] Briefly, hybridoma cells were grown in roller bottles for 15d in IMDM media (Mediatech Inc., Herndon, VA) supplemented with

Primatone, Kanamycin, 2% low Immunoglobin Fetal Calf Serum (Life Technologies Corp., Carlsbad, CA) and 2-mercaptoethanol. The antibody was purified using 5ml HiTrap Protein G column (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) and labeled with Alexa 568 dye (Life Technologies Corp., Carlsbad, CA). The final purified m21G6 was tested for endotoxin and determined to be 0.015 EU/mg (Associates of Cape Cod Inc., Falmouth, MA).

m21G6 Half-life Determination in Swine

Serum samples from swine injected with fluorescent-labeled Mab (21G6 Alexa 568) were collected at different time points (30min, 4h, 8h, 24h, 48h, 72h, 96h and 120h). Circulating levels of mouse anti-non muscle myosin (21G6) were detected by ELISA. Plates were coated with goat anti-mouse IgG (Southern Biotech Inc., Birmingham, AL) and two-fold dilutions of swine serum were incubated for 1h and detected with a goat anti-mouse IgG1 alkaline phosphatase (Southern Biotech Inc., Birmingham, AL). A standard curve was plotted using serial dilutions of purified m21G6.

Immunohistochemistry

Punch biopsies of swine heart were harvested as described above, embedded in Optimal cutting temperature compound (OCT), and frozen in a dry ice-isopentane bath. Sections were cut, fixed in acetone for 10min, blocked with phosphate buffered saline (PBS), 1% bovine serum albumin (BSA), and 0.5% Tween 20 and incubated with mouse anti-pig CD31 FITC (MCA1746 AbD Serotec/Bio-Rad Laboratories Inc., Raleigh, NC). Images were obtained with an Olympus Fluoview FV1000 Confocal System (Olympus America Inc., Center Valley, PA). For all treatment groups, punch biopsies were taken from the liver, lung, kidney, spleen, and thoracic lymph nodes and embedded in OCT and frozen as well as fixed by immersion in 10% neutral buffered formalin. Hematoxylin and eosin (H&E) staining was performed on all biopsied tissue.

Power Calculations

The sample size calculation for the 21d functional arm of this study including all time points (i.e. 1h, 7d, and 21d) was determined by a power analysis of the %Infarct/AAR data in the 5d study.

We assumed a common standard deviation of 9.2 and calculated 80% power for differences in group means of 26.77 (n=3 per group) or 21.53 (n=4 per group). Non-survival experiments were concluded once statistical significance was achieved to prevent the unnecessary use of research animals in accordance with the MGH Institutional Animal Care and Use Committee Regulations.

Supplemental Tables

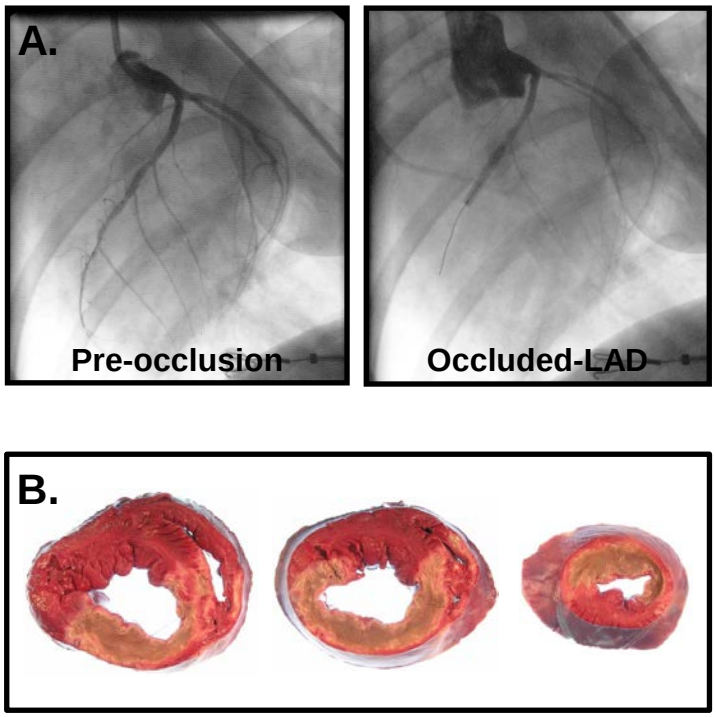
Suppl. Table 1

	Saline			m21G6		
	Baseline	**1h post-reperfusion**	**21d post-reperfusion**	**Baseline**	**1h post-reperfusion**	**21d post-reperfusion**
HR (beats/min)	86.7 ± 5.9	99.0 ± 4.4[a]	88.0 ± 1.2	85.3 ± 4.8	95.3 ± 3.8	81.3 ± 1.8[b]
SBP (mmHg)	132.7 ± 9.8	93.3 ± 2.3[a]	99.7 ± 3.2[a]	111.0 ± 7.1[a]	96.7 ± 3.4	105.7 ± 5.2
DBP (mmHg)	85.0 ± 10.2	63.7 ± 5.8[a]	67.0 ± 5.5[a]	67.7 ± 2.0	66.0 ± 2.1	70.7 ± 4.2
SV (mL)	19.9 ± 4.0	16.8 ± 4.8	20.0 ± 2.1	21.7 ± 0.5	20.8 ± 0.4	19.6 ± 1.8
CO (mL/min)	1678.3 ± 268.6	1635.0 ± 451.7	1761.3 ± 189.4	1850.1 ± 120.0	1979.9 ± 91.3	1586.3 ± 115.7

Suppl. Table 1. Hemodynamic status and LV function. Summary of hemodynamic parameters and 2D TTE left ventricular (LV) functional parameters at baseline (before 1h occlusion), 1h reperfusion, and 21d reperfusion. Data is presented as mean ± SEM and analyzed by two-way repeated measures ANOVA. For post-hoc comparisons, Tukey's HSD test was used (alpha=0.05). **[a]**, $p<0.05$ vs BL saline. **[b]**, $p<0.05$ vs 1h m21G6. HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; SV = stroke volume; CO = cardiac output.

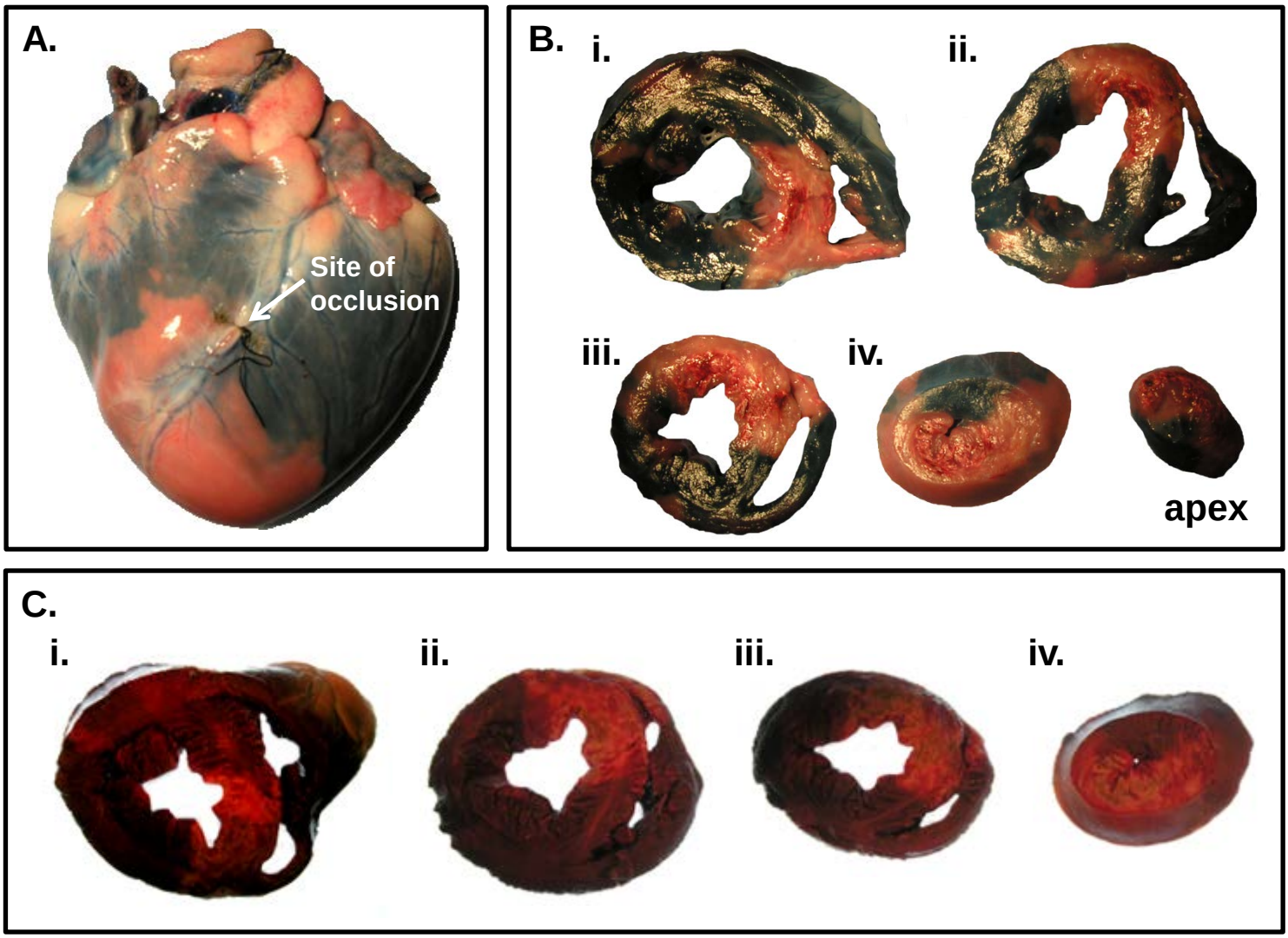
Supplemental Figures and Figure Legends

Suppl. Fig. 1



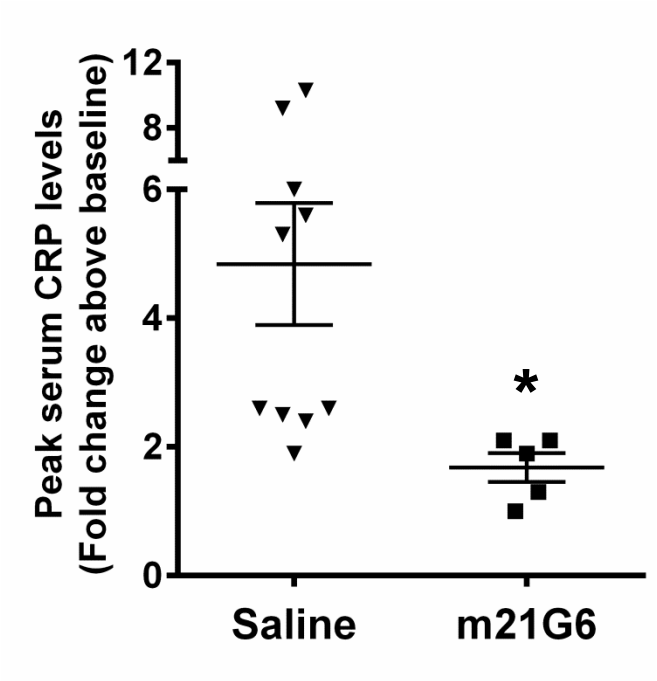
Suppl. Figure 1. Representative angiographic images and TTC-stained heart sections in swine. (A). Angiograms of swine cardiac vasculature were taken prior balloon catheter occlusion of the LAD and at regular intervals throughout occlusion. (B). Swine were subjected to 1h LAD occlusion and 5d reperfusion. Following 5d reperfusion, myocardial sections were staining with Evan's blue and TTC and myocardial infarct size expressed as a percentage of the area at risk.

Suppl. Fig. 2



Suppl. Figure 2. Representative example of TTC-stained swine heart sections. Swine were subjected to 1h LAD occlusion and 5d reperfusion. Hearts were then harvested and perfused with Evan's blue and stained with TTC as described in Methods. (A). Evan's blue perfused heart prior to sectioning. (B). Evan's blue perfused hearts post-sectioning. (C). TTC-stained heart sections. Roman numerals correspond to respective sections in (B) and (C).

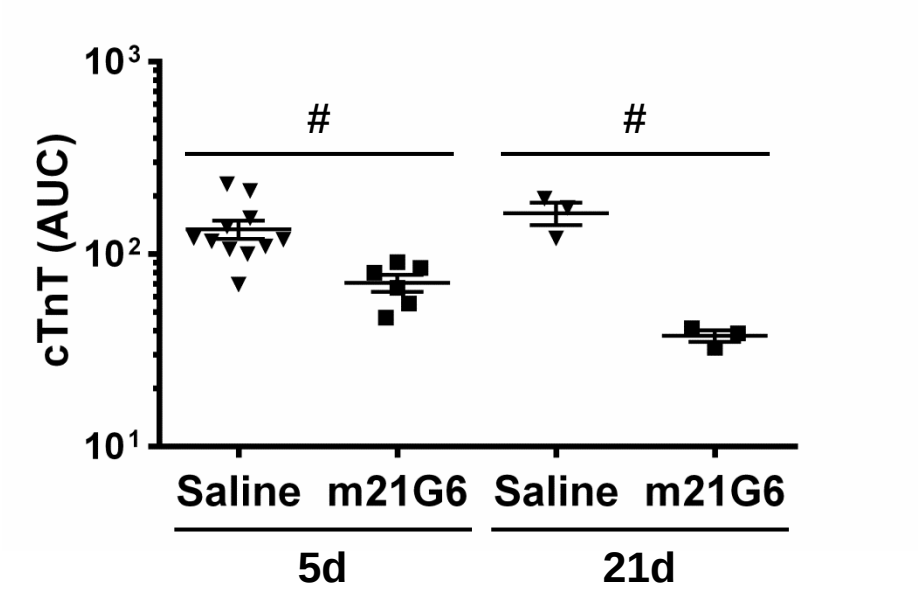
Suppl. Fig. 3



Suppl. Figure 3. Effect of m21G6 on CRP levels. Swine were subjected to 1h LAD occlusion and 5d reperfusion. Prior to reperfusion either saline or m21G6 (2mg/kg) was injected IV. Serum samples were taken at several time points as described in text and analyzed for serum CRP levels. Data is represented as the fold difference between baseline and peak CRP levels in each animal. *, $p<0.01$. N=10 and N=5 for saline and m21G6, respectively.

Suppl. Fig. 4

A



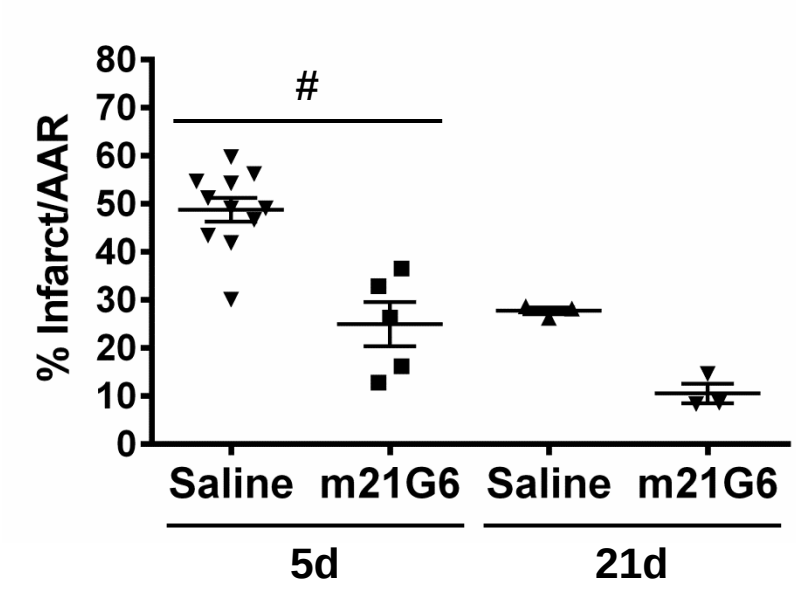
B

cTnT (AUC)					
Source	**Sum Sq.**	**d.f.**	**MeanSq.**	**F**	**Prob>F**
Tx	**4.65**	**1**	**4.65**	**52.12**	**0**
Day	**0.12**	**1**	**0.12**	**1.39**	**0.25**
Tx*Day	**0.66**	**1**	**0.66**	**7.44**	**0.01**
Error	**1.61**	**18**	**0.09**		
Total	**6.63**	**21**			

Suppl. Figure 4. Effect of m21G6 on cTnT (AUC) at 5d and 21d reperfusion. Swine were subjected to 1h LAD occlusion and either 5d or 21d reperfusion. Prior to reperfusion either saline or m21G6 (2 mg/kg) was injected IV. Serum samples were taken at several time points as described in text and analyzed for cTnT (AUC). (A) cTnT (AUC) measurements at 5d and 21d. (B) Two-way ANOVA with interaction summary. Each symbol represents data from one animal. Tx=Treatment. #, $p<0.05$.

Suppl. Fig. 5

A



B

%I/AAR					
Source	**Sum Sq.**	**d.f.**	**MeanSq.**	**F**	**Prob>F**
Tx	**1745.9**	**1**	**1745.9**	**27.9**	**0.0001**
Day	**1315.1**	**1**	**1315.1**	**20.9**	**0.0002**
Tx*Day	**45.4**	**1**	**45.4**	**0.72**	**0.41**
Error	**1130.9**	**18**	**62.8**		
Total	**5672.0**	**21**			

Suppl. Figure 5. Effect of m21G6 on infarct size at 5d and 21d reperfusion. Swine were subjected to 1h LAD occlusion and either 5d or 21d reperfusion. Prior to reperfusion either saline or m21G6 (2 mg/kg) was injected IV. Following 5d or 21d reperfusion, myocardial sections were staining with Evan's blue and TTC and myocardial infarct size expressed as a percentage of area at risk (AAR). (A) %I/AAR measurements at 5d and 21d. (B) Two-way ANOVA with interaction summary. Each symbol represents data from one animal. Tx=Treatment. #, $p<0.05$.

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Supplemental References

1. Haas MS, Alicot EM, Schuerpf F, Chiu I, Li J, Moore FD, Carroll MC. Blockade of self-reactive IgM significantly reduces injury in a murine model of acute myocardial infarction. *Cardiovasc Res* 2010;87:618-627.