Intravenous High Mobility Group Box 1 Fragment Improves Cardiac Function, Fibrosis, and Coronary Flow in Porcine Ischemic Cardiomyopathy Model

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Institutions and affiliations

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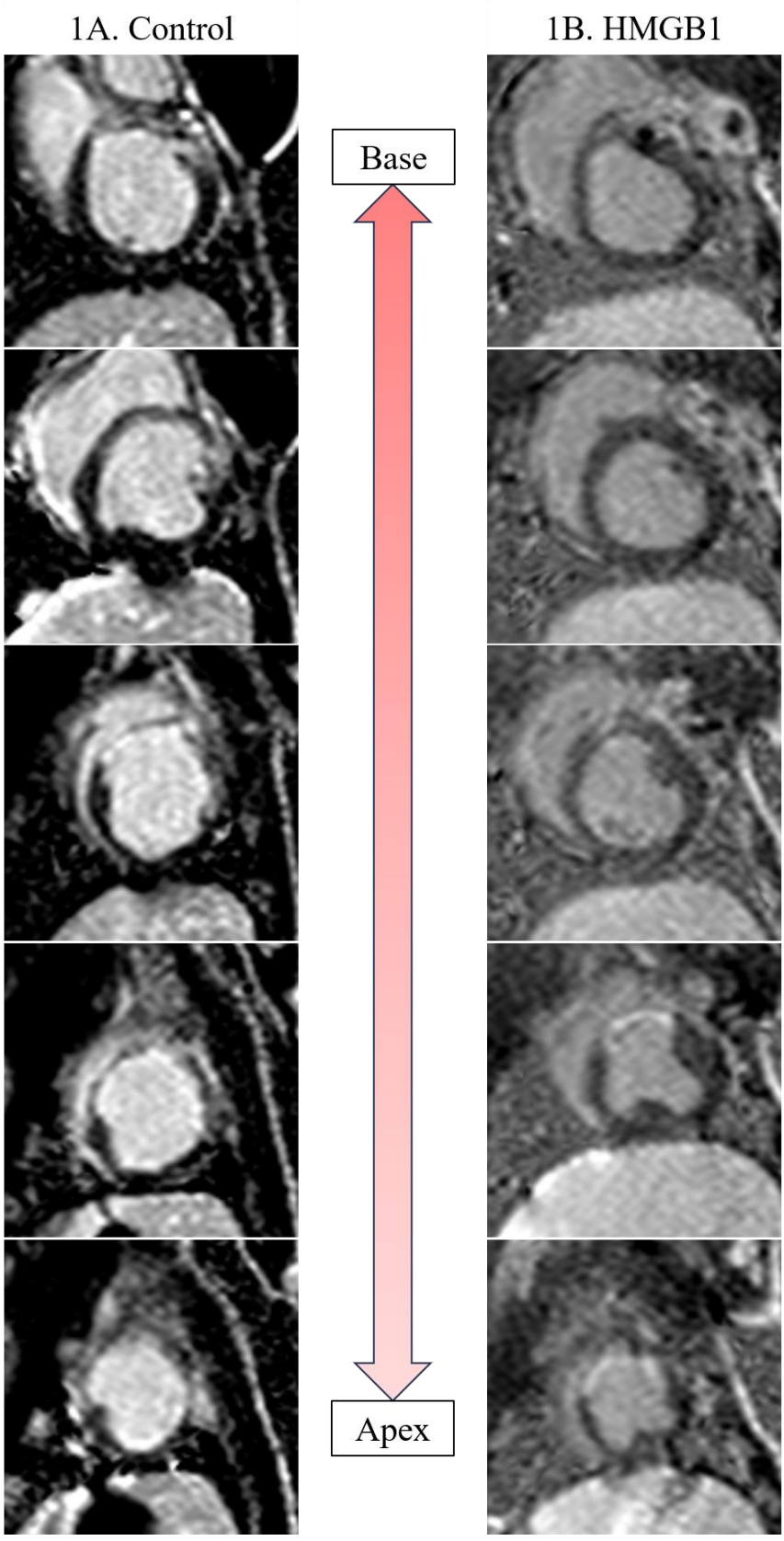
Supplemental Figure 1

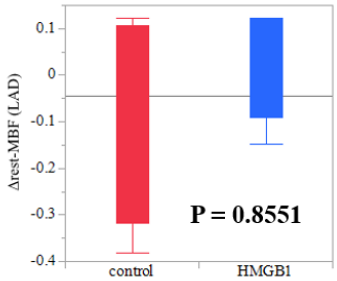


Supplemental Figure 1. Representative consecutive short-axis sections of the LV by MRI with LGE

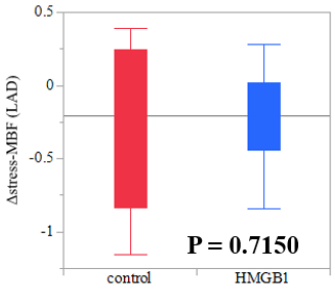
Cardiac MRI images with LGE showing consecutive short-axis sections of the LV in control (1A) and HMGB1-treated (1B) groups following treatment. LGE, late gadolinium enhancement; LV, left ventricle; MRI, magnetic resonance imaging.

Supplemental Figure 2

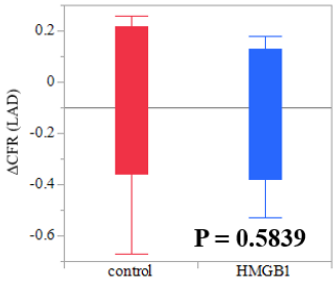
2A. Rest MBF in LAD region



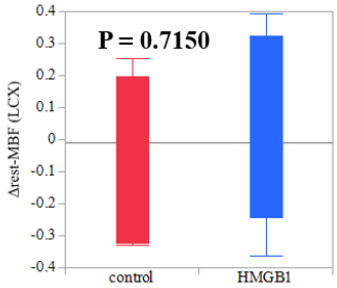
2B. stress MBF in LAD region



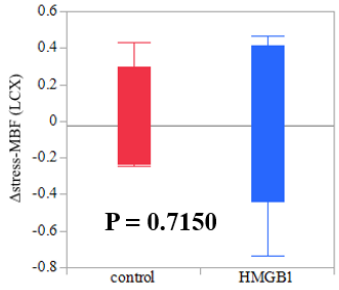
2C. CFR in LAD resion



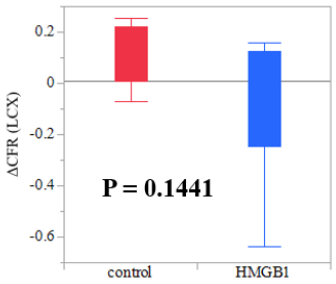
2D. Rest MBF in LCX region



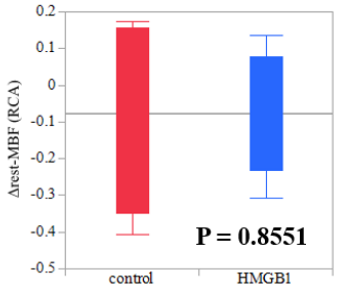
2E. stress MBF in LCX region



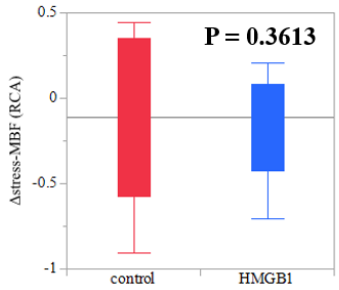
2F. CFR in LCX resion



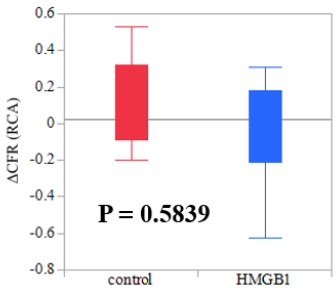
2G. Rest MBF in RCA region



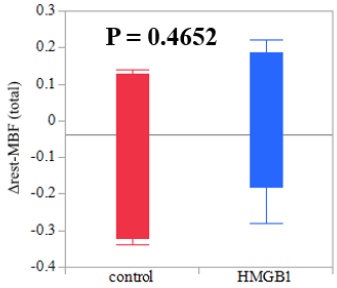
2H. stress MBF in RCA region



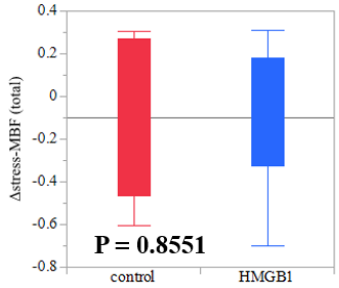
2I. CFR in RCA resion



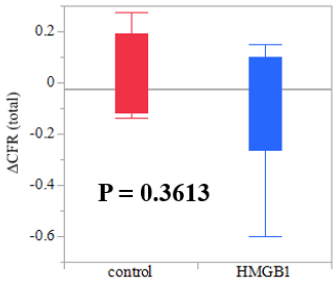
2J. Rest MBF in total



2K. stress MBF in total



2L. CFR in total

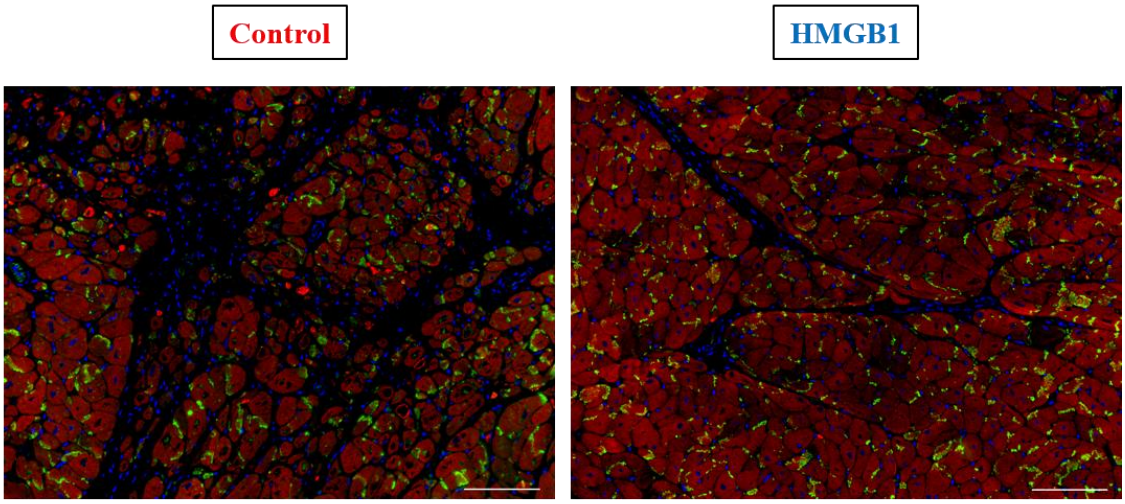


Supplemental Figure 2. Changes of MBF and CFR by ^{13}N-ammonia PET

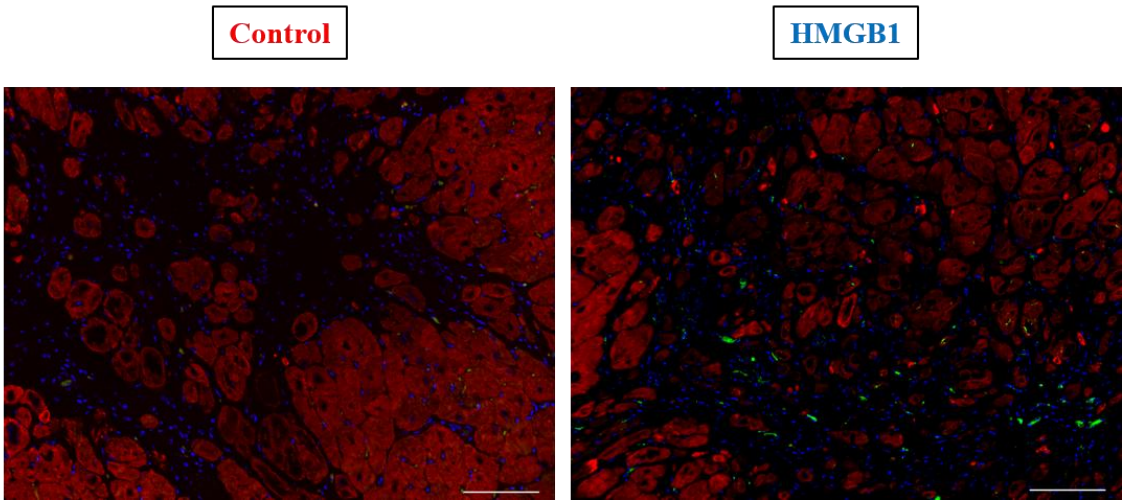
Changes in resting MBF, stress MBF, and CFR in each coronary region by group. (2A-2C) Rest MBF, stress MBF, and CFR in the LAD region. (2D-2F) Rest MBF, stress MBF, and CFR in the LCX region. (2G-2I) Rest MBF, stress MBF, and CFR in the RCA region. (2J-2L) Rest MBF, stress MBF, and CFR. CFR, coronary flow reserve; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; MBF, myocardial blood flow; PET, positron emission tomography; RCA, right coronary artery.

Supplemental Figure 3

3A. Troponin T (red) and Connexin 43 (green) staining



3B. Troponin T (red) and PDGFRα (green) staining

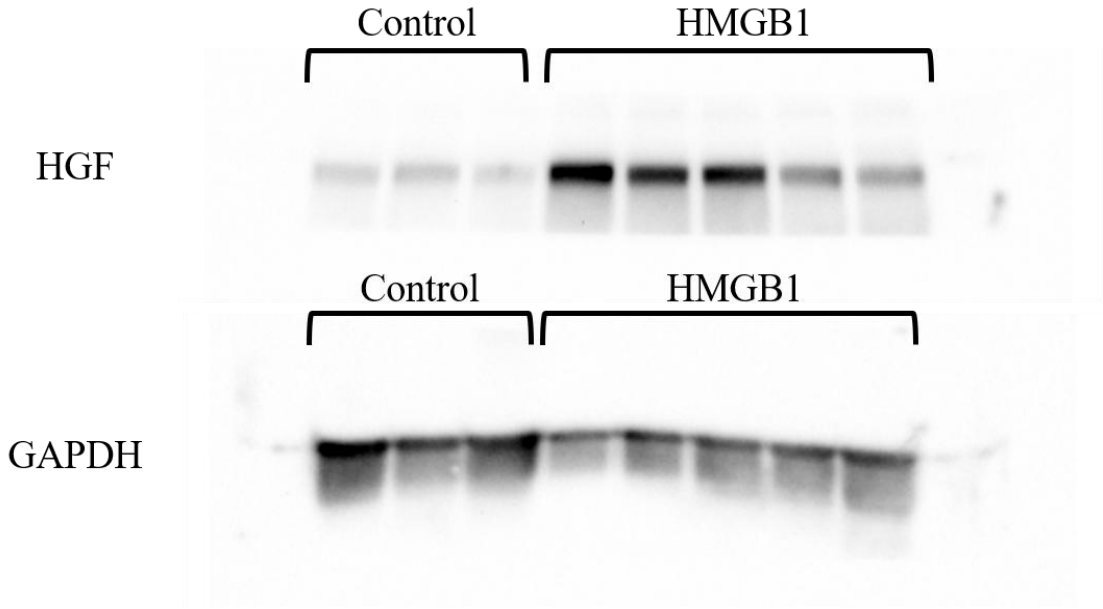


Supplemental Figure 3. Immunofluorescence staining

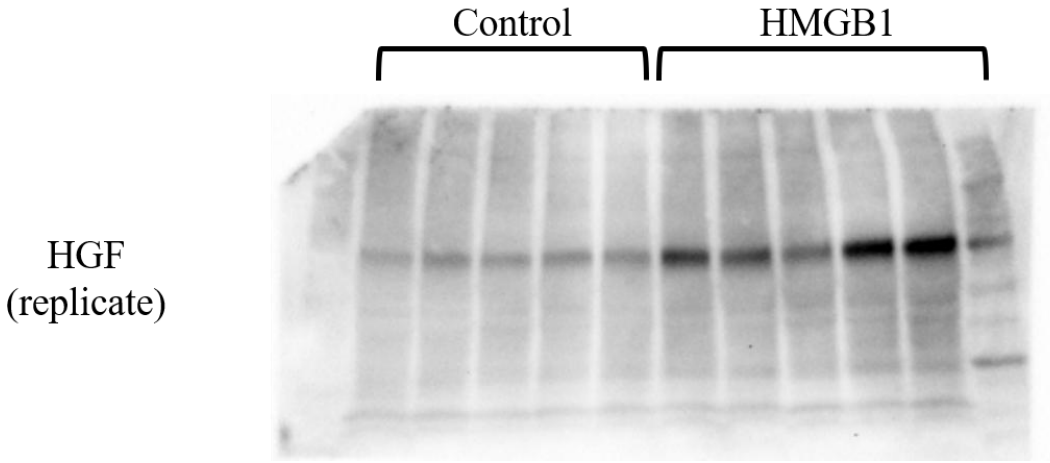
(3A) Representative photographs of Troponin T (red) and Connexin 43 (green) staining in each group. the HMGB1 group showed preserved myocardial cell structure and well-organized alignment of junctional proteins. (3B) Representative photographs of Troponin T (red) and PDGFR α (green) staining in each group. A greater number of PDGFR α positive cells were observed in the HMGB1 group. HMGB1, high mobility group box 1; PDGFRα, Platelet-Derived Growth Factor Receptor α.

Supplemental Figure 4

4A. Western blotting of HGF and GAPDH



4B. Western blotting of HGF (Replicate)



Supplemental Figure 4. Western blotting of HGF

Protein expression level of HGF was measured by Western blotting. (4A) The upper panel shows HGF expression, and the lower panel shows GAPDH as a loading control. The left three lanes represent samples from the control group, and the right five lanes represent samples from the HMGB1 group. HGF tended to express higher in the HMGB1 group. The membranes were cut prior to antibody incubation to allow for probing with different primary antibodies under optimized conditions. As a result, full-length blots are not available. Additionally, in order to optimize the exposure for clear visualization of the target bands, the molecular weight markers on both sides of the membranes became overexposed. Including them in the main figures would have compromised image clarity and hindered interpretation of the data, and therefore they were omitted. (4B) HGF expression from individual replicates. The left five lanes correspond to control group samples, and the right six lanes to HMGB1 group samples. GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; HGF, hepatocyte growth factor.

Supplemental Video.

LV motion in short-axis view by echocardiography before and 8 weeks after HMGB1 fragment administration. LV motion improved eight weeks after HMGB1 fragment administration. HMGB1, high-mobility group box 1; LV, left ventricle.

Supplemental Table 1. Data of Echocardiography

Echocardiography		control	HMGB1	p value
LVEDV (ml)	pre	37 ± 12	42 ± 12	0.4652
	4 weeks	46 ± 16	50 ± 11	0.4652
	8 weeks	49 ± 12	50 ± 10	0.7150
ΔLVEDV (ml)	4 weeks - pre	9 ± 7	8 ± 8	0.8551
	8 weeks - pre	12 ± 7	8 ± 8	0.4102
LVESV (ml)	pre	18 ± 10	22 ± 8	0.4652
	4 weeks	25 ± 11	22 ± 9	0.7150
	8 weeks	26 ± 11	21 ± 8	0.4652
ΔLVESV (ml)	4 weeks - pre	6 ± 4	-1 ± 4	0.0258
	8 weeks - pre	26 ± 11	21 ± 8	0.0106
LVEF (%)	pre	53 ± 14	48 ± 7	0.3613
	4 weeks	48 ± 9	57 ± 9	0.1003
	8 weeks	49 ± 14	59 ± 10	0.1441
ΔLVEF (%)	4 weeks - pre	-5 ± 11	10 ± 5	0.0106
	8 weeks - pre	-5 ± 6	11 ± 5	0.0062

LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume.

Supplemental Table 2. Data of MRI

MRI		control	HMGB1	p value
LVEDV (ml)	pre	43 ± 11	47 ± 11	0.4652
	8 weeks	46 ± 14	48 ± 10	0.7150
ΔLVEDV (ml)	8 weeks - pre	3 ± 3	1 ± 7	0.2722
LVESV (ml)	pre	24 ± 11	31 ± 12	0.4652
	8 weeks	28 ± 13	29 ± 12	0.7150
ΔLVESV (ml)	8 weeks - pre	4 ± 5	-2 ± 8	0.0679
LVEF (%)	pre	47 ± 12	35 ± 13	0.1003
	8 weeks	43 ± 14	42 ± 14	0.4652
ΔLVEF (%)	8 weeks - pre	-4 ± 6	7 ± 10	0.0062
Longitudinal strain (%)	pre	17 ± 7	9 ± 3	0.0446
	8 weeks	11 ± 1	16 ± 4	0.0446
Δlongitudinal strain (%)	8 weeks - pre	-5 ± 7	6 ± 4	0.0176
Circumferential strain (%)	pre	24 ± 11	12 ± 4	0.0679
	8 weeks	19 ± 4	21 ± 7	0.7150
Δcircumferential strain (%)	8 weeks - pre	-5 ± 10	9 ± 6	0.0679
Circumferential strain base/LAD (%)	pre	18 ± 10	8 ± 6	0.0679
	8 weeks	14 ± 11	18 ± 8	0.4652
Δcircumferential strain base/LAD (%)	8 weeks - pre	-4 ± 18	10 ± 8	0.2012
Circumferential strain mid/LAD (%)	pre	20 ± 15	7 ± 6	0.1441
	8 weeks	13 ± 7	16 ± 9	0.5839
Δcircumferential strain mid/LAD (%)	8 weeks - pre	-7 ± 13	9 ± 7	0.0679
Circumferential strain apex/LAD (%)	pre	17 ± 6	11 ± 5	0.1441
	8 weeks	7 ± 6	18 ± 8	0.0446
Δcircumferential strain apex/LAD (%)	8 weeks - pre	-10 ± 2	7 ± 5	0.0062
Circumferential strain base/LCX (%)	pre	25 ± 14	21 ± 3	0.1003
	8 weeks	30 ± 7	27 ± 9	0.5839
Δcircumferential strain base/LCX (%)	8 weeks - pre	5 ± 17	7 ± 9	1.0000
Circumferential strain mid/LCX (%)	pre	29 ± 19	17 ± 3	0.2012
	8 weeks	25 ± 5	25 ± 9	0.5839
Δcircumferential strain mid/LCX (%)	8 weeks - pre	-4 ± 20	8 ± 9	0.3613
Circumferential strain apex/LCX (%)	pre	26 ± 10	12 ± 11	0.0679
	8 weeks	25 ± 8	22 ± 12	0.7150

Δcircumferential strain apex/LCX (%)	8 weeks - pre	-1 ± 9	10 ± 9	0.0679
Circumferential strain base/RCA (%)	pre	22 ± 13	8 ± 5	0.2002
	8 weeks	18 ± 5	16 ± 9	0.8551
Δcircumferential strain base/RCA (%)	8 weeks - pre	-4 ± 17	8 ± 10	0.2733
Circumferential strain mid/RCA (%)	pre	20 ± 9	10 ± 5	0.1441
	8 weeks	20 ± 6	22 ± 10	0.7150
Δcircumferential strain mid/RCA (%)	8 weeks - pre	0 ± 9	12 ± 11	0.2733
Circumferential strain apex/RCA (%)	pre	26 ± 11	9 ± 5	0.0106
	8 weeks	10 ± 8	12 ± 9	0.8551
Δcircumferential strain apex/RCA (%)	8 weeks - pre	-16 ± 3	2 ± 4	0.0062
Scar zone (%)	pre	30 ± 8	42 ± 12	0.1003
	8 weeks	39 ± 11	31 ± 11	0.1441
Δscar zone (%)	8 weeks - pre	9 ± 4	-11 ± 7	0.0062

LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; LV, left ventricle; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; MRI, magnetic resonance imaging; RCA, right coronary artery.

Supplemental Table 3. Data of Pressure Wire Study

Pressure wire study		control	HMGB1	p value
CFR in LCX	pre	1.22 ± 0.11	1.18 ± 0.13	0.7073
	8 weeks	1.22 ± 0.24	1.70 ± 0.25	0.0171
ΔCFR in LCX	8 weeks - pre	0.00 ± 0.22	0.52 ± 0.24	0.0106
CFR in RCA	pre	1.18 ± 0.29	1.18 ± 0.17	0.7052
	8 weeks	1.20 ± 0.19	1.57 ± 0.39	0.0786
ΔCFR in RCA	8 weeks - pre	0.02 ± 0.24	0.38 ± 0.34	0.0980
CFR average	pre	1.20 ± 0.15	1.18 ± 0.13	0.9257
	8 weeks	1.21 ± 0.19	1.63 ± 0.27	0.0135
ΔCFR average	8 weeks - pre	0.01 ± 0.19	0.45 ± 0.27	0.0166
RRR in LCX	pre	1.35 ± 0.13	1.36 ± 0.18	0.7150
	8 weeks	1.55 ± 0.43	2.02 ± 0.31	0.0679
ΔRRR in LCX	8 weeks - pre	0.20 ± 0.44	0.66 ± 0.22	0.1441
RRR in RCA	pre	1.43 ± 0.44	1.39 ± 0.21	0.5839
	8 weeks	1.37 ± 0.27	1.74 ± 0.46	0.1003
ΔRRR in RCA	8 weeks - pre	-0.06 ± 0.26	0.35 ± 0.37	0.1003
RRR average	pre	1.39 ± 0.20	1.37 ± 0.16	0.8551
	8 weeks	1.46 ± 0.22	1.88 ± 0.28	0.0176
ΔRRR average	8 weeks - pre	0.07 ± 0.24	0.50 ± 0.25	0.0176

CFR, coronary flow reserve; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; RCA, right coronary artery; RRR, resistive reserve ratio.

Supplemental Table 4. Data of ^{13}N-ammonia PET

13N-ammonia PET		control	HMGB1	p value
rest MBF in LAD	pre	0.91 ± 0.35	0.94 ± 0.32	0.7150
	8 weeks	0.83 ± 0.20	0.93 ± 0.28	0.7150
Δrest MBF in LAD	8 weeks - pre	-0.08 ± 0.22	-0.01 ± 0.11	0.8551
stress MBF in LAD	pre	1.30 ± 0.69	1.45 ± 0.70	0.7150
	8 weeks	1.08 ± 0.28	1.26 ± 0.38	0.8551
Δstress MBF in LAD	8 weeks - pre	-0.22 ± 0.62	-0.19 ± 0.38	0.7150
CFR in LAD	pre	1.37 ± 0.27	1.51 ± 0.27	0.3613
	8 weeks	1.31 ± 0.21	1.38 ± 0.29	0.5839
ΔCFR in LAD	8 weeks - pre	-0.06 ± 0.37	-0.13 ± 0.27	0.5839
rest MBF in LCX	pre	1.09 ± 0.37	1.12 ± 0.19	0.8551
	8 weeks	1.05 ± 0.21	1.14 ± 0.31	0.7150
Δrest MBF in LCX	8 weeks - pre	-0.04 ± 0.27	0.02 ± 0.30	0.7150
stress MBF in LCX	pre	1.48 ± 0.57	1.71 ± 0.61	1.0000
	8 weeks	1.52 ± 0.39	1.63 ± 0.48	0.7150
Δstress MBF in LCX	8 weeks - pre	0.05 ± 0.29	-0.08 ± 0.45	0.7150
CFR in LCX	pre	1.33 ± 0.19	1.51 ± 0.32	0.2733
	8 weeks	1.45 ± 0.18	1.43 ± 0.06	0.8551
ΔCFR in LCX	8 weeks - pre	0.12 ± 0.12	-0.08 ± 0.29	0.1441
rest MBF in RCA	pre	1.00 ± 0.35	1.06 ± 0.16	1.0000
	8 weeks	0.92 ± 0.20	0.99 ± 0.23	0.4652
Δrest MBF in RCA	8 weeks - pre	-0.08 ± 0.26	-0.07 ± 0.18	0.8551
stress MBF in RCA	pre	1.44 ± 0.66	1.62 ± 0.56	0.4652
	8 weeks	1.40 ± 0.39	1.45 ± 0.36	1.0000
Δstress MBF in RCA	8 weeks - pre	-0.04 ± 0.54	-0.17 ± 0.32	0.3613
CFR in RCA	pre	1.41 ± 0.23	1.53 ± 0.41	0.7832
	8 weeks	1.51 ± 0.22	1.48 ± 0.22	0.8551
ΔCFR in RCA	8 weeks - pre	0.10 ± 0.27	-0.04 ± 0.32	0.5839
rest MBF in total	pre	0.97 ± 0.35	0.99 ± 0.21	0.8551
	8 weeks	0.90 ± 0.19	0.97 ± 0.24	0.7150
Δrest MBF in total	8 weeks - pre	-0.07 ± 0.23	-0.01 ± 0.20	0.4652
stress MBF in total	pre	1.33 ± 0.54	1.52 ± 0.57	0.8551
	8 weeks	1.26 ± 0.30	1.39 ± 0.36	0.8551

Δstress MBF in total	8 weeks - pre	-0.07 ± 0.39	-0.13 ± 0.35	0.8551
CFR in total	pre	1.34 ± 0.15	1.53 ± 0.31	0.3142
	8 weeks	1.39 ± 0.12	1.44 ± 0.19	0.5795
ΔCFR in total	8 weeks - pre	0.05 ± 0.17	-0.09 ± 0.27	0.3613

CFR, coronary flow reserve; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; MBF, myocardial blood flow; RCA, right coronary artery.

Supplemental Table 5. Data of Histology

Histology	control	HMGB1	p value
% fibrosis area (%)	13.9 ± 5.2	9.7 ± 1.8	0.0758
Cardiomyocyte diameter (μm)	38.0 ± 2.7	26.6 ± 0.8	0.0339
CD31+ cell count (/mm^2)	640 ± 57	1033 ± 249	0.0339

Supplemental Table 6. Data of RT-PCR

RT-PCR	control	HMGB1	p value
SDF1	1.82 ± 1.29	3.67 ± 3.03	0.0018
HGF	1.71 ± 1.15	3.01 ± 2.37	0.0264
VEGFα	1.53 ± 0.65	1.87 ± 0.78	0.1103
FGF2	2.75 ± 2.98	4.50 ± 4.16	0.0198
TGFβ3	2.14 ± 1.81	5.13 ± 6.20	0.0139
IL-10	2.10 ± 1.62	3.10 ± 2.41	0.0563
PDGFRα	1.44 ± 0.48	2.43 ± 2.00	0.0612
PDGFRβ	1.40 ± 0.53	1.93 ± 1.19	0.2223