Supplemental Methods:

MyD88 chimeric mice – Wild type (WT) recipient mice (C57BL/6, 6-8 week-old) were irradiated with 10 Gy of cesium-137 radiation. Twenty-four hours later, $1x10^7$ BMC, harvested from the donor WT or $MyD88^{-/-}$ (KO) mice, were injected via tail vein. Two chimeric mouse models, *i.e.,* WT→WT and KO→WT (donor→recipient), were generated. The mortality of the recipient mice after irradiation and bone marrow transfusion was less than 5%. There was a high mortality, however, among $MyD88^{-/-}$ mice subjected to irradiation and BM transfusion. Thus, all recipient mice used in the current study were WT. Eight weeks later, MyD88 expression of both BM-derived and myocardial cells in the recipient mice were examined (Fig. S1).

Real-time RT-PCR – The following primer pairs were used: 18S rRNA, forward primer 5'-TCATGTGGTGTTGAGGAAAGC-3' and reverse primer 5'-TGGCGTGGATTCTGCATAATG-3'; KC, forward: 5'-CCGCGCCTATCGCCAATGAGCTGCGC-3', reverse: 5'-CTTGGGGACACCCTTTTAGCATCTTTTGG-3'; MCP-1, forward: 5'-TTAAAAACCTGGATCGGAACCAA-3', reverse: 5'-GCATTAGCTTCAGATTTACGGGT-3'; ICAM-1, forward: 5'-GTGATGCTCAGGTATCCATCCA-3', reverse: 5'-CACAGTTCTCAAAGCACAGCG-3'; MIP-1, forward: 5'-CACCCTCTGTCACCTGCTCAACATC-3', reverse: 5'-GGTTCCTCGCTGCCTCCA AGACTC T-3'; MIP-2, forward: 5'-

CCGCTGTTGTGGCCAGTGAACTGCG-3', reverse: 5'-

TTAGCCTTGCCTTTGTTCAGTAT-3'.

AAR and MI measurements – To calculate AAR, the LAD was re-ligated using the same sutures left from previous ligation for ischemia and fluorescent microspheres (Molecular Probes) were injected into the left ventricle (LV). Under a fluorescent microscope, the perfused myocardium was identified as the areas filled with red microspheres and the AAR identified as areas devoid of red microspheres. To examine MI, the sections were incubated in 1% (wt/vol) triphenyltetrazolium chloride (TTC) in PBS (pH 7.4) at 37°C for 10 min followed by fixation with 1% paraformadehyde. For each section, LV, AAR, and, MI areas were measured from enlarged digital microscopic images using Adobe Photoshop. The percentage of MI/AAR was calculated as the infarct area (MI/LV x 100%) divided by the AAR (AAR/LV x 100%).

Myocardial leukocyte isolation – Mice were injected with 200 units of heparin (i.p.) and euthanized. The hearts were removed and the ascending aorta fixed to a cannula. The hearts were perfused with perfusion buffer (in mM, NaCl 137, KCl 5.4, HEPES 0.5, $MgCl_2$ 0.5, Glucose 10, Taurine (C2H7NO3S) 5.0, BDM (C4H7NO2) 10, pH = 7.4) for 3 min at a flow rate of 3 ml/min temperature at 37 ^{o}C and then with 25 ml of enzyme buffer (collagenase D 0.3 mg/g, collagenase B 0.4 mg/g, proteinase XIV, 0.05 mg/g body weight) for 10 min. Digested heart was removed and placed in perfusion buffer containing 5 mg/ml BSA. The atria and great vessels were trimmed from the ventricles. The ventricles were cut into 5-6 pieces and gently teased with forceps to release myocardial cells into buffer. Cell suspension and tissue pieces were pipetted several times to further disseminate cells. Cell suspension was

transferred to a 50-ml Falcon tube and filtered through a series of mesh with pore size of 250 μm, 70 μm, and 40 μm to remove most of myocytes. Cells (except myocytes and small red blood cells) were counted, stained with Gr-1 and CXCR2 antibodies, and analyzed for neutrophils with flow cytometry.

Supplemental Results:

Generation of chimeric MyD88 deletion

Eight weeks after bone marrow transfusion, expression of MyD88 protein (Fig.S1*A-B*) and transcript (Fig. S1*C*) were examined. Compared to WT mice, WT→WT chimeric mice, the control for irradiation and bone marrow reconstruction, possessed similar levels of MyD88 protein and mRNA in bone marrow and the heart. Bone marrow cells appeared to have significantly higher MyD88 protein expression than the heart. In contrast, KO→WT chimeric mice had no detectable MyD88 expression in their bone marrow cells but normal protein expression in the heart. Stimulation of bone marrow-derived macrophages with TLR2 (Pam3Cys) or TLR9 (CpG) agonist, both signal through MyD88-dependent pathways (17), did not alter the MyD88 transcript level (Fig.S1*C*). However, as illustrated in Fig. S2, both Pam3cys and CpG induced a robust gene expression of KC, MIP-2, MIP-1, and ICAM in the bone marrow-derived macrophages from WT and WT→WT mice. In contrast, both TLR2 and TLR9 agonists failed to induce any cytokine expression in macrophages isolated from KO or KO→WT mice. Taken together, these data clearly indicated that the KO→WT chimeric mice maintained normal MyD88 expression in the heart but completely lacked MyD88 expression as well as signaling function in their bone marrow cells. Since MyD88$^{-/-}$ mice had a high mortality after irradiation and bone marrow transfusion in our pilot study, a phenomenon that was reported previously (26), we have decided to only employ WT as the bone marrow recipients to construct the two groups of chimeric mice, *i.e.,* WT→WT and KO→WT, in these studies.

Flow cytometry analysis of leukocytes isolated from peritoneal lavage, bone marrow, and myocardium

Since Gr-1 antibody (anti-Ly6G and -Ly6C) recognizes inflammatory cells including neutrophils, monocytes, and dendritic cells, whereas Ly6G antibody is more specific for neutrophils, we compared the two antibodies in detecting neutrophils by flow cytometry. We stained four groups of cells: 1) Peritoneal lavage cells following thioglycollate injection, 2) Bone marrow (BM)-derived neutrophils purified after density gradient, 3) Leukocytes isolated from myocardial lysates of I/R heart, and 4) Sham heart digest mixed with BM-derived neutrophils, and analyzed on flow cytometry gated with both Gr-1 and anti-Ly6G antibodies. As indicated in Table S1 and Fig.S3, 78%, 98%, 99%, and 99% of Gr-1+ cells were Ly-6G+ neutrophils, respectively, from these 4 groups of cells. These data suggest that while Gr-1+ cells were not exclusive neutrophils, overwhelming majority of Gr-1+ cells were Ly-6G+ neutrophils in the experimental models used the current studies.

Effect of enzyme treatment on FACS measurements of neutrophils

To determine if enzyme used during myocardial digestion described above had any effect on neutrophil detection by FACS, we treated the neutrophils isolated from BM in the perfusion buffer with or without the enzymes as noted above for 4 min, 8 min, and 12 min. The cells were then stained and gated with both Gr-1 and Ly-6G antibodies. As indicated in Fig.S4, the enzyme treatment had no effect on neutrophil detection by Gr-1 and Ly-6G antibodies for 8 min. At 12 min of treatment, the number of neutrophils detected was decreased by 27-28% as demonstrated.

Figure Legends

Fig. S1. Characterization of MyD88 chimeric mice. Eight weeks after irradiation and bone marrow transfusion, recipient bone marrow cells and the heart were examined for MyD88 expression. *A.* Representative MyD88 Western blot showing protein expression. *B.* Quantification of MyD88 protein expression. MyD88 protein expression was normalized to GAPDH. Each error bar represents mean ± SD of 3 mice. *C*. MyD88 transcript production in bone marrow macrophages. Bone marrow cells were differentiated into macrophages in RPMI-1640 medium containing 10 μ□/ml of M-CSF. One week later, the macrophages were stimulated with 1 μg/ml of Pam3cys or 100 nM of CpG for 2 hours. Each error bar represents mean ± SD of 3 mice.

Fig. S2. MyD88 signaling function in bone marrow cells. Bone marrow-derived macrophages were stimulated with PBS, Pam3cys, or CpG for 2 hours as described in Fig. S1*C*. Cytokine transcripts were then quantified with qRT-PCR. Each error bar represents mean ± SD of 6-7 mice.

Fig. S3. Flow cytometry measurements of neutrophils by Gr-1 and Ly-6G antibodies. Cells were isolated from 1) peritoneal lavage following thioglycollate injection (upper panels), 2) bone marrow after density gradient purification (middle panels), and 3) myocardial digest after ischemia and reperfusion for 24 hours (low panels). The cells were washed and stained with Gr-1 and Ly-6G antibodies and analyzed by flow cytometry. The numbers in the middle column represent % of Gr-1+ cells among the cells gated in the left

column and the numbers in the right column represent % of Ly-6G+ cells among Gr-1+ cells. Cumulative data of the percentages of Ly-6G+ cells among Gr-1+ cells and vise versa were presented in Table S1.

Fig. S4. Effect of enzyme treatment on neutrophil measurements by flow cytometry. BM-derived neutrophils were purified by density gradient and treated with the Langendorff perfusion buffer containing enzymes as described above at 37 ^{o}C for various time (0, 4, 8, 12 min). The cells were then washed, stained with Gr-1 and Ly-6G antibodies, and analyzed with flow cytometry. Each error bar presents the mean ± SD of 3 samples.

Table S1. Flow cytometry analysis of neutrophils with Gr-1 and Ly-6G antibodies.

Groups	Sources	Ly-6G+/Gr-1+ (%)	Gr-1+/Ly-6G+ (%)
1	**Peritoneal Lavage**	77.7±2.3	98.7±0.6
2	**BMCs**	98.1±0.2	99.0±0.5
3	**I/R Heart**	99.1±0.5	97.7±0.6
4	**Sham Heart + BMCs**	98.5±0.3	97.5±0.6

Four groups of cells were isolated as described in the text. Cells were stained and analyzed with flow cytometry gated by Gr-1 and Ly-6G. The percentages of Ly-6G+ cells among Gr-1+ cells or the percentages of Gr-1+ cells among Ly-6G+ cells were shown, respectively, among the 4 groups of cells. N = 3. Representative flow cytometry plots of group 1-3 are shown in Fig.S3.