Supplemental Digital Content 1

METHODS

Western Blotting
Homogenates of cell pellets were centrifuged at 12,000 g at 4°C for 30 min. Proteins were separated in 4-20% gradient SDS-PAGE and immunoblotted with 1:1000 diluted Toll-like receptor 4 antibody (Cell Signaling Tech, Danvers, MA).

Lactate assay
Plasma samples were harvested from MyD88-loxP control (MyD88fl/fl) mice at 18 h after lipopolysaccharide or Saline administration. Blood level of lactate was measured using L-Lactate assay kit (Cayman Chemical, Ann Arbor, MI) following the instruction.

Generation of tamoxifen-inducible cardiomyocyte-specific MyD88 gene deletion model
Targeted cells constitutively express Cre recombinase flanked by Mutated estrogen receptor (MerCreMer, MCM) ligand-binding domains insensitive to endogenous estrogen but sensitive to tamoxifen. Linkage of MCM under the control of α-myosin heavy chain (α-MHC) promoter (α-MHC-MCM) creates inducible target gene deletion specifically in adult cardiomyocytes (CM).

To generate inducible CM-specific MyD88 deletion mice (α-MHC-MCM-MyD88−/−), α-MHC-MCM transgenic mice (purchased from Jackson Lab, Bar Harbor, ME) were cross-bred with mice with loxP sites flanking exon 3 of MyD88 gene (MyD88fl/fl). Mice were genotyped by polymerase chain reaction using genomic DNA isolated from tail tips and the following primers: Transgenic (Tg) forward, 5’- ATACCGGAGATCATGCAAGC -3’, Tg reverse, 5’-
AGGTGGACCTGATCATGGAG -3’, Control forward, 5’-
CTAGGCCACAGAATTGAAAGATCT -3’, and Control reverse, 5’-
GTAGGTGGAAATTCTAGCATCATCC -3’. To induce the deletion of MyD88 gene, tamoxifen (Sigma, St. Louis, MO) suspension in peanut oil was administrated to α-MHC-MCM-MyD88−/− and control mice (age 6-27 weeks) by intra-peritoneal injection (40 mg/kg/day) for 5 consecutive days.
RESULTS

Figure 1. TLR4 expression on Mϕ. Western blot was used to detect MyD88 and TLR4 protein expression on Mϕ isolated from MyD88^{fl/fl}, α-MHC-MyD88^{−/−}, Lyz-MyD88^{−/−} and systemic MyD88^{−/−} mice. A, Representative picture of Western blot. B, Quantitative data of Western blot. Each error bar represents mean ± SD. * P < 0.05, ** P < 0.01, *** P < 0.001, n = 3 in each group. GAPDH = glyceraldehyde-3-phosphate dehydrogenase; Lyz-MyD88^{−/−} = myeloid-specific MyD88 knockout mice; Mϕ = bone marrow-derived macrophage; (α-) MHC-MyD88^{−/−} = cardiomyocyte-specific MyD88 knockout mice; Mus = skeletal muscle; MyD88 = myeloid
differentiation factor 88; MyD88\(^{-/-}\) = MyD88 knockout mice; MyD88\(^{fl/fl}\) = MyD88-loxP control mice; TLR4 = Toll-like receptor 4.
**Figure 2. Inducible and cardiomyocytes-targeted MyD88 gene deletion.** MyD88 deletion was induced by tamoxifen (40 mg/kg intraperitoneal injection for 5 consecutive days). 8 days after tamoxifen administration, adult cardiomyocytes (CM) were isolated from digested heart together with other non-cardiac tissues for MyD88 gene and protein expression detection. A. polymerase chain reaction detecting of gene deletion. Constitutive Cre expression in Cre-MyD88^{fl/fl} mice caused deletion of MyD88 gene exon-3 flanked by loxP sites and thus resulted in a smaller size of MyD88 gene polymerase chain reaction product in CM (lane 3-4) but not in non-CM cells of cardiac tissue (lane 5-6) or in other non-cardiac tissues (lane 7-12). CM without Cre expression (MyD88^{fl/fl}, lane 1-2) or myocardium of MerCreMer (MCM)-expressing mice without loxP (lane 16-17) had no MyD88 gene deletion. B and D. Quantitatively, tamoxifen administration led to significant reduction in MyD88 transcripts (42% in B) and proteins (36% in D) in CM isolated from Cre-MyD88^{fl/fl} mice compared to that from MyD88^{fl/fl} control mice. Each error bar represents mean ± SD. * P < 0.05, n = 4 in each group. C. Representative picture of MyD88 protein expression in CM of MyD88^{fl/fl} and Cre-MyD88^{fl/fl} mice treated with tamoxifen. E. There was no MyD88 protein reduction in non-cardiac tissues of Cre-MyD88^{fl/fl} and MyD88^{fl/fl} mice subjected to tamoxifen. F. Serial echocardiography images. Constitutive expression of Cre with or without loxP sites resulted in transient dilated cardiomyopathy within a week of tamoxifen injection but completely recovered by 22 days. α-MHC = α-myosin heavy chain; Br = brain; CM = cardiomyocyte; Cre-MyD88^{fl/fl} = inducible cardiomyocyte-specific MyD88 knockout mice; def = deficiency; Flox = flanked with loxP site; FS = fractional shortening; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; Ki = kidney; Li = liver; Lu = lung; LVIDd = left ventricle internal dimension at the end of diastole. MCM = Mutated estrogen receptor (MerCreMer); Mu = skeletal muscle; MyD88 = myeloid differentiation factor 88;
MyD88$^{-/-}$ = MyD88 knockout mice; MyD88$^{+/+}$ = wild type mice; MyD88$^{fl/fl}$ = MyD88-loxP control mice; Sp = spleen.
Figure 3. Blood lactate detection during endotoxemia. MyD88^{fl/fl} mice were injected with 15 mg/kg LPS or Saline. Eighteen hours later, body temperature was measured and plasma was harvested for lactate measurement using an L-Lactate assay kit. A, Blood lactate level in Saline- or LPS-injected MyD88^{fl/fl} mice. Each box and whiskers represents median with minimal to maximal. n = 5 in Saline group, n = 6 in LPS group. B, The relationship between body temperature and lactate level. n = 5 in Saline group (shown in red), n = 6 in LPS group (shown in blue). LPS = lipopolysaccharide; MyD88 = myeloid differentiation factor 88; MyD88^{fl/fl} = MyD88-loxP control mice; Temp. = body temperature.