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 ($MyD88^{fl/fl}$) 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 ($MyD88^{fl/fl}$). 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

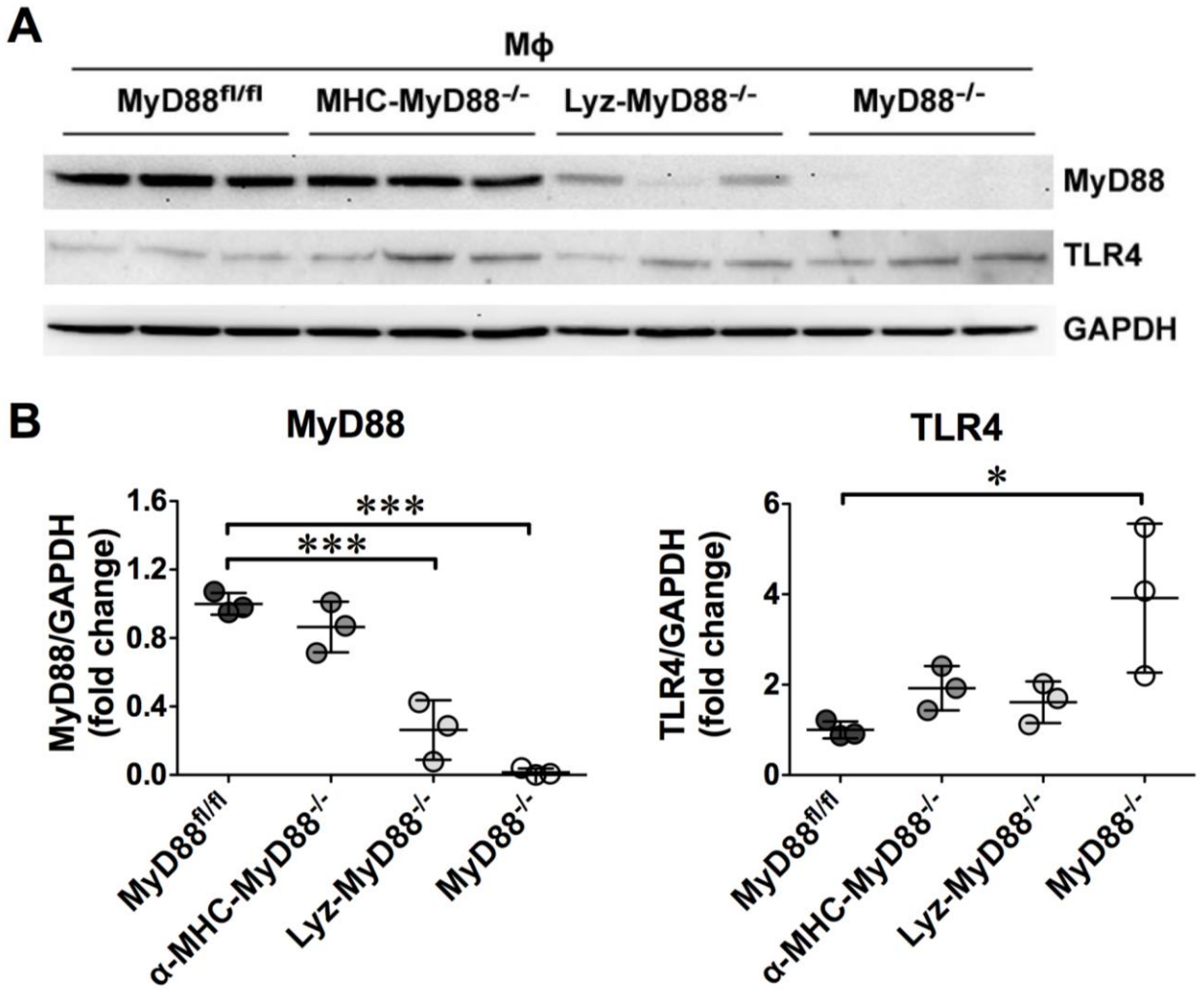

Figure 1. TLR4 expression on Mϕ. Western blot was used to detect MyD88 and TLR4 protein expression on Mϕ isolated from MyD88$^{fl/fl}$, α-MHC-MyD88$^{-/-}$, Lyz-MyD88$^{-/-}$ and systemic MyD88$^{-/-}$ mice. ***A,*** Representative picture of Western blot. ***B,*** Quantitative data of Western blot. 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.

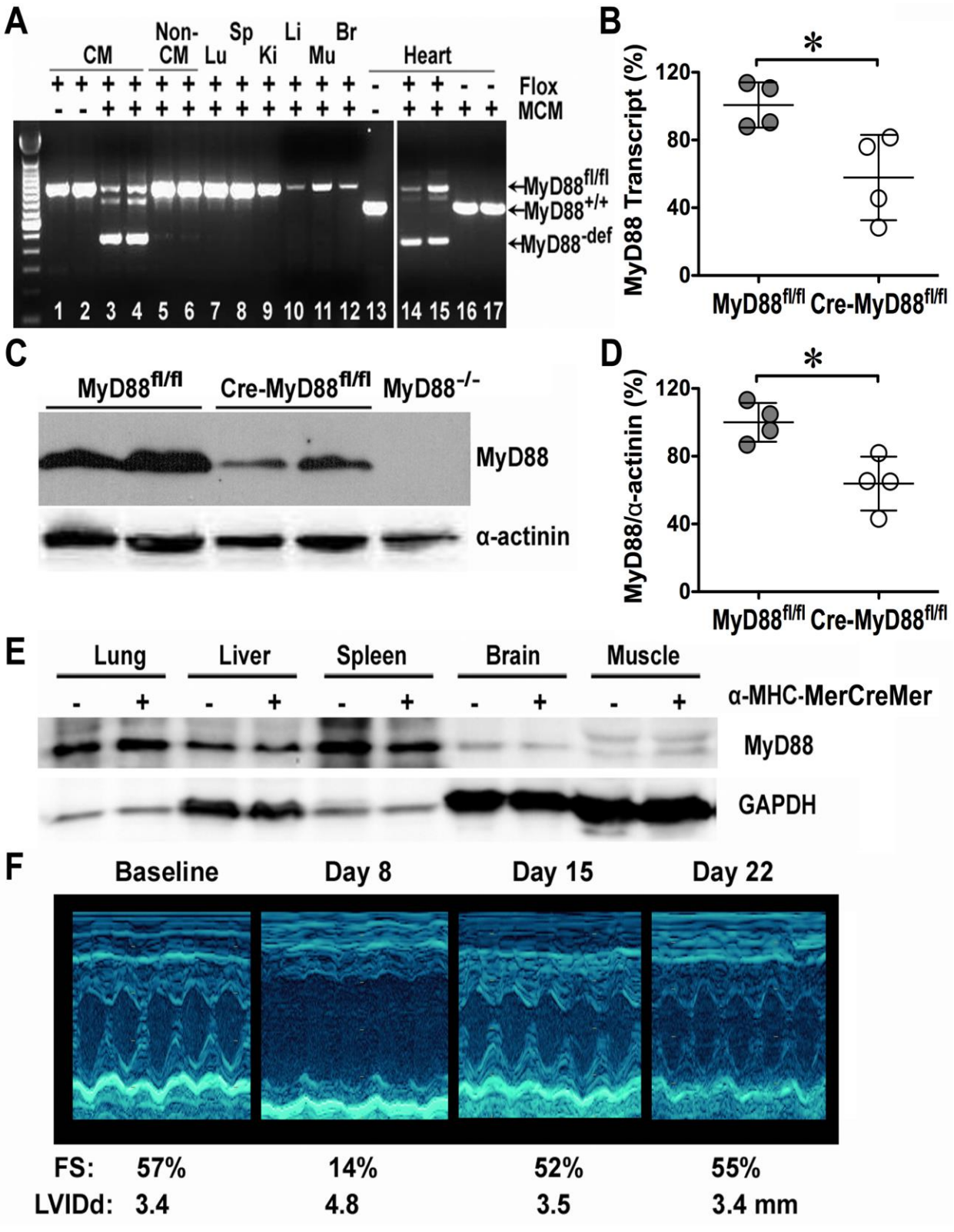
A
CM
Non-CM
Lu
Sp
Ki
Li
Mu
Br
Heart
Flox
MCM
MyD88fl/fl
MyD88+/+
MyD88-def
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
B
MyD88 Transcript (%)
120
80
40
0
*
MyD88fl/fl
Cre-MyD88fl/fl
C
MyD88fl/fl
Cre-MyD88fl/fl
MyD88-/-
MyD88
α-actinin
D
MyD88/α-actinin (%)
120
80
40
0
*
MyD88fl/fl
Cre-MyD88fl/fl
E
Lung
Liver
Spleen
Brain
Muscle
α-MHC-MerCreMer
MyD88
GAPDH
F
Baseline
Day 8
Day 15
Day 22
FS: 57% 14% 52% 55%
LVIDd: 3.4 4.8 3.5 3.4 mm

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.

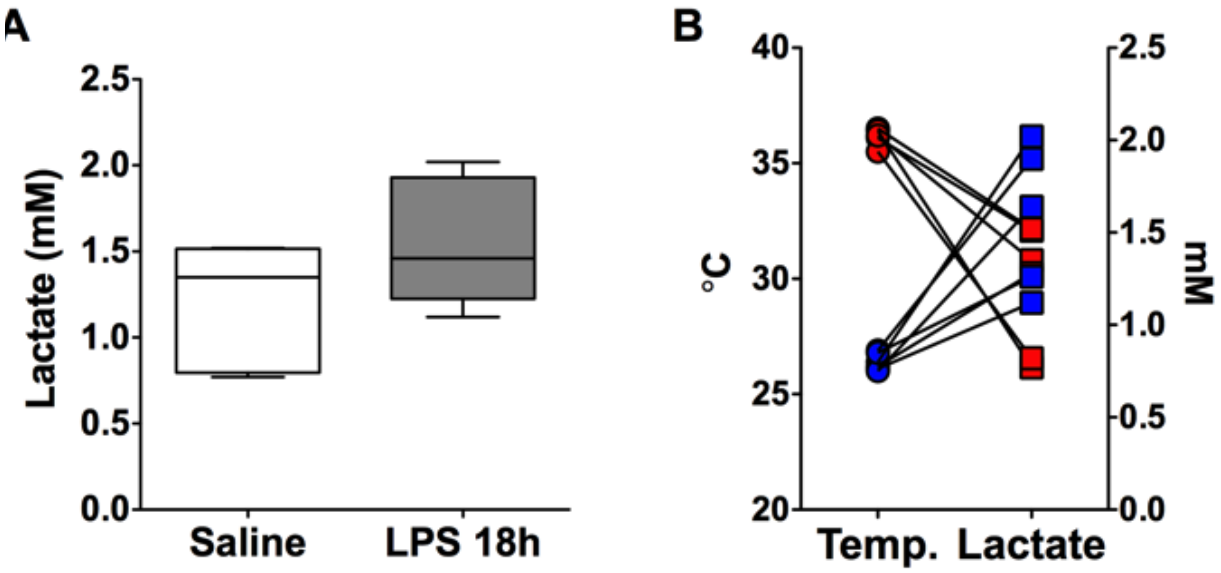

Figure 3. Blood lactate detection during endotoxemia. MyD88$^{fl/fl}$ mice were injected with 15 mg/kg LPS or Saline. Eighteen hours later, body temperature was measured and plasma was harvested for lactate measurement using an L-Lactate assay kit. ***A,*** Blood lactate level in Saline- or LPS -injected 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.