Figure S1

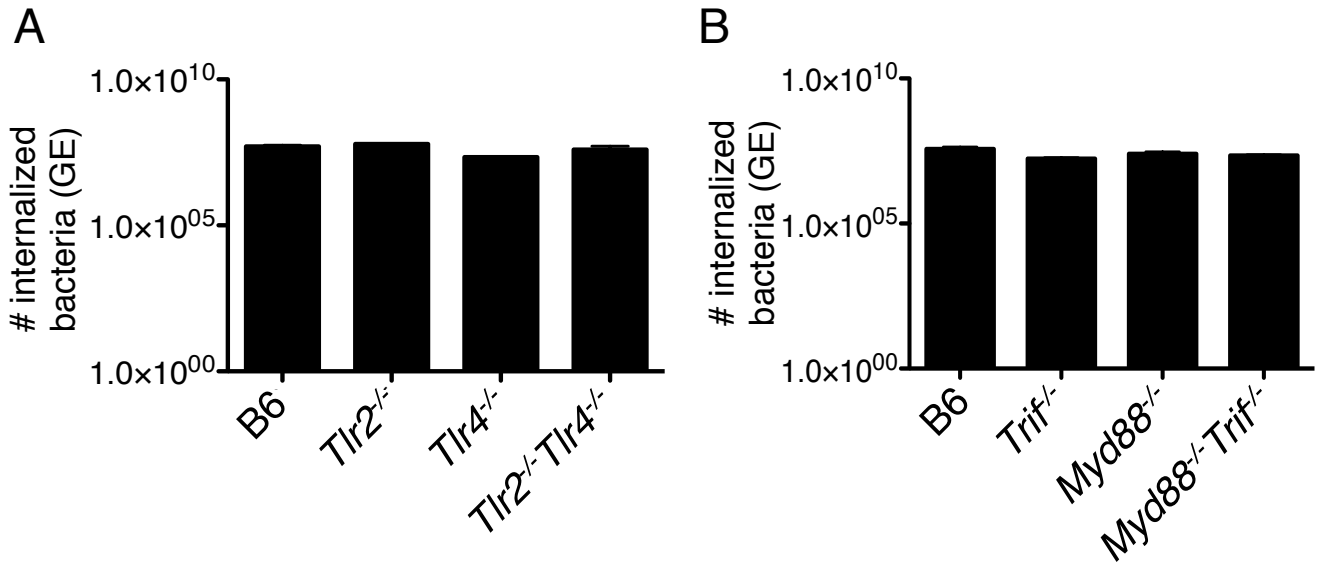

A
internalized bacteria (GE)
1.0×10^10
1.0×10^05
1.0×10^00
B6
Tlr2-/-
Tlr4-/-
Tlr2-/-Tlr4-/-
B
internalized bacteria (GE)
1.0×10^10
1.0×10^05
1.0×10^00
B6
Trif-/-
Myd88-/-
Myd88-/-Trif-/-

Figure S2

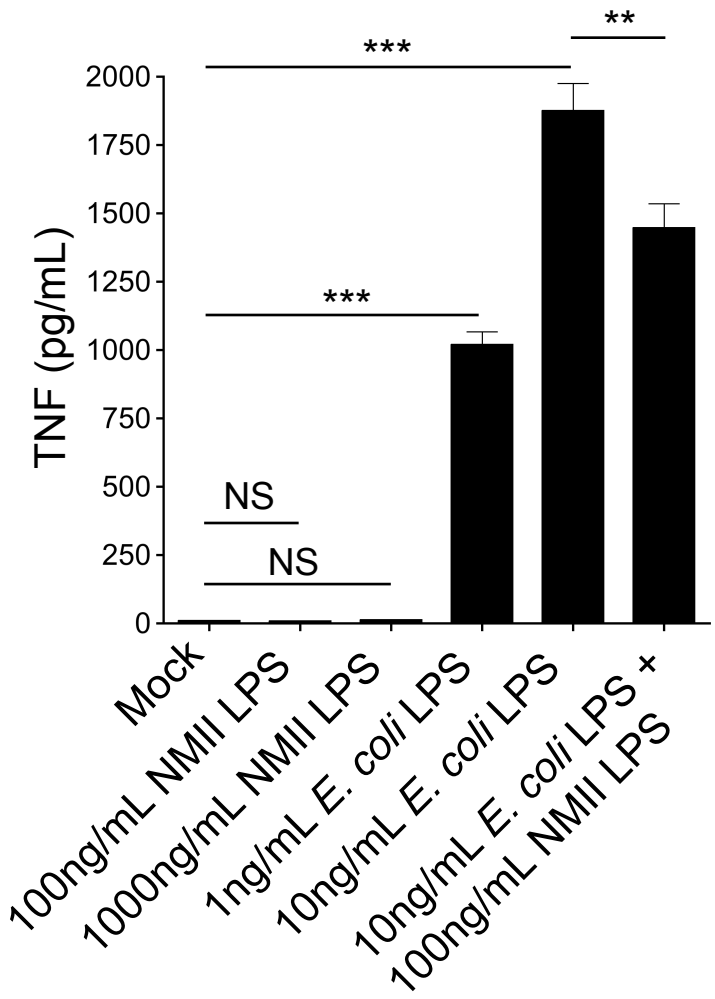

***
**
***
NS
NS
TNF (pg/mL)
2000
1750
1500
1250
1000
750
500
250
0
Mock
100ng/mL NMII LPS
1000ng/mL NMII LPS
1ng/mL E. coli LPS
10ng/mL E. coli LPS
10ng/mL E. coli LPS +
100ng/mL NMII LPS

Figure S3

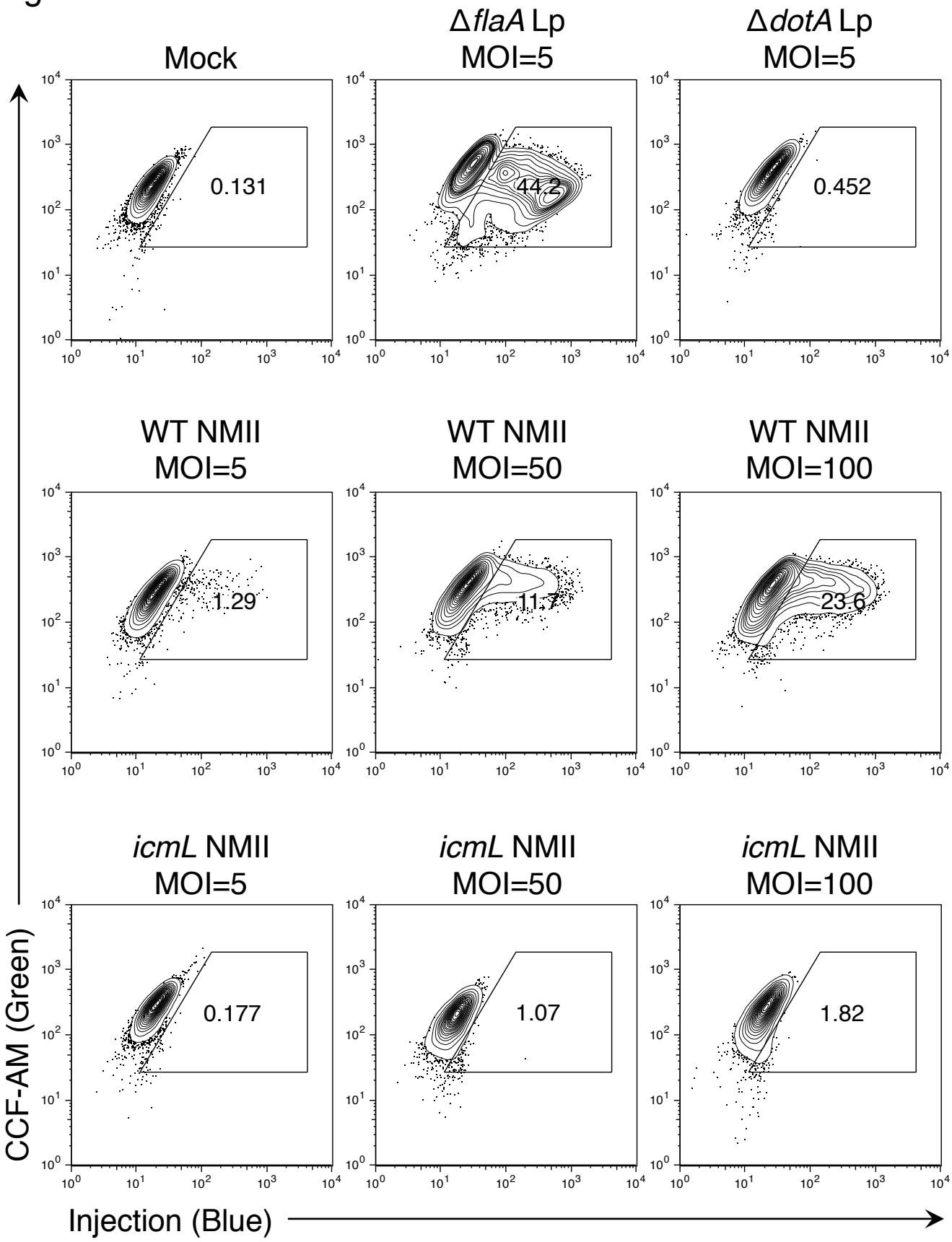

Mock
ΔflaA Lp MOI=5
ΔdotA Lp MOI=5
0.131
44.2
0.452
WT NMII MOI=5
WT NMII MOI=50
WT NMII MOI=100
1.29
11.7
23.6
icmL NMII MOI=5
icmL NMII MOI=50
icmL NMII MOI=100
0.177
1.07
1.82
CCF-AM (Green)
Injection (Blue)

Figure S4

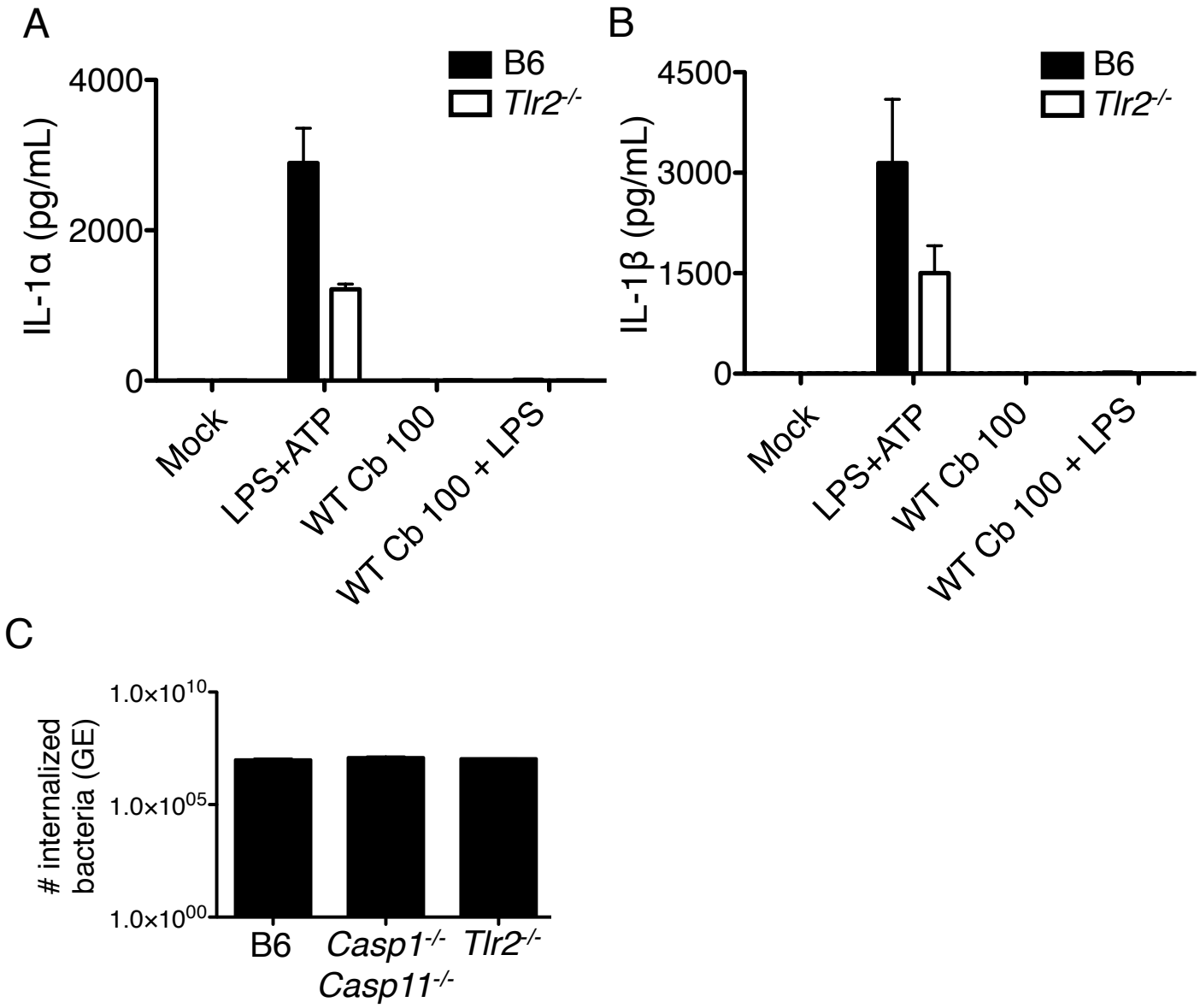

A
B6
Tlr2-/-
IL-1α (pg/mL)
4000
2000
0
Mock
LPS+ATP
WT Cb 100
WT Cb 100 + LPS
B
B6
Tlr2-/-
IL-1β (pg/mL)
4500
3000
1500
0
Mock
LPS+ATP
WT Cb 100
WT Cb 100 + LPS
C
internalized bacteria (GE)
1.0×10^10
1.0×10^05
1.0×10^00
B6
Casp1-/-
Tlr2-/-
Casp11-/-

Figure S5

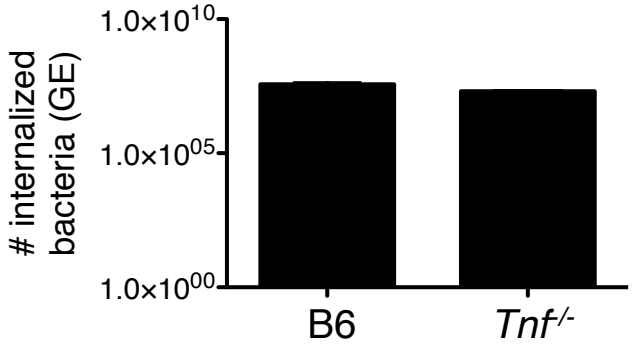


Figure S6

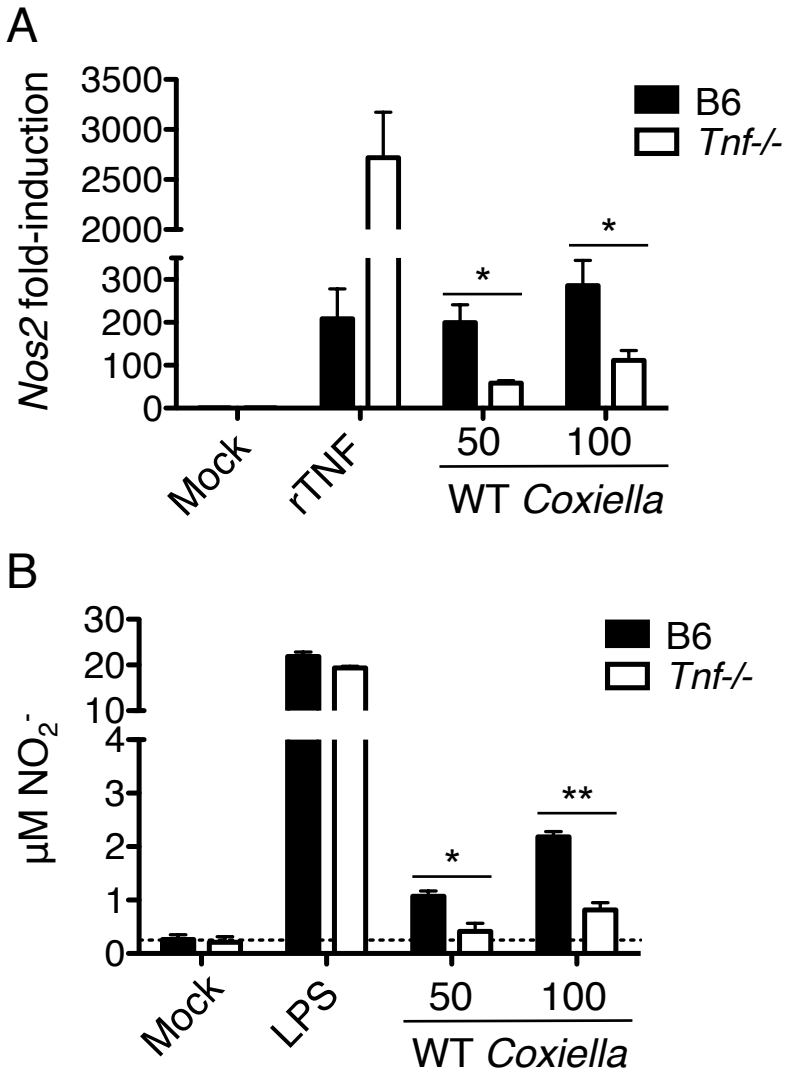
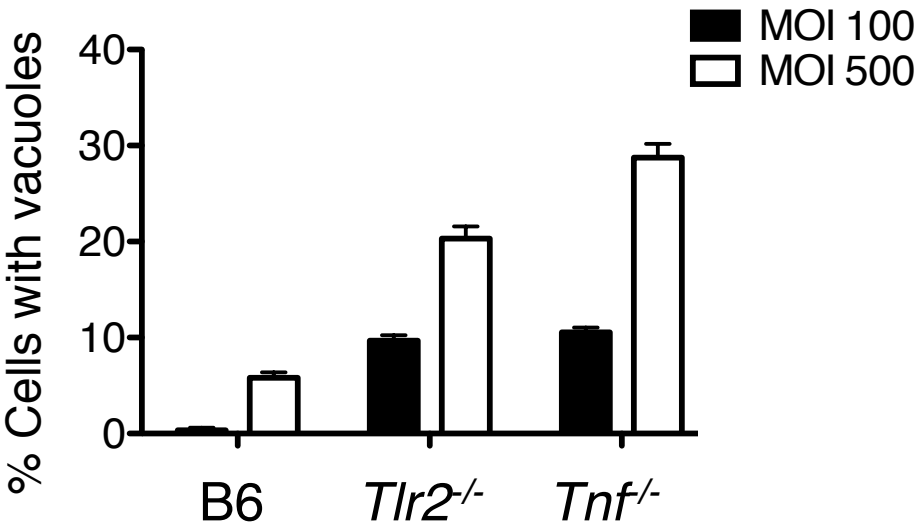

A
Nos2 fold-induction
3500
3000
2500
2000
300
200
100
0
B6
Tnf-/-
*
*
Mock
rTNF
50
100
WT Coxiella
B
µM NO2-
30
20
10
4
3
2
1
0
B6
Tnf-/-
*
**
Mock
LPS
50
100
WT Coxiella

Figure S7



Supplementary Figure Legends

Figure S1: Levels of *Coxiella burnetii* Nine Mile II bacterial uptake are comparable in C57BL/6 macrophages and macrophages deficient in TLR signaling components. (A) C57BL/6, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, and *Tlr2*$^{-/-}$*Tlr4*$^{-/-}$ BMDMs were infected with WT *C. burnetii* NMII at MOI=100. At day 1 post-infection, the number of internalized bacteria was measured as genomic equivalents (GEs) by qPCR. (B) C57BL/6, *Trif*$^{-/-}$, *Myd88*$^{-/-}$, and *Myd88*$^{-/-}$*Trif*$^{-/-}$ BMDMs were infected with WT *C. burnetii* NMII at MOI=100. At day 1 post-infection, the number of internalized bacteria was measured as genomic equivalents (GEs) by qPCR.

Figure S2: Purified *Coxiella burnetii* Nine Mile II LPS does not elicit cytokine production from C57BL/6 macrophages. B6 BMDMs were mock-treated or treated with 100 or 1000ng/mL purified *C. burnetii* NMII LPS or 1 or 10ng/mL purified *E. coli* LPS for 16 hours. To examine LPS antagonism, B6 BMDMs were pretreated with 100ng/mL *C. burnetii* NMII for 30 minutes LPS prior to treatment with 10ng/mL *E. coli* LPS. TNF levels were measured by ELISA. NS is not significant, ** is $p<0.01$, and *** is $p<0.001$ by one way ANOVA with Tukey test.

Figure S3: *Coxiella burnetii* Nine Mile II translocates bacterial effectors into C57BL/6 macrophages in a T4SS-dependent manner. C57BL/6 BMDMs were mock-infected, infected with BlaM-RalF-expressing *L. pneumophila ΔflaA* or *ΔdotA* at MOI=5 or infected with BlaM-CBU_0077-expressing WT or *icmL::Tn C. burnetii* NMII at MOI=5, 50, or 100 for 24 hours. Following infection, BMDMs were loaded with CCF4-AM and then subjected to flow cytometric analysis. Shown are flow cytometric plots representative of two independent experiments.

Figure S4: *Coxiella burnetii* Nine Mile II does not induce inflammasome activation in permissive TLR2-deficient macrophages, and WT and caspase-1- and caspase-11-deficient macrophages display similar levels of bacterial uptake. (A and B) C57BL/6 and *Tlr2*$^{-/-}$ BMDMs were unprimed or primed with 0.5 ug/mL LPS for 4 hours. They were then mock-infected, infected with *L. pneumophila ΔflaA* at MOI=5, and infected with WT *C. burnetii* NMII at MOI=100 for 24 hours or treated with 2.5mM ATP for one hour. Levels of (A) IL-1α and (B) IL-1β were measured in the supernatants by ELISA. Graphs show the mean ± SEM of triplicate wells. Representative of three independent experiments. (C) C57BL/6, *Casp1*$^{-/-}$*Casp11*$^{-/-}$, and *Tlr2*$^{-/-}$ BMDMs were infected with WT *C. burnetii* NMII at MOI=100. At day 1 post-infection, the number of internalized bacteria was measured as genomic equivalents (GEs) by qPCR. Representative of two independent experiments.

Figure S5: Levels of bacterial uptake are comparable in C57BL/6 and TNF-deficient macrophages. (A) C57BL/6 and *Tnf*$^{-/-}$BMDMs were infected with WT *C. burnetii* NMII at MOI=100. At day 1 post-infection, the number of internalized bacteria was measured as genomic equivalents (GEs) by qPCR.

Figure S6: Induction of iNOS and nitric oxide production is decreased in TNF-deficient macrophages following *Coxiella burnetii* Nine Mile II infection. C57BL/6 and *Tnf*$^{-/-}$BMDMs were mock-infected, infected with WT *C. burnetii* NMII at MOI=50 or 100 or treated with 10ng/mL recombinant TNF (rTNF) or 0.5μg/mL LPS for 16 or 24 hours. (A) The fold-induction of *Nos2* mRNA in infected cells relative to mock-treated cells was determined by qRT-PCR at 16 hours post-infection. (B) Nitrite levels in the supernatants of infected cells were measured 24 hours post-infection by Griess assay. Representative of two independent experiments.

Figure S7: Levels of large vacuole formation are comparable in TLR2-deficient and TNF-deficient macrophages. B6, *Tlr2*$^{-/-}$, and *Tnf*$^{-/-}$ BMDMs were infected with mCherry-expressing WT *C. burnetii* NMII at MOI=100 or 500. On day 7 post-infection, cells were fixed, stained with DAPI, and imaged by fluorescence microscopy. The number of mCherry-expressing *C. burnetii*-containing vacuoles was determined and calculated as a percentage of total cell number. Graphs show mean percentage of cells containing *C. burnetii* vacuoles ± SEM of triplicate coverslips. At least 300 cells were counted per coverslip. Representative of two independent experiments. *$p<0.05$, **$p<0.01$.