Supplementary Table 1.

Primers used in this study.

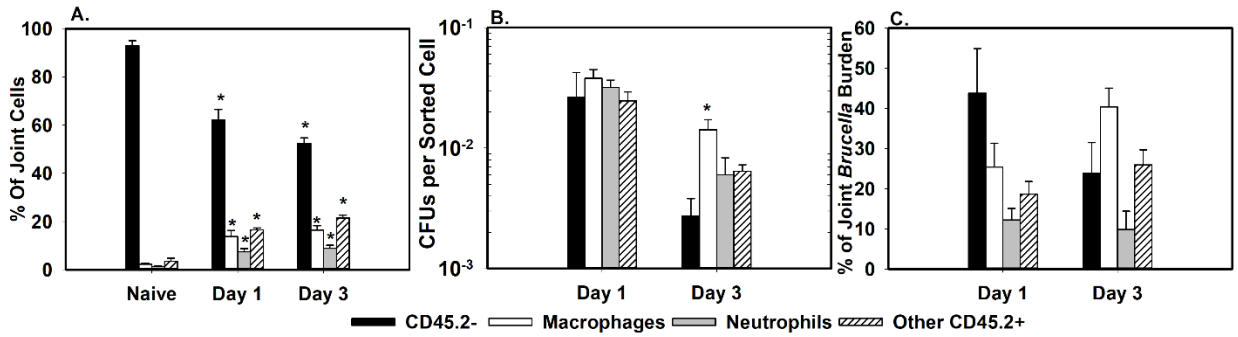
Primer name	5'-3' sequence	Purpose
GAPDH-F	GTG GAC CTC ATG GCC TAC AT	qRT-PCR
GAPDH-R	GGG TGC AGC GAA CTT TAT TG	qRT-PCR
IRG1-F	TACCAAAGAGATTCCACCCTCC	qRT-PCR
IRG1-R	ACTTTGTCAAGCTGAGCCCC	qRT-PCR
gluP upstream-F*	GCGCCAGAAAGCTTCCTGCAGGATATCGTGAACGTCTT CAAACCGGTCATTGCC	Cloning fragment upstream of *gluP*
gluP upstream-R	TGCCATATTTCTCTACAAAC	Cloning fragment upstream of *gluP*
gluP-cat-F^	TCTTGTAATAATTTAGTTTGTAGAGAAATATGGCA GTGTAGGCTGGAGCTGCTTC	Cloning chloramphenicol resistance gene
gluP-cat-R	CATATGAATATCCTCCTTA	Cloning chloramphenicol resistance gene
gluP-downstream F#	GGAATAGGAACTAAGGAGGATATTCATATGAGCTGAAC CGTCGGCCACTTGAAAGC	Cloning fragment downstream of *gluP*
gluP downstream R*	CCAAGCTACGTAATACGACTCACTAGTGGGAATCCGCG TTTCGCTCGATACGGC	Cloning fragment downstream of *gluP*
gluP-recon-F*	GCGCCAGAAAGCTTCCTGCAGGATATCGTGCCGATTAC TTCGCTTCCATTGGCC	Complementation of *gluP*
gluP-recon-R*	CCAAGCTACGTAATACGACTCACTAGTGGGCAGCGCCA TGGCCGCCGCCTGCGC	Complementation of *gluP*
gluP seq F	AGGGTCACTCTCGATCGACGCC	Screening of *gluP* mutant
gluP seq R	GCATCGCCACCAAATATTGGCGG	Screening of *gluP* mutant

*Underlined regions denote homology to pBBR1MCS-2

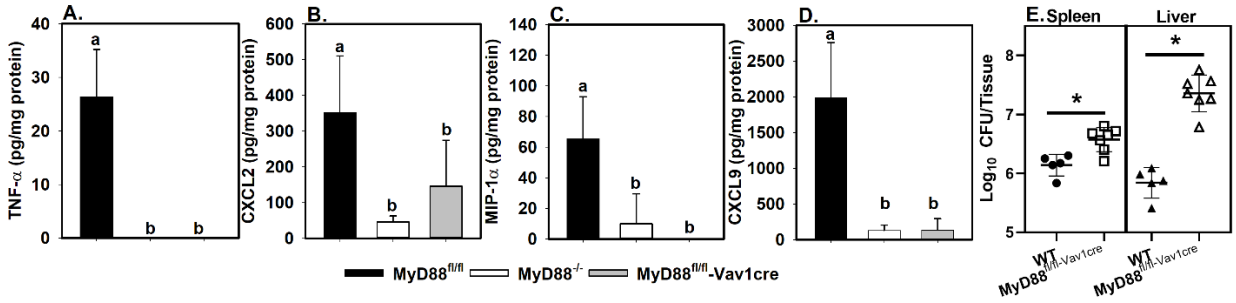
^Underlined region denote homology to *gluP* upstream fragment

#Underlined region denote homology to cloned chloramphenicol resistance gene

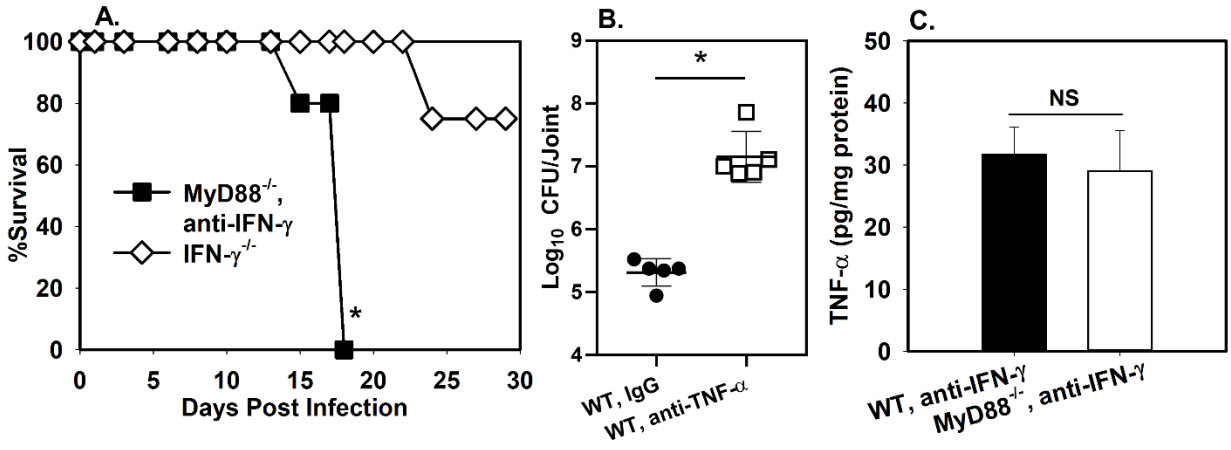
All primers were from IDT, Coralville, IA



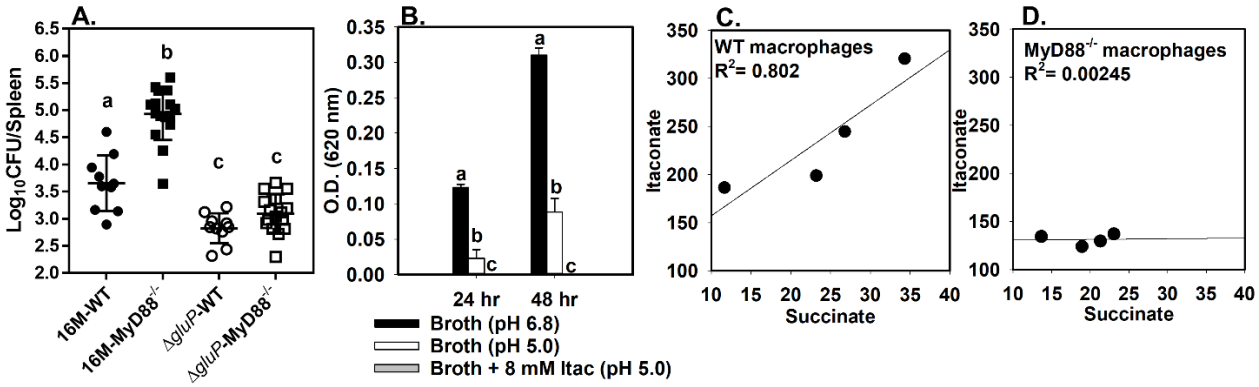
Supplementary Figure 1. A-C) Joints from WT mice one or three days after footpad infection with 10^6 *B. melitensis* were digested and processed for flow cytometry in media containing 50 µg/ml gentamicin to kill extracellular bacteria. After digestion, Fc receptors were blocked, and cells were stained with antibodies against CD45.2, F4/80, and Ly-6G. Cells were then washed in antibiotic free media. After gating for single, live cells, cells were sorted into the following populations: $CD45.2^-$ (non-hematopoietic), $CD45.2^+/F4/80^+/Ly\text{-}6G^-$(macrophages), $CD45.2^+/F4/80^-/Ly\text{-}6G^{HI}$ (neutrophils) and $CD45.2^+/F4/80^-/Ly\text{-}6G^-$ (other hematopoietic/$CD45.2^+$). Sorted cells (~100,000 cells/population, ~95% purity) were lysed, diluted, and plated on agar. The cellular composition of the joint **A)** CFUs per sorted cell **B)**, and proportion of joint burden was calculated **C)**. *P<0.05 as compared to same population in naïve mice in **A)** or as compared to all other cell populations at the same timepoint in **B)**. All error bars depict S.D. of the mean.



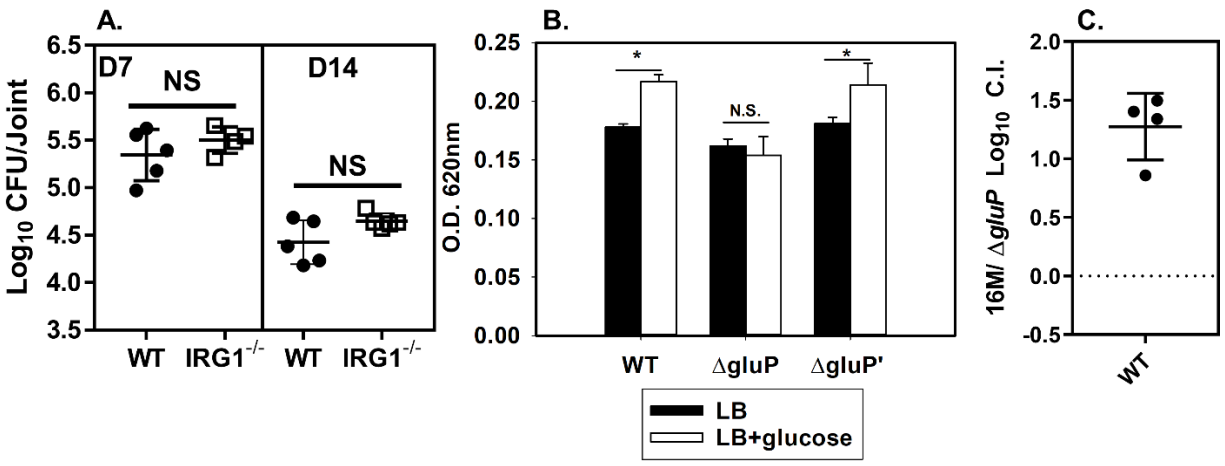
Supplementary Figure 2. A-D). MyD88$^{fl/fl}$, MyD88$^{-/-}$, and MyD88 $^{fl/fl\text{-}Vav1cre}$ mice (n=4-5/group) were footpad infected with 1x10^5 CFU of *B. melitensis* 16M and cytokine levels in joints were measured two days after infection via Luminex. **E)** WT and MyD88 $^{fl/fl\text{-}Vav1cre}$ mice (n=5-7/group) were infected 1x10^5 CFU of *B. melitensis* 16M and spleen and liver CFU levels were determined seven days post-infection. Error bars depict S.D. of the mean. Means with the same letter are not significantly different from each other via ANOVA in **A-D)**. *P<0.05 as compared to WT mice in **E)**.



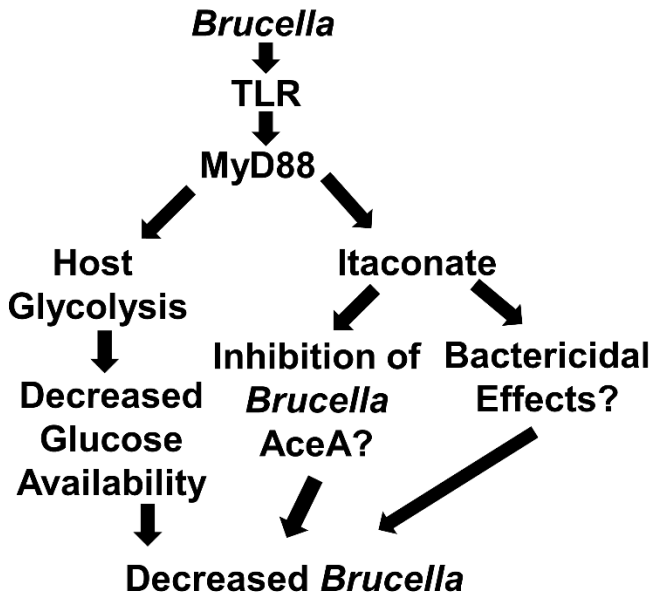
Supplementary Figure 3. A) MyD88$^{-/-}$ mice (n=5) were treated with anti-IFN-γ and infected i.p. with 1x10^3 *B. melitensis* 16M. IFN-γ$^{-/-}$ mice (n=4) were infected i.p. with 1x10^5 *B. melitensis* 16M. Survival was monitored over time. *P<0.05 as compared to IFN-γ$^{-/-}$ mice. **B)** WT mice (n=5/group) were treated with IgG or anti-TNF-α and infected in the footpad with 1x10^5 CFU of *B. melitensis* 16M. Joint CFU levels were determined seven days post-infection. *P<0.05 as compared to IgG-treated mice. **C)** WT or MyD88$^{-/-}$ mice (n=4/group) were treated with anti-IFN-γ and infected in the footpad with 1x10^5 CFU of *B. melitensis* 16M. Joint TNF-α levels were determined seven days post-infection. Error bars depict S.D. of the mean.



Supplementary Figure 4. A) WT or MyD88$^{-/-}$ mice were footpad infected (n=10-16) with 10^5 CFUs containing a 1:1 mix of WT *B. melitensis* "16M" and *B. melitensis*Δ*gluP* "Δ*gluP*". Fourteen days after infection CFU levels of 16M and Δ*gluP* were determined in spleens. Error bars depict S.D. of the mean. Means with the same letter are not significantly different from each other via ANOVA. Data is combined from two experiments. This data was used to calculate the splenic Log_{10} C.I. data in Figure 3C. **B)** *B. melitensis* was cultured in broth (n=5 wells/group) with or without exogenous 8 mM itaconate (pH 5.0). As a comparison, *B. melitensis* was also grown in broth adjusted to pH 5.0 with HCl. Growth was assessed by O.D. at 24 and 48 hours post-infection. Sample means at the same timepoint with the same letter are not significantly different from each other via ANOVA. Macrophages from WT **C)** or MyD88$^{-/-}$ **D)** mice (n=4 wells/group) were infected with *B. melitensis* 16M at a MOI of 100 and intracellular metabolite levels were determined via GC-MS at 48 hours post-infection. Itaconate versus succinate levels in the same macrophage sample were then compared via linear regression.



Supplementary Figure 5. A) WT or IRG1$^{-/-}$ mice (n=4-5/group) were infected in the footpad with 1x10^5 CFU of *B. melitensis* 16M. Joint CFU levels were measured seven (D7) or fourteen days (D14) post-infection. **B)** *B. melitensis* 16M (WT), an isogenic Δ*gluP mutant* and a complemented mutant (Δ*gluP'*) (n=5 wells/group) were grown at 37C/5%CO_2 in LB broth with or without 0.05% glucose. After 48 hours, the O.D. 620nm of the cultures was measured. *P<0.05 as compared growth in LB without glucose. **C)** WT mice were infected i.n. (n=4) with 10^5 CFUs containing a 1:1 mix of WT *Bm* "16M" and *B. melitensis*Δ*gluP*. 7 days post-infection, a Log_{10} Competitive Index (C.I.) was calculated for lungs based on relative strain recovery. Error bars depict S.D. of the mean.



Supplementary Figure 6. In sum, our results indicate that activation of TLR/MyD88 signaling by *Brucella* promotes host cell glycolysis and itaconate production. Host glycolysis reduces glucose availability, which prevents *Brucella* from utilizing its GluP transporter to exploit host glucose. Itaconate production by the host restricts *Brucella* infection perhaps through inhibition of the *Brucella* isocitrate lyase, AceA, or via direct antibacterial effects.