Figure S1

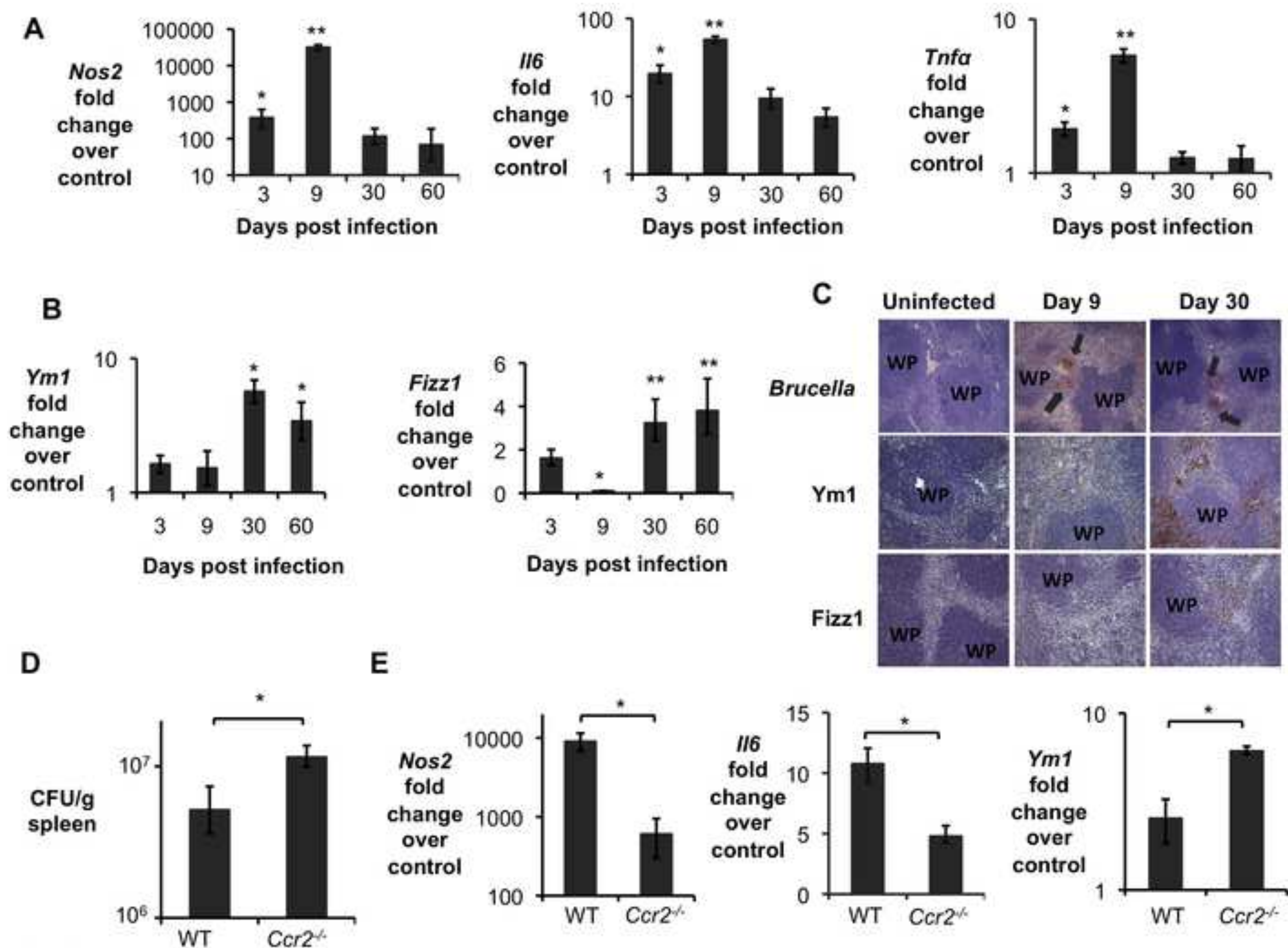

Figure S2

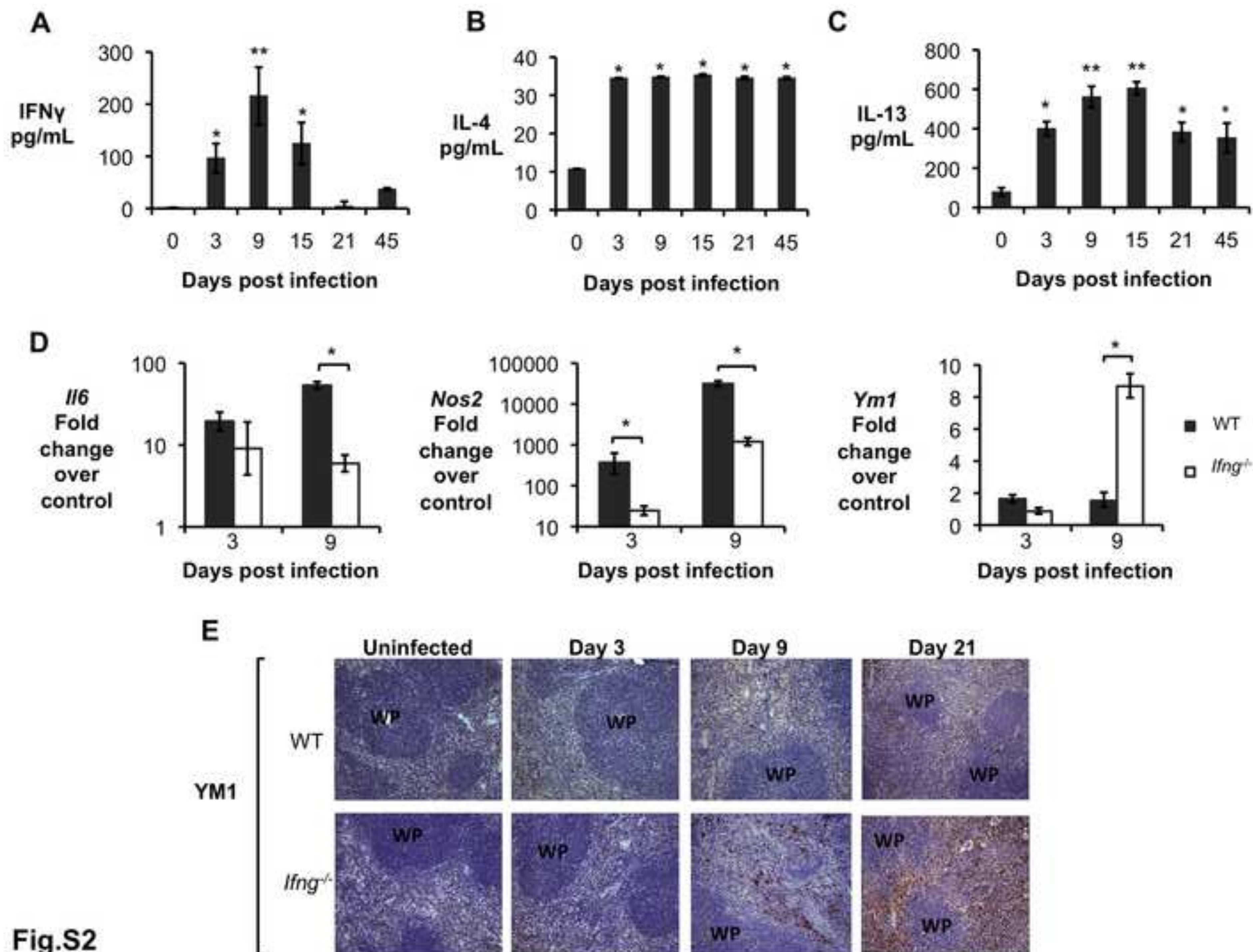

Fig.S2

Figure S3

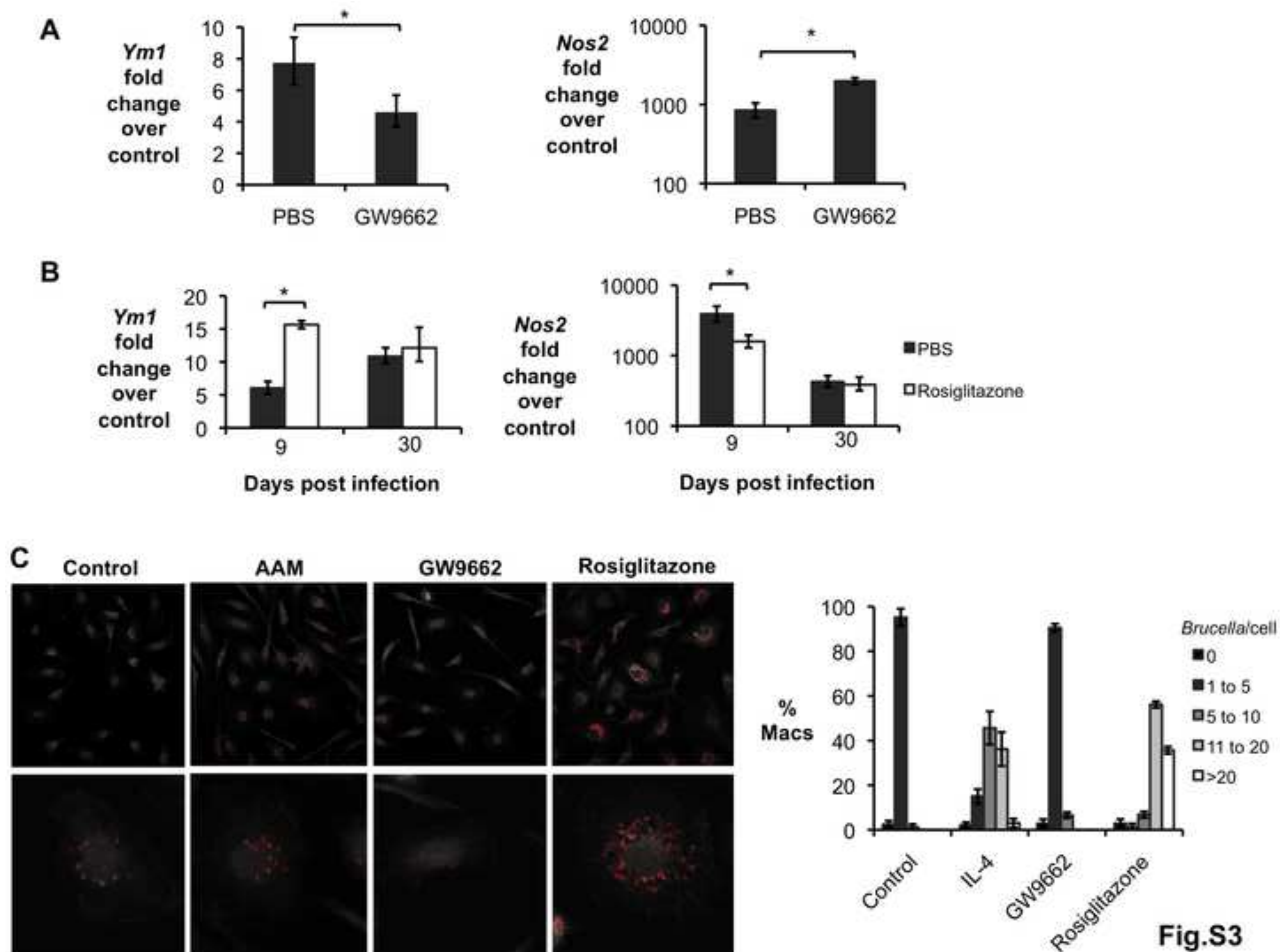

Figure S4

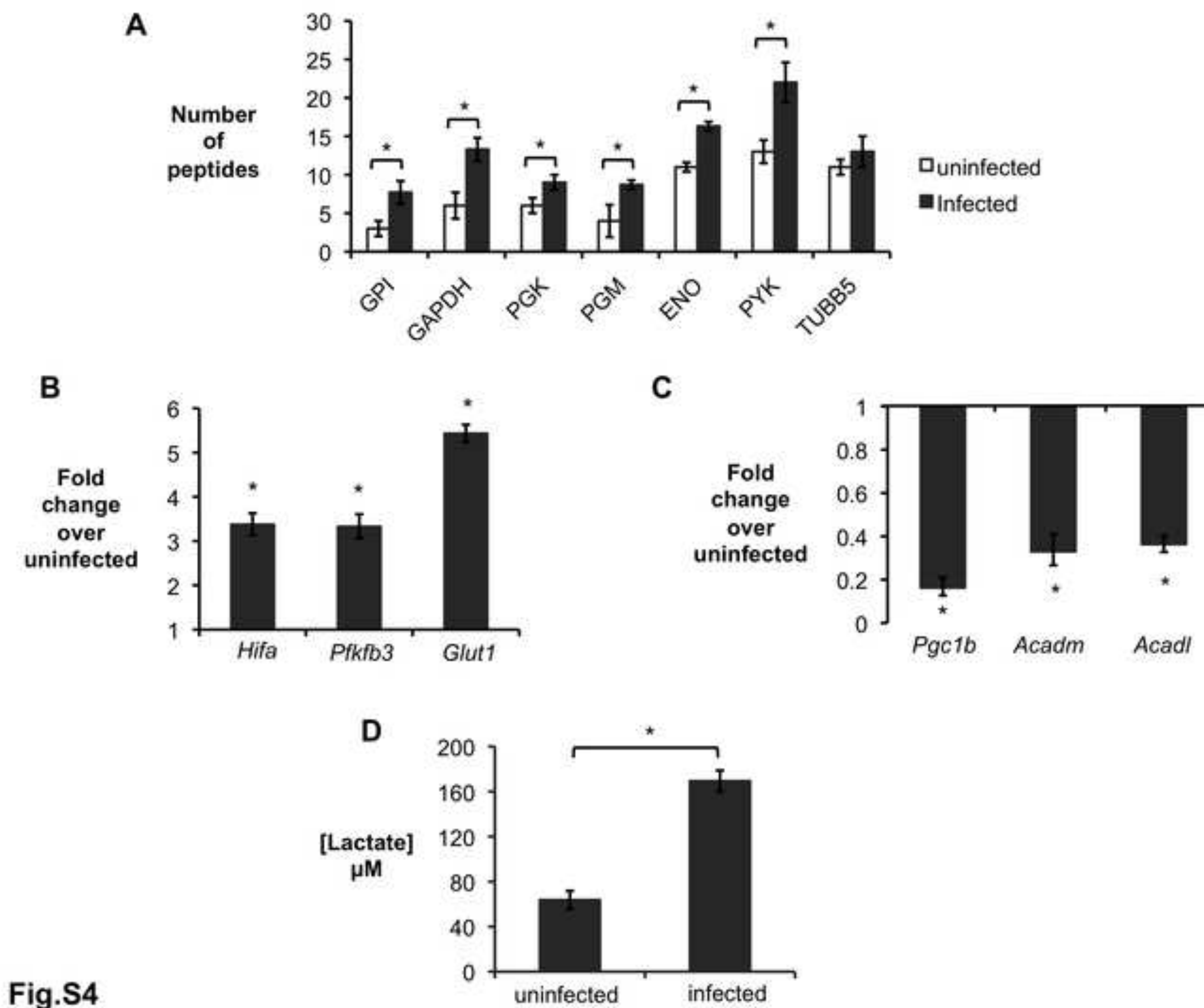

Figure S5

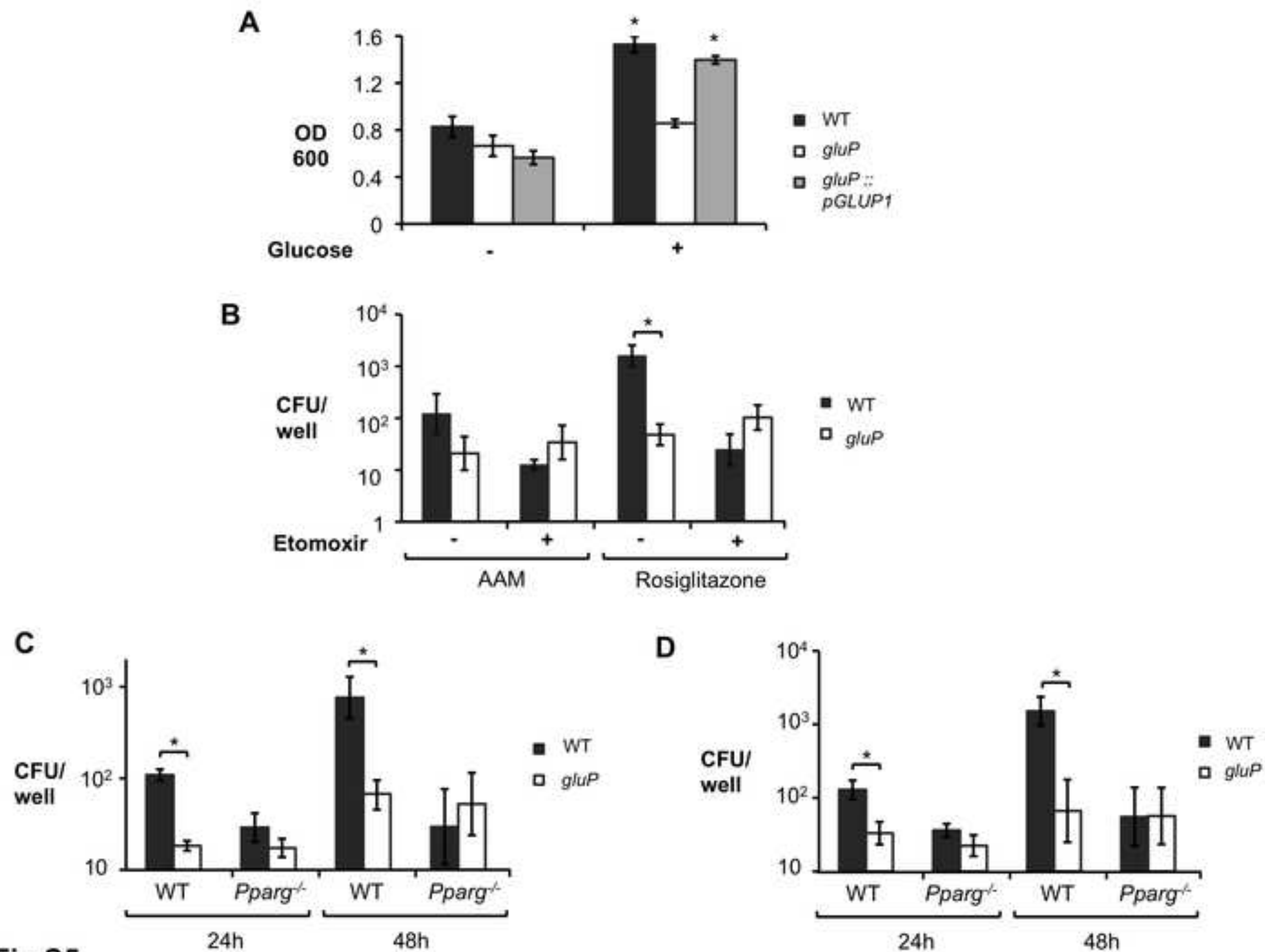

Fig.S5

SUPPLEMENTAL INFORMATION

Supplementary figure 1. Related to Figure 1. (A) Real time RT-PCR gene expression analysis of CAM genes *Nos2, Il6* and *Tnfa* in splenic CD11b$^+$ cells from *B. abortus* infected C57BL/6J mice (n=5) at 3, 9, 30 and 60 d.p.i. **(B)** Real time RT-PCR gene expression analysis of AAM genes *Ym1* and *Fizz1* in CD11b$^+$ splenic cells from *B. abortus* infected C57BL/6J mice (n=5) at 3, 9, 30 and 60 d.p.i. **(C)** Immunolabeling of *B. abortus* (first panel, black arrows) and AAM markers Ym1 and Fizz1 in spleens of *B. abortus* infected mice at 9 and 30 d.p.i. (x20). **(D)** *B. abortus* 2308 CFU counts in spleens from C57BL/6J and CCR2$^{-/-}$ mice (n=6) at 9 days post infection (d.p.i). **(E)** Real time RT-PCR gene expression analysis of CAM genes *Nos2* and *Il6* and AAM gene *Ym1* in CD11b+ splenic cells from *B. abortus* infected C57BL/6J and CCR2$^{-/-}$ mice (n=5) at 9 d.p.i. Values represent mean ± SEM. (*) Represents P<0.05 and (**) represents P<0.01 using one way ANOVA for (A-B) or unpaired t-test analysis for (D-E).

Supplementary figure 2. Related to Figure 3. Detection of IFN-γ **(A)**, IL-4 **(B)** and IL-13 **(C)** in serum from *B. abortus* infected C57BL/6J mice (n=5) at 0, 3, 9, 15, 21 and 45 days post-infection using a multiplex cytokine array. **(D)** Real time RT-PCR gene expression analysis of CAM genes *Il6* and *Nos2* and AAM gene *Ym1* in CD11b$^+$ splenocytes from *B. abortus*-infected C57BL/6J and congenic *Ifng*$^{-/-}$ mice (n=5) at 3 and 9 d.p.i. **(E)** Immunolabeling of AAM marker Ym1 in spleens of *B. abortus*-infected C57BL/6J and congenic IFNγ$^{-/-}$ mice at 3, 9 and 21 d.p.i. (x20). Values represent mean ± SEM. (*) Represents P<0.05 and (**) represents P<0.01 using one way ANOVA for (A-C) or unpaired t-test analysis for (D).

Supplementary figure 3. Related to Figure 4. (A) Real time RT-PCR gene expression analysis of CAM marker *Nos2* and AAM marker *Ym1*. Transcripts were measured in

CD11b^{+} splenocytes obtained at 30 d.p.i.,from *B. abortus*-infected C57BL/6J mice (n=5) that had been treated daily from 18 to 30 d.p.i. with either PPARγ antagonist GW9662 or the diluent (PBS). **(B)** Real time RT-PCR gene expression analysis of CAM marker *Nos2* and AAM marker *Ym1*, measured at 9 and 30 d.p.i., in CD11b+ splenocytes from *B. abortus* infected C57BL/6J mice (n=5) treated daily for 7 days prior to infection with PPARγ agonist Rosiglitazone or PBS control. **(C)** Fluorescence microscopy of BMDM from C57BL/6J non-stimulated (Control), or stimulated with 10 ng/mL of rIL-4 (AAM) or with 10 ng/mL of IL-4 + 3μM of GW9662 or with 5μM of Rosiglitazone and infected with mCherry-expressing *B. abortus* 2308 (MX2; red) for 24h. Nuclei in white are stained with DAPI (left panel). Quantification of intracellular *B. abortus* MX2 in individual BMDM treated as described above (right panel). Values represent mean ± SEM from four independent experiments, each conducted in duplicate. (*) Represents $P<0.05$ using unpaired t-test analysis.

Supplementary figure 4. Related to Figure 5. (A) Mass spectrometry analysis of glycolytic pathway enzymes GPI (glucose phosphate isomerase), GAPDH (glyceraldehyde-3-phosphate-dehydrogenase), PGK (phosphoglycerate kinase), PGM (phosphoglycerate mutase), ENO (enolase 1), PYK (pyruvate kinase) and TUBB5 (Tubulin beta 5; loading control) as control in RAW 264.7 macrophages uninfected or infected with *Brucella* for 4 hours. Results shown are compiled from three independent experiments. **(B)** Real time RT-PCR gene expression analysis of glycolytic pathway genes *Hifa* (hypoxia inducible factor α), *Pfkfb3* (phosphofructokinase-3) and *Glut1* (glucose transporter 1) in BMDM from C57BL/6J infected with *B. abortus* for 8 hours. **(C)** Real time RT-PCR gene expression analysis of fatty acid β-oxidation pathway genes *Pgc1b* (PPARγ coactivator 1 β), *Acadm* (medium-chain acyl-CoA dehydrogenase) and *Acadl* (long-chain acyl-CoA dehydrogenase) in BMDM from C57BL/6J infected with *B.*

abortus for 8 hours. **(D)** Measurement of lactate concentration in supernatant from BMDM from C57BL/6J uninfected or infected with *B. abortus* for 24 hours. Values represent mean ± SEM from four independent experiments, each conducted in duplicate. (*) Represents $P<0.05$ using one way ANOVA for (B-C) or unpaired t-test analysis for (A) and (D).

Supplementary figure 5. Related to Figure 6. **(A)** *In vitro* growth of *B. abortus* 2308 WT, isogenic *gluP* mutant and complemented *gluP* mutant (*gluP*::pGLUP1) in Tryptic Soy Broth formulated with (+) or without (-) glucose (0.25%), as measured by optical density (OD_{600}) at 24h. Data shown are compiled from three independent experiments, each conducted with duplicate samples. **(B)** Recovery of *B. abortus* from C57BL/6J BMDM treated with the β-oxidation inhibitor etomoxir (50 μM) or vehicle (PBS) in the presence of 10 ng/mL of rIL-4 (AAM) or 5μM of PPARγ agonist Rosiglitazone and infected with *B. abortus* 2308 WT or isogenic *gluP* mutant for 24h. **(C)** Recovery of *B. abortus* from WT or congenic *Pparg-/-* BMDM stimulated with 10 ng/mL of rIL-4 (AAM) and infected with *B. abortus* 2308 WT or isogenic *gluP* mutant for 24 and 48h. **(D)** Recovery of *B. abortus* from WT or congenic *Pparg-/-* BMDM stimulated with 5 μM of PPARγ agonist Rosiglitazone and infected with *B. abortus* 2308 WT or isogenic *gluP* mutant for 24 and 48h. Values shown in B-D represent mean ± SEM of four independent experiments conducted with duplicate samples. (*) Represents $P<0.05$ using one way ANOVA for (A) or unpaired t-test analysis for (B-D).

Supplementary table 1. Real-time PCR primers used in the present study:

Target gene	Sequence
Actb	FWD: 5'-AGAGGGAAATCGTGCGTGAC-3’
	REV: 5'-CAATAGTGATGACCTGGCCGT-3’
Ym1	FWD: 5'-GGGCATACCTTTATCCTGAG-3’
	REV: 5'-CCACTGAAGTCATCCATGTC-3’
Fizz1	FWD: 5'-TCCCAGTGAATACTGATGAGA-3’
	REV: 5'-CCACTCTGGATCTCCCAAGA-3’
Pparg	FWD: 5'- CAGGCTTGCTGAACGTGAAG -3’
	REV: 5'- GGAGCACCTTGGCGAACA -3’
Il6	FWD: 5'-GCACAACTCTTTTCTCATTTCCACG-3’
	REV: 5'-GCCTTCCCTACTTCACAAGTCCG-3’
Nos2	FWD: 5'-TTGGGTCTTGTTCACTCCACGG-3’
	REV: 5'- CCTCTTTCAGGTCACTTTGGTAGG-3’
Tnfa	FWD: 5'-CATCTTGTCAAAATTCGAGTGACAA-3’
	REV: 5'-TGGGAGTAGACAAGGTACAACCC-3’
Hifa	FWD: 5'-TCTGGAAGGTATGTGGCATT-3’

	REV: 5'-AGGGTGGGCAGAACATTTAT-3'
Pfkfb3	FWD: 5'-AGCTGCCCGGACAAAACAT-3'
	REV: 5'-CTCGGCTTTAGTGCTTCTGGG-3'
Glut1	FWD: 5'-GCTGTGCTTATGGGCTTCTC-3'
	REV: 5'-CACATACATGGGCACAAAGC-3'
Pgc1b	FWD: 5'-CAAGCTCTGACGCTCTGAAGG-3'
	REV: 5'-TTGGGGAGCAGGCTTTCAC-3'
Acadm	FWD: 5'-GAAAGTTGCGGTGGCCTTGG-3'
	REV: 5'-AAGCACACATCATTGGCTGGC-3'
Acadl	FWD: 5'-GGGAAGAGCAAGCGTACTCC-3'
	REV: 5'-TCTGTCATGGCTATGGCACC-3'

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Multiplex cytokine assays. Detection of IFNγ, IL-4 and IL-13 in the serum of C57BL/6J mice was performed using Multi-Plex cytokine assays (Bio-Rad, Hercules, CA), as previously described (Rolán and Tsolis, 2008). Groups of five C57BL/6 mice were infected i.p. with 5×10^5 CFU of *Brucella abortus* 2308, and serum was obtained at necropsy at days 0, 3, 9, 15, 21 and 45 post infection. Cytokine detection was performed according to the instructions provided by the kit's manufacturer. Multiplex assays were performed in the Animal Resources and Laboratory Services Core of the Pacific

Southwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases.

Immunofluorescence microscopy: Immunofluorescence of *B. abortus* infected BMDM was performed as previously described (Starr et al., 2008). Briefly, *B. abortus* MX2-infected BMDM were grown on 12-mm glass coverslips in 24-well plates. At 24h post-infection, cells were washed three times with PBS, fixed with 3% paraformaldehyde, pH 7.4, at 37°C for 20 min, washed three times with PBS and then incubated for 10 min in 50 mM NH_4Cl in PBS in order to quench free aldehyde groups. Samples were blocked and permeabilized in 10% goat serum and 0.1% saponin in PBS for 30 min at room temperature. Cells were labeled by inverting coverslips onto drops of DAPI (Invitrogen, Grand Island, NY) diluted 1:200 in 10% horse serum and 0.1% saponin in PBS and incubating for 45 min at room temperature. Cells were washed twice with 0.1% saponin in PBS, once in PBS, once in H_2O and then mounted in Mowiol 4-88 mounting medium (Calbiochem). Samples were observed on a Carl Zeiss LSM 510 confocal laser scanning microscope for image acquisition (Carl Zeiss Micro Imaging). Confocal images of 1024 × 1024 pixels were acquired as projections of three consecutive slices with a 0.38-μm step and assembled using Adobe Photoshop CS2 (Adobe Systems). For quantification of intracellular *Brucella* MX2, 50 BMDM/sample were counted. All experiments were performed independently in triplicate.

Growth in Tryptic Soy Broth *in vitro.* Tryptic soy broth (TSB) with or without glucose was prepared by adding 17g of enzymatic digest of casein (Difco/Becton-Dickinson, Sparks, MD), 3g of enzymatic digest of soybean meal (Difco/Becton-Dickinson, Sparks, MD), 5g of sodium chloride and 2.5g of dipotassium phosphate to 1 liter of purified water. The solution was autoclaved at 121°C for 30 min. Once TSB reached room

temperature (RT), 12.5 mL of 20% glucose solution was added to reach a final glucose concentration of 0.25% (TSB + glucose). For *in vitro* growth essay, *B. abortus* 2308, and isogenic mutants BA159 (*gluP*) and MX6 (*gluP*::pGLUP1) overnight cultures were prepared using commercially available TSB containing glucose (Difco/Becton-Dickinson, Sparks, MD) as described in experimental procedures. Then, 1 mL of each overnight culture was washed 3 times in RT sterile PBS and the optical density (OD) was determined and adjusted to OD=1. All initial cultures were prepared by adding 100 µL of OD=1 solution to 9.9 mL of TSB or TSB + glucose, to reach starting OD of 0.01. Strains were further cultured at 37°C on a rotary shaker and the final OD was determined at 24 hours post inoculation. The experiment was performed independently in duplicate at least three times and the standard error for the 24h time point calculated.

Mass spectrometry analysis. RAW 264.7 macrophages were infected with *Brucella melitensis* 16M for 4 hours as described in Experimental procedures, then washed 3 times in 50 mM ammonium bicarbonate (AMBIC) to remove serum proteins. For mass spectrometry analysis, partially lysed cells in AMBIC were heat-treated at 100°C for 5 min, placed into a Barocycler NEP2320 (Pressure Biosciences), then subjected to pressures alternating between 31,000 PSI and atmospheric pressure for 35 cycles (20 min total run time). Samples were centrifuged 10 min at 10,000 rpm on a microcentrifuge to separate soluble and insoluble broken cell components, the supernatant containing 50 mM AMBIC and soluble components (including proteins) was retained, then proteins reduced with 10mM TCEP (Pierce BondBreaker) at 90ºC for 20 min and afterward alkylated with 15 mM iodoacetamide (IAA) at room temperature in the dark for 1 hr. The IAA was deactivated with 5 mM dithiothreitol. Samples were then digested overnight with 1 ug of Promega modified sequencing grade trypsin, after which an equal amount of dichloromethane was added to each tube; each tube was vortexed

vigorously for 2 min, then centrifuged 2 min at 15,000 rpm on a microcentrifuge to separate phases. The upper aqueous phase (containing digested peptides) was retained and the lower phase and interface (lipids and other small hydrophobics) were discarded. Samples were then dried completely in a vacuum centrifuge (Labconco), then resuspended in 2% ACN, 0.1% TFA. Samples were normalized using the A280 program on a ND-1000 Nanodrop spectrometer to roughly quantify, then loaded in equal amounts for LC-MS/MS. Digested peptides were analyzed by LC-MS/MS on a Thermo Finnigan LTQ with Michrom Paradigm LC and CTC Pal autosampler. Peptides were separated using a Michrom 200 µm x 150 mm Magic C_{18}AQ reversed phase column at 2 µl/min. Peptides were directly loaded onto a Agilent ZORBAX 300SB C_{18} reversed phase trap cartridge, which, after loading, was switched in-line with a Michrom Magic C_{18} AQ 200 um x 150 mm column connected to a Thermo-Finnigan LTQ iontrap mass spectrometer through a Michrom Advance Plug and Play nano-spray source. The nano-LC column was run with a 90 min-long gradient using a two buffer system, with Buffer A being 0.1% formic acid and Buffer B 100% acetonitrile. The gradient started with 1-10% buffer B for 5 min, then 10-35% buffer B for 65 min, 35-70% buffer B for 5 min, 70% buffer for 1 min, 1% buffer B for 14 min) at a flow rate of 2 mL min^{-1} for the maximum separation of tryptic peptides. MS and MS/MS spectra were acquired using a top 10 method, where the top 10 ions in the MS scan were subjected to automated low energy CID. An MS survey scan was obtained for the m/z range 375-1400. An isolation mass window of 2 Da was for the precursor ion selection, and a normalized collision energy of 35% was used for the fragmentation. A 2 min duration was used for the dynamic exclusion. Tandem mass spectra were extracted with Xcaliber version 2.0.7. Charge state deconvolution and deisotoping were not performed. All MS/MS samples were analyzed using X! Tandem (The GPM, thegpm.org; version CYCLONE (2013.02.01.1)). X! Tandem was set up to search a Uniprot mouse reference database appended to a decoy reversed database

with the same number of entries (86070 entries total); the decoys allowing for the calculation of protein and peptide false discovery rates. Searches were conducted assuming the digestion enzyme trypsin. X! Tandem was searched with a fragment ion mass tolerance of 0.40 Da and a parent ion tolerance of 1.8 Da. Carbamidomethyl of cysteine was specified in X! Tandem as a fixed modification. Glu->pyro-Glu of the n-terminus, ammonia-loss of the n-terminus, gln->pyro-Glu of the n-terminus, deamidated of asparagine and glutamine, oxidation of methionine and tryptophan, dioxidation of methionine and tryptophan and acetyl of the n-terminus were specified in X! Tandem as variable modifications. Scaffold (version Scaffold_4.0.1, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 80.0% probability by the Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii, AI et al Anal. Chem. 2003;75(17):4646-58). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Proteins sharing significant peptide evidence were grouped into clusters. These parameters yielded a protein decoy false discovery rate (FDR) of 4.1% and a peptide decoy FDR of 0.61%, with 862 proteins identified in total. Differences between *Brucella* infected and control samples were observed using spectral counting and the T-test feature of the Scaffold program, with the weighted quantitative value (total spectra) of each sample as the criterion for differences in samples.