Genome-Wide Screens in Macrophages Identify Host Genes that Support *Brucella* Early Infection

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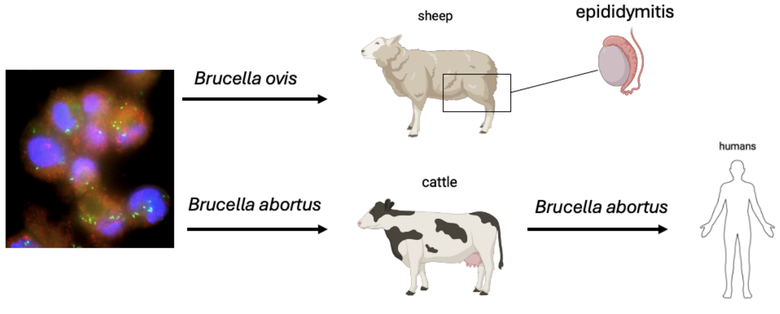
Abstract

Brucellosis is a widespread zoonosis caused by intracellular bacterial pathogens of the genus, *Brucella*. While it is well established that nearly all *Brucella* species infect and replicate within mammalian phagocytes, our understanding of the specific host factors and host-cellular processes that facilitate *Brucella* infection into host cells remain incomplete. To address this gap in knowledge, we conducted a genome-wide forward genetic CRISPR-Cas9 screen in THP-1 human macrophage-like cells to identify host genes that support *Brucella ovis* (*B. ovis*) and *Brucella abortus* (*B. abortus*) early infection. Among the candidate pan-infection host factors identified were C-terminal Src kinase (CSK), RAS-responsive element binding protein 1 (RREB1), and TMEM30A, a regulator of phospholipid homeostasis. Additionally, pathway enrichment analysis highlighted several host cellular pathways, including the mechanistic target of rapamycin (mTOR) signaling pathway, with key genes such as LAMTOR2 and AKT1 emerging as notable hits. We validated the screen by generating targeted knockouts of select genes in THP-1 cells, confirming their roles in promoting *Brucella* infection. These results highlight a diverse set of host factors and cellular pathways that facilitate intracellular replication, offering new insights into *Brucella* infection biology and identifying potential targets for future therapeutic intervention.

Background

- *Brucella* are Gram-negative intracellular pathogens.
- There are currently > 12 species of *Brucella* identified, each having preferred animal hosts.
  - *B. ovis* is a BSL2 pathogen restricted to sheep and rams.
  - *B. abortus* is a BSL3 pathogen that can cause chronic disease in cattle and humans.



What host-genes and pathways are required for *Brucella* macrophage infection?

Results

1. Fluorescently labeled mNeonGreen *Brucella* can be detected in macrophages by flow cytometry

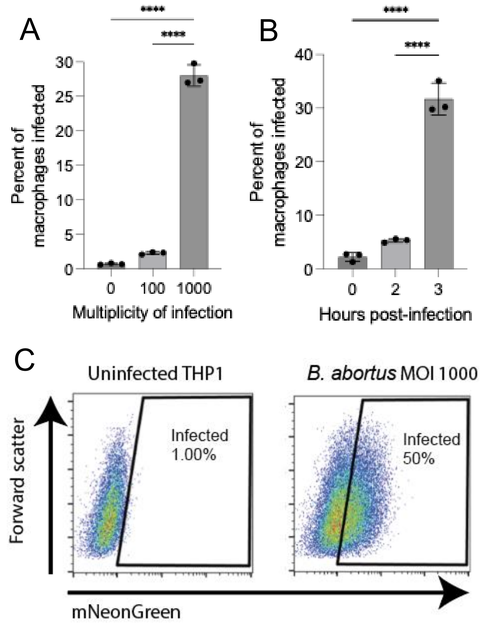


Figure 1. Early interactions with fluorescent *Brucella* and macrophages can be detected by flow cytometry. (A) THP-1 macrophages were infected with mNeonGreen *B. ovis* at multiplicities of infections of 100 and 1,000 for 3 hours. Flow cytometry was used to measure the percentage of cells that were infected (mNeonGreen). (B) THP-1 macrophages were infected with mNeonGreen *B. ovis* at a multiplicity of 1,000 for 2 or 3 hours. The percentage of macrophages infected with mNeonGreen *B. ovis* was determined by flow cytometry. Values are means ± standard deviations (SD) of 3 independent replicates. Statistical significance was calculated using one-way ANOVA, followed by Dunnet's multiple-comparison test (***, P < 0.001; ****; P < 0001). (C) Representative flow cytometry plots of THP-1 macrophages infected with mNeonGreen *B. abortus*.

2. A genome-wide CRISPR-cas9 screen identifies host genes required for early *Brucella* macrophage infection

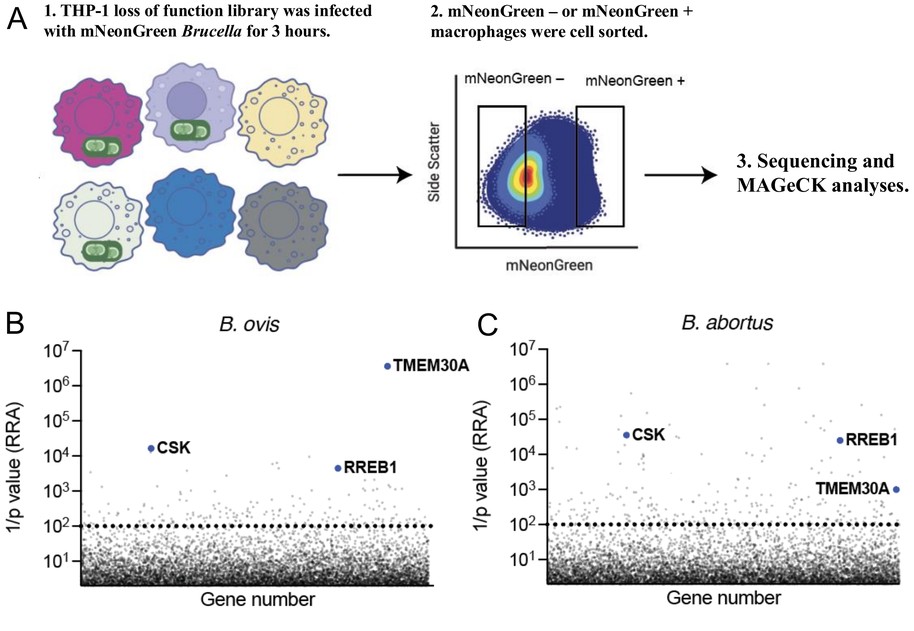


Figure 2. Genome-wide loss-of function screens in THP1 macrophages identify host genes required for early infection of *B. ovis* and *B. abortus*. (A) A schematic of the genome-wide screen to identify host genes that are required for *Brucella* macrophage infection. A genome-wide CRISPR-Cas9 library generated in Cas9+ THP-1-macrophage like cells was infected with mNeonGreen *Brucella* for 3 h. mNeonGreen+ and mNeonGreen- populations were isolated by FACS and the abundance of sgRNAs in each population was determined by sequencing. (B-C) Shown is the score determined by the alpha-robust rank algorithm (α-RRA) in MAGeCK for each gene in the CRIPSR-Cas9 library that passed filtering metrics. Highlighted genes represent host genes that contribute to early infection of (B) *B. ovis* and (C) *B. abortus*.

3. CSK, TMEM30A and RREB1 macrophage mutants are less susceptible to *Brucella* infection

A

Gene name	Function
CSK (C-terminal SRC kinase)	Non-receptor tyrosine protein kinase
TMEM30a (transmembrane protein 30a)	Amino phospholipid flippase
RREB1 (Ras responsive element binding protein)	Transcription factor

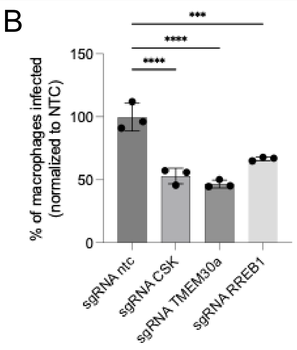
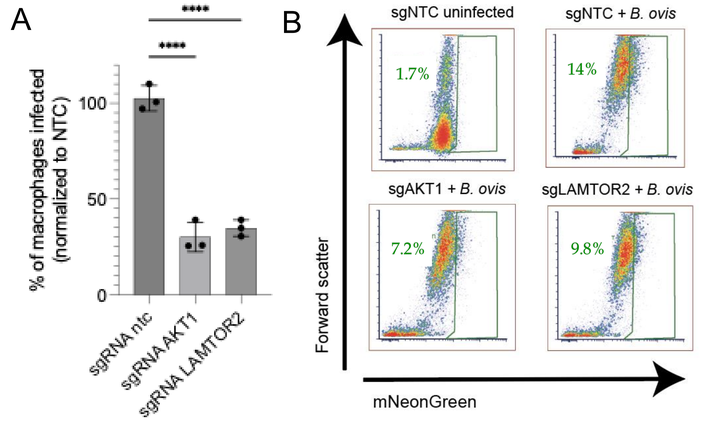


Figure 3. (A) Table of gene names and annotated functions for host factors identified in the genome-wide CRISPR-Cas9 screen. (B) Cas9-expressing THP-1 cells transduced with the indicated sgRNAs were infected with mNeonGreen *B. ovis* for 3 hours. The percentage of infected macrophages was quantified by flow cytometry and normalized to the mean infection rate of non-targeting control cells. Data represent mean ± standard deviation (SD) from three independent experiments. Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparisons test (****P < 0.0001; ***P < 0.001).

4. Positive regulators of mTOR signaling support *Brucella* macrophage infection



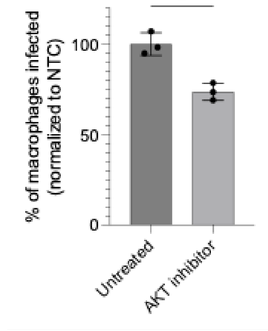


Figure 4. (A) Cas9-expressing THP-1 cells were transduced with the indicated sgRNAs and infected with mNeonGreen-expressing *B. ovis* for 3 hours. The percentage of infected macrophages was quantified by flow cytometry and normalized to the mean infection rate of non-targeting control cells. Data represent mean ± SD from three independent experiments. Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparisons test (****P < 0.0001). (B) Representative flow cytometry plots gated on live, single cells that were mNeonGreen-positive following infection at MOI 1,000.(C) THP-1 cells were pre-treated with 10 μM AKT inhibitor X prior to *B. ovis* infection. Infection levels were quantified by flow cytometry. Data represent mean ± SD from three independent experiments. Statistical significance was determined by unpaired t-test (**P < 0.01).

Acknowledgements & Funding

R01AI177619

Flow Cytometry Core Facility
MICHIGAN STATE UNIVERSITY