

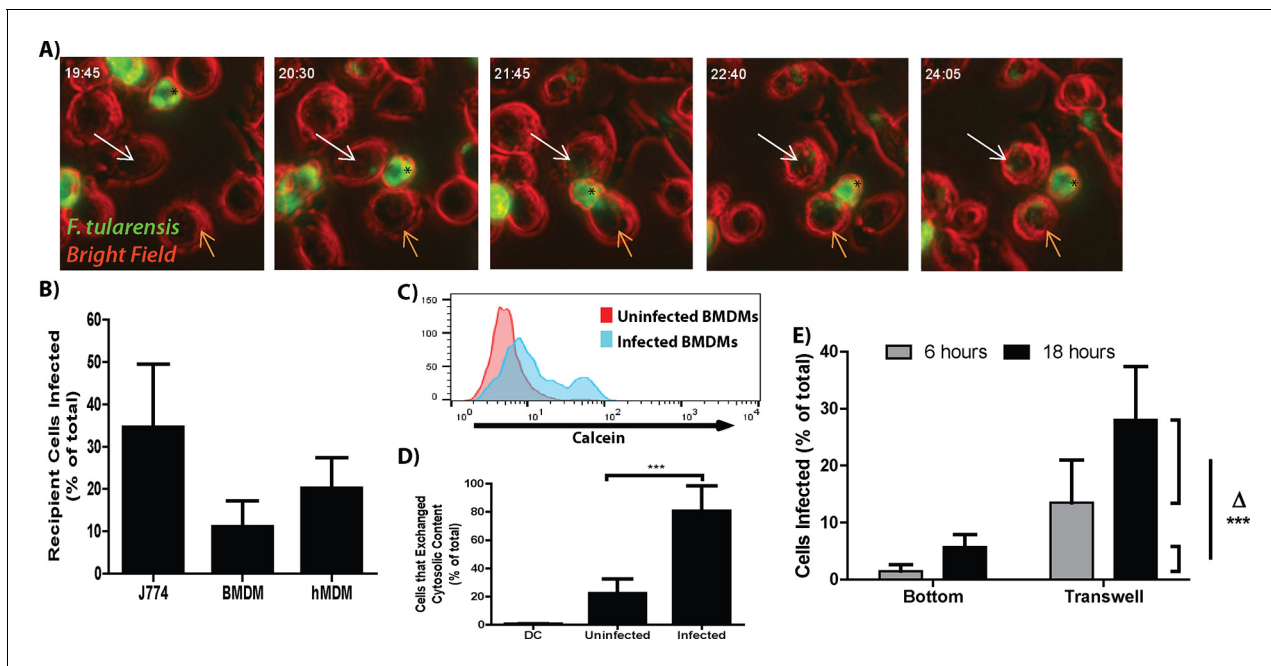


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## Figures and figure supplements

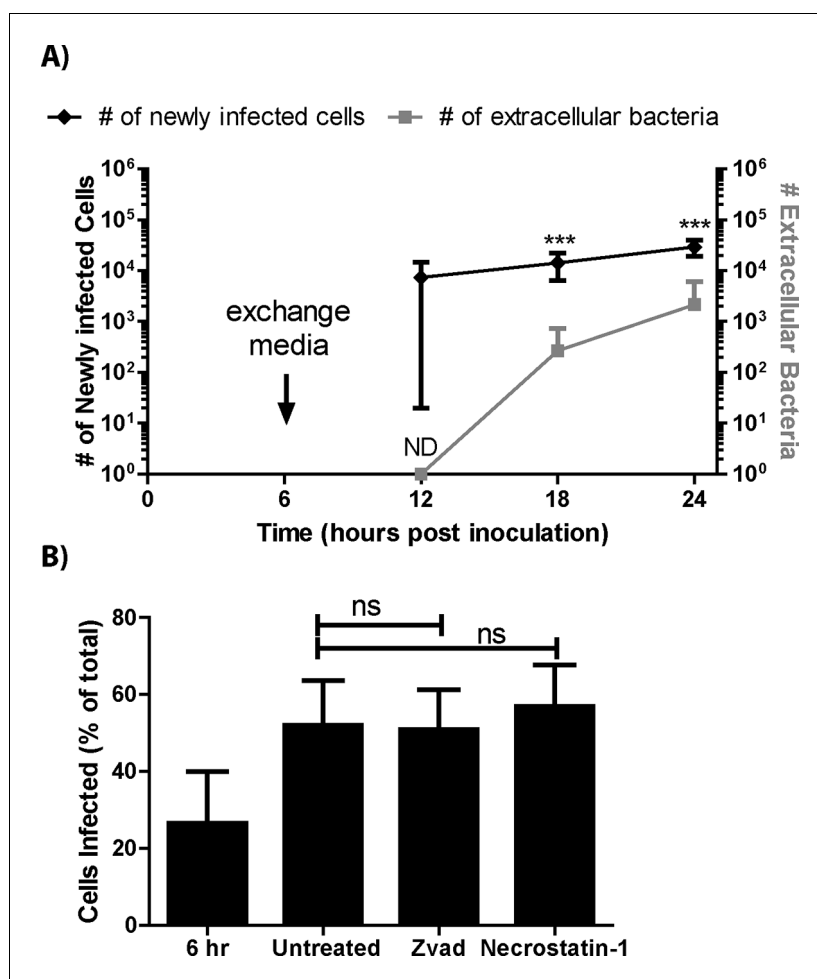
Trogocytosis-associated cell to cell spread of intracellular bacterial pathogens

**Shaun Steele et al**



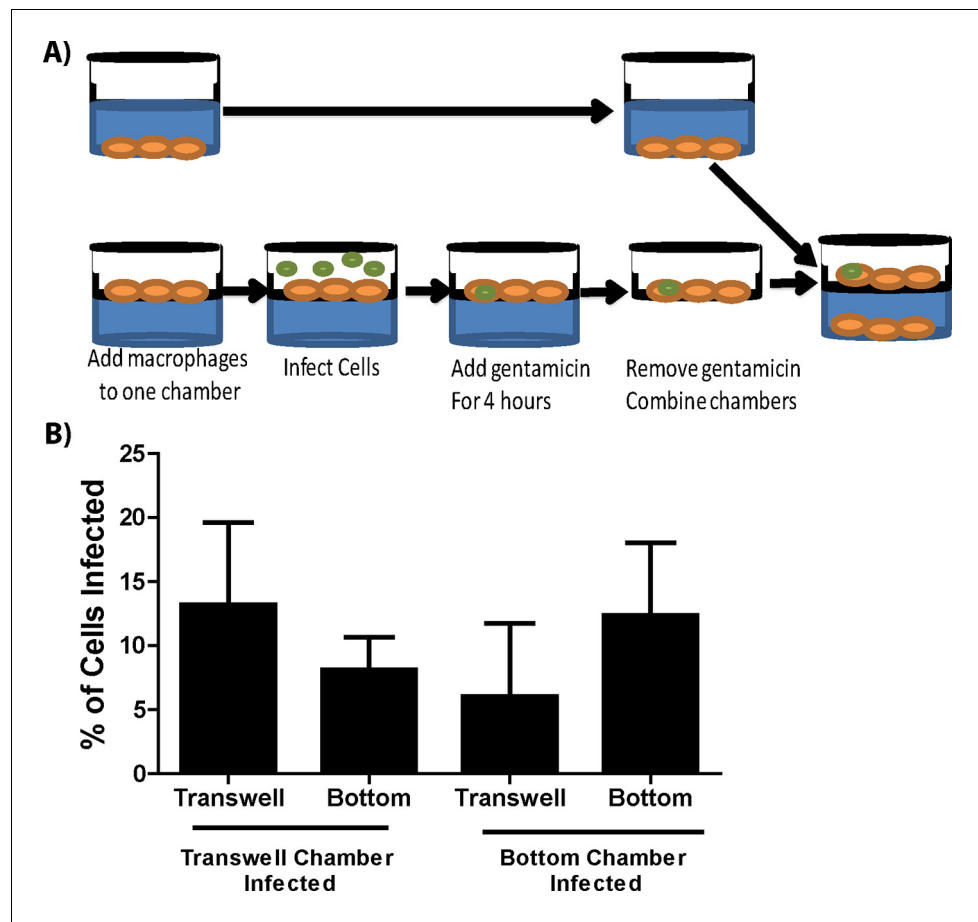
**Figure 1.** *F. tularensis* transfers between macrophages during cytosolic transfer. (A) Representative panels from live cell imaging of *F. tularensis* infected J774 cells transferring bacteria. Time- hour: minutes post inoculation; \* - initially infected cell; White arrow- first bacterial transfer event; Orange arrow- second bacterial transfer event. Movie available as **Video 1**. (B) The proportion of recipient macrophages infected after a 6 hr co-incubation with infected cells of the same type (3 independent experiments performed in triplicate). (C) A representative histogram of the amount of calcein that transferred to recipient cells ( $\log_{10}$  fluorescence) after 6 hr co-incubation. (D) The percent of infected or uninfected cells that exchanged cytosolic content (positive for both Cell Trace Red and calcein) after 6 hr co-incubation. The uninfected population represents cells in the infected well that did not become infected. DC refers to a doublet control (2 independent experiments performed in triplicate) (E) Bacterial transfer to uninfected cells is significantly higher with direct cell to cell contact. Infected BMDMs on a transwell filter were suspended over uninfected BMDMs. The percent of total cells infected on the bottom chamber (bottom) and top filter (transwell) were determined by FACS 6 and 24 hr after suspending the transwell over uninfected cells. Side brackets indicate the change in numbers of infected cells in each chamber from 6 to 24 hr. Transfer to BMDMs separated from the initially infected cells was significantly lower than transfer to BMDMs in contact with the infected cell population. (3 independent experiments performed in triplicate). (Mean  $\pm$  SD). (\*\*\*) $p < 0.001$

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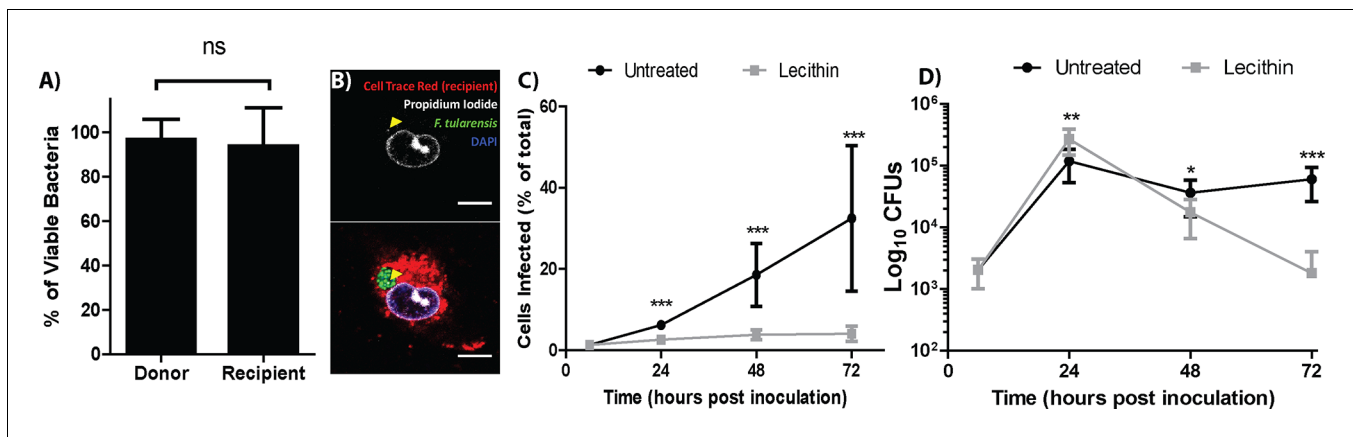
**Figure 1—figure supplement 1.** The extracellular space is not a major source of infectious bacteria. (A) The total number of BMDMs infected at 6 hr intervals compared to the number of extracellular bacteria in 1 milliliter of media. All samples were initially treated with gentamicin between 2 and 6 hr post inoculation to destroy extracellular bacteria from the inoculum. The antibiotic containing media was replaced with antibiotic free media at 6 hr post infection. (B) BMDMs were assessed for the number of infected cells at 6 or 24 hr post inoculation in the presence of specific inhibitors. Z-Vad(OMe)-FMK or necrostatin-1 were added at 6 hr post inoculation. (All experiments from 3 independent experiments performed in triplicate) (Mean  $\pm$  SD). (ND- not determined, bacterial counts below limit of detection, ns  $p > 0.05$ , \*\* $p < 0.05$ , \*\*\* $p < 0.001$ )

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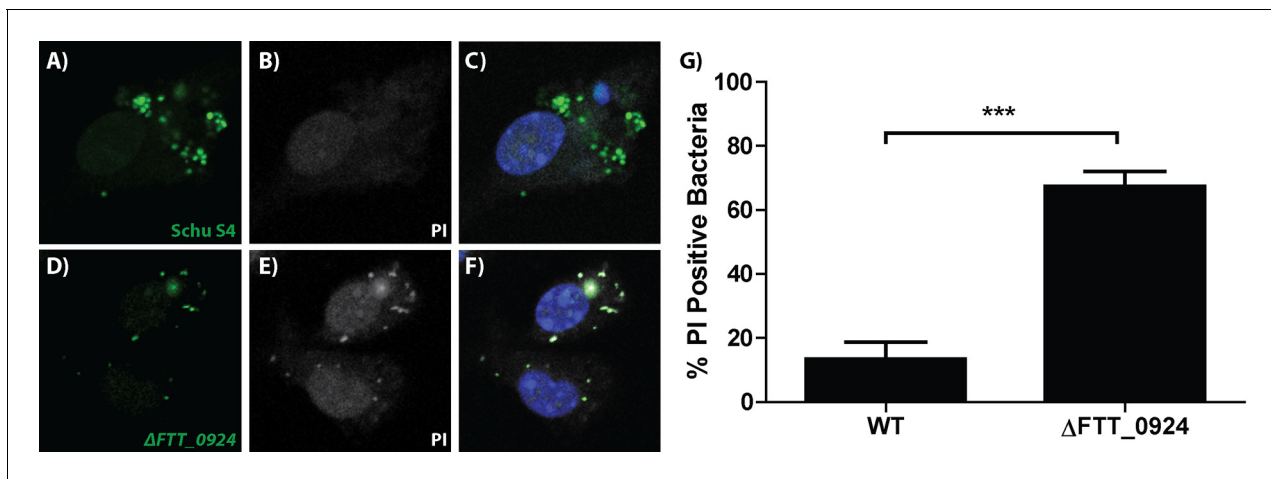
**Figure 1—figure supplement 2.** Experimental design and bacterial motility for transwell assay. (A) Experimental design of the transwell assay performed in **Figure 1E**. (B) Extracellular *F. tularensis* can penetrate and infect BMDMs on both sides of the transwell membrane. *F. tularensis* was added to the indicated chamber for 2 hr and both chambers were assessed for the number of BMDMs that became infected. (3 independent experiments performed with a single replicate) (Mean  $\pm$  SD).

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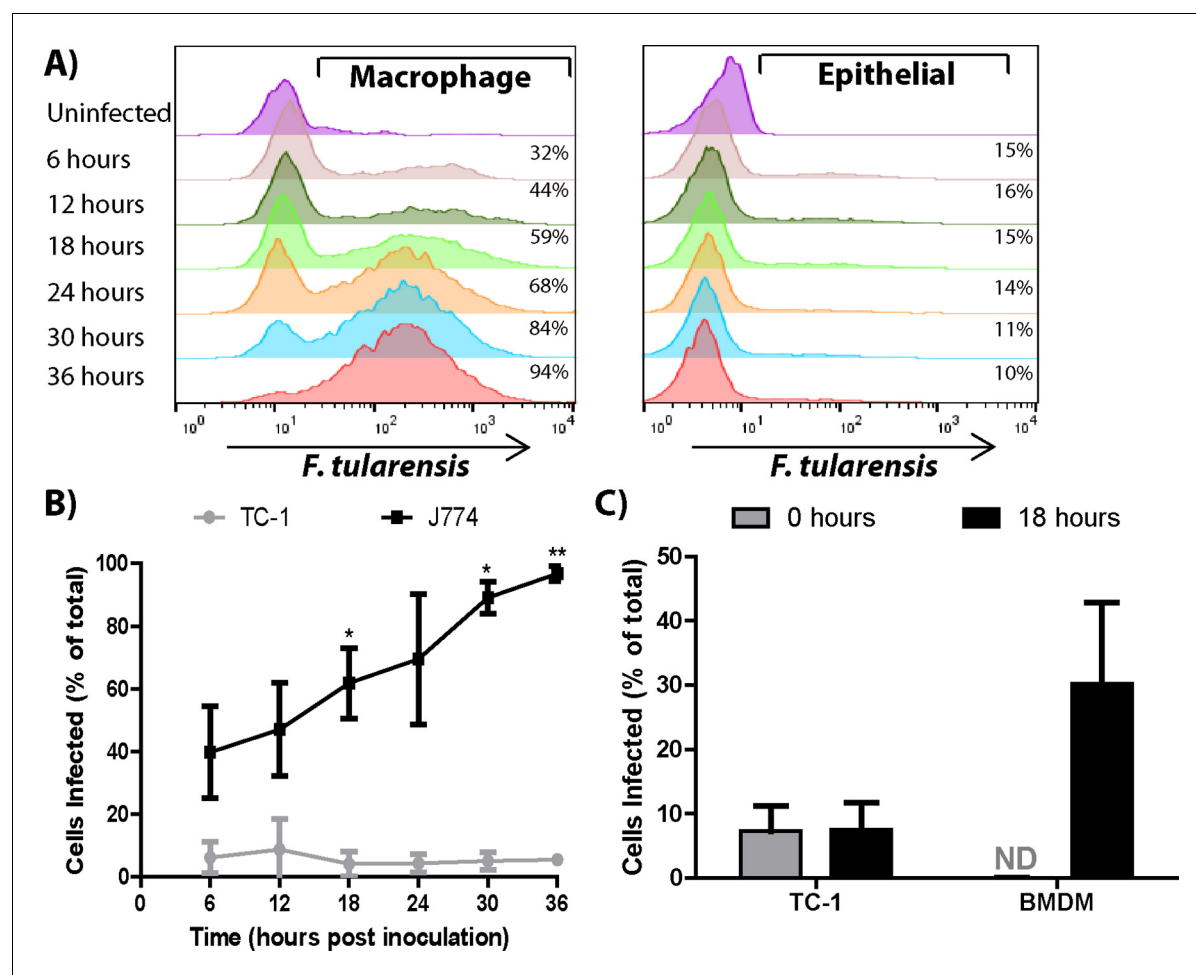
**Figure 2.** Live bacteria transfer to macrophages during bacterial transfer. (A) The percent of viable bacteria (propidium iodide negative) in donor and recipient BMDMs (2 independent experiments, 50 fields of view each) (B) Micrographs of propidium iodide treated permeabilized *F. tularensis* infected BMDMs. Arrow- propidium iodide positive bacterium. Scale bar- 10  $\mu$ M. (C) The number of cells infected in untreated or soy lecithin treated BMDMs. Infected BMDMs in soy lecithin treated (grey) and untreated (black) were quantified by FACS at indicated times post inoculation. Data are presented as number of infected cells regardless of number of bacteria per infected cell. Soy lecithin was added to the treated populations after initial infection with *F. tularensis*. (D) Soy lecithin does not inhibit *F. tularensis* intracellular replication. The number of viable bacteria in untreated or soy lecithin treated BMDMs was quantified at indicated times by dilution plating and calculation of colony forming units. Lecithin was added at 6 hr post inoculation (3 independent experiments performed in triplicate for both lecithin experiments). (Mean  $\pm$  SD). (ns  $p > 0.05$ , \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

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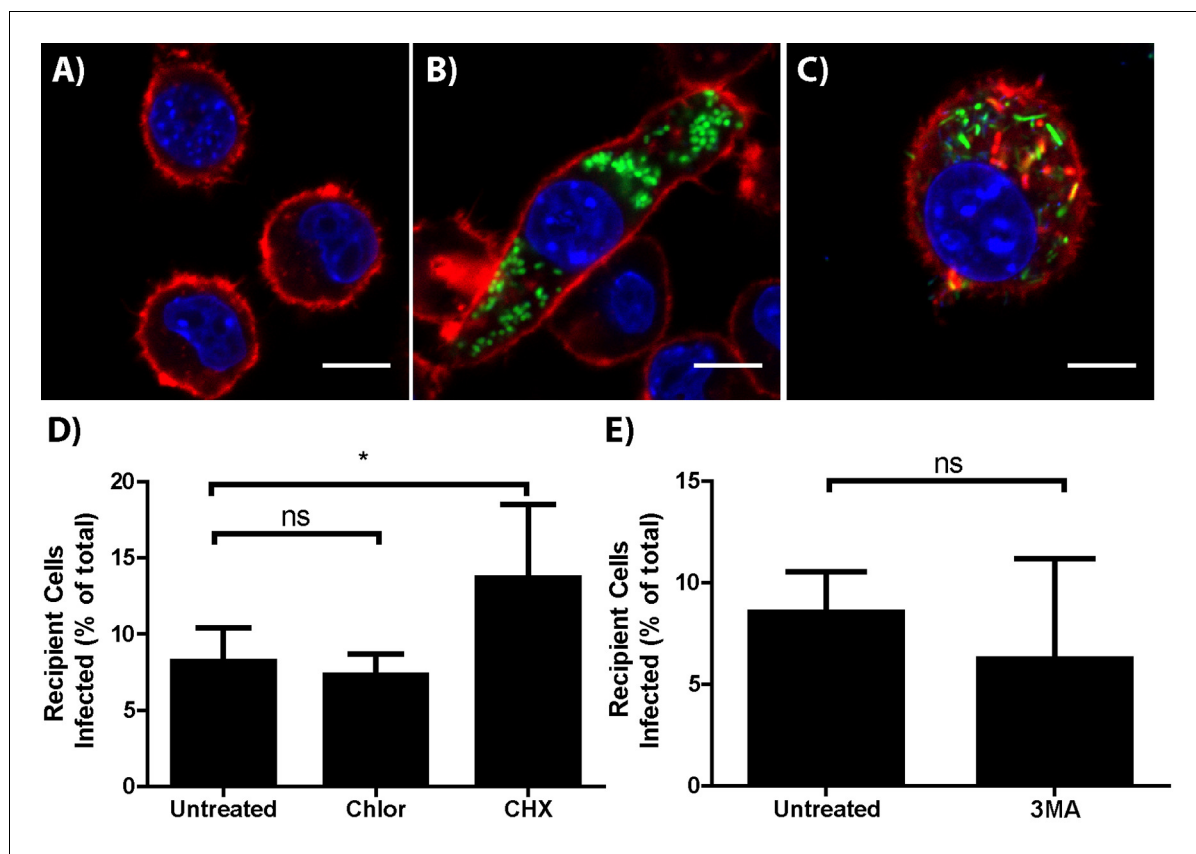
**Figure 2—figure supplement 1.** Propidium iodide can access and bind to dead intracellular bacteria following saponin treatment. Fluorescence microscopy images of BMDMs infected with (A–C) wild type Schu S4-GFP or (D–F) intracellular growth defective mutant  $\Delta$ FTT\_0924-GFP, permeabilized with saponin and treated with propidium iodide (PI). (A,D) GFP positive bacteria (green), (B,E) PI positive bacteria (white), (C,F) merged images. (G) Proportion of PI positive (dead) intracellular wild type (WT) and growth deficient mutant bacteria ( $\Delta$ FTT\_0924). The data were analyzed from n = 100 macrophages per strain from 3 independent experiments. (Mean  $\pm$  SD) (\*\*P<0.001).

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**Figure 3.** Bacterial transfer is cell type specific. (A) Percentage of infected J774 macrophages or TC-1 epithelial cells at the indicated time post inoculation (log<sub>10</sub> fluorescence). (B) A compilation of the number of J774 or TC-1 cells infected over time. Statistics represent test for a significant increase in the number of cells infected compared to the previous 6 hr time point. (C) TC-1 to TC-1 transfer vs TC-1 to BMDM transfer after a 0 or 18 hr co-incubation. (All results from 3 independent experiments performed in triplicate) (Mean +/- SD). (ns p>0.05, \*p<0.05, \*\*p<0.01).

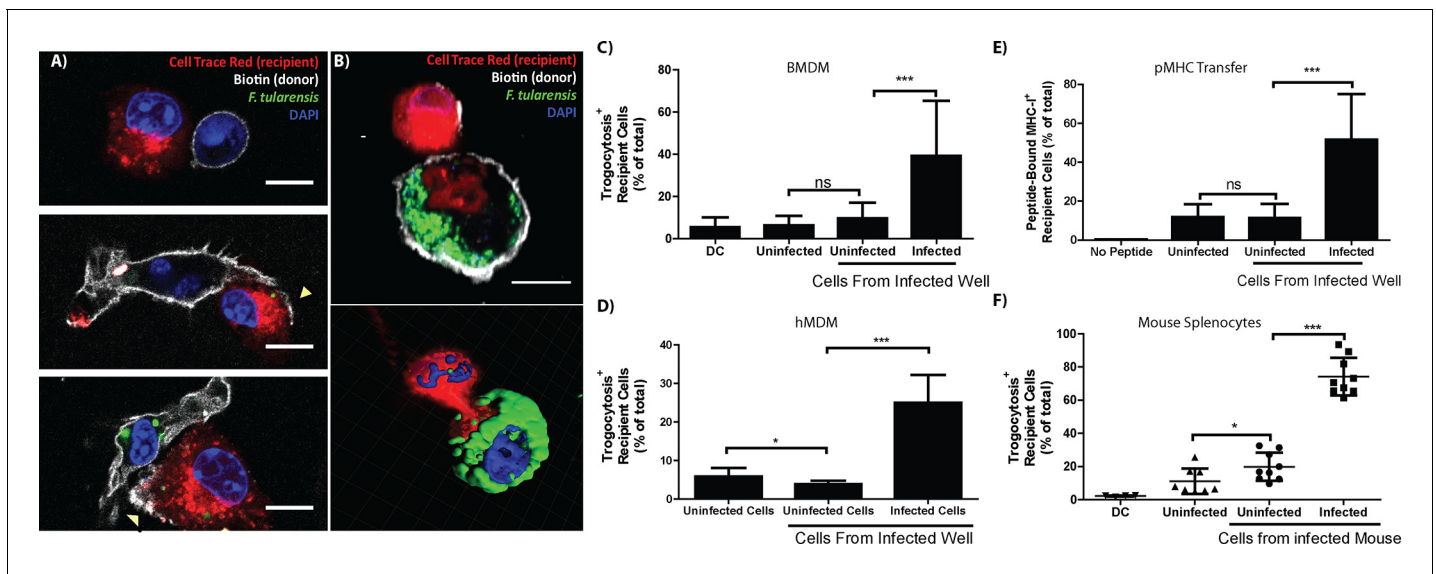
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**Figure 3—figure supplement 1.** *F. tularensis* does not transfer via actin based motility or autophagy. Representative micrographs of (A) uninfected, (B) *F. tularensis* infected, or (C) *Listeria monocytogenes* infected BMDMs 16 hr post inoculation. DAPI is depicted in blue, phalloidin (actin) in red and the bacteria in green. The scale bar represents 10  $\mu$ M. (D) Transfer of bacteria to recipient cells in the presence of host (cycloheximide [CHX]) or bacteria (chloramphenicol [Chlor]) protein synthesis inhibitors. (E) *F. tularensis* transfer to recipient BMDMs after a 6 hr co-incubation in the presence or absence of 3-methyladenine (3 MA). (All experiments from 3 independent experiments performed in triplicate) (Mean  $\pm$  SD). (ns  $p > 0.05$ , \*  $p < 0.05$ ).

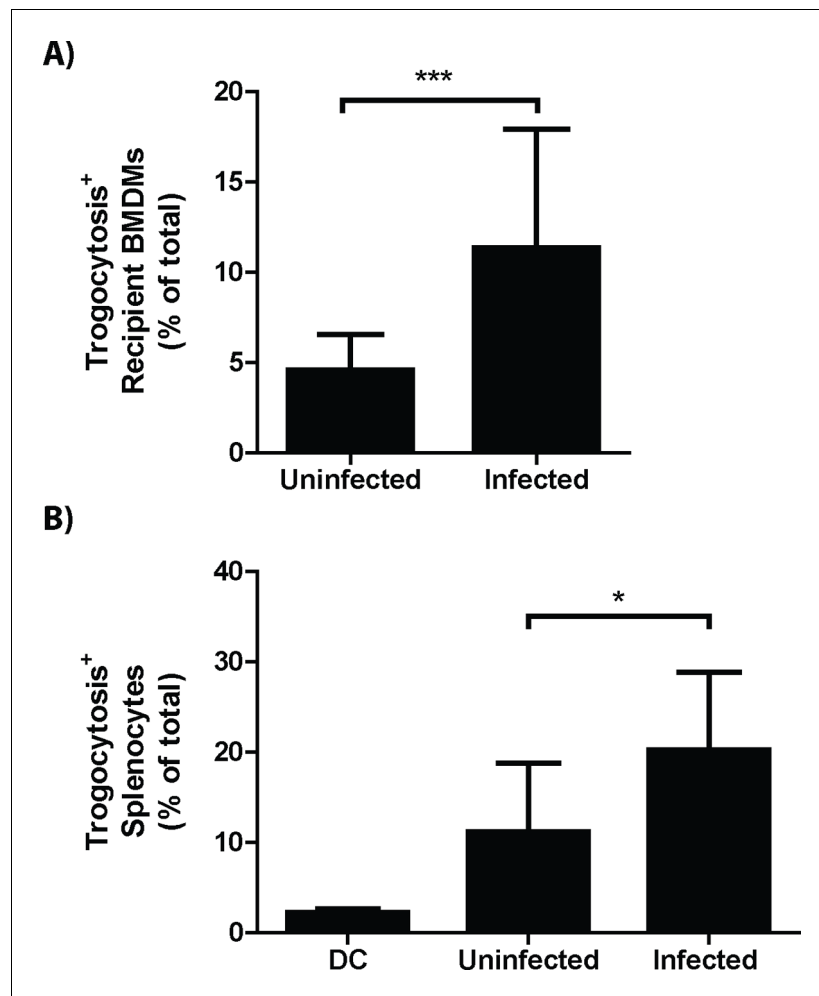
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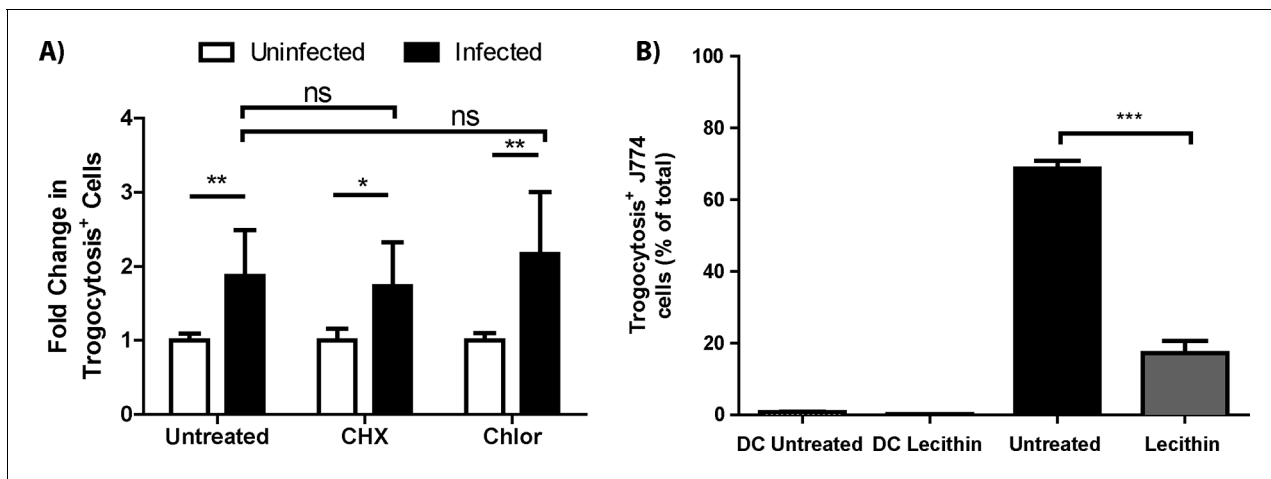
**Figure 4.** Plasma membrane protein transfer correlates with bacterial transfer. (A) Fluorescence micrographs of BMDMs before, during, and after trogocytosis. (B) A donor [white plasma membrane] and trogocytosis positive recipient BMDM [red cell] exchanging cytosolic material and bacteria. The bottom right panel is a 3D rendering of the Z-stack from the cells in the top panel. Percent of trogocytosis positive recipient cells that are (C) BMDMs or (D) hMDMs. (E) The percent of Balb/c recipient BMDMs that acquired SIINFEKL peptide bound MHC-I from B6 BMDMs. (F) The percent of infected splenocytes that underwent trogocytosis in a mouse infection model (8 or 9 mice per group from 4 independent experiments). DC refers to a doublet control. (All tissue culture data are from 3–4 independent experiments performed in triplicate) (Scale bar- 10 um) (Mean +/- SD). (ns  $p > 0.05$ , \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ )

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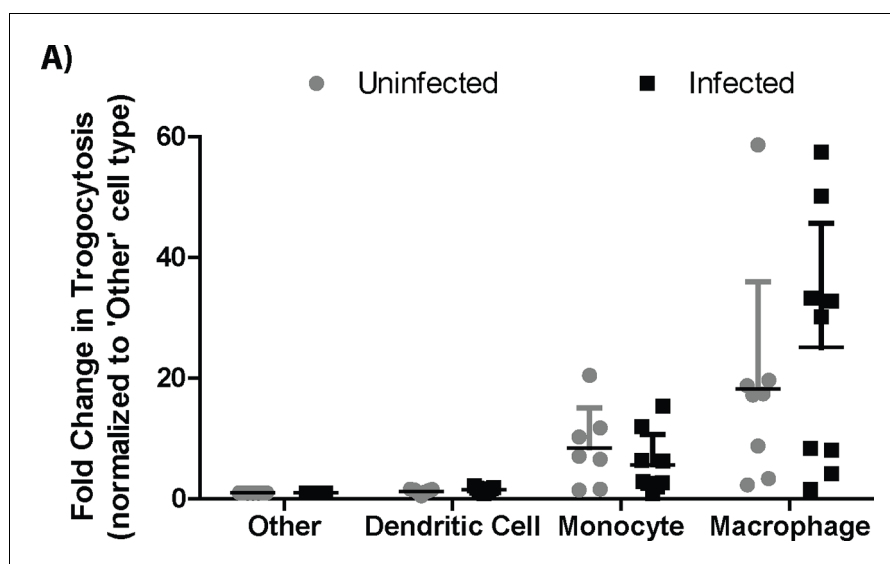
**Figure 4—figure supplement 1.** Plasma membrane protein exchange increases during infection. (A) The percent of Balb/c recipient BMDMs that acquired B6 MHC-I from *F. tularensis* infected or uninfected donor B6 BMDMs. (3 independent experiments performed in triplicate) (B) The percent of splenocytes that exchanged MHC-I in infected or uninfected mice (8 or 9 animals per group from 4 independent experiments). (Mean  $\pm$  SD). (\* $p$  < 0.05, \*\*\* $p$  < 0.001)

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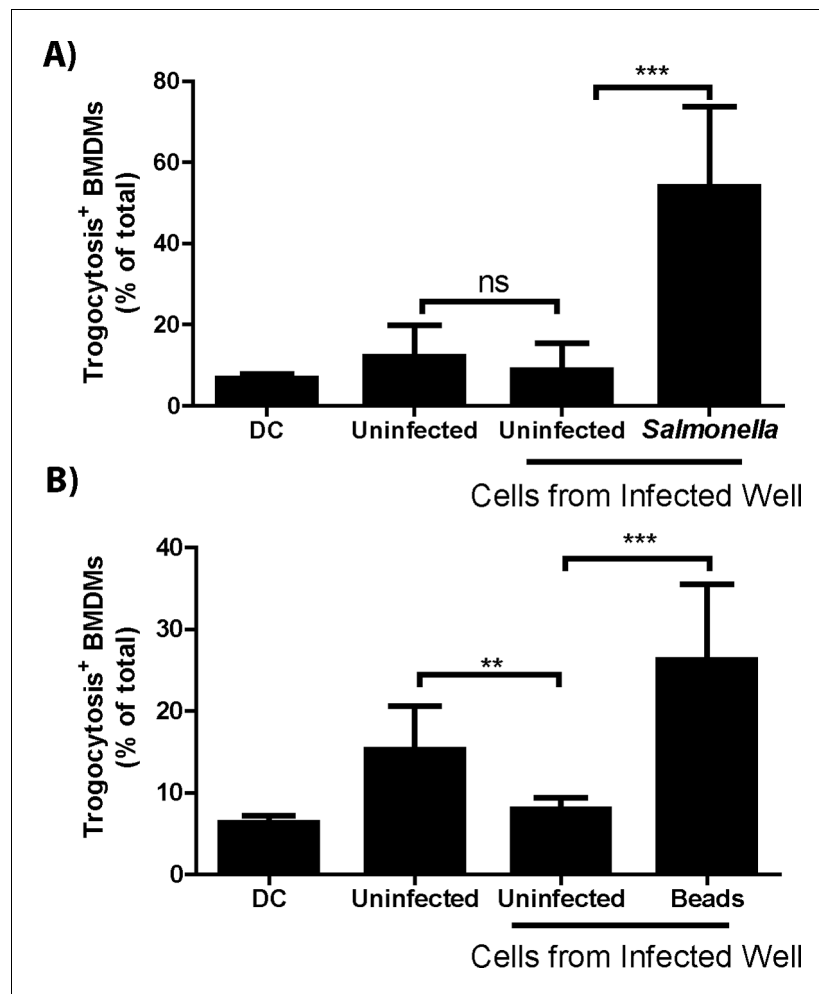
**Figure 4—figure supplement 2.** Trogocytosis does not require *de novo* protein synthesis, but is inhibited by lecithin. (A) The change in MHC-I transfer induced by infection when host (cycloheximide [CHX]) or bacteria (chloramphenicol [Chlor]) protein synthesis is inhibited. The uninfected cells were averaged for each experiment and each infected or uninfected sample was compared to this average because cycloheximide and chloramphenicol altered the basal level of MHC-I exchange. (Mean  $\pm$  SD). (n=2 independent experiments performed in triplicate) (ns  $p > 0.05$ , \* $p < 0.05$ , \*\* $p < 0.01$ ). (B) The percent of J774 recipient cells that underwent plasma membrane transfer in the presence or absence of 0.5% soy lecithin. DC refers to doublet control. Graph is representative of 3 independent experiments performed in triplicate (Mean  $\pm$  SD). (\*\* $p = 0.001$ )

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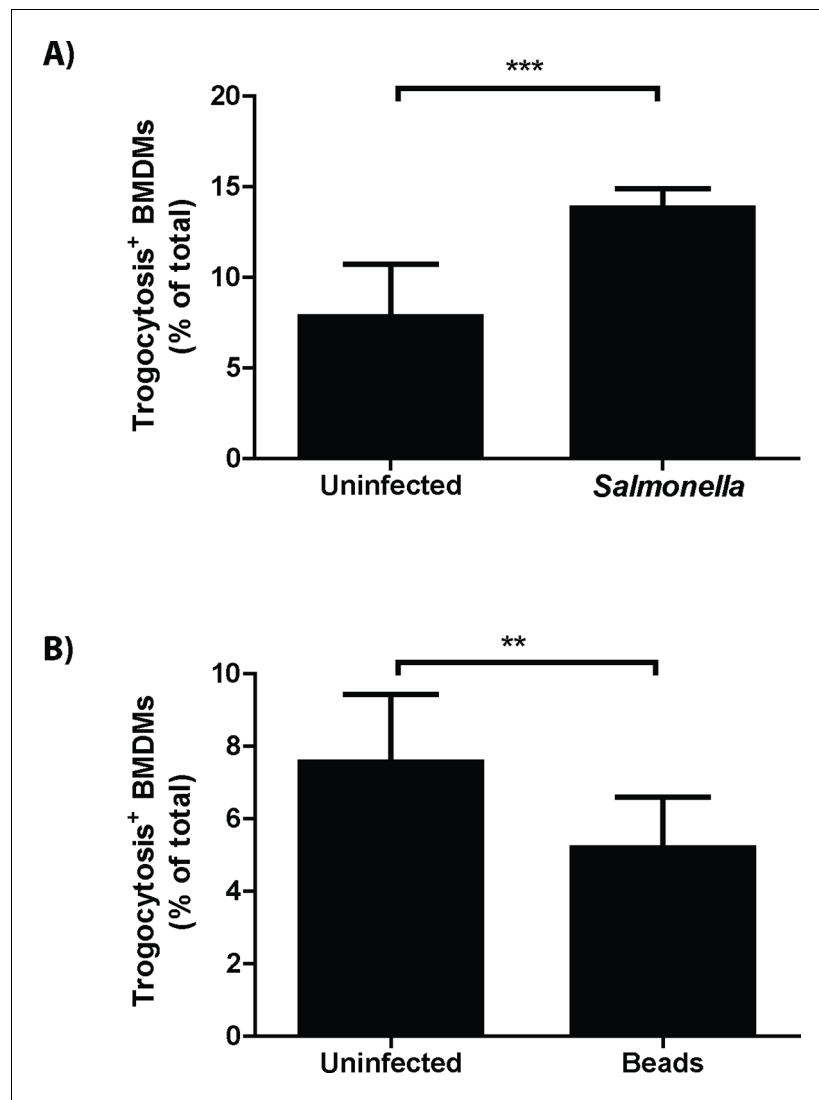
**Figure 4—figure supplement 3.** Trogocytosis in various cell types in mouse splenocytes. The percent of Balb/c H2-Kd positive cells that had surface exposed CD45.1 in each represented population. Macrophage: F4/80+; Monocyte: F4/80-, CD11b+; Dendritic Cell: F4/80-, CD11c+; Other: F4/80-, CD11b-, CD11c-. Results for each cell type were normalized to the 'Other' population in that mouse, so fold change in the 'Other' population is always 1. Dendritic cells fold change: Uninfected = 1.3 fold  $\pm$  0.3, Infected 1.6 fold  $\pm$  0.3 (average  $\pm$  SD). Macrophages and monocytes were significantly more likely to undergo trogocytosis in both the infected and uninfected mice than the 'Other' cell types based on the raw data ( $p < 0.05$ ). Bars represent the mean. (8 or 9 animals per group from 4 independent experiments)

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**Figure 5.** Trogocytosis – associated bacterial transfer is not restricted to *F. tularensis*. The percent of recipient cells that underwent plasma membrane protein transfer in response to (A) *Salmonella typhimurium* or (B) fluorescent beads. The recipient BMDMs that acquired bacteria or beads grouped separately from recipient cells in the same well that did not acquire foreign material. DC refers to a doublet control. (All results from 3–4 independent experiments performed in triplicate) (Mean +/- SD). (ns  $p > 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ )

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**Figure 5—figure supplement 1.** Plasma membrane protein exchange increases during infection. The percent of Balb/c recipient BMDMs that acquired B6 MHC-I from uninfected or (A) *S. typhimurium* or (B) bead infected donor B6 BMDMs. (Mean  $\pm$  SD). (3 independent experiments performed in triplicate) (\*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

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