SUPPLEMENTAL METHODS

Cellular Localization by Confocal Microscopy

For detection of *Francisella* co-localization with LAMP-1 at 8 hours PI, MDM on fibronectin-coated coverslips were infected as described in Methods. Coverslips were treated with 4% paraformaldehyde in DPBS for 1 hour and then stored at 4°C in 70% ethanol. After verifying sterility, coverslips were treated with 0.5 M ammonium chloride in DPBS to quench auto-fluorescence. Coverslips were then washed in DPBS and incubated at room temperature for 1 hour with rabbit antiserum to Francisella and mouse monoclonal IgG anti-human lysosomal-associated membrane protein-1 (LAMP-1) (H4A3, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA). Following DPBS washes, coverslips were stained with Alexa Fluor 488-conjugated donkey anti-rabbit IgG and Alexa Fluor 555-conjugated donkey anti-mouse IgG (Life Technologies, Eugene, OR) in DPBS. Coverslips were mounted in ProLong Gold with DAPI and imaged using the ACS APO 63×/1.30 numerical aperture oil objective of a Leica TCS SPE confocal microscope. Images are presented as maximum projections of 1 μm Z-series stacks spanning the entire monolayer.

For determining percent co-localization of bacteria with LAMP-1, at least 75 infected MDM were analyzed per SCHU S4 strain. Bacteria in each cross-section through an infected MDM were scored as not co-localized (Alexa Fluor 488 positive only; green) or co-localized (Alexa Fluor 488 and Alexa Fluor 555 positive; yellow) with LAMP-1. The average percent LAMP-1 positive bacteria in each infected MDM was calculated.

IL-1β ELISA

MDM were infected as described in the Methods. At 24 hour PI, culture supernatants were collected, 0.22 μm filter sterilized and stored at -20°C. IL-1β concentrations were determined using the Human IL-1β ELISA Set II (BD, San Diego, CA) according to the manufacturer's instructions.

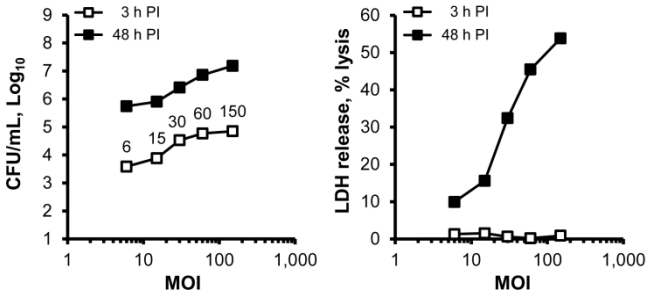

FIG S1 Bacterial burden and MDM death as a function of MOI. MDM were infected with SCHU S4 with various MOIs in the presence of HS. Data labels indicate the actual MOI. Bacterial burden and LDH release were measured at 3 and 48 h PI. The results shown are representative of three independent experiments.

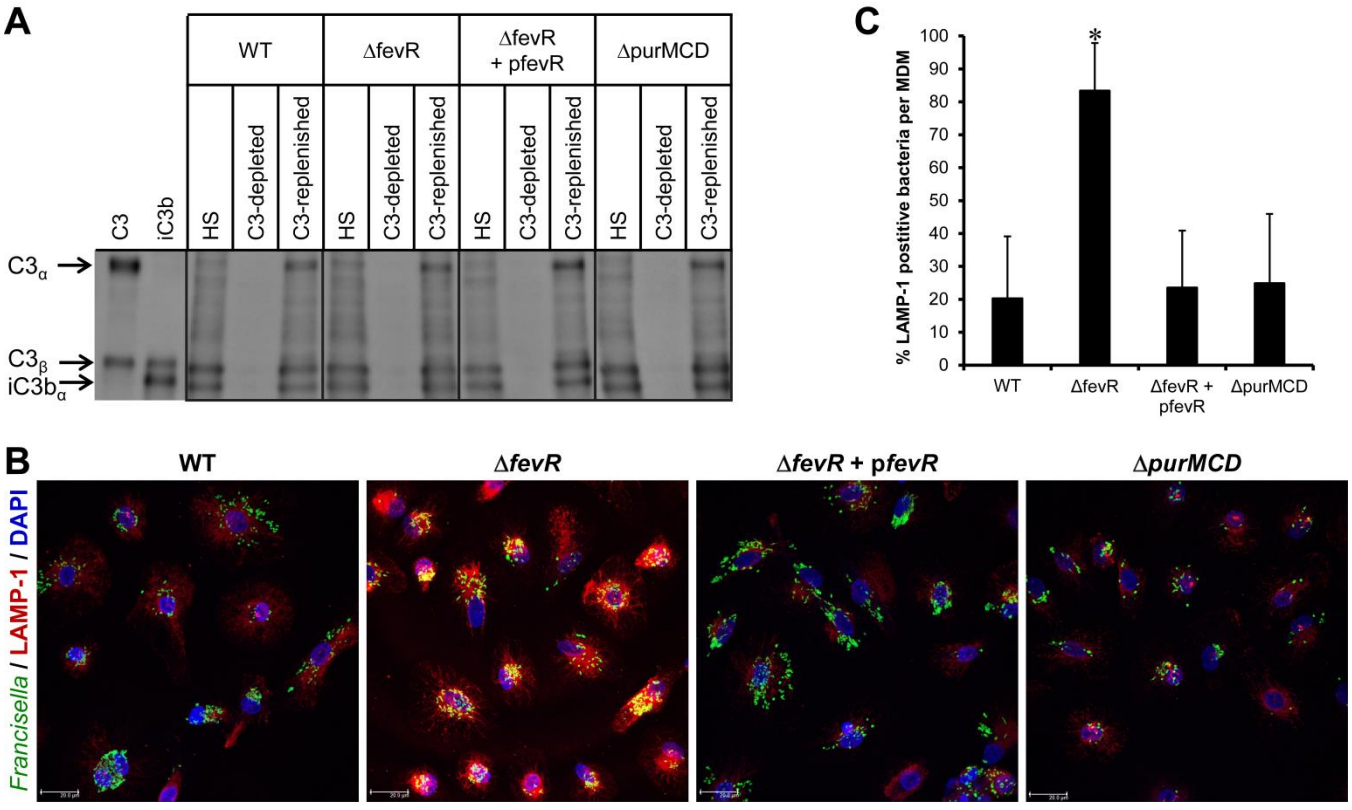

FIG S2 WT and mutant strains of SCHU S4 support similar levels of C3-deposition and traffic to the expected cellular locations in human macrophages. (A) A Western blot was used to detect C3 deposition on WT and mutant strains of SCHU S4 when opsonized with HS, C3-depleted HS, or C3-replenished HS (4 x 10^7 bacteria per lane). Purified C3 and iC3b proteins serve as markers. (B, C) MDM were infected with SCHU S4 WT or mutant strains in the presence of HS and analyzed for LAMP-1 co-localization by confocal microscopy. The MOI were as follows: WT = 49, Δ*fevR* = 31, Δ*fevR* + p*fevR* = 41, Δ*purMCD* = 47. Representative images (B) are presented as maximum projections of 1 μm Z-series stacks spanning the entire monolayer. Scale bars represent 20 μm. The average percent LAMP-1 positive bacteria in each infected MDM is shown in panel C. * denotes significant differences compared to WT. This complete experiment was performed once.

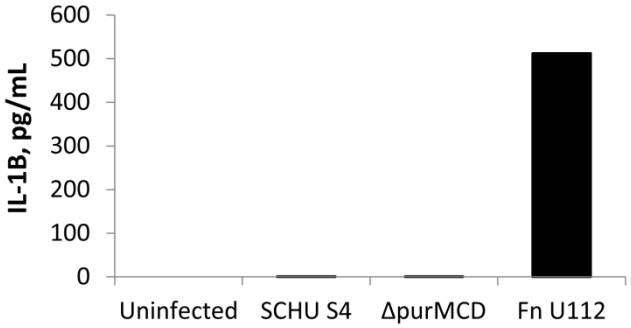

FIG S3 Neither WT nor Δ*purMCD* SCHU S4 induced release of the inflammatory cytokine IL-1β. MDM were infected with WT SCHU S4 (MOI = 36), Δ*purMCD* SCHU S4 (MOI = 50) or *Francisella novicida* strain U112 (MOI = 35) in the presence of HS. IL-1β release into culture supernatants was measured by ELISA at 24 h PI. The results shown are representative of three independent experiments.