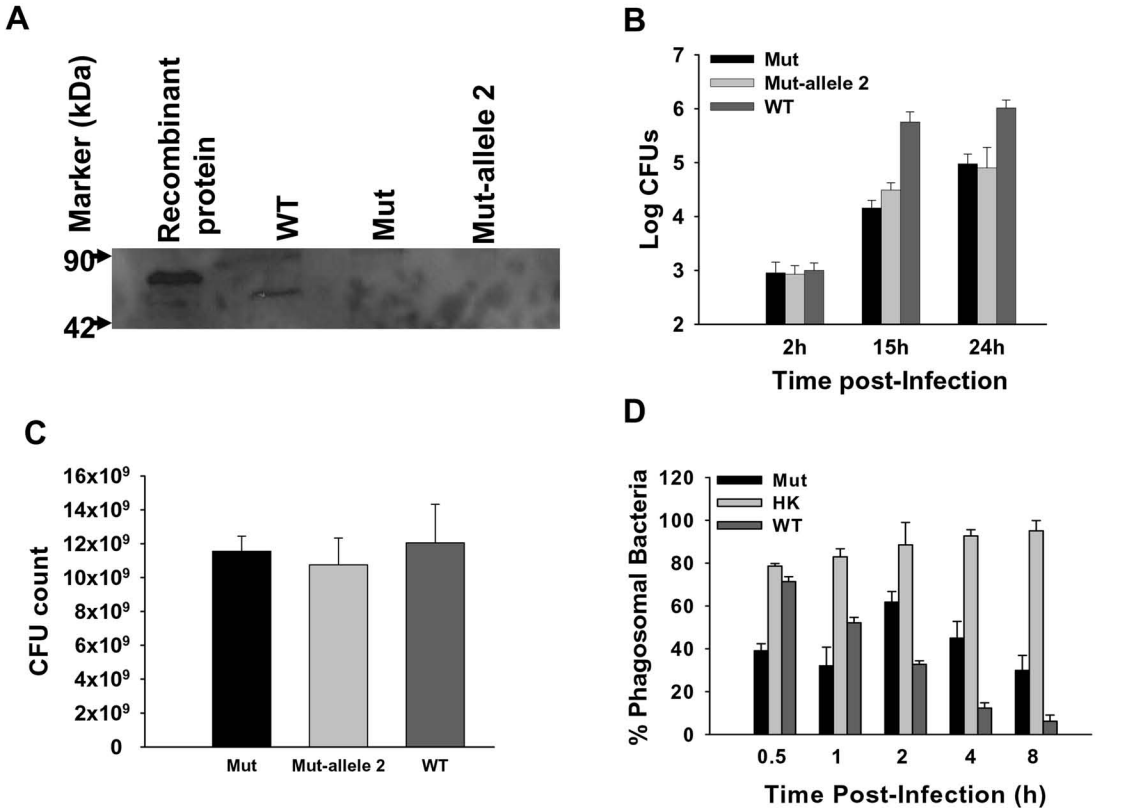
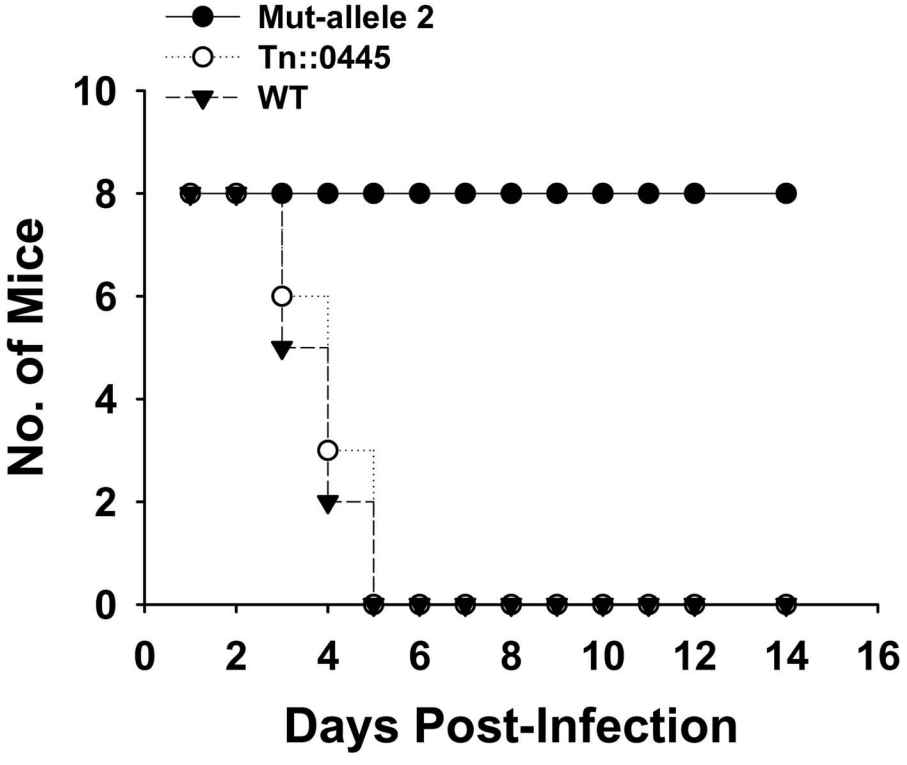


Supplemental Fig. 1. The 58 kDa mutant is attenuated for growth in host cells *in-vitro*. (A) shows western blot analysis of WT, Mut or Mut-allele 2 bacteria for presence/absence of 58 kDa protein. Bactreia (0.2 x10^{10} CFUs) were lysed in SDS buffer and loaded onto polyacrylamide gels. The proteins, after transfer to polyvinylidene difluoride (PVDF) membranes were probed with a mouse polyclonal antibody raised against recombinant 58 kDa protein. Lane 1, recombinant protein, lane 2, WT *F. novicida*, lane 3, Mut, lane 4, Mut-allele 2. (B) represents growth of WT, Mut or Mut-allele 2 bacteria in murine macrophage cell line J774. The cells were infected with bacteria at MOI of 50 and lysed at 2 h, 15 h and 24 h p.i. using 0.01% sodium deoxycholate. Serial dilutions of lysates were plated on TSA plates and incubated at 37 ^{o}C O/N to obtain CFU counts. The counts at various times are represented as CFUs/ ml lysate. Experiment was performed in technical triplicates and average CFU counts from a representative of 3 independent experiments are shown. (C) shows growth of WT, Mut or Mut-allele 2 bacteria in trypticase soy broth (TSB) medium. A single colony of each bacterial strain was inoculated in medium and grown O/N at 37 ^{o}C with shaking at 230 rpm. The CFU count in cultures were obtained by serial dilution and plating on trypticase soy agar (TSA) plates. (D) depicts percentage of phagosomal bacteria in J774A.1 macrophages infected with the Mut or the WT bacteria at MOI of 50:1 as determined by a phagosomal integrity assay. At indicated times p.i., the cells were washed with KHM buffer (110 mM potassium acetate, 20 mM HEPES, 2 mM $MgCl_2$; pH 7.5) and the plasma membrane was permeabilized with digitonin (50 ug/ml in KHM buffer). Bacteria in cell cytosol were labeled with Alexa-647 conjugated monoclonal antibody against Francisella LPS. This was followed by fixation with 3 % paraformaldehyde and permeabilization of all membrane components of the cells with 0.1 % saponin in PBS+ 10 % bovine serum. Finally the cells were treated with Alexa-488 conjugated anti-Francisella antibody. By this method, the bacteria within phagosomes appeared green while the cytoplasmic bacteria stained both blue and green. The samples were analyzed by Olympus Fv-1000 Laser Confocal microscope at Optical Imging Core Facility in Universty of Texas Health Science Center, San Antonio. At least 200 bacteria were counted for each sample, and the results shown are representative of three independent experiments.



Supplemental Fig. 2. Transposon mutation of 58 kDa protein does not have any polar effects. The transposon insertion in another location within the gene FTN_0444 (Mut-allele 2) resulted in attenuation of bacteria for infection *in-vivo* similar to Mut bacteria, whereas transposon mutation of a downstream gene (Tn::locus tag FTN_0445) does not have any effect on virulence of bacteria. The C57BL/6 mice were infected intranasally with 3 x10^2 CFUs of the bacteria and were monitored for 2 weeks for signs of disease.