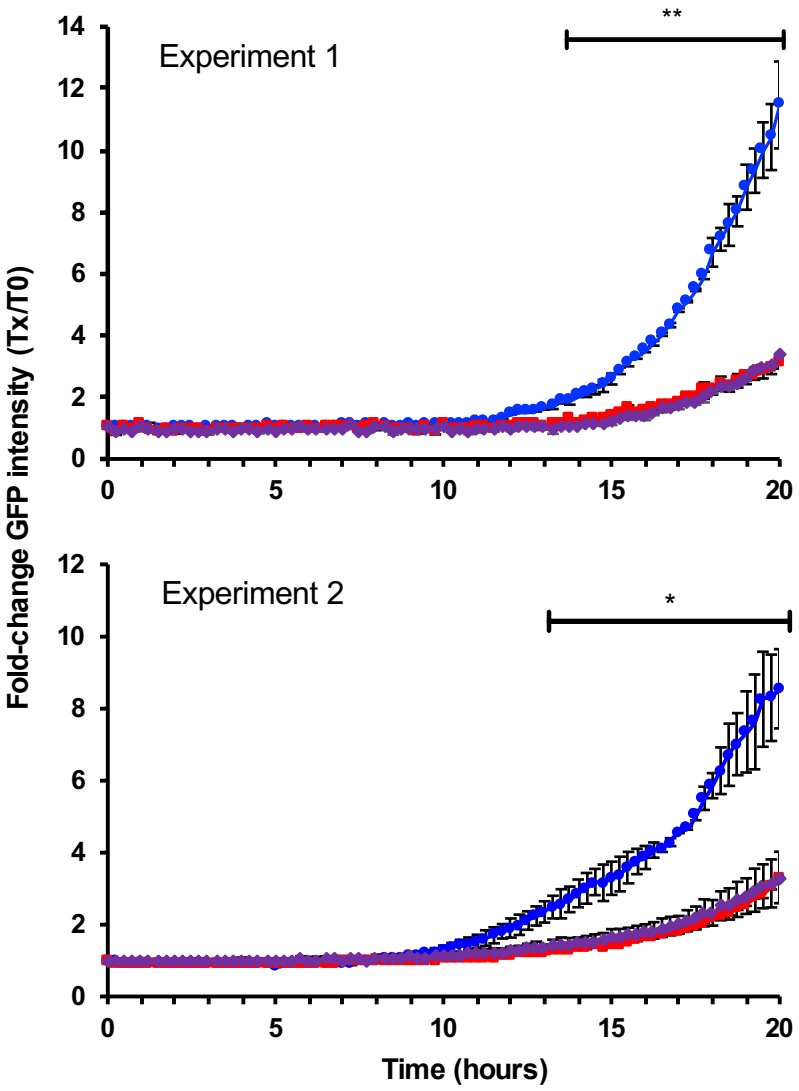

FIG S1. Additional plate reader analysis of bacteria within *L. pneumophila*-infected *A. castellanii*. Monolayers of *A. castellanii* were infected with either wild-type strain 130b expressing plasmid-encoded GFP (WT), *lspF* mutant NU275 expressing plasmid-encoded GFP (*lspF*), or *lspDE* mutant NU258 expressing plasmid-encoded GFP (*lspDE*) at an MOI of 20. After centrifugation and a 1-h incubation to facilitate uptake, gentamicin was added to kill remaining extracellular bacteria. Next, the GFP fluorescence from *L. pneumophila* was monitored kinetically every 15 min for the next 20 h, and the fluorescence values obtained were normalized to the GFP signal at t = 0. The data are presented as means with standard error from three technical replicates for WT (blue lines), *lspF* (red lines), and *lspDE* (purple lines), and the lines with asterisks indicate the time intervals in which significant differences were observed between WT- and mutant-infected amoebae (Student's *t*-test; *, $P < 0.05$; **, $P < 0.01$).

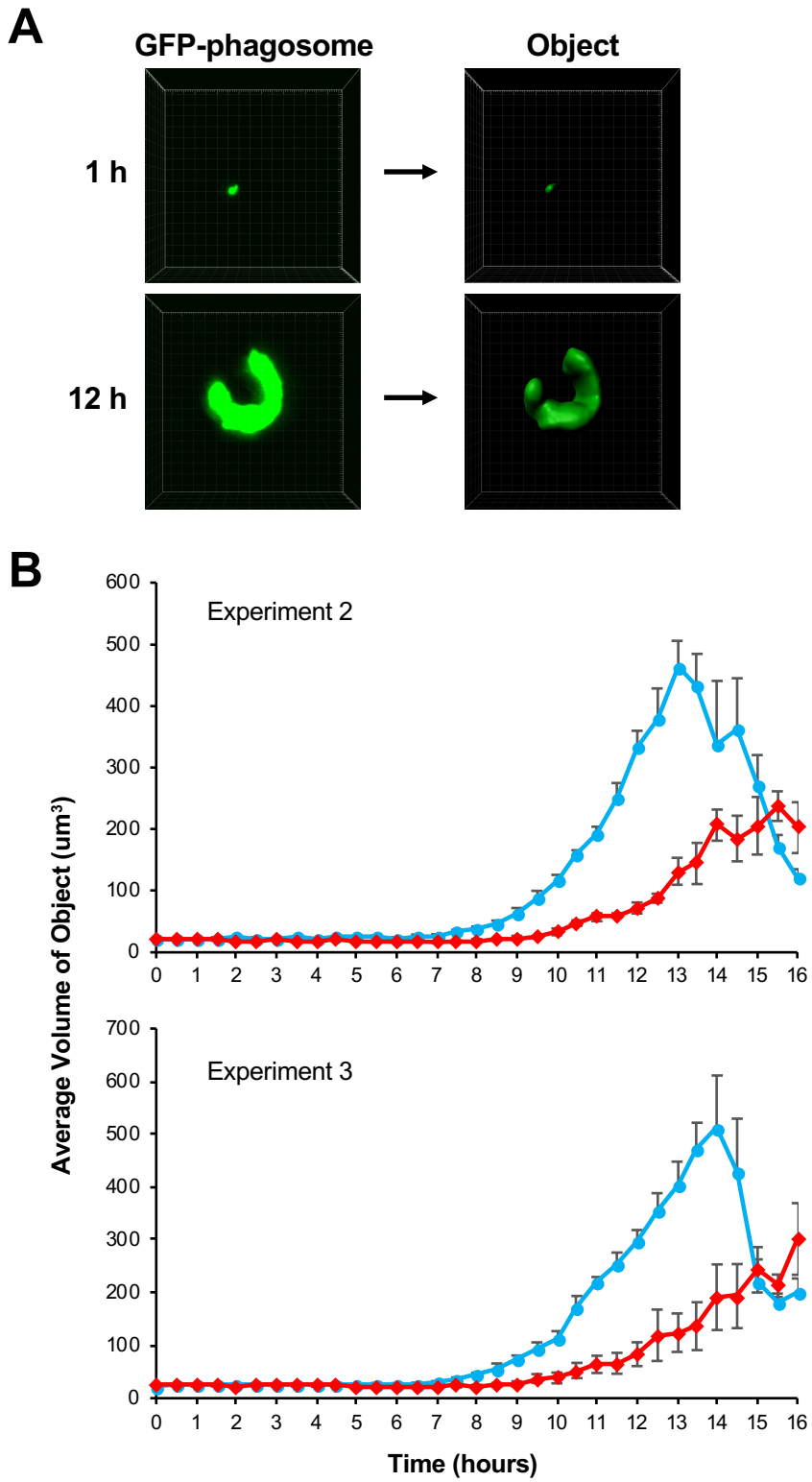

FIG. S2. Additional volume analysis of bacteria and phagosomes within *L. pneumophila*-infected *A. castellanii*. Amoebae were infected with GFP-positive wild-type strain 130b (WT) or *lspF* mutant NU275, and z-stack images from confocal microscopy were generated every 30 min for 16 h. (A) Images were converted into 3-D objects for volume analysis using Imaris image analysis software. Representative images are presented, showing the conversion of a GFP-positive, WT bacterium at 1 h post-inoculation or GFP-positive, WT phagosome at 12 h PI. (B) The average volume of the GFP-positive objects from five fields of view are graphed over time with the SEM, following infection with WT (blue lines) or mutant (red lines) bacteria. Data presented are from two representative experiments, with the data from an additional independent experiment appearing in Fig. 7B. The difference between the average volume of objects over time for WT and mutant was statistically significant (ANOVA; $P < 0.02$).

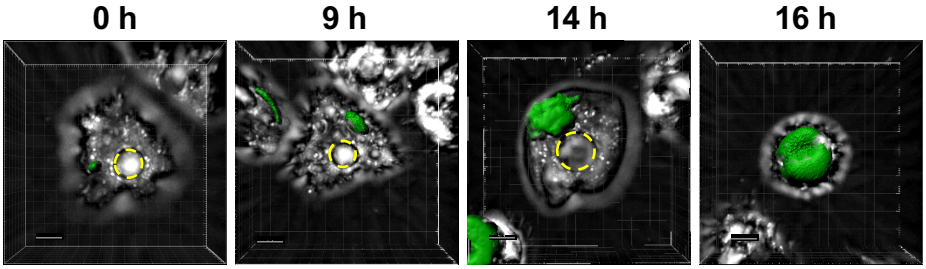

FIG. S3. Shapes of phagosomes that do not localize near the contractile vacuole. *A. castellanii* were infected with GFP-positive wild-type strain 130b (WT) or *lspF* mutant NU275 (*lspF*), and z-stack images were generated every 30 min and converted into 3-D objects. Based upon three independent experiments, the four images presented are representative of the bacterial phagosomes that did not localize near the contractile vacuole (demarcated by yellow dashed lines). Scale bar = 5 μm.

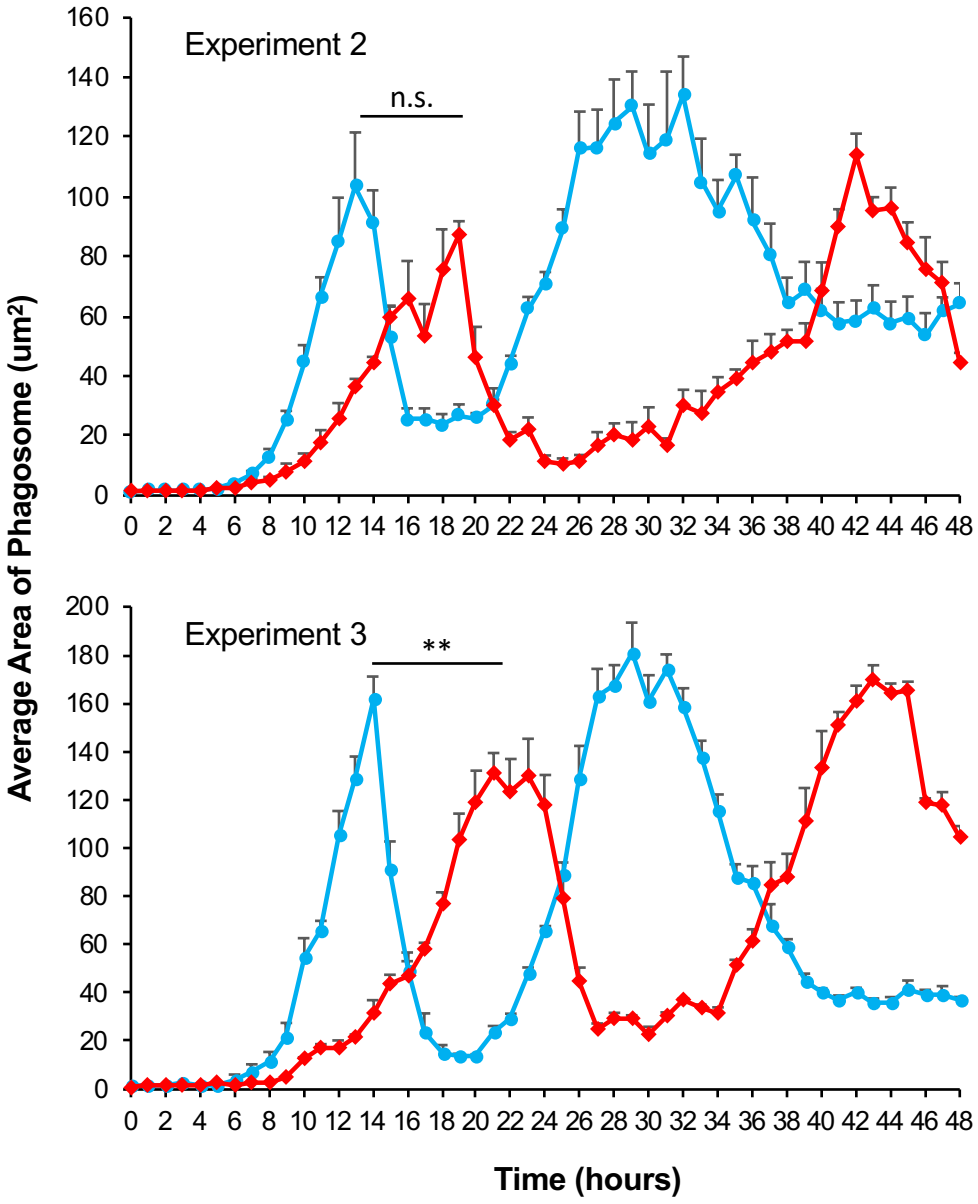

FIG. S4. Additional area analysis of phagosomes within *L. pneumophila*-infected *A. castellanii* over a 48-h time period. Amoebae were infected with GFP-positive wild-type strain 130b (WT) or *lspF* mutant NU275 (*lspF*), and widefield images were generated every hour for 48 h. Analysis of areas of fluorescence was performed using Nikon Elements 5.01 software. The average area of intracellular GFP-positive phagosomes from five fields of view are graphed over time, following infection with WT (blue lines) or T2SS mutant (red lines) bacteria. Data presented are from two representative experiments, with the data from an additional independent experiment appearing in Fig. 8. The asterisks in experiment 3 indicate a significant difference between WT and *lspF* mutant average phagosome area (Student's *t* test; **, $P < 0.01$).

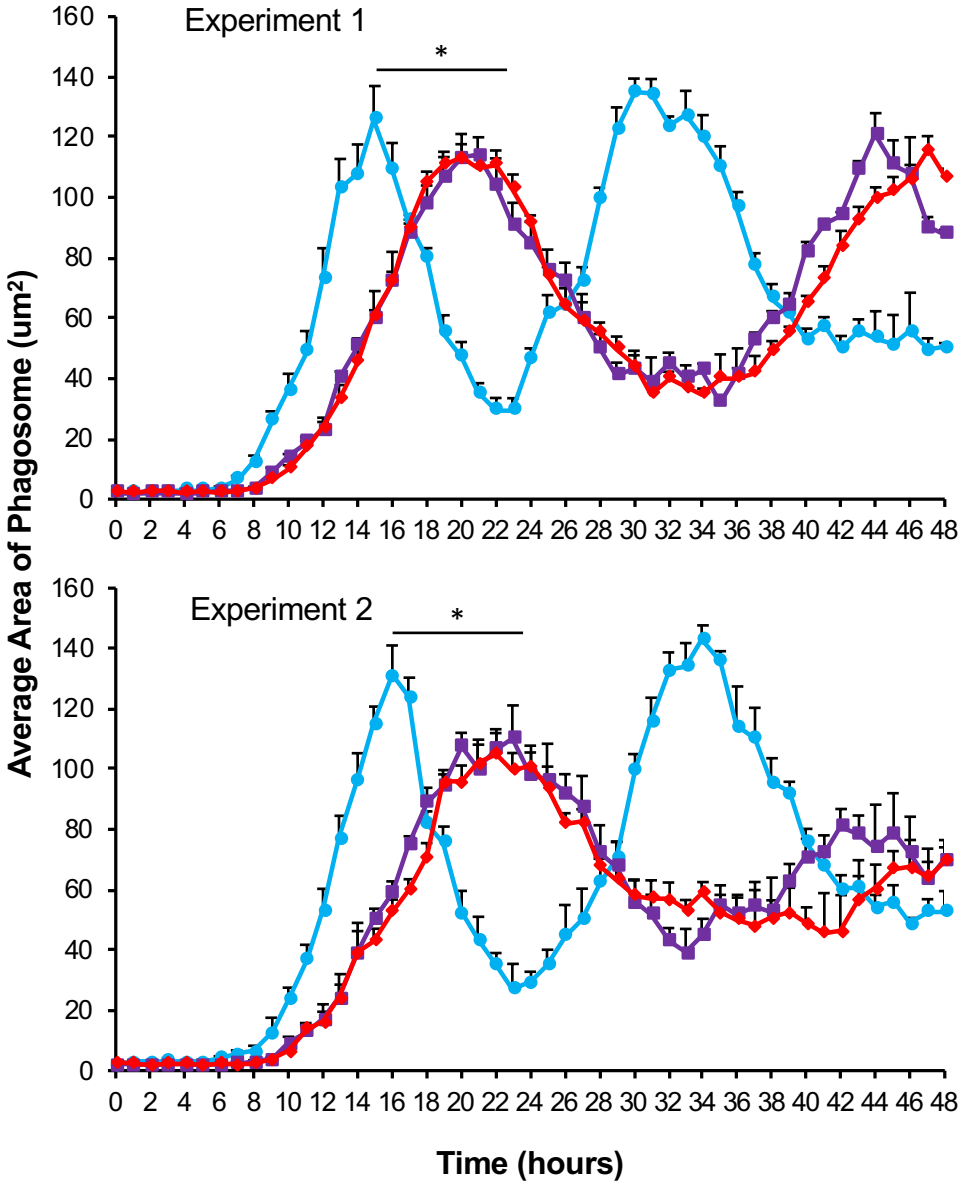

FIG. S5. Additional area analysis of phagosomes within *L. pneumophila*-infected *A. castellanii* over a 48-h time period. Amoebae were infected with GFP-positive wild-type strain 130b (blue line), *lspF* mutant NU275 (red line), or *lspDE* mutant NU258 (purple line), and widefield images were generated every hour for 48 h. Analysis of areas of fluorescence was performed using Nikon Elements 5.01 software. The average area of intracellular GFP-positive phagosomes from five fields of view are graphed over time. Data presented are from two representative experiments. The asterisks indicate a significant difference between WT and *lspF* or *lspDE* mutant average phagosome area (Student's *t* test: *, $P < 0.05$).