Supplementary Figures

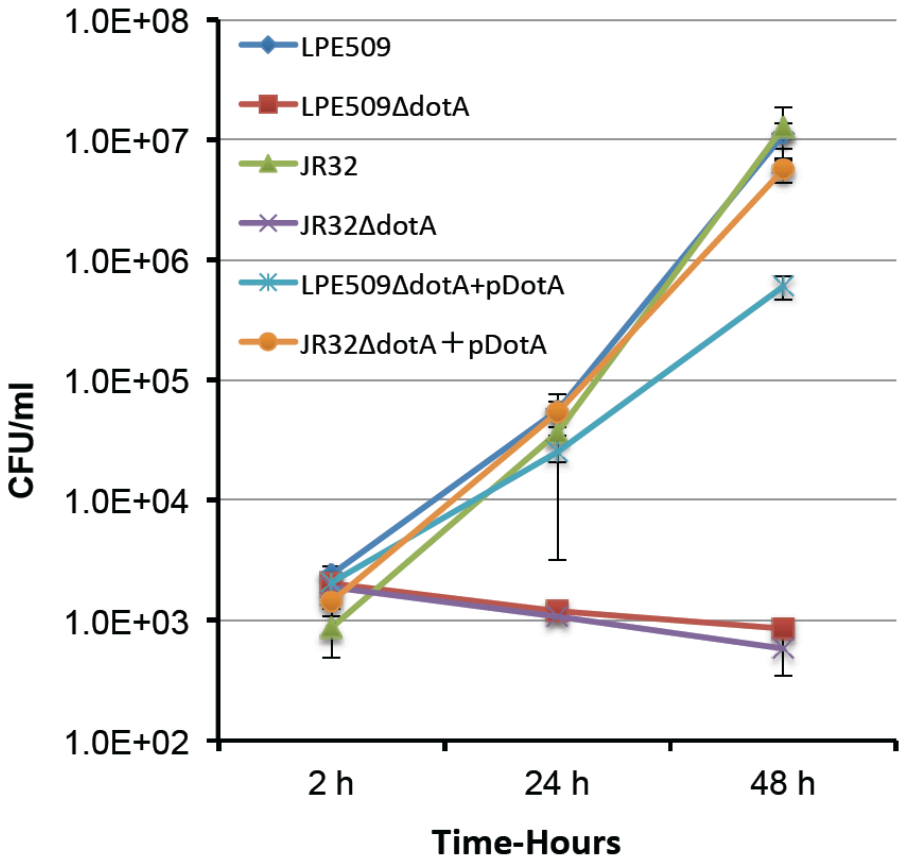

Fig. S1 Complementation of the *dotA* mutant of strain LPE509. Differentiated U937 cells were infected with indicated bacterial strains and bacterial growth was monitored by determining the total colony-forming-unit at the indicated time points. Experiments were performed in triplicate and data shown were from one of the two experiments with similar results.

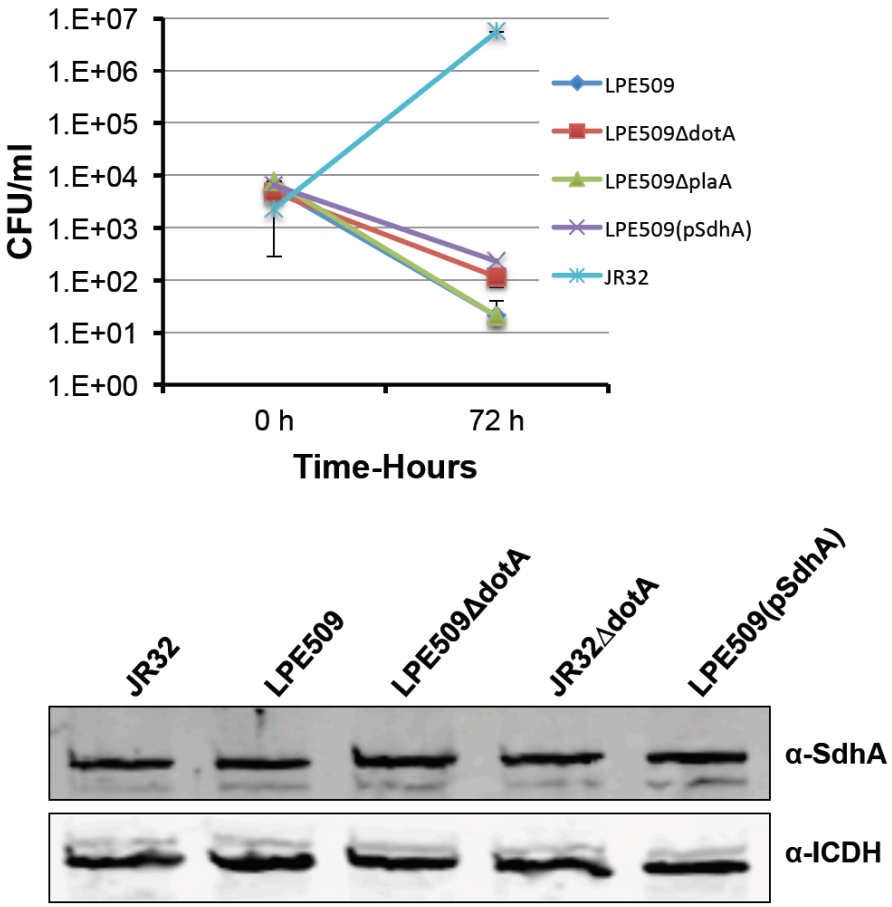

Fig. S2 SdhA is not involved in the cell death induced by strain LPE509. A. Intracellular growth of strains overexprssing SdhA or defective in *plaA* in A/J macrophages. Indicated bacterial strains grown to postexponential phase were used to challenge A/J macrophages in triplicate at an MOI of 0.05 and the total bacterial counts for each sample were determined at indicated time points. All samples were done in triplicate and similar results were obtained in two independent experiments. **B**. Expression of SdhA in the relevant bacterial strains. Bacteria from cultures used for infection in panel A was collected, lysed with SDS-loading buffer and the soluble fraction was resolved by SDS-PAGE prior to detection by a SdhA-specific antibody. Note the presence of similar SdhA in the testing strains, including the one harboring the plasmid capable of complementing the *sdhA* mutant in Lp02ΔsdhA. The isocitrate dehydrogenase (ICDH) was probed as a loading control (low panel).

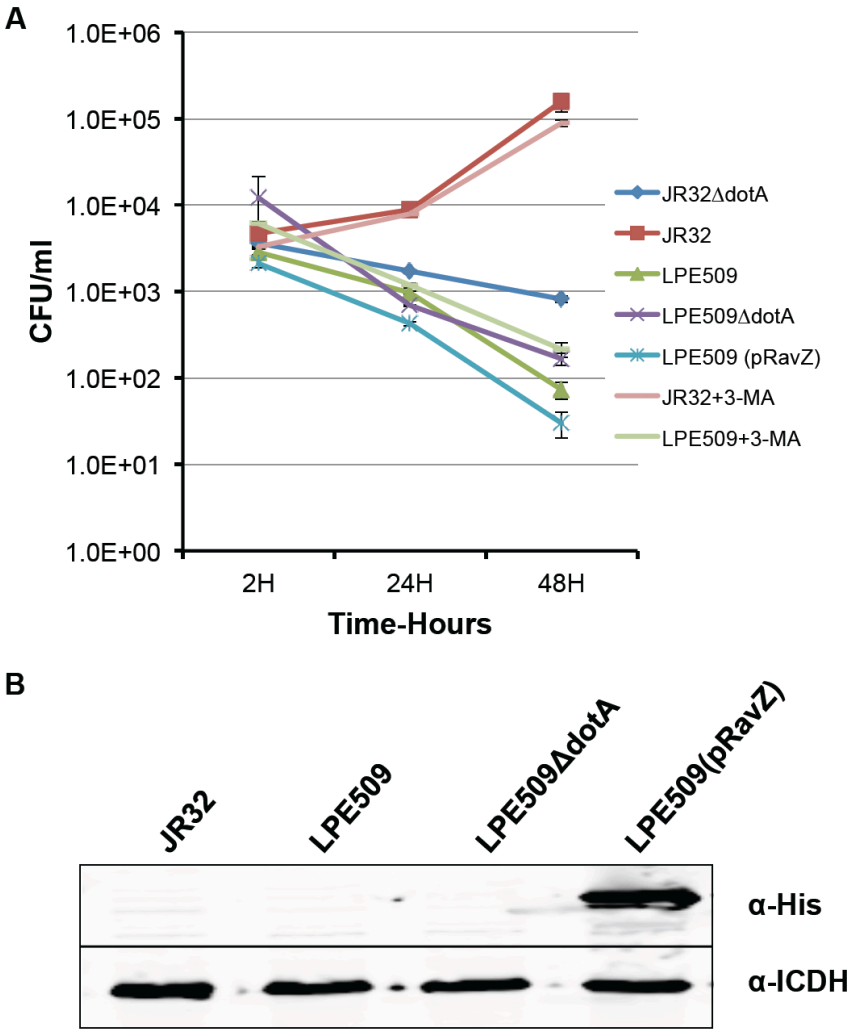

Fig. S3 Evaluation of autophagy in the intracellular growth defect of LPE509 in A/J macrophages. **A**. Overexpression of RavZ or the addition of autophagy inhibitor 3-3-Methyladenine did not rescue intracellular growth of LP509 in A/J macrphages. Indicated bacterial strains grown to postexponential phase were used to infect A/J macrophages at an MOI of 0.05. Total bacterial counts were obtained at indicated time points. Each sample was done in triplicate and similar results were obtained in two independent experiments. **B**. Evaluation of the expression of His_6-RavZ in LPE509. Bacterial cells collected from cultures used for infection in panel A were lysed with SDS sample buffer and was resolved by SDS-PAGE prior to detection of His_6-RavZ by immunoblot.

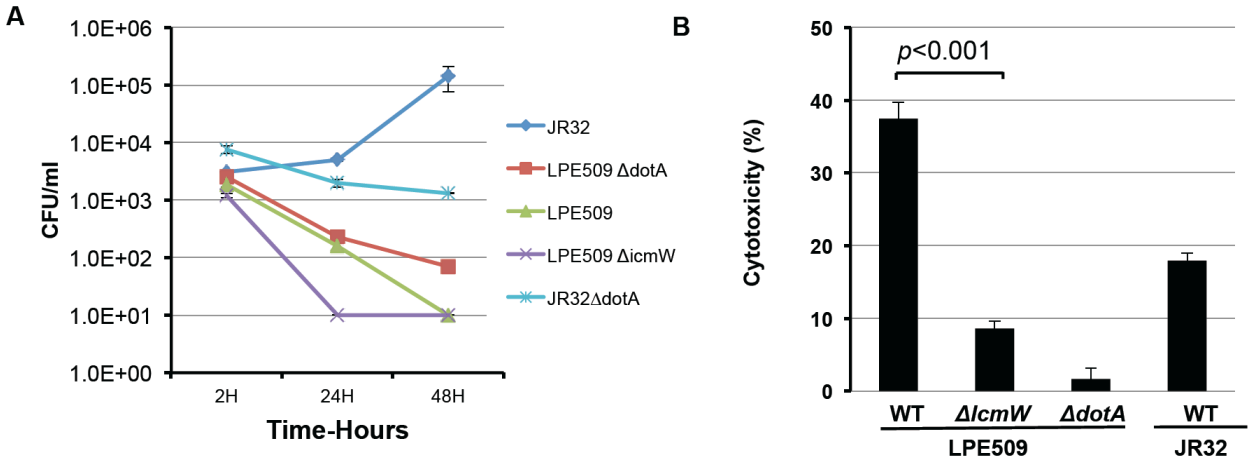

Fig. S4 The IcmS/W chaperone complex is required for the cell death induction by LPE509. **A**. A LPE509 mutant lacking *icmW* is defective in intracellular growth in A/J macrophage. Indicated bacterial strains grown to postexponential phase were used to challenge A/J macrophages at an MOI of 0.05. Total bacterial yields were determined at indicated time points. All samples were performed in triplicate and similar results were obtained in two independent experiments. **B**. The LPE509ΔicmW mutant no longer induced cytotoxicity in A/J macrophages. Indicated bacterial strains grown to postexponential phase were used to challenge A/J macrophages at an MOI of 1 for 4 hrs and the release of lactate dehydrogenase (LDH) was measured. All assays were performed in triplicate and similar results were obtained in three independent experiments.

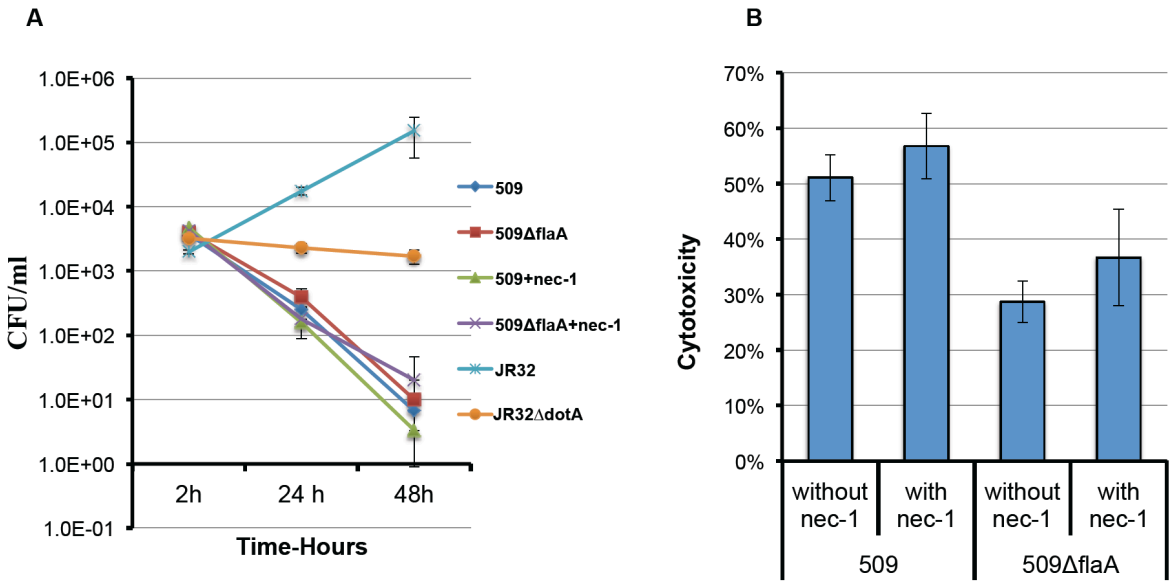

Fig. S5 The necrosis inhibitor necrostatin has no effect on intracellular growth and cytotoxicity of strain LPE509 and its derivatives in A/J macrophages. A. Macrophages in medium containing necrostatin were challenged with indicated bacterial strains at an MOI of 0.05 and the growth of the bacteria was monitored at a 24-hr interval. **B.** Cytotoxicity of strain LPE509 and its *flaA* mutant was determined on macrophages from A/J mice in the presence of necrostatin by measuring LDH release at an MOI of 1. All assays were performed in triplicate and similar results were obtained in three independent experiments.

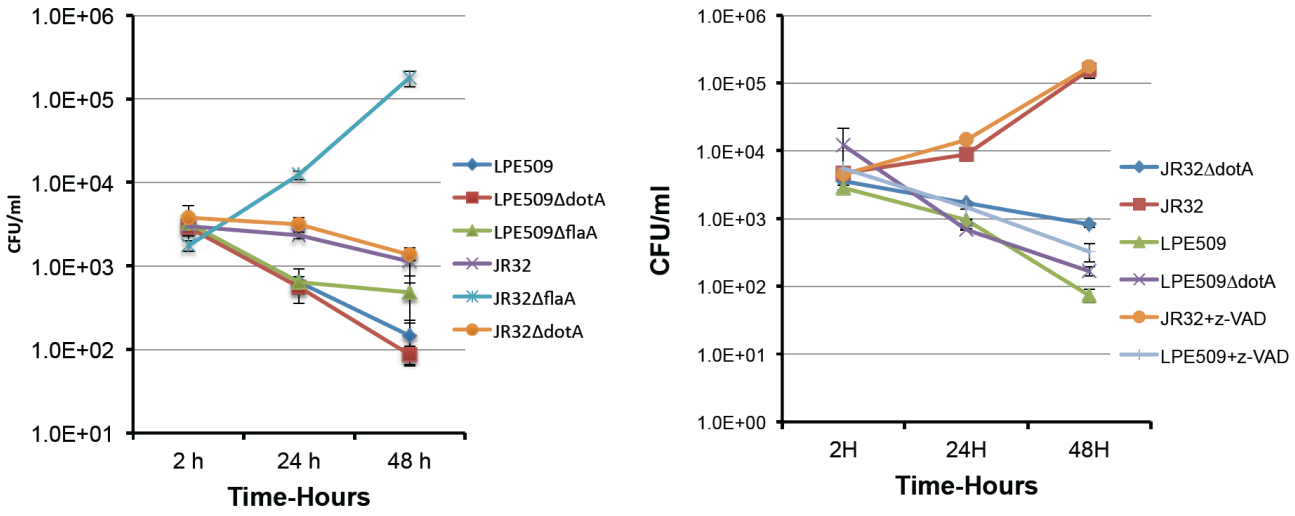

Fig. S6 The effects of caspase 3 and an apoptosis inhibitor on the cell death induced by LPE509 on A/J macrophage. **A**. LPE509 cannot replicate in macrophages from mice deficient in caspase 3. Bone marrow macrophages from caspase $3^{-/-}$ were infected with indicated bacterial strains grown to postexponential phase at an MOI of 0.05 and the total bacterial counts were determined at the indicated time points. Note that only the JR32ΔflaA can productively replicate in these cells. **B**. The apoptosis inhibitor z-VAD does not rescue intracellular growth of LPE509 in A/J macrophages. Macrophages were infected with indicated bacterial strains as described in panel A. The inhibitor z-VAD was included in the indicated samples at a final concentration of 20 mM.

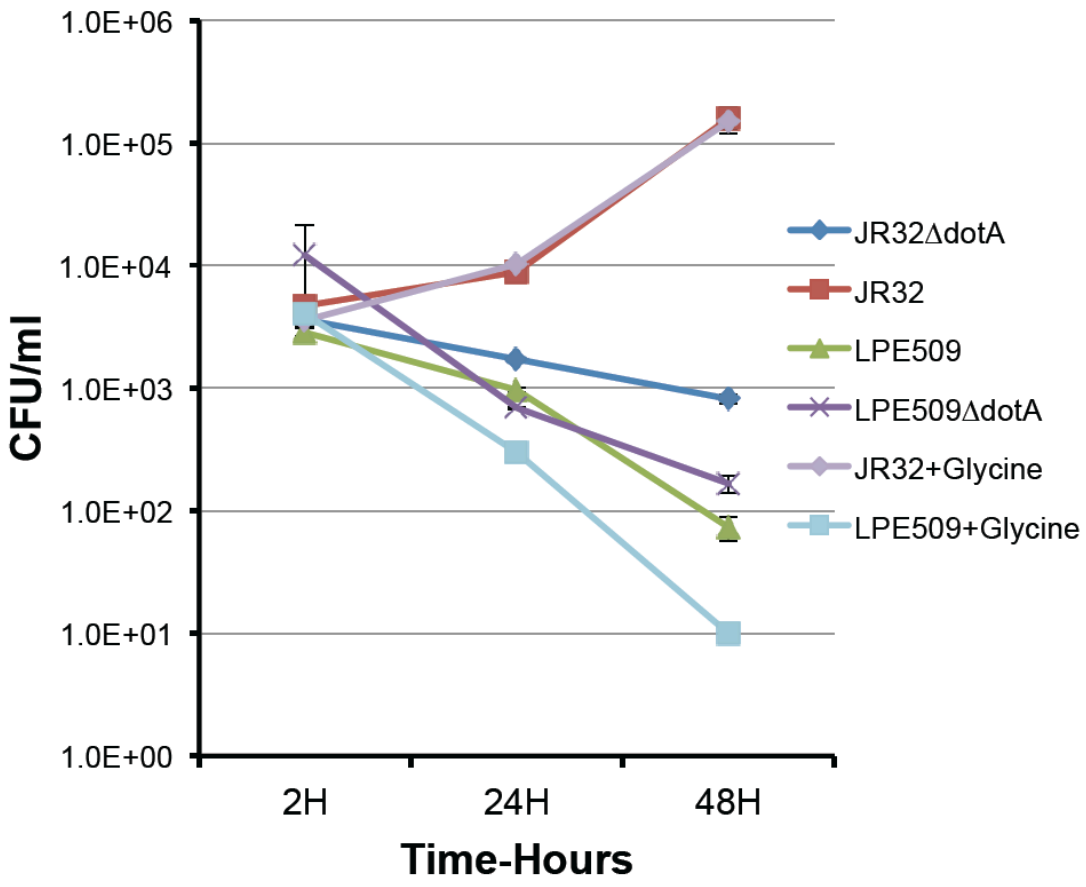

Fig. S7 Glycine cannot rescue the intracellular growth defect of LPE509 in A/J macrophages. A/J macrophages were challenged with indicated bacterial strains grown to postexponential phase at an MOI of 0.05. Glycine was added at a final concentration of 20 mM to a subset of samples throughout the entire experimental duration. Total bacterial counts were determined at indicated time points. Infection was performed in triplicate and similar results were obtained in three independent experiments.