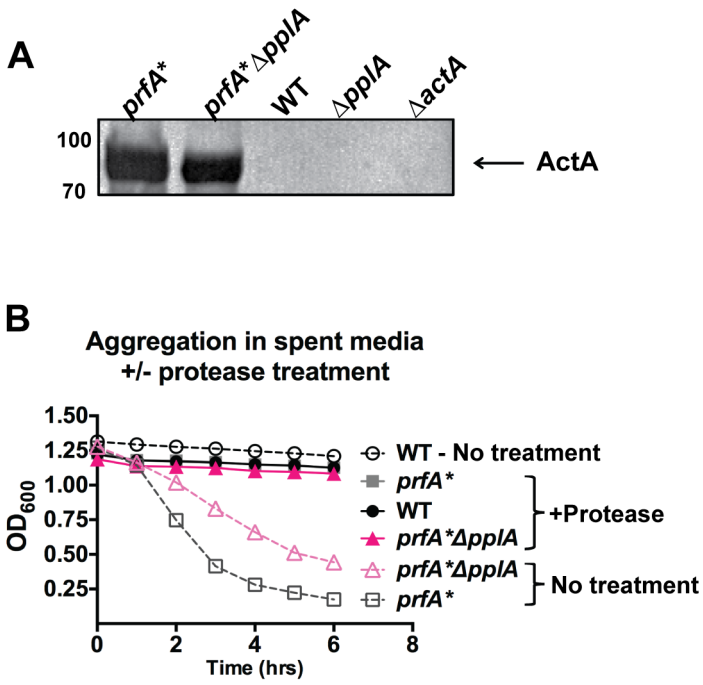

Supplemental Figure S2. Reduced bacterial aggregation in a *prfAΔ*pplA* is not due to decreased levels of ActA protein, and treatment of spent media with protease K eliminates bacterial aggregation. (A)** Measurement of bacterial surface-associated ActA protein levels. Overnight cultures of the indicated strains were grown overnight to stationary phase in BHI at 37°C with shaking, cells were normalized to an optical density at 600nm of 1.5, and non-covalently associated cell surface proteins were extracted by boiling recovered bacteria in SDS-boiling buffer. The presence of ActA was detected using western blot analysis with antibodies directed against ActA. **(B)** Assessment of bacterial aggregation for bacteria incubated in spent media derived from *pplA*-G72$_{STOP}$ codon mutant cultures with and without proteinase K treatment. Prior to measurement of bacterial aggregation, a portion of spent media derived from *pplA*-G72$_{STOP}$ cultures was treated with 50 µg/mL of proteinase K for 30 minutes at 37°C, then protease was heat-inactivated at 65°C. *L. monocytogenes* strains to be assayed for bacterial aggregation were recovered and resuspended in spent media treated or untreated with proteinase K, and the optical-density 600nm was measured over time. For both panels (A) and (B), data is representative of at least two-independent experiments.