Supplemental Figure S2. Reduced bacterial aggregation in a prfA*ΔpplA 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-G72STOP codon mutant cultures with and without proteinase K treatment. Prior to measurement of bacterial aggregation, a portion of spent media derived from pplA-G72STOP 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.