Spermine inhibits *Vibrio cholerae* biofilm formation through the NspS-MbaA polyamine signaling system

Richard C. Sobe, Whitney G. Bond, Caitlin K. Wotanis, Joziah P. Zayner, Marybeth A. Burriss, Nicolas Fernandez, Eric L. Bruger, Christopher M. Waters, Howard S. Neufeld, and Ece Karatan

Figure S1-9.

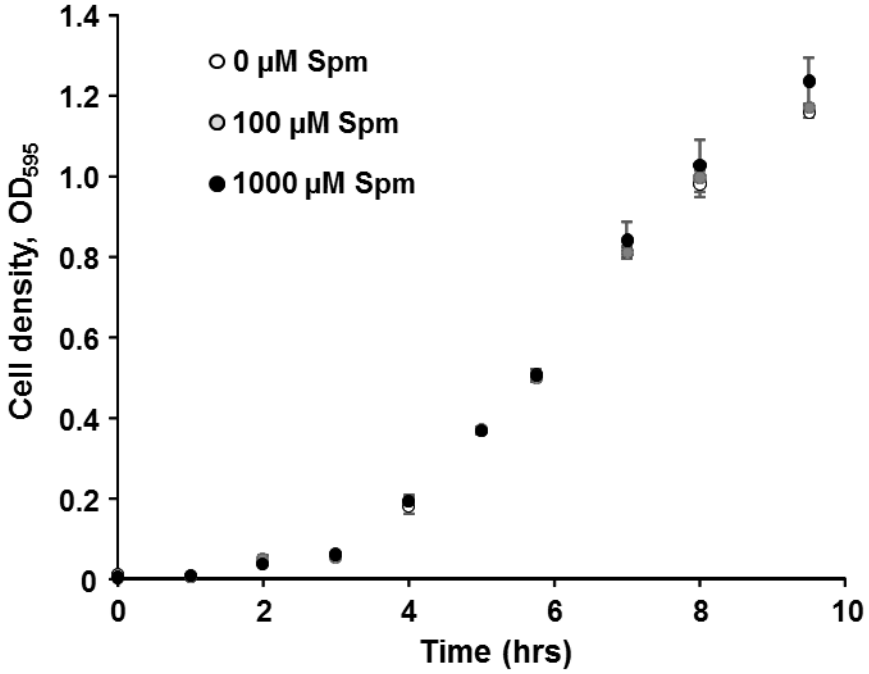

Figure S1. Spermine does not inhibit growth of wild-type *V. cholerae* O139 in shaking culture. Overnight cultures were diluted 1:100 in fresh LB media with increasing concentrations of spermine (Spm) and grown for 9.5 hours at 27˚C with agitation. Growth was monitored by taking OD_{595} measurements every hour to hour and a half. Shown is a representative experiment with three biological replicates.

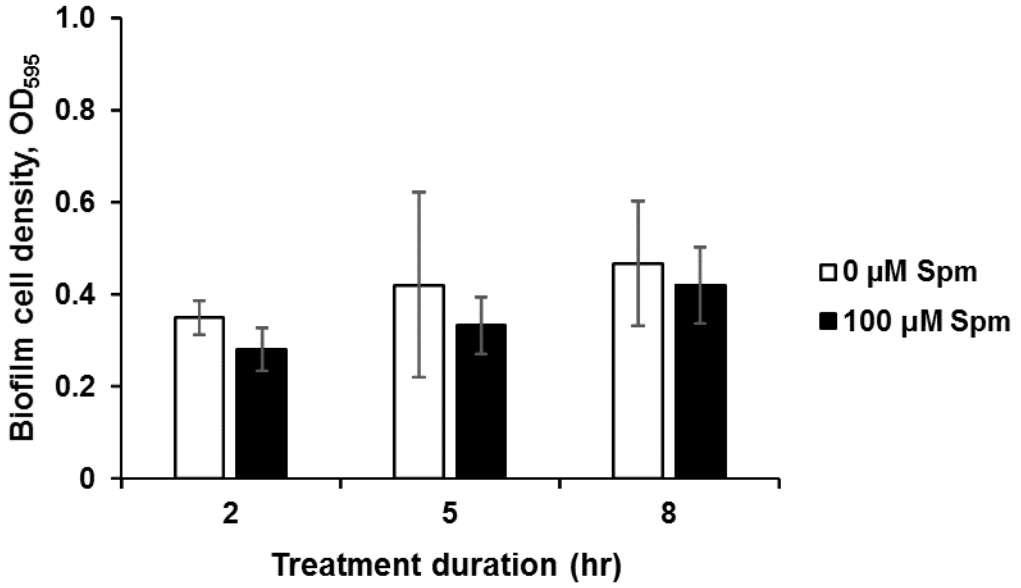

Figure S2. Spermine does not disperse preformed biofilms. Static cultures were incubated for 20 hours at 27˚C in LB only to allow biofilms to form. Planktonic cells were removed and biofilms were washed with PBS prior to adding fresh LB media only or LB with 100 µM spermine (Spm) and incubating at 27˚C for the indicated time. Biofilms were then washed, disrupted, and measured at OD_{595}. Shown are averages and standard deviations of three separate experiments with three technical replicates each. Comparisons between treatments for a given strain and treatment duration were made by a two-way ANOVA followed by Tukey's post-hoc test, using Sigma Plot Ver. 12.5. No significant differences were found at $p \leq 0.05$ between treatments, or for various treatment durations (not indicated for clarity).

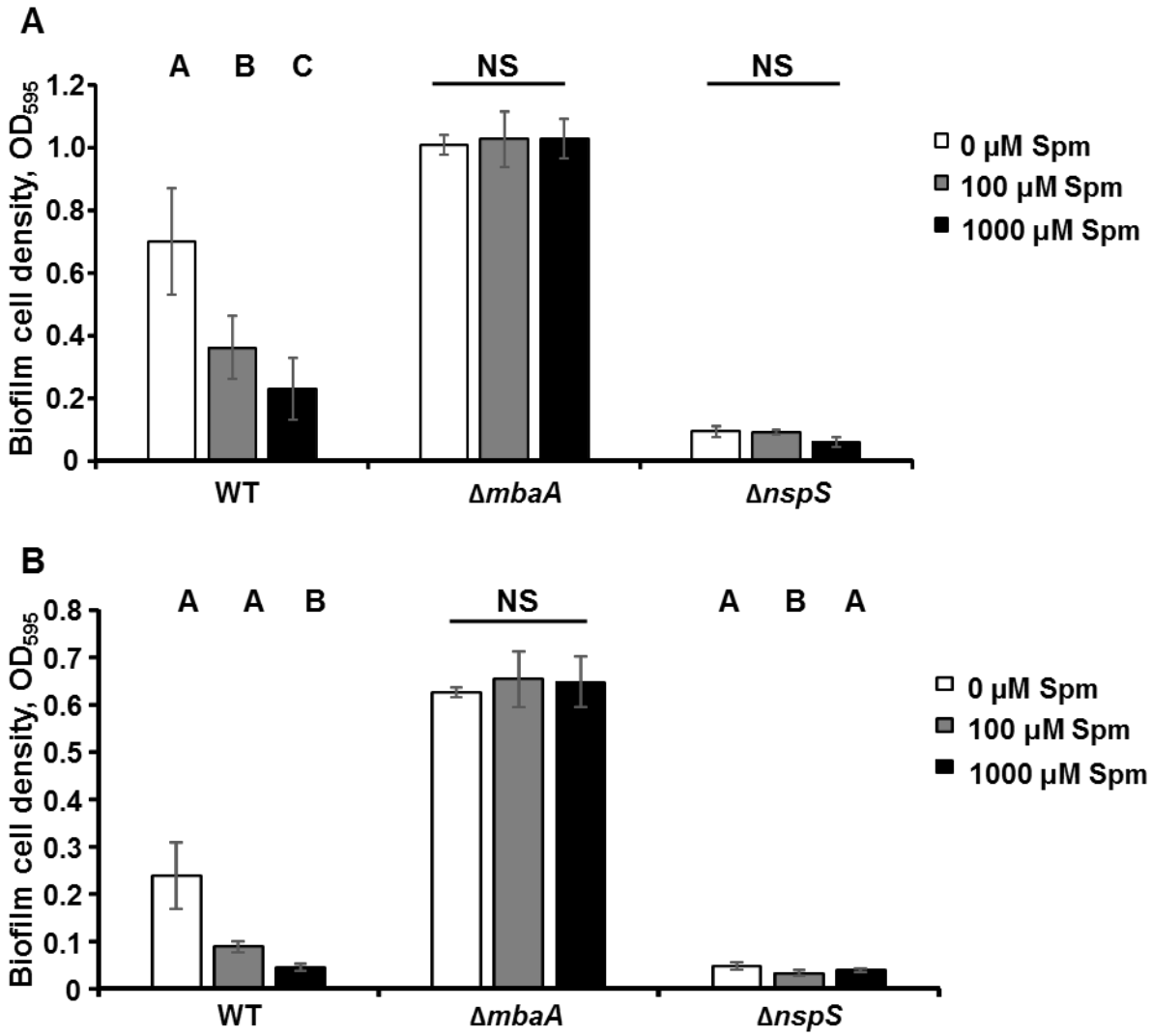

Figure S3. Spermine inhibits *V. cholerae* biofilm formation in a NspS- and MbaA-dependent manner under different environmental conditions. Static cultures were incubated for 20 hours at 27˚C in in glass tubes in TSB (A) or polypropylene tubes in LB (B) with or without spermine to allow biofilms to form. Biofilms were then washed, disrupted, and measured at OD_{595}. Shown are averages and standard deviations of four separate experiments with three technical replicates each. Means not followed by the same letter are statistically different at $p \leq 0.05$ from the untreated samples within a given strain as determined by a one-way ANOVA using Sigma Plot Ver. 12.5 (NS, not significant).

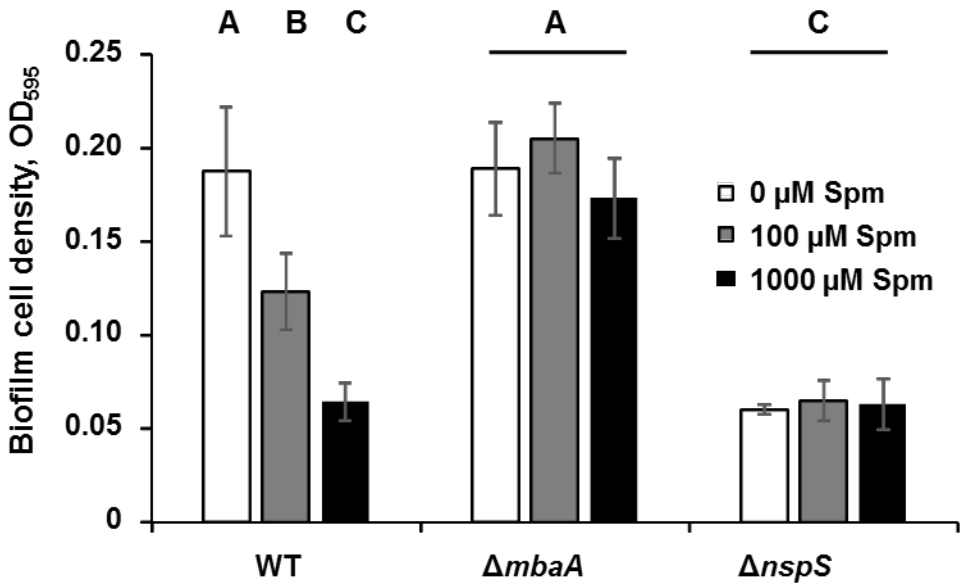

Figure S4. Spermine inhibits *V. cholerae* O1 El Tor biofilm formation in a NspS- and MbaA-dependent manner. Static cultures were incubated for 48 hours at 27˚C in the LB only or in the presence of varying spermine (Spm) concentrations. Biofilms were washed, dispersed, and measured at OD_{595}. Shown are averages and standard deviations of four separate experiments with three technical replicates each. Means not followed by the same letter are statistically different at $p \leq 0.05$ as determined by two-way ANOVA followed by Tukey's post-hoc test, using Sigma Plot Ver. 12.5.

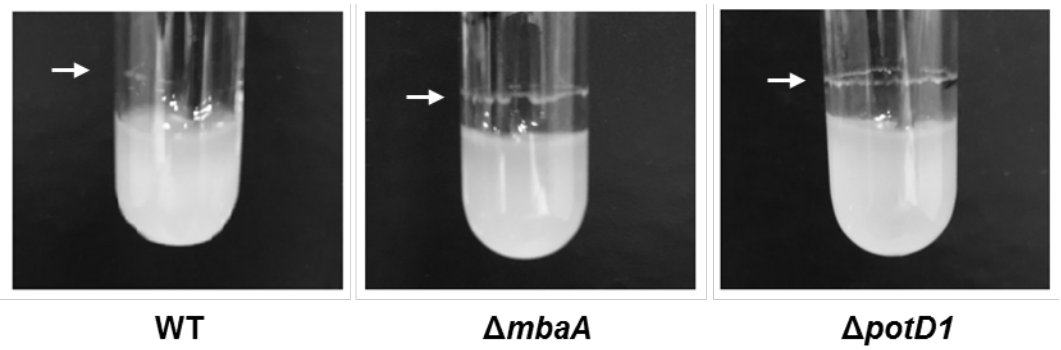

Figure S5. Δ*mbaA* and Δ*potD1* mutants form robust biofilm in shaking culture. Overnight cultures were inoculated with a single isolated colony using a sterile toothpick and growth in LB broth at 27˚C with shaking at 200 RPM. Images were taken using an iPhone 5SE camera. Arrows indicate point of biofilm formation.

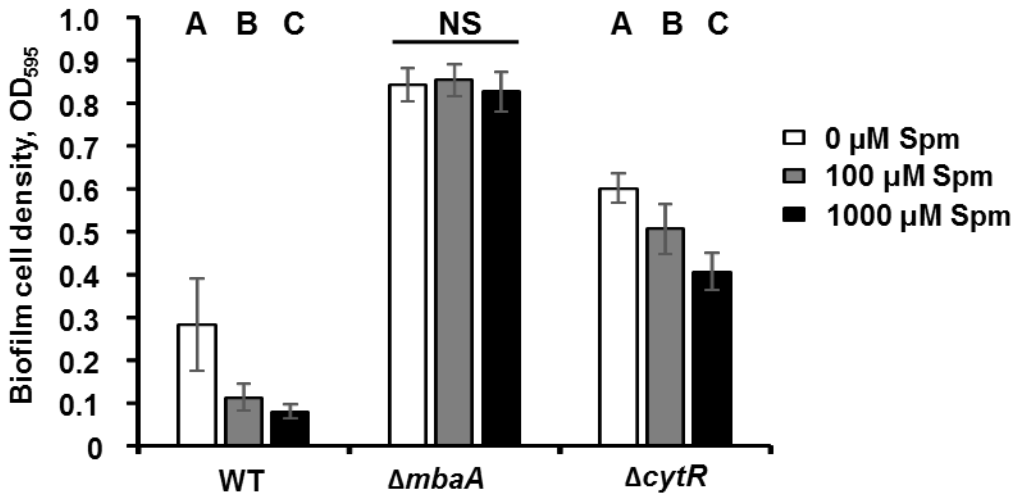

Figure S6. Spermine inhibits *V. cholerae* biofilm formation in a *cytR* mutant. Static cultures were incubated for 16 hours at 27˚C in LB with or without spermine to allow biofilms to form. Biofilms were then washed, disrupted, and measured at OD_{595}. Shown are averages and standard deviations of three separate experiments with three technical replicates each. Means not followed by the same letter are statistically different at $p \leq 0.05$ from the untreated samples within a given strain as determined by one-way ANOVA using Sigma Plot Ver. 12.5 (NS, not significant).

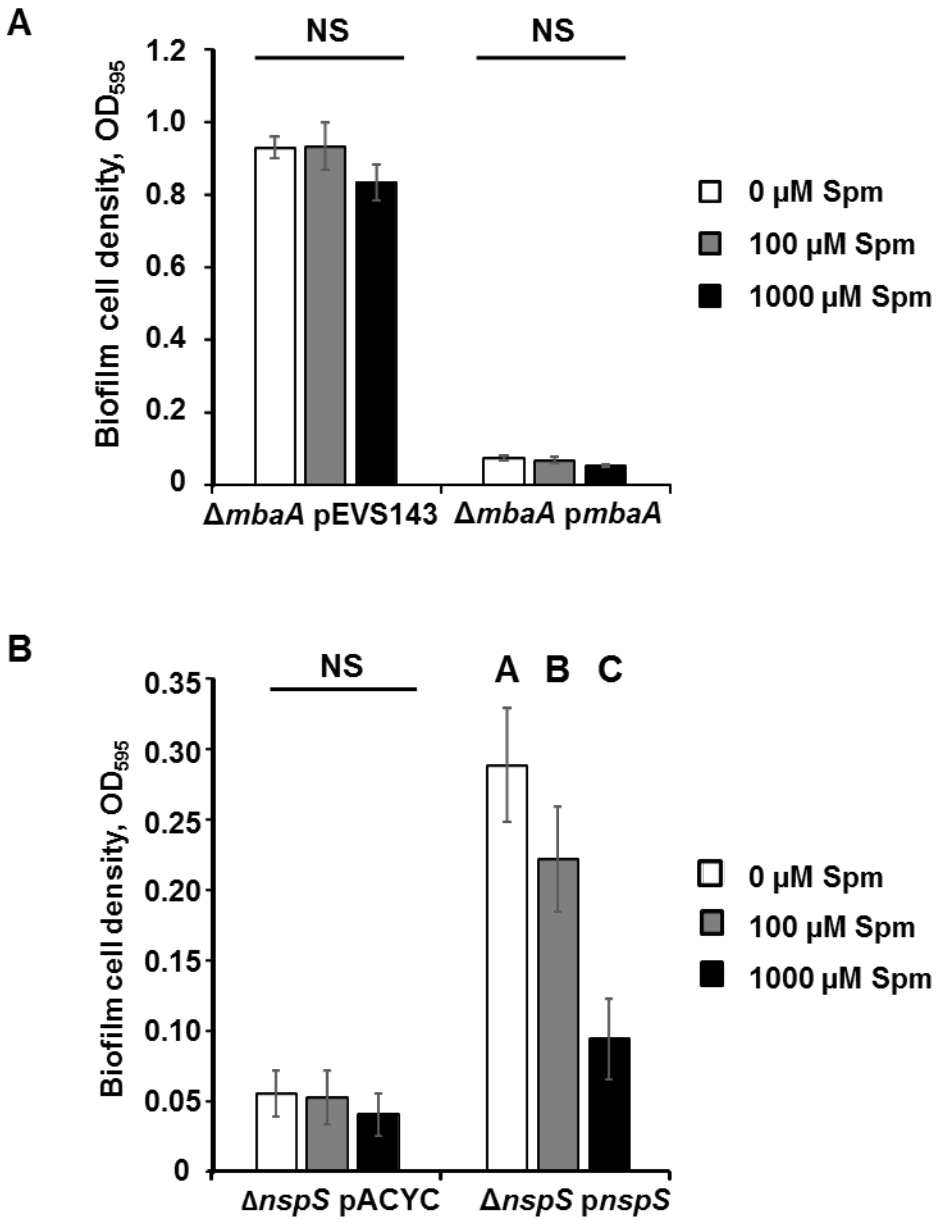

Figure S7. Complementation of *nspS* restores response to spermine. Biofilm cell densities of Δ*mbaA* (A) and Δ*nspS* (B) mutants carrying empty plasmid or plasmid for complementation. Static cultures were incubated for 20 hours at 27˚C in the LB only or in the presence of varying spermine (Spm) concentrations. Biofilms were washed, disrupted, and measured at OD_{595}. Shown are averages and standard deviation of three separate experiments with three technical replicates each. Means not followed by the same letter are statistically different at $p \leq 0.05$ from the untreated samples within a given strain as determined by one-way ANOVA using Sigma Plot Ver. 12.5 (NS, not significant).

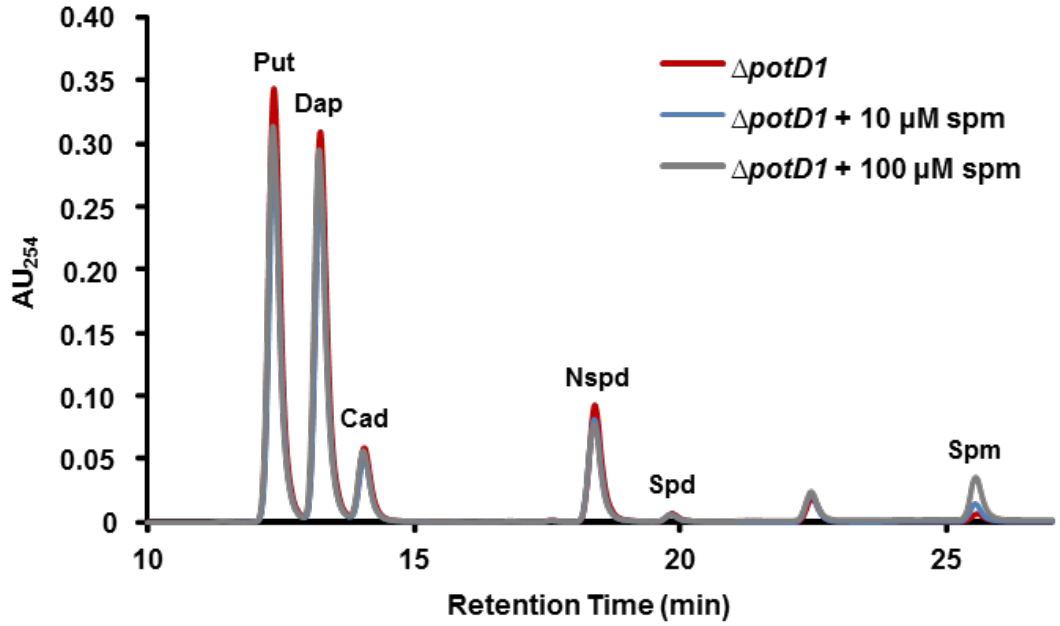

Figure S8. Spermine is imported independently of PotD1. Cells were grown to mid-log phase in LB only or supplemented with the indicated concentration of spermine. Cells were lysed and polyamines were extracted and benzoylated. Benzoylated polyamines were identified using HPLC (Put, putrescine; Dap, diaminopropane; Cad, cadaverine; Spd, spermidine; Nspd, norspermidine; Spm, spermine). The identity of the peak between spermidine and spermine is unknown. Shown are representative HPLC traces for a given treatment. Experiments were repeated four times to confirm reproducibility.

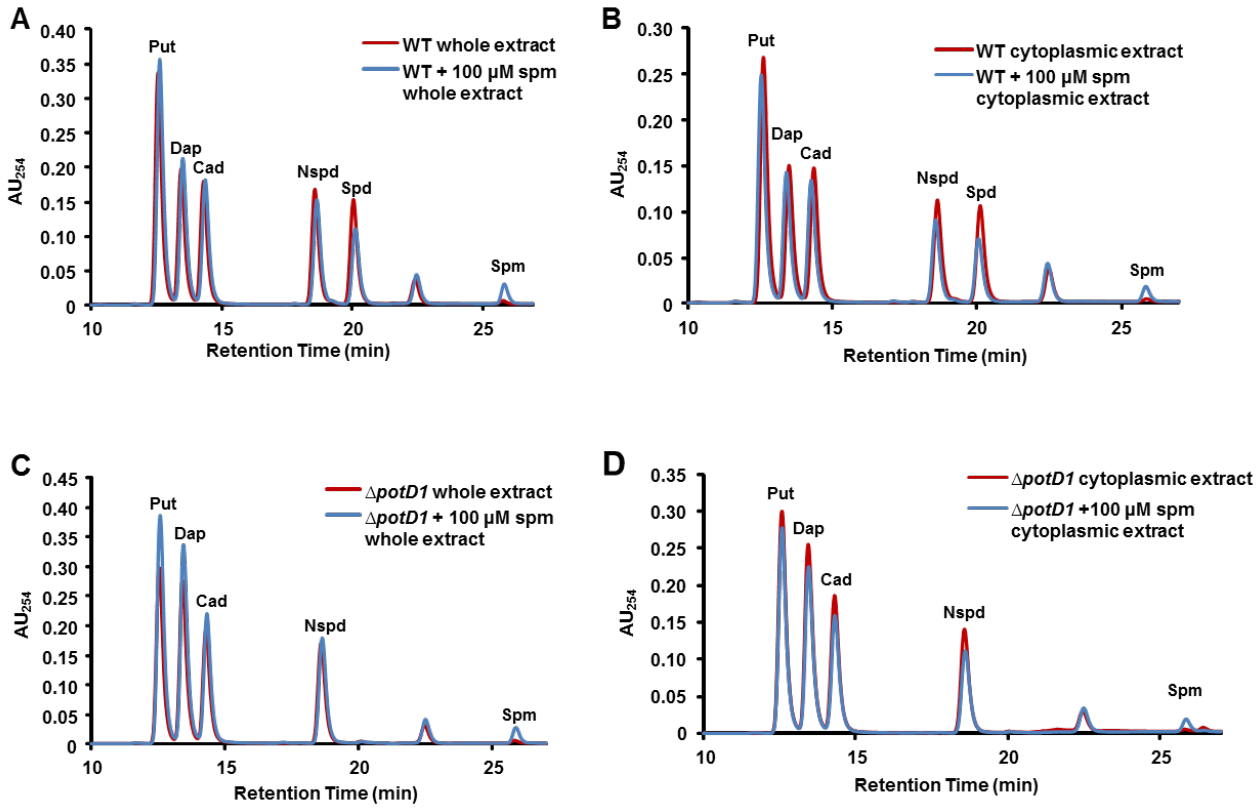

Figure S9. Cytoplasmic and whole cell extracts contain similar levels of spermine. Cells were grown to mid-log phase in LB only or supplemented with the indicated concentration of spermine. Cells were lysed by sonication and polyamines were extracted from either the cell lysates (A and C) or from the cytoplasmic extract after membranes were removed by differential centrifugation (B and D). Polyamines were then benzoylated and identified using HPLC (Put, putrescine; Dap, diaminopropane; Cad, cadaverine; Spd, spermidine; Nspd, norspermidine; Spm, spermine). The identity of the peak between spermidine and spermine is unknown. Shown are representative HPLC traces for a given treatment. Experiments were repeated twice to confirm reproducibility.