

## Supplemental Material

**Supplemental Figure 1. LapA homologs Contain High Identity N- and C-terminal Elements.** LapA homologs from *P. fluorescens* strains were aligned using MUSCLE and amino acid similarities visualized by coloring residues according to ClustalX default parameters in Jalview. See Table S1 for strains, accessions, putative functions, and amino acid lengths.

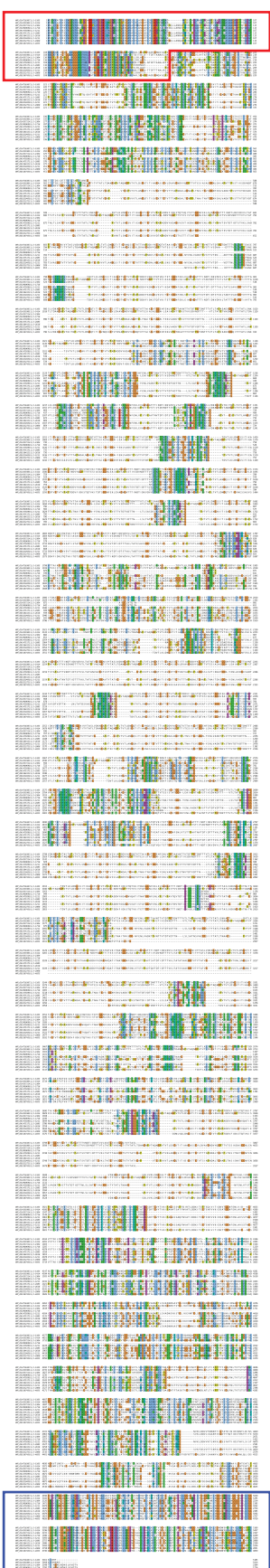
**Supplemental Figure 2. Gly-to-Gly Target Mutation Analysis Suggests Secondary Structures May Play A Role in LapA Retention.** (A) Cartoon representation of LapA secondary structure predictions from PHYRE. Truncation mutations tested are indicated. Orange arrows and green cylinders indicate predicted  $\beta$ -strands and  $\alpha$ -helices, respectively. PHYRE suggests LapA-like N-termini may adopt similar secondary structures. (B) Biofilm formation of the retention module mutants from (A). (C) The N-termini (aa 1-150) from predicted LapA-like proteins detected by our algorithm were aligned using MUSCLE and the alignments visualized using WebLogo (<http://weblogo.berkeley.edu/logo.cgi>) to highlight conserved residues.

**Supplemental Figure 3. RtxA homologs Contain High Identity N- and C-terminal Elements.** RtxA homologs from *L. pneumophila* strains were aligned using MUSCLE and amino acid similarities visualized by coloring residues according to ClustalX default parameters in Jalview. See Table S1 for strains, accessions, putative functions, and amino acid lengths.

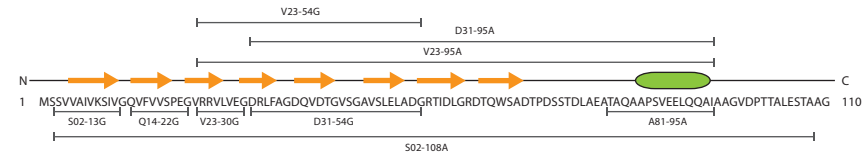
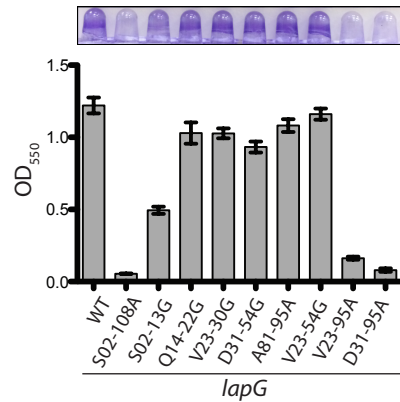
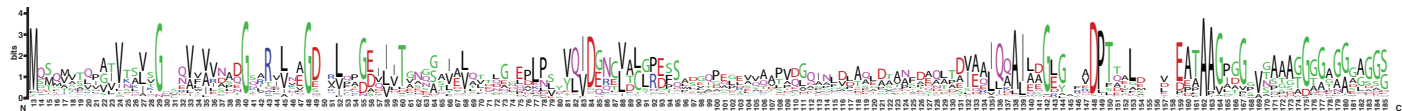
**Supplemental Figure 4. BrtA homologs Contain High Identity N- and C-terminal Elements.** BrtA homologs from *B. bronchiseptica* strains were aligned using MUSCLE and amino acid similarities visualized by coloring residues according to ClustalX default parameters in Jalview. See Table S1 for strains, accessions, putative functions, and amino acid lengths.

## Literature Cited:

1. Newell PD, Boyd CD, Sondermann H, O'Toole GA. 2011. A c-di-GMP effector system controls cell adhesion by inside-out signaling and surface protein cleavage. PLoS Biol. <http://dx.doi.org/10.1371/journal.pbio.1000587>
2. Monds RD, Newell PD, Gross RH, O'Toole GA. 2007. Phosphate-dependent modulation of c-di-GMP levels regulates *Pseudomonas fluorescens* Pf0-1 biofilm formation by controlling secretion of the adhesin LapA. Mol Microbiol 63:656–679. <http://dx.doi.org/10.1111/j.1365-2958.2006.05539>
3. Newell PD, Monds RD, O'Toole GA. 2009. LapD is a bis-(3',5')-cyclic dimeric GMP-binding protein that regulates surface attachment by *Pseudomonas fluorescens* Pf0-1. Proc Natl Acad Sci U S A 106:3461–6. <http://dx.doi.org/10.1073/pnas.0808933106>.



*P. fluorescens*  
LapA Homologs

**A****B****C**



*L. pneumophila*

RtxA Homologs



*B. bronchiseptica*  
BrtA Homologs