SUPPLEMENTAL INFORMATION

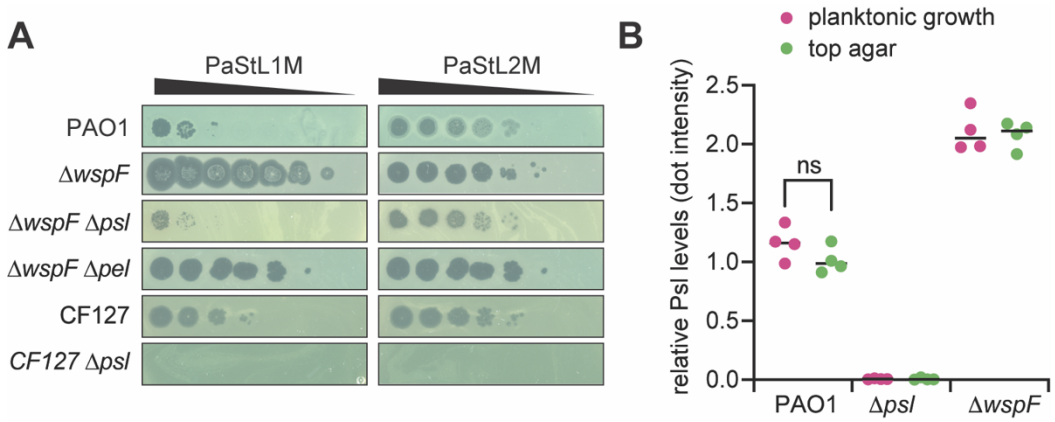

Fig S1. Phages display different abilities to form plaques on different bacterial strains. (A) Phage plaques of two phage stocks, PaStL1M and PaStL2M, on a panel of PAO1 and CF127 bacterial strains. Serial dilutions of the indicated phage stocks were spotted on plates made with the indicated bacterial strain. (B) Psl immunoblots indicate that levels of cell-associated Psl are similar during planktonic and top agar growth.

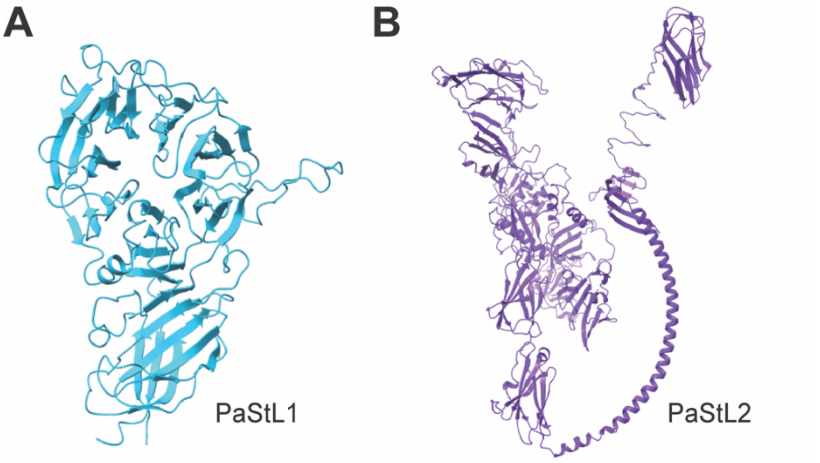

Fig S2. AlphaFold predicted structures of putative depolymerases from (A) PaStL1 and (B) PaStL2.

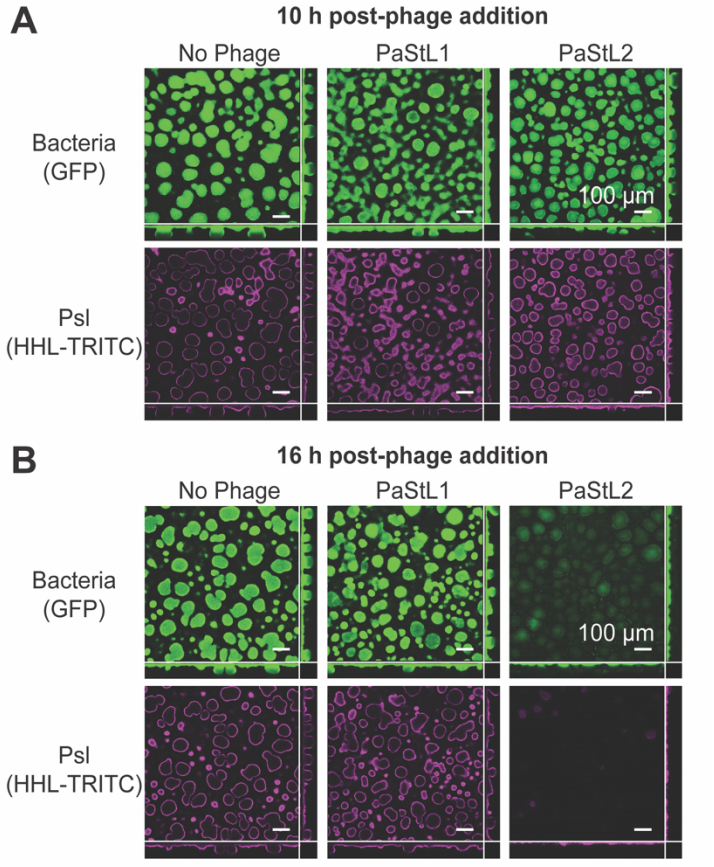

Fig S3. PaStL2 clears mature flow cell biofilms. Phages were added to mature flow cell biofilms of PAO1 Δ*wspF* GFP^{+}, and (A) 10 h or (B) 16 h post-phage addition, confocal microscopy images of the biofilms were collected to determine whether the phages cleared the biofilms. Fluorescence due to GFP (bacteria) is shown in green, and HHL-TRITC (Psl) is shown in magenta. Typical images for each time point are shown.

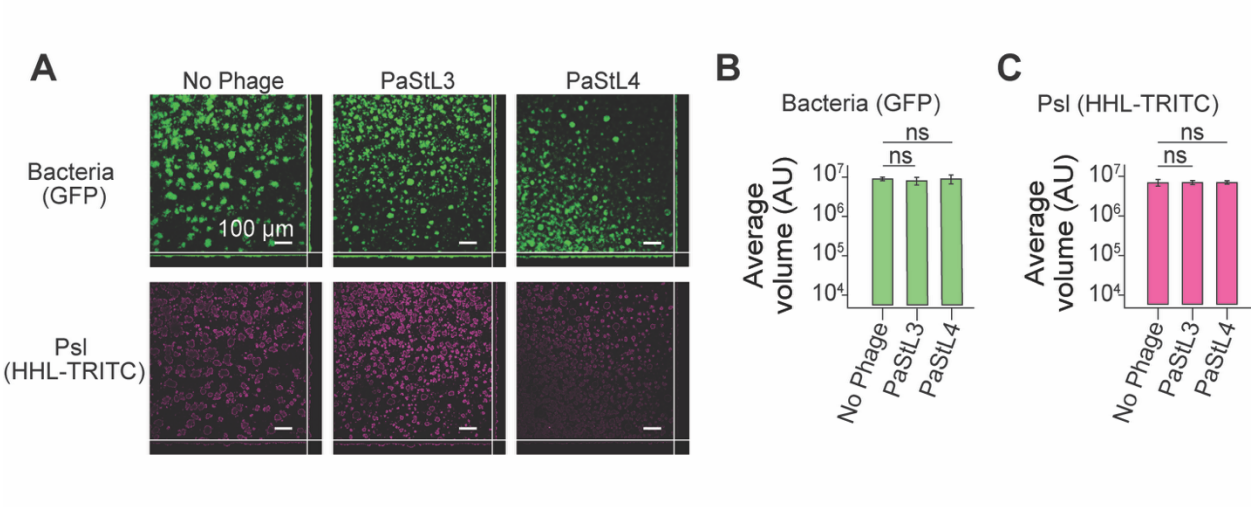

Fig S4. Neither PaStL3 nor PaStL4 clears flow cell biofilms. Phages were added to mature flow cell biofilms of PAO1 Δ*wspF* GFP^{+}, and 26 h post-phage addition, confocal microscopy images of the biofilms were collected to determine whether the phages cleared the biofilms. Fluorescence due to GFP (bacteria) is shown in green, and HHL-TRITC (Psl) is shown in magenta. Typical images for each time point are shown. The volumes of bacteria **(B)** and Psl **(C)** in the flow cells 26 h post-phage addition were determined using BiofilmQ and compared to the volumes in the no-phage control. Data represent the means of results from 3 biological replicates (3 flow cells with 6 images collected per flow cell), and error bars represent standard deviation. Student's *t*-test; $P < 0.005$, ns, not statistically significant.

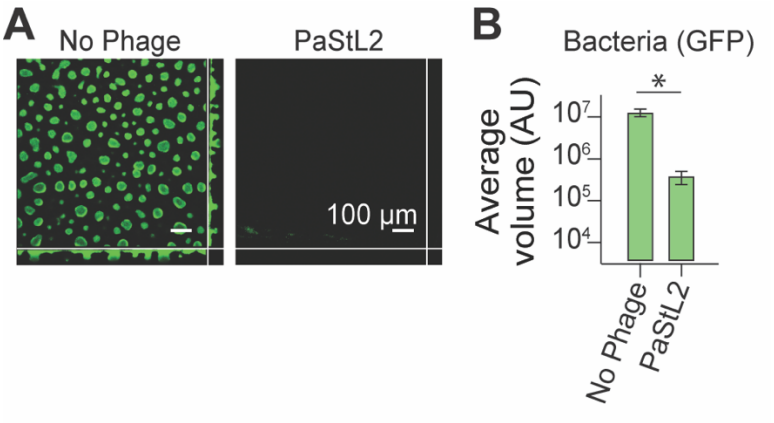

Fig S5. PaStL2 also clears wild-type PAO1 flow cell biofilms. (A) Phages were added to mature flow cell biofilms of PAO1 GFP$^+$, and confocal microscopy images of the biofilms were collected 26 h after the addition of phage. Fluorescence due to GFP (bacteria) is shown in green. Typical images are shown. **(B)** The volume of bacteria in the flow cells 26 h post-phage addition was determined using BiofilmQ and compared to the volume of bacteria in the no-phage control. Data represent the means of results from 3 biological replicates (3 flow cells with 6 images collected per flow cell), and error bars represent standard deviation. Welch's *t*-test; $P < 0.005$.

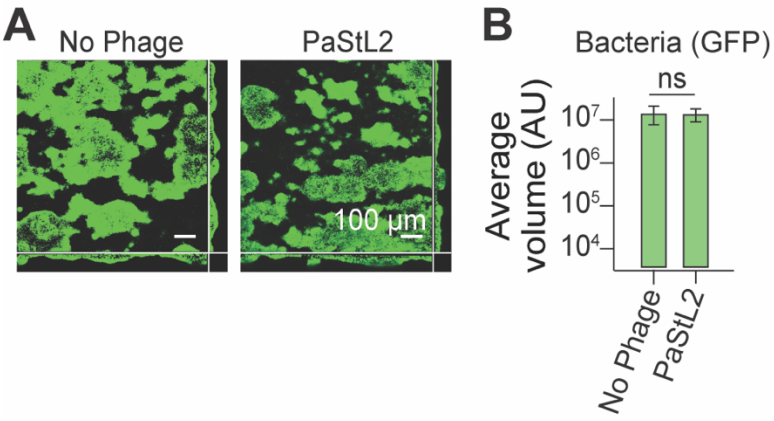

Fig S6. PaStL2 does not clear PAO1 Δ*wspF* Δ*psl* flow cell biofilms. (A) Phages were added to mature flow cell biofilms of PAO1 Δ*wspF* Δ*psl* GFP$^+$, and confocal microscopy images of the biofilms were collected 26 h. Fluorescence due to GFP (bacteria) is shown in green. Typical images are shown. **(B)** The volume of bacteria in the flow cells 26 h post-phage addition was determined using BiofilmQ and compared to the volume of bacteria in the no-phage control. Data represent the means of results from 3 biological replicates (3 flow cells with 6 images collected per flow cell), and error bars represent standard deviation. Welch's *t*-test; $P < 0.005$, ns, not statistically significant.

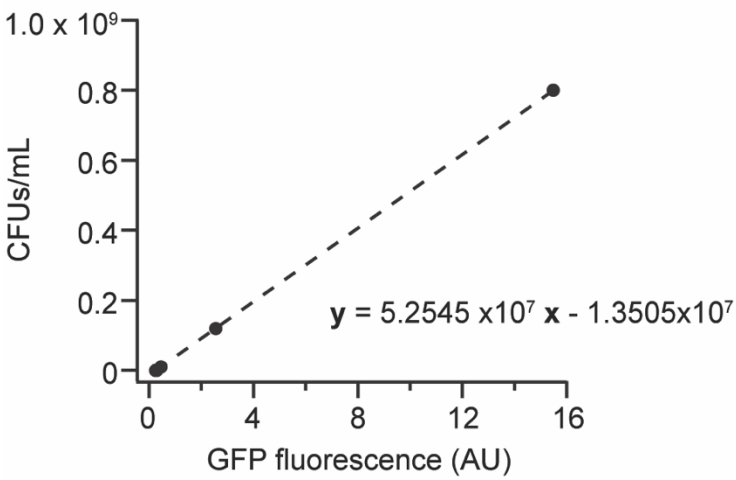

Fig S7. Standard curve of CFUs/mL to GFP fluorescence of planktonic PAO1 Δ*wspF* GFP$^+$ bacteria.

Table S1. Strains used in this study

***P. aeruginosa* strains**		**Reference**
PAO1	Wild type	Holloway, 1955
PAO1 Δ*wspF*	*wspF*, nonpolar mutation	Borlee, 2010
PAO1 Δ*wspF* Δ*psl*	*wspF*, nonpolar mutation*; pslBCD* polar mutant of *psl* operon	Jennings, 2015
PAO1 Δ*wspF* Δ*pel*	*wspF*, nonpolar mutation*; pelA,* polar mutant of the *pel* operon	Jennings, 2015
PAO1 Δ*wspF* Δ*psl* Δ*pel*	*wspF*, nonpolar mutation*; pslBCD* polar mutant of *psl* operon; *pelA,* polar mutant of the *pel* operon	Colvin, 2013
CF127	Cystic fibrosis isolate	Wolfgang, 2003
CF127 Δ*psl*	*pslD,* non-polar mutant of the *psl* operon	Colvin, 2011
PAO1 Δ*wspF* Δ*pel* pBAD*psl*	Arabinose-inducible *psl* operon	Jennings, 2015

Table S2. Additional mutations in Δ*wspF-PaStL2E-f*

Additional mutations that were identified in one of the escape mutants, present in >60% of reads

Protein	locus tag	AA change	Protein Effect
tssK1	PA0079	A278S; L388F	substitution
CynX/NimT family MFS transporter	PA0273	G147C	substitution
batD family protein	PA3075	A396E	substitution
hypothetical protein	PA3684	S67I	substitution
wspR	PA3702		frameshift
pchG	PA4224	D150E; G137C	substitution
ureF	PA4892	A136S	substitution
dguA	PA5084	G376V	substitution

References

Borlee, B. R. *et al. Pseudomonas aeruginosa* uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. *Mol Microbiol* **75**, 827–842 (2010).

Colvin, K. M. *et al.* The Pel polysaccharide can serve a structural and protective role in the biofilm matrix of *Pseudomonas aeruginosa*. *PLOS Pathog* **7**, e1001264 (2011).

Colvin, K. M. *et al.* PelA deacetylase activity is required for Pel polysaccharide synthesis in *Pseudomonas aeruginosa*. *J Bacteriol* **195**, 2329–2339 (2013).

Holloway, B. W. Genetic Recombination in *Pseudomonas aeruginosa*. *Microbiol* **13**, 572–581 (1955).

Jennings, L. K. *et al.* Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the *Pseudomonas* aeruginosa biofilm matrix. *Proc Natl Acad Sci* **112**, 11353 (2015).

Wolfgang, M. C. *et al.* Conservation of genome content and virulence determinants among clinical and environmental isolates of *Pseudomonas aeruginosa*. *Proc Nat Acad Sci* **100**, 8484–8489 (2003).