

## Text S1: Supplementary Materials and Methods for Figure S1

### Crystal Violet Assay for Biofilm Formation

The air-liquid interface method of biofilm development was used with some modification [1]. Overnight cultures of *P. aeruginosa* strains were subcultured at a starting OD<sub>600</sub> of 0.02 into 3 ml of tryptone broth in polypropylene tubes (BD Biosciences) and incubated under static condition at 37°C for 18 h. Biofilms developed at the air-liquid interface were washed with sterile distilled water to remove planktonic cells. The crystal violet assay was conducted as previously described [2-4]. After thorough rinsing with sterile distilled water, the biofilms in the tubes were stained by the addition of 4 ml of 1 % (w/v) crystal violet. After incubation at room temperature for 30 min, the crystal violet solution was discarded. The tubes were then thoroughly washed with sterile distilled water and the stain eluted from the biofilms by the addition of 4 ml of 95 % ethanol. The eluted crystal violet was measured at 595 nm ( $A_{595}$ ). Each experiment was repeated three times.

### References

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2. Déziel E, Gopalan S, Tampakaki AP, Lépine F, Padfield KE, et al. (2005) The contribution of MvfR to *Pseudomonas aeruginosa* pathogenesis and quorum sensing circuitry regulation: multiple quorum sensing-regulated genes are modulated without affecting *lasRI*, *rhlRI* or the production of N-acyl-L-homoserine lactones. *Mol Microbiol* 55: 998-1014.
3. O'Toole GA, Kolter R (1998) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol Microbiol* 30: 295-304.
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