Supplemental Figure

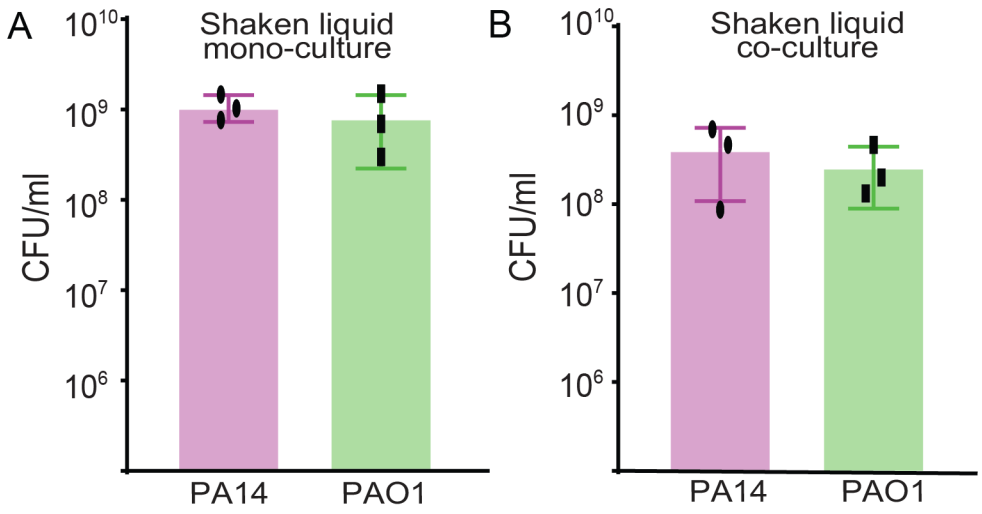

Figure S1. Well-mixed liquid culture growth comparison of PA14 and PAO1 in monoculture and co-culture. PA14 and PAO1 were back-diluted 1:10 from overnight cultures and allowed to regrow into exponential phase for 3 hr prior to being back-diluted once again to OD_{600} = 0.05 either on their own (A) or together at a 1:1 starting ratio (B). Population size in stationary phase was measured by colony-forming unit (CFU) count. $n = 3$; error bars denote the standard deviation. PAO1 and PA14 were not significantly different from each other in mono-culture or co-culture by 2-way ANOVA with a Sidak post-test.

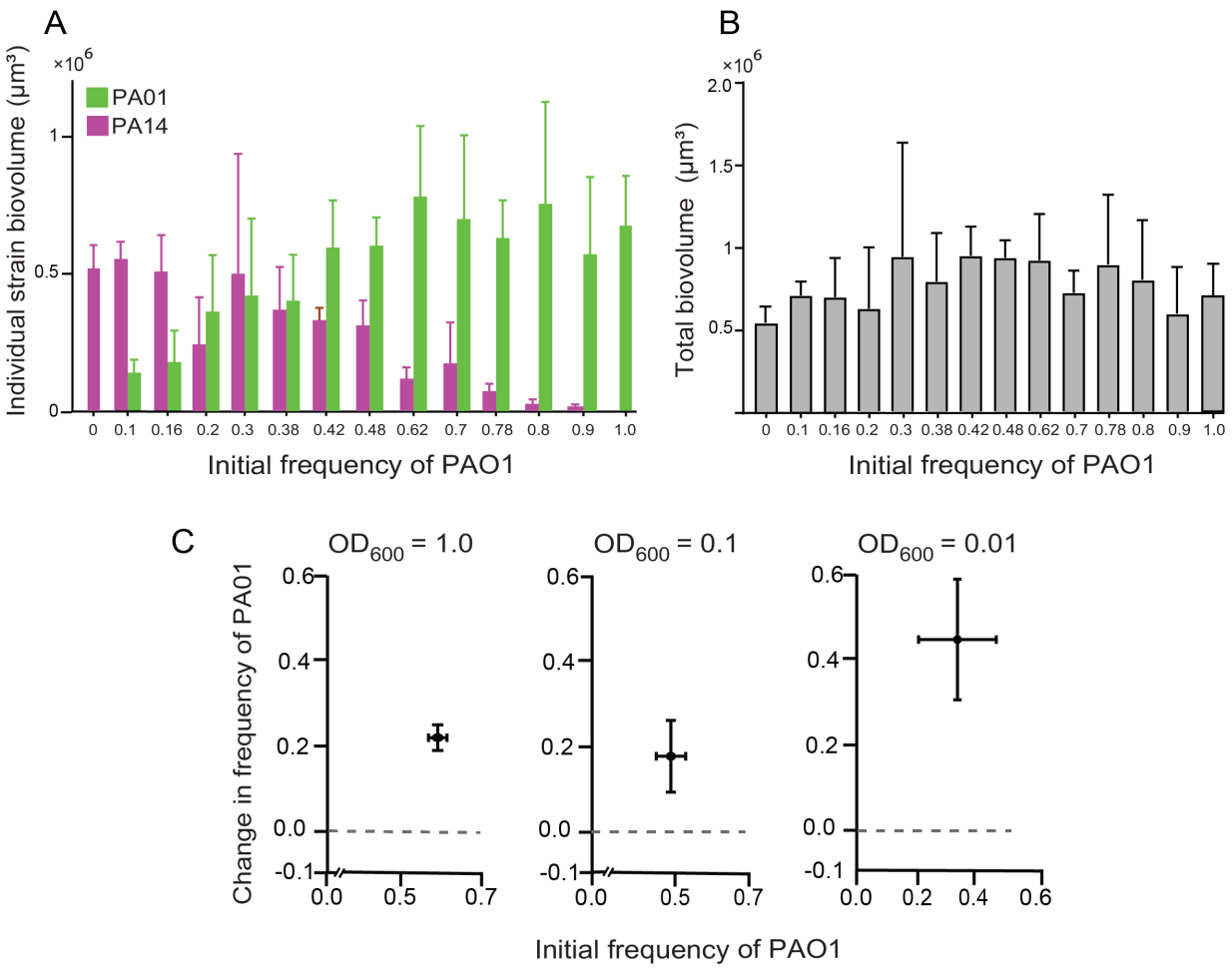

Figure S2. Absolute growth rate dynamics in biofilm co-cultures of PA14 and PAO1, and tests for density-dependence of the PAO1 biofilm competitive advantage. (A) The biovolume accumulation of PA14 and PAO1 in co-culture from a full spectrum of initial frequencies of the two strains. **(B)** The total biovolume of both strains from each initial condition in (**A**). Data from (A) and (B) are the same as those from Figure 1G of the main text, visualized in a different way. $n = 9$ non-overlapping image stacks from 4 separate microfluidic chambers. Error bars denote the standard deviation. **(C)** Competition between PA14 and PAO1 from approximately 1:1 initial inoculation at three different initial culture densities used for inoculation. For each inoculation density, $n = 9$ non-overlapping image stacks from 4 separate microfluidic chambers; error bars denote the standard deviation.

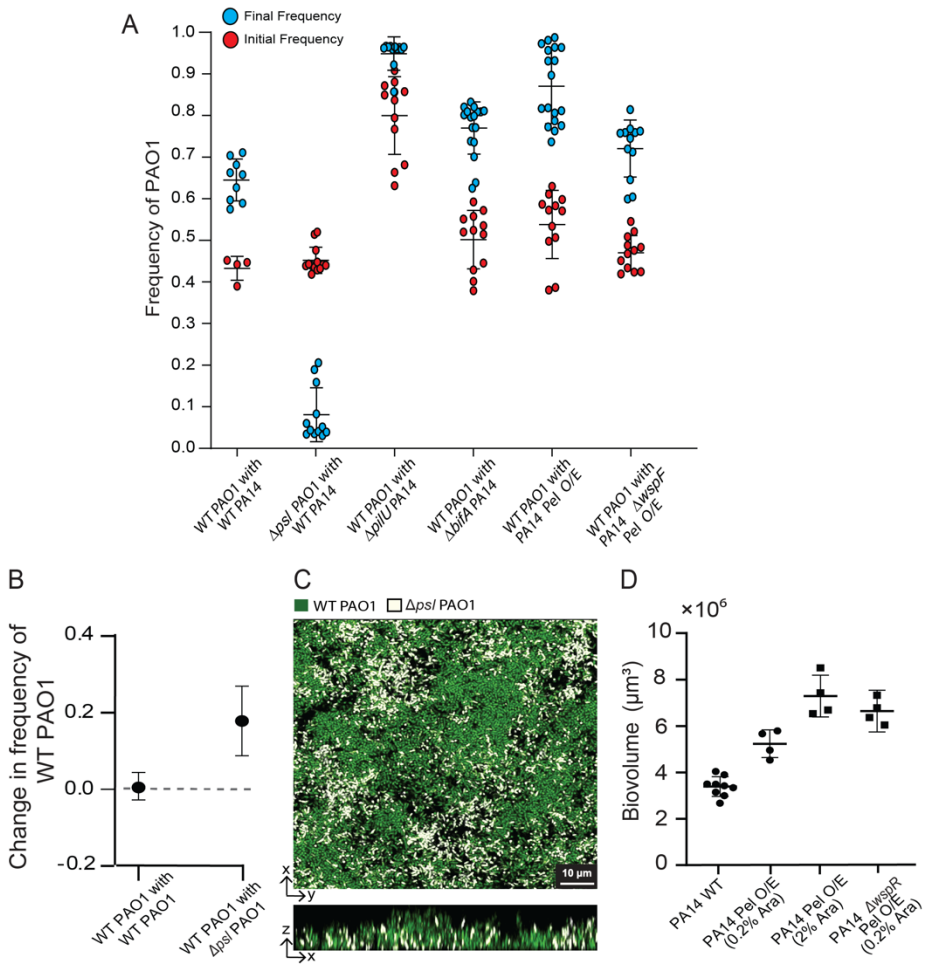

Figure S3. Control biofilm competition experiments with strain PAO1 and Pel over-expression strains of PA14. (A) Absolute initial and final frequencies of PAO1 (and in once case PAO1 Δ*psl*) against PA14 and isogenic strains with altered piliation or matrix production. The change-in-frequency data in Figure 2 are derived from the final and initial frequencies displayed in this panel. *n* = 9-18 non-overlapping image stacks from 4-6 separate microfluidic chambers; error bars denote the standard deviation. **(B,C)** Biofilm competition of PAO1 against itself and against a PAO1 mutant with reduced Psl production. *n* = 4-9 non-overlapping image stacks from 2-4 separate microfluidic chambers; error bars denote the standard deviation. **(D)** Biofilm production of PA14 WT in mono-culture and in three strain backgrounds with augmented Pel production by overexpression in trans for Pel, or with the *wspR* deletion. *n* = 4-9 non-overlapping image stacks from 2-4 separate microfluidic chambers; error bars denote the standard deviation.

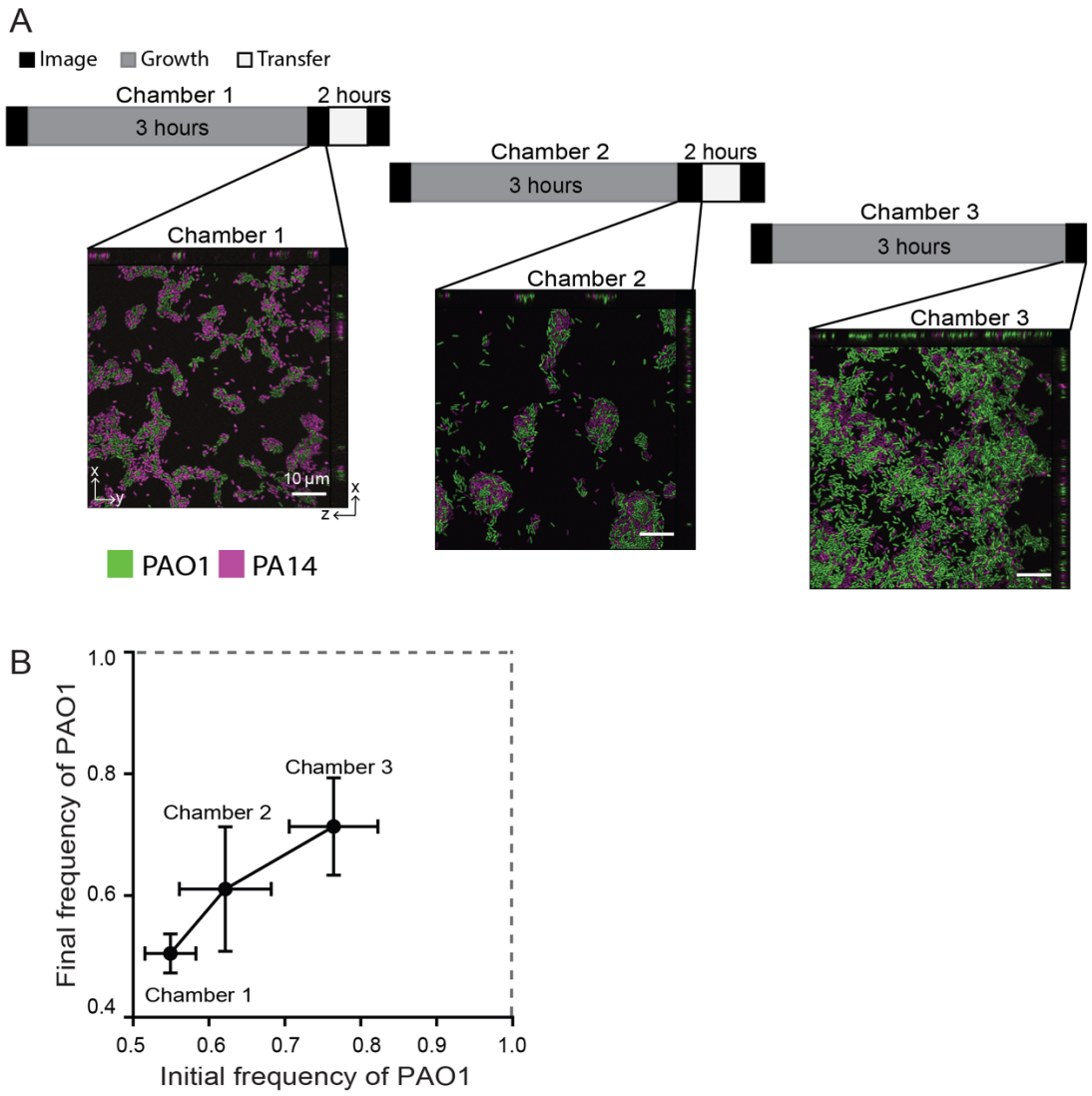

Figure S4. PAO1 outcompetes PA14 in a high-frequency dispersal regime. (**A**) Graphical summary of dispersal experiment regime (top) and representative images of biofilms in serially inoculated chambers (bottom). (**B**) The frequency of PAO1 in dispersal experiments through serially inoculated chambers with intervening 3 hr incubations. All error bars denote standard deviation (n = 9 non-overlapping image stacks from 4 separate microfluidic chambers). Population densities exiting the 3 hr-incubated biofilm chambers were too low to consistently colonize subsequent chambers with sufficient surface coverage. To adjust for this effect, the final frequencies of each strain in chambers following their 3 hr incubation were used to seed downstream chambers, but at higher cell density to obtain sufficient surface coverage for seeding the next chamber's biofilm growth.

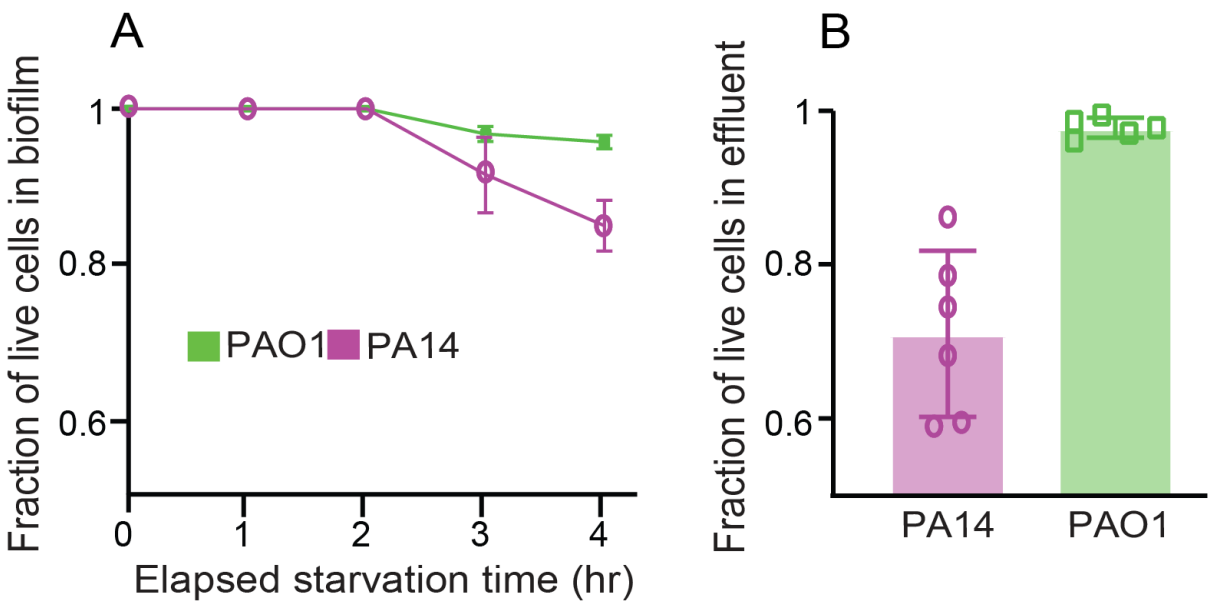

Figure S5. **Tracking cell death following carbon starvation in growing biofilms and dispersed cells in the effluent liquid phase**. Biofilms inoculated with a 1:1 mixture of *P. aeruginosa* PAO1 and PA14 were incubated for 24 h under constant flow, after which the inflow tubing was switched to a new syringe containing carbon-free medium and propidium iodide (2µg/mL) at room temperature. The biofilms were imaged 1/hr for 4 hr, and effluent from these chambers was collected continuously into chilled storage tubes, from which samples were taken for imaging at the end of the experiment. (**A**) The frequency of living PA14 and PAO1 cells (cell with zero propidium iodide staining) within biofilms undergoing carbon starvation for 4 hr. n = 6 non-overlapping image stacks from 3 separate microfluidic chambers. Error bars denote the standard deviation. (**B**) The fraction of living PA14 and PAO1 cells in the effluent collected over 4 hr of carbon starvation. Effluent was collected separately for n = 6 independent microfluidic chambers; error bars denote the standard deviation.