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Supplementary Information for

Heat shock proteases promote survival of *Pseudomonas aeruginosa* during growth arrest

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Datasets S1 and S2

Supplementary Text

The morphology of growth-arrested Δ*ftsH* cells of *P. aeruginosa* was similar to the phenotype of a gain-of-function mutation in *mlaA* (designated *mlaA**) in *E. coli* during growth arrest (1). *mlaA** mutants aberrantly transport phospholipids to the outer leaflet of the OM, which normally comprises almost exclusively LPS (1, 2). The accumulation of phospholipids in the outer leaflet triggers compensatory LPS synthesis via increased activity of LpxC, which ultimately destabilizes the OM and leads to vesiculation and loss of OM lipids (1, 3, 4). During growth, lost lipids can be replenished by ongoing synthesis and transport from the IM to the OM. Upon growth arrest, however, *de novo* lipid synthesis ceases while lipid loss from the OM continues unabated. The continual transport of lipids from the IM to the OM results in shrinkage of the IM and ultimately cell lysis (1). Lysis could be suppressed by adding $MgCl_2$ or $CaCl_2$ to LB, because cations stabilize the negatively charged LPS and prevent vesiculation and lipid loss (1, 2). Importantly, increased LpxC activity in *mlaA** is believed to be caused by reduced FtsH proteolysis (1, 3). Thus, deletion of *ftsH* in *P. aeruginosa* could compromise cell integrity by inducing LPS overproduction, phenocopying the results in *E. coli*.

Although FtsH does not regulate LpxC in *P. aeruginosa* during growth (5), it is possible that it regulates LpxC specifically upon entry into growth arrest. In support of this possibility, LpxC degradation has been shown to inversely correlate with growth rate in *E. coli*, highlighting the importance of tightly coordinating LPS synthesis with the cellular demand (6). Thus, we performed experiments to test the hypothesis that LPS overproduction in Δ*ftsH* results in cell death of *P. aeruginosa* during growth arrest.

Cation supplementation could suppress lysis of Δ*ftsH* in stationary phase in a dose-dependent manner (Fig. S3A and B), although the cation concentration necessary for complete suppression was much greater than that required in *E. coli* (100 mM vs. 5 mM $MgCl_2$) (1). However, suppression of lysis did not suppress cell death (Fig. S3C and D). The high cation concentration simply preserved the “carcasses” of dead cells (Fig. S3E). Furthermore, during carbon starvation cation supplementation had a mildly positive, but transient, effect on viability

(Fig. S3F) and only partially reduced the abundance of “detached” and “ghost” cells (Fig. S3G). Treatment with the LpxC inhibitor CHIR-090 (7) during growth arrest or at sub-inhibitory concentrations during pre-growth of cultures had no effect on survival (Fig. S3H-J). Finally, we measured LPS levels directly and observed no difference between WT and Δ*ftsH* during exponential growth or stationary phase (Fig. S3K). Therefore, we conclude that LPS production is unaltered in growth-arrested *P. aeruginosa* in the absence of FtsH.

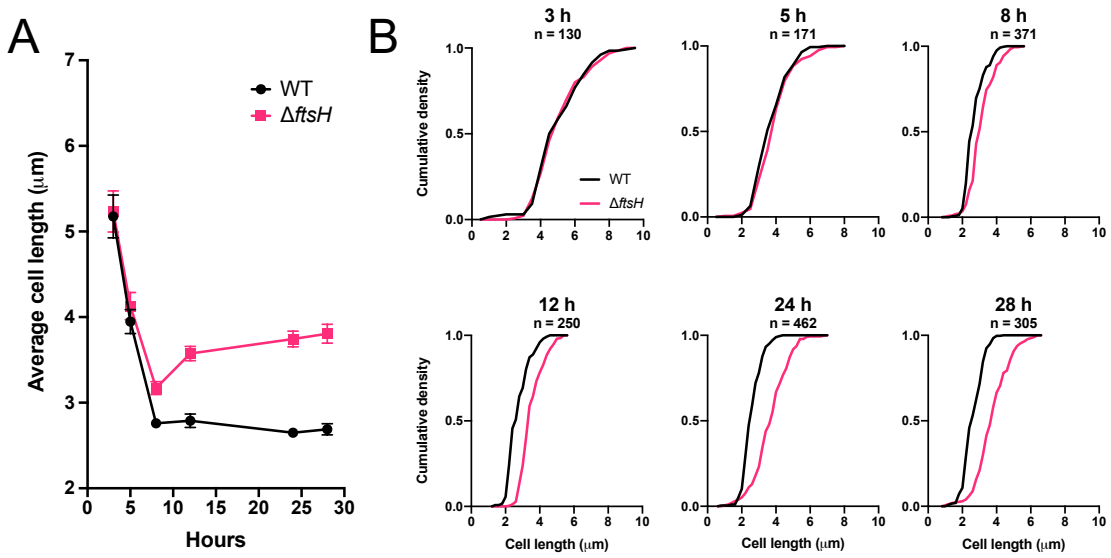

Fig. S1. Cell length diverges between WT and Δ*ftsH* during stationary phase. (A) Overnight cultures grown in LB were diluted to a starting OD_{500} of 0.01 and incubated at 37°C. Samples were taken for microscopy at the indicated time points and the average cell length is plotted over time. Cells entered stationary phase at ~8 h. Time points were taken in conjunction with the viability measurements in Fig. S3C and all data are derived from a single experiment. Error bars show 95% confidence intervals. (B) Empirical cumulative frequency distributions of cell length at each time point in (A).

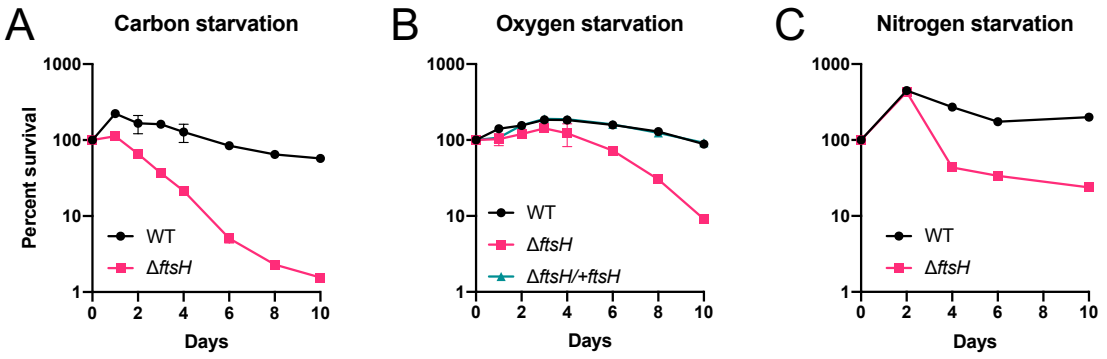

Fig. S2. FtsH is generally required for survival during growth arrest. Survival during carbon starvation (A), oxygen starvation (B), and nitrogen starvation (C). ~1-2% of Δ*ftsH* cells remain viable after extended carbon starvation, in contrast to complete viability loss in stationary phase. Cultures were supplemented with 40 mM pyruvate or 10 mM glucose as a carbon source during oxygen or nitrogen starvation, respectively. Error bars show standard deviation of biological replicates in (A) and (B) (n = 3), and representative data from two independent experiments are shown in (C).

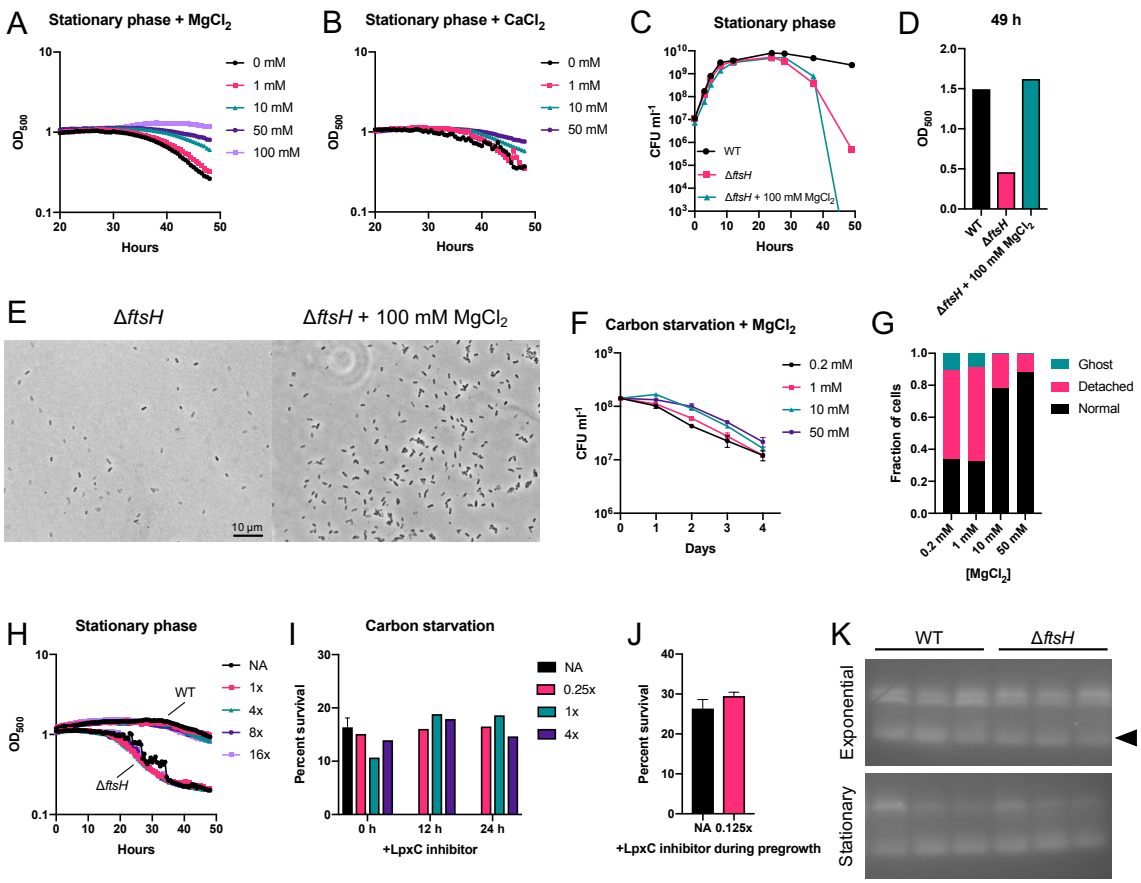

Fig. S3. Cation supplementation and LpxC inhibition does not rescue viability of Δ*ftsH* during growth arrest. Addition of $MgCl_2$ (A) or $CaCl_2$ (B) to LB prevents cell lysis of Δ*ftsH* during stationary phase, as measured by optical density. However, 100 mM $MgCl_2$ does not rescue viability of Δ*ftsH* (C), although it prevents cell lysis (D) and (E). Viability was below the limit of detection (~$3x10^3$ CFU ml^{-1}) for Δ*ftsH* at 49 h in (C), (D), and (E). (F) Addition of $MgCl_2$ mildly rescues viability of Δ*ftsH* during carbon starvation, but the effect is transient. Error bars show standard deviation of biological replicates (n = 3). (G) $MgCl_2$ partially rescues the morphological defects of Δ*ftsH* on day 1 of carbon starvation. A minimum of 300 cells were counted for each $MgCl_2$ concentration. (H) Addition of the LpxC inhibitor CHIR-090 to stationary phase cells of Δ*ftsH* does not prevent cell lysis. The concentration of inhibitor was 1x-16x the minimum inhibitory concentration (MIC) (1 μg ml^{-1}). (I) Addition of CHIR-090 after 0 h, 12 h, or 24 h of carbon

starvation does not rescue viability of Δ*ftsH* on day 3 of carbon starvation. NA = nothing added. Error bar shows standard deviation of biological replicates (n = 3) in the NA condition. (J) Addition of a sub-MIC concentration of CHIR-090 to Δ*ftsH* cells during growth does not rescue viability on day 3 of carbon starvation. Error bar shows standard deviation of biological replicates (n = 3). (K) The amount of LPS is similar between WT and Δ*ftsH* during exponential phase and stationary phase. Cell lysates were separated by SDS/PAGE and stained with Pro-Q Emerald 300 LPS Gel Stain Kit and LPS was visualized by UV transillumination. The arrowhead indicates LPS-core.

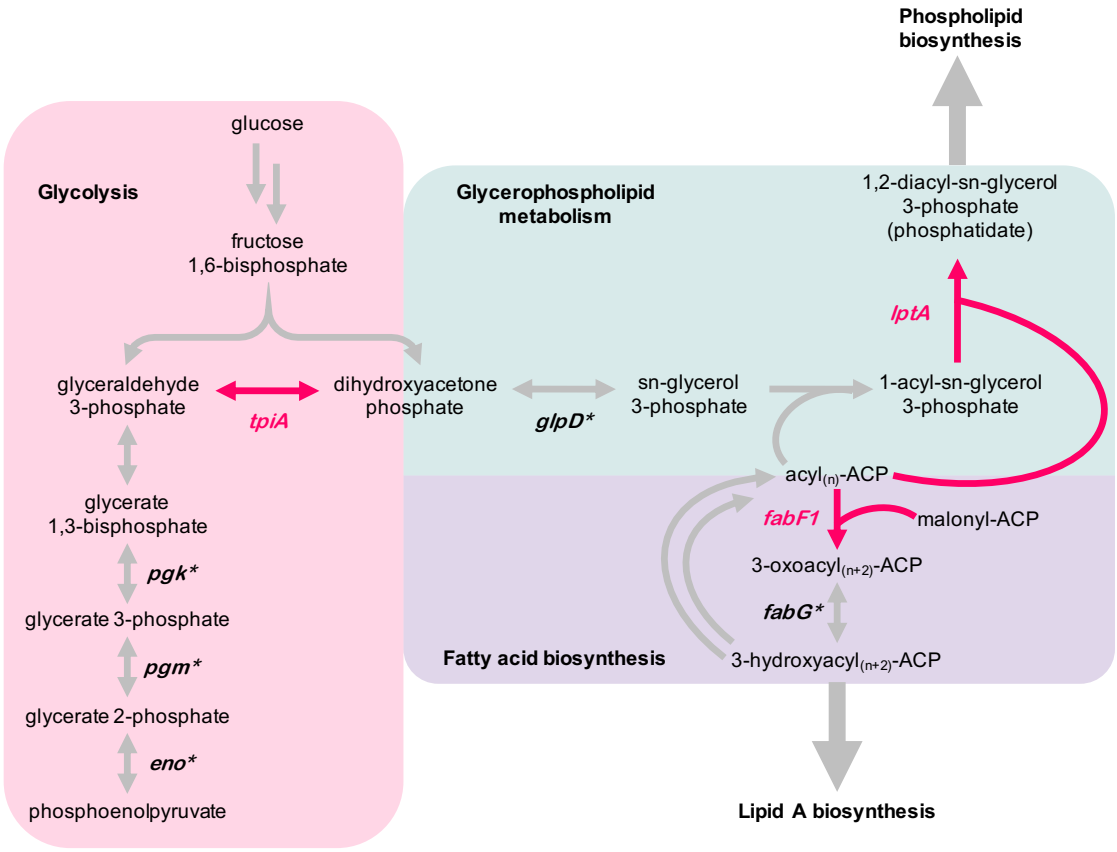

Fig. S4. Genetic interactions with *ftsH* at the intersection of glycolysis, fatty acid biosynthesis, and phospholipid metabolism. Genes catalyzing the reactions in red were >10-fold depleted in reads (P <0.05) in the Δ*ftsH* background compared to the WT background. Genes denoted with an asterisk also had a statistically significant depletion in reads, but the depletion was <10-fold (Dataset S1).

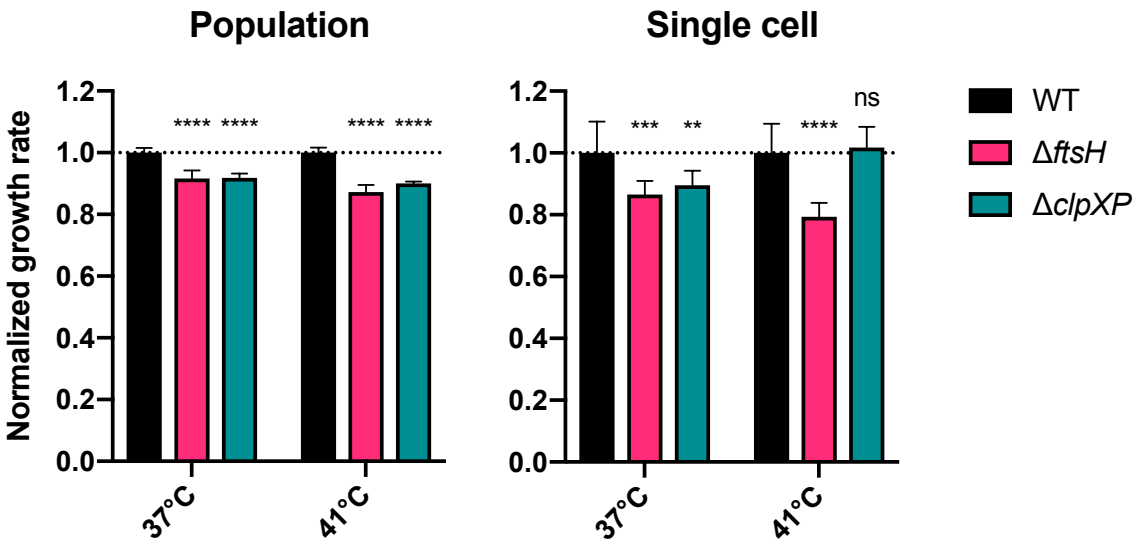

Fig. S5. Comparison of population and single cell growth rates at high temperature. All growth rates were normalized to the WT growth rate in LB at the indicated temperature. The population growth rate was measured using optical density and the single cell growth rate was measured using time-lapse microscopy (see Materials and Methods). High temperature experiments were performed at 41°C, as this was the maximum temperature that could be stably maintained in the time-lapse incubation chamber. Results of one-way ANOVA with Dunnett's multiple comparisons test are denoted as follows: ns = not significant; **, $P < 0.005$; ***, $P < 0.0005$; ****, $P < 0.0001$. Error bars show standard deviation of biological replicates (n ≥ 6 for population and n ≥ 9 for single cell growth rates).

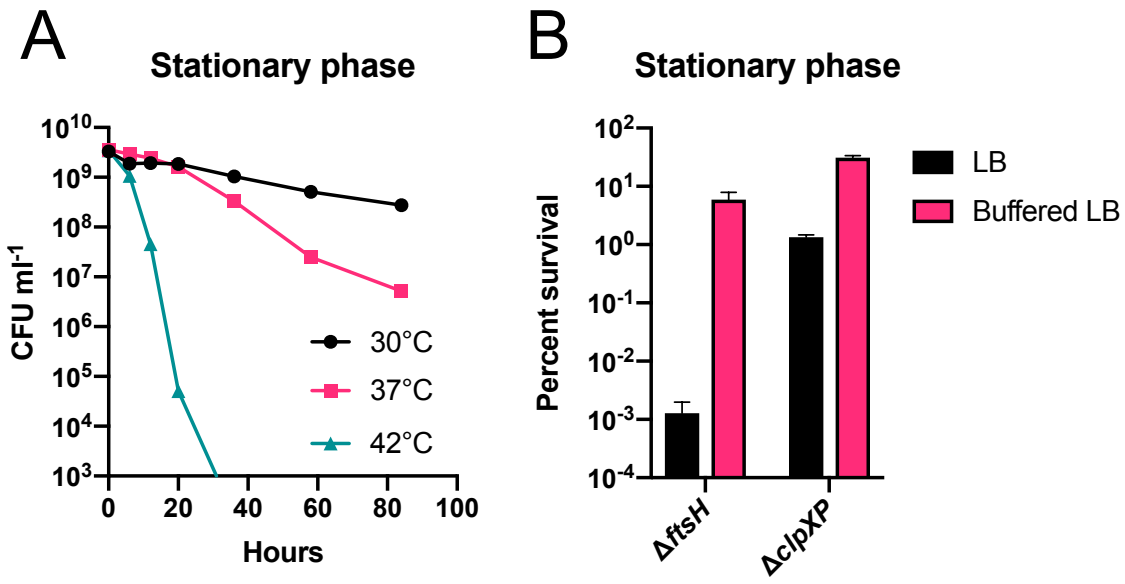

Fig. S6. Heat and alkaline pH exacerbate survival defects of protease mutants. (A) Survival of Δ*clpXP* during stationary phase is reduced at higher temperature. Cultures were grown to stationary phase in LB at 37°C and then incubated at the indicated temperature. Viability was below the limit of detection (~1.7x10^2 CFU ml^{-1}) at 42°C after 36 h. (B) Incubation in MOPS buffered LB at 37°C mitigates cell death of Δ*ftsH* and Δ*clpXP* during stationary phase. Percent survival is measured as the ratio of CFU ml^{-1} after 40 h in stationary phase relative to 16 h in stationary phase. Error bars show standard deviation of biological replicates (n = 3).

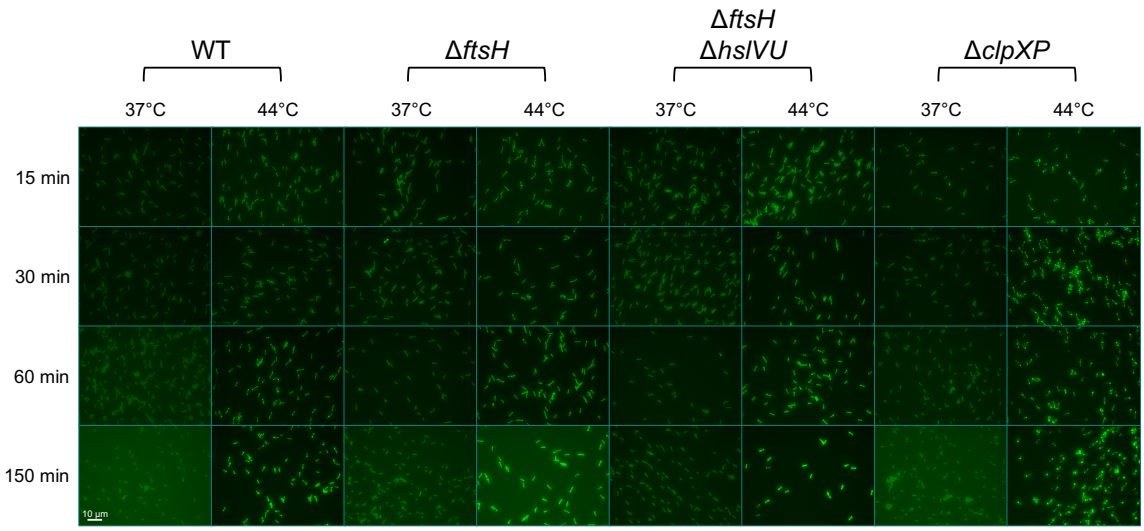

Fig. S7. Heat shock induces rapid protein aggregation. Fluorescence of cells expressing the IbpA-mVenus reporter during exponential growth at 37°C and upon heat shock at 44°C. Duplicate cultures of each strain were grown to exponential phase at 37°C and then one culture of each strain was shifted to 44°C. Samples of each strain/temperature condition were imaged at the indicated time points following the temperature shift. All images were taken using the same exposure time (2000 ms) and are displayed with the same brightness and contrast.

Table S1. Strains used in this study.

***P. aeruginosa* strains**	**Description**	**Reference**
DKN263; PA14 WT	UCBPP-PA14, wild type (WT)	
DKN1992; PA14 Δ*ftsH*	WT with a deletion in *ftsH* (*PA14_62860);* made using pDB36	This study
DKN1993; PA14 Δ*hslVU*	WT with a deletion in *hslVU* (*PA14_66770; 66790);* made using pDB48	This study
DKN1994; PA14 Δ*lon*	WT with a deletion in *lon* (*PA14_41220);* made using pDB54	This study
DKN1995; PA14 Δ*clpXP*	WT with a deletion in *clpXP* (*PA14_41230; 41240);* made using pDB55	This study
DKN1996; PA14 Δ*amgRS*	WT with a deletion in *amgRS* (*PA14_68680; 68700);* made using pDB43	This study
DKN1997; PA14 Δ*fabF1*	WT with a deletion in *fabF1* (*PA14_25690);* made using pDB49	This study
DKN1998; PA14 Δ*tpiA*	WT with a deletion in *tpiA* (*PA14_62830);* made using pDB50	This study
DKN1999; PA14 Δ*lptA*	WT with a deletion in *lptA* (*PA14_00060);* made using pDB51	This study
DKN2000; PA14 Δ*htpX*	WT with a deletion in *htpX* (*PA14_27480);* made using pDB58	This study
DKN2001; PA14 Δ*lon* Δ*clpXP*	WT with a deletion in *lon* and *clpXP* (*PA14_41220; 41230; 41240);* made using pDB56	This study
DKN2002; PA14 Δ*ftsH* Δ*hslVU*	Δ*ftsH* with a deletion in *hslVU;* made using pDB48	This study
DKN2003; PA14 Δ*ftsH* Δ*lon*	Δ*ftsH* with a deletion in *lon;* made using pDB54	This study
DKN2004; PA14 Δ*ftsH* Δ*clpXP*	Δ*ftsH* with a deletion in *clpXP;* made using pDB55	This study
DKN2005; PA14 Δ*hslVU* Δ*lon*	Δ*hslVU* with a deletion in *lon;* made using pDB54	This study
DKN2006; PA14 Δ*hslVU* Δ*clpXP*	Δ*hslVU* with a deletion in *clpXP;* made using pDB55	This study
DKN2007; PA14 Δ*ftsH* Δ*hslVU* Δ*lon*	Δ*ftsH* Δ*lon* with a deletion in *hslVU;* made using pDB48	This study
DKN2008; PA14 Δ*ftsH* Δ*hslVU* Δ*clpXP*	Δ*ftsH* Δ*clpXP* with a deletion in *hslVU;* made using pDB48	This study
DKN2009; PA14 Δ*ftsH* Δ*lon* Δ*clpXP*	Δ*ftsH* with a deletion in *lon* and *clpXP;* made using pDB56	This study
DKN2010; PA14 Δ*hslVU* Δ*lon* Δ*clpXP*	Δ*hslVU* with a deletion in *lon* and *clpXP;* made using pDB56	This study
DKN2011; PA14 ΔftsH Δ*hslVU* Δ*lon* Δ*clpXP*	Δ*hslVU* Δ*lon* Δ*clpXP* with a deletion in *ftsH;* made using pDB36	This study
DKN2013; PA14 Δ*ftsH* Δ*amgRS*	Δ*ftsH* with a deletion in *amgRS;* made using pDB43	This study
DKN2014; PA14 Δ*ftsH* Δ*fabF1*	Δ*ftsH* with a deletion in fabF1*;* made using pDB49	This study
DKN2015; PA14 Δ*ftsH* Δ*tpiA*	Δ*ftsH* with a deletion in tpiA*;* made using pDB50	This study
DKN2016; PA14 Δ*ftsH* ΔlptA	Δ*ftsH* with a deletion in lptA*;* made using pDB51	This study
DKN2017; PA14 Δ*ftsH* Δ*htpX*	Δ*ftsH* with a deletion in htpX*;* made using pDB58	This study
DKN2018; PA14 Δ*ftsH/+ftsH*	Δ*ftsH* complemented at the *att*Tn7 site on the chromosome*;* made using pDB37	This study
DKN2019; PA14 WT::IbpA-mVenus	PA14 expressing ibpA ORF fused to mVenus inserted at the *att*Tn7 site on the chromosome; made using pLREX97	This study
DKN2020; PA14 Δ*ftsH*::IbpA-mVenus	Δ*ftsH* expressing ibpA ORF fused to mVenus inserted at the *att*Tn7 site on the chromosome; made using pLREX97	This study
DKN2021; PA14 Δ*ftsH* Δ*hslVU*::IbpA-mVenus	Δ*ftsH* Δ*hslVU* expressing ibpA ORF fused to mVenus inserted at the *att*Tn7 site on the chromosome; made using pLREX97	This study
***E. coli* strains**	**Description**	**Reference**
DKN89; DH10B	Host for routine cloning and plasmid stocks	(8)
DKN1298; SM10l*pir*	Helper strain for integration at the *att*Tn7 site and for generation of the transposon library in *P. aeruginosa* (carries pTNS1 or pIT2)	(9)
DKN1299; HB101	Helper strain for integration at the *att*Tn7 site and for making unmarked deletions in *P. aeruginosa* (carries pRK2013)	(9)

Table S2. Plasmids used in this study.

Plasmid	Description	Reference
pMQ30	Conjugal vector for making unmarked deletions in *P. aeruginosa*	(10)
pUC18T-mini-Tn7T	Conjugal vector for inserting genes at the *att*Tn7 site in *P. aeruginosa*	(9)
pTNS1	Helper plasmid for integration at the *att*Tn7 site (carried by SM10)	(9)
pRK2013	Helper plasmid for integration at the *att*Tn7 site and for making unmarked deletions in *P. aeruginosa* (carried by HB101)	(9)
pIT2	Vector containing transposon T8 (*ISlacZhah*-tc) for generation of the transposon library in *P. aeruginosa* (carried by SM10)	(11)
pDB36	*ftsH* deletion vector; ~1kb upstream and ~1kb downstream of *ftsH* ORF cloned into pMQ30	This study
pDB48	*hslVU* deletion vector; ~1kb upstream and ~1kb downstream of *hslVU* ORF cloned into pMQ30	This study
pDB54	*lon* deletion vector; ~1kb upstream and ~1kb downstream of *lon* ORF cloned into pMQ30	This study
pDB55	*clpXP* deletion vector; ~1kb upstream and ~1kb downstream of *clpXP* ORF cloned into pMQ30	This study
pDB43	*amgRS* deletion vector; ~1kb upstream and ~1kb downstream of *amgRS* ORF cloned into pMQ30	This study
pDB49	*fabF1* deletion vector; ~1kb upstream and ~1kb downstream of *fabF1* ORF cloned into pMQ30	This study
pDB50	*tpiA* deletion vector; ~1kb upstream and ~1kb downstream of *tpiA* ORF cloned into pMQ30	This study
pDB51	*lptA* deletion vector; ~1kb upstream and ~1kb downstream of *lptA* ORF cloned into pMQ30	This study
pDB58	*htpX* deletion vector; ~1kb upstream and ~1kb downstream of *htpX* ORF cloned into pMQ30	This study
pDB56	*lon* and *clpXP* deletion vector; ~1kb upstream and ~1kb downstream of *lon/clpXP* locus cloned into pMQ30	This study
pDB37	*ftsH* complementation vector; *ftsH* promoter region and ORF cloned into pUC18T-mini-Tn7T	This study
pLREX97	*ibpA-mVenus* expression vector; *ibpA* promoter region and ORF fused to mVenus cloned into pUC18T-mini-Tn7T	This study

Table S3. Primers used in this study.

Primer	Sequence	Description
pDB36		
ftsH Upstream Fwd	taaaacgacggccagtgccaGTGGTGACCGATAGATTTGAACTG	LC: overlap with pMQ30; UC: complementary to 5' end of *ftsH* upstream region
ftsH Upstream Rev	cttccaggttGATGATCAGCCACAGAATCAGATTC	LC: overlap with *ftsH* downstream region; UC: complementary to 3' end of *ftsH* upstream region
ftsH Downstream Fwd	gctgatcatcAACCTGGAAGAGTCGGGC	LC: overlap with *ftsH* upstream region; UC: complementary to 5' end of *ftsH* downstream region
ftsH Downstream Rev	catgattacgaattcgagctACTCGCCAACACGACCAC	LC: overlap with pMQ30; UC: complementary to 3' end of *ftsH* downstream region
pDB48		
hslVU Upstream Fwd	taaaacgacggccagtgccaTGATCAACAACGTCGCGC	LC: overlap with pMQ30; UC: complementary to 5' end of *hslVU* upstream region
hslVU Upstream Rev	cggttcgggaGGGGGAAATCTCCACACTG	LC: overlap with *hslVU* downstream region; UC: complementary to 3' end of *hslVU* upstream region
hslVU Downstream Fwd	gatttcccccTCCCGAACCGGGGTATCC	LC: overlap with *hslVU* upstream region; UC: complementary to 5' end of *hslVU* downstream region
hslVU Downstream Rev	catgattacgaattcgagctAGGTCTGCATGTAGCGCTTG	LC: overlap with pMQ30; UC: complementary to 3' end of *hslVU* downstream region
pDB54		
lon Upstream Fwd	taaaacgacggccagtgccaACGTGCCGTTCACCATCG	LC: overlap with pMQ30; UC: complementary to 5' end of *lon* upstream region
lon Upstream Rev	tacctaccgaAATGTCGGCTCTACAGCGG	LC: overlap with *lon* downstream region; UC: complementary to 3' end of *lon* upstream region
lon Downstream Fwd	agccgacattTCGGTAGGTATTCTTGACACTG	LC: overlap with *lon* upstream region; UC: complementary to 5' end of *lon* downstream region
lon Downstream Rev	catgattacgaattcgagctTGCAGGATCAACTGGTCC	LC: overlap with pMQ30; UC: complementary to 3' end of *lon* downstream region
pDB55		
clpXP Upstream Fwd	taaaacgacggccagtgccaAGTTTCCGATGCTGATGTC	LC: overlap with pMQ30; UC: complementary to 5' end of *clpXP* upstream region
clpXP Upstream Rev	ccctgctcttGTCTTGCGATCACTCCCTAAC	LC: overlap with *clpXP* downstream region; UC: complementary to 3' end of *clpXP* upstream region
clpXP Downstream Fwd	atcgcaagacAAGAGCAGGGGCCTTCGG	LC: overlap with *clpXP* upstream region; UC: complementary to 5' end of *clpXP* downstream region
clpXP Downstream Rev	catgattacgaattcgagctAGCAGCCAGTCTATGTAGGAAC	LC: overlap with pMQ30; UC: complementary to 3' end of *clpXP* downstream region
pDB43		
amgRS Upstream Fwd	taaaacgacggccagtgccaATCATGACTTCGCTTGGGTG	LC: overlap with pMQ30; UC: complementary to 5' end of *amgRS* upstream region
amgRS Upstream Rev	ccgtcgagtaAGAAACTCCCAGAATGAGTCAGG	LC: overlap with *amgRS* downstream region; UC: complementary to 3' end of *amgRS* upstream region
amgRS Downstream Fwd	gggagtttctTACTCGACGGGTTTGTGCCG	LC: overlap with *amgRS* upstream region; UC: complementary to 5' end of *amgRS* downstream region
amgRS Downstream Rev	catgattacgaattcgagctTCGTCCCTCCTCCGACCAGAC	LC: overlap with pMQ30; UC: complementary to 3' end of *amgRS* downstream region
pDB49		
fabF1 Upstream Fwd	taaaacgacggccagtgccaAACCGCTGATCGTGGTCAATAAC	LC: overlap with pMQ30; UC: complementary to 5' end of *fabF1* upstream region

fabF1 Upstream Rev	gggtgccgttTACGACTTCCTCTTTCTCATTTCAC	LC: overlap with *fabF1* downstream region; UC: complementary to 3' end of *fabF1* upstream region
fabF1 Downstream Fwd	ggaagtcgtaAACGGCACCCTGGTGTTC	LC: overlap with *fabF1* upstream region; UC: complementary to 5' end of *fabF1* downstream region
fabF1 Downstream Rev	catgattacgaattcgagctGAAACGTCCAGCAGGCGC	LC: overlap with pMQ30; UC: complementary to 3' end of *fabF1* downstream region
pDB50		
tpiA Upstream Fwd	taaaacgacggccagtgccaTCGAGCTGATGATCGAGGAACTG	LC: overlap with pMQ30; UC: complementary to 5' end of *tpiA* upstream region
tpiA Upstream Rev	gacagatcgcGCTGTACCTCGCGGGGCA	LC: overlap with *tpiA* downstream region; UC: complementary to 3' end of *tpiA* upstream region
tpiA Downstream Fwd	gaggtacagcGCGATCTGTCGTGCCGCG	LC: overlap with *tpiA* upstream region; UC: complementary to 5' end of *tpiA* downstream region
tpiA Downstream Rev	catgattacgaattcgagctACCTCCAGGGTGTACTCGCCG	LC: overlap with pMQ30; UC: complementary to 3' end of *tpiA* downstream region
pDB51		
lptA Upstream Fwd	taaaacgacggccagtgccaCTTTCGGTCCATTCCTTG	LC: overlap with pMQ30; UC: complementary to 5' end of *lptA* upstream region
lptA Upstream Rev	ttgcggccctGTATTCTGACCTTACTGAAGTAATG	LC: overlap with *lptA* downstream region; UC: complementary to 3' end of *lptA* upstream region
lptA Downstream Fwd	gtcagaatacAGGGCCGCAAAGCCCCCT	LC: overlap with *lptA* upstream region; UC: complementary to 5' end of *lptA* downstream region
lptA Downstream Rev	catgattacgaattcgagctACGTCAACGAGCACGCTCCG	LC: overlap with pMQ30; UC: complementary to 3' end of *lptA* downstream region
pDB58		
htpX Upstream Fwd	taaaacgacggccagtgccaAAGGTCTCGACATCCTCG	LC: overlap with pMQ30; UC: complementary to 5' end of *htpX* upstream region
htpX Upstream Rev	tgaaagcggaGGTGTAAAGCTTCTCCTCACG	LC: overlap with *htpX* downstream region; UC: complementary to 3' end of *htpX* upstream region
htpX Downstream Fwd	gctttacaccTCCGCTTTCACACTTGGG	LC: overlap with *htpX* upstream region; UC: complementary to 5' end of *htpX* downstream region
htpX Downstream Rev	catgattacgaattcgagctCGGAAGCGTTGCTCGAGC	LC: overlap with pMQ30; UC: complementary to 3' end of *htpX* downstream region
pDB56		
lon/clpXP Upstream Fwd	taaaacgacggccagtgccaAGTTTCCGATGCTGATGTC	LC: overlap with pMQ30; UC: complementary to 5' end of *lon/clpXP* upstream region
lon/clpXP Upstream Rev	tacctaccgaGTCTTGCGATCACTCCCTAAC	LC: overlap with *lon/clpXP* downstream region; UC: complementary to 3' end of *lon/clpXP* upstream region
lon/clpXP Downstream Fwd	atcgcaagacTCGGTAGGTATTCTTGACACTG	LC: overlap with *lon/clpXP* upstream region; UC: complementary to 5' end of *lon/clpXP* downstream region
lon/clpXP Downstream Rev	catgattacgaattcgagctTGCAGGATCAACTGGTCC	LC: overlap with pMQ30; UC: complementary to 3' end of *lon/clpXP* downstream region
pDB37		
ftsH Fwd	aattcgatcatgcatgagctATCCTCGAGATGGACAGCATCC	LC: overlap with pUC18T-mini-Tn7T; UC: complementary to 5' end of *ftsH* upstream region
ftsH Rev	ttcgcgaggtaccgggcccaGCAAGGCAGTCGGGTCGG	LC: overlap with pUC18T-mini-Tn7T; UC: complementary to 3' end of *ftsH* downstream region
pLREX97		
ibpA Upstream Fwd	aggccttcgcgaggtaccgggcccaagcttTCAGTAGCGAGTGAAGGTCAGGTAGTCGGC	LC: overlap with pUC18T-mini-Tn7T; UC: complementary to 5' end of *ibpA* upstream region
ibpA Rev	cgatccctggccagacggtccctgcccggatccCTGGTTGTCCAGTGCCGGGCGCTGGCC	LC: overlap with 5' end of Linker+*mVenus;* UC: complementary to 3' end of *ibpA*

Linker+*mVenus* Fwd	ggccagcgcccggcactggacaaccagGGATCCG GGCAGGGACCGTCTGGCCAGGGATCG	LC: overlap with 3' end of *ibpA*; UC: complementary to 5' end of Linker+*mVenus*
mVenus Rev	gctaattcgatcatgcatgagctcactagTTACTTGTA CAGCTCGTCCATGCCGAGAGTG	LC: overlap with pUC18T-mini-Tn7T; UC: complementary to 3' end of *mVenus*

LC: Lowercase
UC: Uppercase
Linker: GGATCCGGGCAGGGACCGTCTGGCCAGGGATCGGGGCCAGGATCAGGTCAAGGCTCCGGT

Table S4. Functional categories of genes that have an aggravating interaction with *ftsH* during growth. Insertions are >10-fold depleted (*P* <0.05) in the Δ*ftsH* library background compared to the WT library background.

Locus	Gene name	Description	Functional category	Read ratio	*P* value
PA14_03880	*spuB*	glutamine synthetase	Amino acid transport and metabolism	0.029829957	0.019768744
PA14_52630	*aruE*	succinylglutamate desuccinylase	Amino acid transport and metabolism	0.074124367	0.000667732
PA14_67920	*hisH*	imidazole glycerol phosphate synthase subunit HisH	Amino acid transport and metabolism	0.024450841	0.000734266
PA14_62830	*tpiA*	triosephosphate isomerase	Carbohydrate transport and metabolism	0.004666208	0.006462704
PA14_19170		hypothetical protein	Cell envelope biogenesis, outer membrane	0.064410998	0.00952381
PA14_66120	*ssg*	hypothetical protein	Cell envelope biogenesis, outer membrane	0.007052033	4.24E-05
PA14_66160	*wapH*	glycosyl transferase family protein	Cell envelope biogenesis, outer membrane	0.031246536	5.29E-05
PA14_09300		ABC transporter ATP-binding protein	Defense mechanisms	0.063525034	4.03E-06
PA14_09320		ABC transporter ATP-binding protein	Defense mechanisms	0.037609511	1.42E-06
PA14_70390	*crc*	catabolite repression control protein	DNA replication, recombination, and repair	0.064077863	0.040662005
PA14_66290	*aceE*	pyruvate dehydrogenase subunit E1	Energy production and conversion	0.047988119	6.28E-06
PA14_66310	*aceF*	dihydrolipoamide acetyltransferase	Energy production and conversion	0.049134279	0.038782988
PA14_04070		hypothetical protein	Function unknown	0.071908993	0.012460317
PA14_72930		hypothetical protein	Function unknown	0.041016874	0.039375977
PA14_14250		hypothetical protein	Function unknown	0.049761349	0.008730159
PA14_48400		hypothetical protein	Function unknown	0.006560515	0.028571429
PA14_58040		hypothetical protein	Function unknown	0.013467284	0.028857143
PA14_50710	*shaD*	ShaD	Inorganic ion transport and metabolism	0.005634115	2.51E-05
PA14_70800	*phoU*	phosphate uptake regulatory protein PhoU	Inorganic ion transport and metabolism	0.024783057	0.014679487
PA14_14610	*yajC*	preprotein translocase subunit YajC	Intracellular trafficking and secretion	0.037379051	0.047714286
PA14_00060	*lptA*	acyltransferase	Lipid metabolism	0.026131147	0.006074592
PA14_25690	*fabF1*	3-oxoacyl-ACP synthase	Lipid metabolism	0.010658398	0.00019428
PA14_64220	*purD*	phosphoribosylamine--glycine ligase	Nucleotide transport and metabolism	0.097334294	0.000789115
PA14_70370	*pyrE*	orotate phosphoribosyltransferase	Nucleotide transport and metabolism	0.086529909	0.024300699
PA14_27480	*htpX*	heat shock protein HtpX	Posttranslational modification, protein turnover, chaperones	0.004960439	0.018971617
PA14_57530	*sspA*	stringent starvation protein A	Posttranslational modification, protein turnover, chaperones	0.049522582	0.027922078
PA14_63060	*smpB*	SsrA-binding protein	Posttranslational modification, protein turnover, chaperones	0.086787305	0.042698413

PA14_66770	*hslV*	ATP-dependent protease peptidase subunit	Posttranslational modification, protein turnover, chaperones	0.056683992	0.007936508
PA14_66790	*hslU*	ATP-dependent protease ATP-binding subunit HslU	Posttranslational modification, protein turnover, chaperones	0.070003096	0.000201401
PA14_06200		hypothetical protein	Secondary metabolites biosynthesis, transport, and catabolism	0.05514422	0.028571429
PA14_68680	*envZ*	two-component sensor EnvZ (AmgS)	Signal transduction mechanisms	0.011819731	2.27E-05
PA14_68700	*ompR*	osmolarity response regulator (AmgR)	Signal transduction mechanisms	0.007752306	0.000582751
PA14_72390		two-component sensor	Signal transduction mechanisms	0.046897485	0.000209592
PA14_06680	*nirH*	hypothetical protein	Transcription	0.018169261	0.007936508
PA14_65950		transcriptional regulator	Transcription	0.026108949	0.03021645
PA14_56070	*mvaT*	transcriptional regulator MvaT, P16 subunit	Transcription	0.071169721	0.028571429

Dataset S1 (separate file). Read ratios for each gene and intergenic region in the Tn-seq experiment. Ratios are derived from read counts in the Δ*ftsH* library background divided by the WT library background. IG = intergenic region.

Dataset S2 (separate file). Raw read counts for each gene and intergenic region in each Tn-seq library. The genome is divided into 100 bp windows and total reads within each window are aggregated. IG = intergenic region.

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