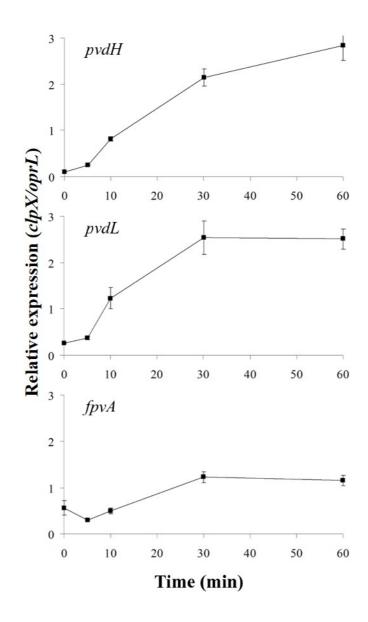
## Supplementary Figures

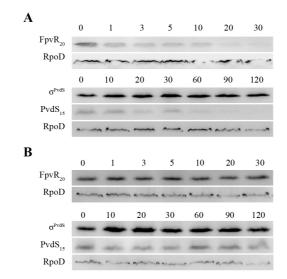
## Activation of a Cell Surface Signaling Pathway in *Pseudomonas aeruginosa* requires ClpP Protease and New Sigma Factor Synthesis

Thomas F. Bishop, Lois W. Martin and Iain L. Lamont\*

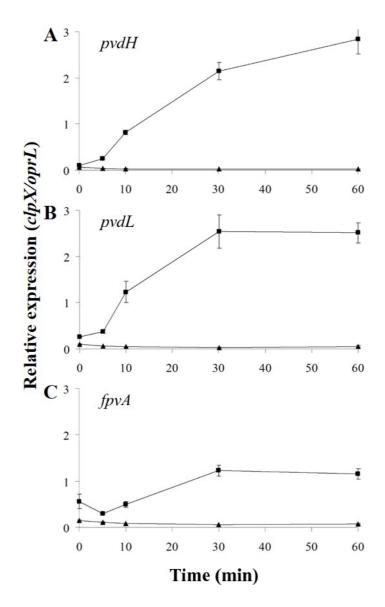
\* Correspondence: Corresponding Author: iain.lamont@otago.ac.nz



**Supplementary Figure S1. Activation of gene expression following addition of pyoverdine.** Pyoverdine was added to *P. aeruginosa* PAO1 *pvdF* bacteria (0 minutes) and samples were collected at intervals and analyzed by RT-qPCR. The amounts of *pvdH*, *pvdL* and *fpvA* transcripts are shown relative to the reference genes *clpX* and *oprL*. Data are means of six technical replicates with standard deviation shown. Similar results were obtained in a replicate experiment (Fig. 2 in the main text).

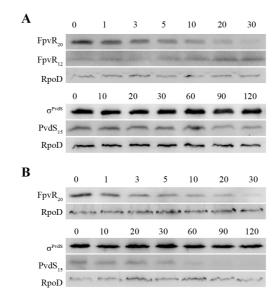


Supplementary Figure S2. Time course of degradation of FpvR and  $\sigma^{PvdS}$  following addition of pyoverdine. Pyoverdine was added to *P. aeruginosa* bacteria (0 minutes). Samples were collected at intervals and analyzed by Western blotting with antibodies against FpvR<sub>20</sub>,  $\sigma^{PvdS}$  or RpoD (loading control). FpvR<sub>20</sub>, PvdS23 (full-size), PvdS<sub>15</sub> and RpoD are indicated. Times are shown in minutes. A. PAO1 *pvdF* B. PAO1 *pvdF fpvA*. Similar results were obtained in a replicate experiment (Fig. 3 in the main text).

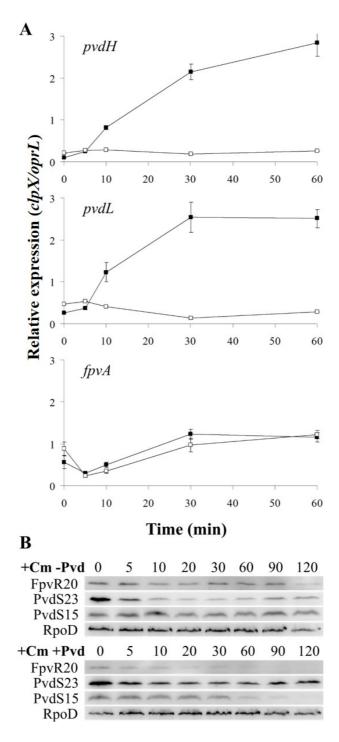


### Supplementary Figure S3. Effect of *clpP* mutation on induction of gene expression.

Pyoverdine was added (0 minutes) to *P. aeruginosa* PAO1 *pvdF clpP* (black triangles). Samples were collected at intervals and analyzed by RT-qPCR. Data are means of six technical replicates with standard deviation shown. Equivalent data from strain PAO1 *pvdF* bacteria (Fig. S1) (black squares) are included for comparison. A. *pvdH*. B. *pvdL*. C. *fpvA*. Similar results were obtained in a replicate experiment (Fig. 4 in the main text).

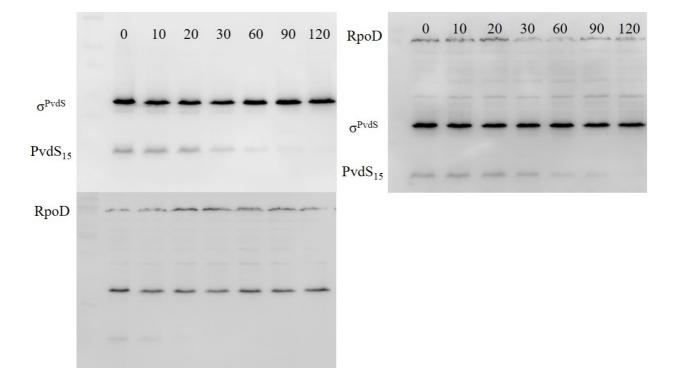


Supplementary Figure S4. Effects of *clpP* mutation on degradation of FpvR<sub>20</sub> and  $\sigma^{PvdS}$ . Pyoverdine was added (0 minutes) to *P. aeruginosa* PAO1 *pvdF* bacteria containing a mutation in the *clpP* gene. Samples were collected at intervals and analyzed by Western blotting. Times are shown in minutes. Times are shown in minutes. A. *P. aeruginosa* PAO1 *pvdF clpP*. B. *P. aeruginosa* PAO1 *pvdF clpP* (minictx::*tig-clpP*). FpvR<sub>20</sub>, FpvR<sub>12</sub>, PvdS23 (full-size), PvdS<sub>15</sub> and RpoD are indicated. FpvR<sub>12</sub> was not detected in *P. aeruginosa* PAO1 *pvdF clpP* (minictx::*tig-clpP*). Similar results were obtained in a replicate experiment (Fig. 5 in the main text).



### Supplementary Figure S5. Effect of chloramphenicol on induction of gene expression.

Chloramphenicol (Cm) and then pyoverdine were added to *P. aeruginosa* PAO1 *pvdF* bacteria (0 minutes) and samples were collected at intervals. A. Samples were analyzed by RT-qPCR for *pvdH*, *pvdL*, and *fpvA*. Open squares, Cm present; Black squares, Cm absent. Data are means of six technical replicates with standard deviation shown. B. Samples were analyzed by Western blotting for  $\sigma^{PvdS}$  or FpvR<sub>20</sub> in the presence or absence of pyoverdine, as shown. FpvR<sub>20</sub>,  $\sigma^{PvdS}$  (full-size), PvdS<sub>15</sub> and RpoD are indicated. Times are shown in minutes. Similar results were obtained in a replicate experiment (Fig. 6 in the main text).



# Supplementary Figure S6. A *lon* mutation does not affect degradation of $\sigma^{PvdS}$ .

Pyoverdine was added (0 minutes) to *P. aeruginosa* PAO1 *pvdF* bacteria containing a mutation in the *lon* gene. Samples were collected at intervals and analyzed by Western blotting. Times are shown in minutes. The positions of  $\sigma^{PvdS}$ , PvdS<sub>15</sub> and RpoD are indicated. Two replicate experiments are shown, with two exposures shown in the left panel.