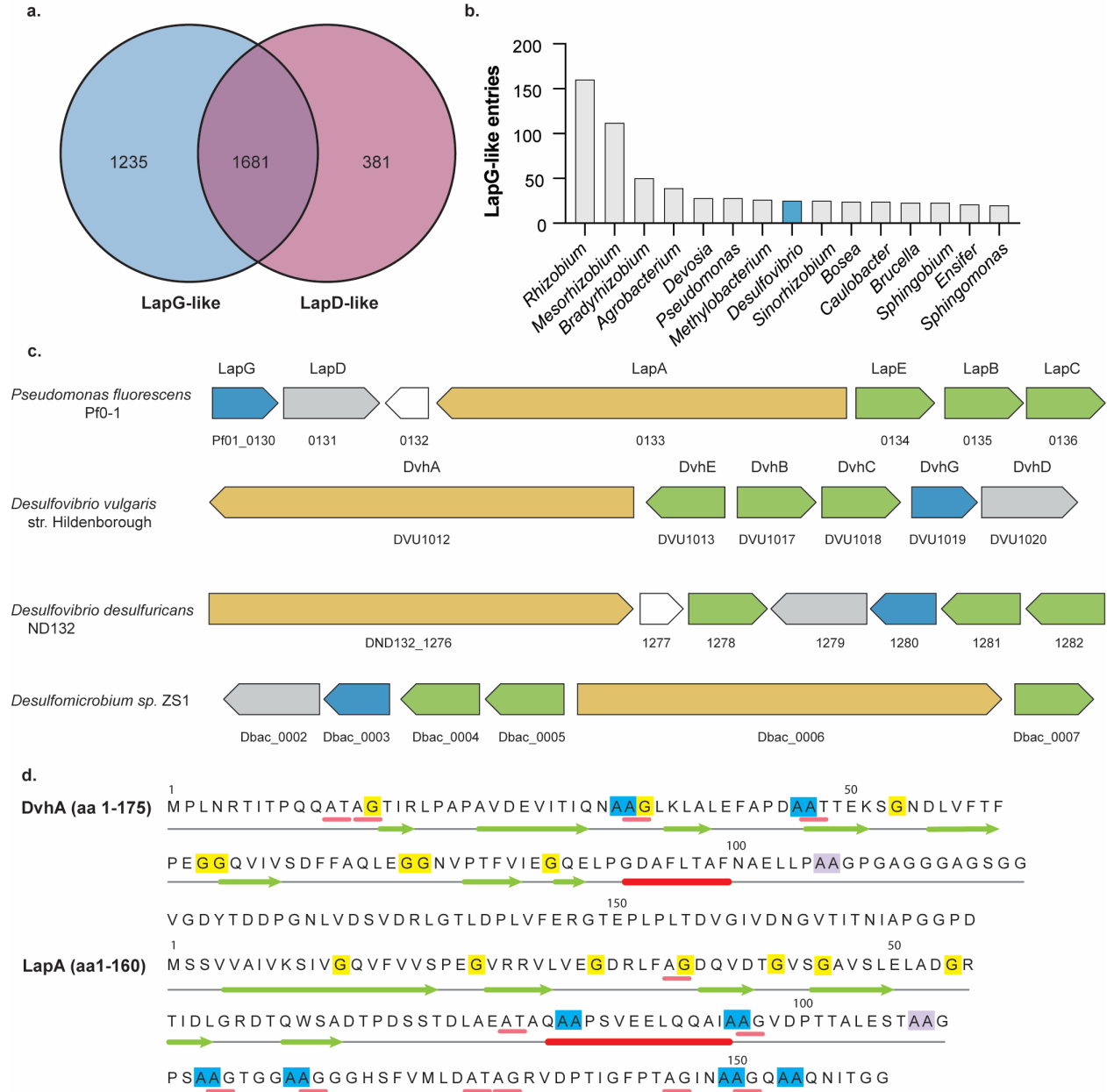
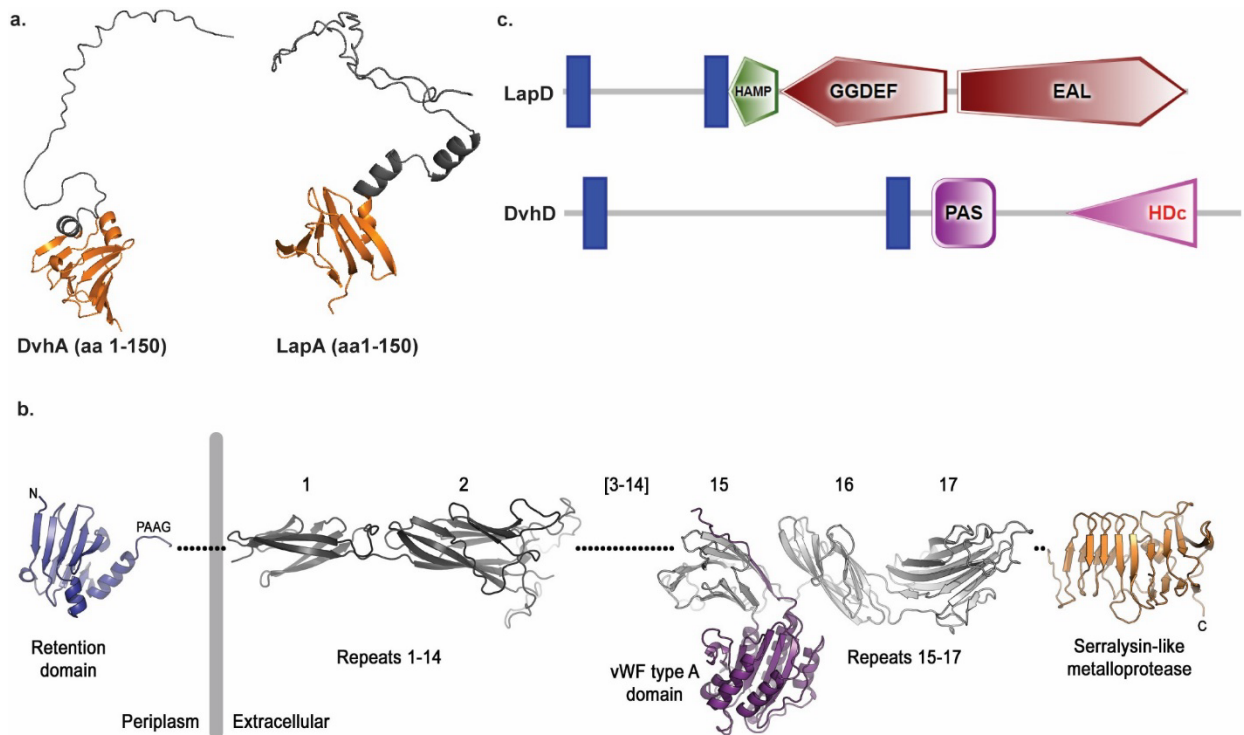


## Supporting Information

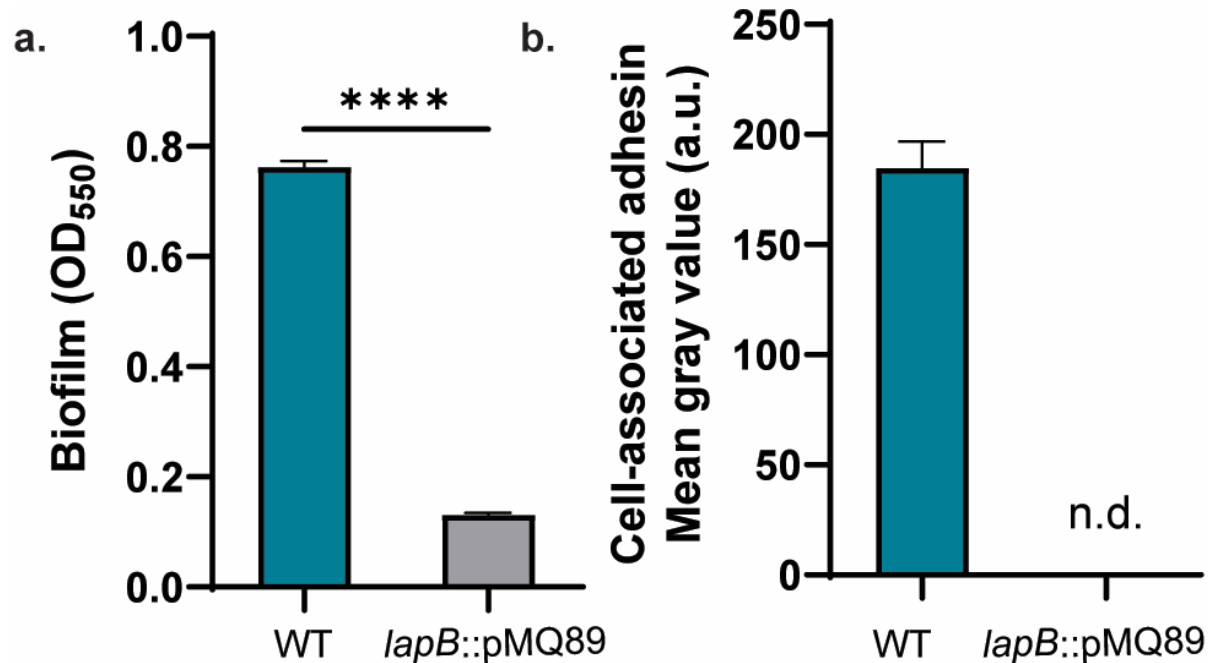
### Figures



**Figure S1: Conservation of LapA- and DvhA-like proteins.** a) The NCBI Conserved Domain Database (CDD) was used to gather organisms encoding LapG-like (pfam06035) and LapD-like (pfam16448) proteins. Each group was separated into a list and the R package VennDiagram was used to find the overlap between the groups. A previous study reported the distribution of Lap-like system across 1000+ bacterial genera (7). b) Counts of LapG-like proteins in various microbial genera. Proteins belonging to the genus *Desulfovibrio* are highlighted in blue. c) Genomic arrangement of Lap components of *P. fluorescens* Pf0-1, *D. vulgaris* Hildenborough, *D. sulfuricans* ND132 and *Desulfomicrobium* sp. ZS1. LapG-like proteins are highlighted in blue, ABC transporters in green, and adhesins in yellow. d) Secondary structure prediction using JPred4 of DvhA aa 1-175 and LapA aa1-160. Green arrows represent beta sheets, red arrows represent alpha-helices. Glycine residues in between the beta sheets are shown in yellow. Primary double alanine residue targets of the LapG/DvhG proteases are highlighted in purple, potential secondary double alanine motifs are highlighted in blue, and potential AT and AG processing sites are underlined in pink.



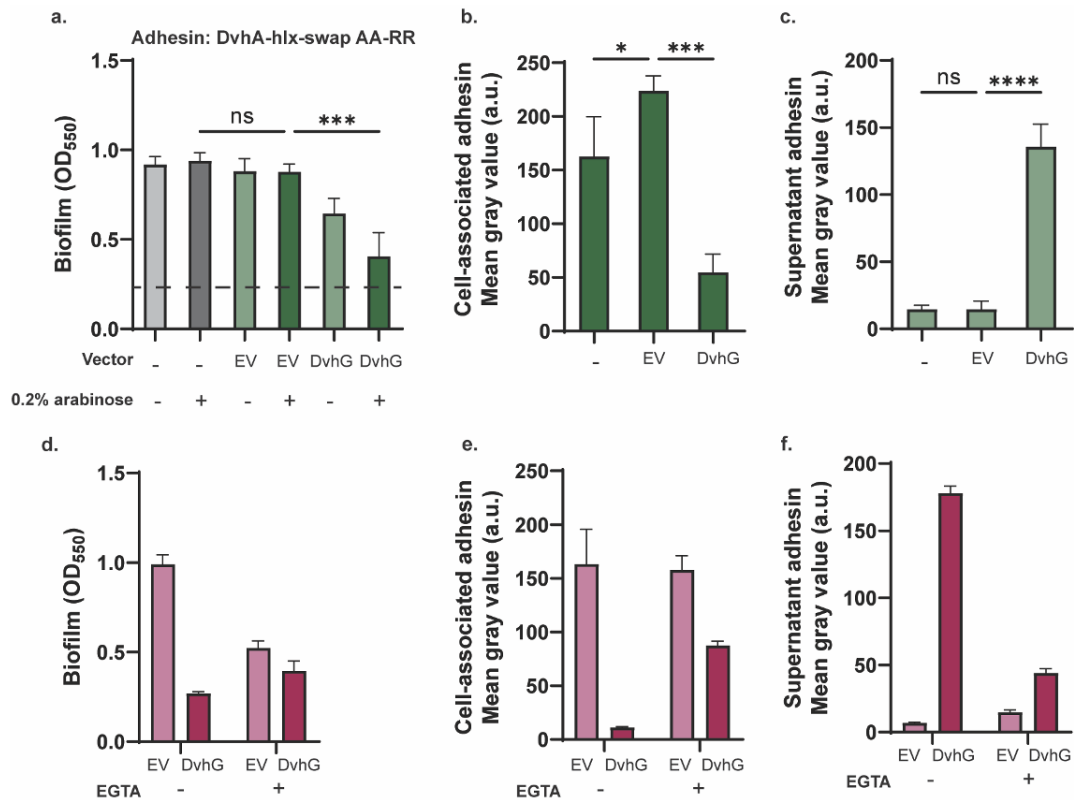
**Figure S2: Domains and predicted structure of DvhA.** a) AlphaFold structure prediction of the first 150 amino acids of LapA and DvhA displaying a folded globular domain at the N-terminus of the two proteins (shown in orange), likely serving as a retention module, followed by a predicted alpha helical region then an unstructured domain. b) AlphaFold prediction of the entire DvhA protein depicting the retention module in the periplasm and other domains such as the vWF type A domain and Serralyisin-like metalloprotease domain along with  $\beta$ -sheet-rich repeats regions in the extracellular space. The vWFA domain is located at the amino acid residues 2241-2441 between repeats 15 and 16. c) Comparison of domain architectures of LapD (top) and DvhD (bottom) and using Simple Modular Architecture Research Tool (SMART).



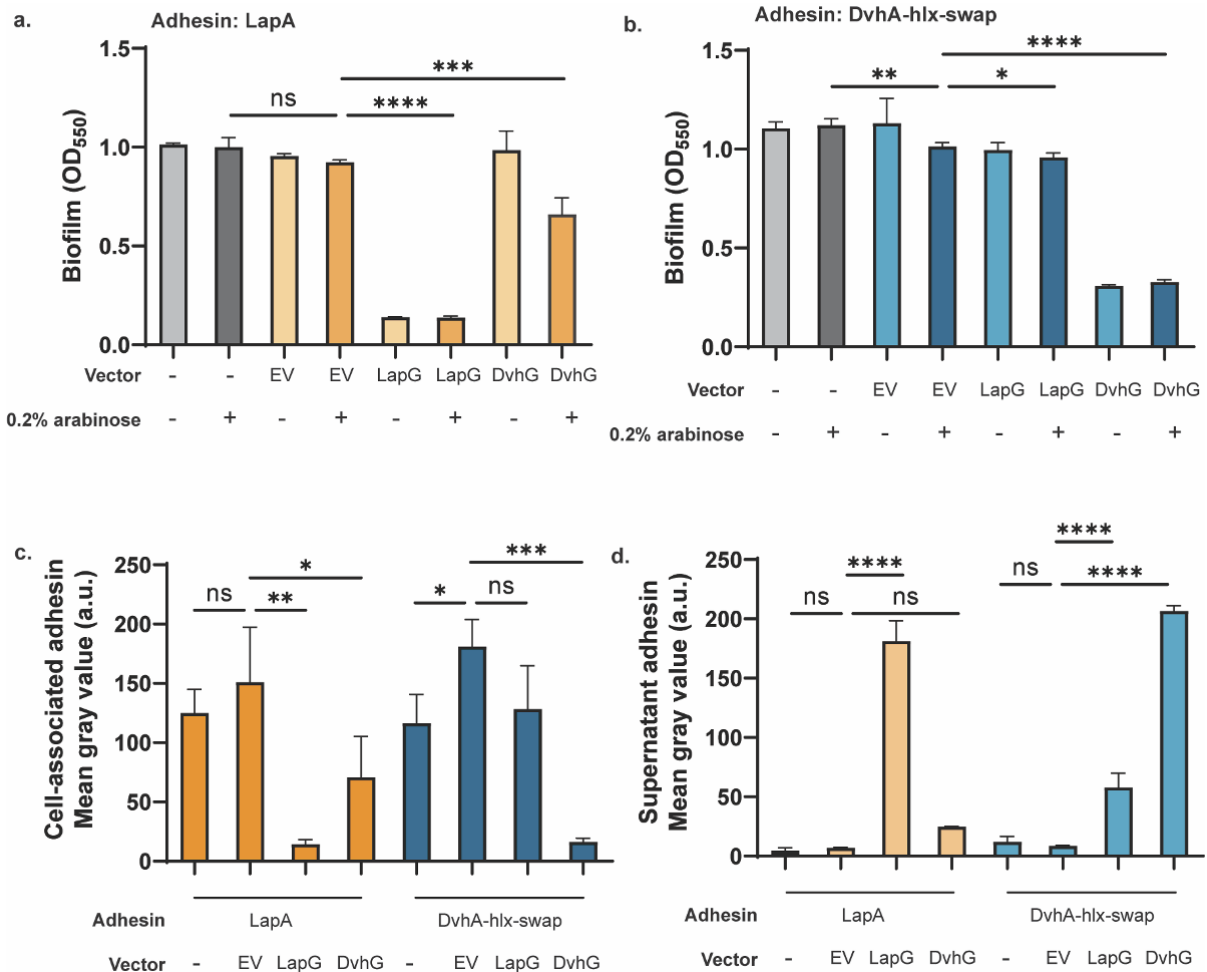
**Figure S3: DvhA-hlx-swap uses the T1SS machinery of *P. fluorescens* Pf0-1.** a) Biofilm formation in K10T-1 medium at 24 hours with DvhA-hlx-swap construct with and without the Pf0-1 T1SS ABC transporter component LapB. The *lapB* mutation was constructed using a single-cross-over mutation represented as *lapB::pMQ89*. b) Quantification of cell-surface associated adhesin in both strains using dot blots. Signal intensity for *lapB::pMQ89* strain was not detected (n.d.), indicating little/no DvhA-hlx-swap protein on the cell surface. Because there was no detectable cell surface signal for the adhesin we could not effectively calculate a P value for these data. Statistical analysis was performed using unpaired two-tailed t-test (ns,  $p > 0.05$ ; \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.01$ ; \*\*\*,  $p \leq 0.001$ ; \*\*\*\*,  $p \leq 0.0001$ ).



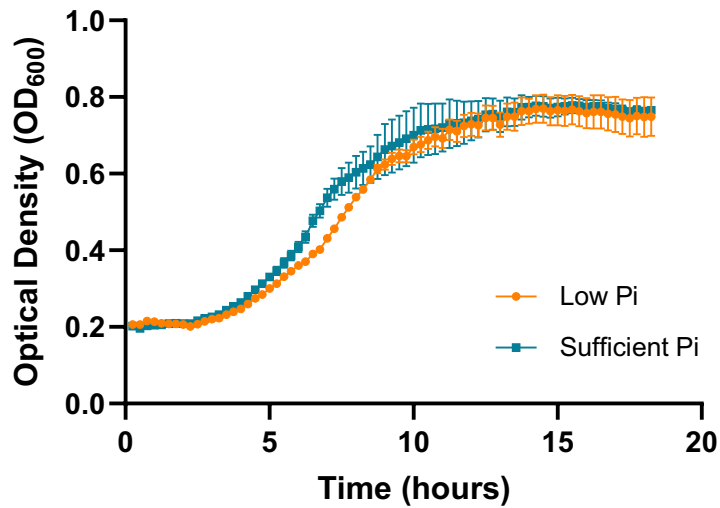
**Figure S4: Multiple sequence alignment of DvhG with LapG-like proteases.** Multiple sequence alignment of DvhG with LapG-like proteases using MUSCLE reveals conserved catalytic triad C-H-D (purple) and conserved calcium binding residues (green). The colors above the sequences represent amino acid consensus for these sequences, with red the most conserved residues and blue the least conserved residues. The sequences used in the alignment are LapG-like proteases from *Desulfovibrio vulgaris* Hildenborough, *Legionella pneumophila*, *Bordetella bronchiseptica*, *Pseudomonas fluorescens* Pf0-1, *Pseudomonas putida* KT2440, *Pseudomonas entomophila*, *Vibrio cholerae* O1 biovar El Tor str N16961, *Shewanella denitrificans* OS217 and *Vibrio vulnificus* CMCP6, respectively.



**Figure S5: DvhG is a calcium ion dependent protease that targets the di-alanine motif of DvhA-hlx-swap.** a) Biofilm formed in K10T-1 medium at 24 hours with *dvhA-hlx-swap* mutated at the dialanine site from 105PAAG108 to 105PRRG108 without a vector, with empty vector (EV) or *dvhG* expressed from an arabinose-inducible plasmid in the presence or absence of arabinose. The dashed line represents the mean biofilm formed when DvhG processes the unmutated di-alanine motif (see **Figure 2**). b) Cell surface levels of DvhA-hlx-swap AA-RR in the presence of 0.2% arabinose. c) DvhA-hlx-swap AA-RR levels in the culture supernatants in the presence of 0.2% arabinose. d) Biofilm formed in K10T medium at 24 hours by the Pf0-1  $\Delta lapG \Delta lapD dvhA-hlx-swap$  strain with arabinose inducible empty vector (EV) or vector expressing *dvhG* in the presence of 0.2% arabinose, with or without 40  $\mu$ M EGTA (a calcium chelator). Note that the chelator lowers the basal level of biofilm formation. e) Quantification of cell-surface associated DvhA-hlx-swap with EV or *dvhG* induced with arabinose, with or without EGTA. f) DvhA-hlx-swap levels associated with the culture supernatants of the corresponding strains. Statistical analysis was performed using one way ANOVA corrected for multiple comparisons (ns,  $p > 0.05$ ; \*,  $p \leq 0.01$ ; \*\*\*).

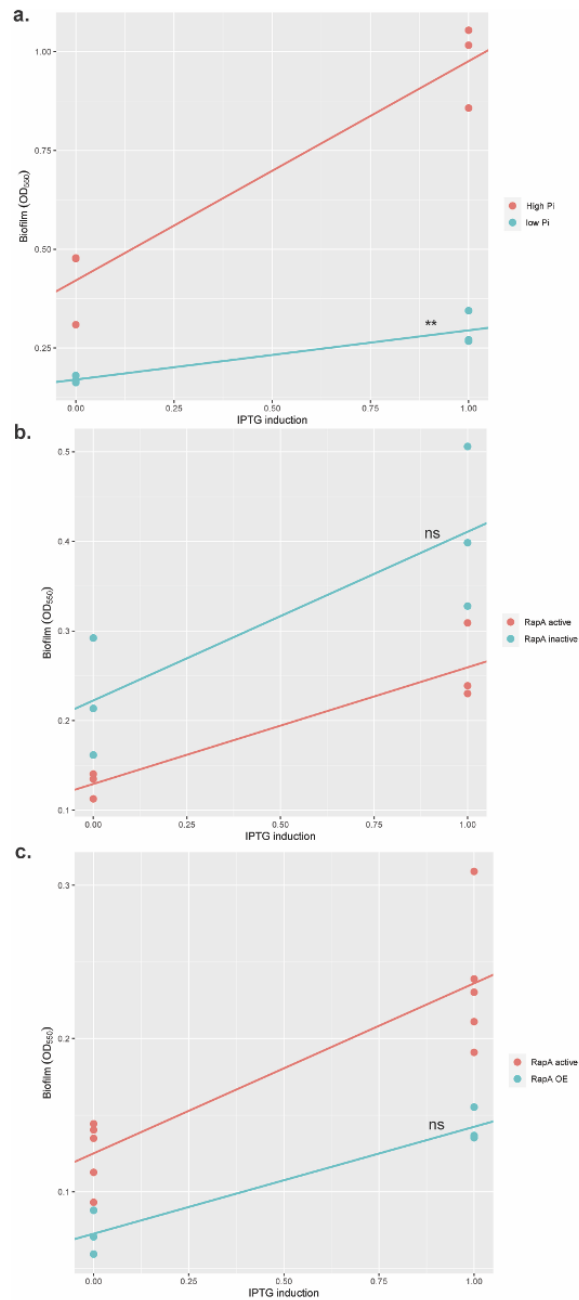


**Figure S6. LapG and DvhG display specificity towards their cognate adhesins.** a) Biofilm formation in K10T medium at 24 hours by the Pf0-1  $\Delta lapG \Delta lapD$  strain expressing the native adhesin LapA. Quantification was performed on the strains carrying no vector, arabinose inducible empty vector (EV), plasmid carrying the *lapG* or *dvhG* gene in the presence or absence of arabinose. b) Biofilm formation in K10T medium at 24 hours by the Pf0-1  $\Delta lapG \Delta lapD$  strain expressing the fusion adhesin DvhA-hlx-swap. Quantification was performed on the strains carrying no vector, arabinose inducible empty vector (EV), plasmid carrying the *lapG* or *dvhG* gene in the presence or absence of arabinose. c) Quantification of cell-surface associated adhesin of the strains in the presence of 0.2% arabinose. The bars in orange represent LapA and the bars in teal represent DvhA-hlx swap. d) Quantification of adhesin levels associated with the culture supernatants of the strains in the presence of 0.2% arabinose. The bars in coral represent LapA and the bars in light blue represent DvhA-hlx swap. Statistical analysis was performed using one way ANOVA corrected for multiple comparisons against the empty vector control (ns,  $p > 0.05$ ; \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.01$ ; \*\*\*,  $p \leq 0.001$ ; \*\*\*\*,  $p \leq 0.0001$ ).



**Figure S7. Growth analysis using optical density (600 nm).** Growth of the Pf0-1  $\Delta lapG \Delta lapD$  *dvhA-hlx-swap-HA attTn7::lacI-P<sub>tac</sub>dvhD-HA* strain over 18 hours in K10T media with high phosphate (teal, 1 mM) or low phosphate (K10T $\pi$ , orange, 0.1 mM). The symbols represent the average OD<sub>600</sub> for three biological replicates measured every 15 minutes and the error bars represent standard error of mean (SEM). The means show a modest but significant difference for the two phosphate concentrations between hours 3 to 8, but the differences are not significant at any other time points. Statistical analysis was performed using multiple unpaired t-tests.





**Figure S8: Statistical analysis to determine if biofilm rescue is dependent on c-di-GMP.**

Plots showing the effect of IPTG induction (0 – no IPTG, 1- 0.01% IPTG) on biofilm formation under a) high phosphate (red) vs low phosphate (blue) conditions. b) RapA-active (WT *rapA*; red) vs RapA-inactive ( $\Delta rapA$ , blue) strain under low phosphate conditions c) RapA-active (WT *rapA*; red) vs RapA overexpression (RapA-OE, blue) strain under low phosphate conditions. Significance in all cases was calculated by linear regression with interaction term in R (<https://github.com/GeiselBiofilm/>). \*\*,  $p \leq 0.01$ .

## **SI Text**

### **Proteolytic activity of DvhG depends upon calcium ions.**

Calcium has been shown to influence biofilm formation by bacteria (1, 2). For *P. fluorescens* Pf0-1, low calcium concentrations result in enhanced biofilm formation, as this lack of calcium negatively affects LapG's Ca-dependent activity and thus proteolysis of the N-terminus of LapA (3). The Multiple Sequence Alignment analysis also revealed conserved calcium-binding sites that could be critical for proteolysis (3, 4) (**Figure S4**).

To test if the activity of DvhG is dependent upon calcium ions, we added a calcium chelator (EGTA) to the K10T-1 medium to sequester extracellular calcium (5). Static biofilm assays were performed at 24 hours. We compared the biofilm formed by strain Pf0-1  $\Delta lapG \Delta lapD$  *dvhA-hlx-swap* expressing DvhG to the strain harboring the empty vector under conditions with and without 40  $\mu$ M EGTA, all in the presence of arabinose (**Figure S5d**). We found that EGTA compromised the ability of even our EV control to form biofilms. Upon calculating the percentage decrease in biofilm formation in the presence or absence of EGTA between the empty vector (EV) and DvhG, we find that this change is much smaller in the presence of EGTA (29%) than the absence (68%).

The dot blot analysis showed us a more convincing picture. Despite the observed reduction in biofilm formation +EGTA, EV controls under EGTA- and EGTA+ conditions were not significantly different ( $p = 0.88$ ; **Figure S5e**), suggesting that the defect in biofilm formation in EV controls was due to a factor besides DvhG activity. For example, RTX proteins like LapA require Ca-binding to facilitate folding (6). Finally, we noted that 92% reduction in adhesin level on the cell surface when expressing DvhG without EGTA, but only 44% reduction with EGTA, implying the added EGTA reduced DvhG activity. The supernatant dot blots also complement these data (**Figure S5f**). Together, these data are consistent with the conclusion that calcium is required for the DvhG proteolytic activity.

### **LapG and DvhG display specificity towards their native adhesins.**

To test whether LapG and DvhG can proteolyze their non-native adhesins, static biofilm assays were conducted at 24 hours comparing a plasmid carrying full length *lapG* (pMQ72-*lapG*), a plasmid carrying full length *dvhG* (pMQ72-*dvhG*) and an empty vector in two background strains: Pf0-1  $\Delta lapG \Delta lapD$  which expresses WT LapA adhesin and  $\Delta lapG \Delta lapD$  *dvhA-hlx-swap* which expresses the fusion protein DvhA-hlx-swap. The plasmids were induced with 0.2% arabinose and compared to un-induced conditions.

Our results show that, when LapG is present, the strain expressing WT LapA has a significantly reduced ability to form a biofilm. With DvhG, on the other hand, the biofilm phenotype is significantly reduced as compared to the EV in the presence of arabinose ( $OD_{550} = 0.66 \pm 0.08$  vs  $0.98 \pm 0.09$ ,  $p = 0.01$ ), however it is also significantly higher than biofilms formed with a strain expressing LapG ( $OD_{550} = 0.13 \pm 0.008$ ,  $p = 0.0004$ ; **Figure S6a**).

When LapG is induced with arabinose in the strain expressing DvhA-hlx-swap, we observe a significant but modest loss in biofilm formation compared to the EV control ( $OD_{550} = 0.95 \pm 0.02$  vs  $1.01 \pm 0.02$ ). With DvhG expressed in this background, as expected, the loss of biofilm phenotype was more striking in comparison to the EV control (**Figure S6b**).

We calculated the percentage decrease in biofilm formation from the EV control with both proteases in arabinose-induced conditions. We noted that LapG caused an 85% decrease with LapA but only 6% decrease with DvhA-hlx-swap. DvhG caused a 68% decrease with DvhA-hlx-swap but only 30% decrease with LapA. Dot blot analysis suggests that changes in biofilm phenotype are due to changes adhesin levels on the cell surface, with a concomitant increase in the levels of adhesin in the supernatant (**Figure S6c-d**). These results suggest that these proteases are proficient in processing their own adhesins but less efficient towards the other adhesin.

### **Additional Materials and Methods**

#### **Static biofilm assays.**

Pf0-1 strains were struck on LB agar plates overnight and a single colony was used to inoculate 5 ml LB for 16-18 hours of growth. 1.5  $\mu$ l of the LB overnight culture was used to inoculate 100  $\mu$ l of K10T-1 (or K10T $\pi$ ) medium in a 96-well round bottom polypropylene plates (Corning Life Sciences, Glendale, AZ). The plate was incubated in a humidified box at 30°C for 24 hours. The planktonic cells were discarded, and the plate was washed with water to remove any loosely bound cells. To stain the biofilms, 125  $\mu$ l crystal violet (0.1% (w/v)) was added to the wells and incubated at room temperature for 20 minutes. Excess stain was washed-off twice with water and the wells were allowed to dry before de-staining with 150  $\mu$ l solution of water, methanol, and glacial acetic acid (45:45:10) for 5 minutes. 100  $\mu$ l of the solubilized crystal violet from the wells was transferred to a 96-well flat bottom plate and the optical density was measured at 550 nm wavelength. Solubilized crystal violet from an uninoculated well was used as a blank for the measurements.

### **Quantitative surface adhesin localization assays using dot blots.**

Pf0-1 strains expressing a WT 3x HA-tagged LapA or LapA variant were struck on LB agar plates overnight and a single colony was used to inoculate 5 ml LB for 16-18 hours. The LB overnight culture was used to inoculate 5 ml of K10T-1 medium (1:100) for growth at 30°C for 24 hours. The cultures were normalized to an OD<sub>600</sub> value of 0.5 and pelleted by centrifugation (13,200 rpm, 1 minute). The supernatants were collected, and filter sterilized using 0.22 µm syringe filters to probe for supernatant associated LapA. The pellets were washed with K10T-1 medium twice by resuspension and centrifugation. The pellets were finally resuspended in K10T-1 medium to an OD<sub>600</sub> of 0.5 to probe for cell-surface associated LapA. 5 µl of cell-surface associated and supernatant fractions were spotted on 0.2 µm nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA) and allowed to dry completely. The membranes were then incubated in a blocking solution containing 3% bovine serum albumin (BSA) in Tris-buffered saline with 1% Tween 20 (TBST) for 1 hour at room temperature. The blots were probed for LapA-HA using anti-HA antibody (BioLegend, San Diego, CA) at 1:2000 dilution in TBST containing 3% BSA overnight at 4°C. The membranes were washed thrice in TBST to remove excess antibody and incubated in horseradish peroxidase conjugated anti-mouse secondary antibody (1:15,000 dilution) in TBST for 1 hour at room temperature. Next, the membranes were washed twice in TBST and once in Tris-buffered saline (TBS) and incubated with Western Lightning Plus-ECL enhanced chemiluminescence substrate (PerkinElmer, Waltham, MA) for 1 minute. The membranes were imaged on a BioRad ChemiDoc MP Imaging System. Images were quantified using ImageJ software (NIH) as previously described in (7).

### **Detection and quantification of the proteins using Western blots.**

Pf0-1 strains of interest were struck on LB agar plates (with antibiotics, if needed) overnight and a single colony was used to inoculate 5 ml LB for 16-18 hours of growth. The LB overnight culture was used to inoculate 5 ml of K10T-1 medium (1:100) at 30°C for 24 hours. DvhG overnights were subcultured (1:100) in 50 ml K10T-1 media. The cultures were OD<sub>600</sub> normalized and pelleted by centrifugation (13,200 rpm, 1 minute). The pellets were resuspended, lysed using French press and quantified for protein using Pierce™ BCA assay (ThermoFischer Scientific, Waltham, MA). The samples were boiled for 5 minutes in Laemmli sample buffer with 2-mercaptoethanol and were resolved on 7.5% (for adhesins) or 10% (for DvhD and DvhG) polyacrylamide gels. After transferring the proteins to a 0.2 µm nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA) and blocking with 3% BSA in TBST for 1 hour, the proteins were incubated in mouse anti-HA antibody in TBST (1:2000 dilution) overnight at 4°C for DvhD and adhesins. For DvhG, the blots

were incubated in Invitrogen mouse anti-His antibody at the same dilution (ThermoFischer Scientific, Waltham, MA). Fluorescently labelled anti-mouse secondary antibody was used to detect the protein bands on Odyssey Clx imager (LICOR Biosciences Inc., Lincoln, NE). The protein levels were quantified using Image Studio Lite software (LICOR Biosciences Inc., Lincoln, NE).

**Cyclic-di-GMP quantification using flow cytometry.** Strains harboring the c-di-GMP-dependent  $P_{cdrA}$ -*gfp* transcriptional reporter plasmid subcultured into K10T-1 medium and incubated at 30°C for 24 hours. Strains without the plasmid were used as a non-fluorescent control to account for any background fluorescence. Cells were then washed, diluted, and analyzed on a Beckman 534 Coulter Cytoflex S, as described previously (8). FlowJo software version 10.8.1 was used to gate on populations of single cells. The GFP fluorescence from the  $P_{cdrA}$  promoter was then measured on the gated population. A workflow of the gating strategy is described previously (8).

**Supplementary Table 1: Strains and plasmids used in this study.**

Strains	SMC Strain #	Description	Reference
<i>Escherichia coli</i>			
S17-1( $\lambda$ -pir)	SMC117	<i>thi pro hsdR- hsdM+ <math>\Delta</math>recA</i> RP4-2::TcMu-Km::Tn7	(9)
<i>Pseudomonas fluorescens</i> Pf0-1			
$\Delta$ lapG $\Delta$ lapD	SMC 6422	Pf0-1 with unmarked deletion of <i>lapG</i> and <i>lapD</i>	(10)
$\Delta$ lapG $\Delta$ lapD <i>lapA</i> -HA	SMC 5116	$\Delta$ lapG $\Delta$ lapD with internal 3x HA epitope tag on <i>lapA</i>	(10)
$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> -RM-swap-HA	SMC 8159	$\Delta$ lapG $\Delta$ lapD <i>lapA</i> -HA with N-terminal of <i>lapA</i> (aa 1-125) swapped with N-terminal of <i>dvhA</i> (aa 1-119) at the native locus.	This study
$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> - <i>hlx</i> -swap-HA	SMC 8176	$\Delta$ lapG $\Delta$ lapD <i>lapA</i> -HA with the known <i>lapG</i> proteolysis residues of <i>lapA</i> (aa 81-123) swapped with predicted <i>dvhG</i> proteolysis residues of <i>dvhA</i> (aa 87-119) at the native locus.	This study
$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> - <i>hlx</i> -swap-HA AA-RR	SMC 9825	$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> - <i>hlx</i> -swap-HA with 105PAAG108 mutated to 105PRRG.	This study
$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> - <i>hlx</i> -swap-HA <i>lapB</i> ::pMQ89	SMC 9826	$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> - <i>hlx</i> -swap-HA with <i>lapB</i> gene disrupted on the <i>P. fluorescens</i> chromosome.	This study
$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> - <i>hlx</i> -swap-HA attTn7:: <i>lacI</i> -P <sub>tac</sub> <i>dvhD</i> -HA	SMC 9827	$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> - <i>hlx</i> -swap-HA with <i>lacI</i> and <i>dvhD</i> -HA under P <sub>tac</sub> promoter inserted at the <i>P. fluorescens</i> attTn7 site.	This study
$\Delta$ lapG $\Delta$ lapD $\Delta$ rapA <i>dvhA</i> - <i>hlx</i> -swap-HA attTn7:: <i>lacI</i> -P <sub>tac</sub> <i>dvhD</i> -HA	SMC 9828	$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> - <i>hlx</i> -swap-HA attTn7:: <i>lacI</i> -P <sub>tac</sub> <i>dvhD</i> -HA with unmarked <i>rapA</i> deletion.	This study
$\Delta$ lapG $\Delta$ lapD P <sub>xutR</sub> - <i>rapA</i> <i>dvhA</i> - <i>hlx</i> -	SMC 9829	$\Delta$ lapG $\Delta$ lapD <i>dvhA</i> - <i>hlx</i> -swap-HA attTn7:: <i>lacI</i> -P <sub>tac</sub> <i>dvhD</i> -HA with <i>rapA</i> under the control of xylose-inducible promoter P <sub>xutR</sub> at its native locus	This study

<i>swap-HA attTn7:: lacI-P<sub>tac</sub>dvhD-HA</i>			
Plasmids		Description	Reference
pMQ30	SMC 2765	Vector for cloning in yeast and allelic exchange in Gram-negatives; Gentamycin-resistance (Gm <sup>r</sup> ) cassette	(11)
pMQ30- <i>dvhA-RM-swap</i>	SMC 8158	Vector to replace aa 1- 125 of <i>lapA</i> with aa 1-119 of <i>dvhA</i> using allelic exchange; Gm <sup>r</sup>	This study
pMQ30- <i>dvhA-hlx-swap</i>	SMC 8175	Vector to replace aa 81-123 of <i>lapA</i> with aa 87-119 of <i>dvhA</i> using allelic exchange; Gm <sup>r</sup>	This study
pMQ30- <i>dvhA-hlx-swap</i> AA-RR	SMC 9831	Vector to replace 105PAAG108 with 105PRRG108 in <i>dvhA-hlx-swap</i> using allelic exchange; Gm <sup>r</sup>	This study
pMQ56-mTn7	SMC 3242	Shuttle vector for insertion of the mini-Tn7 element from pUT18-mTn7-Gm into the attTn7 site on the <i>P. fluorescens</i> chromosome; Carbenicillin-resistance (Cb <sup>r</sup> ) cassette	(11)
pBUX-BF13	SMC 1822	Helper plasmid encoding Tn7 transposition functions; Cb <sup>r</sup>	(12)
pFLP3	SMC 4060	Vector for removal of antibiotic resistance cassettes flanked by FRT sites with flipase; <i>sacB</i> for counterselection; Cb <sup>r</sup>	
pMQ56- <i>lacI</i> P <sub>tac</sub> - <i>dvhD-HA</i>	SMC 9832	Vector for integration of <i>lacI</i> repressor and HA-tagged <i>dvhD</i> under IPTG-inducible Ptac promoter at the attTn7 site on the <i>P. fluorescens</i> chromosome; Cb <sup>r</sup>	This study
pMQ30- <i>rapA</i>	SMC 9833	Vector to produce an unmarked deletion of <i>rapA</i> from <i>P. fluorescens</i> chromosome by allelic exchange; Gm <sup>r</sup>	This study
pMQ72	SMC 2795	Expression vector for cloning in yeast, <i>E. coli</i> and <i>P. fluorescens</i> with arabinose-inducible gene expression system; Gm <sup>r</sup>	(11)
pMQ72- <i>dvhG-His</i>	SMC 9834	Vector for arabinose-inducible expression of full length <i>dvhG</i> with a 6x-His tag at the N-terminal; Gm <sup>r</sup>	This study
pMQ72- <i>dvhG</i> Δ <i>TM-His</i>	SMC 9835	Vector for arabinose-inducible expression of <i>dvhG</i> without aa 1-40 and with a 6x-His tag at the N-terminal; Gm <sup>r</sup>	This study

pMQ72- <i>dvhG</i> -His C317A	SMC 9836	Vector for arabinose-inducible expression of full length <i>dvhG</i> -C317A with a 6x-His tag at the N-terminal; Gm <sup>r</sup>	This study
pMQ72- <i>lapG</i>	SMC 5143	Vector for arabinose-inducible expression of full length <i>lapG</i> ; Gm <sup>r</sup>	(10)
pMQ89	SMC 2952	Suicide vector to generate single-crossover constructs in <i>P. fluorescens</i> chromosome; Gm <sup>r</sup>	(11)
pMQ89- <i>lapB</i>	SMC 5148	Vector to introduce disruption in <i>lapB</i> gene on <i>P. fluorescens</i> chromosome; Gm <sup>r</sup>	(13)
pMQ650	SMC 9396	Expression vector for cloning with xylose-inducible gene expression system; Gm <sup>r</sup>	(14)
pP <sub><i>cdrA</i></sub> - <i>gfpC</i>	SMC 5709	Vector for fluorescence-based quantification of cyclic di-GMP in <i>Pseudomonas aeruginosa</i> and related species	(15)



**Supplementary Table 2: Oligonucleotides used in this study.**

Oligonucleotide Name	Sequence (5' → 3')
DvhA_RMswap_up_F	GTCGACTCTAGAGGATCCCCCGCTGTCGGTGCCTTCAC
DvhA_RMswap_up_R	GATGGTGCGATTGAGAGGCATTGAAGACTCTCCGGGTGTCAC
DvhA_RMswap_down_F	GCAGGTAGCGGCGGTGCCGCTGGTGGCGGC
DvhA_RMswap_down_R	CGAATTCGAGCTCGGTACCCCCTTCGGTCACGCTTGG
DvhA_RMinsert_F	GTGACACCCGGAGAGTCTTCAATGCCTCTCAATCGCACCATC
DvhA_RMinsert_R	GTCGCATCGAGCATCACGAAACCGCCGCTACCTGC
DvhA_hlxswap_up_F	GTCGACTCTAGAGGATCCCCCGCTGTCGGTGCCTTCAC
DvhA_hlxswap_up_R	CCGGGGAGTTCCTGACCGGTGGCTTCGGCCAGGTC
DvhA_hlxswap_down_F	GCAGGTAGCGGCGGT ACTGGCGGTGCCGCT
DvhA_hlxswap_down_R	CGAATTCGAGCTCGGTACCCCCTTCGGTCACGCTTGG
DvhA_hlxInsert_F	GACCTGGCCGAAGCCACCGGTCAGGAACTCCCCGG
DvhA_hlxInsert_R	CCAGCGGCACCGCCACCGCCGCTACCTGC
DvhG_nHis_F	TCGAGCTCGGTACCCGAAGGAGATATACATATGCATCATCACCATCAC CACGCAAAGGCGCGGGAGA
DvhG_R	GACTCTAGAGGATCCCCTCATTTCCTCCCGGCTTTCTTC
DvhG-noTM_nHis_F	TCGAGCTCGGTACCCGAAGGAGATATACATATGCATCATCACCATCAC CACGGAGATACCGAAGCTGGGG
delrapA_up_F	GTCGACTCTAGAGGATCCCCCGGCGAGCGATCCGTTC
delrapA_up_R	GTTTCGTGCATTTGAAGAGGGGCAATCTCTGGCGATAAAAAAAGG
delrapA_down_F	ATCGCCAGAGATTGCCCTCTTCAAATGCACGAAACGGC
delrapA_down_R	CGAATTCGAGCTCGGTACCCGGGCGGAAGTGAAATTTGATAAACG
rapAup_F	GTCGACTCTAGAGGATCCCCACGTAGTCCGGCCGC
rapAup_R	CCAAGAACAACAAGGAGGATTTTATGACCACGACCGAACAG
PxutR_insert_F	GCTGTTTCGGTCGTGGTCATAAAATCCTCCTTGTTGTTCTTGG
PxutR_insert_R	ATCGCCAGAGATTGCCCGAGCCCTATCGGCTGG
rapA_down_F	CCAGCCGATAGGGCTCGGGCAATCTCTGGCGATAAAAAAAGG
rapA_down_R	CGAATTCGAGCTCGGTACCCCGGCGAGCGATCCGTTC
LapB_seqF	GGCCGAGATCAAAAACATTCAGTTAT
LapB_seqR	TGGCCCACAGCATCAGG
Fra1_Ptaclacl-dvhD_mTn7_F	CTCACTAGTGGATCCCCCTCACTGCCCGCTTTCCAGTC
Fra1_Ptaclacl-dvhD_mTn7_R	ACAGGAAACAGACTAGTGCTCTGCAGAAGGAGATATACATATGTACCCATAC

Fra2_Ptaclacl-dvhD _mTn7_F	ACAGGAAACAGACTAGTGCTCTGCAGAAGGAGATATACATATGTACCCATAC
Fra2_Ptaclacl-dvhD _mTn7_R	GGAATTCCTGCAGCCCTCAGCCTTGACGCACATCAAGC
hlxswap_RR_up_F	GTCGACTCTAGAGGATCCCCGGCAACCGGAATGACCGTATG
hlxswap_RR_up_R	CGGCGGTTCGCATCGAGCAT
hlxswap_RR_down_F	ATGCTCGATGCGACCGCCG
hlxswap_RR_down_R	CGAATTCGAGCTCGGTACCCGATGGTGTCTGGTGACCTGAGTC

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