**Supplementary Information**

**Supplementary Table 1. Primers used in this study**

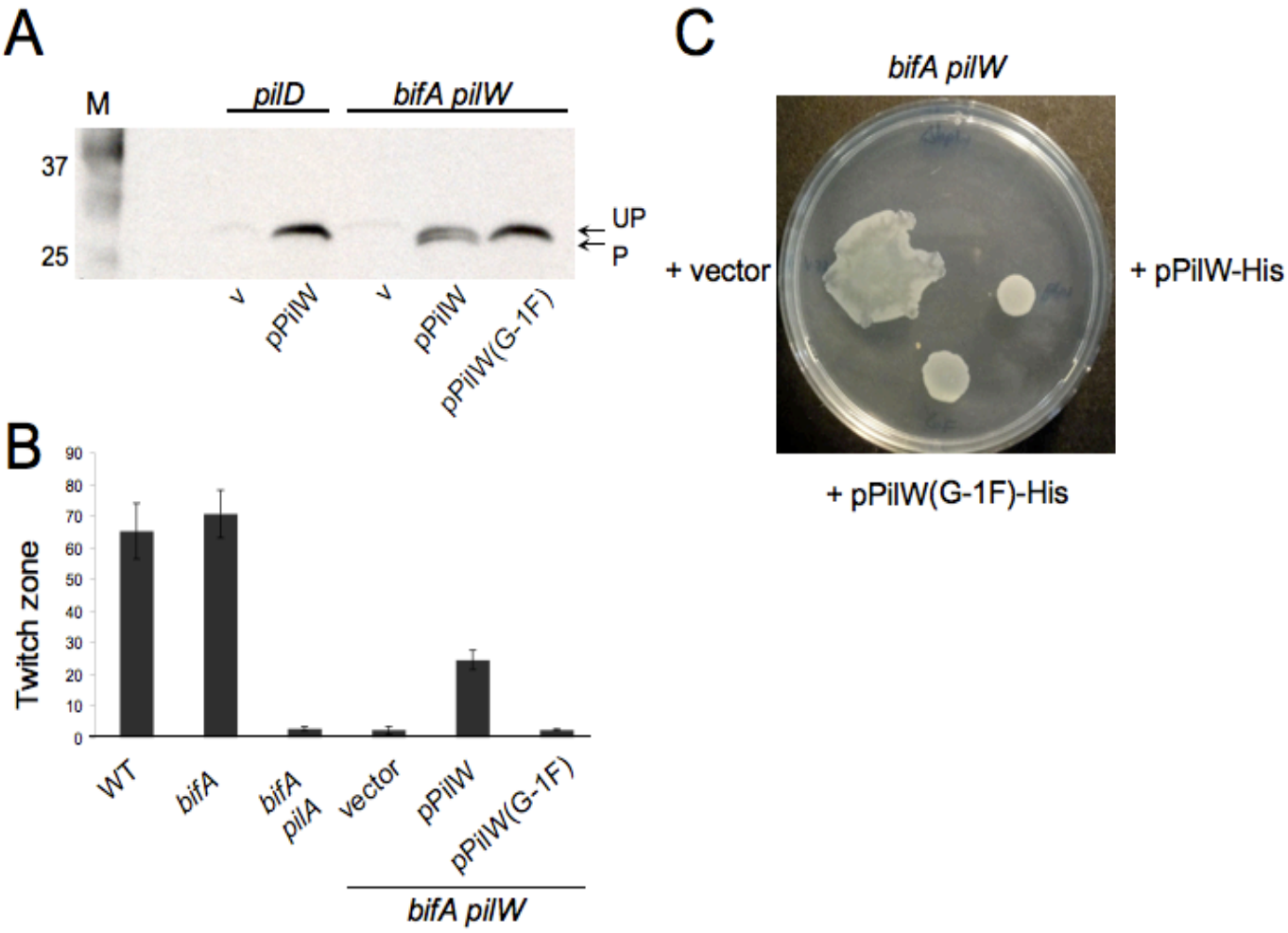
| Primer name | Primer sequence (5'-3')[a] |
|---|---|
| *fimU* KO-1 | tgtaaaacgacggccagtgccaagcttgcatgcctgGTCCAGGTCCAGCCAGTACAG |
| *fimU* KO-2 | GTTGCTTTGAAGATGGGTACAGGCTGCGACAAATAGAGCGCATG |
| *fimU* KO-3 | CATGCGCTCTATTTGTCGCAGCCTGTACCCATCTTCAAAGCAA C |
| *fimU* KO-4 | ccatgattacgaattcgagctcggtacccggggatccTAGGTAGACCAG GCGAACGAC |
| *pilV* KO-1 | tgtaaaacgacggccagtgccaagcttgcatgcctgAGCCGTGCTACTCGTGACAGC |
| *pilV* KO-2 | CAAGGTGGAGTCAGAGGCGTTAACCAGTACTTCGATCATGCTG |
| *pilV* KO-3 | CAGCATGATCGAAGTACTGGTTAACGCCTCTGACTCCACCTTG |
| *pilV* KO-4 | ccatgattacgaattcgagctcggtacccggggatccCGCTTCTGTTCGATGAGATTG |
| *pilW* KO-1 | tgtaaaacgacggccagtgccaagcttgcatgcctgAGTTTCAACGCCTTGATCGAG |
| *pilW* KO-2 | GCTCTCGGGATAAAGGACGATGTAGATCTGGCTGATCCCCAG |
| *pilW* KO-3 | CTGGGGATCAGCCAGATCTACATCGTCCTTTATCCCGAGAGC |
| *pilW* KO-4 | ccatgattacgaattcgagctcggtacccggggatccTCGCCTGACAGTGTCAATTTC |
| *pilX* KO-1 | tgtaaaacgacggccagtgccaagcttgcatgcctgTGCGACAACACCAAAGGCTCG |
| *pilX* KO-2 | *GCTGTTGGTTTCGTAGTAGAA*GATACGGCTTTCCAGTGACAC |
| *pilX* KO-3 | GTGTCACTGGAAAGCCGTATC*TTCTACTACGAAACCAACAGC* |
| *pilX* KO-4 | ccatgattacgaattcgagctcggtacccggggatccTTCCGTCTTCGGTGGTATAAG |
| *pilY2* KO-1 | tgtaaaacgacggccagtgccaagcttgcatgcctgTGGCTGATA ACAACAGCGATG |
| *pilY2* KO-2 | CTCCGATCCGAAGGGAACGAGAGCTGTGGCGAGAAGACGTAAG |
| *pilY2* KO-3 | CTTACGTCTTCTCGCCACAGCTCTCGTTCCCTTCGGATCGGAG |
| *pilY2* KO-4 | ccatgattacgaattcgagctcggtacccggggatccCGAAGCCCTGCACTACGTGAC |
| *pilE* KO-1 | tgtaaaacgacggccagtgccaagcttgcatgcctgTACCTTGGTACGCCATCCTAG |
| *pilE* KO-2 | GCTCTTCTTGCTTCCAGTCCGCATTGTTGCTGCTCCGATCCG |

| | |
|---|---|
| *pilE* KO-3 | CGGATCGGAGCAGCAACAATGCGGACTGGAAGCAAGAAGAGC |
| *pilE* KO-4 | ccatgattacgaattcgagctcggtacccggggatccACCGCCATGCAAATCAAACTC |
| *pilD* KO-1 | tgtaaaacgacggccagtgccaagcttgcatgcctgCTCGGCAGATGTCTACCATGATG |
| *pilD* KO-2 | GAATTGCAGATAGGTCCGGGTAAGGAGGATGGCGCACAAGAC |
| *pilD* KO-3 | GTCTTGTGCGCCATCCTCCTTACCCGGACCTATCTGCAATTC |
| *pilD* KO-4 | ccatgattacgaattcgagctcggtacccggggatccGAGTTGCTGTCGAAGAGCGAC |
| *fimU-pilE* KO-1 | tgtaaaacgacggccagtgccaagcttgcatgcctgACCGATATAGGGGATGCCTTC |
| *fimU-pilE* KO-2 | GCTCTTCTTGCTTCCAGTCCGATGAATACCTCTGCGGGTATG |
| *fimU-pilE* KO-3 | CATACCCGCAGAGGTATTCATCGGACTGGAAGCAAGAAGAGC |
| *fimU-pilE* KO-4 | See *pilE* KO-4 |
| *pilW* comp 5’ | caactctctactgtttctccatacccgttttttt*ggggaaggagatatacat*GTGAGAACAAGCATGCT CTTC |
| *pilW* comp 3’ | taatctgtatcaggctgaaaatcttctctcatccgccTCAgtggtgatggtggtggtgTGGCATGAGATT CCTGATGG |
| *pilX* comp 5’ | ttctccatacccgttttttt*ggggaaggagatatacat*ATGACCCTGCGCCATACCTC |
| *pilX* comp 3’ | atcttctctcatccgccTCAgtggtgatggtggtggtgGTTGGTATACAGGCGTGCATG |
| *pilX* (G-1A) For | CCATACCTCTCGACAGCAG**GCC**TCCACGTTGTTGATCTCGCTG |
| *pilX* (G-1A) Rev | CAGCGAGATCAACAACGTGGA**GGC**CTGCTGTCGAGAGGTATGG |
| *pilX* (G-1D) For | CCATACCTCTCGACAGCAG**GAT**TCCACGTTGTTGATCTCGCTG |
| *pilX* (G-1D) Rev | CAGCGAGATCAACAACGTGGA**ATC**CTGCTGTCGAGAGGTATGG |
| *pilX* (G-1F) For | CCATACCTCTCGACAGCAG**TTC**TCCACGTTGTTGATCTCGCTG |
| *pilX* (G-1F) Rev | CAGCGAGATCAACAACGTGGA**GAA**CTGCTGTCGAGAGGTATGG |
| *pilW* (G-1F) For | CTTCAGCAAAATGCAGAAA**TTC**CTATCGATGGTAGAACTGCTC |
| *pilW* (G-1F) Rev | GCAGTTCTACCATCGATAG**GAA**TTTCTGCATTTTGCTGAAGAG |
| *pilX* KI 5’ | tttcccagtcacgacgttgtaaaacgacggccagtgccCTACATTTGTCGCAGCCCAAC |

| | |
|---|---|
| *pilX* KI 3’ | aacaatttcacacaggaaacagctatgaccatgattacTTGCTCAGGTTGGTGGTGGAG |
| *pilX* KI.2 3’ | aacaatttcacacaggaaacagctatgaccatgattacGGTCTGGTTCTGGTTGTCGAG |
| *pilX* His KI For | TGTATACCAACcaccaccaccatcaccacTGACTGGAGCCAGCGCATGATC |
| *pilX* His KI Rev | GGCTCCAGTCAgtggtgatggtggtggtgGTTGGTATACAGGCGTGCATG |
| *pilX*ΔLP For | CAAACCATCAGGAATCTCATGCCATGAGCACGTTGTTGATCTCGCTG |
| *pilX*ΔLP Rev | CAGCGAGATCAACAACGTGGACATGGCATGAGATTCCTGATGGTTTG |

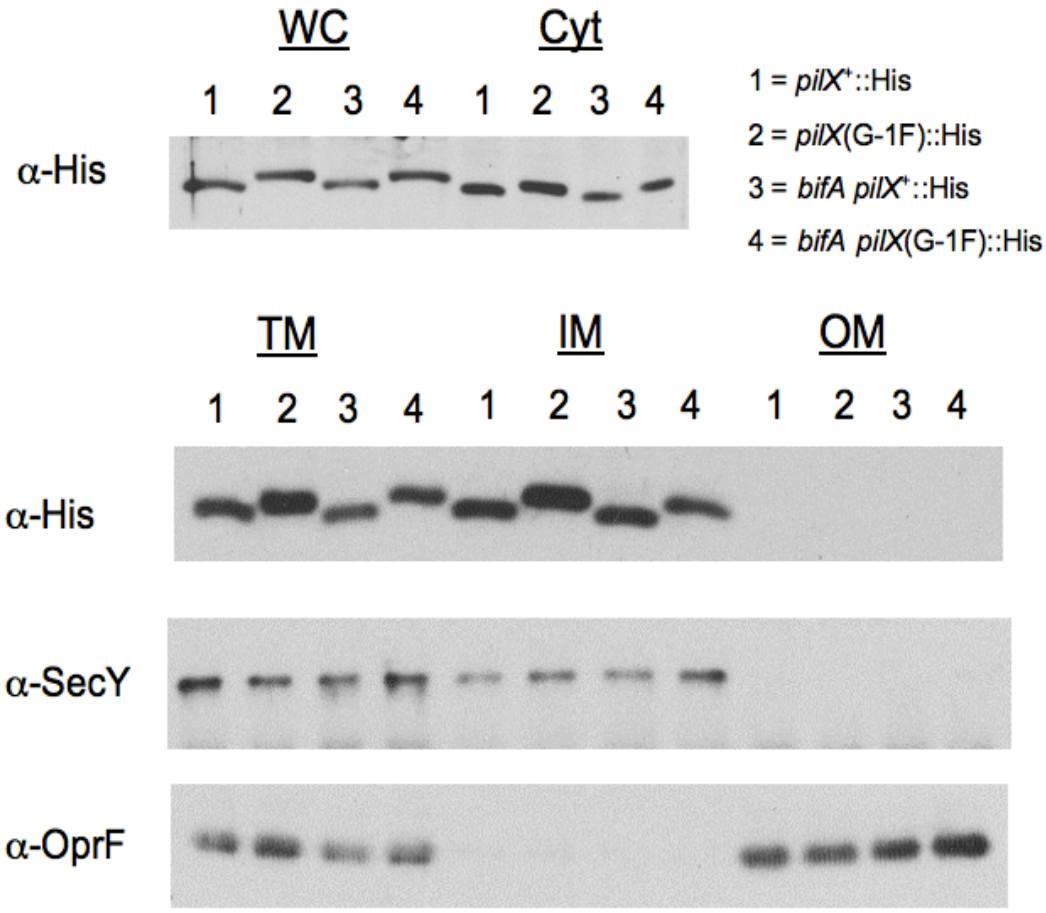
[a]Lowercase letters indicate sequence homology to the cloning vector, uppercase letters indicate a *Pseudomonas*-specific gene sequence, lowercase underlined letters indicate a His epitope tag sequence, lowercase italics indicate ribosome binding site, bold-face letters indicate codon mutations.

# Supp. Fig. 1



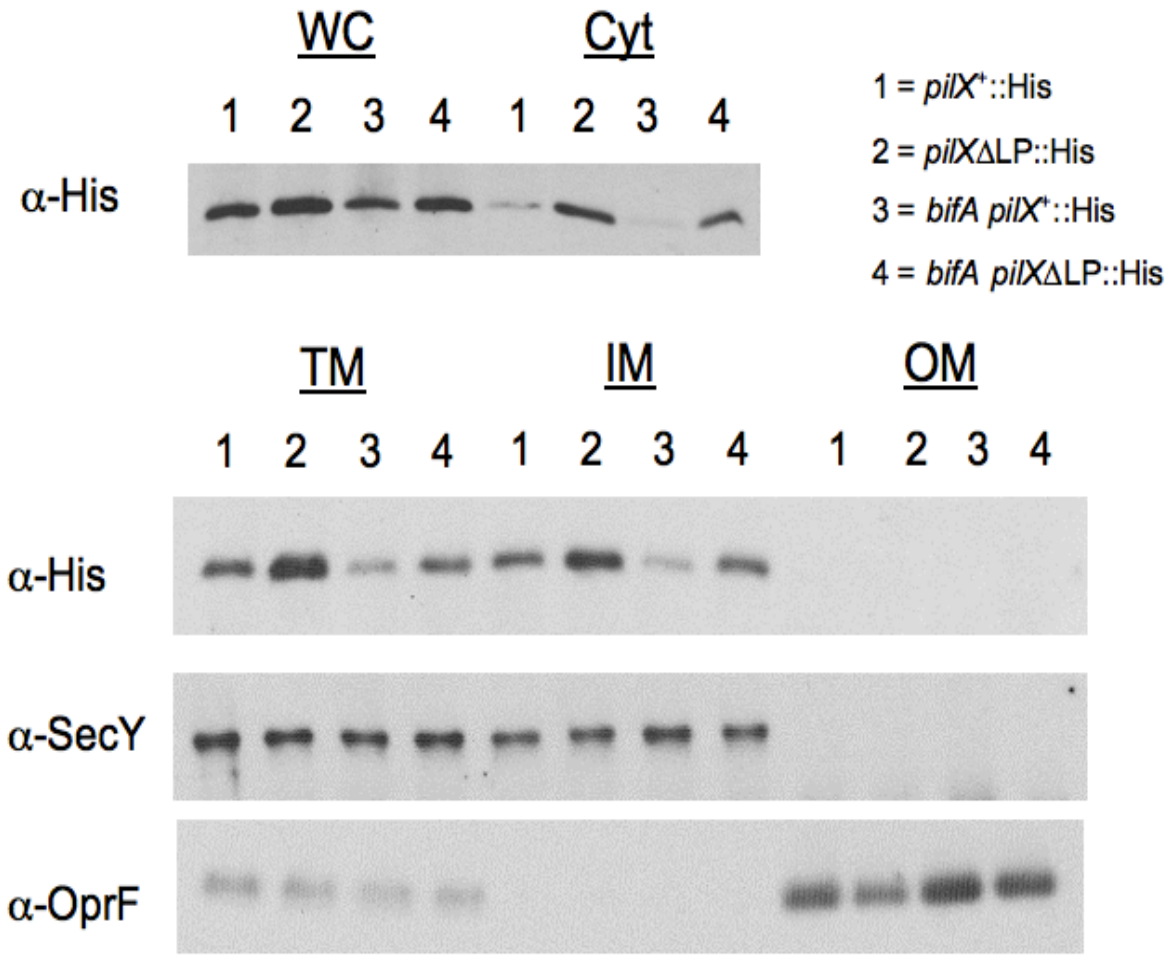

**Supplemental Fig. 1. Analysis of PilW. A.** Western blot showing plasmid-based expression and PilD-mediated processing status of the PilW-His and PilW(G-1F)-His proteins in the *pilD* and *bifA pilW* mutant backgrounds. Equal amounts of total protein from whole cell lysates prepared from each strain were resolved by SDS/PAGE on a 15% polyacrylamide gel. PilW-His and the PilW(G-1F)-His mutant variant were detected using an anti-penta-His antibody. The protein size markers (M) are indicated in kDa. P, processed form; UP, unprocessed form. **B.** Graph depicting quantification of twitch zones for the indicated strains, measured using ImageJ (units are pixels x 1000). Error bars represent standard deviation of averages from three plates. **C.** Representative image of a swarm plate showing swarms of the *bifA pilW* mutant carrying either vector (left), pPilW-His (right) or pPilW(G-1F)-His (bottom). Swarm medium was supplemented with 0.2 % arabinose and plates were grown at 37ºC for 16h.

Supp. Fig. 2




**Supplemental Fig. 2. Cellular localization of the PilX protein in the WT and *bifA* mutant backgrounds.** Western blots showing abundance of chromosomally expressed PilX-His and PilX(G-1F)-His in the cellular fractions depicted as follows: WC, whole cell lysate; Cyt, cytosolic; TM, total membrane; IM, inner membrane; and OM, outer membrane. Lane designations are indicated in the upper right corner. Western blots were probed with one of the following antibodies, as indicated: anti-His antibody to detect PilX-His; anti-SecY antibody to detect the inner membrane-localized SecY protein and anti-OprF antibody to detect the outer membrane-localized OprF protein.

Supp. Fig. 3




**Supplemental Fig. 3. Cellular localization of the PilXΔLP protein.** Western blots showing abundance of chromosomally expressed PilX-His and PilXΔLP-His in the cellular fractions of the WT and *bifA* strains when grown on swarm plates. Fractions are as follows: WC, whole cell lysate; Cyt, cytosolic; TM, total membrane; IM, inner membrane; and OM, outer membrane. Lane designations are indicated in the upper right corner. Western blots were probed with one of the following antibodies, as indicated: anti-His antibody to detect PilX-His; anti-SecY antibody to detect the inner membrane-localized SecY protein, and anti-OprF antibody to detect the outer membrane-localized OprF protein.