Supporting Information S1

The growth conditions. *A. tumefaciens* was grown at 25 °C in 523 (1), whereas LB (2) were routinely used for *E. coli* and *P. aeruginosa* strains at 37 °C unless indicated. The plasmids were maintained by the addition of 50 μg/mL gentamycin (Gm) and 200 μg/mL spectinomycin (Sp) for *A. tumefaciens* and 100 μg/mL ampicillin (Ap), 100 μg/mL spectinomycin (Sp), 20 μg/mL kanamycin (Km), and 50 μg/mL Gm for *E. coli*, and 50 μg/mL Gm, and 2000 μg/mL Sp for *P. aeruginosa.* Growth conditions are as previously described (3,4).

Plasmid construction and generation of in-frame deletion mutants. Plasmid pJQ200KS-Δ*ppkA*Δ*tagF-pppA* (Supplementary Table S1) was created by ligating the *Xba*I/*Bam*HI-digested PCR product 1 (~500 bp DNA fragment upstream of *tagF-pppA* gene) and the *BamH*I/*Xma*I-digested PCR product 2 (~500 bp DNA fragment downstream of *ppkA* gene) into *Xba*I/*Xma*I sites of pJQ200KS (5) and used to generate the *ppkA* and t*agF-pppA* genes deletion mutant (Supplementary Tables S1 and S2). The resulting strain was confirmed by PCR and designated as EML4307 (Δ*ppkA*Δ*tagF-pppA*).

To construct the plasmids for expressing proteins in *A. tumefaciens*, each DNA fragment containing the ribosomal-binding sequence (RBS) and ORF (with stop codon) of *tagF-pppA*, *tagF*, *tagF-Strep*, *tagF*^{GK}*-Strep*, *tagF*^{DF}*-Strep*, *tagF*^{DW}*-Strep*, *tagF*^{SDR}*-Strep*, *tagF*^{FD}*-Strep*, and *pppA* were PCR-amplified with primers described in Supplementary Table S2 and respectively cloned into pTrc200 (6) with appropriate enzyme sites to create the plasmids pTrc-TagF-PppA, pTrc-TagF, pTrc-TagF-Strep, pTrc-TagF^{GK}-Strep, pTrc-TagF^{DF}-Strep, pTrc-TagF^{DW}-Strep, pTrc-TagF^{SDR}-Strep, pTrc-TagF^{FD}-Strep, and pTrc-PppA.

To construct the plasmids for expressing proteins in *P. aeruginosa*, the gene of interest containing its RBS and ORF was cloned to be driven by a *lac* promoter on the broad host range vector pRL662 (7). The PCR products of *P. aeruginosa tagF*, *tagF-Strep*, *tagF*^{GK}*-Strep*, and *tagF*^{SDR}*-Strep* were amplified with primers described in Supplementary Table S2 and respectively digested by *Xho*I/*Xba*I, and cloned into the same sites of pRL662, which resulted in the plasmids pTagF^{Pa}, pTagF^{Pa}-Strep, pTagF$^{Pa\text{-}GK}$-Strep, and pTagF$^{Pa\text{-}SDR}$-Strep. The PCR product of *P. aeruginosa fha1-HA* was amplified with primers described in Supplementary Table S2 and cloned into pTrc200 with appropriate enzyme sites, which resulted in the plasmid pTrc-Fha1^{Pa}-HA.

To construct the plasmid for protein expression in *E. coli*, the DNA fragment containing *tagF* 1-214 was PCR-amplified with primers described in Supplementary Table S2 and cloned into pET28a(+) to create the plasmid pET28a(+)-TagF 1-214.

For the constructs used for yeast two-hybrid, the *fha*, *tagF-pppA*, *tagF*, *tagF*^{GK}, *tagF*^{DF},

tagF^{DW}, *tagF*^{SDR}, *tagF*^{FD}, *tagF*^{Pa}, *tagF*$^{Pa\text{-}GK}$, *tagF*$^{Pa\text{-}SDR}$,and *fha1*^{Pa} ORFs (without stop codon) were PCR-amplified with primers described in Supplementary Table S2 and respectively cloned into pGBKT7 or pGADT7 with appropriate enzyme sites to create the plasmids pGBKT7-TagF-PppA, pGBKT7-TagF, pGBKT7-TagF^{GK}, pGBKT7-TagF^{DF}, pGBKT7-TagF^{DW}, pGBKT7-TagF^{SDR}, pGBKT7-TagF^{FD}, pGBKT7-TagF^{Pa}, pGBKT7-TagF$^{Pa\text{-}GK}$, pGBKT7-TagF$^{Pa\text{-}SDR}$, pGBKT7-Fha1^{Pa}, pGADT7-Fha, pGADT7-TagF, pGADT7-TagF^{Pa}, and pGADT7-Fha1^{Pa}, respectively.

For the constructs used for bacterial two-hybrid, the *tagF*^{Pa} and *fha1*^{Pa} ORFs (without stop codon) were PCR-amplified with primers described in Supplementary Table S2 and respectively cloned into pKT25 (8) or pUT18C (8) with appropriate enzyme sites to create the plasmids pKT25-TagF^{Pa}, pKT25-Fha1^{Pa}, pUT18C-TagF^{Pa}, and pUT18C-Fha1^{Pa}, respectively.

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