PlzR regulates type IV pili assembly in *Pseudomonas aeruginosa* via PilZ binding

Supplementary Information

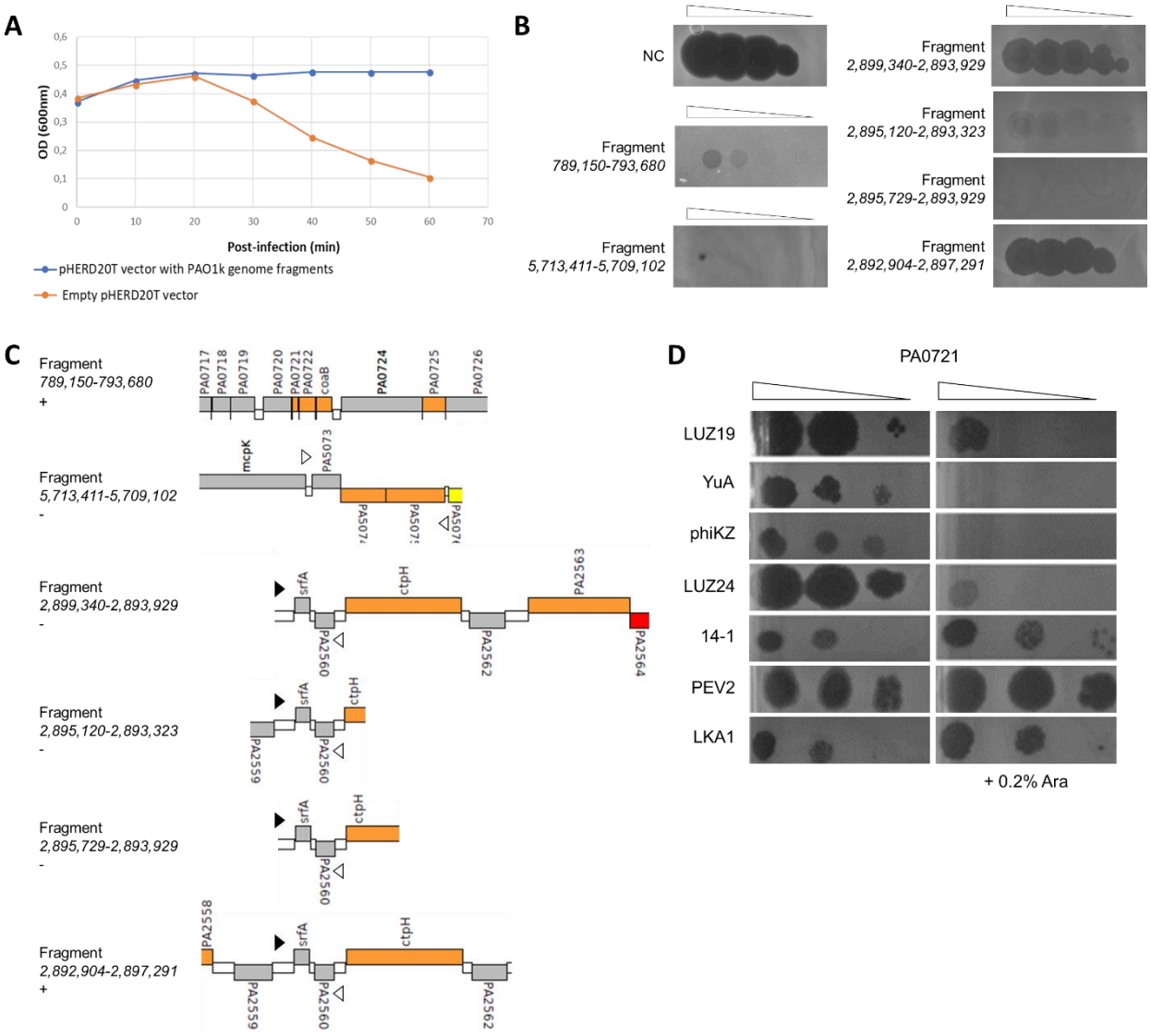

Fig S1. Screen for phage resistance determinants in *P. aeruginosa* PAO1 against infection with the T4P-dependent phage LUZ19. (A) Growth curves of PAO1 cells with an empty pHERD20T vector (orange) or with a library of pHERD20T plasmids containing random PAO1 genome fragments (blue), infected with the lytic phage LUZ19. (B) Serial dilution (10^{-2}-10^{-10}) of phage LUZ19 on lawns of PAO1 host cells containing PAO1 genome fragments overexpressed via pHERD20T-based plasmids. The different fragments are indicated with their genomic location. (C) Overview of the different fragments with enclosing genes visualized using the *Pseudomonas* Genome Database[1]. The color of the bars points to the localization of the gene products: red, cytoplasmic; orange, cytoplasmic membrane; yellow, periplasmic; grey, unknown. The white bars indicate intergenic regions. The full arrows point to a known promoter, while the empty arrows indicate predicted promoters based on visualized RNA-Seq data of *P. aeruginosa* PAO1[2]. (D) Hundredfold dilution series of the different *Pseudomonas* phages (indicated at the left) up to 10^{-6} were spotted on a lawn of *P. aeruginosa* PAO1 carrying the pHERD20T plasmid with PA0721 gene, on top of LB agar with or without 0.2% L-arabinose.

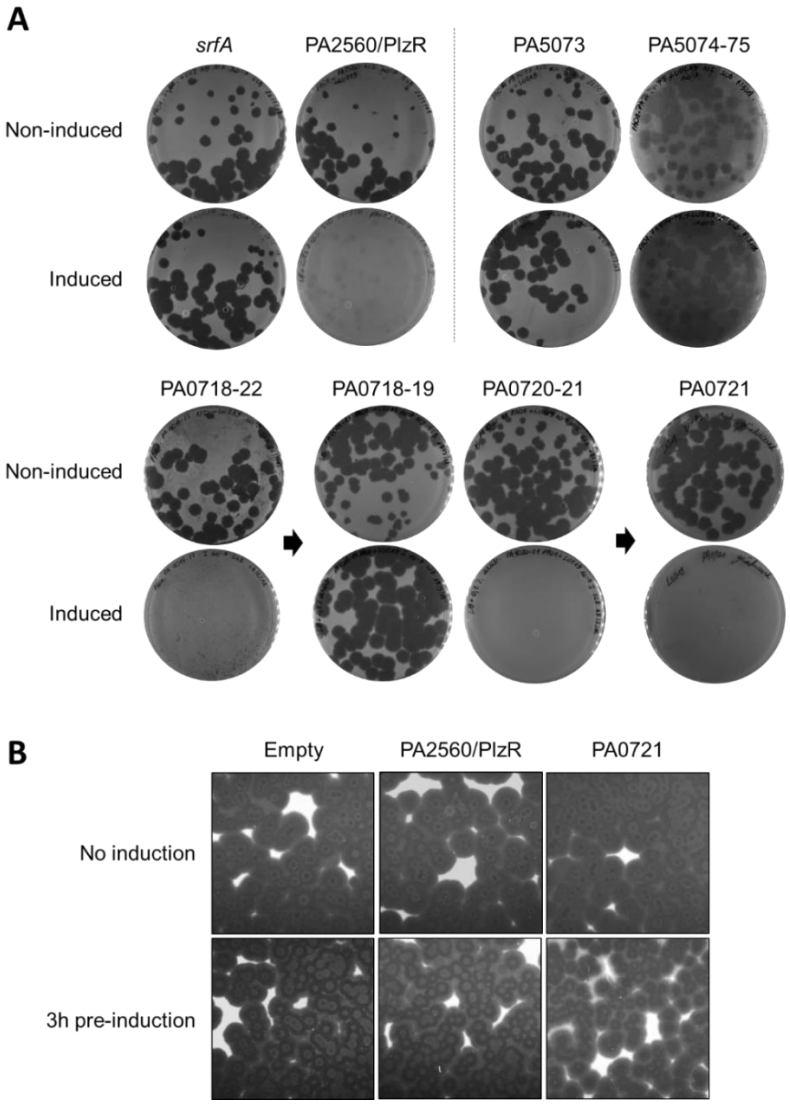

Fig S2. Identification of phage resistance determinants in *P. aeruginosa* PAO1 against the T4P-dependent phage LUZ19. (A) Plaque formation of phage LUZ19 on lawns of PAO1 host cells with genes or gene fragments of PAO1 overexpressed via pHERD20T-based plasmids. The specific PAO1 genes inserted in the pHERD20T plasmid are indicated above the figures. The recombinant strains were infected with LUZ19 using the double agar method, 0.2% arabinose was added for induction and pictures were taken after incubation overnight at 37°C. (B) Reversibility of the activity of the phage resistance determinants. The recombinant PAO1 strains were first exposed to 0.2% arabinose for 3 h to express the phage resistance determinants, and then used in a double agar experiment with LUZ19 in absence of arabinose. The inserts present in a pHERD20T vector in the different recombinant PAO1 strains are indicated on top. LUZ19 infection of PAO1 with an empty pHERD20T plasmid was used as a control.

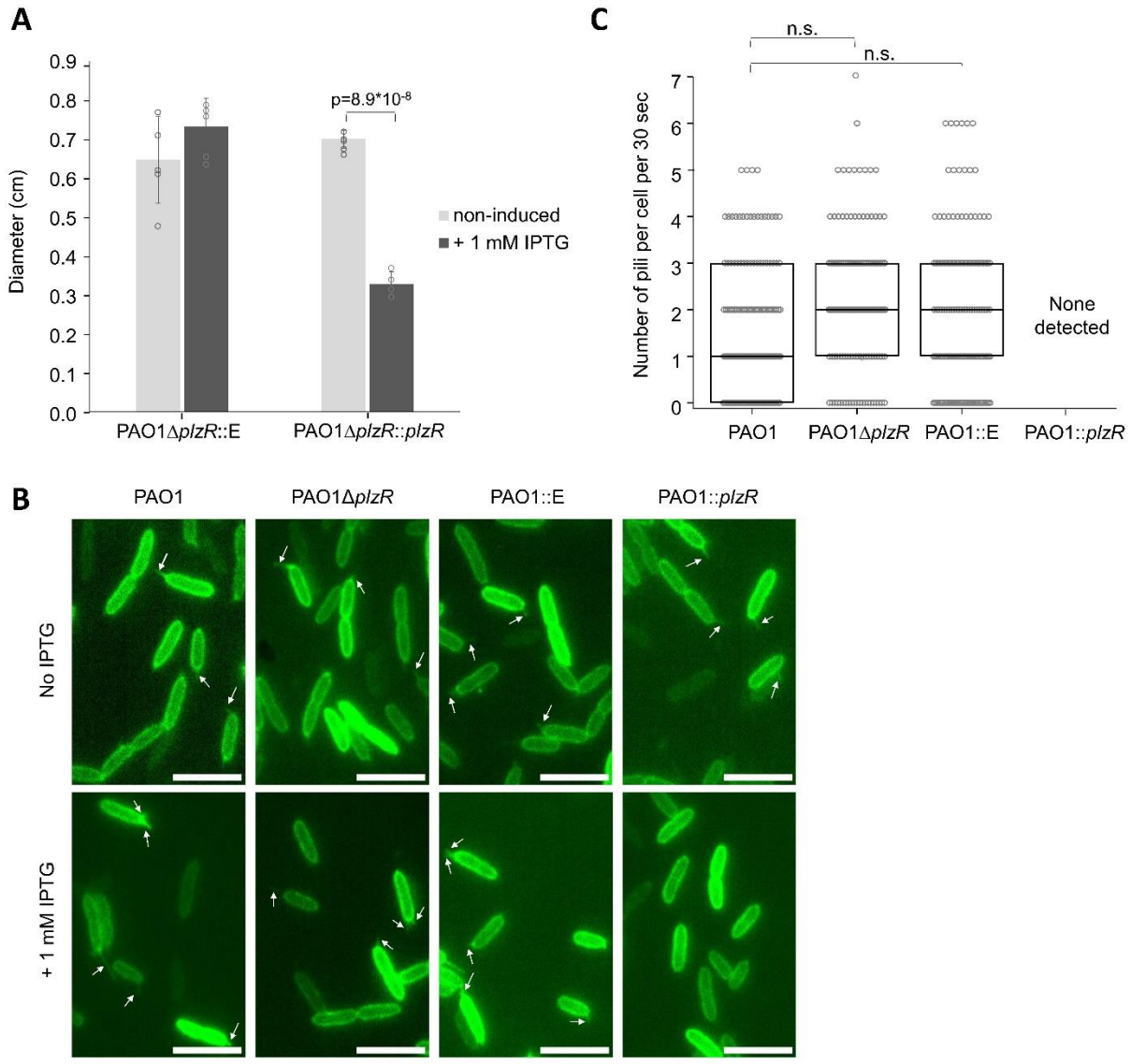

Fig S3. PlzR reduces twitching motility and prevents the assembly of T4P on the bacterial cell surface. (A) Twitching motility assay with the *P. aeruginosa* PAO1 *plzR* deletion mutant harboring the *plzR* gene in the mini-tn7T transposon (PAO1Δ*plzR*::*plzR*) and its empty counterpart (PAO1Δ*plzR*::E). Data are presented as mean values +/- SD of five biological replicates and p-values were calculated using the Student's t-test (two-sided, n=5 colonies). Source data are provided as a Source Data file. (B) Fluorescence microscopy images of fluorescently labeled T4P on the cell surface of the *P. aeruginosa* PAO1 pilA^{A86C} mutant (PAO1), the pilA^{A86C} mutant lacking the *plzR* gene (PAO1Δ*plzR*), the pilA^{A86C} mutant harboring the *plzR* gene in the mini-tn7T transposon (PAO1:*plzR*) and its empty counterpart (PAO1::E) without IPTG induction (top) and upon induction with 1 mM IPTG (bottom). The white arrows point to T4P, and the scale bar represents 5 µm. (C) Number of T4P observed on the cell surface of the *P. aeruginosa* PAO1 pilA^{A86C} mutant (PAO1), the pilA^{A86C} mutant lacking the *plzR* gene (PAO1Δ*plzR*), the pilA^{A86C} mutant harboring the *plzR* gene in the mini-tn7T transposon (PAO1:*plzR*) and its empty counterpart (PAO1::E) upon induction with 1 mM IPTG. For each condition, three biological replicates of 50 cells were analyzed for 30 sec. Abbreviation: n.s. = not significant. Boxes represent the median and 25%/75% quartiles of the distributions of all measured pili for each sample. Source data are provided as a Source Data file.

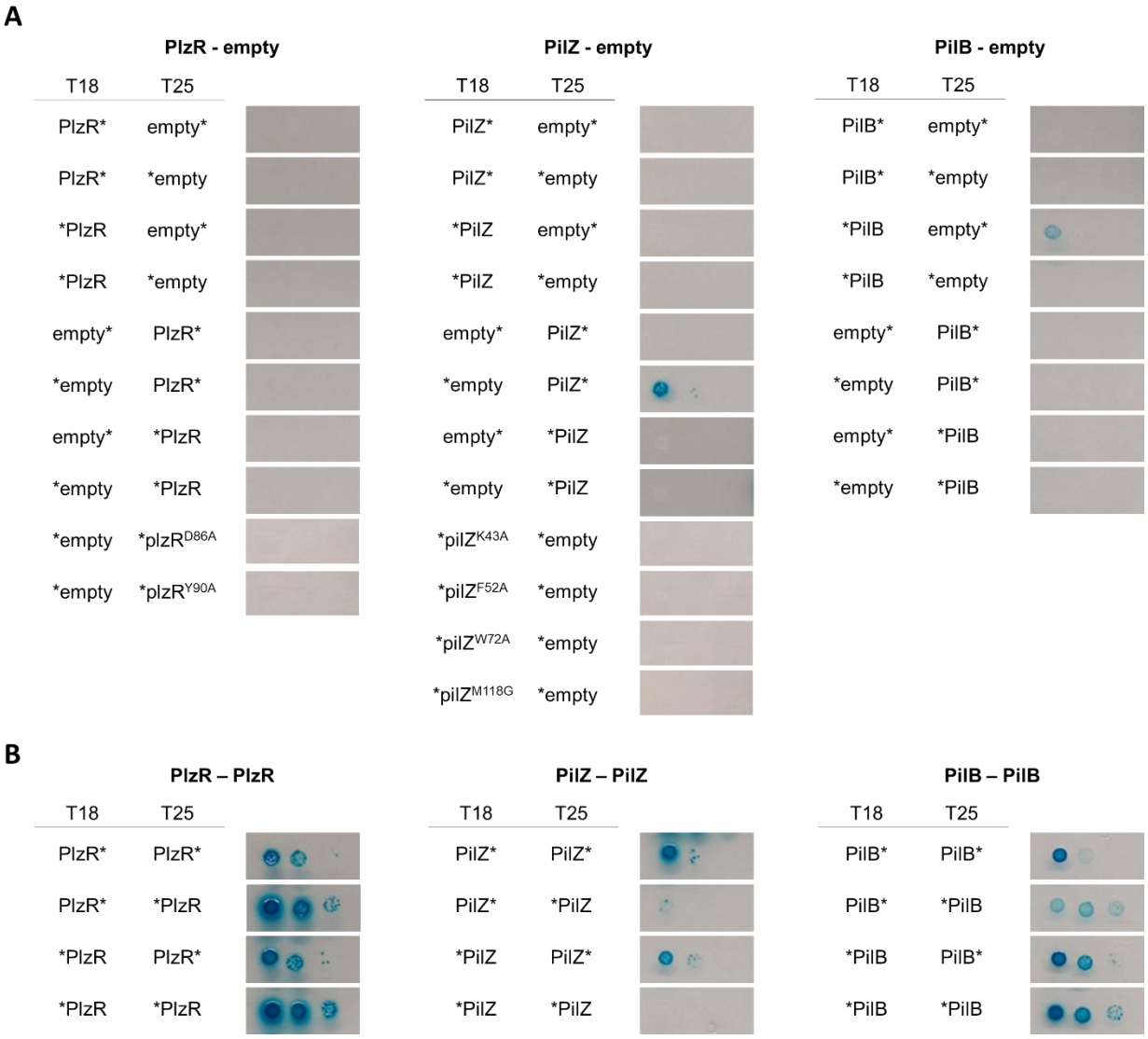

Fig S4. Autoactivation results and dimer formation results of the bacterial two-hybrid. PlzR, PilZ and PilB were both C- (indicated with *PlzR, *PilZ and *PilB) and N-terminally (indicated with PlzR*, PilZ* and PilB*) fused to the T18 and T25 domain of the adenylate cyclase, and checked for (A) activating the reporter system in absence of another fusion product (indicated with empty* and *empty), and for (B) dimer formation.

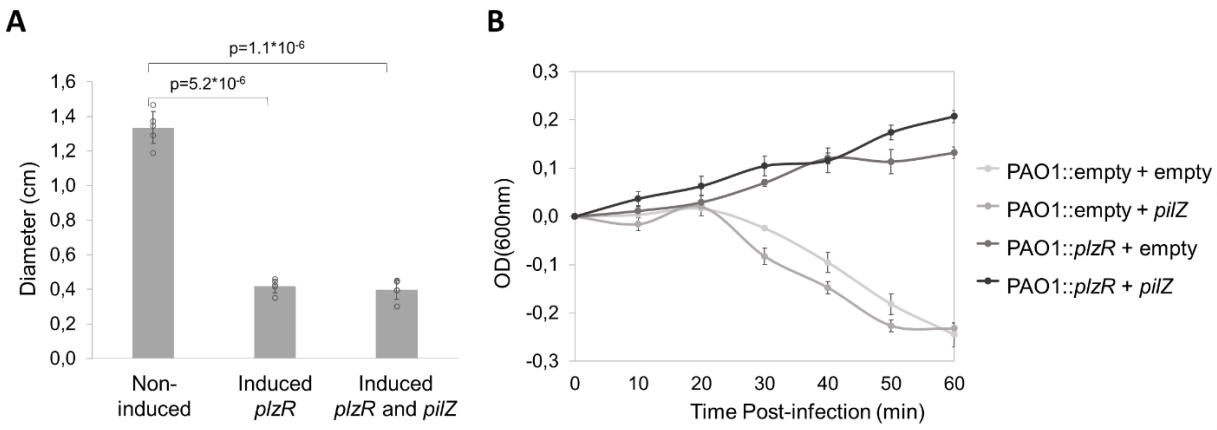

Fig S5. Effect of PilZ overexpression on PlzR activity. (A) Results of twitching activity of PAO1 cells upon overexpression of *plzR* (genomic insertion via the mini-tn7T transposon), and *pilZ* (in the pHERD20T plasmid). The results show clear reduction of twitching activity upon 1 mM IPTG induction of *plzR*, even after additional 0.2% L-arabinose induction of *pilZ*. Data are presented as mean values +/- SD of five biological replicas and p-values were calculated using the Student's t-test (two-sided, n=5 colonies). Source data are provided as a Source Data file. (B) Infection curves of phage LUZ19 infecting *P. aeruginosa* cells harboring *plzR,* present in the mini-tn7T transposon (PAO1::*plzR*), and *pilZ,* present in the pHERD20T vector (PAO1 + *pilZ*). Empty counterparts of both constructs were tested as controls. The strains were induced with 1 mM IPTG and 0.2% L-arabinose at an OD_{600nm} of 0.1, infected with phage LUZ19 (MOI = 30) at an OD_{600nm} of 0.3, and the OD_{600nm} was measured every 10 min for 1 h. Although *pilZ* overexpression slightly improved LUZ19 infection, no effect of *pilZ* overexpression on PlzR antiphage activity was observed (black line). Data are presented as mean values +/- SD of three biological replicas. Source data are provided as a Source Data file.

Supplementary References

1. Winsor, G. L. *et al.* Enhanced annotations and features for comparing thousands of *Pseudomonas* genomes in the *Pseudomonas* genome database. *Nucleic Acids Res* **44**, D646-53 (2016).
2. Blasdel, B.G., Ceyssens, P-J., Chevallereau, A., Debarbieux, L., Lavigne, R. Comparative transcriptomics reveals a conserved Bacterial Adaptive Phage Response (BAPR) to viral predation. *bioRxiv*, 248849 (2018).