Supporting information

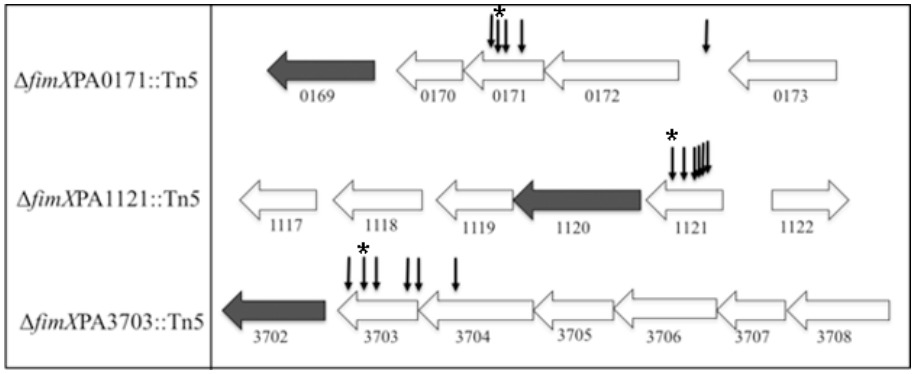

Fig. S1. Sites of transposon insertions in Δ*fimX* suppressors. Sites of Tn insertion mapped by inverse PCR are indicated by black arrows. Gray shading indicates genes encoding putative (PA0169) or confirmed (PA1120/YfiN/TpbB and PA3702/WspR) diaguanylate cyclases. Multiple independent insertions were mapped to PA0171, PA1121 and PA3703. An asterisk indicates the sites of Tn5 insertions PA0171-2, PA1121-1, PA3703-2.

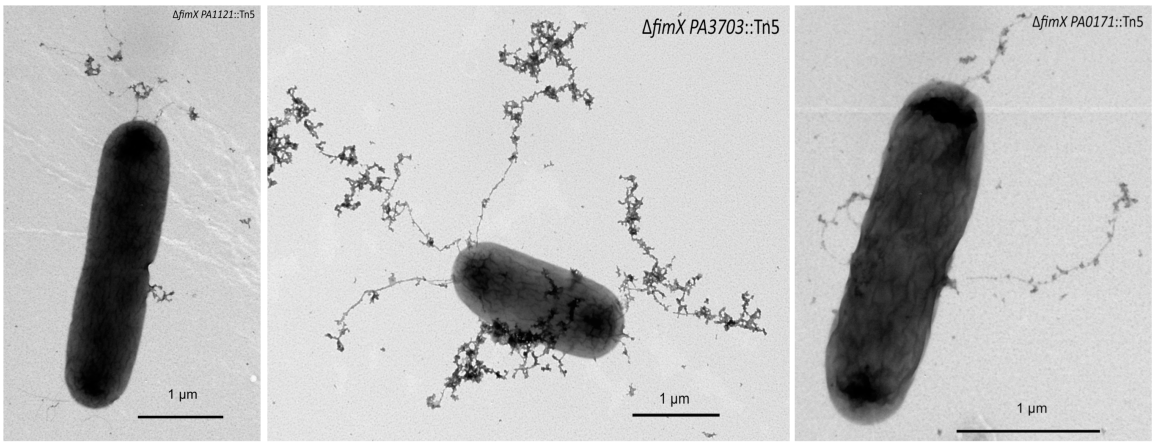

Fig. S2. Visualization of T4P by transmission electron microscopy. Extragenic suppressor strains with Tn insertions in PA1121, PA3703 and PA0171 were stained directly with 1% Uranyl Acetate. Panels show picture of a representative cell for each mutant. Scale bar =1 µm.

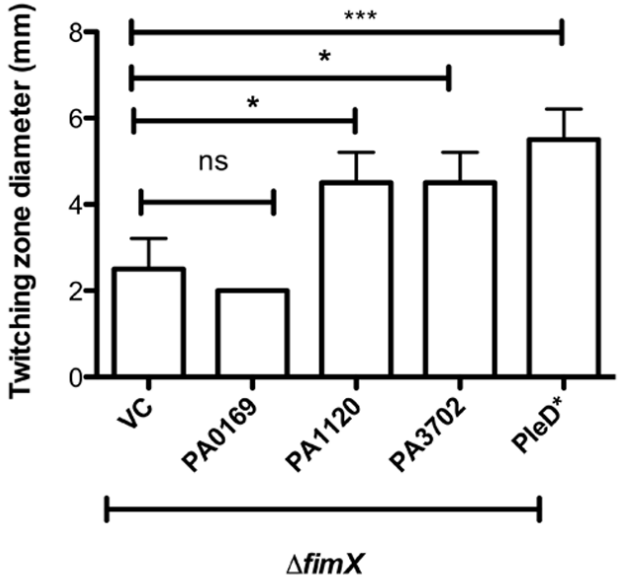

Figure S3: Over-expression of diguanylate cyclases restores twitching motility. PA103Δ*fimX* carrying pMQ72 (VC) or the indicated DGCs cloned under the pBAD promoter were grown on plates with 0.2 % arabinose. Bars represent the twitching zone diameter as measured by the subsurface stab assay.

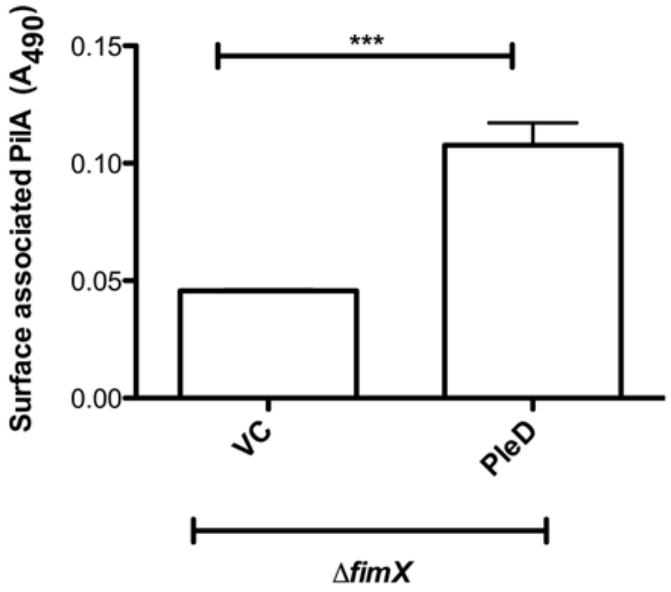

Figure S4: Over-expression of PleD restores surface pilin assembly. Graph depicts the total surface pilin of PA103Δ*fimX* carrying pUCPKS (VC) or PleD cloned in a high copy number plasmid under a constitutively active promoter.

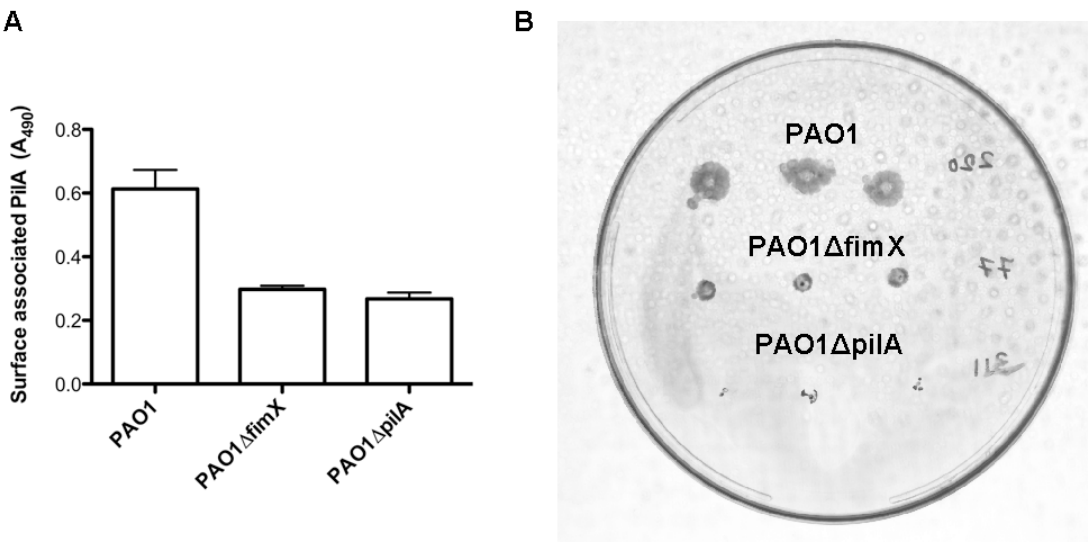

Figure S5: Deletion of FimX in PAO1 results in pilin assembly and twitching motility defects. (A) Graph shows the total surface pilin as assessed by ELISA for PAO1 WT, PAO1Δ*fimX* and PAO1Δ*pilA*. (B) Surface associated twitching motility shown for PAO1 WT, PAO1Δ*fimX* and PAO1Δ*pilA*. Plate was stained using Coomassie Brilliant Blue after 24 hour of incubation at 37°C.

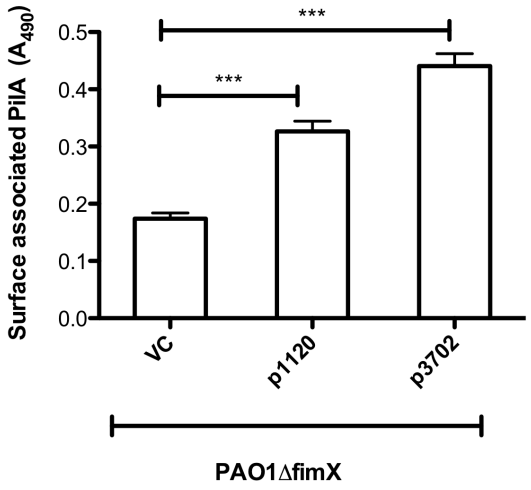

Figure S6: Overexpression of DGC in PAO1Δ*fimX* restores surface pilin assembly. Bars represent the surface associated pilin from plate grown PAO1Δ*fimX* over-expressing either empty vector pMQ72 (VC) or PA1120 or PA3702. Experiment was done in triplicates and bars represent mean ± SD from a representative experiment.

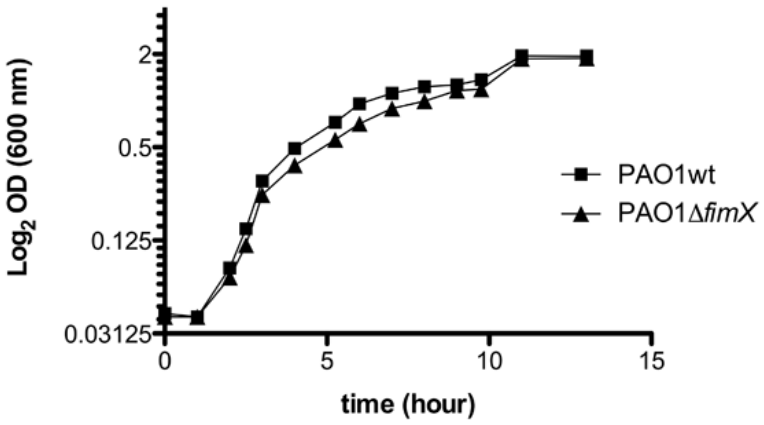

Figure S7: Growth Curve of PAO1 and PAO1Δ*fimX* in M9 minimal media.

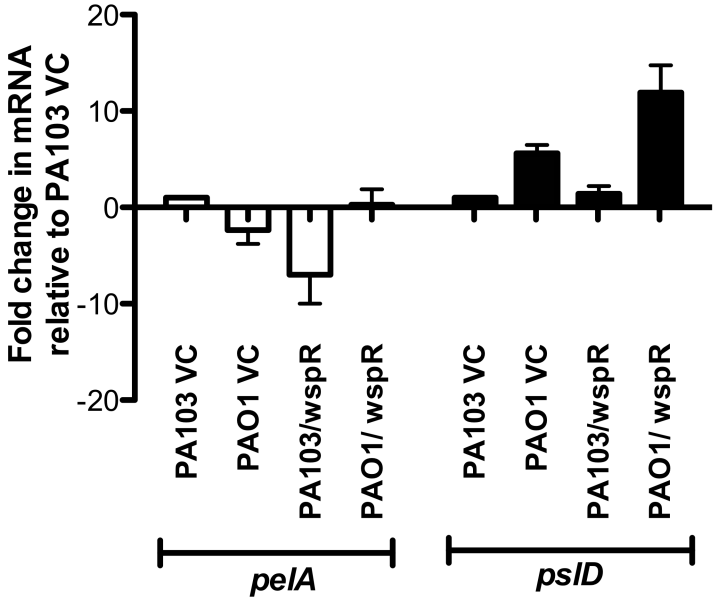

Fig S8: Relative m-RNA expression of *pelA* and *pslD* in PA103 and PAO1. Total RNA was isolated from plate grown bacteria and qRT-PCR was carried out for *pelA* and *pslD* in PA103 or PAO1 carrying pMQ72 (VC) or *wspR* cloned in pMQ72. Fold change of the respective genes was normalized for the expression of *rplU*. Data represented here is compared to PA103 carrying vector alone.