Appendix

TssA forms a gp6-like ring attached to the type VI secretion sheath

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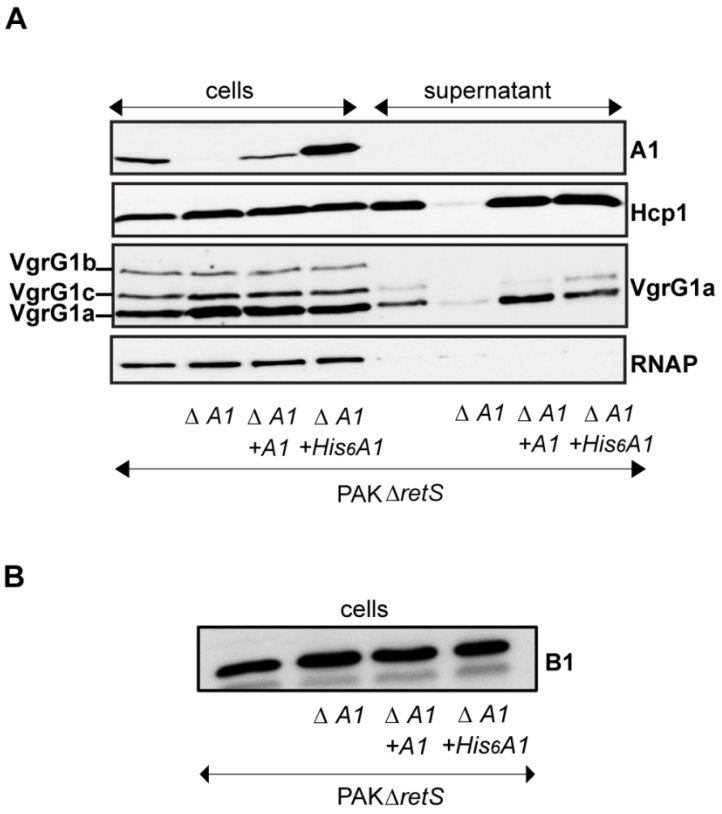
Appendix Table S1. Strains used in this study

Strains	Description	References
E. coli		
One-shot top10	F− *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)	Invitrogen
B834(DE3)	F− *ompT hsdS*B(rB− mB−) *gal dcm met* (DE3)	Laboratory collection
DHM1	*cya*-854 *rec*A1 *gyrA96* (NaI) *thi1 hsdR17 spoT1 rfbD1 glnV44*(AS)	Karimova et al., 1998
C118λpir	Host strain for pKNG101 replication; D (*ara-leu*) *araD DlacX74 galE galK-phoA20 thi-1 rpsE rpoB argE* (Am) *recA1 Rf*^{r} (*lpir*)	Herrero et al., 1990
P. aeruginosa		
PAKΔ*retS*	In-frame deletion of *retS* (PA4856) in *P. aeruginosa* PAK wild-type strain	Goodman et al., 2004
PAKΔ*retS*-pBBR	PAKΔ*retS* strain carrying the empty vector pBBR1MCS-4	This study
PAKΔ*retS*Δ*A1*	In-frame deletion of *tssA1* (PA0082) in *PAKΔretS*	This study
PAKΔ*retS*Δ*A1*-pBBR	PAKΔ*retS*Δ*A1*mutant carrying the empty vector pBBR1MCS-4	This study
PAKΔ*retS*Δ*A1*-pBBR-*A1*	PAKΔ*retS*Δ*A1*mutant carrying the pBBR1MCS-4 vector expressing *tssA1* (pBBR-*A1*)	This study
PAKΔ*retS*Δ*A1*-pBBR-*His$_6$A1*	PAKΔ*retS*Δ*A1*mutant carrying the pBBR1MCS-4 vector expressing the N-terminally His_6 tagged *tssA1* (pBBR-*His$_6$A1*)	This study
PAKΔ*retS*Δ*E1*	In-frame deletion of *tssE1* (PA0087) in *PAKΔretS*	Lossi et al., 2011
PAKΔ*retS*Δ*E1*-pBBR	PAKΔ*retS*Δ*E1*mutant carrying the empty vector pBBR1MCS-4	
PAKΔ*retS*Δ*E1*-pBBR-*E1*	PAKΔ*retS*Δ*E1*mutant carrying the pBBR1MCS-4 vector expressing *tssE1* (pBBR-*E1*)	This study
PAKΔ*retS*Δ*B1::B1-sfGFP*	PAKΔ*retS*Δ*B1* strain having the gene encoding the C-terminal fusion of the sfGFP with tssB1 (*B1-sfGFP*) under a plac promoter integrated into the *att* site	This study
PAKΔ*retS*Δ*A1*Δ*B1::B1-sfGFP*	PAKΔ*retS*Δ*A1*Δ*B1* strain having the gene encoding the *B1-sfGFP* under a plac promoter integrated into the *att* site.	This study
PAKΔ*retS*Δ*B1*Δ*E1::B1-sfGFP*	PAKΔ*retS*Δ*B1*Δ*E1* strain having the gene encoding the *B1-sfGFP* under a plac promoter integrated into the *att* site	This study
PAKΔ*retS*Δ*A1*Δ*B1::B1-sfGFP-pBBR-A1*	PAKΔ*retS*Δ*A1*Δ*B1::B1-sfGFP* carrying the pBBR1MCS-4 vector expressing *tssA1* (pBBR-*A1*)	This study
PAKΔ*retS*Δ*B1*Δ*E1::B1-sfGFP-pBBR-E1*	PAKΔ*retS*Δ*B1*Δ*E1::B1-sfGFP* carrying the pBBR1MCS-4 vector expressing *tssE1* (*pBBR-E1*)	This study

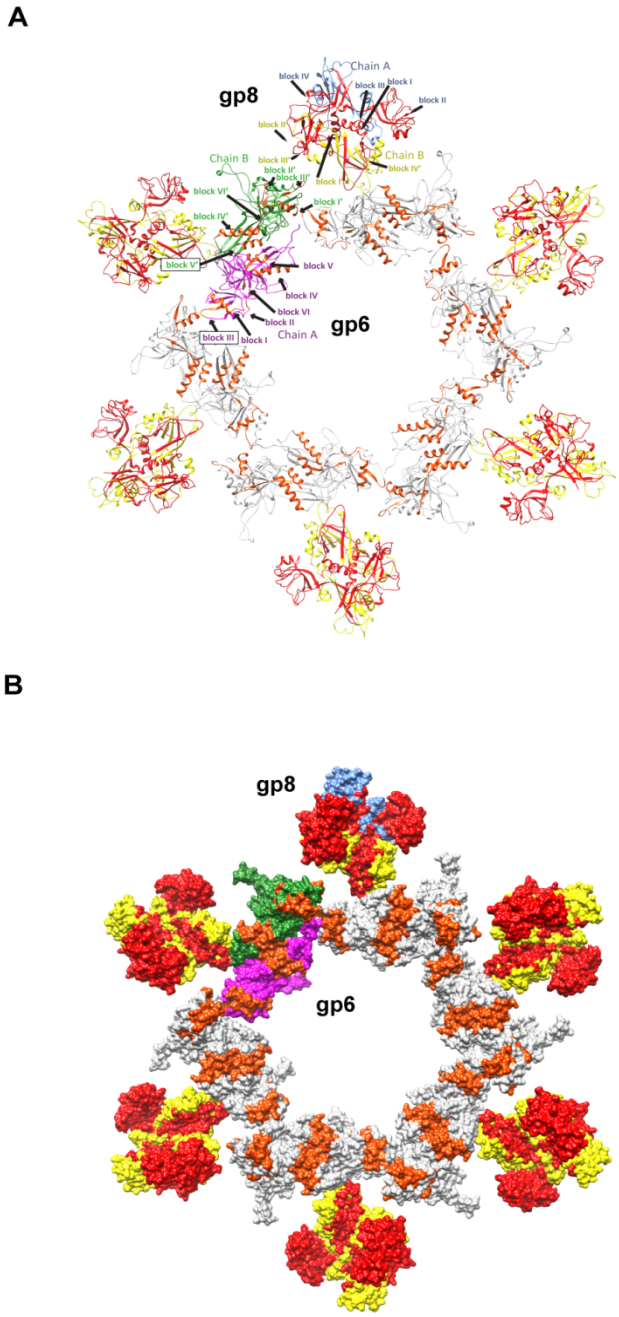
Appendix Table S2. Plasmids used in this study

Plasmids	Description	References
pKNG101-Δ*A1*	pKNG101 containing the mutator fragment for deletion of *tssA1*, Str^R	This study
Mini-CTX-*B1-sfGFP*	Mini-CTX-1 encoding *tssB1-sfGFP* under a plac promoter for integration into the *att* site of the *P. aeruginosa* chromosome, Tc^R	This study
pBBR	pBBR1MCS-4, Broad host range vector pBBR1 replicon, Ap^R	Kovach et al., 1995
pBBR-*A1*	pBBR1MCS-4 containing *tssA1*	This study
pBBR-*His_6A1*	pBBR1MCS-4 containing *His_6tssA1*	This study
pBBR-*E1*	pBBR1MCS-4 containing *tssE1*	This study
pET28	Expression vector used for expression of N-terminal His_6 tagged proteins, Km^R	Novagen
pET26	Expression vector used for expression of untagged proteins, Km^R	Novagen
pACYC	pACYC-Duet vector, Cm^R	Novagen
pET-*His_6A1*	pET28 expressing TssA1with an N-terminal His_6 tag (His_6-TssA1)	This study
pET26-*B1C1*	pET26 expressing untagged TssB1C1	This study
pACYC-*A1-B1C1*	pACYC-Duet-1 expressing His_6-tagged TssA1 and TssB1C1 without tag from two separate T7 promoters	This study
pKT25	BTH vector for fusion of target proteins to *B. pertussis cya* gene T25 fragment; P_{lac}::*cya*$^{1–675}$p15ori, Km^R	Karimova et al., 1998
pUT18C	BTH vector for fusion of target proteins to *B. pertussis cya* gene T18 fragment; P_{lac}::*cya*$^{675-1197}$pUCori, Ap^R	Karimova et al., 1998
pKT25-*A1*	Fusion of *tssA1* to *cya* gene T25 fragment in pKT25, Km^R	Lossi et al., 2012
pUT18C-*A1*	Fusion of *tssA1* to *cya* gene T18 fragment in pUT18C, Ap^R	This study
pUT18C-*Hcp1*	Fusion of *hcp1* to *cya* gene T18 fragment in pUT18C, Ap^R	This study
pUT18C-*E1*	Fusion of *tssE1* to *cya* gene T18 fragment in pUT18C, Ap^R	Lossi et al., 2012
pUT18C-*F1*	Fusion of *tssF1* to *cya* gene T18 fragment in pUT18C, Ap^R	Lossi et al., 2012
pUT18C-*G1*	Fusion of *tssG1* to *cya* gene T18 fragment in pUT18C, Ap^R	Lossi et al., 2012
pUT18C-*K1*	Fusion of *tssK1* to *cya* gene T18 fragment in pUT18C, Ap^R	This study
pUT18C-*VgrG1a*	Fusion of *vgrG1a* to *cya* gene T18 fragment in pUT18C, Ap^R	This study
pUT18C-*ClpV1*	Fusion of *ClpV1* to *cya* gene T18 fragment in pUT18C, Ap^R	Förster et al., 2014

Ap^R, ampicillin-resistant; Cm^R, chloramphenicol-resistant; Km^R, kanamycin-resistant; Str^R, streptomycin-resistant; Tc^R, tetracyclin-resistant.



Appendix Figure S1. The N-terminally His_6-tagged TssA1 is functional. A. Proteins from whole cell extracts and culture supernatants of PAKΔ*retS*, the derivative *tssA1* mutant (Δ*A1*) (both carrying the pBBR plasmid) and the complemented Δ*A1* mutant carrying pBBR-*tssA1* (Δ*A1*+*A1*) or pBBR-*His_6-tssA1*(ΔA1+His_6A1) were analysed by Western blotting. Polyclonal antibodies directed against TssA1 (A1), Hcp1 and VgrG1a were used. The anti-VgrG1a antibody detects VgrG1a, VgrG1b and VgrG1c, as indicated on the left. RNA polymerase (RNAP) was monitored in both whole-cell lysates and culture supernatants using monoclonal antibody directed against its β-subunit. **B.** Western blot analysis showing the production of TssB1 in PAKΔ*retS*, Δ*A1* (both carrying the pBBR plasmid) and the complemented strains Δ*A1*+*A1* and Δ*A1*+*His_6A1*. Polyclonal antibody directed against TssB1 was used.



Appendix Figure S2. The gp6/gp8 baseplate subcomplex of the T4 phage. (A), Cartoon and **(B),** surface representations of the dodecameric gp6 structure (PDB code 3H3W) and the structure of gp8 (PDB code 1PDM) are shown. In both representations, chain A of the gp6 dimers is shown in magenta and chain B in green and the TssA1/gp6 conserved regions are shown in orange in the gp6 ring. The TssA1/gp8 conserved blocks are shown in red in the 3D structure of gp8. The conservation is most significant in regions involved in gp8 dimerization and interaction with gp6.