

Appendix

Tvp complexes support formation of the VgrG-PAAR spike during Type VI secretion system assembly

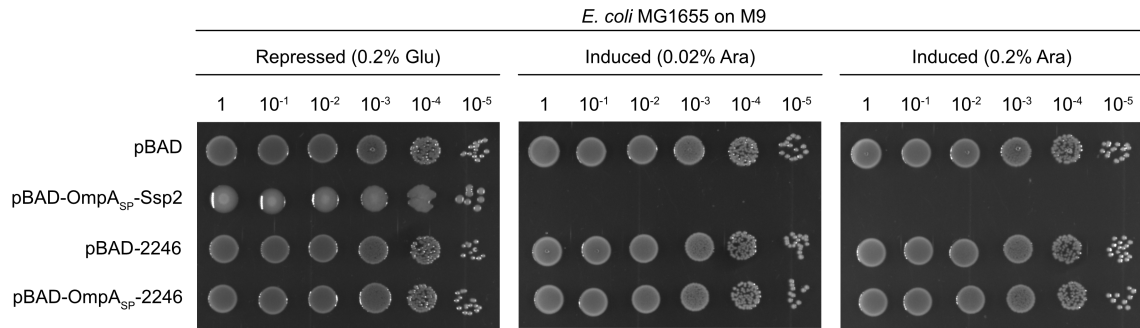
Table of Contents:

Appendix Figure S1	SMDB11_2246 is not toxic upon heterologous expression. p.3
Appendix Figure S2	The VgrG1 C-terminal domain does not display specific toxicity upon heterologous expression. p.4
Appendix Figure S3	Example of flow cytometry experiments from the analysis presented in Figure 1G. p.5
Appendix Figure S4	Epitope-tagged proteins used as bait in affinity purification and immunoprecipitation experiments retain function. p.7
Appendix Figure S5	Conservation of Tvp accessory proteins in representative Gram-negative species. p.8
Appendix Figure S6	Predicted structures of TvpAB, Tap2 and PA0097 proteins. p.10
Appendix Figure S7	Predicted structures of <i>Serratia marcescens</i> and <i>Pseudomonas aeruginosa</i> accessory pre-complexes. p.11
Appendix Figure S8	Predicted structure of the <i>Pseudomonas aeruginosa</i> accessory pre-complex with the full Tse7 effector. p.13
Appendix Figure S9	Predicted structure of the <i>Agrobacterium tumefaciens</i> VgrG2-associated accessory complex. p.14
Appendix Figure S10	Identification of conserved regions of the TvpA and PAAR DUF4150 proteins for phylogenetic analysis of the Tvp system. p.15
Appendix Figure S11	Alignment of an extended selection of PAAR, TvpA sequences for phylogenetic analysis. p.17
Appendix Figure S12	Extended phylogeny and taxonomy. p.18
Appendix Figure S13	Comparison of the AlphaFold3 predicted structure of TvpAB from <i>Serratia marcescens</i> Db10 with the experimentally-determined structure of TvpA of <i>Vibrio xiamenensis</i> . p.19
Appendix Figure S14	Comparison of the orientation of the DUF2169 domain in the TvpA-PAAR complex between the different classes of Tvp systems. p.20
Appendix Figure S15	Interaction between TssK and TvpC by bacterial-two-hybrid assay. p.21
Appendix Table S1	List of strains used in this study. p.22
Appendix Table S2	List of plasmids used in this study. p.24

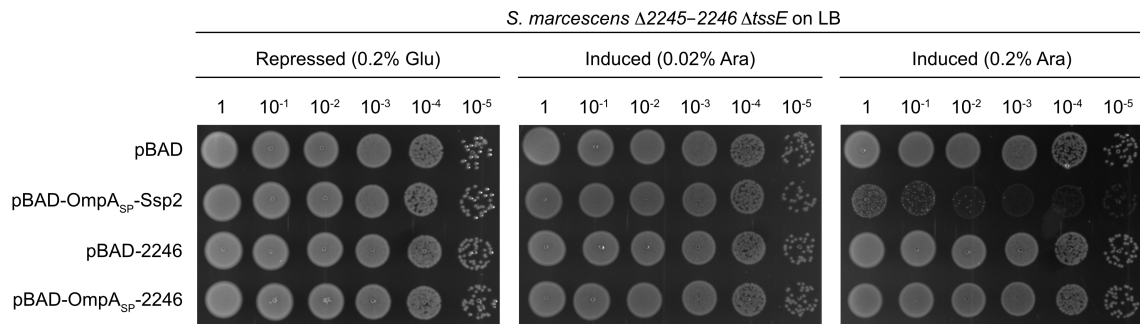
Appendix Table S3	Oligonucleotide primers and synthetic gene fragments used for plasmid construction. p.26
Appendix Table S4	Summary of the confidence scores of the Alphafold predictions presented in Figures 4 and 5. p.31
Appendix Table S5	Summary Table of VgrG β -prism length measurements. p.32
References	References for Appendix. p.34

Appendix Figure S1

A



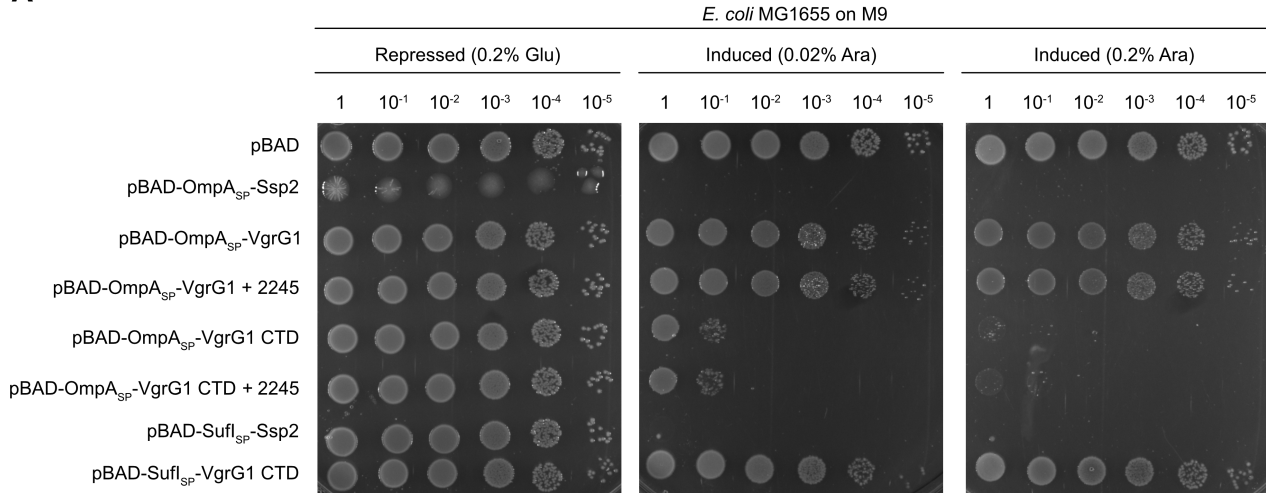
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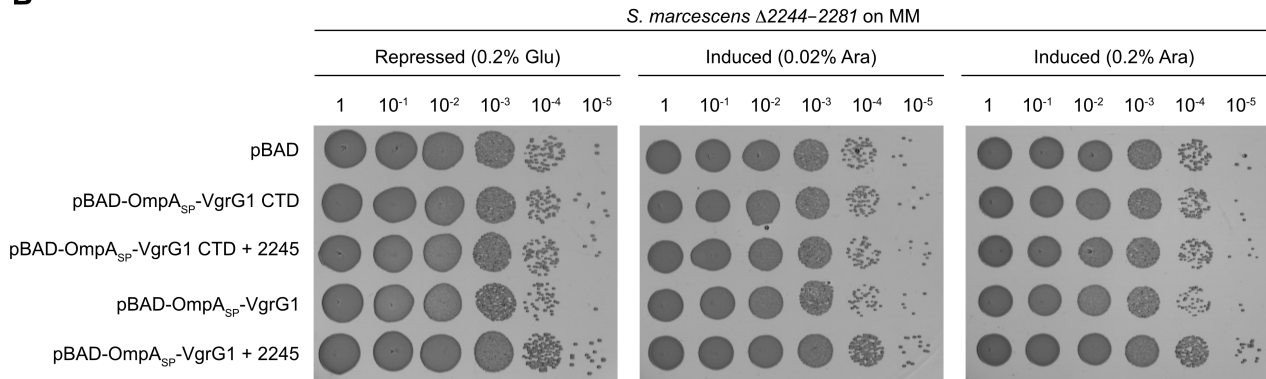
Appendix Figure S1. SMDB11_2246 is not toxic upon heterologous expression. (A) Growth on M9 minimal medium of *E. coli* MG1655 carrying empty vector control (pBAD) or plasmids directing the expression of native SMDB11_2246 (pBAD-2246) or SMDB11_2246 fused with an N-terminal OmpA signal peptide (pBAD-OmpA_{SP}-2246). **(B)** Growth on LB medium of *S. marcescens* $\Delta 2245-2246$ $\Delta tssE$ carrying the same constructs as in a). **(A-B)** Gene expression is repressed by the addition of 0.2% D-glucose or induced by the addition of 0.02% or 0.2% L-arabinose. pBAD-OmpA_{SP}-Ssp2 is used as a positive control for T6SS effector toxicity in the periplasm.

Appendix Figure S2

A



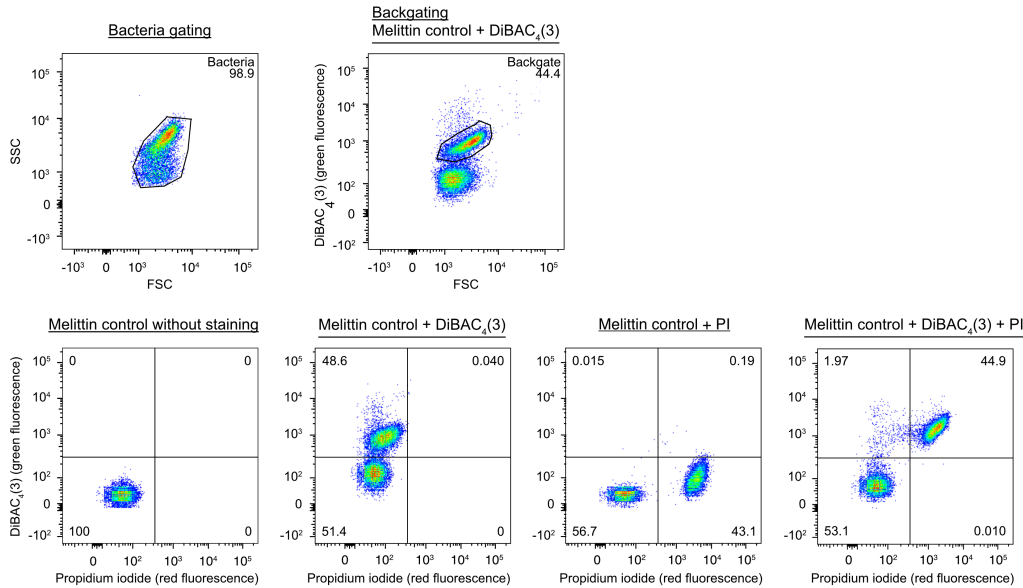
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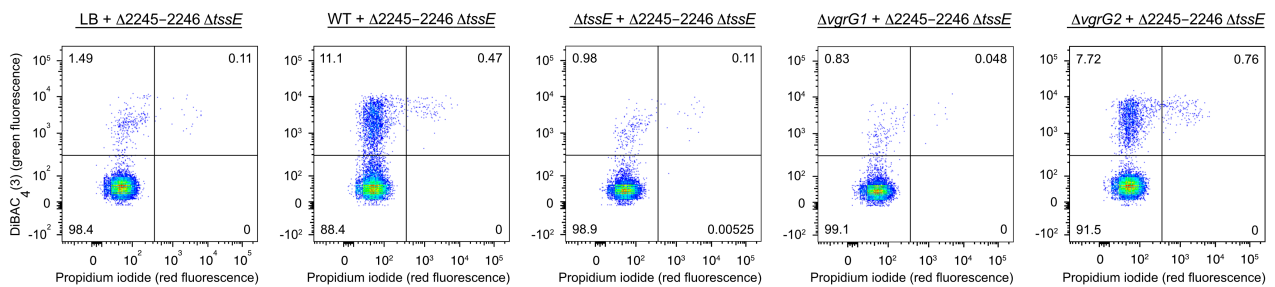
Appendix Figure S2. The VgrG1 C-terminal domain does not display specific toxicity upon heterologous expression. **(A)** Growth on M9 minimal medium of *E. coli* MG1655 carrying empty vector control (pBAD) or plasmids directing the expression of VgrG1 C-terminal domain (VgrG1 CTD, amino acids 598-786) or the full-length VgrG1, both fused with an N-terminal OmpA signal peptide (pBAD-OmpA_{SP}-VgrG1 CTD and pBAD-OmpA_{SP}-VgrG1 respectively). Co-expression of SMDB11_2245 is also tested for both VgrG1 constructs (pBAD-OmpA_{SP}-VgrG1 CTD + 2245 and pBAD-OmpA_{SP}-VgrG1 + 2245, respectively) and VgrG1 CTD expression is also tested with a N-terminal Sufl signal peptide (pBAD-Sufl_{SP}-VgrG1 CTD). pBAD-OmpA_{SP}-Ssp2 and pBAD-Sufl_{SP}-Ssp2 are used as a positive control for T6SS effector toxicity in the periplasm. **(B)** Growth on minimal medium (MM) of *S. marcescens* Δ2244–2281 carrying constructs as in A). **(A-B)** Gene expression is repressed by the addition of 0.2% D-glucose or induced by the addition of 0.02% or 0.2% L-arabinose.

Appendix Figure S3

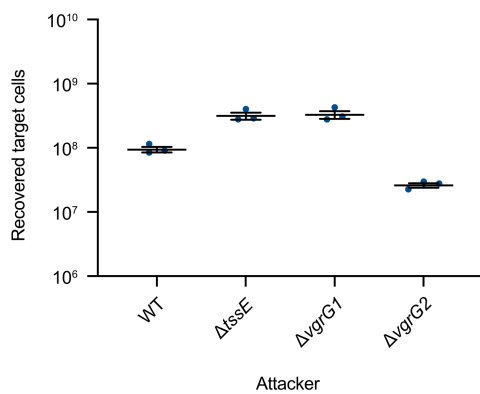
A *S. marcescens* $\Delta 2245-2246 \Delta tssE$ target only



B Competition setting (DiBAC₄(3) + PI staining) - Replicate 1



C Target: *S. marcescens* $\Delta 2245-2246 \Delta tssE$

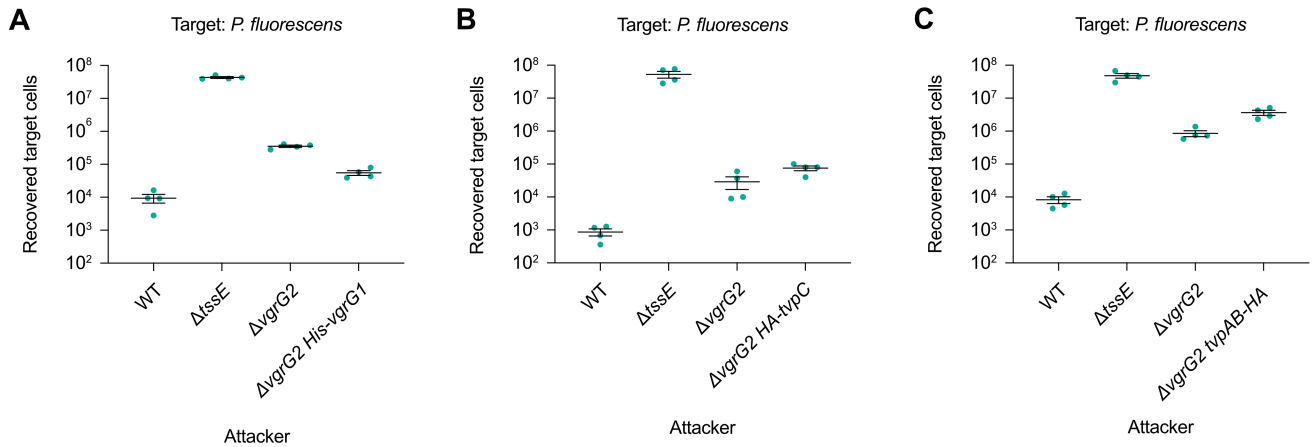


Appendix Figure S3. Example of flow cytometry experiments from the analysis presented in Figure 1G. (A) Analysis of *S. marcescens* Db10 $\Delta 2245-2246 \Delta tssE$ target only. Top panels illustrate the gating strategy. Left: Bacterial cells were selected using side scatter (SSC) vs. forward scatter (FSC). Right: Bacterial cells were treated with melittin, stained with DiBAC₄(3) (green fluorescence) and were selected using DiBAC₄(3) fluorescence vs. FSC. Bottom panels illustrate the quadrant strategy. Cells subjected to melittin treatment and stained with DiBAC₄(3) only define the depolarised cells quadrant (green fluorescence) while those treated with propidium iodide only were used to define the permeabilized cells quadrant (red fluorescence). Cells treated with melittin and stained with both DiBAC₄(3) and propidium iodide were used to establish the quadrant for cells that are simultaneously depolarised and permeabilized (green and red fluorescence). **(B)** Analysis of bacteria following co-culture of *S. marcescens* $\Delta 2245-2246 \Delta tssE$ target with wild type (WT) or

Appendix Figure S3

mutant strains of Db10 for four hours at an initial attacker:target ratio of 1:2. After four hours of co-culture, cells were treated with both DiBAC₄(3) and propidium iodide. Data shown correspond to one of the four replicates presented in Figure 1G. **(C)** Recovery of the *S. marcescens* $\Delta 2245-2246$ $\Delta tssE$ target following co-culture with WT or mutant strains under the conditions described in **(B)**. Data sets are displayed as mean $\pm$ SEM (n=3) with individual data points overlaid.

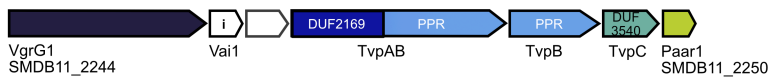
Appendix Figure S4



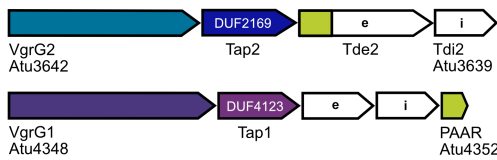
Appendix Figure S4. Epitope-tagged proteins used as bait in affinity purification and immunoprecipitation experiments retain function. (A-C) Recovery of *Pseudomonas fluorescens* target cells following co-culture with wild type (WT) and mutant strains of *S. marcescens* Db10 at an initial ratio of 1:1 for four hours. The functionality of the tagged proteins, namely His-VgrG1 (**A**), HA-TvpC (**B**), and TvpAB-HA (**C**), was assessed in a $\Delta vgrG2$ background where loss of function in the VgrG1 pathway results in a complete loss of T6SS activity. Data sets are displayed as mean $\pm$ SEM (n=4) with individual data points overlaid.

Appendix Figure S5

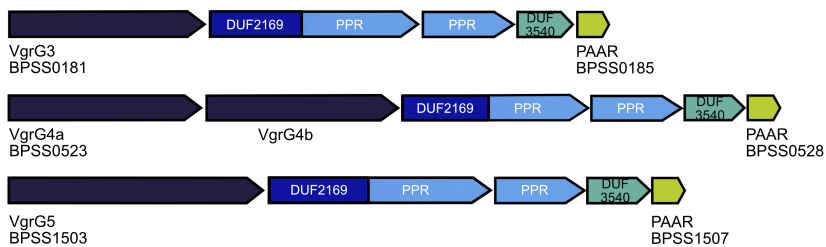
***Serratia marcescens* Db10**



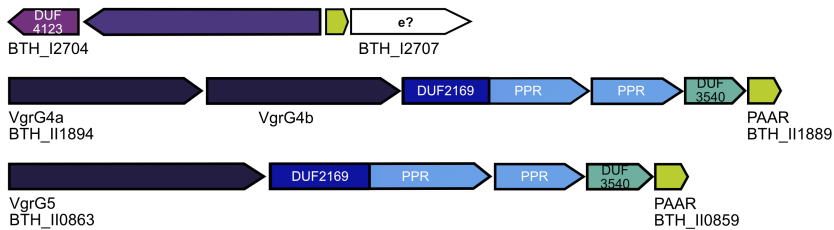
***Agrobacterium tumefaciens* C58**



***Burkholderia pseudomallei* K96243**



***Burkholderia thailandensis* E264**



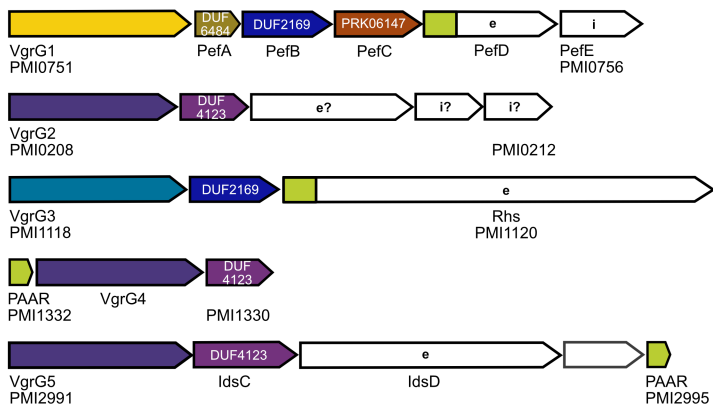
***Cronobacter sakazakii* ES15**



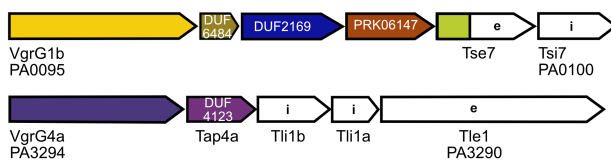
***Pantoea ananatis* PA13**



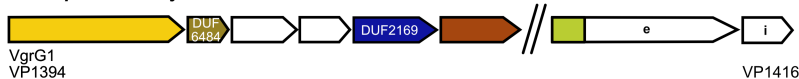
***Proteus mirabilis* HI4320**



***Pseudomonas aeruginosa* PAO1**



***Vibrio parahaemolyticus* RIMD 2210633**

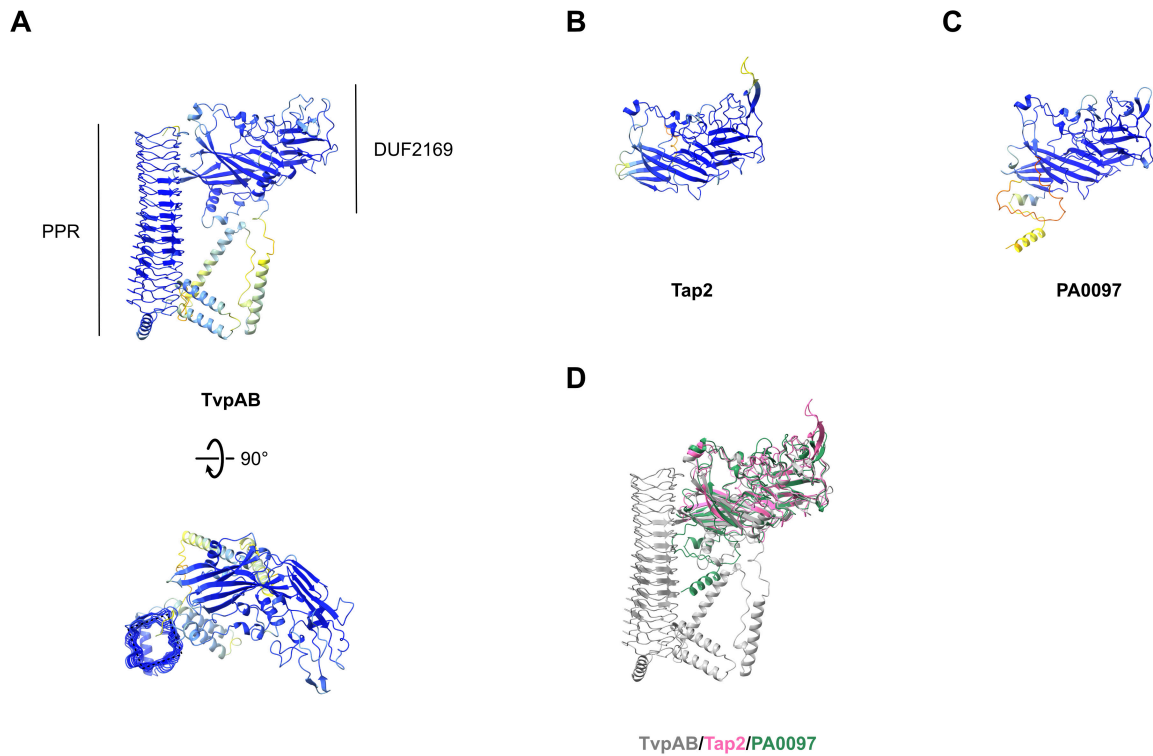


450nt

Appendix Figure S5

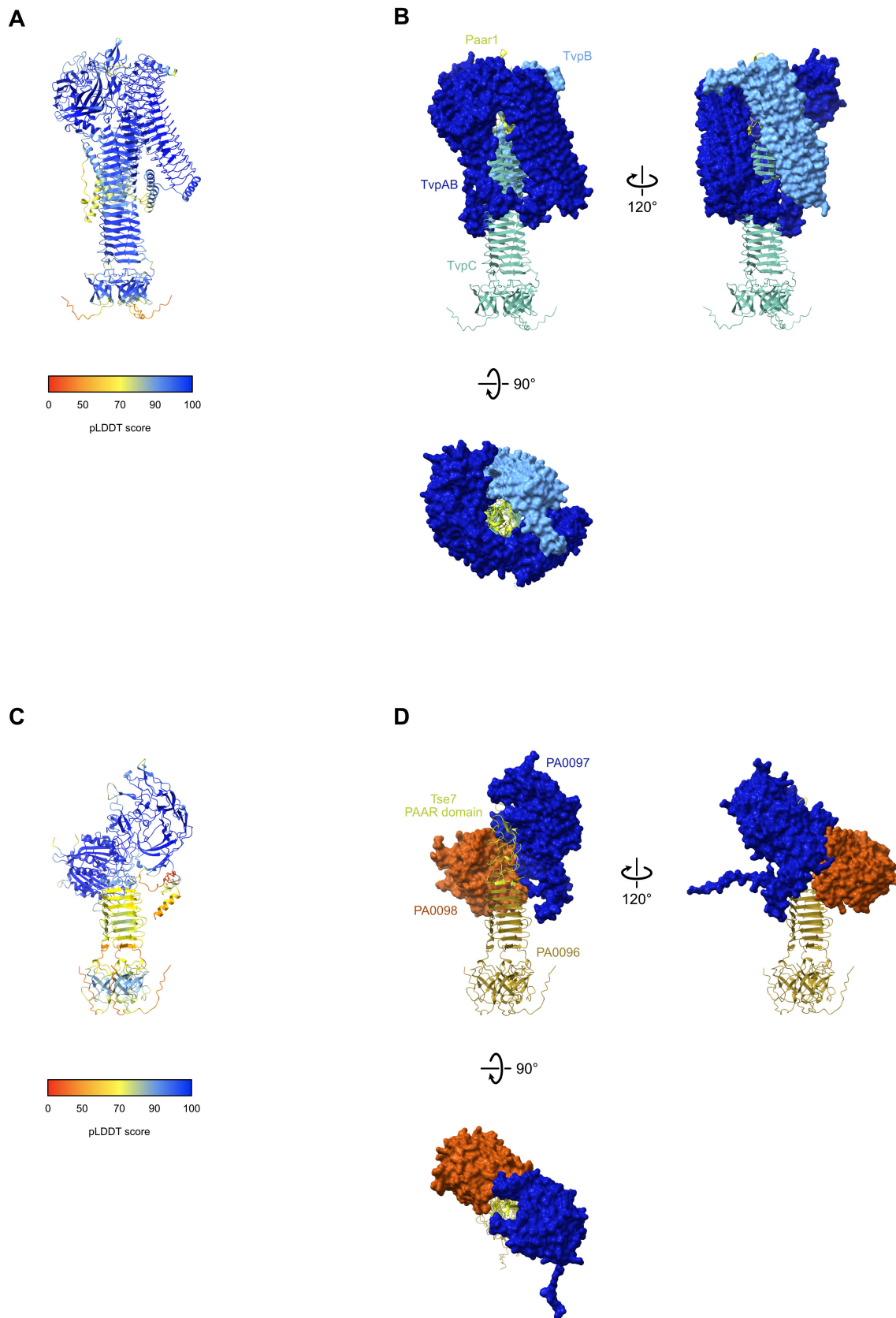
Appendix Figure S5. Conservation of Tvp accessory proteins in representative Gram-negative species. The genetic context of T6SS-associated DUF2169-containing accessory proteins in *Serratia marcescens* Db10 (accession: HG326223), *Agrobacterium tumefaciens* C58 (accession: AE007869.2), *Pantoea ananatis* PA13 (accession: CP003085.1), *Cronobacter sakazakii* ES15 (accession: AFK01427), *Proteus mirabilis* HI4320 (accession: NC_010554.1), *Pseudomonas aeruginosa* PAO1 (accession: AE004091.2), *Burkholderia pseudomallei* K96243 (accession: NC_006350.1 and NC_006351.1) and *B. thailandensis* E264 (accession: CP000085.1 and CP000086.1). If present in these genomes, the genetic context of DUF4123-containing accessory proteins is also depicted. Each open reading frame (ORF) is represented by an arrow whose size is proportional to its gene length. The name and/or the genomic identifier of the ORFs at each end is indicated below each set of genes. When identified, conserved domains (PPR for pentapeptide repeat, DUF3540, DUF6484, PRK06147) are also highlighted inside the ORFs as well as effector (e) and immunity (i) encoding genes.

Appendix Figure S6



Appendix Figure S6. Predicted structures of TvpAB, Tap2 and PA0097 proteins. (A) Ribbon representation of the predicted structure of TvpAB from *S. marcescens* Db10. The DUF2169 and pentapeptide repeat (PPR) domains are highlighted. A 90° rotated view is provided to show the quadrilateral shape of the PPR β -helix. (B) Ribbon representation of the predicted structure of the *Agrobacterium tumefaciens* DUF2169-containing accessory protein Tap2. (C) Ribbon representation of the predicted structure of the *Pseudomonas aeruginosa* DUF2169-containing accessory protein PA0097. (D) Superposition of TvpAB (grey), Tap2 (pink) and PA0097 (green) predicted structures. (A-C) Predicted structures were generated by AlphaFold2 and coloured according to the pLDDT score, from red (pLDDT <50, lowest confidence) to blue (pLDDT >90, highest confidence).

Appendix Figure S7

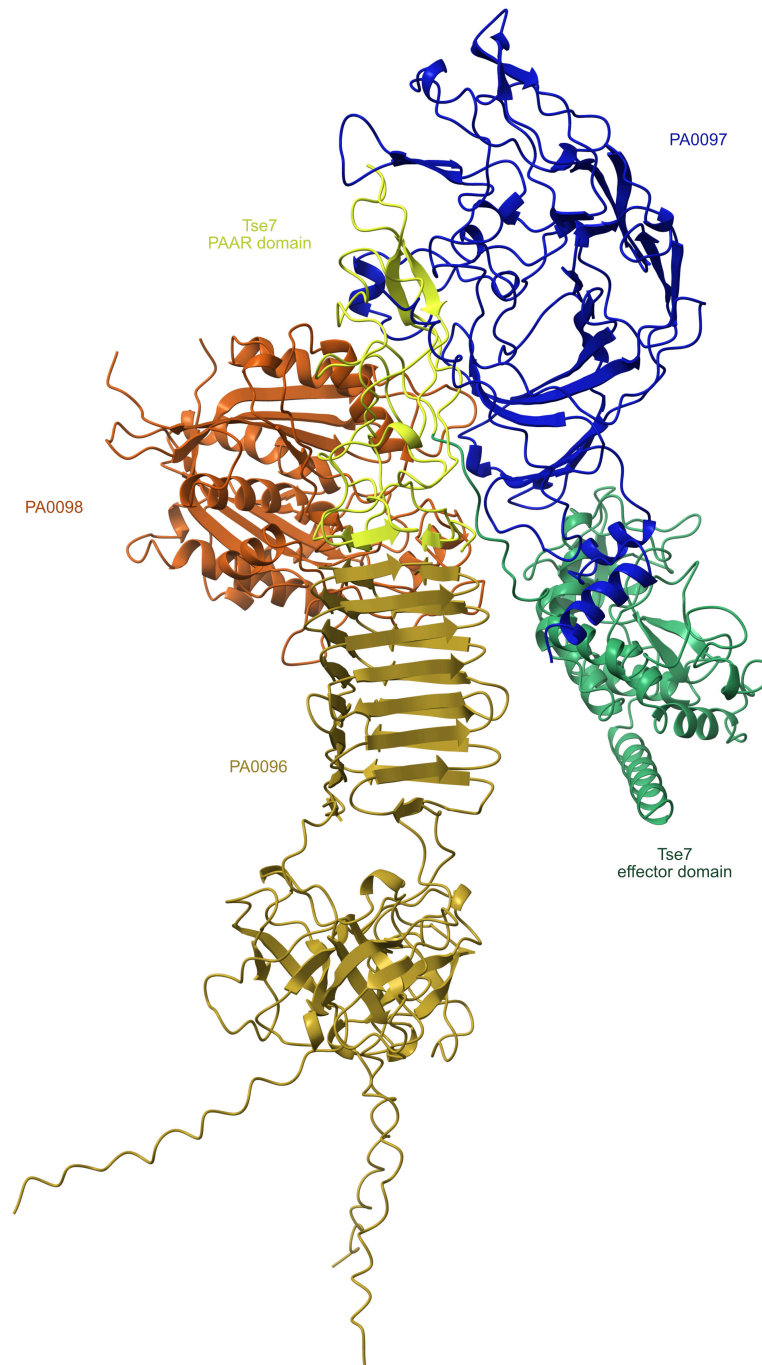


Appendix Figure S7. Predicted structures of *Serratia marcescens* and *Pseudomonas aeruginosa* accessory pre-complexes. (A) Ribbon representation of the *Serratia marcescens* Tvp accessory pre-complex coloured according to the pLDDT score from AlphaFold2 modelling, from red (pLDDT <50, lowest confidence) to blue (pLDDT >90, highest confidence). **(B)** The *S. marcescens* Tvp accessory pre-complex, as in Figure 6a, with the surface of TvpAB and TvpB highlighted to show how the proteins wrap the tip of the pre-complex. **(C)** Ribbon

Appendix Figure S7

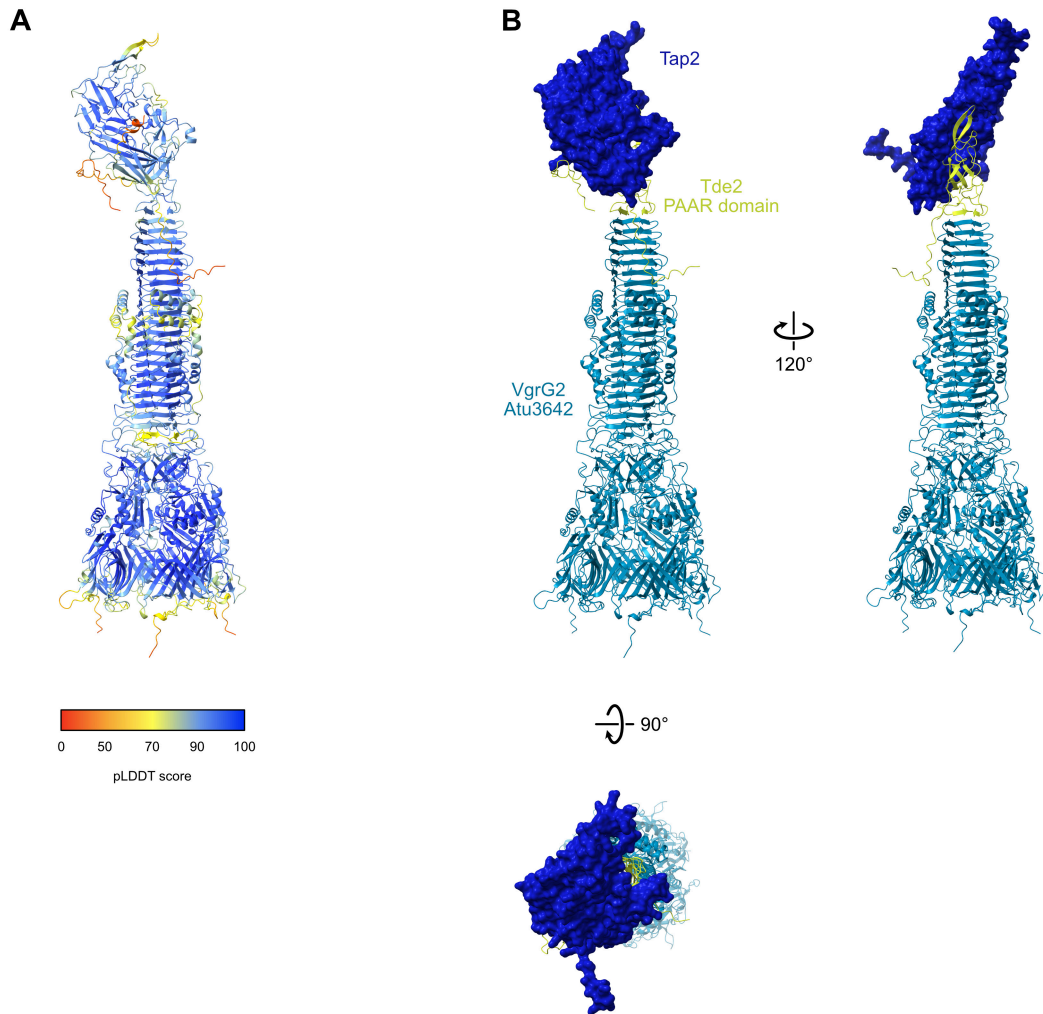
representation of the *Pseudomonas aeruginosa* accessory pre-complex coloured according to the pLDDT score from AlphaFold2 modelling, from red (pLDDT <50, lowest confidence) to blue (pLDDT >90, highest confidence). **(D)** The *P. aeruginosa* accessory pre-complex, as in Figure 6B, with the surface of PA0097 and PA0098 highlighted to show how the proteins wrap the tip of the pre-complex.

Appendix Figure S8



Appendix Figure S8. Predicted structure of the *Pseudomonas aeruginosa* accessory pre-complex with the full Tse7 effector. Ribbon representation of the predicted structure of the *P. aeruginosa* accessory pre-complex generated with AlphaFold2. PA0096 is colored in ginger, PA0097 in medium blue, PA0098 in rust, the PAAR domain of Tse7 effector in light green and the effector domain of Tse7 in sea green

Appendix Figure S9



Appendix Figure S9. Predicted structure of the *Agrobacterium tumefaciens* VgrG2-associated accessory complex. (A) Ribbon representation of the *Agrobacterium tumefaciens* VgrG2-associated accessory complex coloured according to the pLDDT score from AlphaFold2 modelling, from red (pLDDT <50, lowest confidence) to blue (pLDDT >90, highest confidence). **(B)** The *A. tumefaciens* VgrG2-associated accessory complex, as in Figure 6E, with the surface of Tap2 highlighted to show how the protein wraps the tip of the complex.

Appendix Figure S10

A

S_marcescens_Db10/1-375
A_tumefaciens_C58/1-412
P_aeruginosa_PAO1/1-401
P_mirabilis_PMI0755/1-392
P_mirabilis_PMI1120/1-3393
C_sakazakii_ES15/1-375
P_ananatis_PA13/1-375
B_pseudomallei_BPSS0185/1-368
B_pseudomallei_BPSS1507/1-317
B_pseudomallei_BPSS0528/1-358
B_thailandensis_BTH_II0859/1-317
B_thailandensis_BTH_II1889/1-358
V_paraahaemolyticus_RIMD2110633/1-376

PAAR
delimiter
sequence
TvpA

1 DVCLTPSPAPV-----PVPYPDIALAFTAIPNAFNLIFV-GTPAHNMATVPTLTNGDNP---GV--ATGVSAGTVM--GPSRHLTGAFTVLTKGTPATRLTSLSLONSAAAAAAVSVLALADMG-----SPRLRPEPELWOLADELTLTG 134
1 DVCLTPVGSVV-----PIPYVDFGCHDNKYTSVRFT-GKKAMVMRSCTTHVHGDAF---GV--RKGVKSGTVE--SVCEPIGHADOVAEGSHVIRHLDRFHMNSAAAAAAVSVRATFTHVDGDT--MEIARECESFQWEDAYEGDPHQ 138
1 DVCFTPPQAPPTLGLVPIPYNTGLSKDITTKGTRITRIRKEVMLKNKSYKTSYGDPEP---GRABKKGLVTSKIK--GKVYFTSWMNVKFESKNVVRHLDTTHNHAATAAAVIAKGSFDLPDLDGE--ARLLDEQOTLLMVDIFYGEPGF 146
1 DVCWSPGSSVY-----PYNPNTSADALNGTATVEIRNSMVAQEDRSFFSISTGDEPATNGL--AKGVITGVIK--GKAYFRSWSLNVKFGGYGVARHQDLMShNHAATAAAVVIKATYHIPENNQL--PRPIILPSOFLYYSIDIYGEVQK 140
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TvpA binding region

TvpA

S_marcescens_Db10/1-375
A_tumefaciens_C58/1-412
P_aeruginosa_PA01/1-401
P_mirabilis_PMI0755/1-392
P_mirabilis_PMI1120/1-393
C_sakazakii_ES15/1-375
P_ananatis_PA13/1-375
B_pseudomallei_BPSS0185/1-368
B_pseudomallei_BPSS1507/1-317
B_pseudomallei_BPSS0528/1-358
B_thailandensis_BTH_IJ0859/1-317
B_thailandensis_BTH_IJ1889/1-358
V_parahaeemolyticus_RIMD210633/1-376

132 **G** **V** **L** **D** **L** **A** **I** **P** **K** **C** **A** **E** **F** **L** **T** **G** **N** **A** **-** **Y** **T** **H** **Q** **D** **K** **-** **-** **T** **A** **C** **A** **V** **R** **I** **D** **E** **N** **L** **S** **K** **L** **A** **V** **-** **F** **G** **D** **R** **Y** **-** **-** **I** **N** **D** **R** **P** **T** **L** **P** **F** **A** **E** **R** **M** **L** **D** **W** **R** **R** **A** **Y** **G** **G** **T** **F** **-** **A** **D** **-** **N** **P** **H** **G** **I** **G** **-** **A** **T** **P** **E** **T** **F** **P** **O** **G** **R** **I** **-** **H** **R** **L** **P** **N** **V** **-** **231**
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147 **S** **A** **R** **I** **Y** **D** **A** **D** **F** **C** **K** **P** **O** **C** **D** **V** **I** **F** **N** **A** **K** **-** **E** **A** **P** **H** **K** **K** **P** **I** **-** **-** **S** **Q** **L** **I** **S** **W** **O** **V** **D** **I** **M** **K** **I** **A** **V** **-** **C** **R** **N** **W** **C** **K** **-** **E** **G** **L** **Y** **K** **P** **Q** **V** **A** **E** **T** **P** **L** **H** **G** **V** **A** **F** **G** **K** **Y** **-** **-** **Y** **K** **D** **E** **I** **S** **H** **I** **-** **N** **P** **L** **G** **I** **G** **F** **L** **S** **R** **K** **P** **N** **K** **E** **I** **-** **K** **M** **P** **A** **I** **-** **280**
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135 **G** **V** **-** **-** **I** **D** **M** **A** **I** **P** **K** **V** **R** **A** **E** **F** **L** **T** **G** **H** **A** **-** **Y** **T** **H** **Q** **Q** **E** **K** **-** **-** **T** **A** **C** **A** **V** **R** **I** **D** **E** **N** **L** **S** **K** **L** **A** **V** **-** **F** **G** **D** **R** **Y** **-** **-** **A** **G** **S** **R** **M** **T** **A** **P** **R** **P** **F** **E**

TvpA

S_marcescens_Db10/1-375
A_tumefaciens_C58/1-412
P_aeruginosa_PAO1/1-401
P_mirabilis_PM1075/1-392
P_mirabilis_PM11220/1-393
C_sakazakii_ES15/1-375
P_anatis PA13/1-375
B_pseudomallei_BPSS0185/1-368
B_pseudomallei_BPSS1507/1-317
B_pseudomallei_BPSS0528/1-358
B_thailandensis_BTH_I10859/1-317
B_thailandensis_BTH_I11889/1-358
V_parahaeemolyticus_RIMD211063/1-376

282 **T**PLQGLRALSPPGRSQAIP-A-----SFDA~~LD~~LITWPRRFSR--GKNYDADWLKHG**P**FGFANDIDWRLFNMAESDQ**Q**Q**P**QRSDLPPRAAYRIWNMHPSSEPVQ**Q**KG**L**PP-----WRAR**Q**CFINLRGGETH**F**EELIAMR**T**ITWVFF--PHREQMMLVY-375

282 **T**SPGLERLD-WKDAPEP-A-----G**F**GLVLSRWRRS**Q**Q**Y**YTG--GT-YDDAWAERH**P**L**P**DR**P**DFRFQWAAQ**Q**DLVAT--**P**HLKGDEKYELVNLSS**H**EPVAKG**S**LP--**L**T**L**GV**S**CHSREDEDEWHV---**L**KD**G**VO**D**WRD**G**DR**L**LLTWIR412

272 **E**KL**G**EP**V**DS**P**HGRFQT-M-----AL**G**PLGRHQWAQ**V**GF**A**--GR-YDADWLAE**R**FF**L**PA**D**DFER**V**FQSA**P**AD**Q**W**I**---**D**HLRGSEVILLN**L**LT**G**E**R**-RAAFR**V**PR-REVP**V**TF**F**KL**Q**---GG**H**ETA**A**---**R**LD**L**LD**V**L**G**-CDARR**E**VTWIR401

281 **C**IDEQINLT**R**FSQIN**P**-J-----ALS**V**LR**H**RYG**E**RVKY**A**--GT-FDEKWRNETA**P**L**P**DR**F**DER**V**FQSA**P**AD**Q**W**I**---**D**FG**I**NEV**I**LNN**I**HL**R**SK**I**FN**K**L**R**LN**L**---**V**RFY**R**Q**G**---GEV**V**LT**F**---**S**VD**T**LY**F**E**P**---CD**Q**FF**S**VTWIR392

256 **C**IMIDELIKRRLD**L**SLID**T**RQISE**G**MP**I**SRWMAQ**E**RY**A**--GT-Y**G**KEWQEN**Q**FP**F**Y**P**DD**F**DERFFNS**A**HP**S**LR**P**-**G**Y**L**L**G**NE**E**L**L**EG**L**L**P**SSRWVLT**P**D---**V**R**K**IL**L**Q**D**IE**G**EL**S**FL**K**P---**D**LD**T**L**I**D**L**-LDRRLIS**V**K**R**393

239 **E**PFEGRMIS**P**KQ**Q**PEP-A-----S**F**GLD**L**ITWPRRFSR--GKNYDASWLQND**F**PG**F**AK**D**IDW**V**FNAAS**P**DQW**Q**DRD**S**L**P**SOAKWRIWNMH**P**K**P**Q**E**GT**L**PP-----WQAR**Q**CFINR**Q**REDD**T**L**F**EVALR**T**ITWVFF--PHLEQMMLIW-375

239 **E**AMSGRLTQ**K**Q**K**PEP-A-----G**F**GLD**L**ISWPRRFSR--GKKYDSSWLQND**F**PG**F**AK**D**IDW**V**FNAAS**P**DQW**Q**VRD**R**L**P**EQAAWRIWNMH**P**K**P**Q**V**Q**E**KL**P**PP-----WQAR**Q**CFINR**Q**REDD**T**L**F**EELISL**R**ATTWVFF--PHLEQMMLVW-375

234 **E**Y**A**HSMPRFA**H**Q**E**AP-A-----G**F**CPVDAWPAARAG**L**--GA-LDROWQEED**Q**PG**F**RT**R**LPY**N**I**A**PAD**Q**W**I**EL**R**AF**G**YELD**G**AR**E**LTHM**P**PHAT**L**AG**N**LP**A**---LRAR**S**FFV**R**---RGS**D**A**E**EM**P**MR**L**ITAW**F**V--PHRER**V**ILY368

189-----DARDP**A**RV**L**A-----D**F**RAL**P**SH**P**ORV**R**DL--GA-LDRAWLAE**R**W**H**PS**G**RA**E**H**F**HTA**P**DR**I**A**A**---G**F**WRG**D**DE**I**ELV**N**LA**H**Q**H**I**A**GA**L**IP**A**---VRAC**F**CV**E**RSAG**A**TRD**V**CD**M**RA**E**TW**L**F--PGAAC**G**IVLVR317

221 **T**PLQQLR**T**FGV**S**PA**L**A-----AMGA**I**NPDW**E**RAQW**M**TR**F**GT-LDAMAQ**Q**D**H**MGW**P**AD**V**LR**F**FQAA**P**DQW**A**R**G**---E**C**WT**P**GA**R**FE**L**SG**F**PG**R**RG**E**GA**G**EL**P**---LAPALV**T**R---NGR**P**IE**L**RS**F**Q**Q**ITAW**L**F--**P**DR**G**IGVLWV-358

199-----E**F**RAL**P**SH**P**ERS**R**DL--GA-FDERLAA**R**W**H**LPAG**T**RA**E**H**F**HTA**P**DR**I**A**A**---G**F**WRG**D**DE**I**ELV**N**LA**H**DR**P**I**A**GA**L**IP**A**---VRAC**F**CV**E**RWV**G**GVAR**I**CD**M**RA**E**TW**L**F--PGAAC**G**IVLVR317

221 **T**PLQQLR**S**FGV**S**PA**L**A-----AMGA**I**NPDW**E**RAQW**M**TR**F**GT-VDAMAQ**Q**D**H**MGW**P**AD**V**LR**F**FQAA**P**DQW**A**R**G**---E**C**W**M**PGAR**E**LM**S**FG**P**RG**R**GE**G**AL**P**---LAPALV**T**R---NGR**P**IE**L**RS**F**Q**Q**ITAW**L**F--**P**DR**G**IGVLWV-358

248 **F**PK**E**W**S**TSK**N**LR**L**A-----G**F**GPV**G**DFE**A**RQ**T**LA--GT-FDEEW**I**ENR**K**ML**E**DR**D**RF**F**QSA**P**AD**Q**Q**C**K---G**L**FGK**G**GER**L**MM**S**FG**C**H**D**-T**I**SFR**I**PK**E**---E**K**Y**A**IT**F**KG**S**Y---**P**VD**M**AF**T**W**L**D**V**-A**E**K**T**IS**S**Y-376

PAAR binding loop

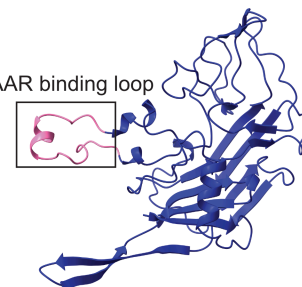
B

TvpA binding
region



C

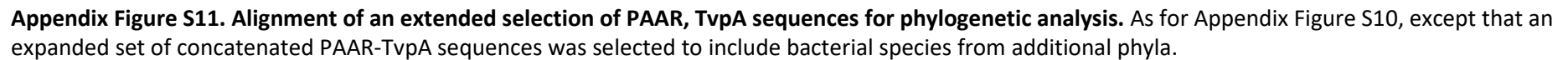
PAAR binding loop



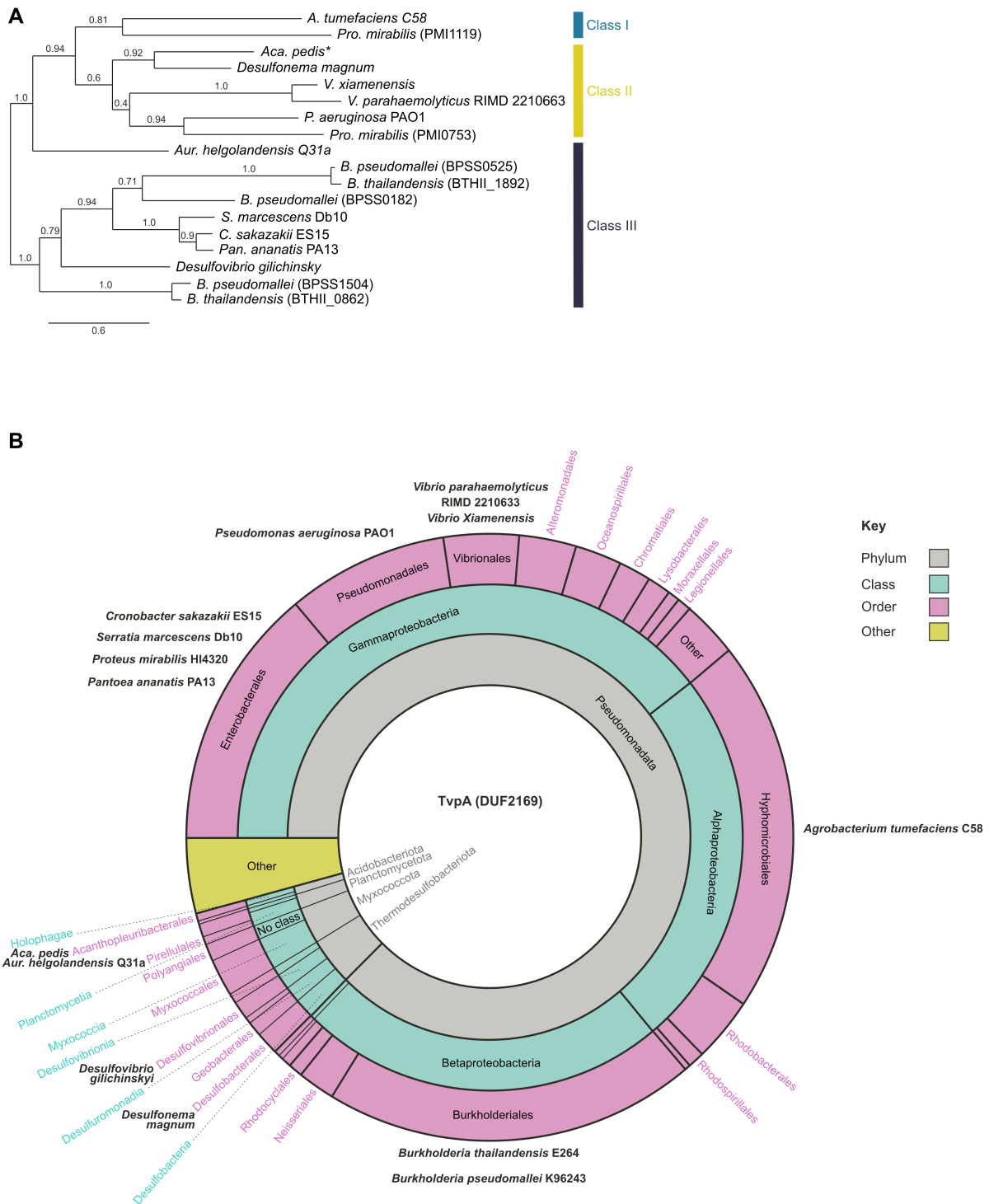
Appendix Figure S10

Appendix Figure S10. Identification of conserved regions of the TvpA and PAAR DUF4150 proteins for phylogenetic analysis of the Tvp system. (A) A concatenated PAAR-TvpA sequence was used to generate a multiple sequence alignment using the MUSCLE algorithm (Edgar, 2004) and coloured using ClustalX (Thompson, 1997). The alignment was also used for the purpose of phylogenetic tree construction. The chosen sequences were validated against structural comparisons between the corresponding AlphaFold3 predicted structures and each represents a conserved structural region. The conserved region is represented by (B) PAAR amino acids 16-110 (shown in green-yellow) and (C) TvpA amino acids 26-299 (shown in medium-blue) both from *S. marcescens* Db10. Highlighted within these regions is the conserved TvpA binding interface of PAAR (black box in A, black ribbon in B) and the corresponding PAAR binding loop from TvpA (pink box in A, pink ribbon in B). Ribbon representations of structures in B) and C) were generated in ChimeraX (Meng *et al*, 2023).

TvpA

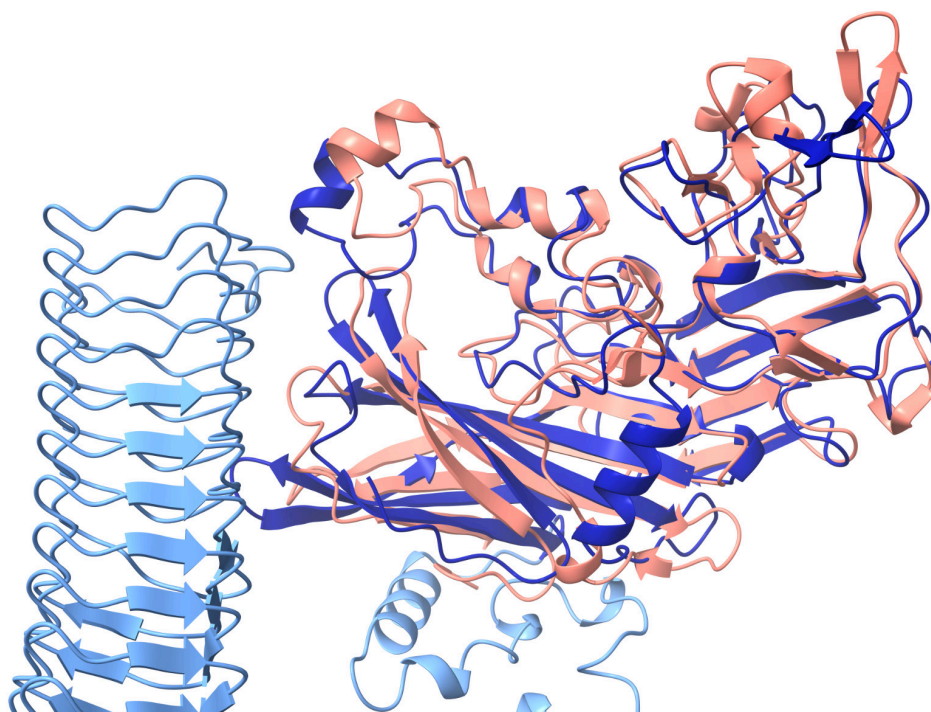


Appendix Figure S12



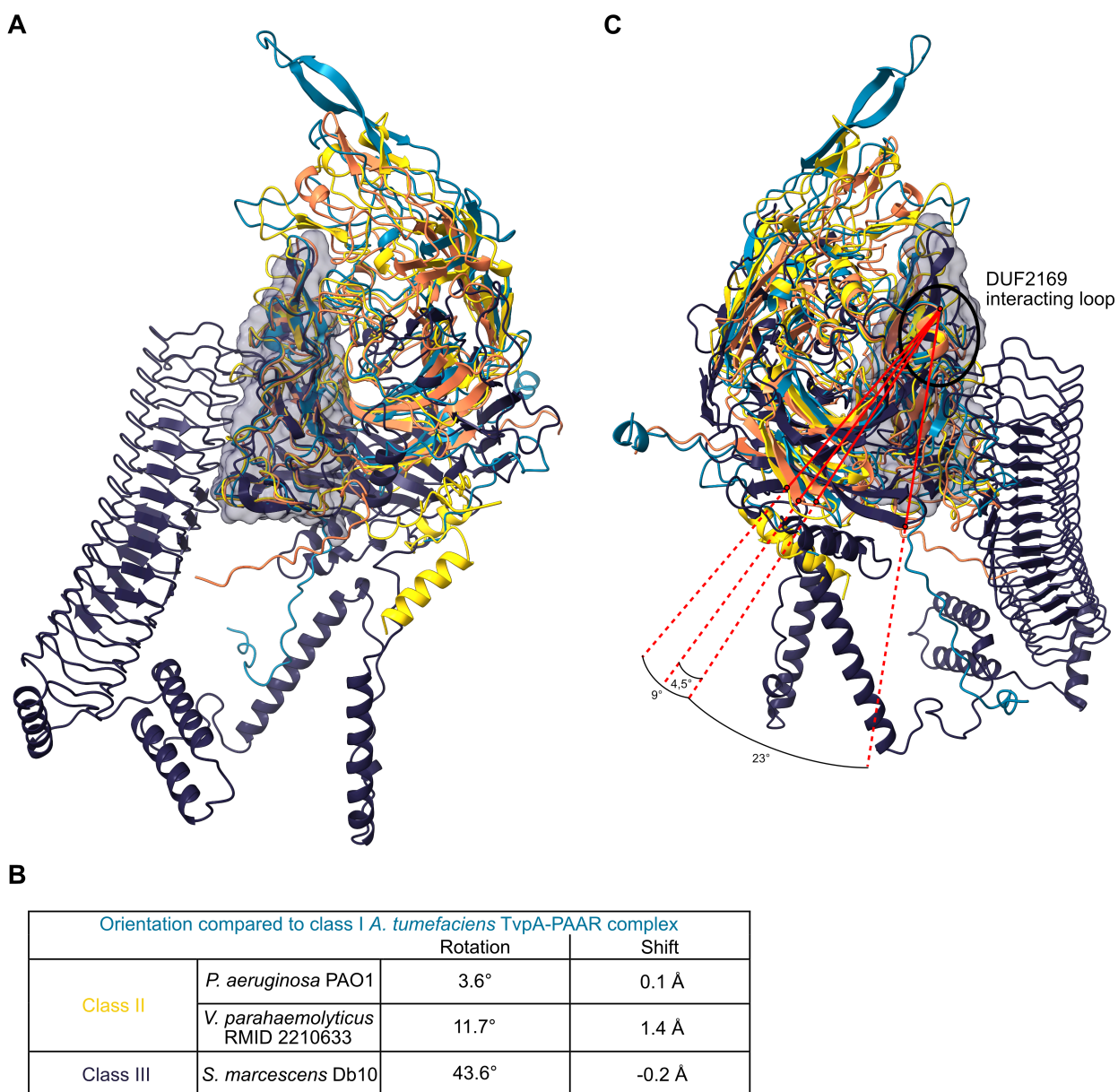
Appendix Figure S12. Extended phylogeny and taxonomy. (A) An extended phylogenetic tree, similar to that in Figure 8A, was constructed from the expanded set of sequences in the extended sequence alignment in Appendix Figure S11. This represents an extension from the *Pseudomonodonta* phylum to include representatives from additional phyla (Dereeper *et al*, 2008). Where strains contain multiple Tvp systems, the accession number for the TvpA protein is enclosed in brackets to distinguish between them. *No syntenous TvpC protein. **(B)** Taxonomic distribution of TvpA (DUF2169) genes (InterPro entry: IPR018683; Pfam: PF09937) (Mistry *et al*, 2021; Blum *et al*, 2025) as in Figure 8D with the positions of *Acanthopleuribacter pedis* (Acidobacteriota), *Aureliella helgolandensis* (Planctomycetota), *Desulfonema magnum* and *Desulfovibrio qilichinskyi* (Thermodesulfobacteriota) indicated.

Appendix Figure S13



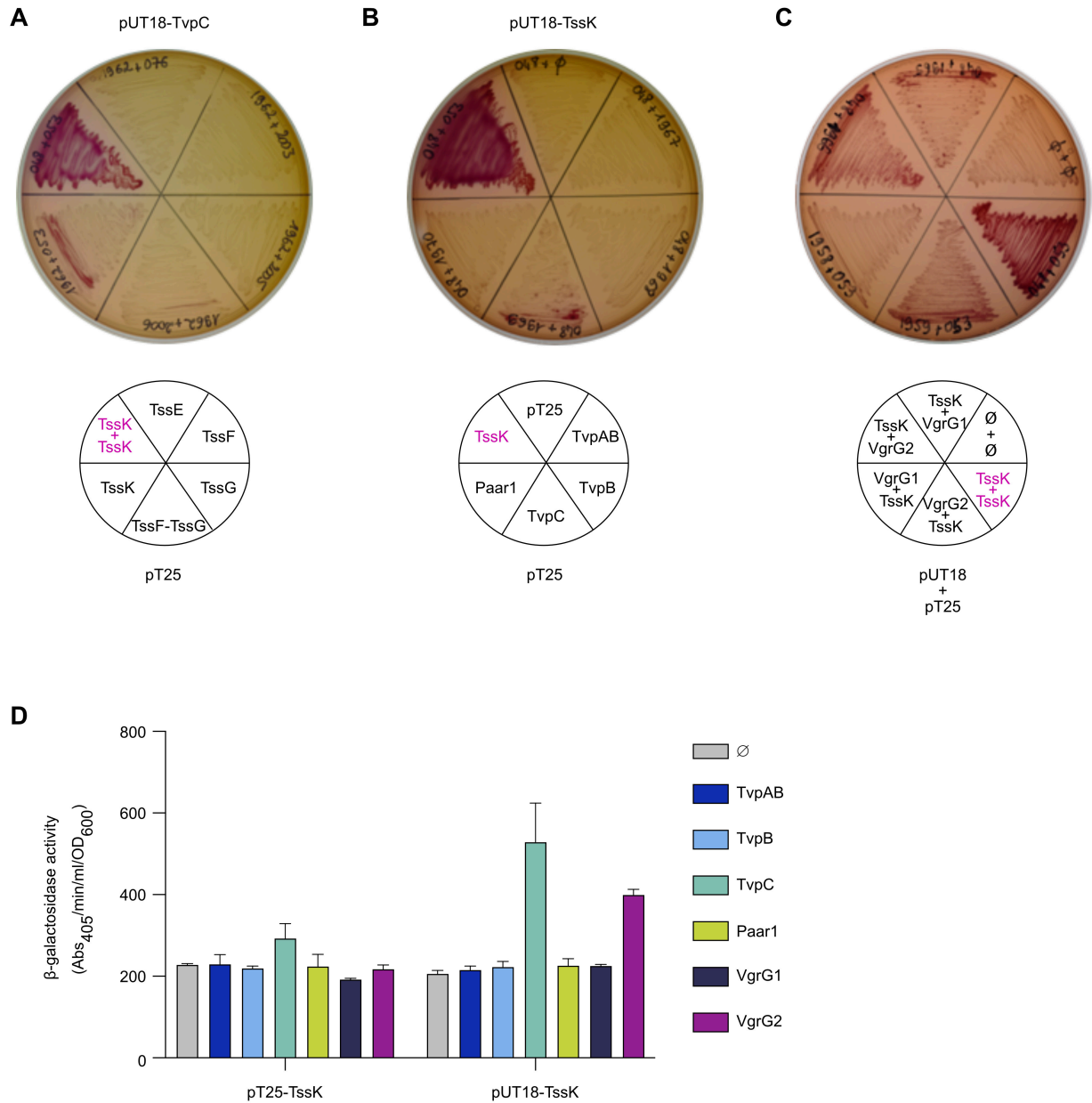
Appendix Figure S13. Comparison of the AlphaFold3 predicted structure of TvpAB from *Serratia marcescens* Db10 with the experimentally-determined structure of TvpA of *Vibrio xiamenensis*. Ribbon representations of the AlphaFold3 model of TvpAB from *S. marcescens* and the crystal structure of *V. xiamenensis* TvpA (PDB: 8VTH). Superimposition of the structures was performed using ChimeraX (Meng *et al*, 2023) (RMSD = 1.076 Å between 140 pruned atom pairs) and the figure focuses on the DUF2169 domain. The structure of the TvpA domain of Db10 (amino acids 1-323) is shown in dark blue while the rest of the structure is shown in light blue. The *V. xiamenensis* TvpA protein is shown in salmon.

Appendix Figure S14



Appendix Figure S14. Comparison of the orientation of the DUF2169 domain in the TvpA-PAAR complex between the different classes of Tvp systems. (A) AlphaFold3 structural predictions of TvpAB-Paar1 from *S. marcescens* Db10 (dark blue), Tap2-PAAR domain of Tde2 from *A. tumefaciens* C58 (cyan), PA0097-PAAR domain of Tse7 from *P. aeruginosa* PAO1 (yellow), and VP1398-PAAR domain of VP1415 from *V. parahaemolyticus* RIMD 2210633 (salmon) are represented as cartoons and superimposed by their PAAR domain. The surface of Db10 Paar1 is highlighted. **(B)** Table summarizing the rotation and shift required to realign the DUF2169 domain of all the TvpA proteins to the DUF2169 domain of Tap2 from *A. tumefaciens* starting from the superimposition presented in A). **(C)** Same structural predictions as in A) but superimposed by the short α -helix located in the interacting loop defined by Sachar *et al.* (Sachar *et al*, 2025). An axis was drawn between the top of this short α -helix and the end of the same β -strand for all the structures (red line). Flexibility of the DUF2169 domain among the different classes is estimated by the rotation angle formed between the axis of *A. tumefaciens* (Class I) and those of Class II (*P. aeruginosa* and *V. parahaemolyticus*) or Class III (*S. marcescens*)

Appendix Figure S15



Appendix Figure S15. Interaction between TssK and TvpC by bacterial-two-hybrid assay.

A-C) Detection of interactions between T6SS components by observing growth of *E. coli* Δ *cya* carrying derivatives of the pUT18 and pT25 vectors directing the expression of T18 and T25 fusions with proteins of interest on MacConkey maltose plates. A red colour indicates positive interaction between proteins in the two-hybrid system. As a positive control, the interaction between TssK monomers was assayed, the negative control was empty pUT18 and pUT25 plasmids ($\emptyset + \emptyset$). **(A)** Testing the interaction between TvpC (in pUT18) and baseplate components (in pT25) from *S. marcescens* Db10 when expressed in *E. coli* Δ *cya*. **(B)** Testing the interaction between TssK (in pUT18) and Tvp accessory proteins (in pT25) from *S. marcescens* Db10 when expressed in *E. coli* Δ *cya*. **(C)** Testing the interaction between TssK and VgrG proteins from *S. marcescens* Db10 (either in pUT18 or pT25) when expressed in *E. coli* Δ *cya*. **(D)** Measurement of β -galactosidase activity to quantify the interactions between TssK and Tvp accessory proteins or VgrG proteins presented in A-C). Measurement of the absorbance at 405 nm was performed following incubation of toluene-permeabilized cells for 30 minutes at 37°C with ONPG substrate.

Appendix Table S1

Name	Description/Genotype	Source/reference
Strains		
<i>Serratia marcescens</i> Db10		
Db10	Wild-type	(Flyg <i>et al</i> , 1980)
SJC11	$\Delta tssE$ (SMDB11_2271)	(Murdoch <i>et al</i> , 2011)
FRA01	$\Delta vgrG2$ (SMDB11_2276)	(Cianfanelli <i>et al</i> , 2016)
FRA02	$\Delta vgrG1$ (SMDB11_2244)	(Cianfanelli <i>et al</i> , 2016)
FRA03	$\Delta vgrG1 \Delta vgrG2$	(Cianfanelli <i>et al</i> , 2016)
DWL05	$\Delta vai1$ (SMDB11_2245)	This study
DP10	$\Delta SMDB11_2246$	This study
DP09	$\Delta vai1 \Delta tssE$	This study
DP15	$\Delta vai1 \Delta tssE$ Sm-resistant derivative	This study
DP13	$\Delta SMDB11_2246 \Delta tssE$	This study
DP16	$\Delta SMDB11_2246 \Delta tssE$ Sm-resistant derivative	This study
DWL03	$\Delta vai1 \Delta 2246 \Delta tssE$	This study
DWL08	$\Delta vai1 \Delta 2246 \Delta tssE$ Sm-resistant derivative	This study
KT13	$\Delta SMDB11_2244-2281$	(English <i>et al</i> , 2012)
LM32	$\Delta vgrG2 \Delta SMDB11_2246$	This study
KK1	$\Delta tvpAB$ (SMDB11_2247)	This study
DP11	$\Delta tvpB$ (SMDB11_2248)	This study
DWL04	$\Delta tvpC$ (SMDB11_2249)	This study
FRC32	$\Delta paar1$ (SMDB11_2250)	(Cianfanelli <i>et al</i> , 2016)
DP02	$\Delta vgrG2 \Delta tvpAB$	This study
CE76	$\Delta vgrG2 \Delta tvpB$	This study
DP03	$\Delta vgrG2 \Delta tvpC$	This study
FRC26	$\Delta vgrG2 \Delta paar1$	(Cianfanelli <i>et al</i> , 2016)
LM33	$\Delta vgrG1 \Delta tvpAB$	This study
YL37	Db10 $\Delta 9$ [$\Delta ssp1$ (SMDB11_2261), $\Delta ssp2$ (SMDB11_2264), $\Delta ssp3/tfe1$ (SMDB11_1112), $\Delta ssp4$ (SMDB11_3980), $\Delta ssp5$ (SMDB11_4628), $\Delta ssp6$ (SMDB11_4673), $\Delta rhs1$ (SMDB11_2278), $rhs2_{H1369A}$ (SMDB11_1610 _{H1369A}), Δslp (SMDB11_0927)]	(Reglinski <i>et al</i> , 2025)
GM131	$\Delta 9 \Delta vgrG1$	This study
CE030	$\Delta 9 \Delta vgrG2$	This study
CE036	<i>vai1</i> -3xFlag (encodes <i>vai1</i> with a C-terminal 3xFLAG tag at native <i>vai1</i> locus in a wild-type background)	This study
CE043	<i>vai1</i> -3xFlag Sm-resistant derivative	This study
CE049	$\Delta vgrG2$ SMDB11_2245-3xFlag (encodes <i>vai1</i> with a C-terminal 3xFLAG tag at native <i>vai1</i> locus in a $\Delta vgrG2$ background)	This study
JAD09	$\Delta rhsI1 \Delta tssH$ Sm-resistant derivative	(Alcoforado Diniz & Coulthurst, 2015)
AO01/JAD06	$\Delta sip4 \Delta ssp4$ Sm-resistant derivative	(Cianfanelli <i>et al</i> , 2016)
LM26	His-VgrG1 (encodes VgrG1 with an N-terminal His ₆ tag in a wild-type background)	This study
LM66	His-VgrG1 $\Delta tvpAB$ (encodes VgrG1 with an N-terminal His ₆ tag in a $\Delta tvpAB$ background)	This study
CE56	His-VgrG1 $\Delta tvpC$ (encodes VgrG1 with an N-terminal His ₆ tag in a $\Delta tvpC$ background)	This study
CE31	TvpAB-HA (encodes TvpAB with a C-terminal HA tag in a wild-type background)	This study

Appendix Table S1

CE68	TvpAB-HA $\Delta tvpC$ (encodes TvpAB with a C-terminal HA tag in a $\Delta tvpC$ background)	This study
CE59	HA-TvpC (encodes TvpC with an N-terminal HA tag in a wild-type background)	This study
CE60	HA-TvpC $\Delta vgrG2$ (encodes TvpC with an N-terminal HA tag in a $\Delta vgrG2$ background)	This study
CE75	HA-TvpC $\Delta vgrG1$ (encodes TvpC with an N-terminal HA tag in a $\Delta vgrG1$ background)	This study
CE70	HA-TvpC $\Delta tvpAB$ (encodes TvpC with an N-terminal HA tag in a $\Delta tvpAB$ background)	This study
CE136	HA-TvpC _{F138E, L152E, I159E} (encodes TvpC with an N-terminal HA tag and amino acid substitutions F138E, L152E, I159E)	This study
CE137	HA-TvpC _{V210Q} (encodes TvpC with an N-terminal HA tag and amino acid substitution V210Q)	This study
CE138	HA-TvpC _{D204R, D199R, E206R} (encodes TvpC with an N-terminal HA tag and amino acid substitutions D204R, D199R, E206R)	This study
CE141	HA-TvpC _{D204R, D199R, E206R} $\Delta vgrG2$ (encodes TvpC with an N-terminal HA tag and amino acid substitutions D204R, D199R, E206R, in a $\Delta vgrG2$ background)	This study
CE142	HA-TvpC _{V210Q} $\Delta vgrG2$ (encodes TvpC with an N-terminal HA tag and amino acid substitution V210Q, in a $\Delta vgrG2$ background)	This study
<i>Pseudomonas fluorescens</i>		
KT02	Sm-resistant derivative of <i>P. fluorescens</i> 55	(Murdoch <i>et al</i> , 2011)
<i>Serratia marcescens</i> SM39 (intrinsically Sm-resistant)		(Iguchi <i>et al</i> , 2014)
<i>E. coli</i>		
CC118 λ pir	Cloning host and donor strain for pKNG101-derived allelic exchange plasmids (λ pir)	(Herrero <i>et al</i> , 1990)
MG1655	Wild type (model K12 strain), used for heterologous toxicity assay	(Blattner <i>et al</i> , 1997)
MG1655	Strain used for bacterial two hybrid assay	F. Sargent
$\Delta cyaA::apr$		

Appendix Table S1. List of strains used in this study

Appendix Table S2

Name	Description/Genotype	Source/reference
pKNG101	Suicide vector for allelic exchange (Sm ^R <i>sacBR mobRK2 oriR6K</i>)	(Kaniga <i>et al</i> , 1991)
pSC3048	Modified version of the recombineering, λ Red recombinase-encoding pORTMAGE plasmid. The dominant negative mutant <i>mutL</i> gene has been removed to prevent off-target effects due to suppression of DNA repair during integration of oligonucleotide CE75. The origin of replication from pBAD Kan-18 was introduced in place of the conjugation and replication machinery to allow for the stable maintenance of the plasmid in <i>S. marcescens</i> Db10 (Kn ^R)	This study
pBAD18-Kn	Arabinose-inducible expression vector (Kn ^R)	(Guzman <i>et al</i> , 1995)
pSUPROM	Vector for constitutive expression of cloned genes under the control of the <i>E. coli</i> <i>tat</i> promoter (Kn ^R)	(Jack <i>et al</i> , 2004)
pUT18	Bacterial Two Hybrid plasmid (for fusion of protein of interest with C-terminal T18 fragment of CyaA; ApR)	(Karimova <i>et al</i> , 2000)
pT25	Bacterial Two Hybrid plasmid (for fusion of protein of interest with N-terminal T25 fragment of CyaA; CmR)	(Karimova <i>et al</i> , 1998)
pSC137	pBAD18 <i>Sufl_{SP}-Ssp2</i>	This study
pSC138	pBAD18 <i>OmpA_{SP}-Ssp2</i>	(English <i>et al</i> , 2012)
pSC934	pBAD18 <i>SMDB11_2246</i>	This study
pSC942	pBAD18 <i>OmpA_{SP}-SMDB11_2246</i>	This study
pSC944	pBAD18 <i>OmpA_{SP}-VgrG1</i>	This study
pSC1940	pBAD18 <i>VgrG1</i> CTD (598-786aa)	This study
pSC1941	pBAD18 <i>OmpA_{SP}-VgrG1</i> CTD (598-786aa)	This study
pSC1943	pBAD18 <i>VgrG1</i>	This study
pSC1944	pBAD18 <i>OmpA_{SP}-VgrG1</i> + <i>SMDB11_2245</i>	This study
pSC1945	pBAD18 <i>OmpA_{SP}-VgrG1</i> CTD (598-786aa) + <i>SMDB11_2245</i>	This study
pSC1951	pBAD18 <i>Sufl_{SP}-VgrG1</i> CTD (598-786aa)	This study
pSC1914	pSUPROM <i>SMDB11_2245</i>	This study
pSC1915	pSUPROM <i>SMDB11_2246</i>	This study
pSC1916	pSUPROM <i>tvpAB</i> (<i>SMDB11_2247</i>)	This study
pSC1917	pSUPROM <i>tvpB</i> (<i>SMDB11_2248</i>)	This study
pSC1918	pSUPROM <i>tvpC</i> (<i>SMDB11_2249</i>)	This study
pSC1919	pSUPROM <i>paar1</i> (<i>SMDB11_2250</i>)	This study
pSC601	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame $\Delta vgrG2$ ($\Delta SMDB11_2276$) deletion	(Cianfanelli <i>et al</i> , 2016)
pSC602	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame $\Delta vgrG1$ ($\Delta SMDB11_2244$) deletion	(Cianfanelli <i>et al</i> , 2016)
pSC921	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame $\Delta SMDB11_2245$ deletion	This study
pSC937	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame $\Delta SMDB11_2246$ deletion	This study
pSC577	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame $\Delta SMDB11_2245-2246$ deletion	This study
pSC902	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame $\Delta tvpAB$ deletion ($\Delta SMDB11_2247$)	This study
pSC945	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame $\Delta tvpB$ deletion ($\Delta SMDB11_2248$)	This study
pSC923	pKNG101-derived allelic exchange plasmid for the generation of chromosomal in-frame $\Delta tvpC$ deletion ($\Delta SMDB11_2249$)	This study

Appendix Table S2

pSC3031	pKNG101-derived allelic exchange plasmid for chromosomal replacement of native <i>vai1</i> with gene encoding Vai1 with 3xFLAG (C-terminal tag)	This study
pSC2026	pKNG101-derived allelic exchange plasmid for chromosomal replacement of native <i>vgrG1</i> gene with gene encoding His-VgrG1 (N-terminal tag)	This study
pSC3027	pKNG101-derived allelic exchange plasmid for chromosomal replacement of native <i>tpvAB</i> gene with gene encoding TvpAB-HA (C-terminal tag)	This study
pSC3088	pKNG101-derived allelic exchange plasmid for chromosomal replacement of native <i>tpvC</i> gene with gene encoding HA-TvpC (N-terminal tag)	This study
pSC4407	pKNG101-derived allelic exchange plasmid for chromosomal replacement of native <i>tpvC</i> gene with gene encoding HA-TvpC with substitutions F138E L152E I159E	This study
pSC4408	pKNG101-derived allelic exchange plasmid for chromosomal replacement of native <i>tpvC</i> gene with gene encoding HA-TvpC with substitutions D204R D199R E206R	This study
pSC4409	pKNG101-derived allelic exchange plasmid for chromosomal replacement of native <i>tpvC</i> gene with gene encoding HA-TvpC with substitution V210Q	This study
pSC048	pUT18 <i>tssK</i> (SMDB11_2253)	(Cianfanelli <i>et al</i> , 2016)
pSC1962	pUT18 <i>tpvC</i> (SMDB11_2249)	This study
pSC1958	pUT18 <i>vgrG1</i> (SMDB11_2244)	This study
pSC1959	pUT18 <i>vgrG2</i> (SMDB11_2276)	This study
pSC053	pT25 <i>tssK</i> (SMDB11_2253)	(Cianfanelli <i>et al</i> , 2016)
pSC076	pT25 <i>tssE</i> (SMDB11_2271)	This study
pSC1967	pT25 <i>tpvAB</i> (SMDB11_2247)	This study
pSC1968	pT25 <i>tpvB</i> (SMDB11_2248)	This study
pSC1969	pT25 <i>tpvC</i> (SMDB11_2249)	This study
pSC1970	pT25 <i>paar1</i> (SMDB11_2250)	This study
pSC1965	pT25 <i>vgrG1</i> (SMDB11_2244)	This study
pSC1966	pT25 <i>vgrG2</i> (SMDB11_2276)	This study
pSC2003	pT25 <i>tssF</i> (SMDB11_2272)	This study
pSC2005	pT25 <i>tssG</i> (SMDB11_2273)	This study
pSC2006	pT25 <i>tssF-tssG</i> (SMDB11_2272-73)	This study

Appendix Table S2. List of plasmids used in this study.

Appendix Table S3

Plasmid	Sequence of relevant primers (5'-3') ^{a,b}	Description
pSC137	TATAGAGCTCAGAGGACGTTGATATGTCACTCAGTCGACGTCAG	Forward primer to clone Sufl (SMDB11_3496) signal peptide and ribosome binding site (RBS) from Ssp2 into pBAD18-Kn (Sacl)
	TATATCTAGACGTTCCGCTCGCTTCAG	Reverse primer to clone Sufl (SMDB11_3496) signal peptide into pBAD18-Kn (Xbal)
	TATATCTAGAAGTCGCCCTTCATTCCAACAAG	Forward primer to clone Ssp2 without ATG into pBAD18-Kn (Xbal)
	TAGAGCATGCGGATTTTATTTAGTAACCATATAGATGCCTCG	Reverse primer to clone Ssp2 into pBAD18-Kn (SphI)
pSC934	TATATCTAGACACCTTGCAAGGAAGTGTACAG	Forward primer to clone SMDB11_2246 including RBS into pBAD18-Kn (Xbal)
	TATAGCATGCTCAAGAGGACAGTGCCTTGTG	Reverse primer to clone SMDB11_2246 into pBAD18-Kn (SphI)
pSC942	TATATCTAGAGGCGCGTTCATTGCCTTTTC	Forward primer to clone SMDB11_2246 into pBAD18-OmpA _{SP} -Kn by replacement of Ssp2 in pSC138 (Xbal)
	TATAGCATGCTCAAGAGGACAGTGCCTTGTG	Reverse primer to clone SMDB11_2246 into pBAD18-Kn (SphI)
pSC944	TATATCTAGAGATCGTCTTATCATTGCGCACAC	Forward primer to clone VgrG1 into pBAD18-OmpA _{SP} -Kn by replacement of Ssp2 (Xbal)
	TATAGCATGCTTACAAGAAGATCGCCAGCG	Reverse primer to clone VgrG1 into pBAD18-Kn (SphI)
pSC1940	TATAGAATTCCTAAAGCGGGGAATAGTATGGCGCAGACGCAAAACATTAC	Forward primer to clone VgrG1 CTD (598-786aa) including VgrG1 RBS and ATG into pBAD18-Kn (EcoRI)
	TATAGCATGCTTACAAGAAGATCGCCAGCG	Reverse primer to clone VgrG1 CTD (598-786aa) into pBAD18-Kn (SphI)
pSC1941	TATATCTAGAGCGCAGACGCAAAACATTAC	Forward primer to clone VgrG1 CTD (598-786aa) into pBAD18-OmpA _{SP} -Kn by replacement of Ssp2 (Xbal)
	TATAGCATGCTTACAAGAAGATCGCCAGCG	Reverse primer to clone VgrG1 CTD (598-786aa) into pBAD18-Kn (SphI)
pSC1943	TATAGAATTCCTAAAGCGGGGAATAGTATGGATCG	Forward primer to clone VgrG1 into pBAD18-Kn (EcoRI)
	TATAGCATGCTTACAAGAAGATCGCCAGCG	Reverse primer to clone VgrG1 into pBAD18-Kn (SphI)
pSC1944	TATATCTAGAGATCGTCTTATCATTGCGCACAC	Forward primer to clone VgrG1 into pBAD18-OmpA _{SP} -Kn by replacement of Ssp2 (Xbal)
	TATACTGCAGTCAAAGCCGGGAAAGCTCTTC	Reverse primer to clone VgrG1 with SMDB11_2245 into pBAD18-OmpA _{SP} -Kn by replacement of Ssp2 (PstI)
pSC1945	TATATCTAGAGCGCAGACGCAAAACATTAC	Forward primer to clone VgrG1 CTD (598-786aa) with SMDB11_2245 into pBAD18-OmpA _{SP} -Kn by replacement of Ssp2 (Xbal)
	TATACTGCAGTCAAAGCCGGGAAAGCTCTTC	Reverse primer to clone VgrG1 CTD (598-786aa) with SMDB11_2245 into pBAD18-OmpA _{SP} -Kn by replacement of Ssp2 (PstI)
pSC1951	TATATCTAGAGCGCAGACGCAAAACATTAC	Forward primer to clone VgrG1 CTD (598-786aa) into pBAD18-Sufl _{SP} -Kn by replacement of Ssp2-Rap2ab in pSC143(Xbal)
	TATAGCATGCTTACAAGAAGATCGCCAGCG	Reverse primer to clone VgrG1 CTD (598-786aa) into pBAD18-Kn (SphI)
pSC1914	TATAGGATCCATGTGCAAGGCCAAAAATGTTTTCA	Forward primer to clone SMDB11_2245 into pSUPROM (BamHI)
	TATATCTAGATCAAAGCCGGGAAAGCTCTTCGATC	Reverse primer to clone SMDB11_2245 into pSUPROM (Xbal)
pSC1915	TATAGGATCCATGGGCGCTTCATTGCCTTTTCGA	Forward primer to clone SMDB11_2246 into pSUPROM (BamHI)
	TATATCTAGATCAAAGAGGACAGTGCCTTGTCTTC	Reverse primer to clone SMDB11_2246 into pSUPROM (Xbal)

Appendix Table S3

pSC1916	TATAGGATCCATGAAAGTCGTAAACCTCTGCGCC	Forward primer to clone <i>tvpAB</i> (SMDB11_2247) into pSUPROM (<u>BamHI</u>)
	TATATCTAGATCATACTTCCTTCCCGTTGTGGCGA	Reverse primer to clone <i>tvpAB</i> (SMDB11_2247) in pSUPROM (<u>XbaI</u>)
pSC1917	TATATCTAGAATGACCTCAAAACCAGAGCAGATAC	Forward primer to clone <i>tvpB</i> (SMDB11_2248) in pSUPROM (<u>XbaI</u>)
	TATAAAGCTTTCAAATTCGGCTCTGGCGCTGTGCG	Reverse primer to clone <i>tvpB</i> (SMDB11_2248) in pSUPROM (<u>HindIII</u>)
pSC1918	TATAGGATCCATGAGCGTTGCCAACACAACCTGCG	Forward primer to clone <i>tvpC</i> (SMDB11_2249) in pSUPROM (<u>BamHI</u>)
	TATATCTAGATTATCCGACGTGGATTGTCTGAA	Reverse primer to clone <i>tvpC</i> (SMDB11_2249) in pSUPROM (<u>XbaI</u>)
pSC1919	TATAGGATCCATGTTTGCCAACAGCCAAATGATCG	Forward primer to clone <i>paar1</i> (SMDB11_2250) in pSUPROM (<u>BamHI</u>)
	TATATCTAGATCAGGGCGCAAGCAGCAACACTTTT	Reverse primer to clone <i>paar1</i> (SMDB11_2250) in pSUPROM (<u>XbaI</u>)
pSC1962	TATAAAGCTTGTAAAGAGGAGGAGTAATGAGCGTTGCCAACACAAC	Forward primer to clone <i>tvpC</i> (SMDB11_2249) in pUT18 (<u>HindIII</u>)
	TATATCTAGAGATCCGACGTGGATTGTCTG	Reverse primer to clone <i>tvpC</i> (SMDB11_2249) without stop in pUT18 (<u>XbaI</u>)
pSC1958	TATACTGCAGGTAAAGAGGAGGAGTAATGGATCGTCTTATCATTGC	Forward primer to clone <i>vgrG1</i> (SMDB11_2244) in pUT18 (<u>PstI</u>)
	TATATCTAGAGACAAGAAGATCGCCAGCGCCT	Reverse primer to clone <i>vgrG1</i> (SMDB11_2244) without stop in pUT18 (<u>XbaI</u>)
pSC1959	TATAAAGCTTGTAAAGAGGAGGAGTAATGTTGGACCGCATTATTGC	Forward primer to clone <i>vgrG2</i> (SMDB11_2276) in pUT18 (<u>HindIII</u>)
	TATATCTAGAGAGGATTTCGGGAACAGCTGAT	Reverse primer to clone <i>vgrG2</i> (SMDB11_2276) without stop in pUT18 (<u>XbaI</u>)
pSC076	TATAGGATCCAATGAACGATAAACGCCACC	Forward primer to clone <i>tssE</i> (SMDB11_2271) in pT25 (<u>BamHI</u>)
	TATAGGTACCTTACCCTATGTCTTTCAGATCGAAGTG	Reverse primer to clone <i>tssE</i> (SMDB11_2271) in pT25 (<u>KpnI</u>)
pSC1967	TATAGGATCCGAAAGTCGTAAACCTCTGCG	Forward primer to clone <i>tvpAB</i> (SMDB11_2247) without start in pT25 (<u>BamHI</u>)
	TATACCCGGGTCATACTTCCTTCCCGTTGT	Reverse primer to clone <i>tvpAB</i> (SMDB11_2247) in pT25 (<u>SmaI</u>)
pSC1968	TATACTGCAGGAACCTCAAAACCAGAGCAGAT	Forward primer to clone <i>tvpB</i> (SMDB11_2248) without start in pT25 (<u>PstI</u>)
	TATAGGTACCTCAAATTCGGCTCTGGCGCT	Reverse primer to clone <i>tvpB</i> (SMDB11_2248) in pT25 (<u>KpnI</u>)
pSC1969	TATAGGATCCGAGCGTTGCCAACACAACCTCG	Forward primer to clone <i>tvpC</i> (SMDB11_2249) without start in pT25 (<u>BamHI</u>)
	TATACCCGGGTTATCCGACGTGGATTGT	Reverse primer to clone <i>tvpC</i> (SMDB11_2249) in pT25 (<u>SmaI</u>)
pSC1970	TATAGGATCCGTTTGCCAACAGCCAAATGAT	Forward primer to clone <i>paar1</i> (SMDB11_2250) without start in pT25 (<u>BamHI</u>)
	TATACCCGGGTCAGGGCGCAAGCAGCAACA	Reverse primer to clone <i>paar1</i> (SMDB11_2250) in pT25 (<u>SmaI</u>)
pSC1965	TATACTGCAGGAGATCGTCTTATCATTGCGCA	Forward primer to clone <i>vgrG1</i> (SMDB11_2244) without start in pT25 (<u>PstI</u>)
	TATAGGTACCTTACAAGAAGATCGCCAGCG	Reverse primer to clone <i>vgrG1</i> (SMDB11_2244) in pT25 (<u>KpnI</u>)
pSC1966	TATAAGATCTGTTGGACCGCATTATTGCGC	Forward primer to clone <i>vgrG2</i> (SMDB11_2276) without start in pT25 (<u>BglII</u>)
	TATAGATATCTCAGGATTTCGGGAACAGCT	Reverse primer to clone <i>vgrG2</i> (SMDB11_2276) in pT25 (<u>EcoRV</u>)

Appendix Table S3

pSC2003	TATAGGATCCGGACAGTAACTGTTAGATTA	Forward primer to clone <i>tssF</i> (SMDB11_2272) without start in pT25 (<u>Bam</u> HI)
	TATACCCGGGTCATATCAGCGTCCTTTTGC	Reverse primer to clone <i>tssF</i> (SMDB11_2272) in pT25 (<u>Sma</u> I)
pSC2005	TATAGGATCCGAACGCGGAGATGATCGCCAC	Forward primer to clone <i>tssG</i> (SMDB11_2273) without start in pT25 (<u>Bam</u> HI)
	TATACCCGGGTCAGAACGGGTTTCCAGCG	Reverse primer to clone <i>tssG</i> (SMDB11_2273) in pT25 (<u>Sma</u> I)
pSC2006	TATAGGATCCGGACAGTAACTGTTAGATTA	Forward primer to clone <i>tssF-tssG</i> (SMDB11_2272-73) without start for <i>tssF</i> in pT25 (<u>Bam</u> HI)
	TATACCCGGGTCAGAACGGGTTTCCAGCG	Reverse primer to clone <i>tssF-tssG</i> (SMDB11_2272-73) in pT25 (<u>Sma</u> I)
pSC921	TATATCTAGAAATCAGTCTTTCAAGCTTGACGG	Forward primer to clone upstream region of SMDB11_2245 for allelic exchange (<u>Xba</u> I)
	TATAACTAGTCGACATGGGAGTCCCTTTACAAG	Reverse primer to clone upstream region of SMDB11_2245 for allelic exchange (<u>Spe</u> I)
	TATAACTAGTGAGCTTTCCCGGCTTTGAG	Forward primer to clone downstream region of SMDB11_2245 for allelic exchange (<u>Spe</u> I)
	TATAGGGCCCAAGTGCCTTGTCTTCCC	Reverse primer to clone downstream region of SMDB11_2245 for allelic exchange (<u>Apa</u> I)
pSC937	TATATCTAGACCAATTTCAAGCTCAGTAATGATCC	Forward primer to clone upstream region of SMDB11_2246 for allelic exchange (<u>Xba</u> I)
	TATAACTAGTGCCCATCGTGACACTTCC	Reverse primer to clone upstream region of SMDB11_2246 for allelic exchange (<u>Spe</u> I)
	TATAACTAGTAACGCACTGTCCTCTTGATCATCC	Forward primer to clone downstream region of SMDB11_2246 for allelic exchange (<u>Spe</u> I)
	TATAGGGCCCGGATTATCGGCGAACTGG	Reverse primer to clone downstream region of SMDB11_2246 for allelic exchange (<u>Apa</u> I)
pSC577	TATATCTAGAAATCAGTCTTTCAAGCTTGACGG	Forward primer to clone upstream region of SMDB11_2245 for allelic exchange (<u>Xba</u> I)
	TATAACTAGTCGACATGGGAGTCCCTTTACAAG	Reverse primer to clone upstream region of SMDB11_2245 for allelic exchange (<u>Spe</u> I)
	TATAACTAGTAACGCACTGTCCTCTTGATCATCC	Forward primer to clone downstream region of SMDB11_2246 for allelic exchange (<u>Spe</u> I)
	TATAGGGCCCGGATTATCGGCGAACTGG	Reverse primer to clone downstream region of SMDB11_2246 for allelic exchange (<u>Apa</u> I)
pSC902	TGTATCTAGACTGTTACCGTCTTTGGCATCC	Forward primer to clone SMDB11_2247 upstream region for allelic exchange (<u>Xba</u> I)
	TGTAGGATCCGACTTTCATTGTGTGTAAAGCTTCC	Reverse primer to clone SMDB11_2247 upstream region for allelic exchange (<u>Bam</u> HI)
	TATAGGATCCACATTGCCTCGCCACAACG	Forward primer to clone SMDB11_2247 downstream region for allelic exchange (<u>Bam</u> HI)
	TATAGGGCCCAATTGGTTATCGGTAACTGGC	Reverse primer to clone SMDB11_2247 downstream region for allelic exchange (<u>Apa</u> I)
pSC945	TATATCTAGACACGCTGGCCAACATTACC	Forward primer to clone upstream region of SMDB11_2248 for allelic exchange (<u>Xba</u> I)
	TATAACTAGTTGAGGTCATACTTCTTCCCG	Reverse primer to clone upstream region of SMDB11_2248 for allelic exchange (<u>Spe</u> I)
	TATAACTAGTAGCCGAATTTGAATGCATTGAC	Forward primer to clone downstream region of SMDB11_2248 for allelic exchange (<u>Spe</u> I)
	TATAGGGCCCGTAATCTTCGCCTGATAATCC	Reverse primer to clone downstream region of SMA2248 for allelic exchange (<u>Apa</u> I)
pSC923	TGTATCTAGAAGTTTGAAGCGTGAACTTCG	Forward primer to clone upstream region of SMDB11_2249 for allelic exchange (<u>Xba</u> I)
	TATAACTAGTAACGCTCATGGTGATCCCCTC	Reverse primer to clone upstream region of SMDB11_2249 for allelic exchange (<u>Spe</u> I)

Appendix Table S3

	TATA <u>ACTAGT</u> ATCCACGTCGGATAACCTGC	Forward primer to clone downstream region of SMB11_2249 for allelic exchange (<u>SpeI</u>)
	TATA <u>GGGCCG</u> GTTGACTTCACTGCTGATG	Reverse primer to clone downstream region of SMDB11_2249 for allelic exchange (<u>Apal</u>)
pSC2026	TATA <u>AAAGCTT</u> CATCATCATCATCAC GATCGTCTTATCATTGCGC AC	Fwd primer to incorporate Nter His Tag on VgrG1 (SMDB11_2244) by allelic exchange (<u>HindIII</u>)
	TATAG <u>TCGAC</u> GTGAGAAACGCTCCAGCTATTG	Reverse primer within VgrG1 coding region to incorporate Nter His Tag on VgrG1 (SMDB11_2244) by allelic exchange (<u>Sall</u>)
pSC3088	<u>gggcccggt</u> accagctt	Forward primer for pBluescript to integrate HA-SMDB11_2249 encoding sequence at the <u>Apal</u> site
	<u>tctagagcggccg</u> ccacc	Reverse primer for pBluescript to integrate HA-SMDB11_2249 encoding sequence at the <u>XbaI</u> site
	gcggtggcggccgct <u>ctaga</u> CAGTTTGAAGGCGTGAAC	Forward primer for upstream region of SMDB11_2249. Overlap with pBluescript shown as lower case.
	TGCATAATCAGGAACATCATAAGGATACATGGTGATCCCTCGTG	Reverse primer for upstream region of SMDB11_2249. HA tag encoding sequence shown in bold.
	TATCCTTATGATGTTCTCTGATTATGCA AGCGTTGCCAACACAATC	Forward primer for coding region of SMDB11_2249. HA tag encoding sequence shown in bold.
	aaaagctgggtacc <u>gggcc</u> GGTGATGTCTTTGAGGTAATGAG	Reverse primer for coding region of SMDB11_2249. Overlap with pBluescript shown as lower case.
Primer CE75	T* <u>T</u> *CGAAACCGTTGGTCAAACGCACACGGCAAACCTTACGCAGTG CGGAGTTTGGTTTGTTCGGGGTAGTGGTATATACGCGGGTGCATA* C*G	Amplifies the SNP conferring streptomycin resistance from Db11 to introduce into target strains. Stars denote phosphothiorate bonds to increase stability and resistance to nucleases
Plasmid	Details of synthetic insert	
pSC3031	Synthetic insert of DNA sequence encoding a C-terminal 3xFLAG tag (shown in bold) on Vai1 (SMDB11_2245) to be introduced into the native genomic locus by allelic exchange (containing <u>XbaI</u>/<u>Apal</u> sites (underlined) for sub-cloning into pKNG101). Produced by GeneArt (ThermoFisher): <u>TCTAGACACACGATGCTGGATATCTCGCATAACCCGCTGTCGATCAGCAAAACCAATTTCAAGCTCAGTAATGATCCCATGACGTTCACTCAGCTTGCGC</u> <u>CGCTGGTACCAACCGTGCCTTACGATTGAGCACCAAGGCGTGCGGATCTTCTGTAAAGGGAAGTCCCATGTCGAAGGCCAAAAATGTTTTACCGGTT</u> <u>ATCGGGTTGATTTTATGGCTCCCATCTCAGTCTGGTGGGTTTTTCAAATTCATACGGTCGATATCTGGGGTATTTCAAACATATCGGTTCTTCCT</u> <u>CAGCGTGATGGAGAACGGTATTTCGCTTCCGGGCAAAATCGTCAGCATTCGCCAGACCAACCTGTGGGACGGAATCGGCCGGTCTGCGAAGTCGAA</u> <u>GTCAAGTACATCGCCCAAGACGGGAAAAACCATACGGCCGTGGCGAAAGGCCGATCAGCGTCTGGATCTGCCGCTTATCAGCCGGCCATTTTAC</u> <u>CGCGATAAAGTACGATCCGAAGAACCCAAAAAGCGGTGATCGAAGAGCTTTCCGGCTTGACTACAAAGACCATGACGGTGATTATAAAGATCAT</u> <u>GATATCGATTACAAGGATGACGATGACAAGT</u>GAGCGAAAAGGATCCCTCAATTACGCGATCCGATTTCAACGTTCTGAAAACGGCTTATCGTTTCATC AGTTCGCTAAATCACCTGCATAAGGAAGTGCACGATGGCGCGTTCATTGCCTTTTCGACGACGAGACGCTCTGGGACAGATCTGTTTACCGTC TTTGGCATCTGGTGGCTGCGTATTGCTCGCTGCGATCGGCAAAACCTTTTGTGCGCAAGCCGAGCGGCCCTTACCATGCGGTTGCTGATCTTT GTGCGCTGATGCTGGCGCTCTGAAATACAATCAGCAGGTGATGCTGCGGTGAATGCTCCCGCAGAGCTGATCGGGTCTCTGATTTTGCCCGGTTTT TTGATGCGTTACCTCGTGGCGGACACCCGAGACTGACGCTGGCGATAGAGTTGCCCTGTTTGGCGCGCTGCTGCGCTGCTGTTCTGATGAGCAC CGCCGGCGTGGATTTTTGCGCCCTATCACTGGAGAGCACCGGCCAATTTACCGCAAGGCGATCGCCGCTCATTGTCCGTCGGGGCC	
pSC3027	Synthetic insert of DNA sequence encoding a C-terminal HA tag (shown in bold) on TvpAB (SMDB11_2247) to be introduced into the native genomic locus by allelic exchange (containing <u>XbaI</u>/<u>Apal</u> sites (underlined) for sub-cloning into pKNG101). Produced by GeneArt (ThermoFisher): <u>TCTAGAGACATTAGCCACGCCGCGCTCGACAAAGTCAGCTTCGTCAAATGCCGGTTGCTGGCGGTGAACCTCAGTCAGGCGCGGCTGGAAGTTGCG</u> <u>CCTGGGTTGACACCGAGACGCAAAAGCCTCAGCTTCACGCGCAGCTTCACGGCTTGCAGCGAAGACACTGTTCCGCGAGGCGGATTTCC</u> <u>AGCGACGCGACGCTAAACAGTGCAATTTACGCCAGATGCCGTTGACGCGGGCAATTTAGCCGGCGCGTCTCAATAATTGCGATCTGTCGGAAAC</u> <u>CCGATTGAACGAGGCGGATTTCCGCCAGGCCAACGGCAGTGGCAGCCTGTTATCCGACGATTTGTCTGCGCCAGCCTGCGCGATGCCAATTTTAT</u> <u>TGCGGCTATCTGCAAAATGCGTGTCTGAGCGCCGACCTGACGGGCGCAATCTGTTCCGGGCGGACCTGTGCGAGTCGCAAGTGGATCAGGCA</u> <u>ACCCGGCTCGAGAGCGCTATACGCCAGGGTGAAACATTGCCTCGCCACAACGGGAAGGATATATCCTTATGATGTTCTCTGATTATGCA</u>TGACA ACGGGAAGGAAGTATGACCTCAAACAGAGCAGATACGCCAGCGCTAAAACGCGCGAGCCGATCGCCGAGAGGACTTGCACGGCTGTCTT CGCCGGTCTGGATTGCGCGCGGCTGTTCAACGAACTGAACCTGAACGCGGTGAACCTCTGACTGCGATCTGCGGACAGCGTTTTCAGCGATT GCCAGCTCGAACACGCACAATTTACGCGGGCGGATCTGAAGCAAACGGCCTTCAATCAGTGCGCCATGTCGGCCAGCGCTTACGCGAGAGCCATATC GAACTGACGATGTTCAACGATTGCGGCTTGAACAGAGCGACTTACGCCGCTGTCGCTCAACCAAGTCACTGGATGTCGTGCCAATTGGCCGGCGC GAATTTTCCGCCACTCAGCAGATCGCACTACCTTTTACGAAAGCCGCTGACGGCGCCATGTTGAATCAGGCGCGCTTATGCCTGTGACGTTCTT CGATTAATCTCTGTAAACGCAAGTTGAAGCGTGAACCTCGACCGCTACGTTTTGGGCC	
pSC4407	Synthetic insert of DNA sequence encoding an N-terminal HA tag (shown in bold) on TvpC with substitutions F138E I152E I159E (in red) to be introduced into the native genomic locus by allelic exchange (replacing a <u>ΔtvpC</u> allele) (contains <u>BglII</u>-<u>SpeI</u> sites (underlined) for sub-cloning into pKNG101). Produced by GenScript : <u>AGATCT</u>CAGTTTGAAGGCGTGAACCTCGACCGCGTCACTGTTTTTCAATGTGACCACCGCGGCAAAAGTTACGCCGGGCGAGCCTGATCGCCTGCCA GTTTACCATAACCAATTGGACGACGTCGACTTCAGCCAGGCGACGCTGCGCCAGAGCAATTTCAAGGGCGCTTCGCTGCGGCGCGCAACCTGACCA GCGTACAGGCGCAGCAGTCTGCTGGCTGGAGGCAATCTGACTCAGGCGCAGTGTGCGGCGGGCAGTTCGATCAGCGCATCTTACGGAAGCGAC GTTGGATGCCGCCAATTCAGCCAGGCGCGCTGTATCAATGCGTATTTTACGACAGCCGATCGGCGCGCTGTGATTACAGCGACAGCGACCTGACCT ACGCCGACTTCTGTTATGCCGACATTGGCGCCCGGATTTCGGCGCGCGCTTATGCGCACCCGTATGCATCGCGCCACCAGCAGCAACGCGCT	

Appendix Table S3

	GGAGCGATCGCAGCGGGATCCTCGAGAGCGACGAAGAGCTGTACGCCGCCGAAACCTGGAGCGCACAGCGCCAGAGCCGAATTTGAATGCATTGAC CACTTCACGAGGGGATCACCATGTATCCTTATGATGTTCTCTGATTATGCAAGCGTTGCCAACACAACCTCGCGCCGTCAACATCGACCGCCCCAACAGG CCGCTGGCCAGGTCGTCAACCTGTTGCCGACGGCAGTCTGGTGGTGAATGTGAAGGTCGTGGCTGGCATTGTCGCCGGGCGGGCAGCTGCCTGCT GACGCCGGCCCTCGGCGATAACGTGCTGGTCGCCGGCTCGCGCCACCAGCTGTGGGCGATCGCCGTCTCGAGCGGGCAGAGCCGCAAAGCACGGC CGCGCTCTCGTCGACGGCGATCTGCAGATTGAAACGCCGAACGGCTCGCTGTCGTTGCACAGCGCGCAGGCGCTGAAACTGAGCGGCGACGCCATG ACGCTGCAGGCGAACAGCGGCGACTGCCAGTCGACAAGATGAAATACAGCGGCGAAGAGCTGTGGGCGAAGTGACGATCAACCGCTTTGGTCGGTA AACGCTGCGAGTCGAATGGCATTGGTCAGCCAGGAAAGCCATTGCTGTTCCGCAAAGTGCGCCAAACCGAGCACGTGCGCGCCGGGCGAGCTGGA TTATCAGGCGGAAGATTACGCCCGCATTATGCCCGCAACACGCTCATTACCTCAAAGACATACCAAGCTGGATTAGAACAATCCACGTCGGATA ACCTGCGGAGGCCTTTATGTTTGCCAACAGCCAAATGATCGGCGTGGAACCTGGCGTTTCCGACGCTGTGCTGACGCCAAGCCCGGCGCCGTGCC GTACCTTACCCGATATCGCCTCGCGCCGACCGCCATCCGAACCGCTTCAACATTCTGTTCTGTCGGTACCCCGGCCACAACATGGGCACGGTAACG CCCCTGACCAACGGCACAACCCGCGTAGCCACCGGTGTCGCTTCCGGGACGGTGATGGGGCCATCCCGCACTGACCGCGCTTTACCGTGT GCTCAAAGGCACTCCGGCCACCCGGCTGACCAGCCTAGCCTGCAGAATTCCACCAACGCGCTCGGCATGCGCATCGTGCCGAGCCAATTAAGTGT TGCTGCTTGCCTGATGACGGCGCCTATTACAGCCATTCAATCAAGAAAAAGAGGACATTGTGAAAACAGGATTACCCTGACGCTGCTGACCG CCCTGACGCTTCCGGTGTGTTCCACCCCGCCGCCACCGCCGCCGGCGCTCAATAACGACGCCCTCATCAGCAGTGAAGTCAACGGCGTCACCTGCAGC ACCGCGCCGCACTAGT
pSC4408	Synthetic insert of DNA sequence encoding an N-terminal HA tag (shown in bold) on TvpC with substitutions D204R D199R E206R (in red) to be introduced into the native genomic locus by allelic exchange (replacing a ΔtvpC allele) (contains <i>BglIII-SpeI</i> sites (underlined) for sub-cloning into pKNG101). Produced by GenScript : <u>AGATCT</u>CAGTTTGAAGGCGTGAACCTCGACCGCTCACGTTTTTCGAATGTGACCACCGCGGCAAAAGTTACGCCGGGCAGAGCCTGATCGCTGCCA GTTTACCATAACCAATTGGACGACGTCGACTTCAGCCAGGCGACGCTGCGCCAGAGCAATTTCAAGGGCGCTTCGCTGCGGCGCGCCAACCTGACCA GGTACAGGCGCAGCAGTCGCTGTGGCTGGAGGCCAATCTGACTCAGGCGCAGTGTGCGGCGCGGCGCTTCGATCAGGCGATCTTCAGCGAAGCGAC TGTGGATTGCCCAACTTCAGCCAGCGCGCTGTATCAATCGTATTTACGACAGCAGCTGCGGCGCTGATTACGACGACAGCACTGACCTGACCT ACGCCGACTTCTGTTATGCCGACATTGGCGCCCGATTTCGGGCGCGCGCTTATGCGCACCCGATATGCATCGCGCCACCAGCAGCAAAACGCGCT GGAGCGATCGCAGCGGGATCCTCGAGAGCGACGAAGAGCTGTACGCCGCCGAAACCTGGAGCGCACAGCGCCAGAGCCGAATTTGAATGCATTGAC CACTTCACGAGGGGATCACCATGTATCCTTATGATGTTCTCTGATTATGCAAGCGTTGCCAACACAACCTCGCGCCGTCAACATCGACCGCCCCAACAGG CCGCTGGCCAGGTCGTCAACCTGTTGCCGACGGCAGTCTGGTGGTGAATGTGAAGGTCGTGGCTGGCATTGTCGCCGGGCGGGCAGCTGCCTGCT GACGCGCGCCTCGGCGATAACGTGCTGGTGCAGCGCTGCGGCCACCAAGCTGTGGGCGATCGCCGTCTCGAGCGGGCAGAGCCGCAAGACAGCGG CGGCTCTCGCTGCGCAGGCGATCTGCAGATTGAAACGCCGAACGGCTCGCTGTGCTTGACAGCGCGCAGCGCTGATTACGACGACAGCGCCATG ACGCTGCAGGCGAACAGCGGCGACTGCCACGTGCAGAAGATGAAATACAGCGGCGAAGAGCTGTGCGCCTTCGTGACGATCAGCCGTTTGGTCGGTA AACGCTGCGAGTCGCTGTGGCATTGGTCAGCCAGATCAGCCATTGCTGTTCCGCAAAGTGCGCCAAACCGAGCACGTGCGCGCCGGGCGAGCTGGA TTATCAGGCGGAAGATTACGCCCGCATTATGCCCGCAACACGCTCATTACCTCAAAACGTATCACCAAGCTGCGTTCACCGTCAAATCCACGTCGGATA ACCTCGGGAGGCCTTTTATGTTTGCCAACAGCCAAATGATCGGCGTGGAACCTGGCGTTTCCCGACGCTGTGCTGACGCCAAGCCCGGCGCCGTGCC GTACCTACCCGCTATGCGGCGACCGCCATCCGAACGCGTTCAACATTCTGCTGCTGCTGACCCCGGCCACAACCTGACCGCGCGCTTTACCGTGT CCCCTGACCAACGGCGACAACCCCGCGTAGCCACCGGTGTGCTTCCGGGACGGTGATGGGGCCATCCCGCACTGACCGCGCGCTTTACCGTGT GCTCAAAGGCACTCCGGCCACCCGGCTGACCAGCCTAGCCTGCAGAATTCCACCAACGCGCTCGGCATGCGCATCGTGCCGAGCCAATTAAGTGT TGCTGCTTGCCTGATGACGGCGCCTATTACAGCCATTCAATCAAGAAAAAGAGGACATTGTGAAAACAGGATTACCCTGACGCTGCTGACCG CCCTGACGCTTCCGGTGTGTTCCACCCCGCCGCCACCGCCGCCGGCGCTCAATAACGACGCCCTCATCAGCAGTGAAGTCAACGGCGTCACCTGCAGC ACCGCGCCGCACTAGT
pSC4409	Synthetic insert of DNA sequence encoding an N-terminal HA tag (shown in bold) on TvpC with substitution V210Q (in red) to be introduced into the native genomic locus by allelic exchange (replacing a ΔtvpC allele) (contains <i>BglIII-SpeI</i> sites (underlined) for sub-cloning into pKNG101). Produced by GenScript : <u>AGATCT</u>CAGTTTGAAGGCGTGAACCTCGACCGCTCACGTTTTTCGAATGTGACCACCGCGGCAAAAGTTACGCCGGGCAGAGCCTGATCGCTGCCA GTTTACCATAACCAATTGGACGACGTCGACTTCAGCCAGGCGACGCTGCGCCAGAGCAATTTCAAGGGCGCTTCGCTGCGGCGCGCCAACCTGACCA GGTACAGGCGCAGCAGTCGCTGTGGCTGGAGGCCAATCTGACTCAGGCGCAGTGTGCGGCGGGCAGCTTCGATCAGGCGATCTTCAGCGAAGCGAC GTTGGATTGCCCAACTTCAGCCAGCGCGCTGTATCAATCGTATTTACGACAGCCGATCGGCGCGCTGTGATTACGCGACAGCACTGACCTGACCT ACGCCGACTTCTGTTATGCCGACATTGGCGCCCGATTTCGGGCGCGCGCTTATGCGCACCCGATATGCATCGCGCCACCAGCAGCAAAACGCGCT GGAGCGATCGCAGCGGGATCCTCGAGAGCGACGAAGAGCTGTACGCCGCCGAAACCTGGAGCGCACAGCGCCAGAGCCGAATTTGAATGCATTGAC CACTTCACGAGGGGATCACCATGTATCCTTATGATGTTCTCTGATTATGCAAGCGTTGCCAACACAACCTCGCGCCGTCAACATCGACCGCCCCAACAGG CCGCTGGCCAGGTCGTCAACCTGTTGCCGACGGCAGTCTGGTGGTGAATGTGAAGGTCGTGGCTGCGATTTGCGCGGGGCGGCGAGCTGCCTGCT GACGCGGCGCTCGGCGATAACGTGCTGGTGCAGCGCTGCGGCCACCAAGTGTGAAGGTCGTGGCTGCGATTTGCGCGGGGCGGCGAGCTGCCTGCT GACGCGGCGCTCGGCGATTAACGTGCTGGTGCAGCGCTGCGGCCACAGCTGTGGGCGCATGCGAGCGGCAGAGCCGCAAGACAGCAGCGC GCGGCTCTCGCTGCGAGGCGATCTGCAGATTGAAACGCCGAACGGCTCGCTGTGCTTGACAGCGCGCAGGCGCTGAAACTGAGCGGCGACGCCATG ACGCTGCAGGCGAACAGCGGCGACTGCCACGTGCAGAAGATGAAATACAGCGGCGAAGAGCTGTGCGCCTTCGTGACGATCAGCCGTTTGGTCGGTA AACGCTGCGAGTCGCTGTGGCATTGGTCAGCCAGATCAGCCATTGCTGTTCCGCAAAGTGCGCCAAACCGAGCACGTGCGCGCCGGGCGAGCTGGA TTATCAGGCGGAAGATTACGCCCGCATTATGCCCGCAACACGCTCATTACCTCAAAGACATACCAAGCTGGATTAGAACAATCCACCAAGGATA ACCTCGGAGGCCTTTTATGTTTGCCAACAGCCAAATGATCGGCTGGAACCTGGCGTTTCCCGACGCTGTGCTGACGCCAAGCCCGGCGCGCTGCC GTACCTTACCCGATATCGCCTCGCGCCGACCGCCATCCGAACGCGTTCAACATTCTGTTCTGTCGGTACCCCGGCGCACAACTGGCAGCGGTAAACG CCCCTGACCAACGGCGACAACCCCGCGTAGCCACCGGTGTGCTTCCGGGACGGTGATGGGGCCATCCCGCACTGACCGCGCGCTTTACCGTGT GCTCAAAGGCACTCCGGCCACCCGGCTGACCAGCCTAGCCTGCAGAATTCCACCAACGCGCTCGGCATGCGCATCGTGCCGAGCCAATTAAGTGT TGCTGCTTGCCTGATGACGGCGCCTATTACAGCCATTCAATCAAGAAAAAGAGGACATTGTGAAAACAGGATTACCCTGACGCTGCTGACCG CCCTGACGCTTCCGGTGTGTTCCACCCCGCCGCCACCGCCGCCGGCGCTCAATAACGACGCCCTCATCAGCAGTGAAGTCAACGGCGTCACCTGCAGC ACCGCGCCGCACTAGT

^a Incorporated restriction sites for cloning into the respective vector are underlined.

^b Sequences encoding protein epitope/affinity tags are in bold.

Appendix Table S3. Oligonucleotide primers and synthetic gene fragments used for plasmid construction.

Appendix Table S4

Protein/Complex	pLDDT	pTM	ipTM
<i>Serratia marcescens</i> Db10			
VgrG1	88	n.a.	n.a.
VgrG2	91.3	n.a.	n.a.
TvpC	93.1	n.a.	n.a.
TvpAB	92.8	n.a.	n.a.
Db10 VgrG1-PAAR	86.5	0.792	0.776
Db10 pre-complex (TvpAB-TvpB-TvpC-PAAR)	92.3	0.898	0.903
<i>Agrobacterium tumefaciens</i> C58			
VgrG2 (Atu3642)	92.1	n.a.	n.a.
Tap2	93.1	n.a.	n.a.
VgrG2 full complex (VgrG2-Tap2-PAAR Tde2)	87.9	0.796	0.778
<i>Pseudomonas aeruginosa</i> PAO1			
VgrG1b (PA0095)	93.4	n.a.	n.a.
PA0096	77	n.a.	n.a.
PA0097	89.8	n.a.	n.a.
PAO1 pre-complex (PA0096-PA0097-PA0098-PAAR Tse7)	84.3	0.789	0.753

Appendix Table S4: Summary of the confidence scores of the Alphafold predictions presented in Figures 4 and 5. Overall confidence scores of each AlphaFold prediction (pLDDT) and specific confidence scores for complexes (pTM and ipTM) are indicated. The PAAR domain of Tde2 corresponds to amino acids 1-172, and the PAAR domain of Tse7 corresponds to amino acids 1-140.

Appendix Table S5

Protein	β prism length (Å)	pLDDT
<i>Serratia marcescens</i> Db10		
VgrG1	186	88
VgrG2	81	91.3
<i>Agrobacterium tumefaciens</i> C58		
VgrG2 (Atu3642)	96	92.1
VgrG1 (Atu4348)	118	84.9
<i>Pseudomonas aeruginosa</i> PAO1		
VgrG1b (PA0095)	154	93.4
VgrG1a (PA0091)	76	89.6
VgrG1c (PA2685)	117	86.1
VgrG2a (PA1511)	110	83.7
VgrG2b (PA0262)	116	80.1
VgrG3 (PA2373)	117	88.2
VgrG4a (PA3294)	80	82.1
VgrG4b (PA3486)	80	76.8
VgrG5 (PA5090)	80	78.9
VgrG6 (PA5266)	80	82.5
<i>Burkholderia pseudomallei</i> K96243		
VgrG3 (BPSS0181)	95	80.2
VgrG4a (BPSS0523)	X	71.6
VgrG4b (BPSS0524)	X	71.5
VgrG5 (BPSS1503)	165	65.6
VgrG (BPSS2056)	148	86.1
VgrG (BPSS0958)	89	86.9
VgrG2 (BPSS0105)	95	73
VgrG6 (BPSS2093)	152	87.6
<i>Burkholderia thailandensis</i> E264		
VgrG4a (BTH_II1894)	X	72.6
VgrG4b (BTH_II1893)	X	70.3
VgrG5 (BTH_II0863)	163	66.3
VgrG (BTH_II1436)	89	86.9
VgrG (BTH_I2705)	105	75.6
VgrG2 (BTH_II0129)	95	75.6
VgrG6 (BTH_II0265)	151	87.8
VgrG (BTH_II1531)	105	77.1
VgrG (BTH_I2693)	109	71.9
VgrG (BTH_I2697)	105	76.4
<i>Pantoea ananatis</i> PA13		
VgrG (PAGR_g1676)	110	80.6
VgrG (PAGR_g1684)	75	83.6
<i>Cronobacter sakazakii</i> ES15		
VgrG (ES15_3822)	159	82.1
VgrG (ES15_3808)	76	87.1
VgrG (ES15_3826)	99	86
VgrG (ES15_2015)	109	86.5
VgrG (ES15_2809)	100	76.4
<i>Proteus mirabilis</i> HI4320		

Appendix Table S5

VgrG1 (PMI0751)	85	80.3
VgrG2 (PMI0208)	80	83.5
VgrG3 (PMI1118)	76	81.2
VgrG4 (PMI1331)	80	83.8
VgrG5 (PMI2991)	99	82.5
<i>Vibrio parahaemolyticus</i> RIMD 2210633		
VgrG1 (VP1394)	125	88.9
VgrG (VPA1026)	n.d.	n.d.

Appendix Table S5: Summary Table of VgrG β -prism length measurements. Structural models of all the VgrG proteins in the set of eight representative bacteria (*S. marcescens* Db10, *A. tumefaciens* C58, *P. ananatis* PA13, *C. sakazakii* ES15, *P. mirabilis* HI4320, *P. aeruginosa* PAO1, *B. pseudomallei* K96243 and *B. thailandensis* E264) and in *V. parahaemolyticus* RIMD 2210633 were generated using AlphaFold3. Each model was visualized with PyMol to measure the length of the β -prism when possible. These measurements were used to generate Figure 8C. Confidence scores (pLDDT) for each AlphaFold prediction are also indicated. n.d : not determined.

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