Supplementary Information

The accessory protein TagV is required for full Type VI secretion system activity in *Serratia marcescens*

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TagV            ---------------MKTRITLTLLTALTLAGCSTPPPPPPALNNDALISSEVNGVTLQH    45
PAGR_g2450      ---------------MKKNLAFILLAGLLLSGCKTPP---PAVNDDTLVTSDVNGVKLVH    42
EBc_18420       --------MKCMRTNVKISIAFTLLAVLSLSGCKAPP---PPITDDTIVTSEVDGVKFTH    49
PAGR_g1655      -------MELNMKKMVNFRIALSLMFVFAVAGCKTP----PKMTDDTMVSSTVDGTTLIH    49
ES15_3853       ---------------MNMRIVLALLFVLGVAGCKAPP---PAVTDDTIVTSVVNGVTLTH    42
EAM_0571        --------------MVKIRIALSLLLVMSVVGCKTPP---PQITDDTVVSSTVDGVKLSY    43
EBc_05760       MSYAFHSKKNFMRIMVKIRIALSLLFVLTVAGCKAPP---PAMTDDTIISSTVDGVKLTY    57
                              ::  :.: *:  : : **.:*    * :.:*::::* *:*..: :

TagV            RAAVSAPKQFKPIGEEYRSLYAASIMSSPNYTGTAVGSLDNAAAFYALGEVENNWLAISA    105
PAGR_g2450      RHAVAAPTTFTPINQTWRALYKASVMTQPDYGGKVVRYLEAGKSFEVLGSVENHWLAIAD    102
EBc_18420       RHAVQAPTSFTPVNETYRALYNASVMTSPDFSGKNVRYLENGKPFTVLGEVENHWLAIAD    109
PAGR_g1655      RHAVAAPAQFSALNESYRALYPASVMTRPDFGGKVVETLKTGETYTVVGQVENNWLALGE    109
ES15_3853       RHAVTPPKEFEPVNEEYRAMYPASVMTRPDFGGKVVRQLETGKTYNVLGQVEGHWLAIAD    102
EAM_0571        RHAITPPQSFTPVNEEYRALYAASVMSRPDFGGKLVRHLDNGQTFTVLGSVENNWFAIAD    103
EBc_05760       RHAVQPPTSFTPVNEEYRALYDASVMNRPDFGGKLVSNLENGKPYTVLGSVENNWFAIAE    117
                * *:  *  *  :.: :*::* **:*. *:: *. *  *. .  : .:*.**.:*:*:.

TagV            IRG--------------------------GDLMGYIQANAGVPEARYKSTLRKDLPRRAR    139
PAGR_g2450      KPD--------------------------GMLTGYVPLKAGVERHRYDDTLRNDRPRPRK    136
EBc_18420       PDQ--------------------------EQLIGYVPFKAGVKTDLYDATIKSDRPRPRK    143
PAGR_g1655      EAQAEAPPAASDAKATDSKAAPAQATTSAIKVVGYVPFRAVVKSDLYDQTVKADQPKRRA    169
ES15_3853       QGE--------------------------EQLIGYVPLRAAVKSSLYDATVSKEIPK--L    134
EAM_0571        AGQ--------------------------EQLIGYVPLRAGVKSDLYNQTLKADQRRKRV    137
EBc_05760       QGQ--------------------------EQLIGYVPLRAVVKSDLYDKTVKADSRRKRV    151
                                           : **:  .* *    *. *:  :  :

TagV            ---AAKQDCVKVGGDSKACKNAGSATWILQ 166
PAGR_g2450      ---T-KQVCVDVGGDSKACRNTATATWILN 162
EBc_18420       ---NAKKVCVDVGGQSKACRNNATATWILE 170
PAGR_g1655      RAAAKKKTCVSVDGNSQACQNKNNGTWIIN 199
ES15_3853       RRKTAKANCVTVDGNSKACKNTKSGTWVID 164
EAM_0571        RAPAKKKTCVAVDGDSKACQNNNNGTWIID 167
EBc_05760       RAASK-KTCV-AAGDSKACQNSNSGTWIID 179
                       ** . *:*:**:*  ..**:::
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Figure S1. Multiple sequence alignment of TagV and its homologues. Sequence titles for TagV homologues refer to their genomic identifiers as presented in Figure 1.

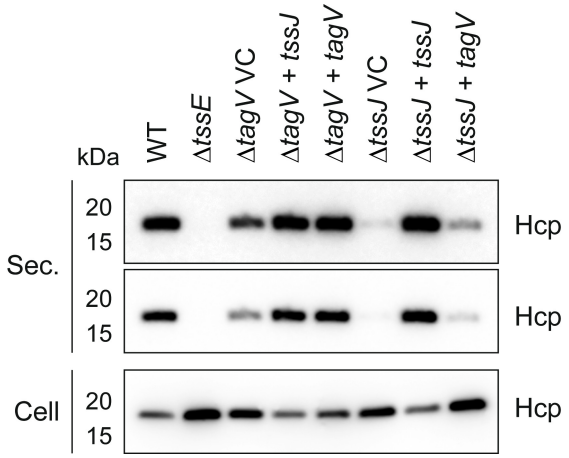

Figure S2. The reduced Hcp secretion observed in the Δ*tagV* and Δ*tssJ* mutants can be complemented by expression of the respective gene *in trans*. Levels of Hcp in the total cellular (Cell) and secreted (Sec.) protein fractions from wild type (WT) and mutant strains of *S. marcescens* carrying the vector control plasmid (pSUPROM, VC) or plasmids directing the expression of *tagV* (+ *tagV*) or *tssJ* (+ *tssJ*) as detected by immunoblot. Two different images of the same blot are shown for the secreted fractions to aid clarity of all bands.

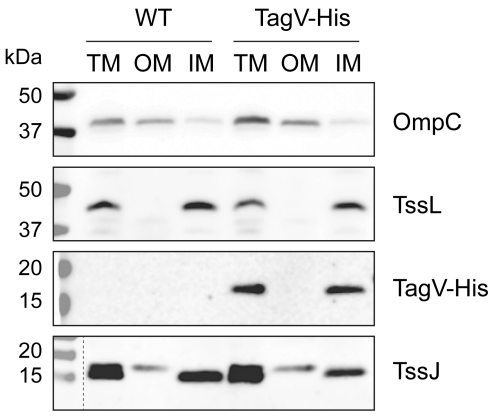

Figure S3. The outer membrane lipoproteins TagV and TssJ solubilise with the inner membrane of *S. marcescens* in selective detergent solubilisation experiments. Total membrane fractions (TM) of wild type (WT) and TagV-His were separated into outer membrane (OM) and inner membrane (IM) fractions by selective detergent (sarkosyl) solubilisation. Fractionated membranes were probed for the presence of OmpC (outer membrane control), TssL (inner membrane control), TagV-His or TssJ by immunoblot.

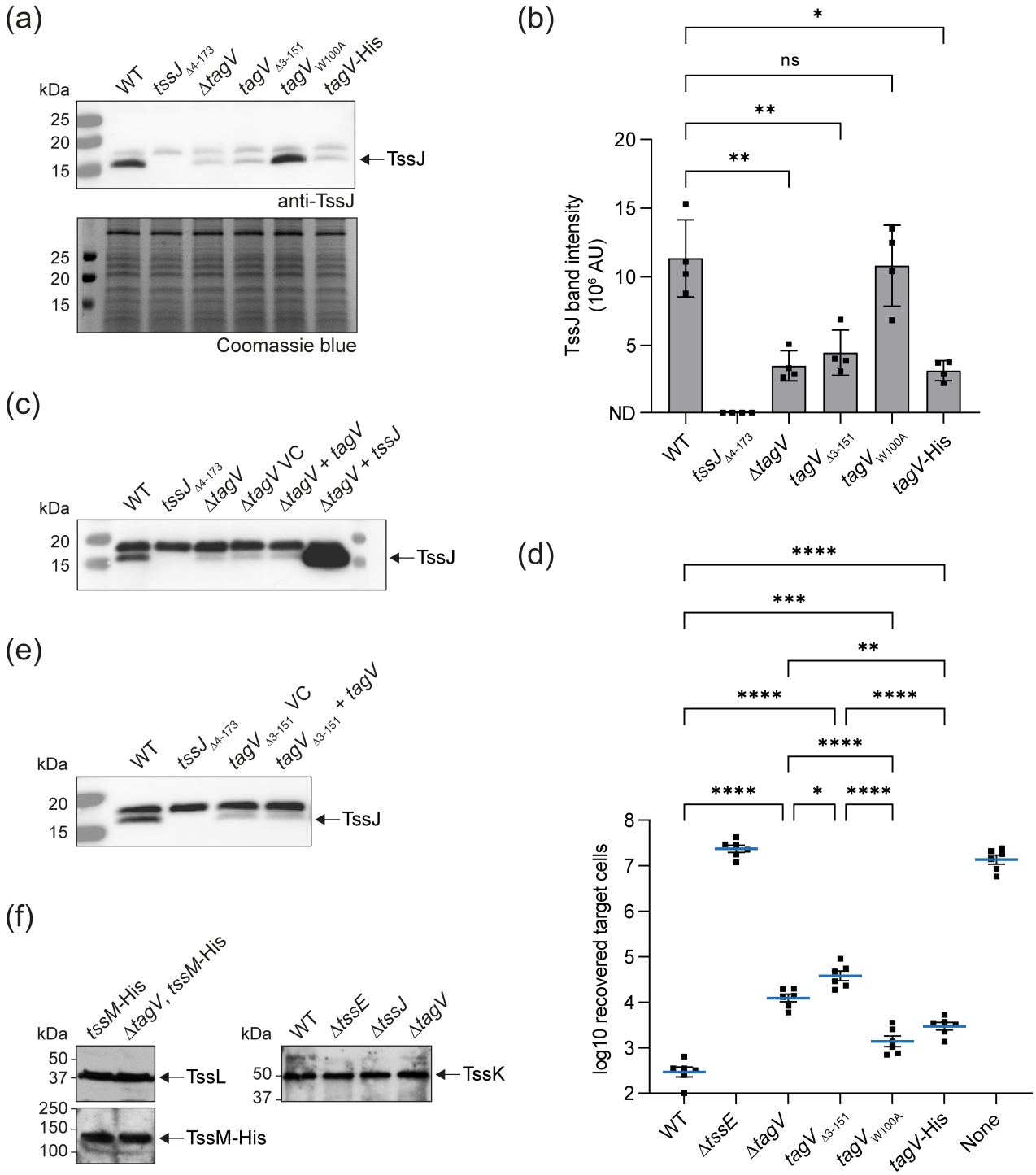

Figure S4. Genetic manipulation of *tagV* results in decreased TssJ production which cannot be restored by complementation with *tagV*. (a) Total cellular protein samples from wild type (WT) and mutant strains of *S. marcescens* were analysed for TssJ production by immunoblot. (b) TssJ band intensity as measured by densitometry. Data are displayed as mean +/- SEM from four independent experiments with individual data points overlaid. One-way ANOVA with Dunnett's multiple comparison test was performed (** P<0.01; * P<0.05; ns, not significant). (c) As in panel a except the Δ*tagV* mutant is carrying either the vector control plasmid (pSUPROM, VC) or plasmids directing the expression of *tagV* (+ *tagV*) or *tssJ* (+ *tssJ*) *in trans*. (d) Recovery of *P. fluorescens* KT02 target cells following co-culture with WT and mutant strains of *S. marcescens* at an initial ratio of 1:1 for four

hours. Data are displayed as mean +/- SEM (n=6) with individual data points overlaid. One-way ANOVA with Tukey's multiple comparison test was performed (**** *P*<0.0001; *** *P*<0.001; ** *P*<0.01; * *P*<0.05; for clarity, only selected comparisons are displayed). None, sterile media only. (e) As in panel c except the *tagV*$_{\Delta 3\text{-}151}$ mutant is carrying either the vector control plasmid (pSUPROM, VC) or plasmids directing the expression of *tagV* (+ *tagV*) *in trans*. (f) Total cellular protein samples from WT and mutant strains of *S. marcescens* were analysed for TssL, TssM and TssK production by immunoblot (TssM levels were detected by anti-His immunoblot of strains encoding TssM-His$_6$ at the normal chromosomal location, *tssM*-His).

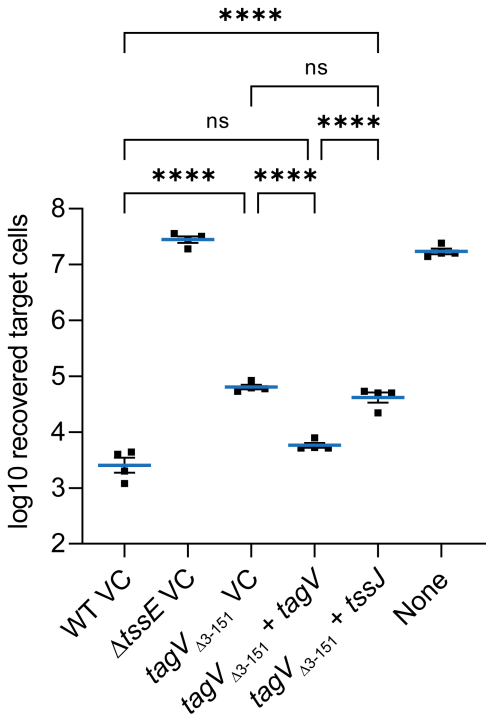

Figure S5. Expression of *tagV* but not *tssJ in trans* is able to restore T6SS activity in the *tagV*$_{\Delta 3\text{-}151}$ mutant. Recovery of *P. fluorescens* KT04 following co-culture with WT and mutant strains of *S. marcescens* carrying either the vector control plasmid (pSUPROM, VC) or plasmids directing the expression of *tagV* (+ *tagV*) or *tssJ* (+ *tssJ*) *in trans*. Data are displayed as mean +/- SEM (n=4) with individual data points overlaid. One-way ANOVA with Tukey's multiple comparison test was performed (**** P<0.0001; ns, not significant; for clarity, only selected comparisons are displayed). None, sterile media only.

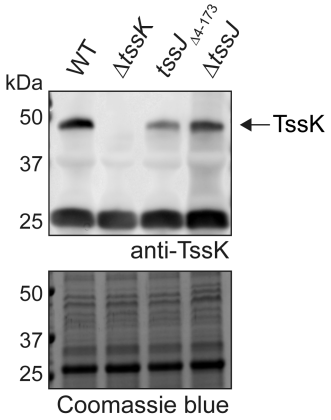

Figure S6. TssK production in strains carrying two different deletions in *tssJ*. Levels of TssK in total membrane protein fractions from wild type (WT) and mutant strains of *S. marcescens* were determined by immunoblot (*tssJ*$_{\Delta 4\text{-}173}$, in-frame deletion of amino acids 4-173 of 176; Δ*tssJ*, in-frame deletion of amino acids 4-28).

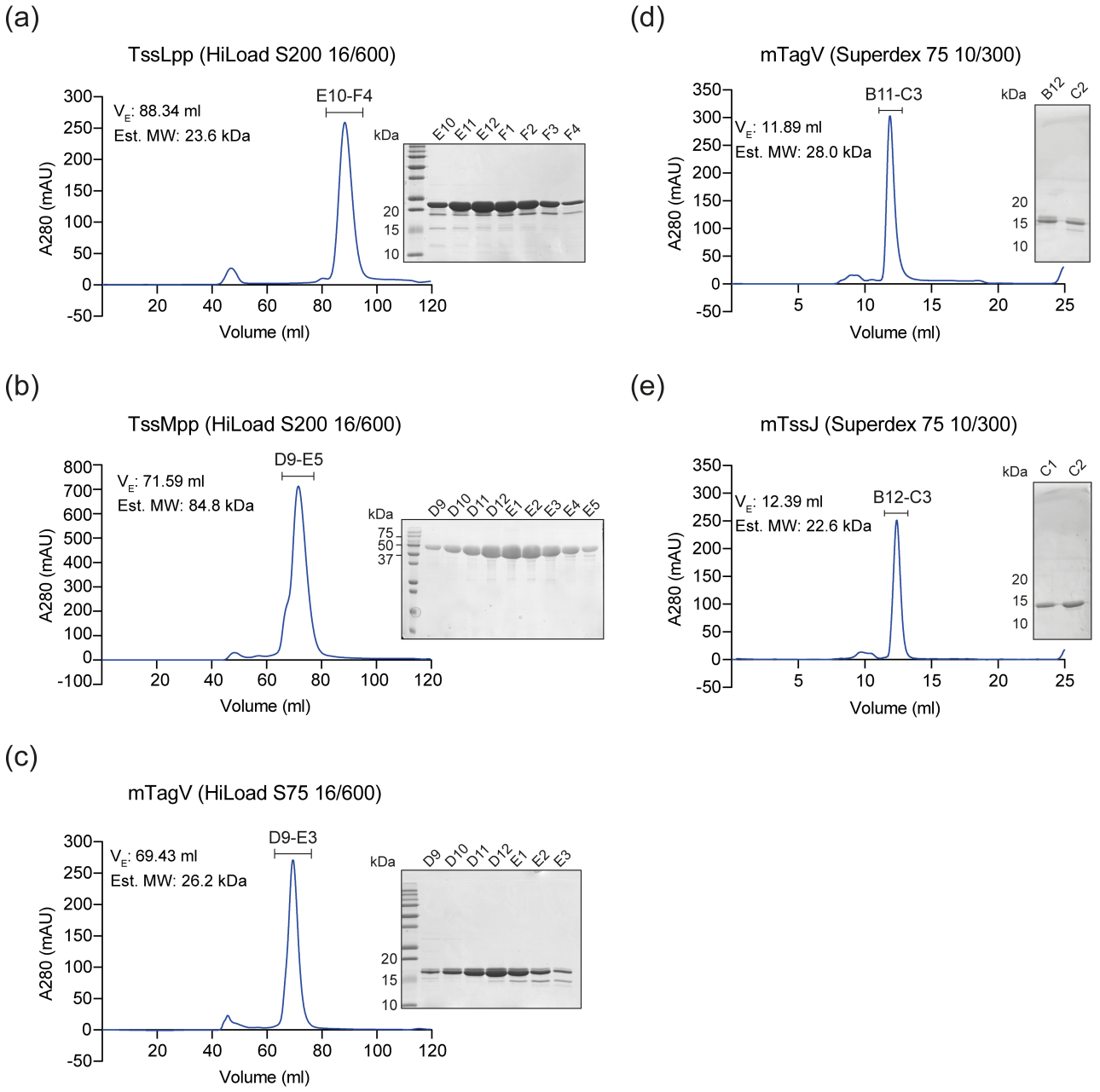

Figure S7. Integrity of proteins used for *in vitro* complex formation experiments. Affinity purified periplasmic domains of TssL (TssLpp, panel a) and TssM (TssMpp, panel b), and mature TagV (mTagV, panels c and d) and TssJ (mTssJ, panel e) proteins were separated by size exclusion chromatography using the column indicated. Protein fractions were visualised by SDS-PAGE and Coomassie staining. The elution volume (Ve) and estimated molecular weight (Est. Mw) of each peak, based on calibration of the column with protein standards, is noted in each case. Note that the data in panels d and e is the same as that used in Figure 4c for the individual mTagV and mTssJ proteins.

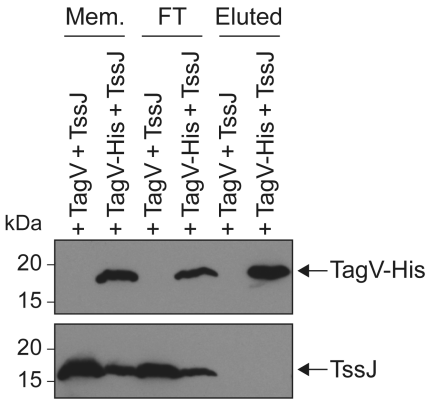

Figure S8. TssJ does not co-purify with TagV-His when both are expressed from a plasmid. Total membrane fractions prepared from *S. marcescens* Db10 Δ*tagV*Δ*tssJ* carrying plasmids directing the expression of TagV and TssJ (pSC2017) or TagV-His and TssJ (pSC2040) were incubated with Ni^{2+} beads and the membrane (input, Mem,), flowthrough (FT) and eluted fractions were probed for the presence of TagV-His and TssJ by immunoblot.

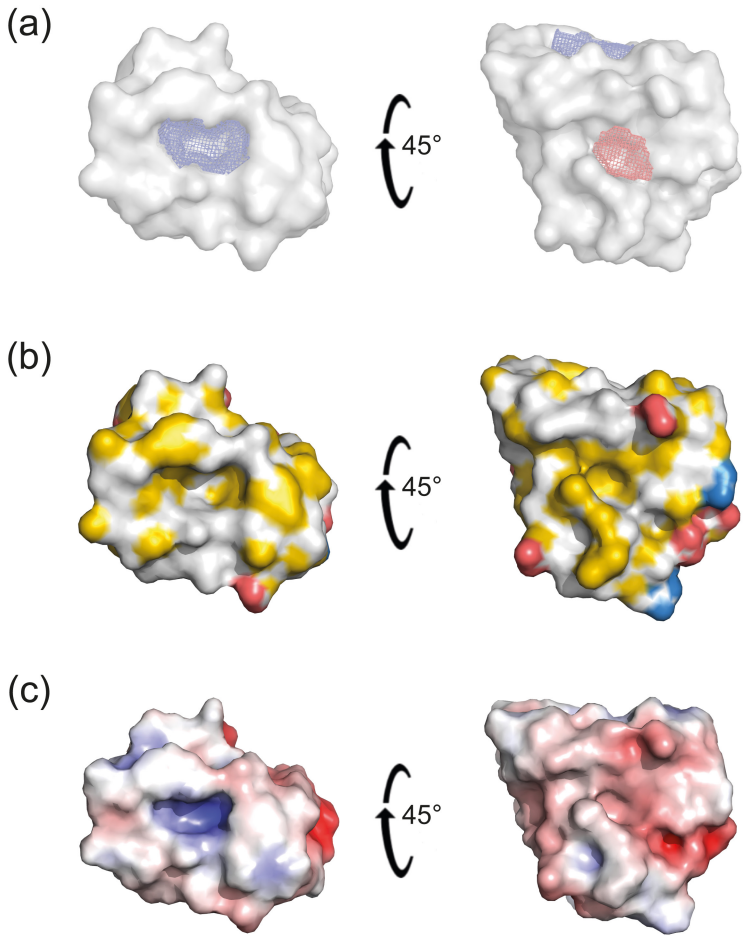

Figure S9. The predicted surface topology of the putative TagV SH3b domain. (a) Pockets P0 (blue mesh) and P1 (red mesh) identified by the DogSiteScorer algorithm (Volkamer *et al.*, 2012). (b) Surface hydrophobicity as determined using the YRB scheme proposed by Hagemans and colleagues (Hagemans *et al.*, 2015). Hydrophobic regions are highlighted in yellow, negatively charged groups of glutamate and aspartate are highlighted in red and positively charged functional groups of lysine and arginine are highlighted in blue. (c) Electrostatic potential as determined using the Adaptive Poisson-Boltzmann Solver for PyMol 2.0 (Baker *et al.*, 2001) and scaled from +5 kT/e (positive, blue) to −5 kT/e (negative, red).

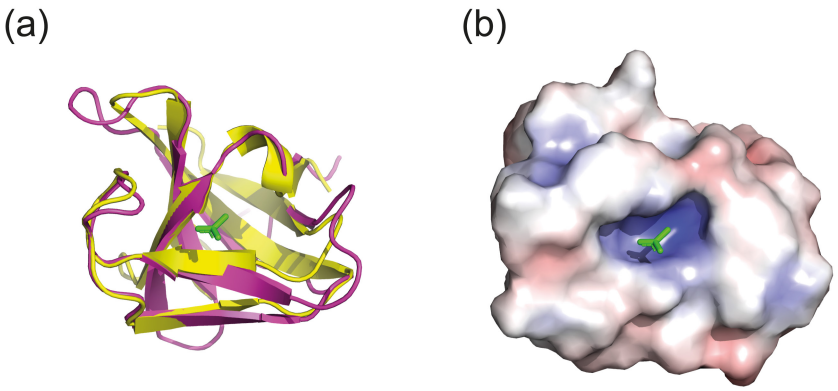

Figure S10. Superimposition of the putative TagV SH3b domain and Psm. (a) The putative TagV SH3b domain (yellow ribbon) is superimposed onto the structure of Psm (PDB 4krt, purple ribbon) in complex with an acetic acid molecule (green stick) with an RSMD of 0.719 Å. (b) The surface electrostatic potential of the superimposed TagV SH3b domain was determined using the Adaptive Poisson-Boltzmann Solver for PyMol 2.0 (Baker *et al.*, 2001) and is scaled from +5 kT/e (positive, blue) to −5 kT/e (negative, red).

Table S1. Previously-described Type VI associated genes (Tags).

Name	Description	Reference
TagA	DUF2169 domain-containing protein often encoded in T6SS gene clusters and originally named by Shalom and colleagues. Subsequently proposed to be an adaptor/chaperone protein for PAAR-containing effectors.	(Shalom *et al.*, 2007, Bondage *et al.*, 2016)
TagA / TsmA	ImpA_N domain-containing protein. Terminates sheath polymerisation and maintains 'long' TssA ($TssA_L$ or TsaC) in extended conformation.	(Santin *et al.*, 2018, Schneider *et al.*, 2019)
TagB	Pentapeptide repeat protein often encoded in T6SS gene clusters and originally named by Shalom and colleagues. Function unknown.	(Shalom *et al.*, 2007)
TagB / TsmB*	Sheath stabilisation in some T6SSs containing 'short' TssA ($TssA_S$ or TsaB). Also known as TagB1 and previously as TagX1. We propose it be named TsmB to fit with the nomenclature of Schneider *et al*.[7]	(Bernal *et al.*, 2021)
TagC	Protein of unknown function often encoded in T6SS gene clusters.	(Lennings *et al.*, 2018, Shalom *et al.*, 2007)
TagD	Class of PAAR protein, containing DUF4150 domain. Part of "PAAR_E" phylogenetic clade defined by Zhang and colleagues.	(Lennings *et al.*, 2018, Zhang *et al.*, 2021)
TagE	Usually known as PpkA. Thr protein kinase that phosphorylates Fha or TssL, activating T6SS assembly.	(Jiang *et al.*, 2019, Lin *et al.*, 2014, Fritsch *et al.*, 2013)
TagF	Negative post-translational regulator of T6SS assembly.	(Lin *et al.*, 2018, Silverman *et al.*, 2011)
TagG	Usually known as PppA. Thr protein phosphatase that dephosphorylates Fha, repressing T6SS assembly.	(Mougous *et al.*, 2007, Jiang *et al.*, 2019, Fritsch *et al.*, 2013)
TagH	Usually known as Fha. FHA domain-containing protein required for T6SS assembly in many T6SS. Can be substrate for PpkA phosphorylation.	(Mougous *et al.*, 2007, Jiang *et al.*, 2019, Ostrowski *et al.*, 2018)
TagI	Putative peptidoglycan-binding lipoprotein, function unknown.	(Shalom *et al.*, 2007)
TagJ	Sheath stabilisation in some T6SSs containing 'short' TssA (also known as $TssA_S$ or TsaB).	(Bernal *et al.*, 2021, Forster *et al.*, 2014)
TagK	Function unknown.	(Shalom *et al.*, 2007)
TagL	Also known as SciZ. Peptidoglycan-binding inner membrane protein, anchors membrane complex to the cell wall in T6SSs with 'short' TssL.	(Santin *et al.*, 2019, Nguyen *et al.*, 2021)
TagM	Putative outer membrane lipoprotein with peptidoglycan-binding domain, function unknown.	(Spiewak *et al.*, 2019, Shalom *et al.*, 2007)
TagN	Putative peptidoglycan-binding protein, function unknown.	(Aschtgen *et al.*, 2010, Ringel *et al.*, 2017)
TagO	Also known as VasI, function unknown.	(Zhang *et al.*, 2020, Moriel *et al.*, 2021)
TagP	Putative peptidoglycan-binding homologue of TssM. Thought to bind TssL.	(Aschtgen *et al.*, 2010)
TagQ	Anchors TagR to the outer membrane.	(Casabona *et al.*, 2013)
TagR	Positive regulator of PpkA kinase activity and thus Fha phosphorylation.	(Hsu *et al.*, 2009, Casabona *et al.*, 2013)
TagS	Integral membrane protein forming a membrane associated complex with TagT which co-operates with TagQR to regulate Fha phosphorylation by PpkA. TagQRST detects incoming T6SS attacks.	(Casabona *et al.*, 2013, Basler *et al.*, 2013)

TagT	ATPase forming a membrane associated complex with TagS which co-operates with TagQR to regulate Fha phosphorylation by PpkA. TagQRST detects incoming T6SS attacks.	(Casabona *et al.*, 2013, Basler *et al.*, 2013)
TagU	Unknown, variably described as a lipoprotein in *P. fluorescens* Pf29Arp and *P. chlororaphis* O6 and as a truncated ORF in *P. fluorescens* MFE01.	(Marchi *et al.*, 2013, Gallique *et al.*, 2017)
TagV	Not defined to date.	
TagW	Putative peptidoglycan binding protein, function unknown.	(Aschtgen *et al.*, 2010)
TagX	Also known as AsaE, peptidoglycan hydrolase required for T6SS transport across cell wall.	(Weber *et al.*, 2016, Spiewak *et al.*, 2019)
TagY	Transmembrane domain containing protein, function unknown.	(Spiewak *et al.*, 2019)
TagZ	Also known as AsaB, putative cytoplasmic membrane protein, function unknown.	(Spiewak *et al.*, 2019)
AsaA	Also known as TslA. Interacts with TssM and directs T6SS assembly to cell-cell contact sites.	(Li *et al.*, 2019, Ringel *et al.*, 2017, Weber *et al.*, 2016, Lin *et al.*, 2022)
RtkS	Interacts with PpkA and regulates T6SS firing.	(Ostrowski *et al.*, 2018)
TasL	Mediates cell-cell attachment which facilitates T6SS intoxication.	(Speare *et al.*, 2022)

*Name proposed here

Table S2. Strains and plasmids used in this study.

Name	Details	Reference
Bacterial strains		
Serratia marcescens		
Db10	Wild type strain	(Flyg *et al.*, 1980)
SJC11	Db10 Δ*tssE* (*SMDB11_2271*)	(Murdoch *et al.*, 2011)
LM04	Db10 Δ*tagV*; in-frame deletion in *tagV* (*SMDB11_2251*) ΔT3-T162	This study
LM31	Db10 Δ*tssJ*; in-frame deletion in *tssJ* (*SMDB11_2252*) ΔT4-S28	This study
LM40	Db10 Δ*tagV* Δ*tssJ*; in-frame deletions *tagV* ΔT3-T162, *tssJ* ΔT4-S28	This study
LM37	Db10 ΔtssE Δ*tssJ*; in-frame deletions Δ*tssE*, *tssJ* ΔT4-S28	This study
LM70	Db10 $tagV_{\Delta 3–151}$; in-frame deletion in *tagV* ΔT3-D151	This study
SJC10	Db10 $tssJ_{\Delta 4–173}$; in-frame deletion in *tssJ* ΔT4-K173	(Murdoch *et al.*, 2011)
BH03	Db10 *tagV*-His; encodes C-terminally His_6-tagged version of TagV at the native chromosomal location	(Mariano *et al.*, 2018)
MR10	Db10 $tagV_{W100A}$	This study
GM80	Db10 *tssM*-His; encodes C-terminally His_6-tagged version of TssM (SMDB11_2255) at the native chromosomal location	(Mariano *et al.*, 2018)
LM45	Db10 Δ*tagV*, *tssM*-His	This study
GE04	Db10 Δ*tssK* (*SMDB11_2253*)	(English *et al.*, 2014)
Pseudomonas fluorescens		
KT02	Sm^R derivative of *P. fluorescens* 55	(Murdoch *et al.*, 2011)
KT04	Sm^R Kan^R derivative of *P. fluorescens* 55	(Murdoch *et al.*, 2011)
Escherichia coli		
CC118λ*pir*	Donor strain for pKNG101-derived allelic exchange plasmids	(Herrero *et al.*, 1990)
HH26 pNJ5000	Mobilising strain for conjugal transfer	(Grinter, 1983)
Plasmids		
Mutagenesis		
pKNG101	Suicide vector for allelic exchange (Sm^R, *sacBR, mobRK2, oriR6K*)	(Kaniga *et al.*, 1991)
pSC1921	pKNG101-based allelic exchange plasmid for deletion of *tagV* (T3–T162)	This study
pSC2031	pKNG101-based allelic exchange plasmid for deletion of *tssJ* (T4–S28)	This study
pSC3430	pKNG101-based allelic exchange plasmid to introduce $tagV_{W100A}$ mutation	This study
pSC2069	pKNG101-based marker exchange plasmid for deletion of *tagV* (T3–D151)	This study
pSC1989	pKNG101-based marker exchange plasmid for addition of His_6 tag to C-terminus of *tagV*	(Mariano *et al.*, 2018)
Protein production		
pET15b-TEV	Protein overexpression vector for fusion with N-terminal His_6 tag under the control of T7 promoter. Derived from pET15b (Amp^R)	(Rao *et al.*, 2011)
pSC102	Protein overexpression vector for fusion with N-terminal His_6 tag under the control of T5 promoter (Ap^R, Kn^R).	(English *et al.*, 2014)
pSC534	TssLpp (G218-K406) cloned in pSC102	This study
pSC055	TssMpp (L527-P1211) cloned in pET15b-TEV	This study

pSC1952	mTagV (S19–Q166) cloned in pET15b-TEV	This study
pET15b-TEV_SMA2252T	mTssJ (A30-D176) cloned in pET15b-TEV	(Rao *et al.*, 2011)
Expression *in trans* in *S. marcescens*		
pSUPROM	Vector for constitutive expression of cloned genes under the control of the *E. coli tat* promoter (Kn^R)	(Murdoch *et al.*, 2011)
pSC066	Full length *tssJ* cloned in pSUPROM	(Murdoch *et al.*, 2011)
pSC1920	Full length *tagV* cloned in pSUPROM	This study
pSC2017	Full length *tagV* and *tssJ* cloned in pSUPROM	This study
pSC2040	Full length *tagV* with C-terminal His_6 tag and *tssJ* cloned in pSUPROM	This study
pSC1920 (W100A)	Full length *tagV* containing W100A point mutation cloned in pSUPROM	This study
pSC1920 (Q116E)	Full length *tagV* containing Q116E point mutation cloned in pSUPROM	This study
pSC1920 (Q116P)	Full length *tagV* containing Q116P point mutation cloned in pSUPROM	This study

Table S3. Primers used in this study.

Primer	Sequence (5'-3')	Description
TagV mutant construction		
LM070	TGTGTCTAGATGCGCCAAACCGAGCACGTGCGCGC	Forward primer to clone upstream region of *tagV* into pKNG101 for Δ*tagV* production in Db10
LM071	TATAAAGCTTTTTCACAATGTCCTCTTTTTCTTGA	Reverse primer to clone upstream region of *tagV* into pKNG101 for Δ*tagV* production in Db10
LM072	TATAAAGCTTTGGATCCTCCAGTAGTCAGCACGCC	Forward primer to clone downstream region of *tagV* into pKNG101 for Δ*tagV* (T3–T162) production in Db10
LM073	TATAGGGCCCTACTTCGCTTCAGCGCAGCGGTAGG	Reverse primer to clone downstream region of *tagV* into pKNG101 for Δ*tagV* production in Db10
LM326	TATACTCGAGTTTCACAATGTCCTCTTTTTCTTGA	Reverse primer to clone upstream region of *tagV* into pKNG101 for Δ*tagV* production in Δ*tssJ*
LM327	TATACTCGAGTGGATCCTCCAGTAGTCAGCACGCC	Forward primer to clone downstream region of *tagV* into pKNG101 for Δ*tagV* (T3-T162) production in Δ*tssJ*
LM383	TATAAAGCTTAGCAAGGCGTGTAAAAACGC	Forward primer to clone downstream region of *tagV* into pKNG101 for Δ*tagV* (T3-D151) production in Db10
LM216	TATAAAGCTTCATCATCATCATCATCACTAGTCAGCACGCCTGCGGGC	Forward primer to incorporate a C-terminal His_6 tag into *tagV*
MR009	ACAGGATCCAACGCGTTCAACATTCTGTTCGTC	Forward primer to clone upstream region of *tagV* into pKNG101 for $tagV_{W100A}$ production in Db10
MR004	GGAGATCGCCAGCGCGTTGTT	Reverse primer to clone upstream region of *tagV* into pKNG101 for $tagV_{W100A}$ production in Db10
MR005	AACAACGCGCTGGCGATCTCC	Forward primer to clone downstream region of *tagV* into pKNG101 for $tagV_{W100A}$ production in Db10
MR006	ACATCTAGAAGAACTGATCGCTGTCCAGCAG	Reverse primer to clone downstream region of *tagV* into pKNG101 for $tagV_{W100A}$ production in Db10
TssJ mutant construction		
LM214	TGTATCTAGACTCGGCATGCGCATCGTGCC	Forward primer to clone upstream region of *tssJ* into pKNG101 for Δ*tssJ* (T4-S28) production in Db10 and SJC11

SC2053	TATAAAGCTTCGTCATCATAGGAATCTCGTCG	Reverse primer to clone upstream region of *tssJ* into pKNG101 for Δ*tssJ* (T4-S28) production in Db10 and SJC11
LM310	TATAAAGCTTTCCGCCAAAAGCGTGCCGTC	Forward primer to clone downstream region of *tssJ* into pKNG101 for Δ*tssJ* (T4-S28) production in Db10 and SJC11
SC2469	TATAGGGCCCTATAGTTACTTCGCTTCAGCGC	Reverse primer to clone downstream region of tssJ into pKNG101 for Δ*tssJ* (T4-S28) production in Db10 and SJC11
Protein expression		
LM068	TATATCTAGAGTGAAAACACGGATTACCCTGACGC	Forward primer to clone *tagV* into pSUPROM
LM069	TATAAAGCTTCTACTGGAGGATCCAGGTGGCTGAG	Reverse primer to clone *tagV* into pSUPROM
LM293	TATAAAGCTTTCAGTCGACCTTTTTTACGG	Reverse primer to clone *tagV* - *tssJ* in pSUPROM
LM305	TATACTCGAGCTAGTGATGATGATGATGATG CTGGAGGATCCAGGTGGCTG	Reverse primer to clone *tagV* with C-term His_6 tag (with TssJ) in pSUPROM
LM306	TATACTCGAGTCAGCACGCCTGCGGGCGAC	Forward primer to clone *tssJ* (with *tagV*-His) in pSUPROM
SC2137	TATACATATGTTGGGCAACGGCGACATGTTC	Forward primer to clone *tssM*pp into pET15b-TEV
SC2120	TATAGGATCCCTACGGGCACGAGAAGGC	Reverse primer to clone *tssM*pp into pET15b-TEV
GE034	TATAGGATCCGGCGACAACACCAGCCC	Forward primer to clone *tssL*pp into pSC102
GE035	TATAGTCGACTTACTTTCCGGTTCCTTGCGG	Reverse primer to clone *tssL*pp into pSC102
Site-directed mutagenesis		
W100A_F	GGAAAACAACGCGCTGGCGATCTCCGCC	Site directed mutagenesis of pSC1920 to introduce W100A mutation
W100A_R	ACTTCGCCCAGCGCGTAG	Site directed mutagenesis of pSC1920 to introduce W100A mutation
Q116E_F	GGGGTATATCGAGGCCAACGCCG	Site directed mutagenesis of pSC1920 to introduce Q116E mutation
Q116E_R	ATCAGATCGCCCCCGCGG	Site directed mutagenesis of pSC1920 to introduce Q116E mutation
Q116P_F	GGGGTATATCCCGGCCAACGCCG	Site directed mutagenesis of pSC1920 to introduce Q116P mutation
Q116P_R	ATCAGATCGCCCCCGCGG	Site directed mutagenesis of pSC1920 to introduce Q116P mutation

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