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Supplementary Information for

An on-board checking mechanism ensures effector delivery of the type VI secretion system in *Vibrio cholerae*

Running title: Effector-loading ensures secretion efficiency

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Supplementary text
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Other supplementary materials for this manuscript include the following:

Datasets S1

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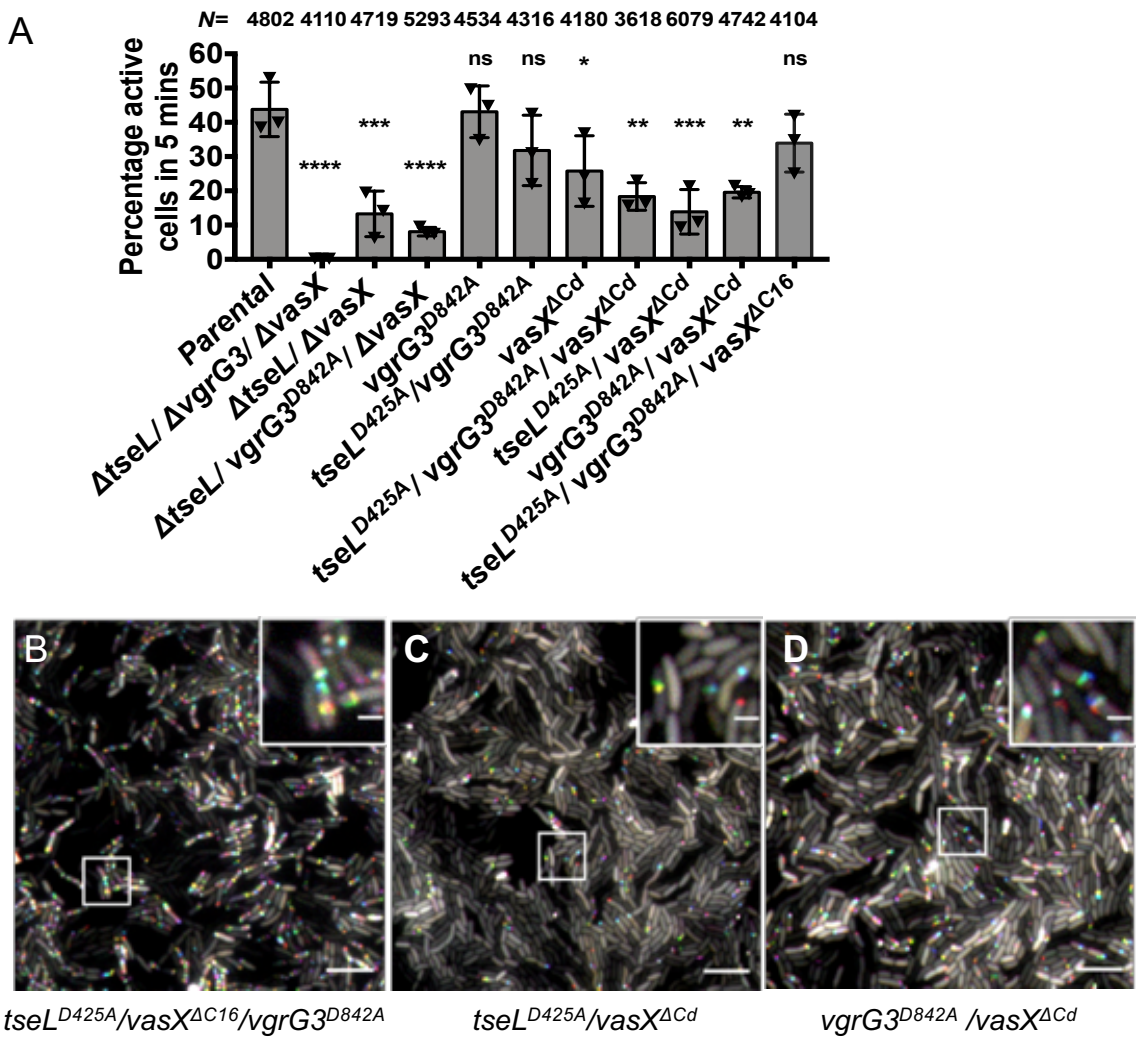


Fig. S1. Quantification and comparison of sheath formation in all mutants. A, *N* indicates the number of cells counted for each strain. Error bars indicate the mean +/- standard deviation (n= 3). One-way ANOVA with Dunnett's multiple comparisons test compared to parental ****$P <$ 0.001; ***$P <$ 0.001; **$P <$ 0.005; *$P <$ 0.05; ns, not significant. B-D, 40 X 40 µm representative field of cells with a 3X magnified 5 X 5 µm inset (marked by box) is shown. VipA-mCherry signal was followed for 5 minutes and temporally color coded. Representative images are shown with a close up of a selected region as an inset. Scale bar for large field of view represents 5 µm and for the inset represents 1 µm. Genotypes are as indicated.

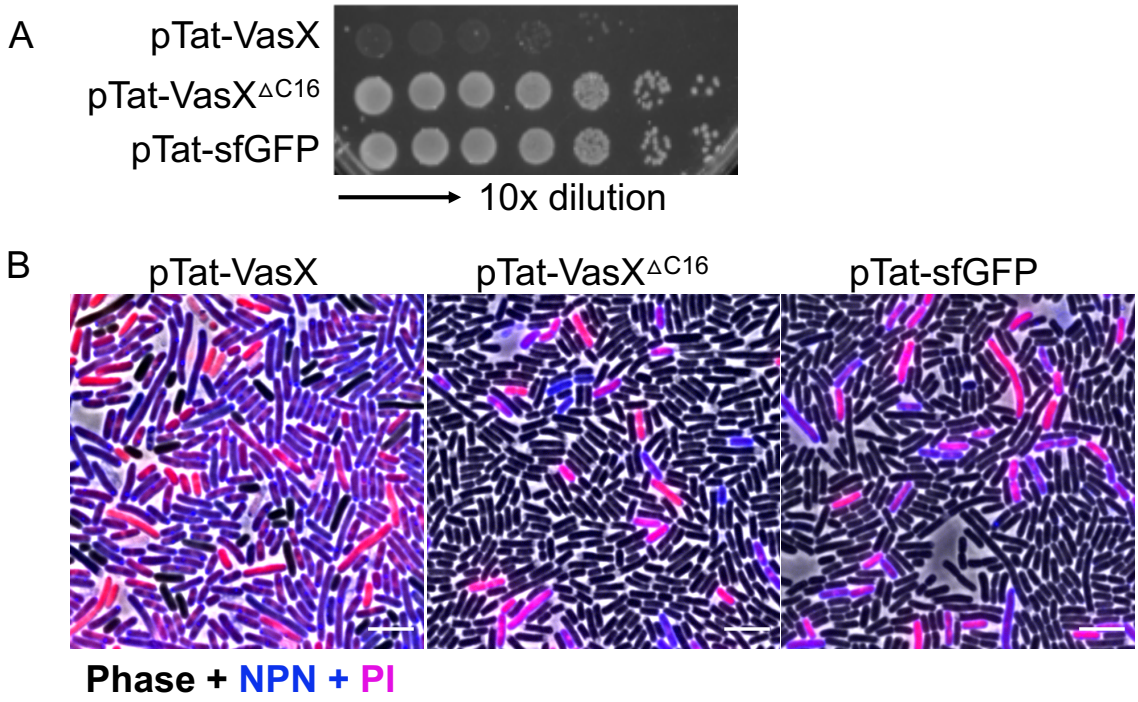


Fig. S2. Periplasmic expression of VasX results in membrane damage and cell death. A, survival of cells expressing pTat-VasX and pTat-VasX$^{\triangle C16}$ by serial dilution. B, merged images of cells expressing pTat-VasX and pTat-VasX$^{\triangle C16}$. The phase-contrast, NPN and PI channels are merged and representative images shown. Fluorescence signals for NPN and PI are depicted in blue and red respectively.

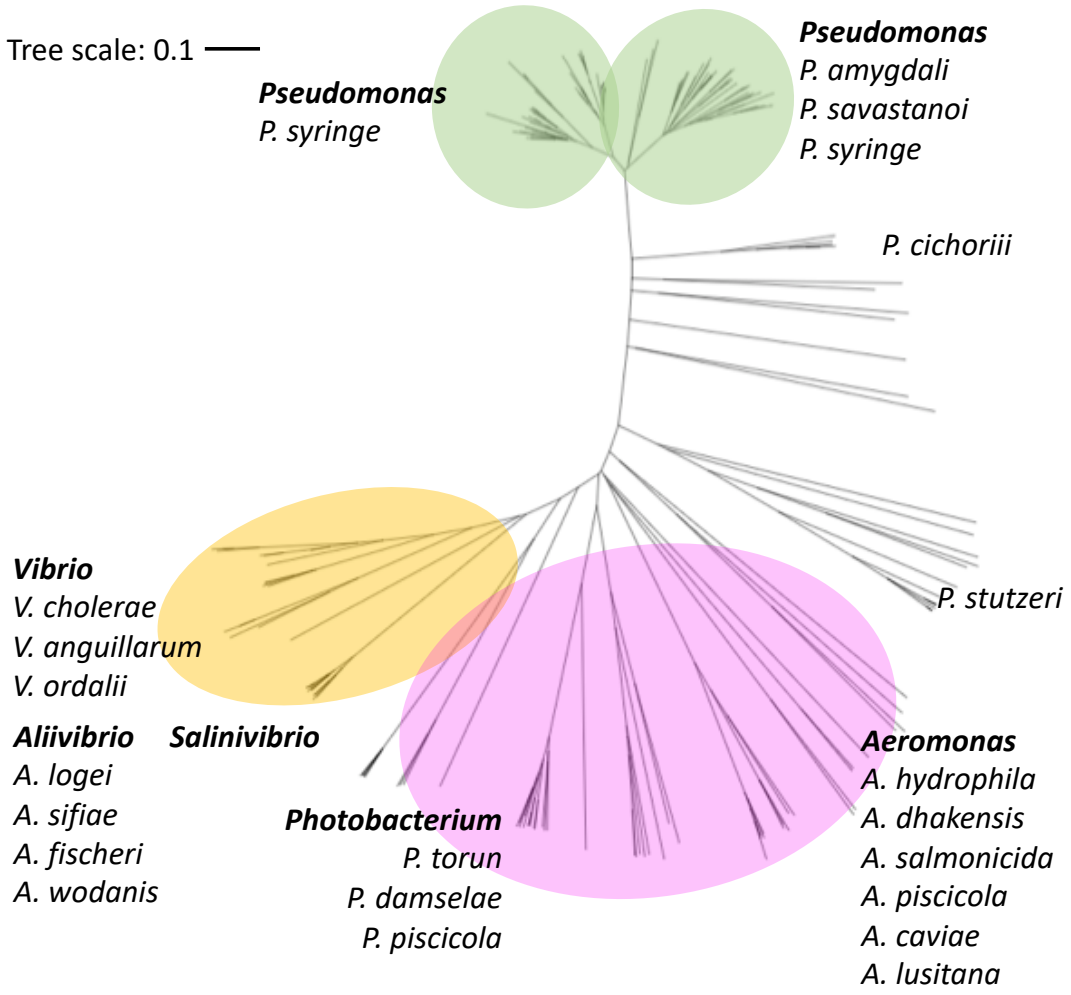


Fig. S3. Neighbor-joining tree of VasX homologs. Sequences of 198 VasX homologs were downloaded from the HMMER database and analyzed using Jalview and EMBL-EBI. The graphic view is generated by iTOL. Representative species and their relative clusters are indicated.

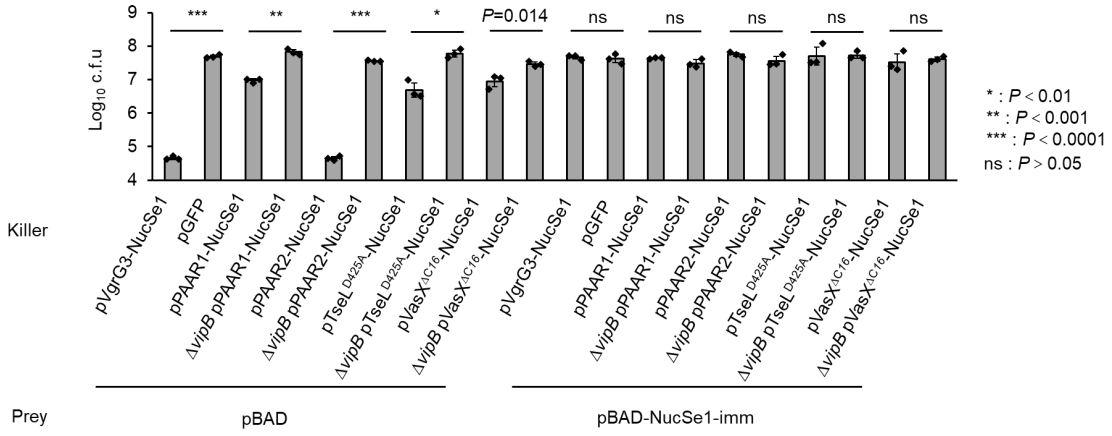
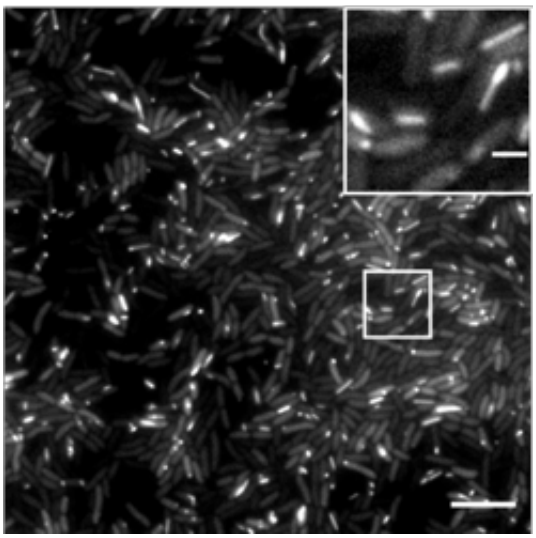


Fig. S4. Delivery of chimeric cargo by T6SS. Please also see Figure 5 for a representative assay plate and detailed description. Data were obtained from independent triplicate samples. Error bars show standard deviation. Statistical analysis was performed using the Student's *t*-test and *P*-values indicated.



vipA-N3-sfgfp ΔtseL/ΔvasX/ ΔvgrG3

Fig. S5. Non-contractile sheath formation in the *ΔtseL/ΔvasX/ΔvgrG3* mutant. 40 X 40 μm representative field of *V. cholerae vipA-N3-sfgfp* effector deletion mutant with a 3X magnified 5 X 5 μm inset (marked by box) is shown. VipA-N3-sfGFP signal was followed for 5 minutes. Scale bar for large field of view represents 5 μm and for the inset represents 1 μm.

Table S1. Strains and plasmids used in this study.

Strain	Genotype	Description	Source
V. cholerae V52 rhh	Parental	Deletion in *rtxA hlyA hapA*, parental strain	(1)
	Δ*vasK*	T6SS null, in-frame deletion of VCA0120	(1)
	vipA-mCherry2	C-terminal chromosomal fusion of mCherry2 to VipA	(2)
	vipA-mCherry2, $tseL^{D425A}$	Chromosomal mutation of the TseL catalytic residue D425	This study
	vipA-mCherry2, $vgrG3^{D842A}$	Chromosomal mutation of the VgrG3 catalytic residue D842	This study
	vipA-mCherry2, $vasX^{\Delta Cd}$	Chromosomal deletion of the colicin domain (R775-I915) of VasX	This study
	vipA-mCherry2, $tseL^{D425A}/vgrG3^{D842A}$	Double mutations in *tseL* and *vgrG3*	This study
	vipA-mCherry2, $tseL^{D425A}/vasX^{\Delta Cd}$	Double mutations in *tseL* and *vasX*	This study
	vipA-mCherry2, $vgrG3^{D842A}/vasX^{\Delta Cd}$	Double mutations in *vgrG3* and *vasX*	This study
	vipA-mCherry2, $tseL^{D425A}/vasX^{\Delta Cd}/vgrG3^{D842A}$	Triple inactive mutations of VgrG3, TseL, and VasX	This study
	vipA-mCherry2, Δ*tseL*/Δ*vasX*/Δ*vgrG3*	In-frame deletion of the three effectors	This study
	vipA-mCherry2, Δ*tseL*/Δ*vasX*/ $vgrG3^{D842A}$	In-frame deletion of *tseL* and *vasX*, point mutation in *vgrG3*	This study
	vipA-mCherry2, $vasX^{\Delta C16}$	Deletion of a 16-aa loop (A852 to F867) in the colicin domain	This study
	vipA-mCherry2, $tseL^{D425A}/vasX^{\Delta C16}/vgrG3^{D842A}$	Triple effector inactive mutations including the loop deletion in VasX	This study
	vipA-mCherry2, Δ*vipB* $tseL^{D425A}/vasX^{\Delta C16}/vgrG3^{D842A}$	T6SS null of the triple effector- inactivated mutant, in-frame deletion of *vipB*	This study
	vipA-N3-sfgfp, ΔtseL/ΔvasX/ΔvgrG3	Non-contractile sheath mutant	(3)

	Δ*tsiV1*	Immunity mutant of TseL, deletion of VC1417-21	(4)
	Δ*tsiV2*	Immunity mutant of VasX, deletion of VCA0019-21	(4)
	Δ*tsiV3*	Immunity mutant of VgrG3, deletion of VCA0123-24	(4)
E. coli			
PIR1	F- Δ*lac169 rpoS(Am) robA1 creC510 hsdR514 endA recA1 uidA(ΔMluI)::pir-116*	Strain used for cloning	Invitrogen
SM10 (λ pir)	Km^r , *thi-1, thr, leu, tonA, lacY, supE, recA::RP4-2-Tc::Mu, pir*	Strain used for conjugation	Mekalanos lab
CC114		A transposon mutant used as prey for T6SS killing	Mekalanos lab
DH5alpha	*F- Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 phoA supE44 thi-1 gyrA96 relA1 λ-*	Strain used for cloning	Invitrogen

Plasmid

Plasmid	Description	Source
pDS132	Suicidal conjugation vector for all chromosomal allelic changes	(5)
pDS132- *tseL*^{D425A}	Suicidal vector to construct chromosomal *tseL*^{D425A}	This study
pDS132- *vgrG3*^{D842A}	Suicidal vector to construct chromosomal *vgrG3*^{D842A}	This study
pDS132- *vasX*$^{\Delta Cd}$	Suicidal vector to construct chromosomal *vasX*$^{\Delta Cd}$	This study
pDS132- *vasX*$^{\Delta C16}$	Suicidal vector to construct chromosomal *vasX*$^{\Delta C16}$	This study
pDS132- *vipA-mCherry2*	Suicidal vector to construct chromosomal insertion VipA-mCherry2	(2)
pBAD18	Arabinose-induced expression vector	(6)
pBAD18-VasX-3V5	For expressing VasX with a C-terminal 3 x V5 epitope tag	This study
pBAD18-VasX$^{\Delta Cd}$-3V5	For expressing VasX$^{\Delta Cd}$ with a C-terminal 3 x V5 epitope tag	This study

pBAD18-VasX$^{\Delta C16}$-3V5	For expressing VasX$^{\Delta C16}$ with a C-terminal 3 x V5 epitope tag	This study
pPSV37-Tat-VasX	For expressing VasX in the periplasm, IPTG inducible	This study
pPSV37-Tat-VasX$^{\Delta C16}$	For expressing VasX$^{\Delta C16}$ in the periplasm, IPTG inducible	This study
pPSV37-Tat-sfGFP	For expressing sfGFP in the periplasm, IPTG inducible	This study
pBAD33-VgrG3-NucSe1	For expressing chimeric VgrG3-NucSe1 fusion	(7)
pBAD33-NucSe1-imm	For expressing NucSe1 immunity protein	(7)
pBAD33-PAAR1-NucSe1	Derivative of VgrG3-NucSe1 for expressing chimeric VCA0105-NucSe1	This study
pBAD33-PAAR2-NucSe1	Derivative of VgrG3-NucSe1 for expressing chimeric VCA0284-NucSe1	This study
pBAD33-TseL^{D425A}-NucSe1	Derivative of VgrG3-NucSe1 for expressing chimeric TseL^{D425A}-NucSe1	This study
pBAD33-VasX$^{\Delta C16}$-NucSe1	Derivative of VgrG3-NucSe1 for expressing chimeric VasX$^{\Delta C16}$-NucSe1	This study

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