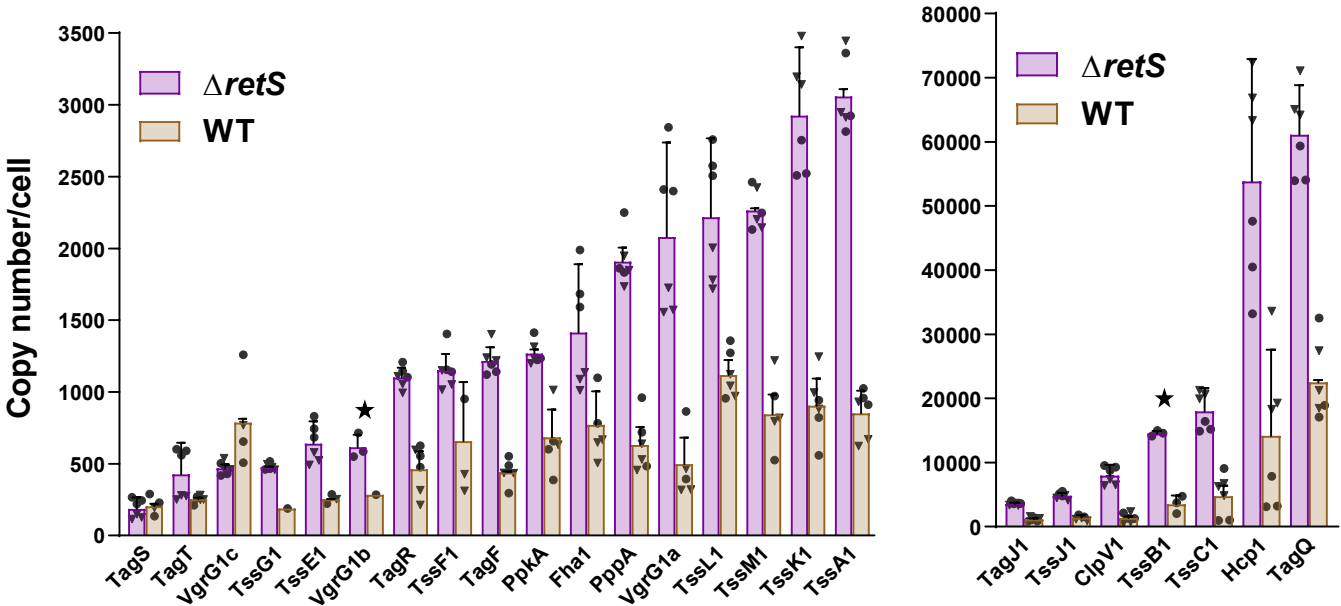


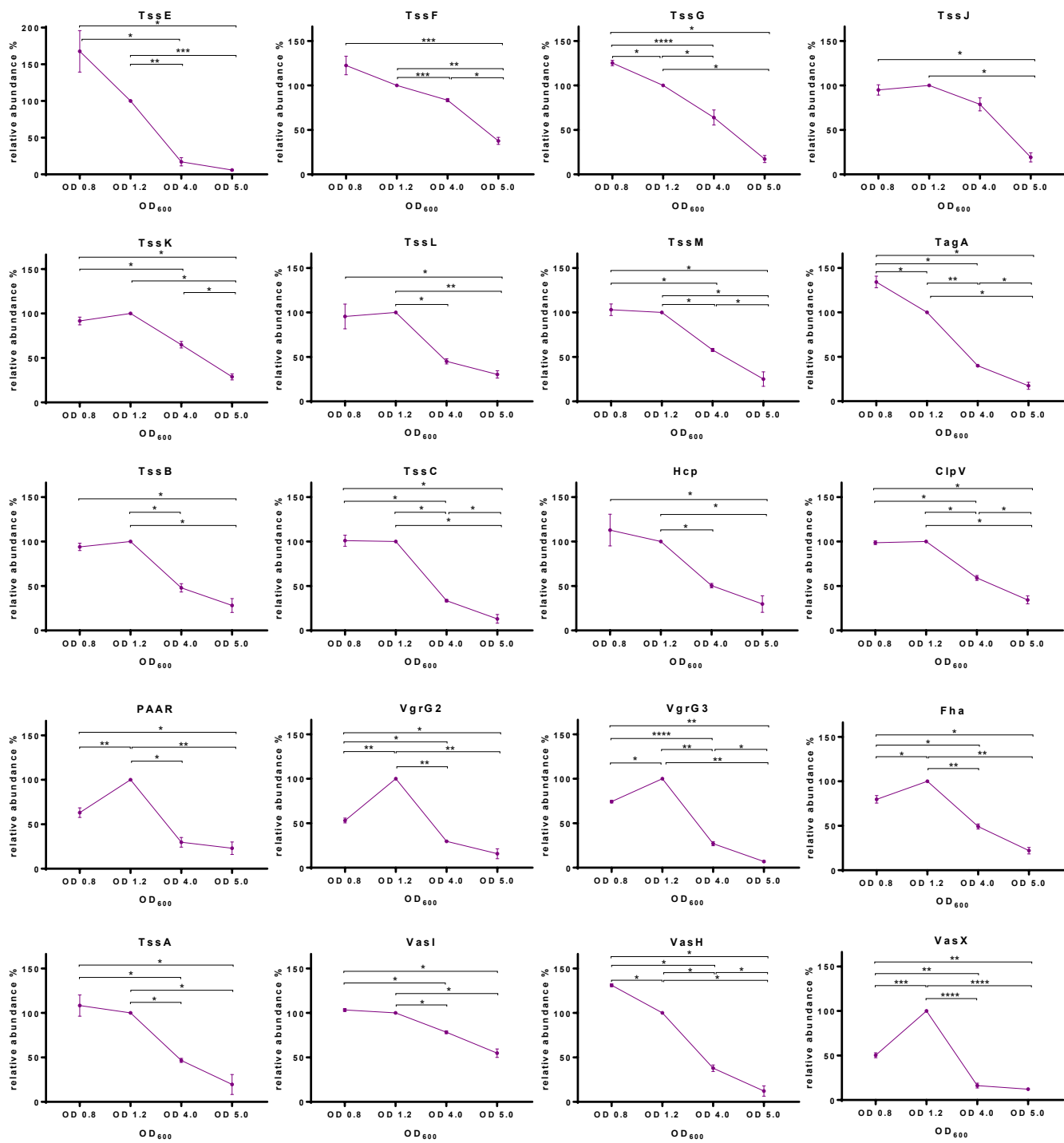
Abundance of bacterial Type VI secretion system components measured by targeted proteomics.

Lin et al.



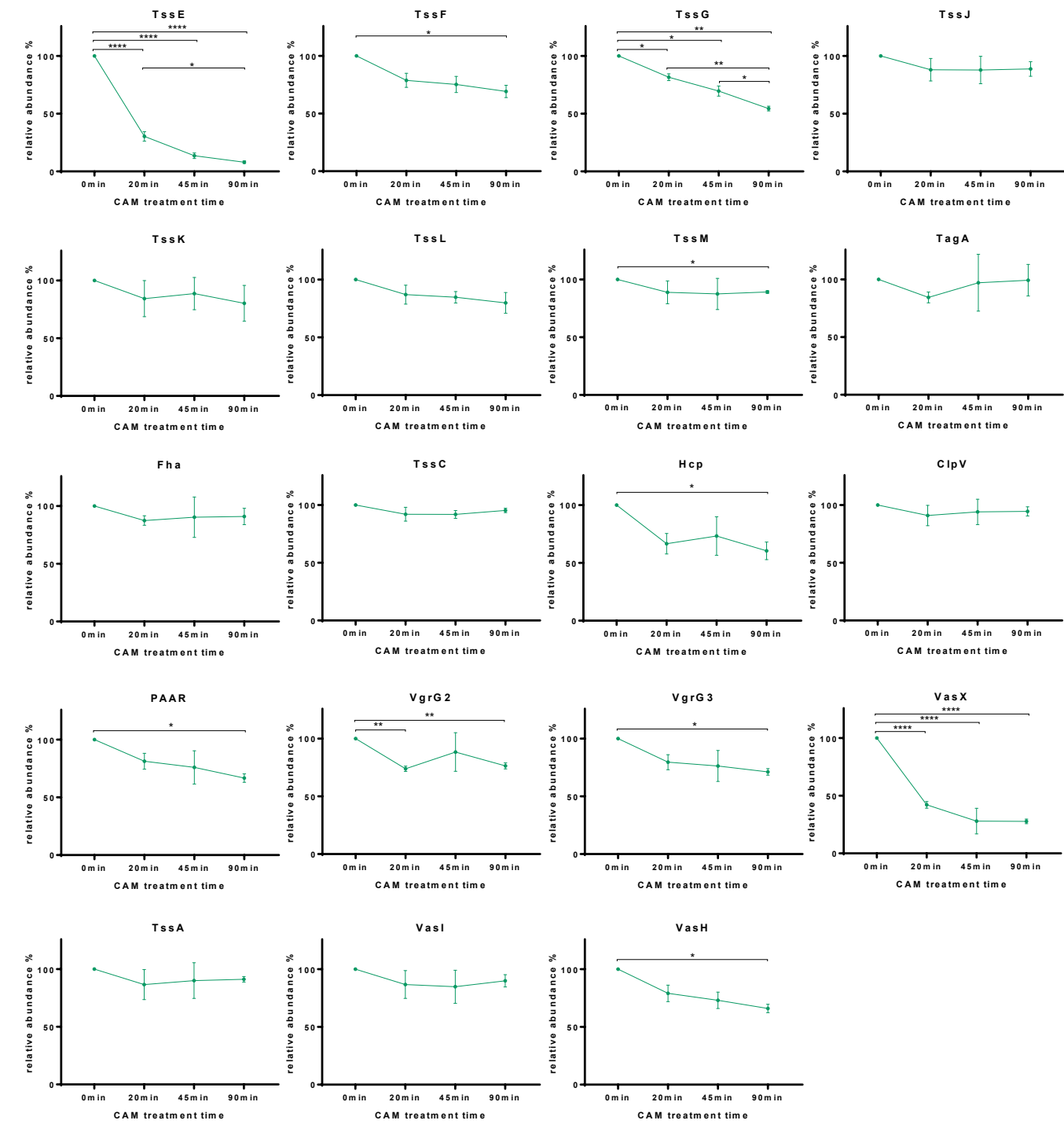
Supplementary Figure 1. The abundance of T6SS components in *P. aeruginosa* in the presence or absence of RetS. The absolute copy number of each selected component of H1-T6SS in whole cell lysate of *P. aeruginosa* PAO1 ($\Delta retS$ background or WT background) grown to $OD_{600} \sim 1.5$. Bar graph shows the average of copy number determined by two peptides (individual measurements shown with dots for peptide 1 or triangles for peptide 2), with error bar as the SD between measurements from both peptides if available; otherwise, SD between biological replicates is shown (highlighted with a star). Note the y-axis is shown in linear scale. The detailed quantification for this figure can be found in Supplementary Data 1&6. Source data are provided as a Source Data file.

Supplementary Figures



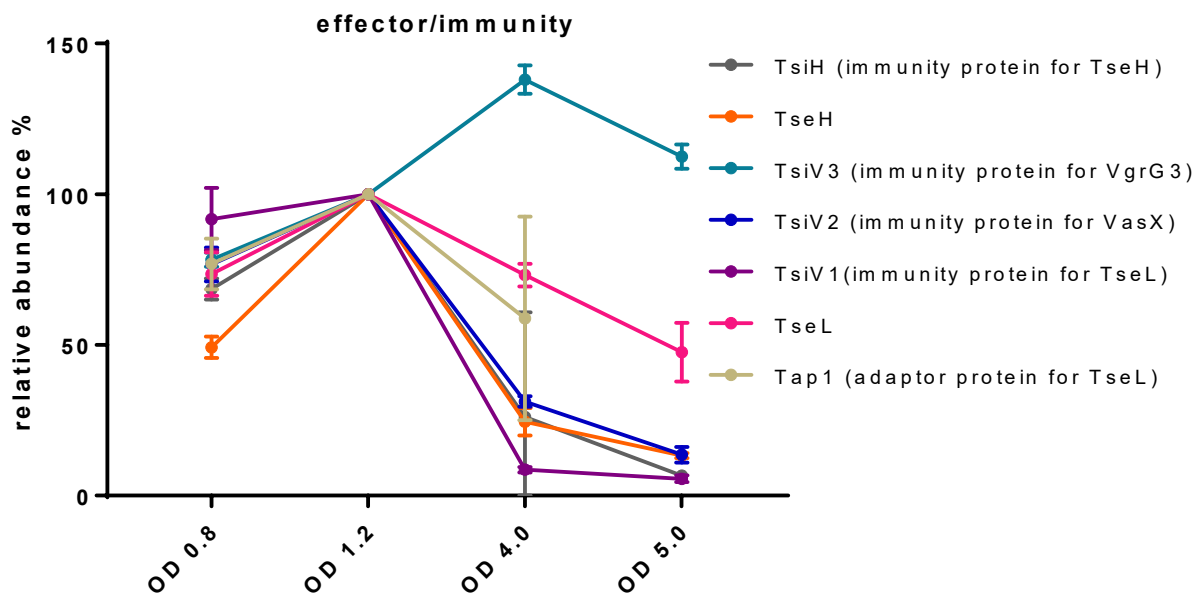
Supplementary Figure 2. The change in abundance of *V. cholerae* T6SS proteins during growth phases. The relative abundance levels of each component during 4 growth phases (OD₆₀₀ ~0.7, ~1.2, ~4, and ~5, respectively). Note the y-axis is shown as % abundance related to the protein levels at T2 (OD₆₀₀ ~1.2). The error bar represents the standard deviation of measurements from three replicates. For statistical analysis, the one-way ANOVA with multiple comparison using Tukey correction was performed. *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$. The detailed quantification for this figure can be found in Supplementary Data 2. Source data are provided as a Source Data file.

Supplementary Figures

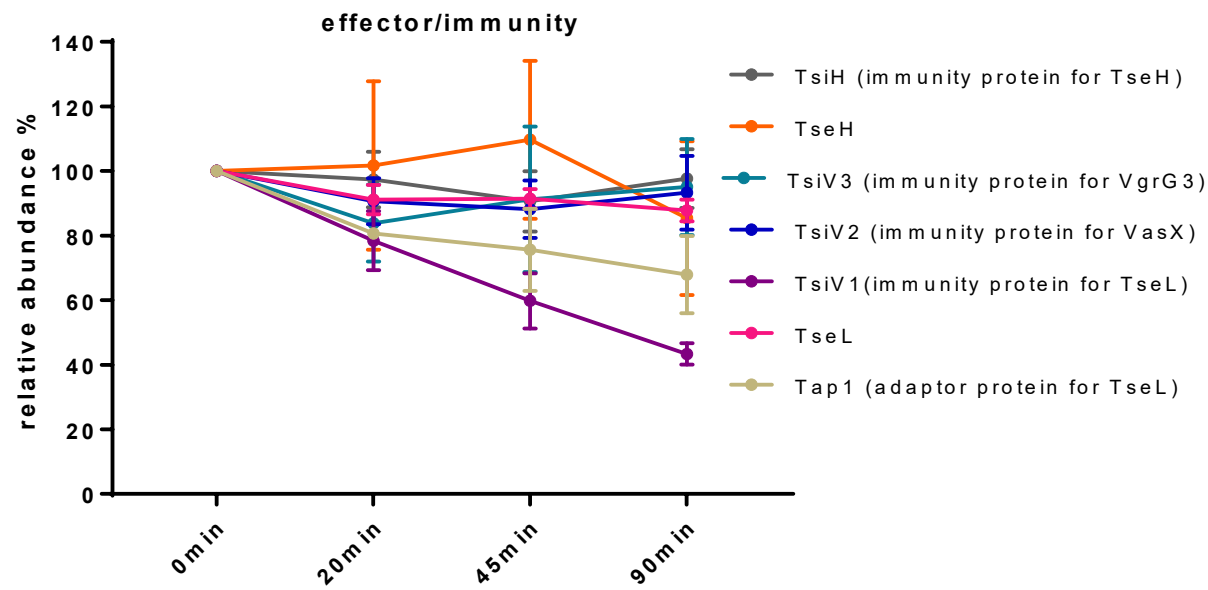


Supplementary Figure 3. The change of abundance of *V. cholerae* T6SS components in response to chloramphenicol treatment. The relative abundance levels of each component at 4 time points following the CAM treatment (0 min, 20 min, 45 min, and 90 min, respectively). Note the y-axis is shown as % abundance normalized to TssB related to the protein levels at T0 (0 min; pre-treatment). The error bar represents the standard deviation of measurements from three replicates. For statistical analysis, the one-way ANOVA with multiple comparison using Tukey correction was performed. *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$. The detailed quantification for this figure can be found in Supplementary Data 3. Source data are provided as a Source Data file.

A

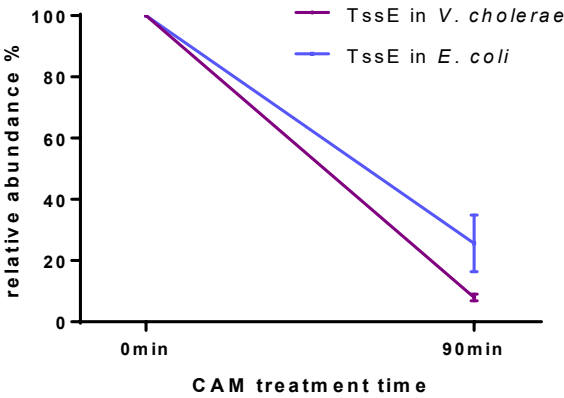


B

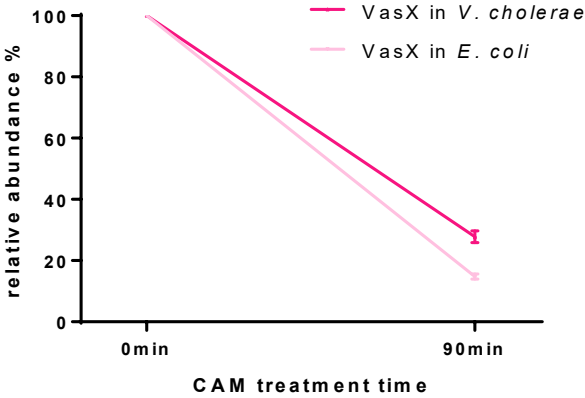


Supplementary Figure 4. The change in abundance of *V. cholerae* T6SS effector and immunity proteins under different conditions. (A) The relative abundance levels of each component during 4 growth phases (OD₆₀₀ ~0.7, ~1.2, ~4, and ~5, respectively). Note the y-axis is shown as % abundance related to the protein levels at T2 (OD₆₀₀ ~1.2). The error bar represents the standard deviation of measurements from three replicates. (B) The relative abundance levels of each component at 4 time points following the CAM treatment (0 min, 20 min, 45 min, and 90 min, respectively). Note the y-axis is shown as % abundance normalized to TssB related to the protein levels at T0 (0 min; pre-treatment). The error bar represents the standard deviation of measurements from three replicates. Source data are provided as a Source Data file.

A

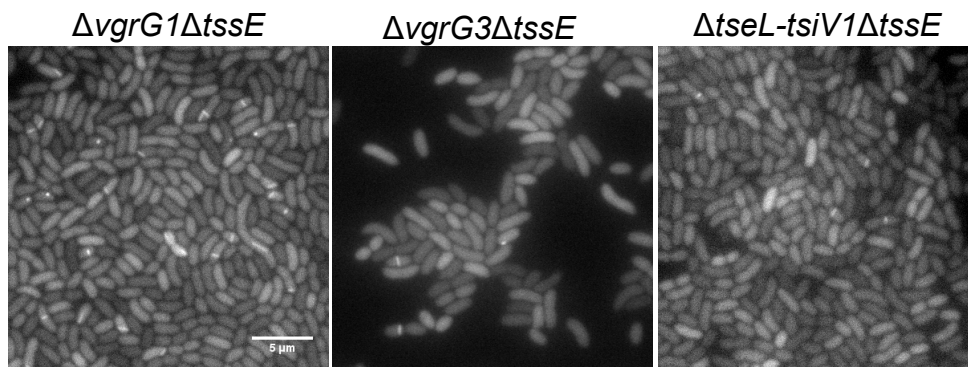


B

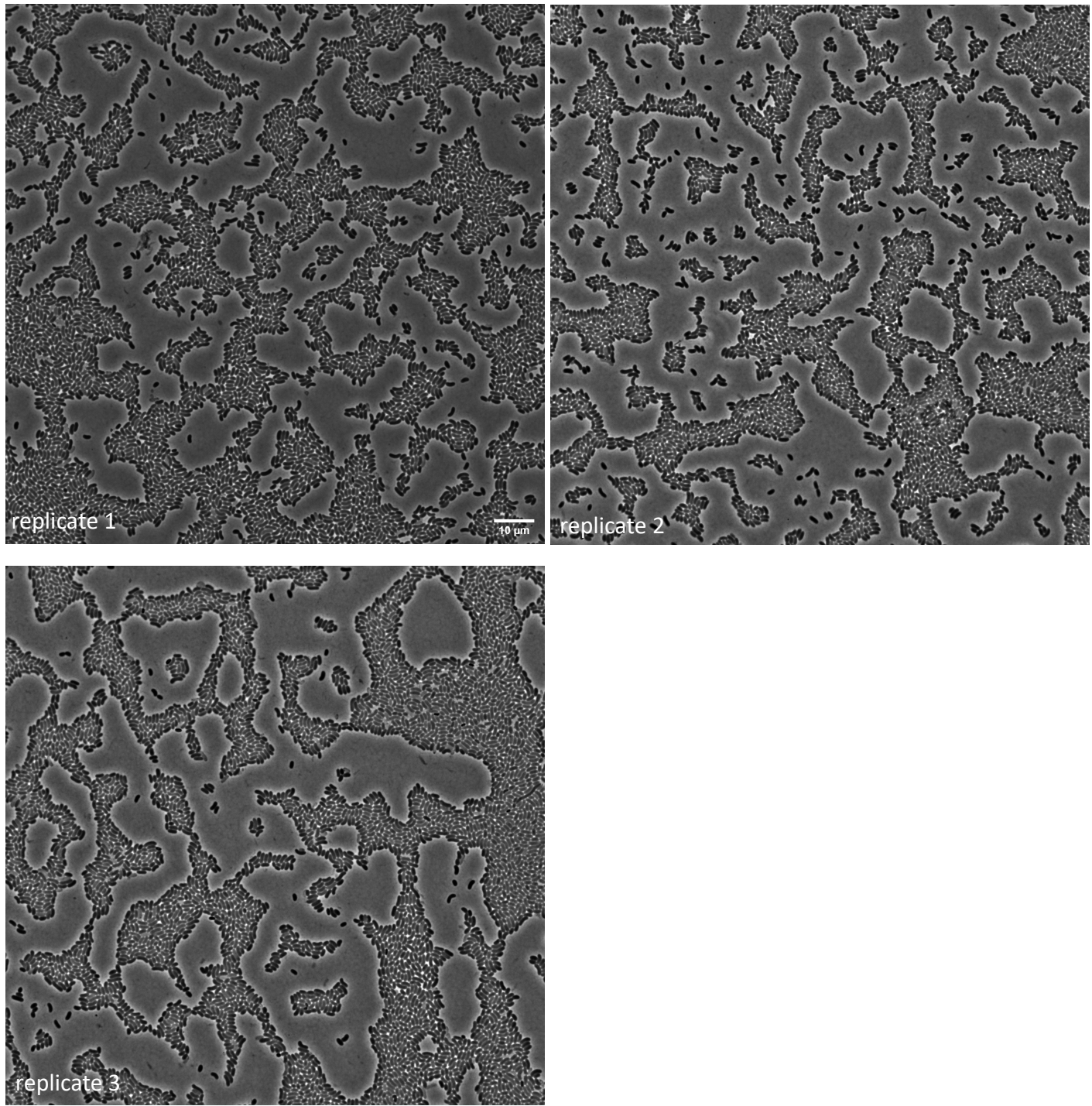


Supplementary Figure 5. The change of abundance of TssE and VasX when overproduced in *E. coli* in response to chloramphenicol treatment. (A) The relative abundance levels of TssE in *E. coli* or *V. cholerae* following the CAM treatment (0 min and 90 min). (B) The relative abundance levels of VasX in *E. coli* or *V. cholerae* following the CAM treatment (0 min and 90 min). Note the y-axis is shown as % abundance normalized to the protein levels at T0 (0 min; pre-treatment). The error bar represents the standard deviation of measurements from three replicates. The detailed quantification for this figure can be found in Supplementary Data 7. Source data are provided as a Source Data file.

Supplementary Figures

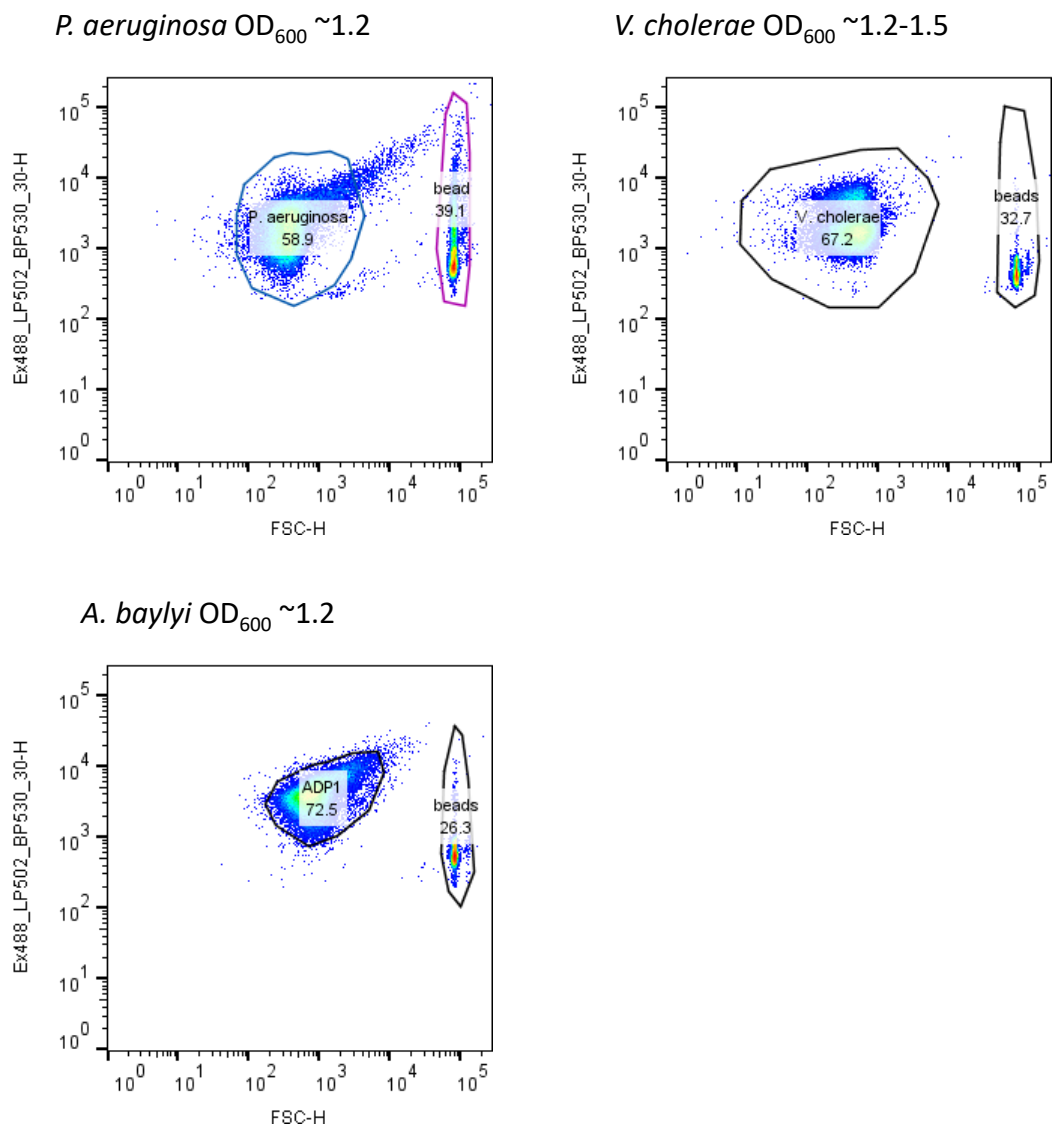


Supplementary Figure 6. *V. cholerae* lacking TssE and one of the other effector proteins harbors residual T6SS assembly. The sheath assembly was monitored by TssB-msfGFP localization in $\Delta tssE$ $\Delta vgrG1$, $\Delta tssE$ $\Delta vgrG3$ or $\Delta tssE$ $\Delta tseL-tsiV1$ cells. Representative images of GFP channel are shown for each indicated strain (all *tssB-msfGFP* background). Scale bar: 5 μ m. Time-lapse movies with a larger field of view can be found in Supplementary Movie 6.



Supplementary Figure 7. Large field of view of *V. cholerae* cells after 90 min of 1 mg/ml chloramphenicol treatment. 3 replicates are shown. Scale bar: 10 μm.

Supplementary Figures



Supplementary Figure 8. Flow cytometry gating strategy for bacterial cell counting. One example plot per bacterial strain is shown. 1 ml of sample containing ~10⁶ cells was stained with SYTO BC bacterial DNA stain and then mixed with 10 µl of microsphere standard beads. The stained mixtures were subjected to the analysis in a flow cytometer (BD Bioscience) equipped with ex488-LP502-BP530/30 laser (green).

Supplementary Table 1. Cell enumeration by FACS for *P. aeruginosa*, *V. cholerae* and *A. baylyi*.

strain	OD ₆₀₀	cell number/1ml OD ₆₀₀ 1	CV for cell number estimation	fg/cell	CV for fg/cell estimation
<i>P. aeruginosa</i> PAO1 $\Delta retS$	~1.2	5.47×10^8	25.58	278.48	5.02
<i>V. cholerae</i> 2740-80	~0.5	4.44×10^8	12.75	259.06	5.36
<i>V. cholerae</i> 2740-80	~1.5	1.00×10^9	13.90	182.21	7.45
<i>V. cholerae</i> 2740-80	~4.0	1.77×10^9	13.39	156.39	9.08
<i>A. baylyi</i> ADP1	~1.2	8.73×10^8	7.46	208.60	7.34

Supplementary Table 2. Strains and plasmids used in this work.

Bacterial strains	Genotypes	Plasmids	References
B131	<i>P. aeruginosa</i> $\Delta retS$		(Mougous et al., 2006) ¹
B133	<i>P. aeruginosa</i>		(Mougous et al., 2006; Stover et al., 2000) ^{1,2}
B274	<i>V. cholerae</i> 2740-80		(Basler et al., 2012) ³
B278	<i>A. baylyi</i> ADP1, rpsL'-K88R		(Basler et al., 2013) ⁴
B475	<i>P. aeruginosa</i> $\Delta retS$ clpV-sfGFP		(Vettiger et al., 2016) ⁵
B625	<i>V. cholerae</i> 2740-80, tssB-msfGFP		(Kudryashev et al. 2015) ⁶
DH022	<i>A. baylyi</i> ADP1, rpsL'-K88R, tssB-sfGFP, clpV-mCherry		(Ringel et al., 2017) ⁷
B630	<i>V. cholerae</i> 2740-80, tssB-msfGFP, $\Delta tssE$		(Vettiger et al., 2016) ⁵
AV029	<i>V. cholerae</i> 2740-80, tssB-msfGFP, $\Delta vgrG3$		(Vettiger et al., 2016) ⁵
AV034	<i>E. coli</i> SM10 λpir	pWM91- $\Delta vasX$	(Vettiger et al., 2016) ⁵
B294	<i>E. coli</i> SM10 λpir	pWM91- $\Delta tssE$	(Vettiger et al., 2016) ⁵
AV041	<i>E. coli</i> SM10 λpir	pWM91- $\Delta vgrG1$	(Vettiger et al., 2016) ⁵
AV060	<i>E. coli</i> SM10 λpir	pWM91- $\Delta tseL$ -tsiV1	(Vettiger et al., 2016) ⁵
LLB382	<i>V. cholerae</i> 2740-80, tssB-msfGFP, $\Delta tssE$ $\Delta vasX$		this work
LLB386	<i>V. cholerae</i> 2740-80, tssB-msfGFP, $\Delta vasX$		this work
LLB390	<i>V. cholerae</i> 2740-80, tssB-msfGFP, $\Delta tssE$ $\Delta vasX$	pBAD33-tssE	this work
LLB402	<i>V. cholerae</i> 2740-80, tssB-msfGFP, $\Delta tssE$ $\Delta vasX$	pBAD24-vasX	this work
B348	<i>E. coli</i> DH5 α λpir	pBAD24-tssE	this work
XB029	<i>E. coli</i> DH5 α λpir	pBAD33-tssE	this work
LLB397	<i>E. coli</i> DH5 α λpir	pBAD24-vasX	this work
LLB442	<i>V. cholerae</i> 2740-80, tssB-msfGFP, $\Delta tssE$ $\Delta vgrG1$		this work
LLB443	<i>V. cholerae</i> 2740-80, tssB-msfGFP, $\Delta tssE$ $\Delta tseL$ -tsiV1		this work
LLB383	<i>V. cholerae</i> 2740-80, tssB-msfGFP, $\Delta tssE$ $\Delta vgrG3$		this work

Supplementary Table 3. primers used in this work.

oligos	sequence 5'-3'
vasX-SpeI-for	TCAGTAACTAGTatgagtaatcccaatcaagc
vasX-XmaI-rev	TCAGTACCCGGGttaaccttttctacaacga
tssE_SpeI_For	TCAGTAACTAGTATGACGTACATCGCACCTGA
tssE-XmaI-rev	TCAGTACCCGGGTAAAACACTCGATATTTTCTGC
tssE_pBAD33_For	TCAGTAGCTAGCAGGAGGAATTCACCATGACGTACATCGCACCTGAAG

Supplementary References

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4. Basler M, Ho BT, Mekalanos JJ. Tit-for-tat: type VI secretion system counterattack during bacterial cell-cell interactions. *Cell* **152**, 884-894 (2013).

5. Vettiger A, Basler M. Type VI Secretion System Substrates Are Transferred and Reused among Sister Cells. *Cell* **167**, 99-110 e112 (2016).

6. Kudryashev M, *et al.* Structure of the type VI secretion system contractile sheath. *Cell* **160**, 952-962 (2015).

7. Ringel PD, Hu D, Basler M. The Role of Type VI Secretion System Effectors in Target Cell Lysis and Subsequent Horizontal Gene Transfer. *Cell Rep* **21**, 3927-3940 (2017).