Supplementary Information Table 1a: Strains used in this study

Organism	Strain	Name used in this study	Genotype	Description	Reference/Source
Vibrio cholerae	V52			Parental V52 strain. Constitutively active T6SS. Serotype O37. Used as *V. cholerae*	
		Wild-type	$\Delta hlyA$ $\Delta hapA$ $\Delta rtxA$	strain unless otherwise indicated.	Pukatzki *et al.* (2006)
		ΔT6SS	$\Delta vasK$ (*tssM*)	Deletion of VCA0120, T6SS-negative mutant	Pukatzki *et al.* (2006)
		ΔtsiV1&2	$\Delta tsiV1$ $\Delta tsiV2$	Deletion of VC1417-VC1421 and VCA0019-VCA0021 (including immunity genes for both TseL and VasX)	This work
		ΔtsiV3	$\Delta tsiV3$	Deletion of VCA0123-VCA0124 (including immunity gene for VgrG3)	Dong *et al.* (2013)
		ΔtsiV1&2 ΔvpsA	$\Delta tsiV1$ $\Delta tsiV2$ $\Delta vpsA$	Deletion of vpsA (VC0917) in ΔtsiV1&2 background	This work
		ΔtsiV3 ΔvpsA	$\Delta tsiV3$ $\Delta vpsA$	Deletion of vpsA (VC0917) in ΔtsiV3 background	This work
		TseH^{WT}	VipA(tssB)-mcherry, TseL^{D425A}, VgrG3^{D842A}, VasX$^{\Delta852-867}$	Catalytic mutations in all anti-bacterial effectors except TseH	Liang *et al.* (2019)
		TseH^{H64A}	VipA(tssB)-mcherry, TseL^{D425A}, VgrG3^{D842A}, VasX$^{\Delta852-867}$, TseH^{H64A}	Catalytic mutations in all anti-bacterial effectors including TseH	This work
		$\Delta PAAR2$	VipA(tssB)-mcherry, TseL^{D425A}, VgrG3^{D842A}, VasX$^{\Delta852-867}$, ΔPAAR2	Deletion of VCA0284 in TseH^{WT} background	This work
		$\Delta tsiH$	$\Delta tseH$ $\Delta tsiH$	Deletion of VCA0285 and VCA0286	Altindis *et al.* (2015)
		$\Delta wigR$	$\Delta wigR$ (*vxrB*)	Deletion of VCA0566	This work
		$\Delta tsiH$ $\Delta wigR$	$\Delta tsiH$ $\Delta wigR$	Deletion of VCA0566 in ΔtsiH background	This work
		Δimm	$\Delta tsiV1$ $\Delta tsiV2$ $\Delta tsiV3$	Deletion of VC1417-VC1421, VCA0019-VCA0021, and VCA0123-VCA0124 (including immunity genes for TseL, VasX, and VgrG3)	This work

Species	Strain	Genotype	Description	Reference
	Δimm ΔwigR	ΔtsiV1 ΔtsiV2 ΔtsiV3 ΔwigR	Deletion of VCA0566 in Δimm background	This work
	ΔT6SS ΔvpsA	ΔvasK ΔvpsA	Deletion of *vpsA* (VC0917) in ΔT6SS background	This work
	ΔT6SS ΔwigR	ΔvasK ΔwigR	Deletion of VCA0566 in ΔT6SS background	This work
	ΔT6SS ΔwigR ΔvpsA	ΔvasK ΔwigR ΔvpsA	Deletion of *vpsA* (VC0917) in ΔT6SS ΔwigR background	This work
	C6706	C6706	Serotype O1 *El Tor* pandemic strain C6706. T6SS is silent under standard laboratory conditions.	Lab of Dr. John Mekalanos
Aeromonas dhakensis	SSU	*A. dhakensis*	T6SS-positive *A dhakensis* strain SSU.	Liang *et al.* (2015)
		ΔvasK (*tssM*)	T6SS-negative mutant of *A dhakensis* SSU. Used as A. dhakensis prey strain throughout this study.	Liang *et al.* (2015)
Aeromonas hydrophila	AH-1	AH-1	*A. hydrophila* isolate	Lab of Dr. John Mekalanos
	AH-3	AH-3	*A. hydrophila* isolate	Lab of Dr. John Mekalanos
	LL1	LL1	*A. hydrophila* isolate	Lab of Dr. John Mekalanos
	Aer-184	Aer-184	*A. hydrophila* isolate	Lab of Dr. John Mekalanos
	Aer-205	Aer-205	*A. hydrophila* isolate	Lab of Dr. John Mekalanos
	Aer-19	Aer-19	*A. hydrophila* isolate	Lab of Dr. John Mekalanos
	L31	L31	*A. hydrophila* isolate	Lab of Dr. John Mekalanos
Edwardsiella sp.	PPD130/91	PPD130/91	*E. piscicida* isolate	Lab of Dr. John Mekalanos
	AL9739	AL9739	*Edwardsiella sp.* isolate	Lab of Dr. John Mekalanos

Organism	Strain			Description	Source
	E381	E381		*E. piscicida* isolate	Lab of Dr. John Mekalanos
	NUF251	NUF251		*E. piscicida* isolate	Lab of Dr. John Mekalanos
	SU226	SU226		*Edwardsiella sp.* isolate	Lab of Dr. John Mekalanos
Acinetobacter baylyi ADP1	ADP1	ΔT6SS		*Deletion of large T6SS cluster in A. baylyi isolate ADP1*	Lab stock
Vibrio parahaemolyticus	RIMD2210633	RIMD2210633		*V. parahaemolyticus* isolate	Lab of Dr. Jun Zheng
Escherichia coli	MG1655	MG1655		K-12 wild-type strain	Lab of Dr. Herb Schellhorn
	DH5α-T1[R]	DH5α		Cloning strain	ThermoFisher Scientific
	BL21 (DE3)	BL21		T7 expression strain	New England Biolabs
	BW25113			Keio collection parental strain derived from MG1655. Used as *E. coli* strain unless otherwise indicated.	
		Wild-type			Baba *et al.* (2006)
		Δ*rcsB*	Δ*rcsB::kan*[R]	Keio collection strain JW2205	Baba *et al.* (2006)
		Δ*baeR*	Δ*baeR::kanR*	Keio collection strain JW2064	Baba *et al.* (2006)
		Δ*cpxR*	Δ*cpxR::kanR*	Keio collection strain JW3883	Baba *et al.* (2006)
		Δ*pspF*	Δ*pspF::kanR*	Keio collection strain JW1296	Baba *et al.* (2006)
		Δ*rpoS*	Δ*rpoS::kanR*	Keio collection strain JW5437	Baba *et al.* (2006)
		Δ*soxR*	Δ*soxR::kanR*	Keio collection strain JW4024	Baba *et al.* (2006)
		Δ*degP*	Δ*degP::kanR*	Keio collection strain JW0157	Baba *et al.* (2006)
		Δ*osmB*	Δ*osmB::kanR*	Keio collection strain JW1275	Baba *et al.* (2006)
		Δ*wza*	Δ*wza::kanR*	Keio collection strain JW2047	Baba *et al.* (2006)
		Δ*bdm*	Δ*bdm::kanR*	Keio collection strain JW5239	Baba *et al.* (2006)
		Δ*mdtC*	Δ*mdtC::kanR*	Keio collection strain JW2061	Baba *et al.* (2006)
		Δ*spy*	Δ*spy::kanR*	Keio collection strain JW1732	Baba *et al.* (2006)
		Δ*tolB*	Δ*tolB::kanR*	Keio collection strain JW5100	Baba *et al.* (2006)
		Δ*ddlB*	Δ*ddlB::kanR*	Keio collection strain JW0090	Baba *et al.* (2006)
		Δ*degQ*	Δ*degQ::kanR*	Keio collection strain JW3203	Baba *et al.* (2006)

Δ*rseA*	Δ*rseA::kanR*	Keio collection strain JW2556	Baba *et al.* (2006)
Δ*bepA*	Δ*bepA::kanR*	Keio collection strain JW2479	Baba *et al.* (2006)
Δ*ycaL*	Δ*ycaL::kanR*	Keio collection strain JW0892	Baba *et al.* (2006)
Δ*mrcA*	Δ*mrcA::kanR*	Keio collection strain JW3359	Baba *et al.* (2006)
Δ*pagP*	Δ*pagP::kanR*	Keio collection strain JW0617	Baba *et al.* (2006)
Δ*surA*	Δ*surA::kanR*	Keio collection strain JW0052	Baba *et al.* (2006)
Δ*rcsF*	Δ*rcsF::kanR*	Keio collection strain JW0192	Baba *et al.* (2006)
Δ*rcsC*	Δ*rcsC::kanR*	Keio collection strain JW5917	Baba *et al.* (2006)
Δ*wcaA*	Δ*wcaA::kanR*	Keio collection strain JW2044	Baba *et al.* (2006)
Δ*wcaJ*	Δ*wcaJ::kanR*	Keio collection strain JW2032	Baba *et al.* (2006)
Δ*mliC*	Δ*mliC::kanR*	Keio collection strain JW1631	Baba *et al.* (2006)
Δ*osmY*	Δ*osmY::kanR*	Keio collection strain JW4338	Baba *et al.* (2006)
Δ*osmC*	Δ*osmC::kanR*	Keio collection strain JW1477	Baba *et al.* (2006)
Δ*ivy*	Δ*ivy::kanR*	Keio collection strain JW0210	Baba *et al.* (2006)
Δ*ymgD*	Δ*ymgD::kanR*	Keio collection strain JW5177	Baba *et al.* (2006)
Δ*ybgS*	Δ*ybgS::kanR*	Keio collection strain JW0736	Baba *et al.* (2006)
Δ*mdtA*	Δ*mdtA::kanR*	Keio collection strain JW5338	Baba *et al.* (2006)
Δ*tolC*	Δ*tolC::kanR*	Keio collection strain JW5503	Baba *et al.* (2006)
Δ*gadE*	Δ*gadE::kanR*	Keio collection strain JW3480	Baba *et al.* (2006)
Δ*gadA*	Δ*gadA::kanR*	Keio collection strain JW3485	Baba *et al.* (2006)
Δ*gadB*	Δ*gadB::kanR*	Keio collection strain JW1488	Baba *et al.* (2006)
ΔgadC	ΔgadC::kanR	Keio collection strain JW1487	Baba *et al.* (2006)
Δ*argH*	Δ*argH::kanR*	Keio collection strain JW3932	Baba *et al.* (2006)
Δ*lysA*	Δ*lysA::kanR*	Keio collection strain JW2806	Baba *et al.* (2006)
Δ*leuB*	Δ*leuB::kanR*	Keio collection strain JW5807	Baba *et al.* (2006)
Δ*bglJ*	Δ*bglJ::kanR*	Keio collection strain JW5955	Baba *et al.* (2006)
Δ*pspA*	Δ*pspA::kanR*	Keio collection strain JW1297	Baba *et al.* (2006)
Δ*soxS*	Δ*soxS::kanR*	Keio collection strain JW4023	Baba *et al.* (2006)

Supplementary Information Table 1b: Plasmids used in this study			
Vector	**Insert**	**Description**	**Reference/Source**
pDS132		Suicidal conjugation vector for generating in-frame knockouts in *V. cholerae*. *Chloramphenicol resistant.*	Philippe *et al.* (2004)
	PAAR2	For generating knockout of VCA0284	This work
	WigR	For generating knockout of VCA0566	This work
pBAD33		Arabinose-inducible expression plasmid. Chloramphenicol resistant.	Guzman *et al.* (1995)
	TsiH (VCA0286)	Expression of immunity gene to protect against TseH	Altindis *et al.* (2005)
	TAT-TseH	TseH overexpression plasmid including TAT (Twin Arginine Translocation)-tag for periplasm translocation.	Altindis *et al.* (2005)
pPSV37	TAT-VgrG3	VgrG3 overexpression plasmid including TAT (Twin Arginine Translocation)-tag for periplasm translocation.	This work
	TAT-TseL	TseL overexpression plasmid including TAT (Twin Arginine Translocation)-tag for periplasm translocation.	This work
pBAD33k		Kanamycin resistant variant of pBAD33. Used in T6SS competition assay for resistance marker to select for prey.	This work
	TseH (VCA0285)	Exogenous TseH expression for complementation	This work
	WigR (VCA0566)	Exogenous WigR expression for complementation	This work
	TAT-TseH	TseH overexpression plasmid including TAT (Twin Arginine Translocation)-tag for periplasm translocation. (As in pBAD33 but with different antibiotic resistance)	This work
pCA24N		ASKA collection plasmid. Includes N-terminal 6His tag. Chloramphenicol resistant.	Kitagawa *et al.* (2005)
	RcsA	Exogenous RcsA for overexpression to induce colanic acid synthesis	Kitagawa *et al.* (2005)
	RcsB	Exogenous RcsB expression for complementation	Kitagawa *et al.* (2005)
	BaeR	Exogenous BaeR expression for complementation	Kitagawa *et al.* (2005)
	Spy	Exogenous Spy expression for complementation	Kitagawa *et al.* (2005)
	pSpy$^{\Delta Sec}$	pCA24N-Spy with Sec secretion tag (first 22 codons of *spy* ORF) and N-terminal 6His tag deleted	This work
	DegP	Exogenous DegP for overexpression	Kitagawa *et al.* (2005)
	YeeN	Exogenous YeeN for overexpression	Kitagawa *et al.* (2005)
	PhoB	Exogenous PhoB for overexpression	Kitagawa *et al.* (2005)
	YnjA	Exogenous YnjA for overexpression	Kitagawa *et al.* (2005)
	YiaD	Exogenous YiaD for overexpression	Kitagawa *et al.* (2005)

	CreD	Exogenous CreD for overexpression	Kitagawa *et al.* (2005)
	MdtC	Exogenous MdtC for overexpression	Kitagawa *et al.* (2005)
	TolC	Exogenous TolC for overexpression	Kitagawa *et al.* (2005)
pET-SUMO	TseH	For purification of TseH for crystallography	Altindis *et al.* (2005)

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