Supporting Information

Quorum regulatory small RNAs repress type VI secretion in *Vibrio cholerae*

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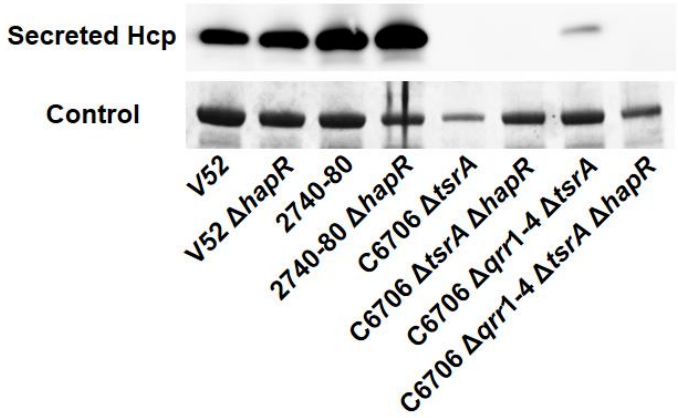

Figure S1

Western blot showing secreted Hcp protein in *V. cholerae* V52, V52 Δ*hapR* (YS2030), 2740-80, 2740-80 Δ*hapR* (YS2029), O1 El Tor C6706 Δ*tsrA* (YS2031), C6706 Δ*tsrA*, Δ*hapR* (YS2034), C6706 Δ*qrr*1-4, Δ*tsrA* (YS2039), and C6706 Δ*qrr*1-4, Δ*tsrA*, Δ*hapR* (YS2040). When comparing these results to those in the main text, note that the amount of secreted Hcp by the pandemic strain C6706 ∆*qrr*1-4, ∆*tsrA* is lower than that secreted by the non-pandemic strains V52 and 2740-80.

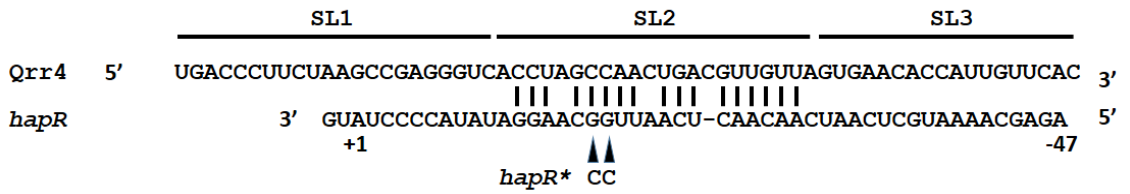

Figure S2

Putative base pairing between *V. cholerae* Qrr4 and the *hapR* 5'UTR was predicted by RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/). The *hapR* translational start site is denoted +1. *hapR** contains the indicated GG to CC double nucleotide change. Sequences encoding stem-loops are shown with over-lines.

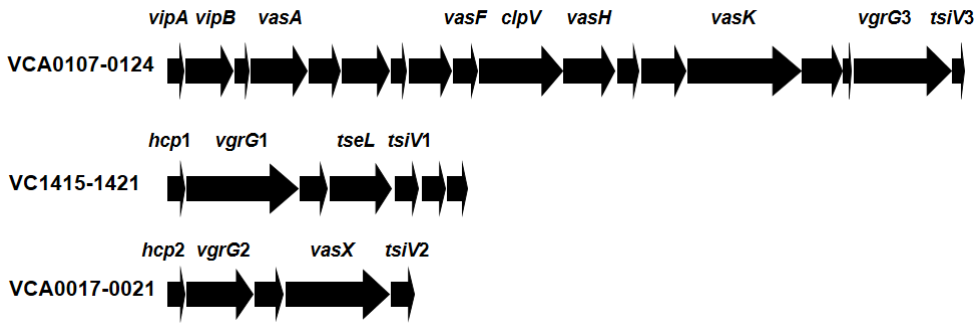

Figure S3

Schematic representation of the T6SS gene clusters in *V. cholerae*.

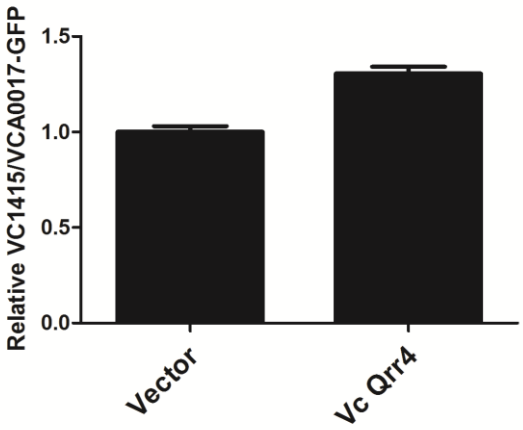

Figure S4

Fluorescence from *E. coli* carrying a plasmid with an IPTG inducible translational GFP fusion to VC1415/VCA0017 (pYS347) was measured in the presence of an empty vector (pLF253) or a vector expressing tetracycline inducible *V. cholerae qrr*4 (Vc Qrr4, pYS245). The 5’ UTR is identical upstream of VC1415 and VCA0017. A plasmid harboring a GFP translational fusion to this region was constructed. It reports on the first gene in both of the two small clusters. GFP production from three independent cultures was measured and the means and SEMs are shown, with all measurements normalized to the mean of the vector controls.

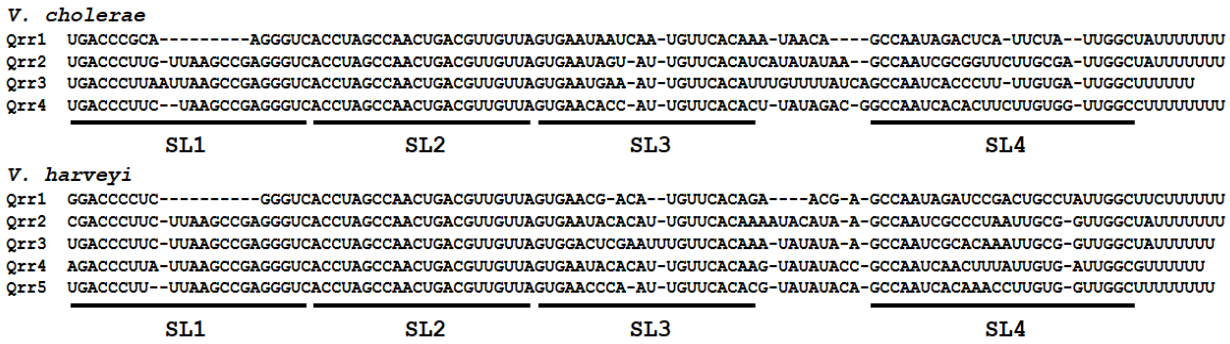

Figure S5

RNA sequence alignment of *V. cholerae* Qrr1-4 and *V. harveyi* Qrr1-5. Sequences corresponding to predicted stem-loops are indicated with underlines.

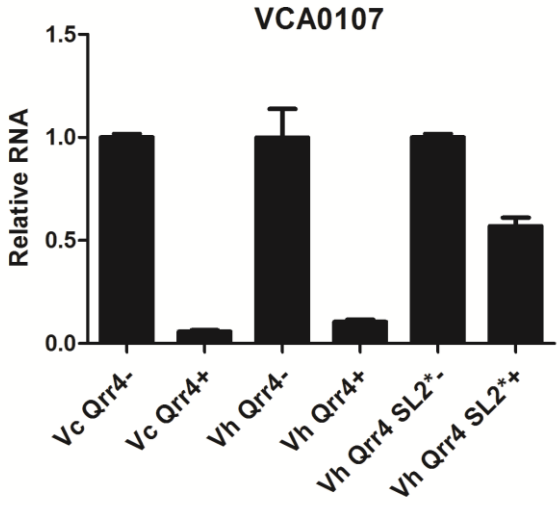

Figure S6

The level of VCA0107 mRNA in *V. cholerae* O1 El Tor C6706 Δ*qrr*1-4, Δ*tsrA, hapR** (YS2048) was measured by qRT-PCR before (-) and after (+) 1 hour induction of *V. cholerae* Qrr4 (Vc Qrr4, pYS249), *V. harveyi* Qrr4 (Vh Qrr4, pLF575), or the *V. harveyi* Qrr4 SL2 inversion variant (Vh Qrr4 SL2*, pYS348). Means and SEMs of triplicate samples are shown, with all measurements normalized to the mean of the uninduced controls.

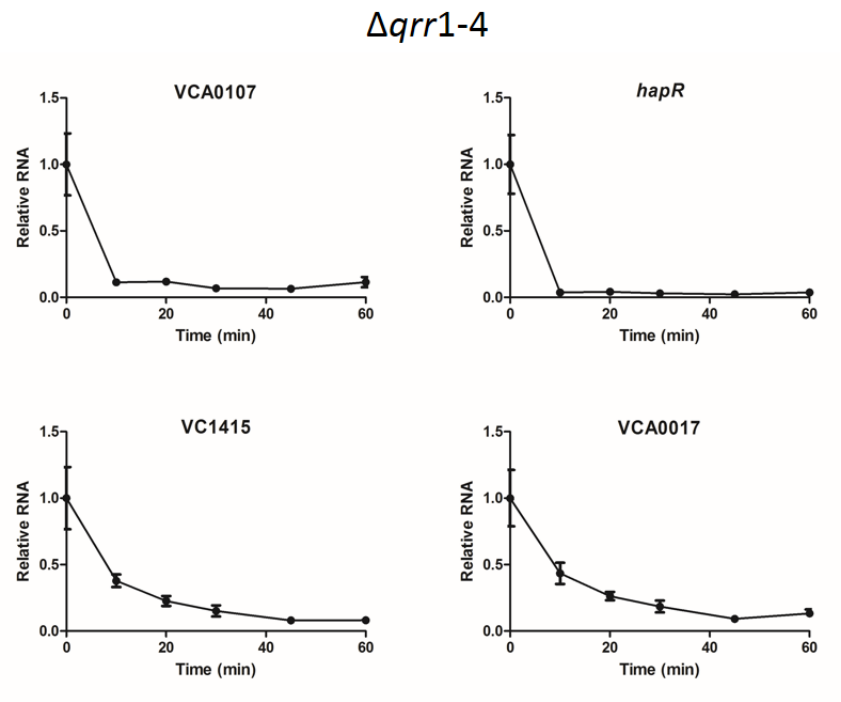

Figure S7

mRNA levels of VCA0107, *hapR*, VC1415, and VCA0017 in *V. cholerae* O1 El Tor C6706 Δ*qrr*1-4 (SLS456) following pulse induction of *V. cholerae* Qrr4 (pYS249) were measured by qRT-PCR. Samples were collected at different time points, and means and SEMs of triplicate samples are shown.

Strain	Relevant Genotype	Source
E. coli		
S17λpir	wild type	(de Lorenzo and Timmis, 1994)
BW-RI	wild type	(Levine *et al.*, 2007)
V. cholerae		
C6706	wild type	(Thelin and Taylor, 1996)
2740-80	wild type	(Basler *et al.*, 2013)
V52	wild type	(Pukatzki *et al.*, 2006)
SLS456	C6706 Δ*qrr*1-4	(Svenningsen *et al.*, 2009)
YS2029	2740-80 Δ*hapR*	this study
YS2030	V52 Δ*hapR*	this study
YS2031	C6706 Δ*tsrA*	this study
YS2032	C6706 *luxO*D47E Δ*tsrA*	this study
YS2033	C6706 Δ*luxO* Δ*tsrA*	this study
YS2034	C6706 Δ*tsrA* Δ*hapR*	this study
YS2037	C6706 *luxO*D47E Δ*qrr*1-4 Δ*tsrA*	this study
YS2039	C6706 Δ*qrr*1-4 Δ*tsrA*	this study
YS2040	C6706 Δ*qrr*1-4 Δ*tsrA* Δ*hapR*	this study
YS2047	C6706 *luxO*D47E Δ*qrr*1-4 Δ*tsrA hapR**	this study
YS2048	C6706 Δ*qrr*1-4 Δ*tsrA hapR**	this study
P. aeruginosa		
PAO1	wild type	(Holloway, 1955)

Table S1 Strains used in this study.

Plasmid	Description	Source
pEVS143	vector	(Dunn *et al.*, 2006)
pZA31-lucNB	vector	(Levine *et al.*, 2007)
pZE12	vector	(Levine *et al.*, 2007)
pSLS155	pEVS143-Vc Qrr4 (constitutive promoter)	(Svenningsen *et al.*, 2009)
pKAS32	vector for chromosomal mutation in *V. cholerae*	(Skorupski and Taylor, 1996)
pMM1162	pKAS32 for *hapR* deletion	(Miller *et al.*, 2002)
pLF253	pZA31-lucNB empty vector	(Shao *et al.*, 2013)
pLF127	pZA31-lucNB-Vh Qrr4	(Shao *et al.*, 2013)
pLF575	pEVS143-ara-Vh Qrr4	(Shao *et al.*, 2013)
pYS230	pZA31-lucNB-Vh Qrr4 SL1*	(Shao *et al.*, 2013)
pYS231	pZA31-lucNB-Vh Qrr4 SL2*	(Shao *et al.*, 2013)
pYS232	pZA31-lucNB-Vh Qrr4 SL3*	(Shao *et al.*, 2013)
pYS245	pZA31-lucNB-Vc Qrr4	this study
pYS249	pEVS143-ara-Vc Qrr4	this study
pYS254	pZE12-VCA0107-GFP	this study
pYS335	pKAS32 for *tsrA* deletion	this study
pYS345	pKAS32 for *hapR**	this study
pYS347	pZE12-VC1415/VCA0017-GFP	this study
pYS348	pEVS143-ara-Vh Qrr4 SL2*	this study
pYS350	pZE12-VCA0107(BP-)-GFP	this study

Table S2 Plasmids used in this study.

Primer	Sequence	Use
YS757	ATAGGATCCATCAAATAAAACGAAAGGCTC	pYS245
YS758	GTGCTCAGTATCTCTATCACTGATAGG	pYS245
YS765	TGACCCTTCTAAGCCGAGGGTC	pYS245/pYS249
YS766	GCGGGATCCCAAGATGCTATGGCGAATGTGGTG	pYS245
YS767	CCGGGTACCAGGATCCGGTGATTGATTGAGCAAG	pYS249
YS768	GGAGAAACAGTAGAGAGTTGCGATAAAAAG	pYS249
YS772	GCGGGTACCCAAGATGCTATGGCGAATGTGGTG	pYS249
YS794	GCTGCTAATGATAAGTTTGCATAATAAGCC	pYS254
YS796	AATAGGTACCTTTGGGAGCTACACTTCCTTCTTTAGA	pYS254
YS997	GTGCTCAGTATCTTGTTATCCGCTC	pYS254/pYS347
YS998	GCGGGTACCATGTCTAAAGGTGAAGAAC	pYS254/pYS347
YS811	GCGGGTACCATGTTAATGGCTCGGTTGCTACAAGG	pYS335
YS812	GGTGTTTTGGATTCCAAACGGTTAGTC	pYS335
YS813	CGTTTGGAATCCAAAACACCTCGAACGCTCATTCCCTATATCGCC	pYS335
YS814	GCGCCTAGGCAGTCCCTGTAACTTGCCATTGCAG	pYS335
YS1146	GCGGGTACCGTTTTGTCTACGGTATCGAGGATGG	pYS345
YS1147	GCGCCTAGGCCGTAAGAAAGGCGAAATGGTGC	pYS345
YS1148	GGAATTGAGTTGTTGATTGAGCATTTTGCTC	pYS345
YS1149	GCTCAATCAACAACTCAATTCCCAAGGATATACCCCTATGGACGC	pYS345
YS1195	ACGGTGCGCAGAGCGCGTTAA	pYS347
YS1197	AATAGGTACCTTCGATAGAGATATAACATGGAGTTGGCAT	pYS347
YS733	CCTTATTAAGCCGAGGGTCATTGTTGCAGTCAACCGATCCAGTGAATACACATTGTTCAC	pYS348
YS734	GTGAACAATGTGTATTCACTGGATCGGTTGACTGCAACAATGACCCTCGGCTTAATAAGG	pYS348
YS1200	TGATTGAATGATTTCAATCAACTGTAAGTAACTGTTGCAATGGCATAGGTATTGGAGACGTAATA	pYS350
YS1201	TATTACGTCTCCAATACCTATGCCATTGCAACAGTTACTTACAGTTGATTGAAATCATTCAATCA	pYS350
STR0382	CTAAGGGGCAATCTCTACAAG	*hfq* qRT-PCR
STR0383	AATTGATCAAATGATTCGATCTGA	*hfq* qRT-PCR
YS1084	AAATCGCGTTGGAAGTGTTT	*hapR* qRT-PCR
YS1085	CGAGTTGGGAAGTAGTTGAA	*hapR* qRT-PCR
YS1153	ATTGATTGAGTTGCGTGAAG	VCA0107 qRT-PCR
YS1154	TCTCTCGACTCTTCTGAGTT	VCA0107 qRT-PCR
YS1206	GAAAACCGTTGAGCTGAAGT	VC1415 qRT-PCR
YS1207	TGGATGTCGACGATAGACGC	VC1415 qRT-PCR
YS1165	GAAAACCGTTGAGCTGAAAT	VCA0017 qRT-PCR
YS1166	TGGATATCAACGATAGACGC	VCA0017 qRT-PCR

Table S3 Primers used in this study.

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