Supplemental Information for

Species-specific quorum sensing represses the chitobiose utilization locus in *Vibrio cholerae*

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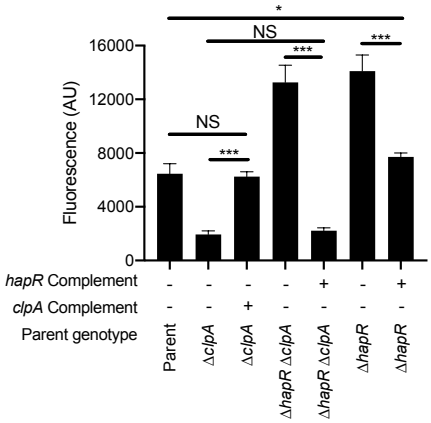

Figure S1. *Complementation of ΔhapR and ΔclpA mutant strains.* Expression of a P_{chb}-GFP transcriptional reporter was determined in the indicated strains. The parent strain contained a chromosomally-encoded P_{chb}-GFP reporter and a Δ*cbp* mutation. Strains were complemented with a chromosomally-encoded single copy of the indicated gene driven by its native promoter at an ectopic site (*hapR* or *clpA* Complement). Fluorescence of cultures was determined on a plate reader from at least three independent biological replicates and is shown as the mean ± SD. Statistical comparisons were made by one-way ANOVA with Tukey's post-test. NS, not significant. *, $p = 0.0104$. ***, $p < 0.001$. The P_{chb}-GFP fluorescence data for "Δ*clpA*", "Δ*hapR* Δ*clpA*", and "Δ*hapR*" are identical to the data presented in **Fig. 1** and were included here for ease of comparison.

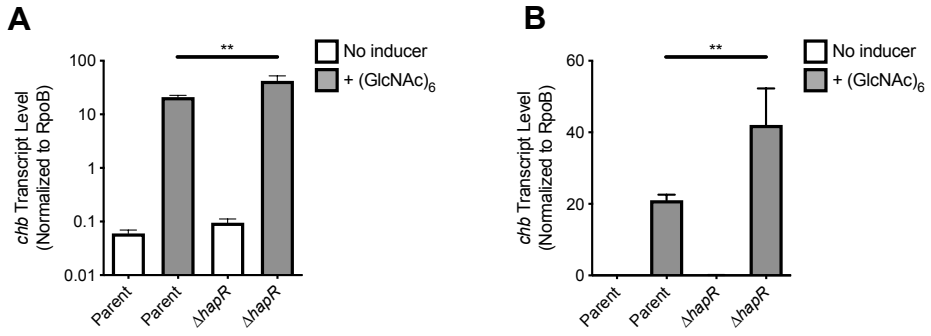

Figure S2. *HapR is a repressor of native chb expression.* The indicated strains were induced with sterile water (no inducer) or chitin oligosaccharides (+ $(GlcNAc)_6$), then assessed for *chb* transcript abundance by qRT PCR. The parent strain used was *V. cholerae* E7946 WT. Data are shown on **A**) a log scale or **B**) a linear scale. The latter plot helps accentuate that deletion of *hapR* results in a two-fold increase in native *chb* transcripts in response to chitin oligosaccharides, which is similar to what is observed using transcriptional reporters. Data are from at least three independent biological replicates and is shown as the mean ± SD. Statistical comparisons were made by one-way ANOVA with Tukey's post-test. **, p = 0.0064.

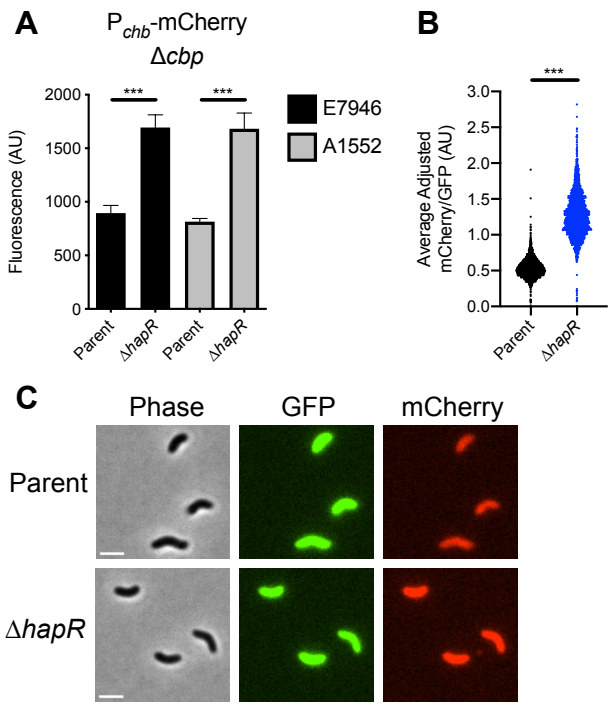

Figure S3. *HapR is a repressor of P_{chb} in V. cholerae E7946 and V. cholerae A1552.* **A**) Expression of a P_{chb}-mCherry reporter was determined in the indicated mutant strains. Each respective parent strain contained a P_{chb}-mCherry reporter and a Δ*cbp* mutation to activate ChiS. **B**) Scatter plot showing the relative expression of a P_{chb}-mCherry reporter in the indicated *V. cholerae* A1552 strains that have *cbp* intact when using chitin oligosaccharides to induce cells. The parent strain background contains both a P_{chb}-mCherry reporter, a P_{const2}-GFP construct (which exhibits constitutive GFP expression), and *cbp* intact. n = 2189 for Parent; n = 2164 for Δ*hapR.* Data shown are from two independent biological replicates. **C**) Representative images of *V. cholerae* A1552 strains analyzed in **B**. Scale bar = 2 μm. Statistical comparisons in **A** were made using a one way ANOVA with Tukey's post test and in **B** were made using Student's t-test. ***, $p < 0.001$.

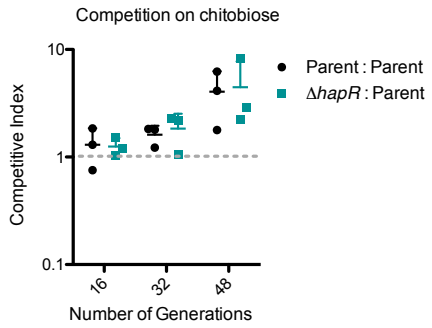

Figure S4. A Δ*hapR mutant does not have a fitness advantage for growth on chitobiose.* The indicated *V. cholerae* strains were mixed and co-cultured in M9 minimal media supplemented with 0.2% chitobiose + 10 μM CAI-1 and grown for the indicated number of generations. Each data point represents an independent biological replicate.

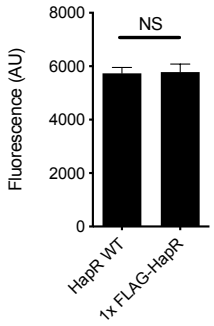

Figure S5. *N-terminally FLAG-tagged HapR is functional for regulation of P_{chb}.* Strains expressing the indicated HapR allele at the native locus were assessed for expression of a P_{chb}-GFP reporter. Both strains also harbored a Δ*cbp* deletion to activate ChiS. Statistical comparisons were made using Student's t-test. NS, not significant.

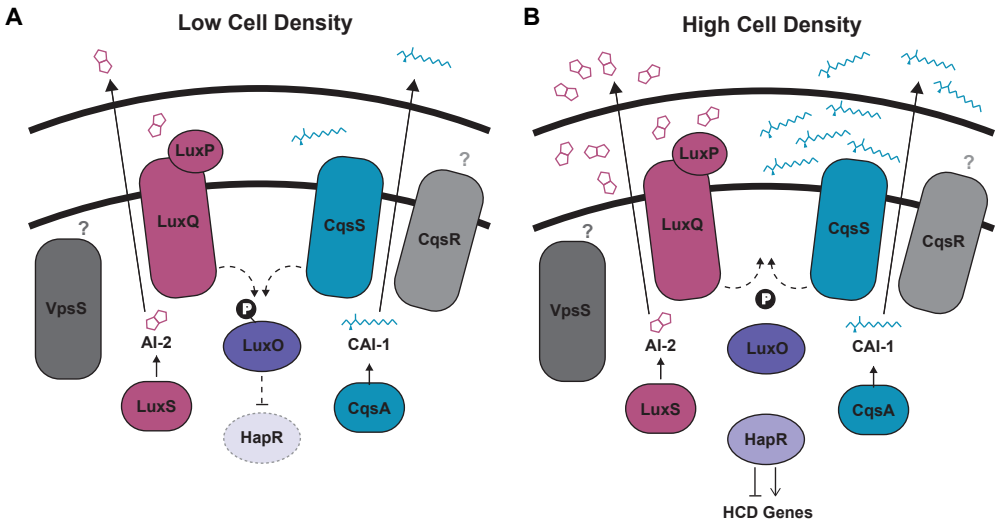

Figure S6. Model of quorum sensing regulation in *V. cholerae*. There are four parallel sensor kinases that contribute to QS in *V. cholerae*: LuxQ, CqsS, VpsS, and CqsR. The signals for VpsS and CqsR are unknown. LuxPQ senses the interspecies autoinducer AI-2 and CqsS senses the *V. cholerae*-specific autoinducer CAI-1. AI-2 is produced by the LuxS synthase and CAI-1 is produced by the CqsA synthase. (**A**) At low cell density, the LuxQ and CqsS sensors act as kinases, resulting in phosphorylation of LuxO, which subsequently decreases expression of HapR. (**B**) At high cell density, LuxQ and CqsS act as phosphatases, which results in dephosphorylation of LuxO, and subsequent activation of HapR expression. Dashed lines indicate indirect effects of one protein on another.

Table S1. *Other hits from P_{chb} activator screen and ΔclpA counter-screen.*

Activator or Δ*clpA* counter-screen?	Gene	VC gene designation	Putative function	Number of unique insertions
Activator	*treC*	VC0911	Trehalose-6-phosphate hydrolase	5
Activator	*cyaA*	VC0122	Adenylate cyclase	1
Δ*clpA* counter-screen	*nagC*	VC0993	GlcNAc transcriptional repressor protein	4
Δ*clpA* counter-screen	*cytR*	VC2677	Cytidine repressor protein	1