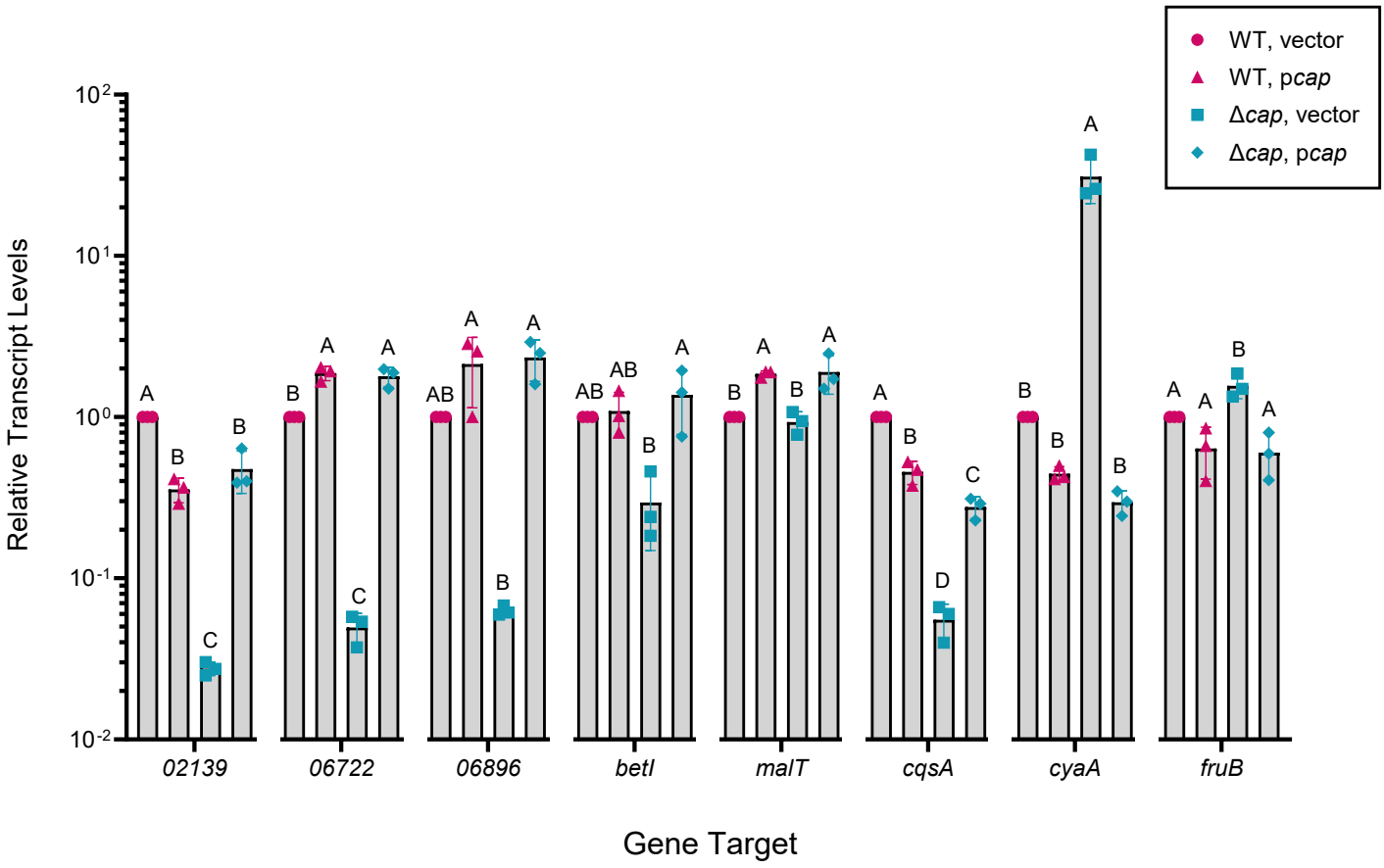
Supporting Information for Catabolite Activator Protein and quorum sensing cross-control group behaviors in *Vibrio campbellii*

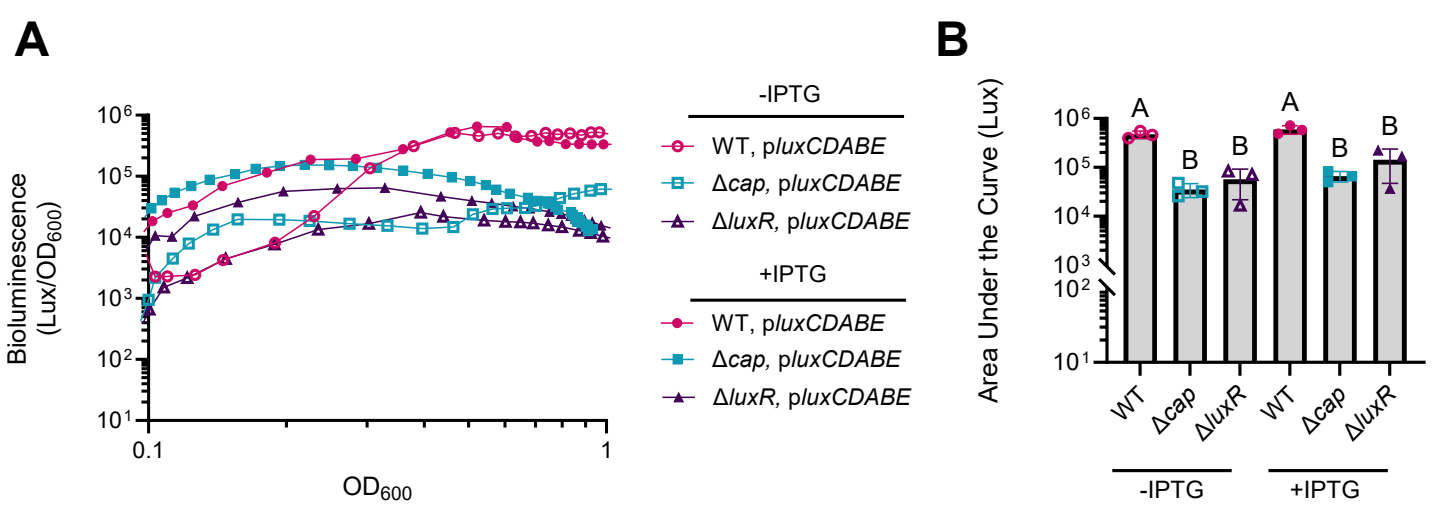
Chase M. Mullins[1], Alyssa S. Ball[1,2], Logan J. Geyman[1], Lydia K. Lukich[1], Lydia K. Hermann[1,3], Ram Podicheti[4], Zhongqing Ren[1,5], Xindan Wang[1], Douglas B. Rusch[4], Julia C. van Kessel[1*]

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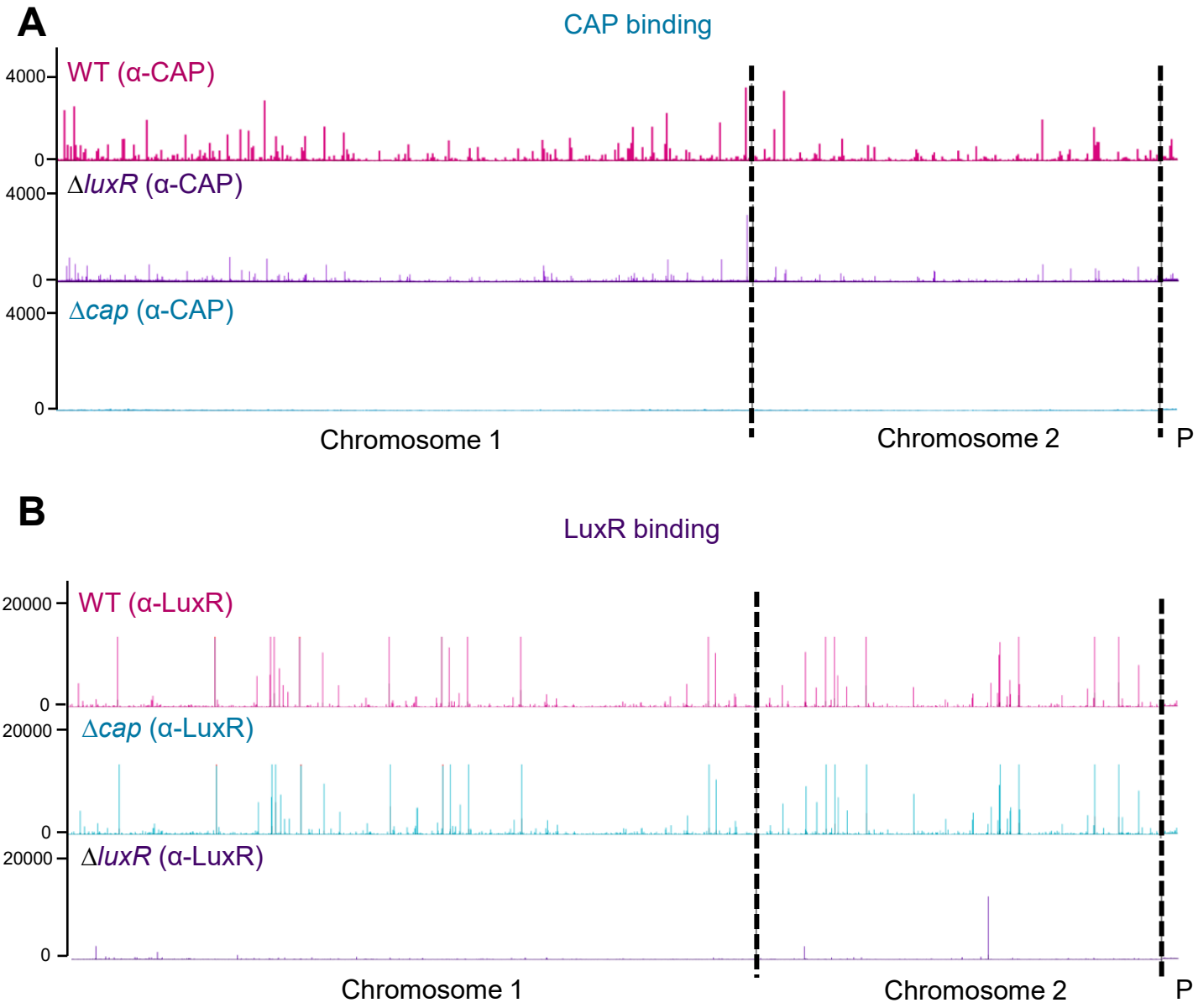
Supplemental Figures (Figures S1-S7)
Supplemental Tables (Table S1-S7)
Legends for Supplemental Datasets S1-S4
Additional References



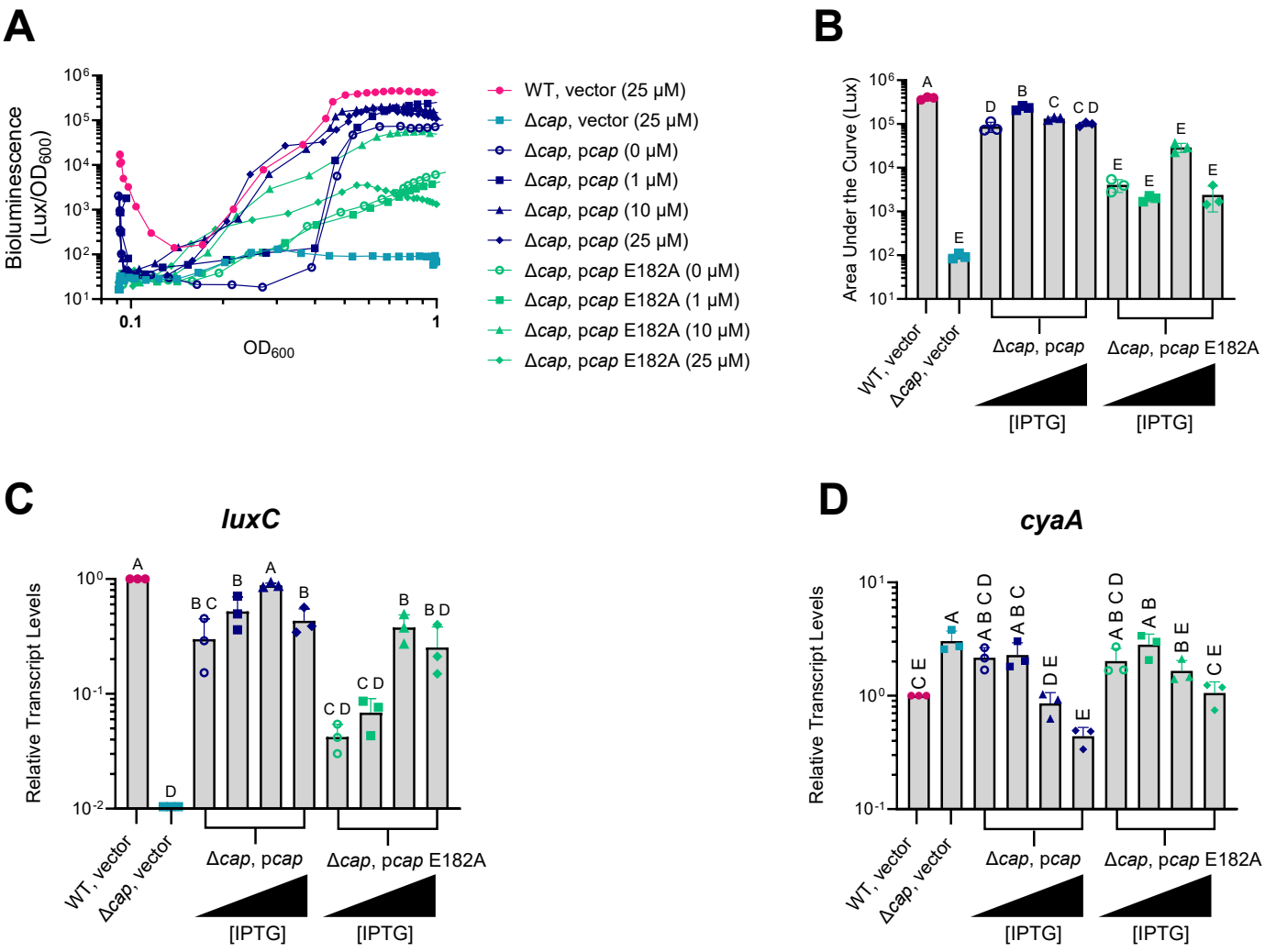
Supplemental Figure 1. RT-qPCR analysis of RNA collected at an OD_{600} of 1.0 from WT and Δ*cap V. campbellii* strains harboring either an empty vector (pCS027) or a plasmid with an IPTG-inducible (25 μM) copy of *cap* (pAB100). Relative transcript levels were measured for *VIBHAR_02139, VIBHAR_06722, VIBHAR_06896, betI, malT, cqsA, cyaA,* and *fruB* using the $\Delta\Delta C_T$ method and *hfq* as an internal control. Three biological replicates were performed with two technical replicates averaged together per biological replicate. A one-way ANOVA test was performed on normally distributed data (Shapiro-Wilk test), followed by Tukey's multiple comparisons test between strains within one gene target. Different letters above bars indicate differences in significance between only the strains in each gene target ($p<0.05$; $n=3$). Error bars represent the standard deviation.



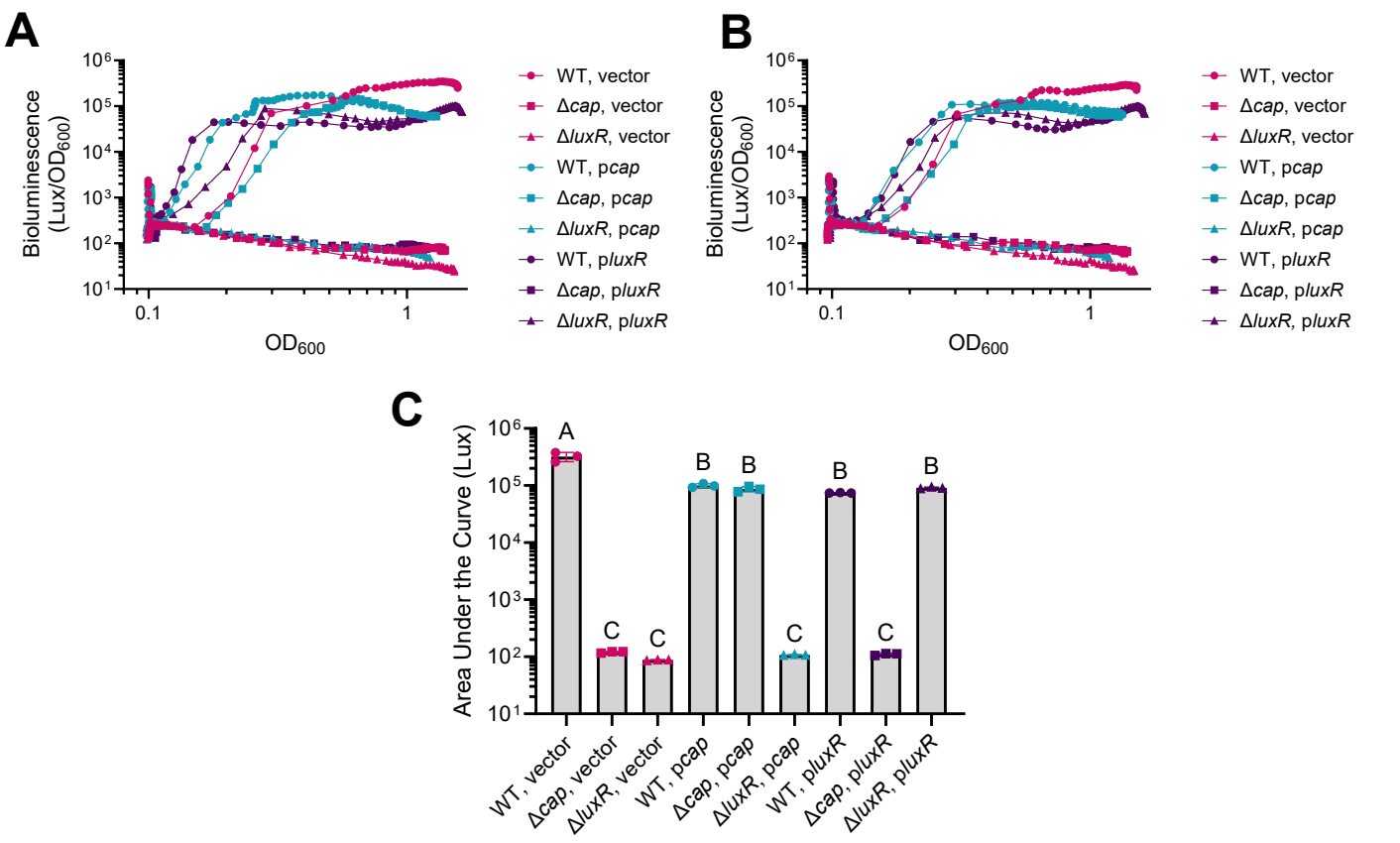
Supplemental Figure 2. (A) Bioluminescence production and OD_{600} were measured over 24 hours for Wild-type (WT), Δ*luxR*, and Δ*cap V. campbellii* strains harboring an episomal plasmid expressing *luxCDABE* from an IPTG-inducible promoter (25 μM; pCM024). The graph shown is a representative image from three biological replicates and each line on the graph is an average of two technical replicates. (B) Area under the curve (Lux) was measured from the bioluminescence vs OD_{600} plots in (A) for three biological replicates. A one-way ANOVA test was performed on normally distributed data (Shapiro-Wilk test), followed by Tukey's multiple comparisons test. Different letters above bars indicate differences in significance between strains in pairwise comparisons (p<0.05; n=3). Error bars represent the standard deviation.



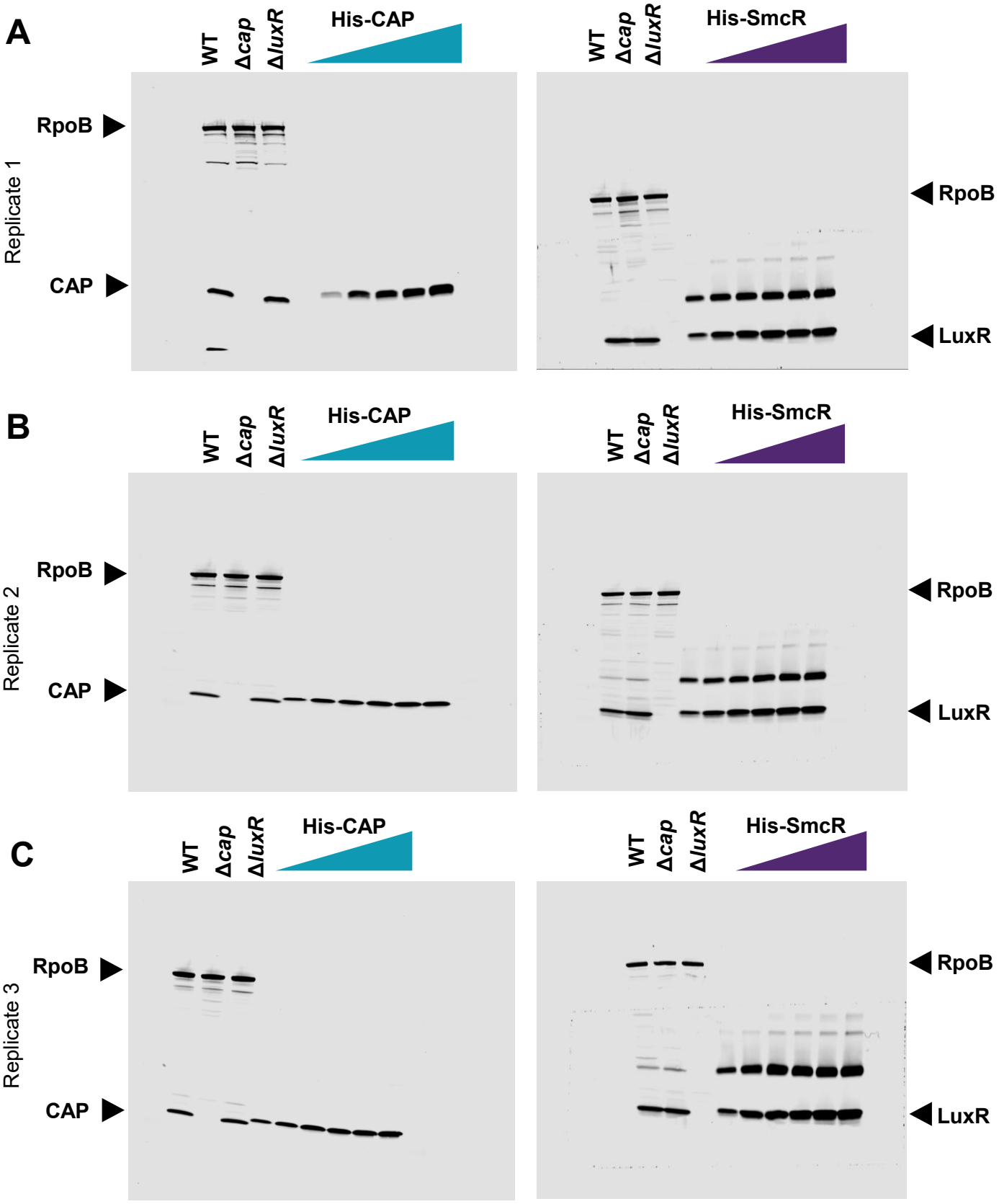
Supplemental Figure 3. (A-B) ChIP-seq was performed in WT, Δ*cap*, and Δ*luxR V. campbellii* strains with either α-CAP (A) or α-LuxR (B) antibodies at an OD_{600} of 1.0. Sequenced DNA was mapped to the *V. campbellii* BB120 (ATCC BAA-1116) chromosome and is graphed as enrichment over chromosomal location. The chromosomes are segmented by the black dashed lines. In addition to two chromosomes, *V. campbellii* contains a plasmid which is denoted with a P. The Y-axis represents peak height. Screenshots of ChIP peaks were captured using JBrowse 2.



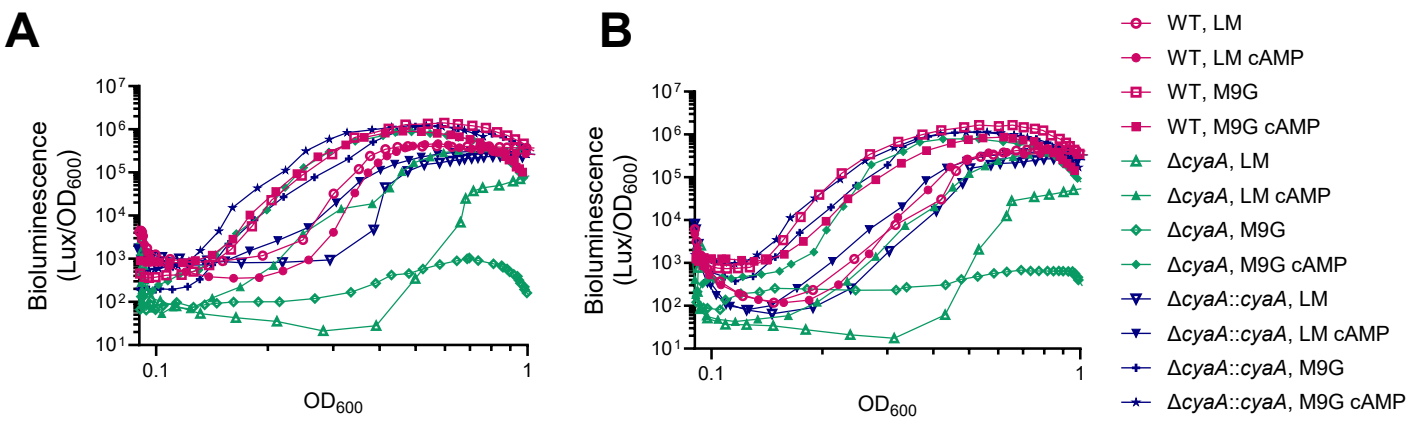
Supplemental Figure 4. (A) Bioluminescence production and OD_{600} were measured over 24 hours for Wild-type (WT) and Δ*cap V. campbellii* strains harboring either an empty vector (pCS027) or an episomal plasmid expressing *cap* (pAB100) or *cap* E182A (pCM026) from an IPTG-inducible promoter. IPTG was titrated to final concentrations of 0, 1, 10, or 25 μM as indicated on the graph. The figure is a representative graph from three biological replicates and each line on the graph is an average of two technical replicates. (B) Area under the curve (Lux) was measured from bioluminescence vs OD_{600} plots shown in panel A for three biological replicates. (C, D) RT-qPCR analysis of RNA collected at an OD_{600} of 1.0 from the same strains and conditions as panel A. Three biological replicates were performed with two technical replicates averaged together per each biological replicate. Relative transcript levels were measured for *luxC* (C) and *cyaA* (D) using the $\Delta\Delta C_T$ method and *hfq* as an internal control. (B-D) A one-way ANOVA test was performed on normally distributed data (D'Agostino-Pearson test on the residuals), followed by Tukey's multiple comparisons test between strains. Different letters above bars indicate differences in significance between strains in pairwise comparisons ($p<0.05$; $n=3$). Error bars represent the standard deviation.



Supplemental Figure 5. (A, B) Bioluminescence production and OD_{600} were measured for 24 hours for Wild-type (WT), Δ*luxR*, and Δ*cap V. campbellii* strains harboring either an empty vector (pCS027) or an episomal plasmid expressing *cap* (pAB100) or *luxR* (pJV388) from an IPTG inducible promoter (25 μM). Each line on the graph is an average of two technical replicates. A representative from three biological replicates was presented in the main text as figure 2A; panels A and B constitute the two remaining biological replicates. (C) Area under the curve (Lux) was measured from the bioluminescence vs OD_{600} plots from the three biological replicates presented in panels A-B and Figure 2A. A one-way ANOVA test was performed on normally distributed data (Shapiro-Wilk test), followed by Tukey's multiple comparisons test. Different letters above bars indicate differences in significance between strains in pairwise comparisons (p<0.05; n=3). Error bars represent the standard deviation.



Supplemental Figure 6. (A-C) Full membrane images from a western blot analysis of WT, Δ*cap*, and Δ*luxR* lysates collected from cells grown to an OD_{600} of 1.0. A representative, cropped image was presented in figures 2E and 2F in the main text. Lysates were probed using either CAP monoclonal antibodies (α-CAP; left panels) or LuxR polyclonal antibodies (α-LuxR; right panels). RNA polymerase subunit B (α-RpoB) monoclonal antibodies were used to detect RpoB as a loading control in all samples. Increasing concentrations of His-CAP were loaded onto gels to determine a linear range (10 ng, 30 ng, 50 ng, 70 ng, 90 ng, 110 ng for panel A and 20 ng, 40 ng, 60 ng, 80 ng, 100 ng, 120 ng for panels B-C) and are represented by teal triangles. Likewise, increasing concentrations of His-SmcR (the LuxR homolog in *Vibrio vulnificus*; 93% amino acid identity) were loaded onto gels to determine a linear range (50 ng, 100 ng, 150 ng, 200 ng, 250 ng, 300 ng) and are represented by purple triangles. Panel A represents replicate 1 blots, panel B represents replicate 2 blots, and panel C represents replicate 3 blots.



Supplemental Figure 7. (A-B) Bioluminescence production and OD_{600} were measured over 24 hours for WT or Δ*cyaA V. campbellii* strains grown in either a rich, undefined medium (LM; LB + NaCl) or M9 minimal medium supplemented with 20 mM glucose (M9G). The Δ*cyaA* strains were complemented with an IPTG-inducible (10 μM) copy of *cyaA* inserted on the chromosome at a non-polar site (Δ*cyaA*::*cyaA*). Exogenous cyclic-AMP (cAMP) was added to the medium at a final concentration of 1 mM. Each line on the graph is the average of two technical replicates. A representative figure was shown in figure 3A in the main text; these are the additional two biological replicates.

Table S1. MEME Suite-FIMO using *E. coli* CAP consensus sequence identified $P_{luxCDABE}$ CAP Binding Sites.

Name	Sequence
E. coli CAP Consensus	5'-AAA**TGTGA**TCTAGA**TCACA**TTT-3'
CAP Binding site 1	5'-AAG**TGCTA**TCTAGG**TGAGA**GGA-3'
CAP Binding site 2	5'-TAT**AGTGT**TACAAA**TAACA**TTA-3'
($p < 0.05$)	

a. Bolded nucleotides indicate where the most important CAP-DNA contacts are made as determined by Gartenberg and Crothers 1988 (1) and Benoff *et al.* 2002 (2).

Table S2. MEME Suite-FIMO using *V. cholerae* CAP consensus sequence identified $P_{luxCDABE}$ CAP Binding Sites.

Name	Sequence
V. cholerae CAP Consensus	5'-WK**TGA**NNNNNV**TCA**MW-3'
CAP Binding site 2	5'-AG**TGT**TACAAA**TAA**CA-3'
CAP Binding site 3	5'-TT**TGA**TCTGCT**TAA**TA-3'
($p < 0.05$)	

a. Bolded nucleotides indicate where the most well-defined base pairs in the *V. cholerae* CAP consensus sequence as determined by Manneh-Roussel et al., 2018 (3).
b. W= Adenine/Thymine, K= Guanine/Thymine, V=Guanine/Cytosine/Adenine, M= Adenine/Cytosine, N= any DNA nucleotide

Table S3. MEME Suite-FIMO using *V. campbellii* CAP consensus sequence identified false-positive $P_{luxCDABE}$ CAP Binding Site.

Name	Sequence	*p*-value	*q*-value
V. campbellii CAP Consensus	5'-WKTGANNNNNNYCWYA-3'		
CAP $P_{luxCDABE}$ Binding site 3	5'-TTTGATCTGCTTAATA-3'	0.00357	0.616
CAP binding site within *cyaA* promoter (positive control)	5'-TGTGACTGGATTCTCA-3'	1.2e-05	0.00317
CAP binding site within *rpoD* promoter (negative control)	5'-TGGGACATTCCCCTCG-3'	0.00481	1

a. W= Adenine/Thymine, K= Guanine/Thymine, Y= Thymine/Cytosine, H=Adenine/Cytosine/Thymine, S= Guanine/Cytosine N= any DNA nucleotide

Table S4. Strains used in this study.

Strains	Genotype	Reference
***E. coli* strains**		
S17-1λ*pir*	wild-type	(4)
DH10B	wild-type	Life Technologies
BL21(DE3)	wild-type	NEB
RLG849	MG1655 wild-type	Gourse Lab unpublished
RLG4771	MG1655 Δ*cap* Cm^R	(5)
***V. campbellii* strains**		
BB120	wild-type	(6)
KM669	Δ*luxR*	(7)
AB003	Δ*cap*	This study
CM056	Δ*cap* Δ*luxR*	This study
CM058	Δ*cyaA*	This study
CM075	Δ*cyaA*::P_{tac}-*cyaA*	This study

Table S5. Plasmids used in this study.

Name	Description	Reference
pMMB67EH	P_{tac} promoter empty vector, IncQ origin, kan^R	(8)
pJV021	P_{tac} promoter vector, p15a origin, kan^R	(9)
pRE112	Gene deletion vector, cm^R, *sacB* (sucrose intolerant), R6Kg origin	(10)
pJMP1339	dCas9, R6Kg origin, kan^R	(11)
pJMP1039	Tn7 Transposase, R6Kg origin, amp^R	(11)
pCS027	Empty vector pMMB67EH, kan^R	(12)
pAB100	*V. campbellii* P_{tac}-*cap* in pMMB67EH, kan^R	This study
pJV388	*V. campbellii* P_{tac}-*luxR* in pMMB67EH, kan^R	(13)
pAB105	*V. campbellii* P_{tac}-6xHis-CAP in pET28b, kan^R	This study
pCM022	P_{tac}-*cyaA* in pJMP1339, kan^R	This study
pCM024	P_{tac}-*luxCDABE* in pMMB67EH, kan^R	This study
pCS019	P_{luxC}-*gfp* in pMMB67EH, kan^R	(12)
pAB101	$P_{-216luxC}$-*gfp* in pMMB67EH, kan^R	This study
pAB102	$P_{-156luxC}$-*gfp* in pMMB67EH, kan^R	This study
pCS042	P_{luxC}-*gfp* in pMMB67EH, $gent^R$	This study

pAB118	$P_{-216luxC}$-GFP in pMMB67EH, *gent*^{R}	This study
pAB119	$P_{-156luxC}$-GFP in pMMB67EH, *gent*^{R}	This study
pLAFR2	Empty vector cosmid, *tet*^{R}	(7, 14)
pKM699	P_{luxR}-*luxR* (2.3 kbp fragment) in pLAFR2 cosmid, *tet*^{R}	(7, 15)
pCM026	*V. campbellii* P_{tac}-*cap* E182A in pMMB67EH, *kan*^{R}	This study

Table S6. RT-qPCR and qPCR primers used in this study.

Name	Sequence	Notes
JCV737	TGGTTAACAGTGTTCTTCAATAGGATC	*hfq* reverse
JCV738	ATGGCTAAGGGGCAATCTC	*hfq* forward
CM128	AACACGGCAACATTACTCATG	*VIBHAR_02139* forward
CM129	CGAAACAACGTCACACCAAG	*VIBHAR_02139* reverse
CM126	TGCACTTATCGAAGACGACGA	*VIBHAR_06722* forward
CM127	CTAAGAATGGCGGCTTTGCC	*VIBHAR_06722* reverse
CM124	CTGCAACGTATTTACTTGCTG	*VIBHAR_06896* forward
CM125	GGTTGATAGAGGAATCCCCA	*VIBHAR_06896* reverse
CM122	GGTTGGGATGCCTGAAATCA	*betI* forward
CM123	GCTGATCAACGAGATACTCG	*betI* reverse
CM120	GGATTCCATCAAAACTTACTCG	*malT* forward
CM121	GTACCAGTTTGTAGTAAGGCG	*malT* reverse
CM118	CCACTTCCTTCCTTTGTTGAGG	*cqsA* forward
CM119	TGGTCGCTTCCCTAATACCA	*cqsA* reverse
CM104	GCTAAGTAAGAACGACATTACCC	*fruB* forward
CM105	TTCAACCAGACCTTTGTCTGTC	*fruB* reverse
CM114	GCGATTAGATAACCTGAACCAG	*cyaA* forward
CM115	GTTCAACAAGGCAGGTATCAAG	*cyaA* reverse
JCV745	GCAAAGAGACCTCGTACTAGG	*luxR* forward
JCV746	GCGACGAGCAAACACTTC	*luxR* reverse
luxC right	TGTTCATTTAACTCAGATGGTGACT	*luxC* right
luxC left	TTCTTCTTGAATACTCTTCGCTCTT	*luxC* left
AB336	TAAACCTCAAACCGACCCAA	*cap* forward
AB337	ACGATGTAGTACAAAGTCTCTGC	*cap* reverse
CM167	AGTGTGACTGGATTCTCATTTAGAC	F P_{cyaA} ChIP-qPCR primer (-232 bp from start of gene)
CM168	CCACACTAGTCGCAAAAATTGATAG	R P_{cyaA} CHIP-qPCR primer (-136 bp from start of gene)
CM169	GCTGCTAGCTTTGATGCTAGA	F P_{rpoD} ChIP-qPCR primer (-133 bp from start of gene)
CM170	GGAATCAGTATGCGAAATGCA	R P_{rpoD} ChIP-qPCR primer (-30 bp from start of gene)

Table S7. Genes mentioned in this study and their associated locus tags.

Gene Name	Locus Tag
cap	*VIBHAR_RS00360*
luxR	*VIBHAR_RS16180*
hfq	*VIBHAR_RS00455*
luxC	*VIBHAR_RS23945*
luxD	*VIBHAR_RS23940*
luxA	*VIBHAR_RS23935*
luxB	*VIBHAR_RS23930*
luxE	*VIBHAR_RS23925*
VIBHAR_02139	*VIBHAR_RS10035*
VIBHAR_06722	*VIBHAR_RS25890*
VIBHAR_06896	*VIBHAR_RS26600*
betI	*VIBHAR_RS23655*
malT	*VIBHAR_RS17930*
cqsA	*VIBHAR_RS23255*
cyaA	*VIBHAR_RS01465*
fruB	*VIBHAR_RS22725*
rpoD	*VIBHAR_RS04015*
focA	*VIBHAR_RS08700*
glpT	*VIBHAR_RS15475*
VIBHAR_06505	*VIBHAR_RS25000*
malE	*VIBHAR_RS19540*
malF	*VIBHAR_RS19545*
malG	*VIBHAR_RS19550*
cqsS	*VIBHAR_RS23260*
luxM	*VIBHAR_RS12950*

Legends for Supplemental Datasets:

Supplemental Dataset S1: Genes ≥ 4-fold regulated by CAP and LuxR
Supplemental Dataset S2: Unfiltered RNA-seq hits with all normalized read counts
Supplemental Dataset S3: ChIP-seq peaks ≥ 2-fold enriched by CAP and LuxR
Supplemental Dataset S4: Genes directly regulated by CAP and LuxR

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