Supplemental Material

Supplemental Tables

Table S1. Strains used in this study.

Strain	Genotype	Reference
***E. coli* strains**		
S17-1λ*pir*	Wild-type	(1)
BL21(DE3)	Wild-type	Life Technologies
DH10B	Wild-type	Life Technologies
***V. harveyi* strains**		
BB120	Wild-type	(3)
BB721	Δ*luxO::kan*^{R} (Kanamycin resistance gene)	(4)
JAF548	*luxO* D47E*::kan*^{R}	(4)
KM669	Δ*luxR*	(2)
JC2212	Δ*betI*	This study
JC2216	Δ*luxO::kan*^{R} Δ*betI*	This study
JC2214	*luxO* D47E*::kan*^{R} Δ*betI*	This study
JC2194	Δ*qrr*1-5 Δ*luxR* Δ*betI*	This study
JS202	Δ*qrr*1-5 Δ*luxR*	(5)
JV48	Δ*aphA*	(5)

Table S2. Plasmids used in this study.

Name	Description	Reference
pET28b	Expression vector, 6x-His, *kan*^{R}	Novagen
pEVS143	P_{tac} promoter vector, p15a origin, *kan*^{R}	(7)
pJC420	*betI* region (~2.5 kbp upstream and 2.5 kbp downstream of the *betI* ORF) in pLAFR2	This study
pJC430	*betI* deletion cosmid constructed from pJC420. The *betI* gene was deleted by replacement with a chloramphenicol cassette (*cm*^{R}) using recombineering (8). The *cm*^{R} was removed by FLP-mediated recombination (9). An ~100 bp scar remains at the site.	This study
pJV021	*cm*^{R} removed from the *gfp-cm*^{R} expression cassette in pEVS143	This study
pJV036	P_{tac} promoter vector, ColE1 origin, *kan*^{R}	(10)
pJV079	LuxR in pET28b for expression	(10)
pJV239	P_{tac}-*luxR*, in pJV036	(10)
pJV298	*cm*^{R} cloned in place of *kan*^{R} in pJV021	This study
pJV299	*cm*^{R} cloned in place of *kan*^{R} in pSQ005	This study
pJV302	*betIBA-proXWV* operon and promoter region in pLAFR2	This study
pJV305	LuxR BS1 deleted in pJV300	This study
pJV306	LuxR BS2 deleted in pJV300	This study
pKM699	pLAFR2 containing *luxR* from a 2.3-kbp HindIII fragment	(11)
pLAFR2	Cosmid vector used for constructing mutant strains of *V. harveyi*	(12-14)
pSQ004	*His-betI* in pET28b for expression	This study
pSQ005	P_{tac}-*betI* in pEVS143	This study

Table S3. Oligonucleotides used in this study.

Name	Sequence	Notes
hfq qRT-PCR Forward	CGTGAGCGTATCCCGGTATCTAT	
hfq qRT-PCR Reverse	TTGCAGTTTGATACCGTTCACAAG	JCV737
qrr1 qRT-PCR Forward	CTCGGGTCACCTATCCAACTGA	
qrr1 qRT-PCR Reverse	TCGGATCTATTGGCTCGTTCTG	
qrr2 qRT-PCR Forward	CAATTAGGGCGATTGGCTTATGT	
qrr2 qRT-PCR Reverse	CTTAAGCCGAGGGTCACCTAGC	
qrr3 qRT-PCR Forward	ACAAATTCGAGTCCACTAACAACGT	
qrr3 qRT-PCR Reverse	CTTAAGCCGAGGGTCACCTAGC	
qrr4 qRT-PCR Forward	GTTGATTGGCGGTATATACTTGTG	
qrr4 qRT-PCR Reverse	CCTTATTAAGCCGAGGGTCAC	
qrr5 qRT-PCR Forward	GACGTTGTTAGTGAACCCAATTGTT	
qrr5 qRT-PCR Reverse	CACAAGGTTTGTGATTGGCTGTATA	
aphA qRT-PCR Forward	GATTGAAGACATGTCATTACCACAC	JCV743
aphA qRT-PCR Reverse	GAAGTAACCGATGCTAGCTGA	JCV744
luxC qRT-PCR Forward	TGTTCATTTAACTCAGATGGTGACT	
luxC qRT-PCR Reverse	TTCTTCTTGAATACTCTTCGCTCTT	
luxO qRT-PCR Forward	GCATTCCTGATCTTATTCTGCTCG	
luxO qRT-PCR Reverse	TCCATCCCCGTCATATCAGGTA	
luxR qRT-PCR Forward	GCAAAGAGACCTCGTACTAGG	JCV745
luxR qRT-PCR Reverse	GCGACGAGCAAACACTTC	JCV746
qRT-PCR 06176 Forward	ATGGATGCAATTACGATTGAAAACC	SQ096
qRT-PCR 06176 Reverse	TAAATCCAGCCTCCTTCGTG	SQ097
qRT-PCR 06177 Forward	GTGAATTTTATTACGGACAACAAACTTCC	SQ098
qRT-PCR 06177 Reverse	TACACCGCAGCATTCCTTT	SQ099
qRT-PCR 06178 Forward	ATGACAAAAATCTTTTCATCTATCGCG	SQ100
qRT-PCR 06178 Reverse	TAACTGGATGCCAACCATGG	SQ101
qRT-PCR 06179 Forward	GACAAGAGCATCTTCATTCAAATGC	SQ021
qRT-PCR 06179 Reverse	GTCTAACCCCTCTTCCGC	SQ022
qRT-PCR 06180 Forward	GCGAATGTGAAGCAGGC	SQ019
qRT-PCR 06180 Reverse	TTTGCCCGTATCTGCCA	SQ020

qRT-PCR 06181 Forward	GTTGGGATGCCTGAAATCAGA	JCV339
qRT-PCR 06181 Reverse	GCTTCTTTGCTGATCAACGAG	JCV340
P_{betI} Reverse	CAACCTTGGGCATTTTACATCCTTGTTATTTTTAATTG	SQ94
P_{betI} Forward	CCTTCACTCAATTGACAATTCTAAAAGTAAAACGTGCTGAAATTAT	SQ113
P_{betI} A	CCTTCACTCAATTGACAATTCTAAAAGTAAAACGTGCTGAAATTA	JCV977
P_{betI} A	TAATTTCAGCACGTTTTACTTTTAGAATTGTCAATTGAGTGAAGG	JCV978
P_{betI} B	GCTGAAATTATTTGAGTTCATTGTATTTGTCTTTATTTATACTTT	JCV979
P_{betI} B	AAAGTATAAATAAAGACAAATACAATGAACTCAAATAATTTCAGC	JCV980
P_{betI} C	TTTATACTTTTGATGCGCACGAATTGCTCATGAGCTGGTATCTCT	JCV981
P_{betI} C	AGAGATACCAGCTCATGAGCAATTCGTGCGCATCAAAAGTATAAA	JCV982
P_{betI} D	TGGTATCTCTATAACTTGCATTAAATGTAGTTTAGTTTCTGATTA	JCV983
P_{betI} D	TAATCAGAAACTAAACTACATTTAATGCAAGTTATAGAGATACCA	JCV984
P_{betI} E	TTTCTGATTATTATGGTTTTTGTTTTTCTGTTTTTACGTGTTAAA	JCV985
P_{betI} E	TTTAACACGTAAAAACAGAAAAACAAAAACCATAATAATCAGAAA	JCV986
P_{betI} F	ACGTGTTAAATCTTCGTAAAATCTATCTTTTCTCAAGTTTTAGTA	JCV987
P_{betI} F	TACTAAAACTTGAGAAAAGATAGATTTTACGAAGATTTAACACGT	JCV988
P_{betI} G	AGTTTTAGTAATGATTGTAGTCGAAATGATTCGGCTGTATCTGTC	JCV989
P_{betI} G	GACAGATACAGCCGAATCATTTCGACTACAATCATTACTAAAACT	JCV990
P_{betI} H	TGTATCTGTCATTTTTTCTAGATAATTAGTTTGAGTTAGAACTAA	JCV991
P_{betI} H	TTAGTTCTAACTCAAACTAATTATCTAGAAAAAATGACAGATACA	JCV992
P_{betI} I	TTAGTTTGAGTTAGAACTAAATTTAAGGCATTTTA	JCV993
P_{betI} I	TAAAATGCCTTAAATTTAGTTCTAACTCAAACTAA	JCV994
P_{betI} J	AGGCATTTTATATTTAACTAACCGTTCAATTAAAAATAACAAGGA	JCV995
P_{betI} J	TCCTTGTTATTTTTAATTGAACGGTTAGTTAAATATAAAATGCCT	JCV996
P_{betI} K	ATAACAAGGATGTAAAATGCCCAAGGTT	JCV997
P_{betI} K	AACCTTGGGCATTTTACATCCTTGTTAT	JCV998
recA Forward	CAATAAACGCACAAGTTTTGCCTTC	SQ110
recA Forward	ATGAATAAATCGGAGAAAGTAATGGACGA	SQ104
P_{qrr3} Forward	ATCATTAGCATTATAATTACAAATTGCA	STR725
P_{qrr3} Reverse	ATGATGCAGTTAGTGTGCCAACTT	STR726
P_{qrr4} Forward	TTTCTTATTAAAACGCCATTTTTCTGATA	STR727
P_{qrr4} Reverse	AGCACGATGCGTGCCAACTTT	STR422

P_{betI} LuxR binding site 1	CACTCAATTGACAATTCTAAAAGTAAAACGT	JCV424
P_{betI} LuxR binding site 1	ACGTTTTACTTTTAGAATTGTCAATTGAGTG	JCV425
P_{betI} LuxR binding site 2	TTTTATATTTAACTAACCGTTCAATTAAAAAT	JCV426
P_{betI} LuxR binding site 2	ATTTTTAATTGAACGGTTAGTTAAATATAAAA	JCV427

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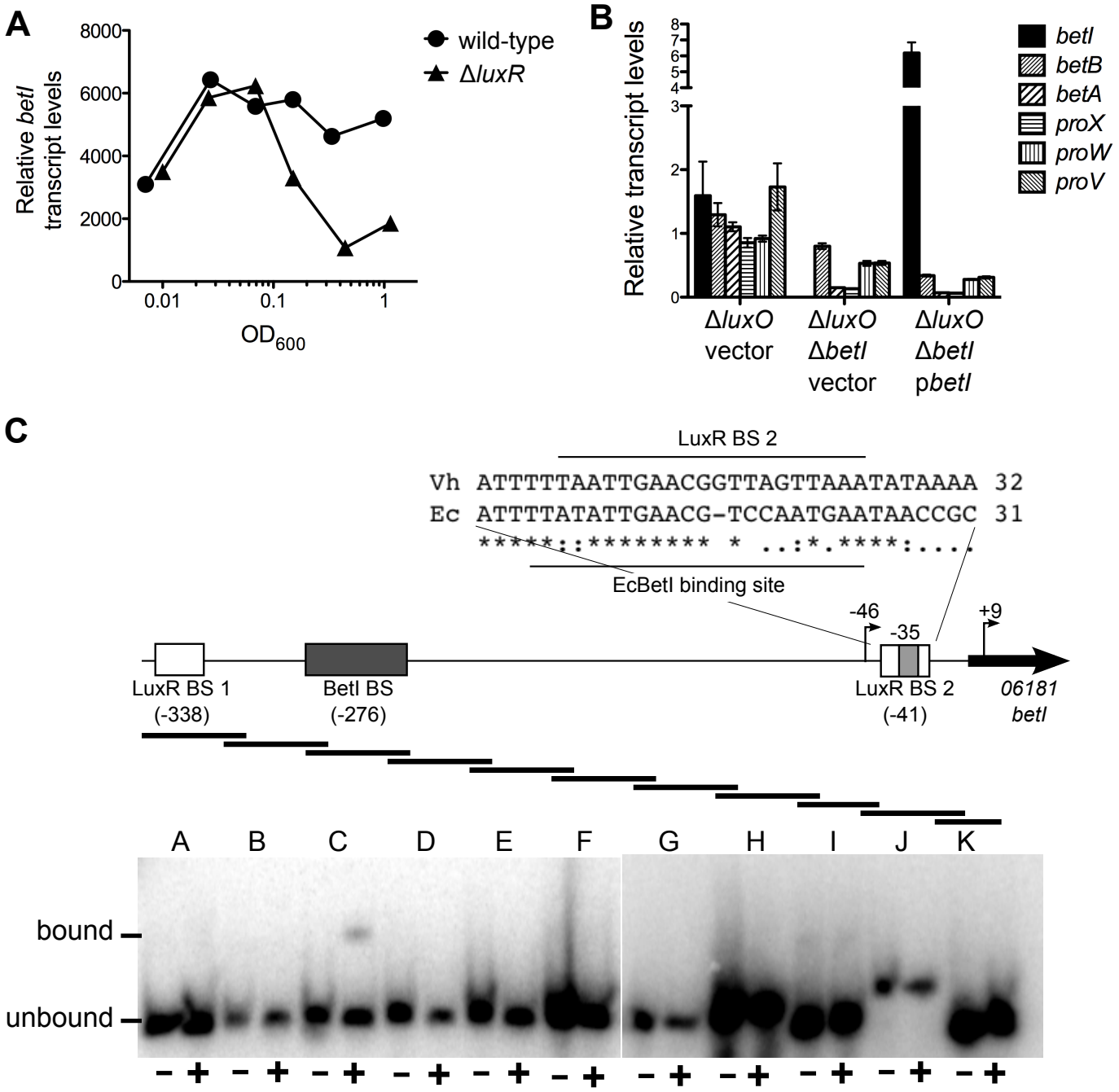

Figure S1. (*A*) Transcript levels of *betI* measured by microarrays are shown for *V. harveyi* wild-type (BB120) and *V. harveyi* Δ*luxR* (KM669) and were determined previously (8). (*B*) Transcript levels of *betIBA-proXWV* genes were assayed by qRT-PCR in *V. harveyi* in the following strains induced with 10 μM IPTG: Δ*luxO* carrying a control vector (BB721::pJV298), Δ*luxO* Δ*betI* carrying a control vector (JC2216::pJV298), and Δ*luxO* Δ*betI* carrying a vector expressing *betI* from an IPTG-inducible promoter (JC2216::pJV299). Error bars represent the standard error of measurement from three biological replicates, and these data represent two independent experiments. (*C*) Alignment of the predicted BetI binding site sequences for *V. harveyi* (Vh) and *E. coli* (Ec) with EMSAs. The location of the predicted BetI binding site overlaps LuxR binding site 2 (BS2, see Figure 2, main text). The positions of the binding sites, transcription start sites, and -35 site (gray box) are indicated relative to the translation start codon of *betI*. Asterisks indicate nucleotide identity. EMSAs contain 0 nM (-) or 1000 nM (+) His-BetI incubated with radiolabeled DNA substrates corresponding to the fragments A through K in the diagram.

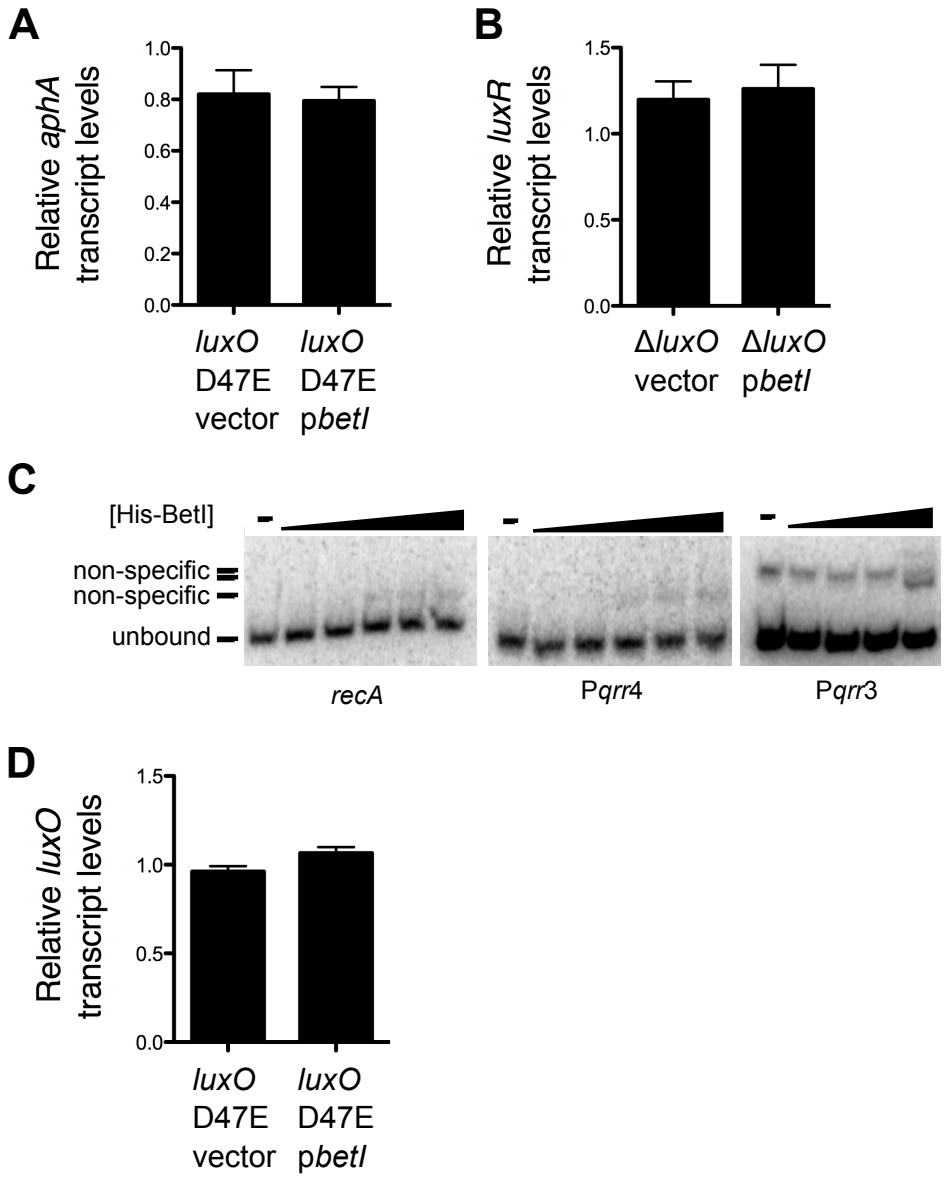

Figure S2. (*A, B, D*) Transcript levels of *aphA* (*A*), *luxR* (*B*), and *luxO* (*D*) were assayed by qRT-PCR in *V. harveyi* strains induced with 1 mM IPTG. Error bars represent the standard error of measurement from three biological replicates, and these data represent two independent experiments. The strains are: (*A,D*) *V. harveyi luxO* D47E carrying a control vector (JAF548::pJV298) and carrying a vector expressing *betI* from an IPTG-inducible promoter (JAF548::pJV299); (*B*) The *V. harveyi* Δ*luxO* strain carrying a control vector (BB721::pJV298) and carrying a vector expressing *betI* from an IPTG-inducible promoter (BB721::pJV299). (*C*) EMSAs with His-BetI incubated with radiolabeled DNA substrates corresponding to the *recA* ORF (300 bp), the *qrr*3 promoter (P*qrr*3; 133 bp), or the *qrr*4 promoter (P*qrr*4; 177 bp). The reactions for *recA* and P*qrr*4 contained 0, 1000 nM, 2000 nM, 4000 nM, 6000 nM, or 8000 nM His-BetI, and the reactions for *qrr*3 contained 0, 250 nM, 500 nM, 1000 nM, and 5000 nM His-BetI.

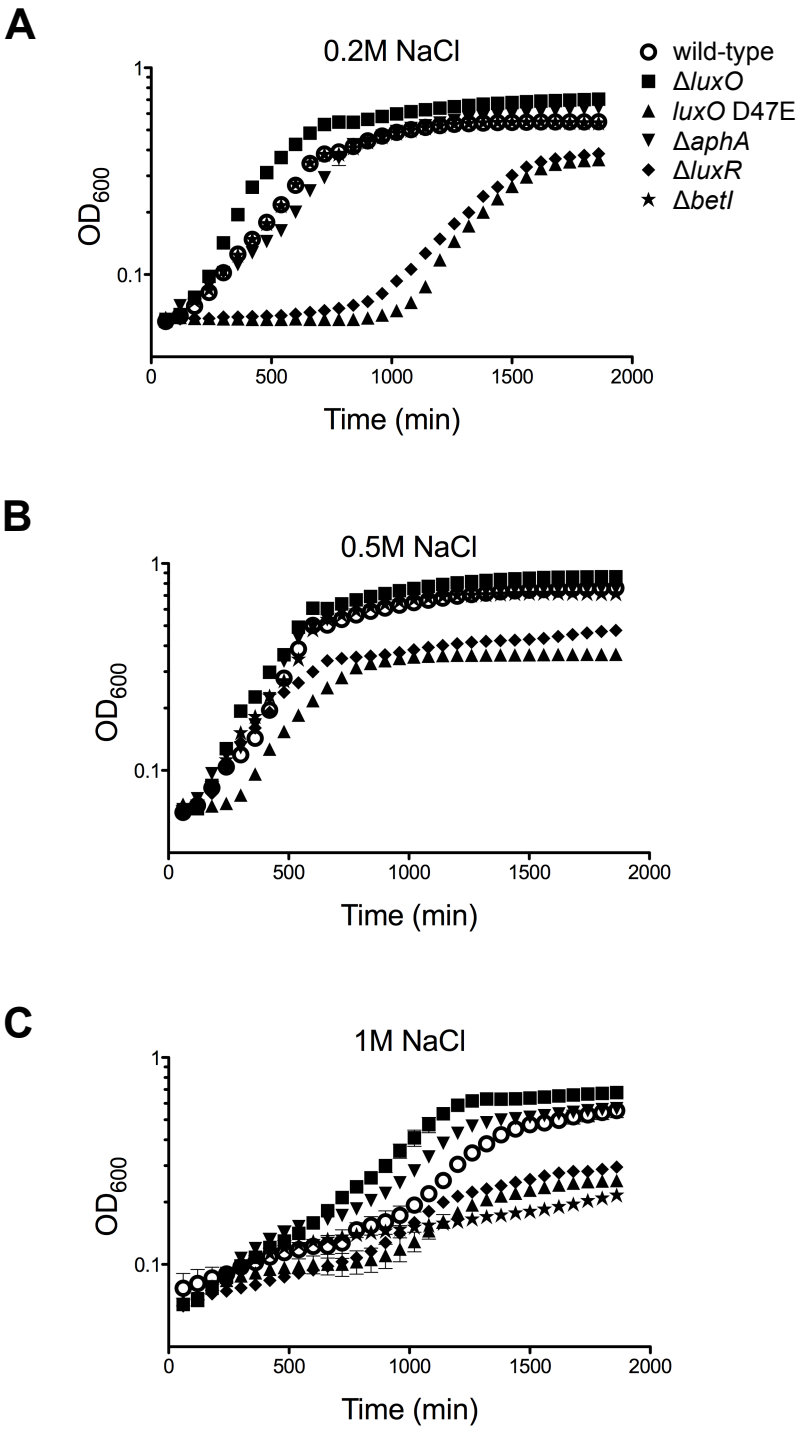

Figure S3. *V. harveyi* strains were grown in LOM containing choline and 0.2 M, 0.5 M, or 1 M NaCl. Strains are *V. harveyi* wild-type (BB120), Δ*luxO* (BB721), *luxO* D47E (JAF548), Δ*aphA* (JV48), Δ*luxR* (KM669), and Δ*betI* (JC2212).