1   Supplementary Table S1. Primers used in this study.

Primers	Nucleotide sequences
Phospho-transfer cloning primers	
*cqsS*_PTP_HK_F	CACCTTGTTCCGTAACCAAATCTCTC
*cqsS*_PTP_HK _R	TTATTCTGTTTTTATCTCGTTAACCGTATC
*cqsS*_PTP_RD_F	CACCTTGCGACGTATTTTGGTG
*cqsS*_PTP_RD _R	TTACACCCAAGCTGCCACTTTAT
*luxQ*_PTP_HK_F	CACCTTGCAAGCCGCAAGGCGC
*luxQ*_PTP_HK_R	TTACGGCGCATCCGGTTTTTCTA
*luxQ*_PTP_RD_F	CACCTTGCTGCGTGTACTGTTGGTG
*luxQ*_PTP_RD_R	TTAATTTAAGCCAGCGTTTTTTTGGC
*luxO*_PTP_RD_F	CACCTTGGTAGAAGACACGGCGTC
*luxO*_PTP_RD_R	TTATTTGAGTTTCGAGGCTTTGC
*luxO*_PTP_R	TTACCGTTCCTTCTCTTTTTCTT
*luxU*_PTP_F	CACCTTGAGAGAATGGATCAACCA
*luxU*_PTP_R	TTAATGAACTAAATCGGAGTAGCATTG
*vpsR*_PTP_RD_F	CACCTTGAGCACTCAATTCCGTATGG
*vpsR*_PTP_RD_R	TTACTTTTCCAACTTCAGCATGC
*vpsR*_PTP_R	TTAGAAGTTTTCATCGGTGATCA
*vpsS*_PTP_HK_F	CACCTTGCGTTTGGCGCA
*vpsS*_PTP_HK_R	TTACGGTTTAATATTTTTCAAGCGAG
*vpsS*_PTP_RD_F	CACCTTGCATCACATTCTTGTTGTG
*vpsS*_PTP_RD_R	TTATGGATTAAGGAAACGTTTCA
*vpsS*_PTP_F	CACCTTGAAAATGGATTCTGAATACAC
VC0303_PTP_RD_F	CACCTTGCTGCAACATCTGCGTGTGCT
VC0303_PTP_RD_R	TTAAGACAGACTATTCAGTAAGG
*chiS*_PTP_RD_F	CACCTTGCCACTTCTGTTGGTCGCCGAT
*chiS*_PTP_RD_R	TTAGAGGTGAGCGACAATGCGC
*vieS*_PTP_RD_F	CACCTTGGGTCACGTATTAGTGGCCGATGAT
*vieS*_PTP_RD_R	TTAGAGTACTTTGCGCAAATTGGCTAAG
VC2369_PTP_RD_F	CACCTTGCTGAATATTTTCATGGTCGAAGACATTGA
VC2369_PTP_RD_R	TTACGTCATCTTCGCAATCACTTCTCG
VC1831_PTP_RD_F	CACCTTGAAAGCCAAAACGTATCCCGG
VC1831_PTP_RD_R	TTACTCTACCGATGGTAAAGATG
*varS*_PTP_RD_F	CACCTTGCTGTGCGTCATGGCGGTCG
*varS*_PTP_RD_R	TTAGCTCCAATGTACCAACACTTGTTGCA
Overexpression primers	
VC0303_pBAD_F	GATCCTCGAGGATGCAAGGTTGGTTGGTAATTCCC
VC0303_pBAD_R	GATCGAATTCCAGACAGACTATTCAGTAAGGCTCTCA
VC1831_pBAD_F	GATCCTCGAGGTTGGTTATGGCTATTCGCTCCTCG
VC1831_pBAD_R	GATCGAATTCCCTCTACCGATGGTAAAGATGGTTCG
VCA0709_pBAD_F	GAGCTCGAGAGTGCAAACGCAGATTTGGCATGA
VCA0709_pBAD_R	TCGAATTCCTTCTTTACTCAGCAAATCCAATGC
VCA0719_pBAD_F	GATCCTCGAGCATGAGTGCCGAAGCGCAATA
VC A0719_pBAD_R	GATCGAATTCCGATCGCAGGGAACAGTAAGGTAAA
*chiS*_pBAD_F	GATCCTCGAGTTTGCGTCTATGCCCACGCTT
*chiS*_pBAD_R	GATCGAATTCCTTCACTGGTCAGGAGTTTTTGCAA
*cqsS*_pBAD_F	GATCCTCGAGAGTGATAGTGAGCATGGATGTAATAAAGAGA
*cqsS*_pBAD_R	GATCTCTAGAAACACCCAAGCTGCCACTTTATTTAG
*luxQ*_pBAD_F	GATCCTGCAGGCTTGAACATTCGACCTAGCCAAATC
*luxQ*_pBAD_R	GATCTCTAGAAAATTTAAGCCAGCGTTTTTTTGGCC
*varS*_pBAD_F	GATCCTCGAGAATGACTCAAAGATATGGCTTGCGC
*varS*_pBAD_R	GATCTCTAGAAAGTTCAGATAGTCGCGAGAGGCTTC
*vpsS*_pBAD_F	GATCCTCGAGCATGAAAATGGATTCTGAATACACA
*vpsS*_pBAD_R	GATCGAATTCCTGGATTAAGGAAACGTTTCACAAC

*vpsV*_pBAD_F	GATCCTCGAGAGTGTCATCTCACGTATACAAAACTCGTT
*vpsV*_pBAD_R	GATCTCTAGAAATGCCTCATAAAATACAACAGAAGAAAT

Deletion primers

*vpsS*_del_A	CATGCCATGGGTGGATTGGGGATCACTCAGG
*vpsS*_del_B	TGCTGCGGATTTCAGAATCCATTTTCATGCCTC
*vpsS*_del_C	GGATTCTGAAATCCGCAGCAAAGACATCATGG
*vpsS*_del_D	GCTCTAGAGAAGGACTTCCCCACGGCTT
*vpsV*_del_A	GCTCTAGACACGAGGAACCCGCGTTCAT
*vpsV*_del_B	TACCGTGCGCTAGCCGCATTGAACGAGTTTTG
*vpsV*_del_C	AATGCGGCTAGCGCACGGTAACTTAATGATTTC
*vpsV*_del_D	CGAGCTCCGCTGATTTGAGCTTGAATCCC
*vpsSV*_del_A	GATCTCTAGAGCCGAAAAACTCACAAAGC
*vpsSV*_del_B	TAATGGGGATGGTTGTTTTTAGCCGCATTG
*vpsSV*_del_C	AAAAACAACCATCCCCATTATCGCACTCAC
*vpsSV*_del_D	GATCGAGCTCGAAGCGTCAATCATGAGCAA
*luxU*_del_A	CATGCCATGGCCGCTGCATTTACCGCCATT
*luxU*_del_B	ATTGATGCGTGATCCATTCTCTCATCGCTTACC
*luxU*_del_C	AGAATGGATCACGCATCAATGCTACTCCGA
*luxU*_del_D	GCTCTAGACCATCCGATCTGGCAGCCCT
VC1080_del_A	GATCGAGCTCAGATGGACGATCAACGGATG
VC1080_del_B	GATTACTCACTGTCGAAATGTTAAATGCGACACTC
VC1080_del_C	CATTTCGACAGTGAGTAATCGATGACAGTAGAAGC
VC1080_del_D	GATCCCATGGATACTGCAATTTGGCGAAGC
VC2038_del_A	CATGCCATGGGAAACCATCGCCAAACTGTTC
VC2038_del_B	GCACAGACCTTGCCTTCGGATGAATTATCAA
VC2038_del_C	TCCGAAGGCAAGGTCTGTGCCATGCCTAAC
VC2038_del_D	GCTCTAGAGAATCAAAGCGGGTGATGAAG
VCA0939_del_A	CGAGCTCGGGCAGCACTGGTGTACGCCTTG
VCA0939_del_B	CGGAGCGTCGCATAGGCTCACCTCTCACTG
VCA0939_del_C	TGAGCCTATGCGACGCTCCGATCAAGCACTG
VCA0939_del_D	GATCTCTAGACTAGGCGAGCGATGGCTTCTGGCG
*luxQ*_del_A	ATCGCCATGGATATGAACGCGGTGCACC
*luxQ*_del_B	TAAGCCTTGGCTAGGTCGAATGTTCAA
*luxQ*_del_C	AGCCAAGGCTTAAATTGAGCCAGCGAG
*luxQ*_del_D	CGATTCTAGACCTGAGGATACTTATGCG
*cqsS*_del_A	ATCGCCATGGTGGATTTGATGGTCGAAG
*cqsS*_del_B	CACTTTTACATCCATGCTCACTATCAC
*cqsS*_del_C	GATGTAAAAGTGGCAGCTTGGGTGTAG
*cqsS*_del_D	CGATGAGCTCGCATTTAGATCGTATGGC

Site-directed mutation primers

*vpsR*_5'_F	ATCGCCATGGACGATGCTGAAGACCAAG
*vpsR*_5'_R	CTGCTACTTGAGTACAGC
*vpsR*_3'_F	CGTGATGCACTGCTGCTG
*vpsR*_3'_R	CGATTCTAGACCGCAGCCTAATAAGAGG
*vpsR*_5'	GATCGGATCCATGAGCACTCAATTCCGTATGG
*vpsR*_3'	CGATCTCGAGTTAGAAGTTTTCATCGGTGATC
*vpsR*_D59A_F	GGTATTGTGGCTTTAAGCCATGATGAATTTAGC
*vpsR*_D59A_R	ATGGCTTAAAGCCACAATACCAATACATGGCCC
*vpsR*_D59E_F	GGTATTGTGGAATTAAGCCATGATGAATTTAGC
*vpsR*_D59E_R	ATGGCTTAATTCCACAATACCAATACATGGCCC

Promoter cloning primers

vpsT promoter F	GCTCTAGAGCGCTATCTTTTGTTTACTTGACGC
vpsT promoter R	CATGCCATGGCTCCTAACACATCAAGGCTAACATG
vpsR promoter F	GCTCTAGATGAACGATGCTGAAGACCAAGAT
vpsR promoter R	CATGCCATGGGTACTGAATCCATACGGAAT

1
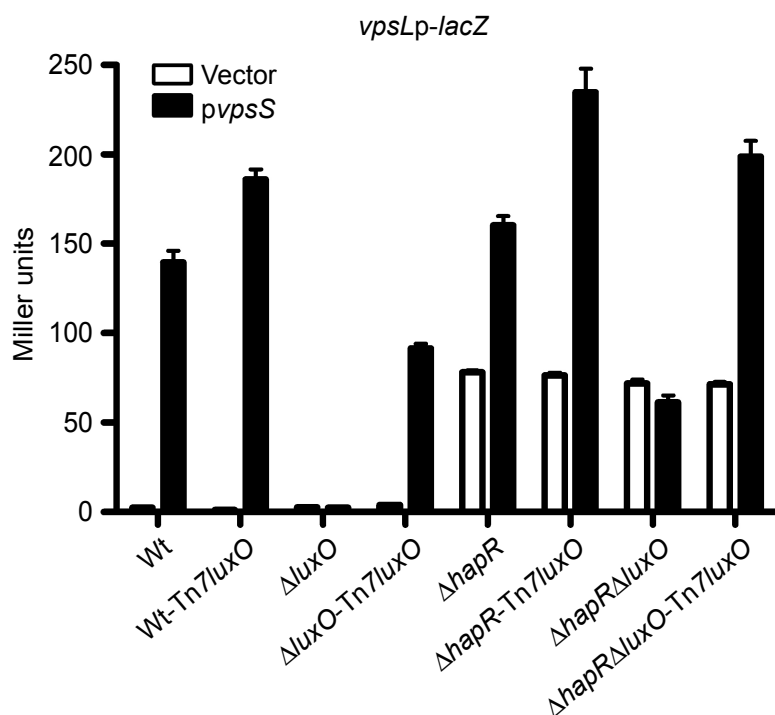
Supplementary Fig. S1. Complementation of *vpsL* expression in Δ*luxO* and Δ*luxO*Δ*hapR*. VpsS activation of *vpsL* expression was only observed in Δ*luxO* and Δ*hapR*Δ*luxO* strains carrying a chromosomally-inserted copy of *luxO* (Δ*luxO*-Tn7*luxO* and Δ*hapR*Δ*luxO*-Tn7*luxO*). Wild-type (Wt) and Δ*hapR* strains, with or without Tn7*luxO* (Wt,Wt-Tn7*luxO*, Δ*hapR* and Δ*hapR*-Tn7*luxO*), exhibited increased *vpsL* expression when *vpsS* is overexpressed. Furthermore, while wild-type strains (with or without Tn7*luxO*) carrying the vector exhibited low basal level of *vpsL* expression, Δ*hapR* strains (with or without Tn7*luxO*) carrying the vector exhibited a higher level of *vpsL* expression, consistent with the negative role of HapR on *vpsL* expression. Strains were grown in the presence of amplicillin and 0.2% arabinose. Error bars indicate standard deviations of 8 technical replicates. One representative experiment of 3 biological replicates is shown.

Supplementary Fig. S2. Shikuma et. al.
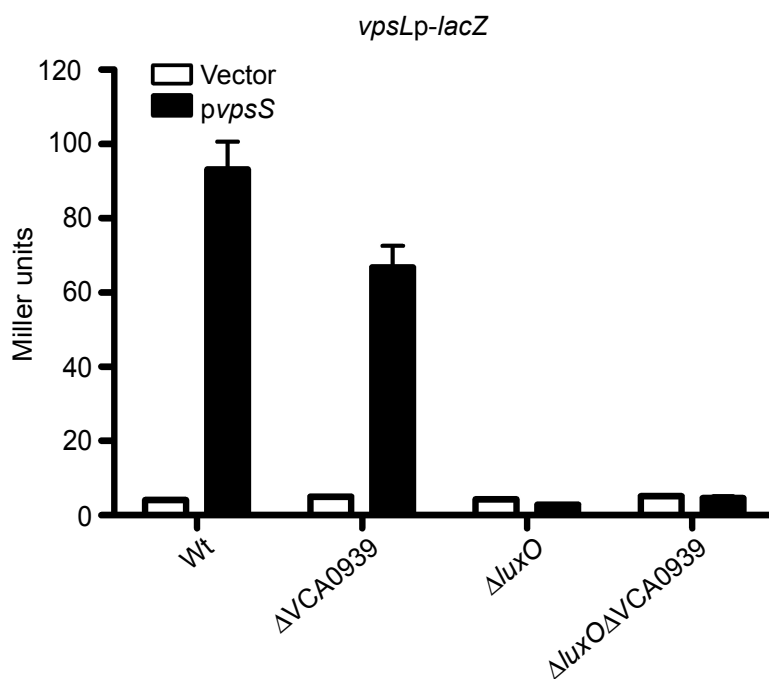
Supplementary Fig. S2. VCA0939 does not regulate *vpsL* expression. VCA0939, which encodes a protein predicted to contain a GGDEF domain and whose expression is regulated by LuxO but not HapR, is not involved in regulating *vpsL* expression. A ΔVCA0939 strain, carrying the overexpression plasmid p*vpsS,* exhibited VpsS activation of *vpsL* expression, similar to that of wild type (Wt). As expected, deletion of *luxO* in both wild-type and ΔVCA0939 strains abolished VpsS activation of *vpsL* expression to the basal level observed in all strains carrying the control vector. Strains were grown in the presence of ampicillin and 0.2% arabinose. Error bars indicate standard deviations of 8 technical replicates. One representative experiment of 4 biological replicates is shown.

A

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                10        20        30        40        50        60        70        80        90       100
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
VC1445  ---MKMDSEYTEKVNELTLELQRSIERGKQLAEENRVILAAISAFSVATNKNHIFEELKTLLNRYIQFDDFIVISKDKNGHQFRTAIASNASFNDVVWPN  97
VP1547  MKDKYLDTYQQEALQEALVELKQTKQREKLLADENKAILSAISAMSEAKNRNEIFSGLNSVLKKYISFEDFIVITRDDNRYPFKTLISTNSVFDKVEWLH 100
VF1296  ----MKNHSEKEQFQEILLELKRSKEREAQLHKENQVILRGVSAIDAQTKEDIFNGLLNVIKEFVEFDNAIVVS-STDKDSFSVLASTNPYFDHLTWRY   95
VV12622 MKVNNMTTFEKEALHDALVELKKSRELEKRLAEENKAILSAISAMSEAKNRHEIFLGLNNVLKKYINFDDFVVLTRHCDKESFATLLTTNQVFNNTTWCD 100
Consensus        *  .::   :**::: :    *  .**:.** .:**:* * .:..** *  * .::*:**::.:  *:* :*  .  .  ::* *:. *


               110       120       130       140       150       160       170       180       190       200
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
VC1445  GVKFQRVLNGECILLFEPCKLDEFACLMGSIDKPVYSALITGIQAQISDSVLILLGQNKGQFSLEAKETLSRFRPLLERAISDIEHKEELQKLVEIRTRE 197
VP1547  GNTMDRALNGECILLFEPAKLLEFENLNSFVKTHVNSVILTGIRSEVTQSIILLIGAQKGHFSIENKETLRRFRPLIERAVIDIETKEKLQRIVEVRTTQ 200
VF1296  NDLFHRACHDETIILFQPHKTNEFLFKNTELQSLFSSVAICGLKSHSGYAVIILACSKFGAYSTLEKEKIERFMPLVERAVIDIDYKERLNAMVSLKTKE 195
VV12622 GKKFSRVLNGECIILFEPTELSEFKQLNSFIKSHINSVLLTGIDAQVTQSVILLIGNTKGQFSIEAKETLKRFLPLIERAVIDIENKEKLQRIVELRTRE 200
Consensus .  : *. :.* *:**:* :  **    :...  *. : *: :.  ::::*    :*   **:.** **:***: **.*:**:*:**:* :  98


               210       220       230       240       250       260       270       280       290       300
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
VC1445  LRLAQK------------------------------------------------------------------------------------------------ 203
VP1547  LARARE------------------------------------------------------------------------------------------------ 206
VF1296  LFLSKERFQDFAETVGDWFWETDPQFNFTYLSDTKINNTSIISQNLLSLISNDFINSTIIESEKRLVPFHEVEWNPDIFNNKVWLSLSGKPFFDEFGTFV 295
VV12622 LALARE------------------------------------------------------------------------------------------------ 206
Consensus *  :::                                                                                              102


               310       320       330       340       350       360       370       380       390       400
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
VC1445  -----------------------LAEQANQSKSNFLAMMSHELRTPLNAVLGLIDILRGESTS-YQVELLEQMENSAELLLIINDILDLSRIESGHFS 278
VP1547  -----------------------EAELANQSKSEFLAMMSHEIRTPLNSVLGMLDILRQSTLSDEQFLIDSGSRLKNIKPSTSSSQQIVNVKNNHILV 282
VF1296  GYRGTAKDISARKKRIRDIQTAKTEAEKANKAKSQFLAMMSHEIRTPLNSILGLVDVFHSSLLSEEQLDWLNQMESSQLLLTIISDVLDISRIEAGTFV 395
VV12622 -----------------------ESERANRAKSEFLAMMSHELRTPLNSVLGMLDVLKQSDMSAGHINLLSHMESSAGLLLAIISDILDLSKIESGEFS 282
Consensus                        :* **::**:*******:*****::**:::**::  .  *  .:: *.:** *: *** **.*:**:*:**:* *


               410       420       430       440       450       460       470       480       490       500
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
VC1445  LHCHWLDLEKKLDQSLVYHRQIAQDKGVHFYVQSNKNPTLEYYTDSARLTQILFNIVGNAIKFTKQGQVDVVLDYT-KDGVVFRVKDTGIGIEKNRVEQL 377
VP1547  LNEQWIHLNDTVTFVISQQKQVAITKNLSFNFDCQISSDKQYWIDSTRLSQILFNLIGNAIKFTDSGSVSVSVAEE-NDEVVVSISDTGIGISRAKQAHL 381
VF1296  LNEQPIDLLRSLNTSINFLDTRAKDKGITLTVTASQNVPKYVFADATRLSQVLFNLVGNAIKFTNHGHVAVKIEKLPNNSISISVSDTGIGIASTTLEQL 495
VV12622 LSPQWININDTVTFVVSQQQLVAATKGLNIELSIELDDKRHYWLDPSRLSQVLFNLIGNAVKFTQQGQIDIRVNLE-NDELSISVIDTGIGIEKDKISSL 381
Consensus *  : :.. .:  :    *  *.: :  .. .        : *.:**::**:::***:***.* . :    :: .. .: ****** *  * 206


               510       520       530       540       550       560       570       580       590       600
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
VC1445  FTPFIQADNSITRNYGGTGLGLAITKHLVELMGGTIQVESEFGIGTTFTLYIPLQSRLKNIKPSTSSSQQIVNVKNNHILVVEDTKTNQMVIQLLLNKLG 477
VP1547  FTAFHQGDRSITRRFGGTGLGLAITKHLVEMMRGEISVKSRENEGSDFTIRIPVLTRYNQSRPVKIEHNRPS--KALNLLIVEDTQSNQLVIKLILNKLG 479
VF1296  FKPFVQADSSITRKYGGTGLGLAIIKRLVELMQGTITVESRLNIGSTFKITLPLKEISDLPKQLNNEAKELVNQRSLNILLAEDNKANQAVLQILLEKQG 595
VV12622 FTAFKQADSSITRKFGGTGLGLAITKHLVELMSGTVRVSSQFGKGSTFVVKIPVKSRKLIRLSQDGTTNKRD--RALNVLVVEDTHSNQMVIQLLLNKLG 479
Consensus *..* *.* ****.:********** *:***:* * : *.*. .*: *  :*:             :.   :  ::*:.**.::** *::::*:* *  263


               610       620       630       640       650       660       670       680       690
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....
VC1445  YDVTIAENGLQAVELLEKN---HVFDLVLMDISMPIMDGIAATKILRDKHIEIPIIALTAHTAGSDKQNCIDAGMNDIVLKPIRSKDIMEVVKRFLNP- 572
VP1547  HNVHIASHGAEALTFLEEND--TRIDMILMDVSMPVMDGITATRLIRKKGITIPIVALTAHALESDKDKCLDAGMDSFVSKPVRRQDIYEAIQSLIETA 576
VF1296  HHVSIVSNGEEAISIFKHQTNGCFFDVVLMDVSMPVKDGISATTELRKLGIELPIIAITAHAMETEKEMCLEAGMNDFISKPIRAKDLSDILIKYSC-- 692
VV12622 HNVFIANNGSEAIEFIESNT--ESLDVVFMDVSMPVMDGLTATKILRAKGFEVPIIALTAHALASDKQNCLDVGMDSFVAKPVRKQELANAIEALIV-- 574
Consensus :.* *..:* :*: ::: :    :*::**:***: **::** :*    : :**:*:***: ::*: *::.**:.:: **:* ::: : :  327
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B

Vibrio cholerae N16961 — VC_1445

Vibrio parahaemolyticus RIMD 2210633 — VP1547

Vibrio vulnificus CMCP6 — VV1_2622

Vibrio fischeri ES114 — VF1296

Supplementary Fig. S3. Aligment of VC1445, VP1547, VF1296, VV12622 and their genomic contexts. Protein (A) and genomic context (B) alignment of VC1445, VP1547, VV12622 and VF1296 from *Vibrio cholerae* N16961, *Vibrio parahaemolyticus* RIMD 2210633, *Vibrio vulnificus* CMCP6 and *Vibrio fischeri* ES114, respectively.