Supplemental Material

Identification of a novel matrix protein that promotes biofilm maturation in *Vibrio fischeri*

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Fig. S1A

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BmpA            MYQFFKITMLILLLVSSKV-----FAVAFDSCPSKAYLFQGKPVS--VYGINLVTGTNSL 53
BmpB            MKSKHSISYLATLSIALGVSTQLQAAVPFESCPSQAFMLQNPSGTPIAYGISLDVGSYST 60
BmpC            MKYKQLIPIIALATASIPIH--------------------------------ATIVDLD 27
                *     *. :     :  :                                   .

BmpA            LQDDTGLSSNINGVGFNETDRYIYGFDTTNYNVVRLGQNFQATTLNVNGLPS-DKTFYVG 112
BmpB            LSWEVG-SGKINAVGYSVHDDFIYGWDYGAQSLTKIGSDFISNPLSVSNLNIGPTSFYVG 119
BmpC            FSNHIEDTNLNNSFGPSYDGPVMHFLNVGVHNGKTIDAKISSRIIGDATFLYHTPNYKEG 87
                :. .   :.  *..* .  .  ::  :    .   :. .: :  :.   :     .:  *

BmpA            DVYDHH--YYVYRSGTG-----LYKIDLSPLDSNVNSTLTAQLITSTASVSLTDFAFHPS 165
BmpB            DVSTNESAWYGYRINNG-----LFKIDIDTLTMSQVATS-----TTIGKLRILDMAFHPD 169
BmpC            STQPSGDIGFLYQTNSPGPAGLIYTFEFFDGTDGLSGTFSIPYTIPEFEMIGYDIDGEPV 147
                ..       : *: ..      ::.:::     .  .*       .  .:   *:  .*

BmpA            NSRLYGVDNGSGGLYEFDINTGAATYIG------DTGELGTFGAMYFDVDGYLYLSRNQD 219
BmpB            DGIIYSVDN-YGYLYQIDPTTGASTQLNQVISSSGVGHSLSFGAQYFDVDGTLYLSNNGN 228
BmpC            QSEQVRVYKNEGFFSYQTGSAGASLTAEESPDGLSVLFTGPGTNYNETDTSGAVKFTYKN 207
                :.    * :  * :     .:**:          ..    .         .        :

BmpA            GQIYRVNLSTQAIIDSGVVPAIKFADGPYSNQNDGARCANAPLIDTDEPATIDFGDAPDT 279
BmpB            GYIYAVDING---MNS---SSEFFAYGPSSNSNDGARCAFAAVGQNDN---SDYGDAPDS 279
BmpC            TSIVTLQFETVTASNSPLPNPIFSAFDGNWDLDD----FTPPIGSSDE---SDFGDAPDS 260
                  *  :::.     :*    .   * .   : :*      ..: ..*:    *:*****:

BmpA            NYGTTLAANGARHEMDGVTWLGASVDGDYY--AAQSPDSDDS-ITSDDEDGVGFVTAIEP 336
BmpB            -YGTLYDSMGARHGVSDLR-LGDVVDGESD--AYVYPLSDDASDSSNDDDGISFPVPIEI 335
BmpC            -YNTLKSNDGAEHAMTSTLYLGSSVDADTDGQPGIASNGDDLDVDGNDDDGITILTSLEK 319
                 *.*     **.* : .   **  **.:    .   . .**    .:*:**: : ..:*

BmpA            GLDSVITVNAS---TTGYLSAWFDWNDDGDFSDDGEQVITDKTLVAGSNNLVISVPFGAT 393
BmpB            QETSFIYANVNGAEGEGVLSAWIDWDQDGQFNDD-EIILNSEWVDDGQNQLYFNVPSWAL 394
BmpC            GLDALINVNAS---GSGYLQAWADWDMNGSFDEG-EQILTNHPIVSGVQIVPIRVSDSAT 375
                   :.* .*..     * *.** **: :*.*.:. * ::... :  * : : : *.  *

BmpA            VGETWSRFRFSQQTGLAYYGGSTSGEVEDHVVTIVDANTSQRHFPSADGYVTIAYEDNWP 453
BmpB            AGTTWARFRLSRTYDLGPNGGVSMGEVEDYQVTVTDQGVSVELYPSGAAYTTFAYEDQYP 454
BmpC            IGSVQTRFRLSSNPNIPSSGYVGDGEVEDYVFDVTDPGTTIQHS----GYYTAAFEDNWP 431
                 * . :***:*   .:   *    *****: . :.* ..: .      .* * *:**::*

BmpA            ETADYDMNDMVLRYRITETLKDGDVAKVSISGQLVAVGASYHSGFAVRLAGIDATNIDSD 513
BmpB            KVGDYDMNDVLMNVKYTEYSHSNKVIQLRIDGQVAALGGTYRSGFAIRLPGISPSKIKSA 514
BmpC            EIGDFDLNDVVAYYRTTIVSKDGEVLRFDITGTIMAYGASYGNGLGWKLNGFSESDINLS 491
                : .*:*:**::   : *   :...* :. * * : * *.:* .*:. :* *:. :.*.

BmpA            KTRLYYNSALQDGNAQESGMTEASFIVINDAIEVSSYN----------CYFFRTLDDCRE 563
BmpB            SVKLIIDGELQNTEVVESGTTDAVLIVHEDLWSFTESGE------EEGCYMFRTQLGCGT 568
BmpC            LARLEKNGVTRANISPFTGEDKSIASPGGDLVVVASLNLREDIIINEECKFHRTNPSCSA 551
                 .:*  :.  :      :*  .:      *   .:. .          * :.**  .*

BmpA            DVG---VEFELNFSLIT---PVTTGSMPAMPYDPFLYAT-PGFYHGPS----FAQAPGRS 612
BmpB            QHR---PSWTLIIPFEQ---PISQSQMPDFPYDPFIFAT-PGYYHGNDGLLVSGGHPGRG 621
BmpC            SLESEQMTFSISLPFNDGSEPSVSSLLPLNGADPFIFAAGYGLYHGDS----FSTAPGTD 607
                .       : : :.:     *   . :*    ***::*:  * *** .     .  ** .
```

```
BmpA        YEVHLADQAPTEK--FDTNFYQLAEDTSDPNTSRYFKTSNNMPWALLIYDEWKWPRERVD 670
BmpB        LEVHLKNKTPTSK--FNYGYIGRSDDATNTAAGTYFHTSNGLPWAIELPLDWKHPIESIS 679
BmpC        LEIHTADFPPTSRGTLVSSFYGIAQDDSDPATSKYYRTTGNLPWGILISSPWNHPSEYID 667
             *:*  : .**.:  :  .:   ::* ::. :. *::*:..:**.: :   *: * * :.

BmpA        LAVAYPQFADYTTSG-GGTHTDWYDITNAIANKYY-- 704
BmpB        ILDAYPQFANFAQDETGQTATDWYVTP--ITGKAYTD 714
BmpC        IGDAYPDFAEWATSG-GTEKTTWYLNPTASNTWSTAD 703
            :  ***:**::: .  *   * **  .
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Fig. S1B

```
BalA        MTSLMQLRTNTIKKGVIMKKVIMISMTSLASLFLIVSCSDSGGFSSGGTAPTVAEEPE-- 58
BalB        --------MSTIVN--KMGRICLI----LGLMILIAACNSDSSGSGDSTYSGGSSSTPSV 46
BalC        -----------------MKRINIL--LSIAIITFLSACSDSSSGTSGGAAPAAADVPKKE 41
                             * :: ::    :. : :: :*..... :...: .  :. .

BalA        -SVKTQDLVAPEGFSFNPIEEQRLIVDLSGSLPARAHLSVYSNFSEKDEGEYRVDYGSKI 117
BalB        QSVMDLEISAEN--NLDSVYSVDVDIDISSISTKRAFVSICNNSDAKG-DLSKLDFDMCF 103
BalC        SGIKTSELSVPEGFTFSTEREVTFDLSVASSQSDRGFMSIYTEFNEG-----AVDYTSQI 96
             .:   :: . :  .:..  .  . :.::.  . *..:*: .: .        :*:   :

BalA        IDVPLANG-AVDIEFAIANSLEESVVEIWLYD--GSDPQQKKFVVDGTQWEWY--- 167
BalB        VKGNLEQG-IGEFDLRVANHNDELITIIWVMEK-DREPLTYTFSHNKQKESYWLIN 157
BalC        ILTPMNDSTEFKSSMMLPNHVDKVWVEIWYPSALGSEIKQMIDIVDNTVVATL--- 149
            :   : :.   . .: :.*  ::  . **  .  . :        :
```

Figure S1. Alignment of the Bmp and Bal proteins, respectively. Protein alignment (1-3) of (A) BmpA, BmpB, and BmpC or (B) BalA, BalB, and BalC. Residues in red are conserved between all three proteins, while those in blue are conserved between two of the three proteins. In (A) the bold black residues indicate the predicted Sec-dependent cleavage site.

Fig. S2

A **BmpA** MYQFFKITMLILLLVSSKVFA VAFDSCPSK

BmpB MKSKHSISYLATLSIALGVSTQLQA AVPFE

BmpC MKYKQLIPIIALATASIPIHA TIVDLDFSN

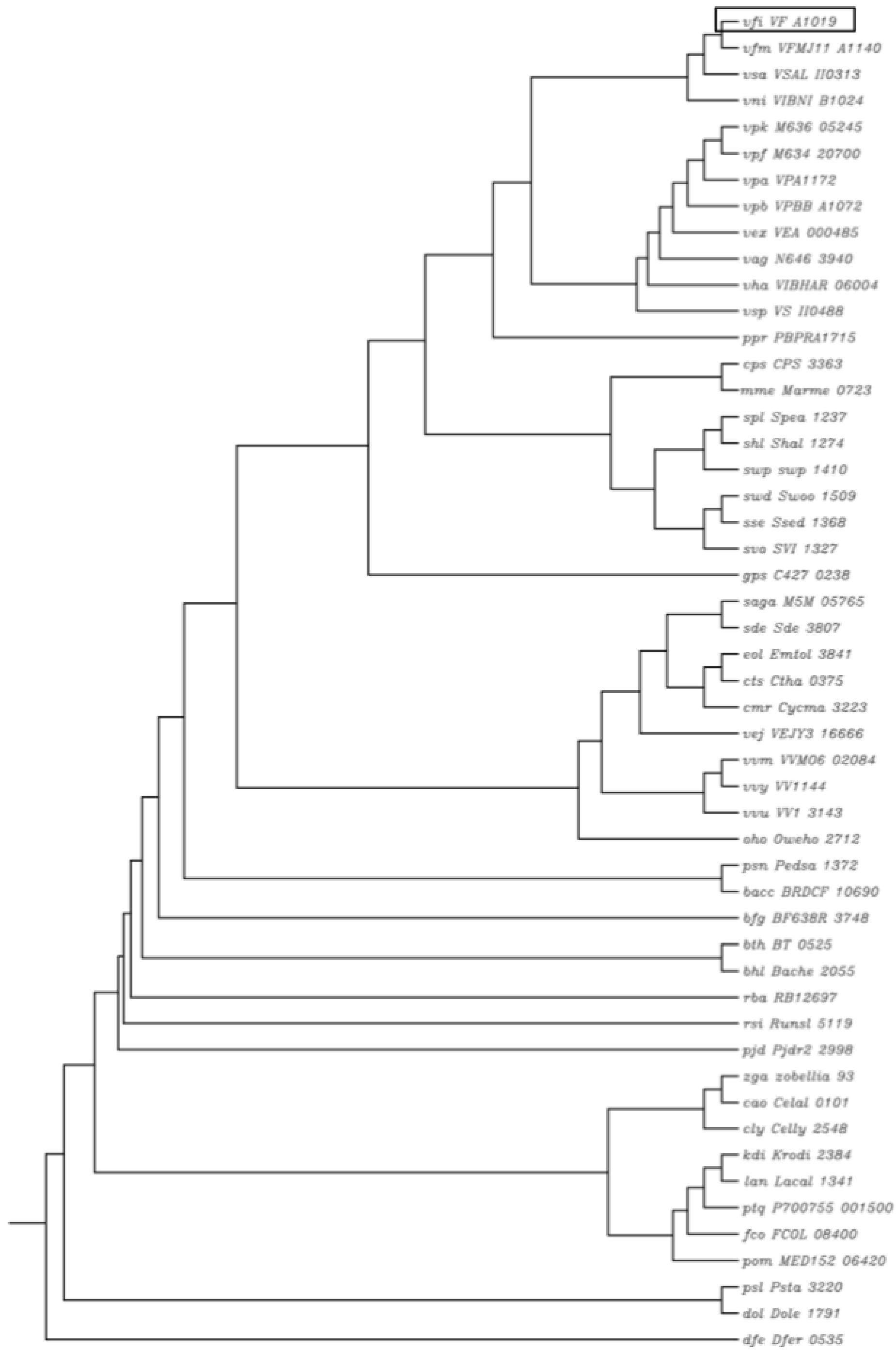
B **BalA** MTSLMQLRTNTIKKGVIMK**KVIMISMTSLASLFL IVSC SDSGG**

BalB MSTIVNKMG**RICLILGLMIL IAAC NSDSS**

BalC MK**RINILLSIAIITF LSAC SDSSS**

Figure S2. Putative signal sequences of the Bmp and Bal proteins. (A) Signal sequence prediction program SignalP (4) predicts the presence of a signal sequence at the N-termini of BmpA, BmpB, and BmpC. The inverted triangle indicates the predicted site of cleavage. (B) The Database of Bacterial Lipoproteins (DOLOP) (5) predicts that the Bal proteins are lipoproteins based on the presence of the following sequences: a charged residue (blue), followed by a hydrophobic stretch of amino acids (green), and a lipobox sequence ending in an cysteine residue (red); bold black sequences after the invariant cysteine do not appear to contain an inner membrane retention signal (6).

Fig. S3A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vex VEA 000485
vag N646 3940
vha VIBHAR 06004
vsp VS II0488
ppr PBPRA1715
cps CPS 3363
mme Marme 0723
spl Spea 1237
shl Shal 1274
swp swp 1410
swd Swoo 1509
sse Ssed 1368
svo SVI 1327
gps C427 0238
saga M5M 05765
sde Sde 3807
eol Emtol 3841
cts Ctha 0375
cmr Cycma 3223
vej VEJY3 16666
vvm VVM06 02084
vvy VV1144
vvu VV1 3143
oho Oweho 2712
psn Pedsa 1372
bacc BRDCF 10690
bfg BF638R 3748
bth BT 0525
bhl Bache 2055
rba RB12697
rsi Runsl 5119
pjd Pjdr2 2998
zga zobellia 93
cao Celal 0101
cly Celly 2548
kdi Krodi 2384
lan Lacal 1341
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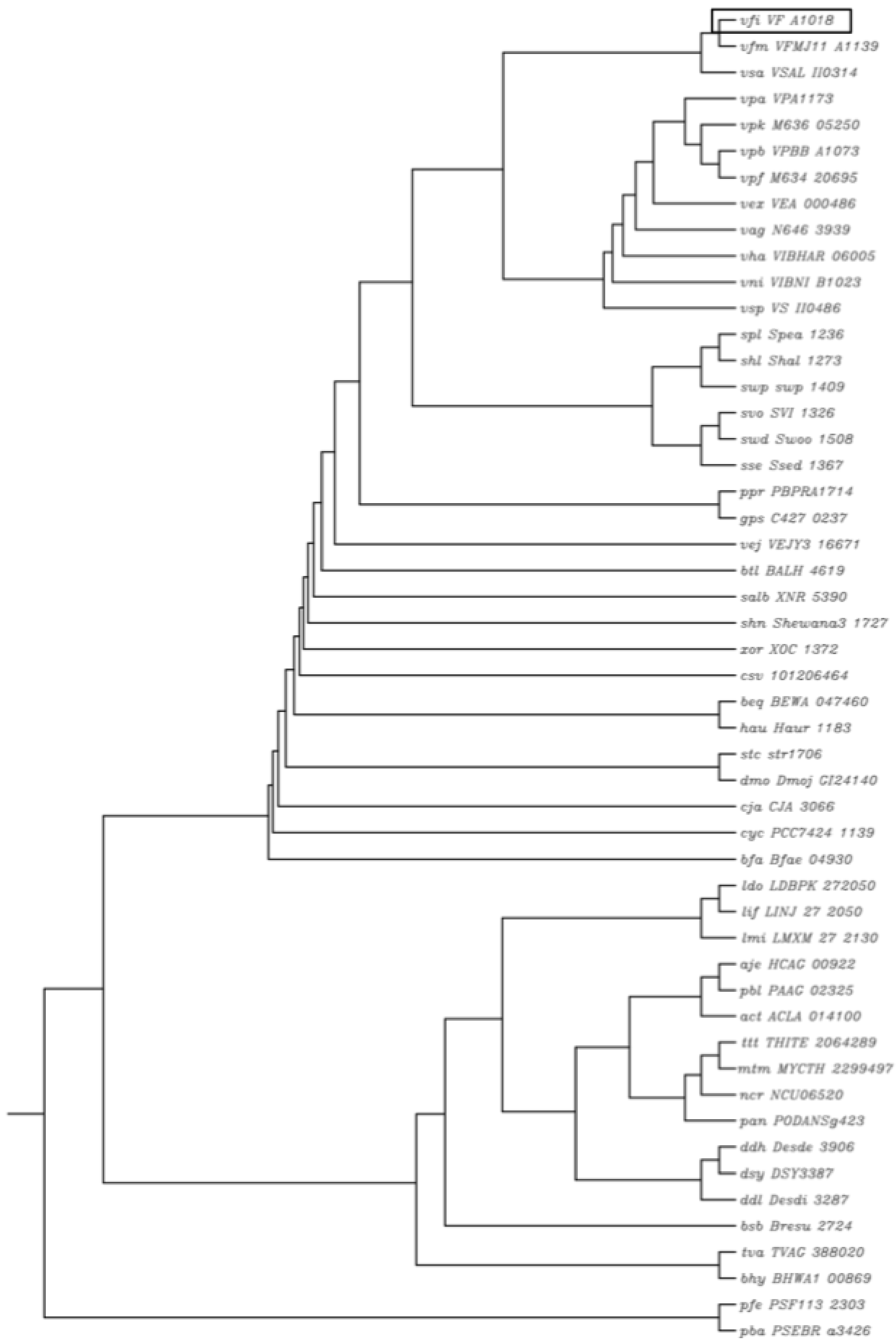
fco FCOL 08400
pom MED152 06420
psl Psta 3220
dol Dole 1791
dfe Dfer 0535

Fig. S3B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vpb VPBB A1073
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vex VEA 000486
vag N646 3939
vha VIBHAR 06005
vni VIBNI B1023
vsp VS II0486
spl Spea 1236
shl Shal 1273
swp swp 1409
svo SVI 1326
swd Swoo 1508
sse Ssed 1367
ppr PBPRA1714
gps C427 0237
vej VEJY3 16671
btl BALH 4619
salb XNR 5390
shn Shewana3 1727
xor XOC 1372
csv 101206464
beq BEWA 047460
hau Haur 1183
stc str1706
dmo Dmoj GI24140
cja CJA 3066
cyc PCC7424 1139
bfa Bfae 04930
ldo LDBPK 272050
lif LINJ 27 2050
lmi LMXM 27 2130
aje HCAC 00922
pbl PAAC 02325
act ACLA 014100
ttt THITE 2064289
mtm MYCTH 2299497
ncr NCU06520
pan PODANSg423
ddh Desde 3906
dsy DSY3387
ddl Desdi 3287
bsb Bresu 2724
tva TVAC 388020
bhy BHWA1 00869
pfe PSF113 2303
pba PSEBR a3426

Figure S3. Conservation of Bmp and Bal in other bacteria. Conservation of the (A) Bmp protein and (B) the Bal protein in other bacteria. Sequences of BmpA and BalA, respectively, were submitted to KEGG (7, 8) for comparison with other proteins. The indicated trees were generated with a subset of the conserved proteins with the *V. fischeri* protein boxed for reference in each tree.

Fig. S4

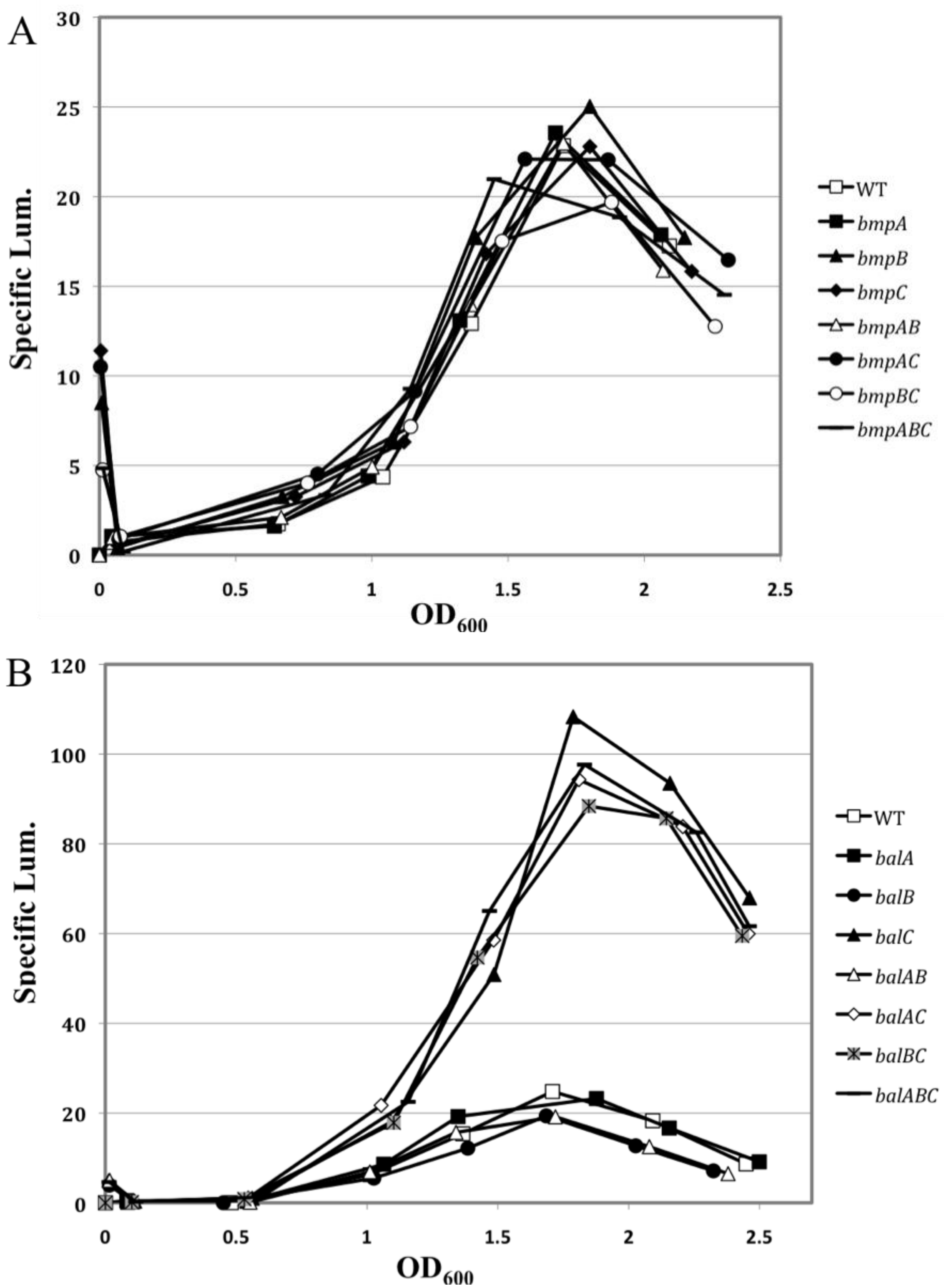
A
Specific Lum.
OD600
WT
bmpA
bmpB
bmpC
bmpAB
bmpAC
bmpBC
bmpABC
B
Specific Lum.
OD600
WT
balA
balB
balC
balAB
balAC
balBC
balABC

Figure S4. Luminescence of the *bmp* and *bal* mutants. The impact of *bmp* and *bal* mutations on bioluminescence was assessed by growing single, double, and triple *bmp* mutants and the wild-type control in SWTO, monitoring luminescence over time, and calculating specific luminescence as described in Materials and Methods. (A) The strains assessed are as follows: wild-type (ES114), Δ*bmpA* (KV6886), Δ*bmpB* (KV6638), Δ*bmpC* (KV6787), Δ*bmpAB* (KV7078), Δ*bmpAC* (KV7079), Δ*bmpBC* (KV6712), and Δ*bmpABC* (KV6897). (B) The strains assessed are as follows: wild-type (ES114), Δ*balA* (KV6890), Δ*balB* (KV6924), Δ*balC* (KV6923), Δ*balAB* (KV7080), Δ*balAC* (KV7081), Δ*balBC* (KV7369), and Δb*alABC* (KV7128). These data are representative of at least two independent experiments.

Fig. S5

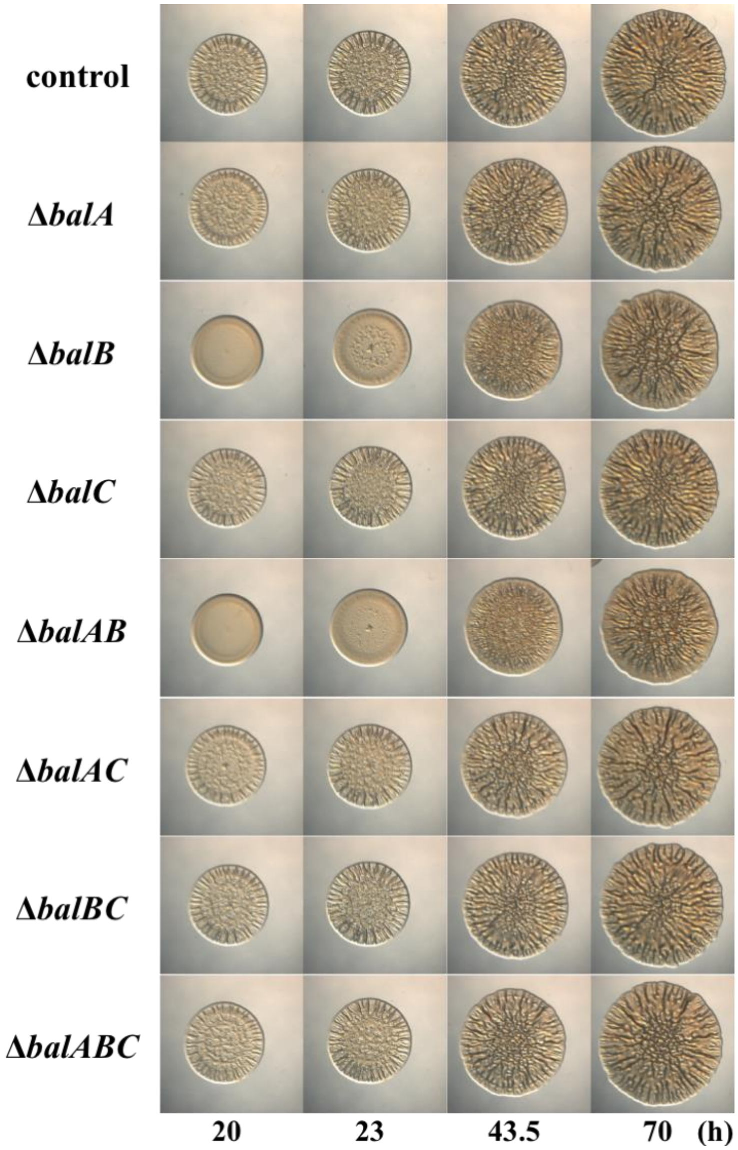

Figure S5. Impact of *bal* mutations on biofilm formation. To assess the impact of *bal* on biofilm formation, we spotted cultures of various *bal* mutants onto LBS medium containing Tc and incubated them at room temperature. All strains overexpressed *rscS*. Images were collected up to 70 h for the following strains: wild-type control (ES114), Δ*balA* (KV6890), Δ*balB* (KV6924), Δ*balC* (KV6923), Δ*balAB* (KV7080), Δ*balAC* (KV7081), Δ*balBC* (KV7369), and Δb*alABC* (KV7128). The images are representative of at least two independent experiments.

Fig. S6

Figure S6. The biofilm defect of the *bmp* mutant can be complemented by *bmpA*. The ability of the *bmpA-balA* operon or *bmpA-FLAG* to complement the triple *bmp* mutant was assessed via wrinkled colony formation. In the experiment shown, cultures were spotted onto LBS medium containing Tc and incubated at room temperature for 22 h. The strains assessed were pKG11-containing derivatives of: wild-type (control; ES114), Δ*bmpABC* (KV6897), Δ*bmpBC* (KV6712), Δ*bmpABC att*Tn*7::bmpA-balA* (KV7062), and Δ*bmpABC att*Tn*7*::*bmpA-FLAG* (KV7274). The images are representative of at least two independent experiments.

Fig. S7

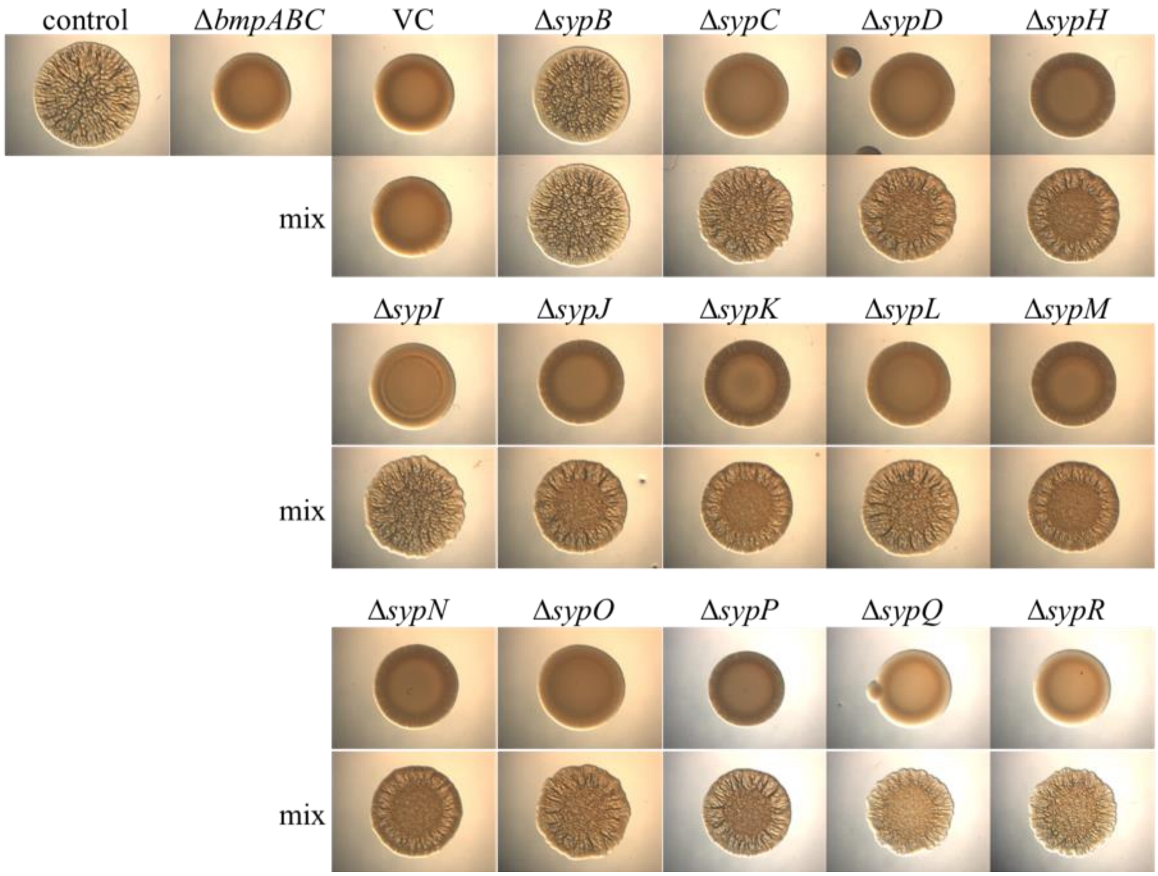

Figure S7. A mixture of biofilm-defective *bmp* and *syp* strains permits wrinkled colony formation. We assessed the ability of each of the biofilm-defective *syp* mutants to complement the *bmp* mutant for wrinkled colony formation by spotting, onto LBS medium containing Tc, a mixture of the pKG11-containing Δ*bmpABC* mutant with each of the following pKG11-containing *syp* mutant strains: Δ*sypB* (KV5145), Δ*sypC* (KV5192), Δ*sypD* (KV5067), Δ*sypH* (KV5193), Δ*sypI* (KV5068), Δ*sypJ* (KV5664), Δ*sypK* (KV5097), Δ*sypL* (KV5069), Δ*sypM* (KV5194), Δ*sypN* (KV5098), Δ*sypO* (KV5146), Δ*sypP* (KV5044), Δ*sypQ* (KV5099), and Δ*sypR* (KV5195). As controls, we spotted each strain separately as well as the biofilm-proficient pKG11-containing wild-type strain ES114. In the experiment shown, cultures were spotted onto plates and incubated at room temperature for 48 h.

Fig. S8

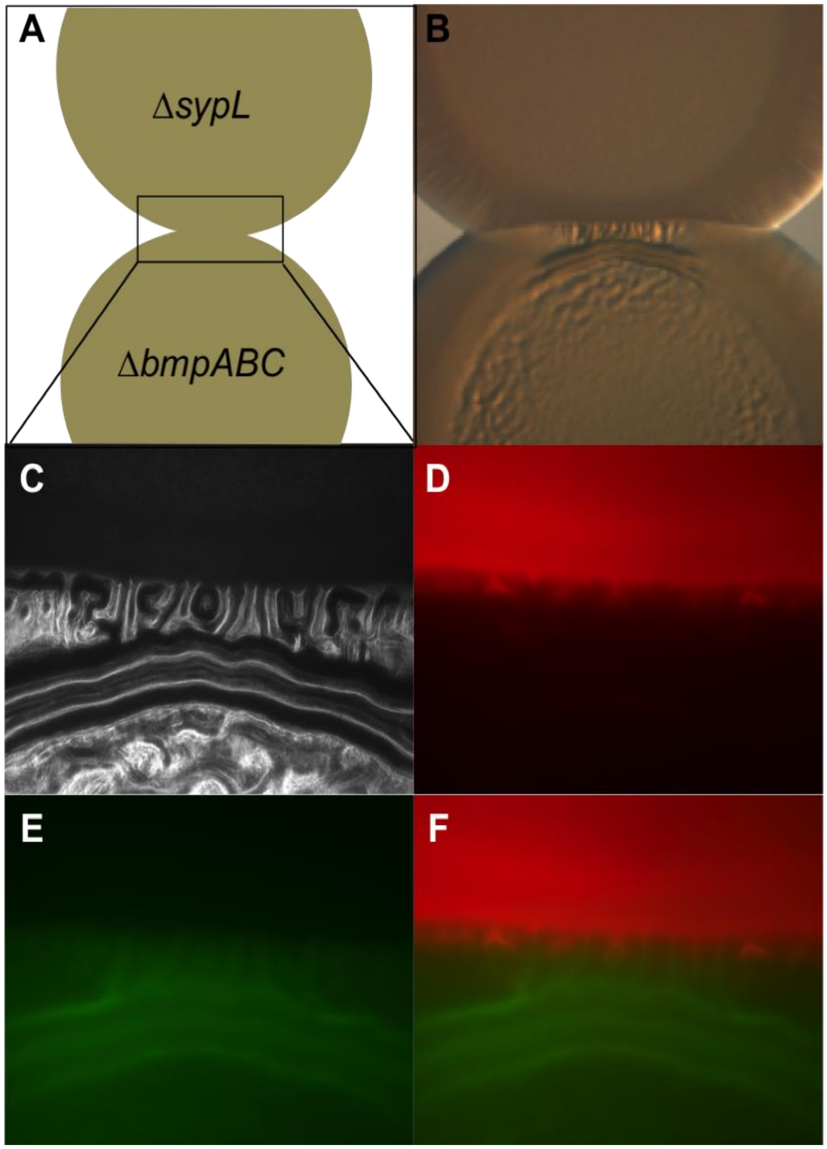

Figure S8. Evaluation of the interface of touching spots using epifluorescence. To further examine the interface of touching spots, we utilized epiflurescence microscopy and the pARM7-containing Δ*sypL* (KV5069) and Δ*bmpABC* (KV6897) strains also harboring either constitutively expressed RFP (pVSV208) or GFP (pESY37) plasmid, respectively. Cultures were spotted onto plates and incubated at room temperature for 43 h. (A) A diagrammatic representation of the experiment and (B) the touching spots used to collect the epifluorescence data. (C) A DIC image of the interface, (D) the RFP channel only, (E) the GFP channel only, and (F) a merge of the RFP and GFP channels. Some mixing of the two strains is observed at the interface (F), but there does not appear to be a massive invasion. Images are representative of 2 independent experiments. Magnification for images (C-F) is at 40x.

Table S1. Strains used in the supplemental material.

Strains	Genotype	Reference
KV3299	Δ*sypE*	(9)
KV4715	Δ*sypA*	(10)
KV5044	Δ*sypP*	(11)
KV5067	Δ*sypD*	(11)
KV5068	Δ*sypI*	(11)
KV5069	Δ*sypL*	(11)
KV5097	Δ*sypK*	(12)
KV5098	Δ*sypN*	(11)
KV5099	Δ*sypQ*	(11)
KV5145	Δ*sypB*	(11)
KV5146	Δ*sypO*	(11)
KV5192	Δ*sypC*	(11)
KV5193	Δ*sypH*	(11)
KV5194	Δ*sypM*	(11)
KV5195	Δ*sypR*	(11)
KV5664	Δ*sypJ*	(11)
KV6890	Δ*balA*	This study
KV6923	Δ*balC*	This study
KV6924	Δ*balB*	This study
KV7080	Δ*balA* Δ*balB*	This study
KV7081	Δ*balA* Δ*balC*	This study
KV7128	Δ*balA* Δ*balB* Δ*balC*	This study
KV7369	Δ*balB* Δ*balC*	This study

Table S2. Plasmids used in this study.

Plasmid	Description	Relevant Primers[1]	Reference
pARM7	EcoRI partial digest of pKG11 (*rscS*); tetR	N/A	(13)
pEAH73	pKV69 carrying wild-type *sypG*; Cm^R Tc^R	N/A	(9)
pEAH121	pEVS107 + P*sypI* - *lacZ* EmR (FL + SE-I)	N/A	(14)
pESY37	pVSV105 (KpnI) + 1.3 bp BamHI/XmnI fragment from pKV111 containing *gfp*; Cm^R	N/A	(15)
pEVS104	Conjugal helper plasmid (*tra trb*); Kn^R	N/A	(16)
pEVS107	Mini-Tn7 delivery plasmid; *oriR6K*, *mob*; Kn^R, Em^R	N/A	(17)
pKG11	pKV69 carrying *rscS1* allele; Cm^R Tc^R	N/A	(15)
pKV69	Cm^R, Tc^R, *mob*, *oriT*	N/A	(18)
pKV363	Cm^R, *oriT*, *oriR6K*, *ccdB*	N/A	(11)
pKV485	pKV363 containing 1.2 kb sequencing flanking *bmpB*	1536, 1537, 1538, 1539	This study
pKV486	pKV363 containing 1.2 kb sequencing flanking *bmpC*	1540, 1541, 1542, 1543	Thus study

pVAR77	pKV363 containing 1.2 kb sequencing flanking *bmpA*	1532, 1533, 1675, 1535	This study
pVAR78	pKV363 containing 850 bp sequencing flanking *balA*	1659, 1676, 1661, 1662	This study
pVAR80	pKV363 containing 1.2 kb sequencing flanking *balC*	1665, 1666, 1667, 1668	This study
pVAR81	pKV363 containing 1.2 kb sequencing flanking *balB*	1669, 1670, 1671, 1672	This study
pVAR88	pEVS107 containing ~2.8 kb fragment with the *native bmpA* promoter and *bmpA* and *balA*	1754, 1755	This study
pVAR94	pEVS107 containing ~2.5 kb fragment with *bmpA* with a C-terminal FLAG-tag	1754, 1644, 1830	This study
pVAR95	pEVS107 containing ~2.5 kb fragment with *bmpA*	1754, 1833	This study
pVAR96	pEVS107 containing ~3.3 kb fragment with the *native bmpA* promoter fused to *lacZ*	1754, 1821, 1824, 1827	This study
pVAR97	pEVS107 containing ~3.3 kb fragment with the *native bmpC* promoter fused to *lacZ*	1758, 1823, 1826, 1827	This study
pVSV105	Mobilizable vector, Cm^R	N/A	(19)
pVSV208	Cm^R, *rfp*	N/A	(19)

[1]Relevant primers for plasmids generated in this study; N/A, not applicable.

Table S3. Primers used in this study.

Number	**Primer[1]**
1532	AAGGCTACGTGTGATAAATCG
1533	taggcggccgcacttagtatgTGAGCTGACTAATAAAAGTATTAG
1535	CCATCTCACGAATCTAACTCTTC
1536	TCACGTTGCCACCTAGTGC
1537	taggcggccgcacttagtatgCGTTGCAAGATAGGATATTGAGTG
1538	catactaagtgcggccgcctaGGCAAGGCTTATACAGATTAAGGAG
1539	CGTGGCAACTTCTGTGTGG
1540	CGATTATGGCTCGGAAGCC
1541	taggcggccgcacttagtatgCGCAGTAGCGAGGGCAATAATCGG
1542	catactaagtgcggccgcctaAATACTTGGTCAACAGCTGACTAAC
1543	GAGCTCCTTGTATTGCTTGG
1644	ggtaccttatttatcatcatcatctttataatcATAGTATTTGTTCGCAATTGCATTAG
1659	GATTGTCGAGAAGATGTCGG
1661	catactaagtgcggccgcctaGAATGGTATTAACTTAAAATAATAC
1662	GCCGAATTATCCGTTACAATTG
1665	GCATCACTTGAATCAGAGCAG
1666	taggcggccgcacttagtatgGATACTTAGTAATATATTAATTCG
1667	catactaagtgcggccgcctaGCCACTTTATAAACATCATTAAGC
1668	CCCAGTTGGACGTACACGC
1669	GGTTGTGGTACTCAACATCG
1670	taggcggccgcacttagtatgACAAATGCGACCCATTTTATTTAC

1671	catactaagtgcggccgcctaATTAATTAAAGAGACAAAAATGCC
1672	GAGATCGGTGTTGATGGGATC
1675	catactaagtgcggccgcctaGCAGACTATACGACGTCAGGCGGT
1676	taggcggccgcacttagtatgCATAATAACTTTTTTCATAATAAC
1754	gatctactagtggccaggtaccCGATGATATATTCTCAATTTGCAAT
1755	ccagtctagttctagagggcccCTCACATGTATTATTTTAAGTTA
1758	gatctactagtggccaggtaccGTAAAATTTATATTCTCATATTGATTC
1798	gccttgcgtataatatttgcccatggAAGTCGATTCTCATTCTGCAAA
1799	gccttgcgtataatatttgcccatggCTGCATTGCAAATTGAGAATATA
1800	gattacgccaagcttgcatgcCTGCAGGAATTCGAGCTCGGTACC
1821	tcctgtgtgaTGAGCTGACTAATAAAAGTATTAG
1823	tcctgtgtgaCGCAGTAGCGAGGGCAATAATCGG
1824	agtcagctcaTCACACAGGAAACAGCTATGAC
1826	cgctactgcgTCACACAGGAAACAGCTATGAC
1827	ccagtctagttctagagggcccGTACATAATGGATTTCCTTACGC
1827	ccagtctagttctagagggcccGTACATAATGGATTTCCTTACGC
1830	ccagtctagttctagagggcccTTATTTATCATCATCATC
1833	ccagtctagttctagagggcccCTTTTTAATAGTATTTGTTCGCAAT

[1]Non-native sequences are indicated with lower-case letters

Supplemental Methods

Luminescence assays. *V. fischeri* cultures were grown in LBS overnight at 24˚C with shaking, then diluted to an optical density at 600 nm (OD_{600}) of ~0.01 in 30 ml of SWTO and incubated at 24˚C with vigorous shaking. Samples were taken every 30-60 minutes. At each time point, bioluminescence (using a Turner Designs TD-20/20 luminometer at the factory settings and a large, clear scintillation vial) and OD_{600} (using a cuvette) were measured for each sample. Maximum luminescence was observed at OD_{600} measurements between 1.5 and 2 for all strains. Specific luminescence was calculated as relative luminescence (the relative light units of 1 ml of culture integrated over a 6-second count) divided by the OD_{600}.

Epifluorescent microscopy. Spots were made per Materials and Methods in the main body of the text. DIC and epifluorescent images were captured using an Optronics MagnaFire S60800 CCD Microscope Camera attached to a Leica DMIRB with a Prior Lumen 200 light source. Images are at 40x magnification (4x objective lens and 10x eyepiece). Images were processed using ImageJ.

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