## 1 **SUPPLEMENTAL MATERIAL:**

## Table S1: Primers utilized in this study

Primer Name	Sequence (5' to 3')	Additional notes
Primers used to	amplify promoters for EMSA assays	Used to amplify promoter of
p480-F	CTGACACGCAGGCCGGGGGCACC	CKS-1750
p480-R	CTGTGCCGGACATCCGGCTG	
p711-F	CGTATGACGGTGTTCTGTCTGG	CKS-5296
p711-R	GCGCTATCTGTTGATTCTTGG	
PACCC-F	GGTCGTTAATCGCATCCCTGTG	accC
PACCC-R	GGCTTTTAGTCGGCTTCATCG	
PAHPF-F	GCAAAATCTAATTTCCGCATCGC	ahpF
PAHPF-F	GGTATCAAGCATAGTGAGTTCC	
PDEGP-F	GGCGGTGGAATAACCCGAG	degP
PDEGP-R	CTAAGCGCCAGGGCGCTTAAC	
PDKGA-F	GCTGACACAGTAAGTCGTGG	dkgA
PDKGA-R	CGCTGTGCTTAAGTCTAGC	
PESAR28F <sup>a</sup>	TCTTGCCTGTACTATAGTGCAGGTTAAG	esaR (as positive
PESAR28R <sup>a</sup>	CTTAACCTGCACTATAGTACAGGCAAGA	control)
PFABF-F	GGAAGAAGTGACCAACTCTGC	fabF
PFABF-R	CGCTTAGACACGTTCGTC	
PFABI-F	GCGTGGCAACGCCAGCGTTG	fabI
PFABI-R	CCTTATGGTCATGGTAGTTGGC	č
PFKPA-F	GTCAGCAGACCTCGGCAGAGC	fkpA
PFKPA-R	CGGACTGAGGGCCCACAC	

FUSAF	GGCCTGGAAGCTTTTGAGGTTGC	fusA
FUSAR	GTA CGA GCC ATT TTA TTC CTC G	
PGALF-F	GAACCGTGGCTGTAAGTGTC	galF, galE
PGALF-R	GACATGCGTCAATTATGCCG	0 0
PGALU-CDN	CTTACCCGCAAGACAGTGCCAGAAGTTAGC	galU, CKS 2738
PGALU-F	CGTCTGAGCAATAAACACCG	C
PGLM-F	CGTTCTGGCCGATACCGCTATTCG	glmU
PGLM-R	CTGAGGAACGTTAAACGATTTAGC	0
PGLPF-F	CCTGCAAGGCGTTGATGATGC	glnF. glnK
PGLPF-R	GTTGTTCCTGAAGCGAGG	81 , 81
PHTPG-F	TGACATCAGCAAAGCCGATCG	htnG
PHTPG-R	CTGCACGTAGAGTTTCAGACC	mp o
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PLRHA-F	CAGAGAGCATCTTTAGTACAGG	lrhA
PLRHA-R	GCGGGAAGCTGTGTGTAGTTGTC	
PMGLB-F	GCGATTCCGCGATGTAACCG	mglB
PMGLB-R	GCTGCGTCAAGCCGCGGAAG	
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PNEMR-F	CTGTCGCTGTATTGCAGCC	nemR
PNEMR-R	CATCCTGGTAGACCAATCG	
PNHAR-F	CCGTCCATTGCGCACCACTG	nhaR
PNHAR-R	GACATGCGTCAATTATGCCGGATG	
<b>POMPF-F</b>	CGCCTATGTCACAGGCGTAC	ompA, down-stream
POMPF-R	CCAGAATGTTGCGCTTCATC	of ompF
POSMY-F	CGCGTTTTCAGGGCCATTCTC	osmY
POSMY-R	CAACGCTACTGCGGCACAGG	35001

PPGM-F	CGTCTGGATACGGCCGCGTTTGC	pgm
PPGM-R	GGCCATTGGCGCTTCTCC	
PPHES-F	CATACTTTACGGGCAGGCATAGC	pheT, downstream
PPHES-R	GTTCTGCTAGTTGGGACATG	of pheS
PRPSB-F	GAGATTGCCATTAAATTATTCCGC	rpsB
PRPSB-R	GTATCCCATACAACCGACCTC	
Primers for a	mplifying coding regions for qRT-PCR	Gene amplified

1		1
DKGA-CDNF	GGCAGCCTGAAAATTGTTAGC	dkgA
DKGA-CDNR	CGTATTAGCCGATAAACGTATCCG	
FABF-CDNF	GGAGGACGAACGTGTCTAAGC	fabF
FABF-CDNR	CGCCAGGTTTAGATTTTACGG	
RPSB-CDNF	GAGGTCGGTTGTATGGGATAC	rpsB
RPSB-CDNR	CAGAGCAAACCTTAATTAAGC	
LRHA-CDNF	CTTGTAATAACACCAGGATAGTAG	lrhA
LRHA-CDNR	GAAGACGGGATTTACTCTTCATCG	
GLPF-CDNF	CAACTATCATGAGTCAGACTACAACC	glpF
GLPF-CDNR	GAACTTACGCTTTACGTTCATGC	
MGLB-CDNF	CTTACCCGATGTTCTTCCGC	mglB
MGLB-CDNR	CTGAGACAGGTTTTCCTGATC	
27F	AGAGTTTGATCATGGCTCAG	16S rRNA
1429RLONG	ACCTTGTTACGACTTCACC	
qRT-PCR Prim	ers	Target gene
DKGA-RTF	GCCCGGAGAAAGATCAGTACGTTG	dkgA
DKGA-RTR	GCTTTTGACCAGGCCTTGTTGC	

FABF-RTF FABF-RTR	CGTCAATATGGTGGCGGGGACATC	fabF
RPSB-RTF	GCTCAAGGCTGGTGTTCACTTCG	rpsB
RPSB-RTR	ACGCGCACCGAAAATGAATGG	
LRHA-RTF	CGTCCGGTGGAGATGATGA	lrhA
LRHA-RTR	ATCCTTCTGCTGCACCCATT	
GLPF-RTF	TCTGGCCGGCATTTTCTC	glpF
GLPF-RTR	CCTGGCCCACGGAAATC	
MGLB-RTF	GATACGGCGATGTGGGATACCG	mglB
MGLB-RTR	GCGTTAGGGCCTGACAGC	-
168-RTF	GCCAGCAGCCGCGGTAAT	16S rRNA
16S-RTF 16S-RTR	GCCAGCAGCCGCGGTAAT CGCTTTACGCCCAGTAATTCC	16S rRNA
16S-RTF 16S-RTR DNase I footprintin	GCCAGCAGCCGCGGGTAAT CGCTTTACGCCCAGTAATTCC ng assays	<i>16S rRNA</i> <b>Promoter amplified</b>
16S-RTF 16S-RTR DNase I footprintin FAM-PDKGA-R2	GCCAGCAGCCGCGGGTAAT CGCTTTACGCCCAGTAATTCC ng assays /6-FAM/-TTGGTCTGCCATATCCTGC	<i>16S rRNA</i> <b>Promoter amplified</b> P <sub>dkgA</sub>
16S-RTF 16S-RTR DNase I footprintin FAM-PDKGA-R2 PDKGAF	GCCAGCAGCCGCGGTAAT CGCTTTACGCCCAGTAATTCC ng assays /6-FAM/-TTGGTCTGCCATATCCTGC GCTGACACAGTAAGTCGTGG	<i>16S rRNA</i> <b>Promoter amplified</b> P <sub>dkgA</sub>
16S-RTF 16S-RTR DNase I footprintin FAM-PDKGA-R2 PDKGAF FAM-PLRHA-R2	GCCAGCAGCCGCGGTAAT CGCTTTACGCCCAGTAATTCC g assays /6-FAM/-TTGGTCTGCCATATCCTGC GCTGACACAGTAAGTCGTGG /6-FAM/-TCTGTCATACACACACGCTG	<i>16S rRNA</i> <b>Promoter amplified</b> P <sub>dkgA</sub> P <sub>lrhA</sub>
16S-RTF 16S-RTR DNase I footprintin FAM-PDKGA-R2 PDKGAF FAM-PLRHA-R2 PLRHA-F2	GCCAGCAGCCGCGGTAAT CGCTTTACGCCCAGTAATTCC g assays /6-FAM/-TTGGTCTGCCATATCCTGC GCTGACACAGTAAGTCGTGG /6-FAM/-TCTGTCATACACACACGCTG GCCGTGCATTAATCGTTAATACGG	16S rRNA <b>Promoter amplified</b> Р <sub>dkgA</sub> Р <sub>lrhA</sub>
16S-RTF 16S-RTR DNase I footprintin FAM-PDKGA-R2 PDKGAF FAM-PLRHA-R2 PLRHA-F2 PGLPF-FAMF	GCCAGCAGCCGCGGTAAT CGCTTTACGCCCAGTAATTCC g assays /6-FAM/-TTGGTCTGCCATATCCTGC GCTGACACAGTAAGTCGTGG /6-FAM/-TCTGTCATACACACACGCTG GCCGTGCATTAATCGTTAATACGG /6-FAM/-CCTGCAAGGCGTTGATGATGC	16S rRNA Promoter amplified P <sub>dkgA</sub> P <sub>lrhA</sub>
16S-RTF 16S-RTR DNase I footprintin FAM-PDKGA-R2 PDKGAF FAM-PLRHA-R2 PLRHA-F2 PGLPF-FAMF PGLPF-R	GCCAGCAGCCGCGGTAAT CGCTTTACGCCCAGTAATTCC g assays /6-FAM/-TTGGTCTGCCATATCCTGC GCTGACACAGTAAGTCGTGG /6-FAM/-TCTGTCATACACACACGCTG GCCGTGCATTAATCGTTAATACGG /6-FAM/-CCTGCAAGGCGTTGATGATGC GTTGTTCCTGAAGCGAGG	16S rRNA Promoter amplified P <sub>dkgA</sub> P <sub>lrhA</sub> P <sub>glpF</sub>



Figure S1: Two-dimensional SDS PAGE experiments of *P. stewartii* DC283 strains. Panel A (i)
ESN51 and (ii) ESN51 supplemented with 10 µM AHL; panels B and C (i) ESAIR pBBR1MCS3 (*esaR<sup>-</sup>/esaI*) and (ii) ESAIR pSVB60 (*esaR* complemented) depicting the three trials
identifying differentially expressed protein spots. Locations of protein spots of DkgA, GlpF and
LrhA are marked as examples in panel A, B and C, respectively. The positions of molecular
weight standards are indicated on the left (in kDa) and the isoelectric pH (pI) gradient is
indicated at the top from left to right.





Figure S2: Electrophoretic mobility shift assays on the promoters of genes in the QS regulon.
Concentration of DNA probe in all lanes is 1 nM. Lanes within each panel labeled with gene
promoters consist of (left to right): DNA probe, DNA probe with 50 nM HMGE, DNA probe
with 100 nM HMGE, and DNA probe with 100 nM HMGE plus 100 nM unlabeled DNA probe.
The identity of the second band produced during PCR amplification of the gene promoters P<sub>pgm</sub>,
P<sub>degP</sub>, P<sub>pheT</sub>, P<sub>CKS-1750</sub>, P<sub>fkpA</sub>, and P<sub>ompA</sub> is not known.



Figure S3: Relative positions of *esa* box on the promoters of the direct targets of EsaR. Numbers

- above or below the *esa* boxes denote base pairs from translational (ATG) start sites of the
- 26 downstream open reading frames. The 'x' in front of the directional arrows indicates repression,
- absence of 'x' indicates activation. Model not drawn to scale.