Supplementary Information

Induction of a quorum sensing pathway by environmental signals enhances group A streptococcal resistance to lysozyme.

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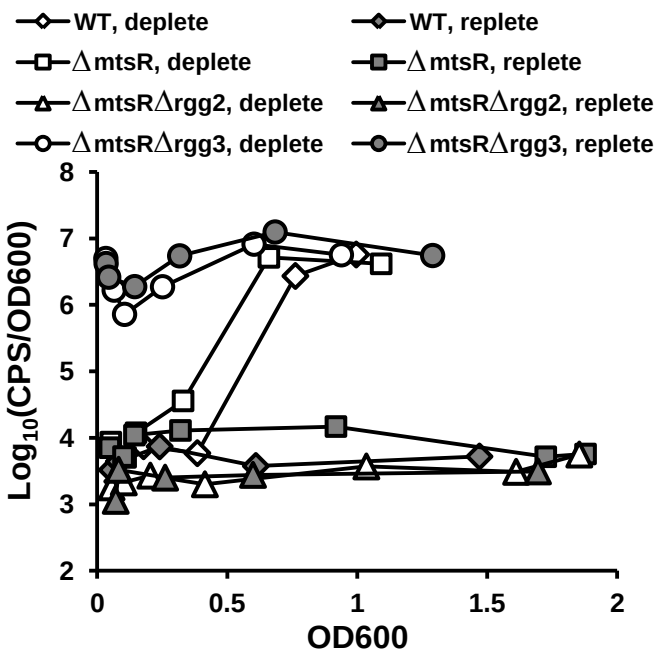

Figure S1. Metal depletion induces Rgg-SHP quorum sensing. Luciferase activity of wild-type NZ131 and isogenic P_{shp3}-*luxAB* reporter strains grown in metal-deplete or replete conditions. Cells were grown to mid-log in CDM+1% glucose, washed three times with CxCDM containing 100 μM dipyridyl, then diluted into CxCDM supplemented with 50 μM $CaCl_2$and 1000 μM $MgSO_4$ (deplete) or 50 μM $CaCl_2$, 1000 μM $MgSO_4$, 20 μM $FeSO_4$, and 30 μM $MnSO_4$ (replete). Data are representative of experiments performed at least three times.

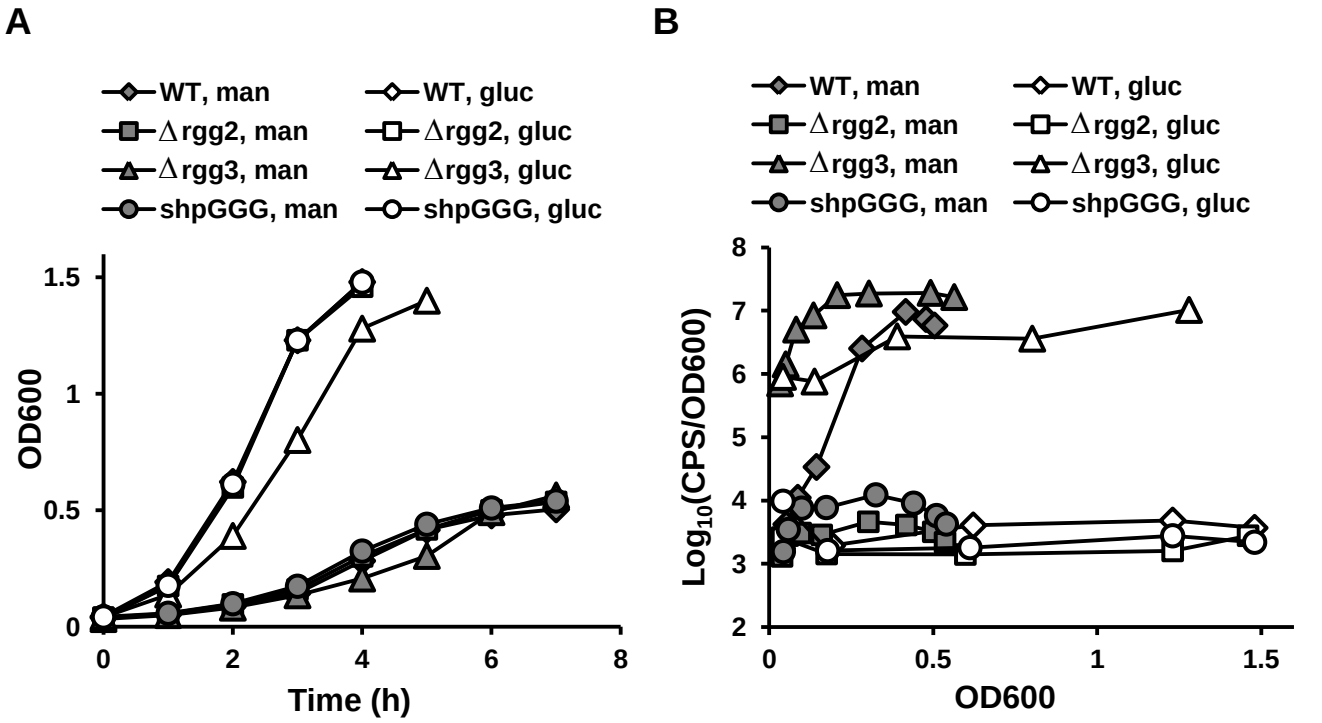

Figure S2. **Mannose-dependent induction of P_{shp3}-*luxAB* requires *rgg2* and *shp*s. (A)** Growth or **(B)** luciferase activity from P_{shp3}-*lux* of WT, Δ*rgg2*, Δ*rgg3*, and shp_{GGG} strains grown in CDM with 1% mannose (man) or 1% glucose (gluc). Data shown are representative of experiments performed at least three times.

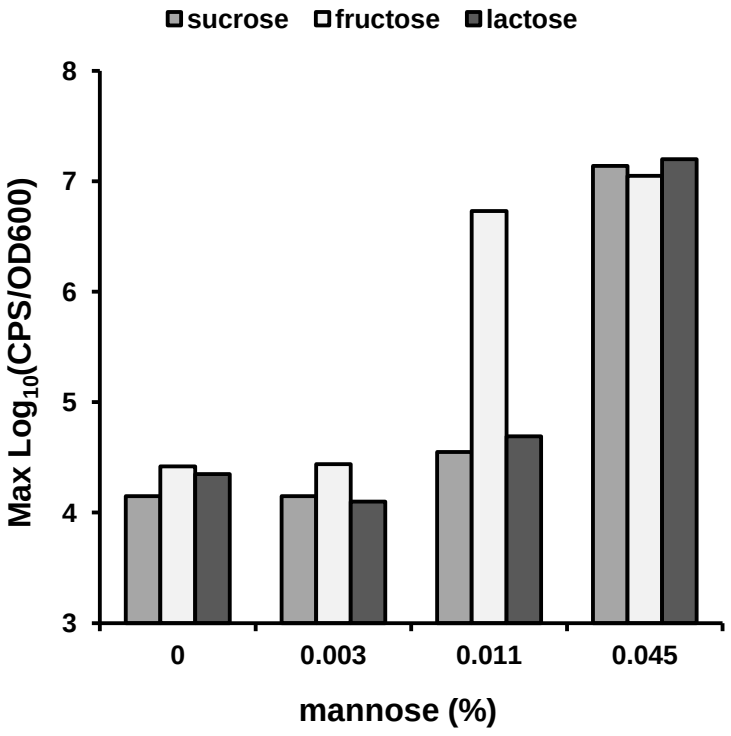

Figure S3. **Low concentrations of mannose are sufficient to induce QS when the primary carbon source is not glucose.** P_{shp3} reporter expression of a strain grown in CDM containing 1% sucrose (medium gray), 1% fructose (light gray) or 1% lactose (dark gray) with mannose supplementation as indicated.

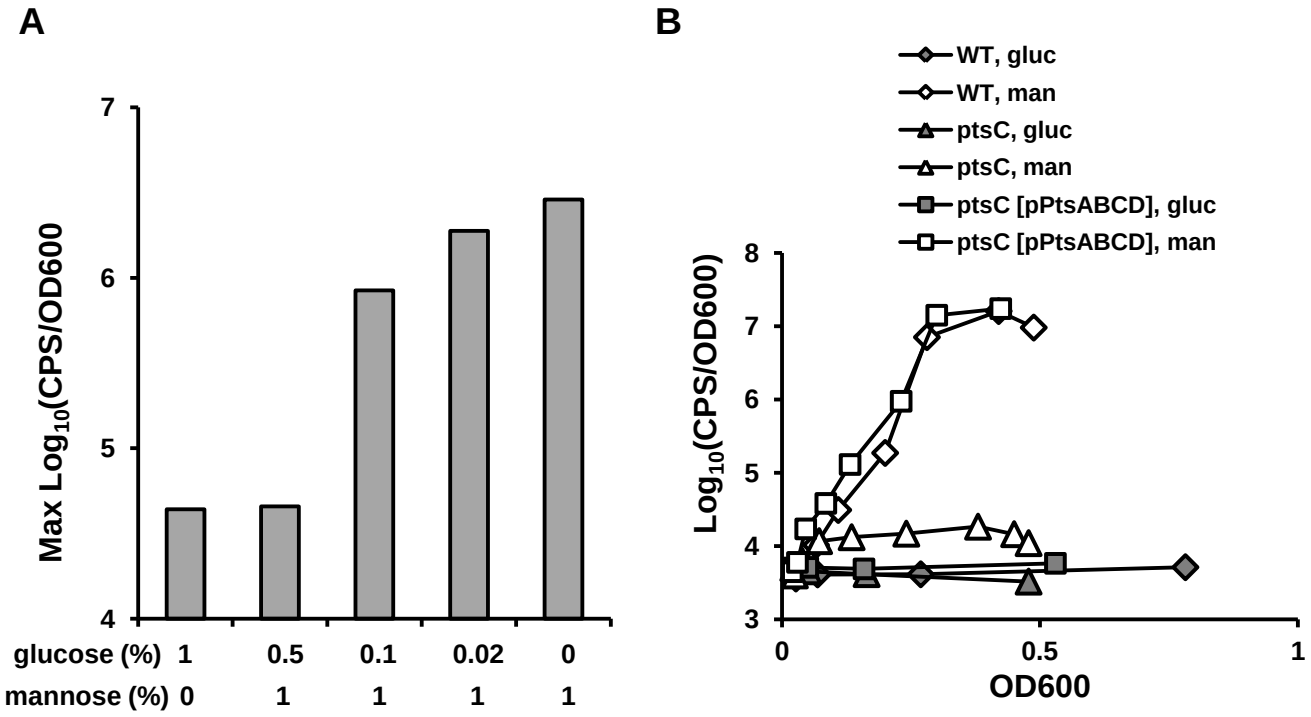

Figure S4. **The mannose response requires *ptsABCD* and is repressed by the carbon catabolite response**. **(A)** Luciferase expression from a P_{ptsA}-*luxAB* reporter strain grown in 1% mannose and decreasing concentrations of glucose. **(B)** P_{shp3}-*luxAB* expression in a *ptsC* complementation strain during growth in 1% glucose (gluc) or 1% mannose (man).

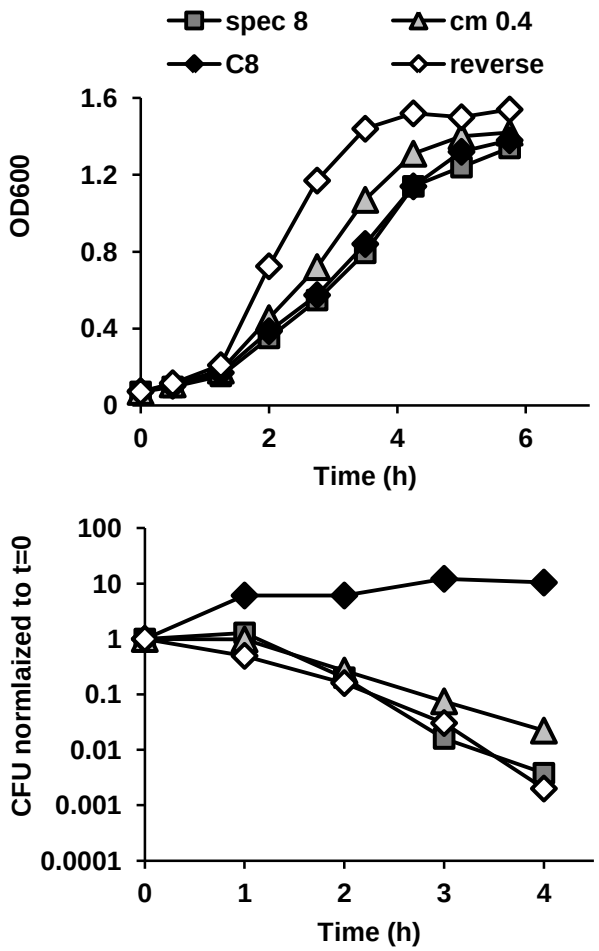

Figure S5. **Effect of artificially-slowed growth on lysozyme resistance**. Wild-type NZ131 grown in sub-inhibitory concentrations of the bacteriostatic antibiotics spectinomycin (8 μg mL^{-1}) or chloramphenicol (0.4 μg mL^{-1}) exhibits reduced growth rates similar to SHP-induced (C8) cultures (top panel) but does not exhibit increased survival after lysozyme challenge (bottom panel). Cells were grown with antibiotics or peptides for two hours before back-dilution into 2 mg mL^{-1} lysozyme. At each time point, an aliquot was removed, diluted and plated to enumerate CFUs.

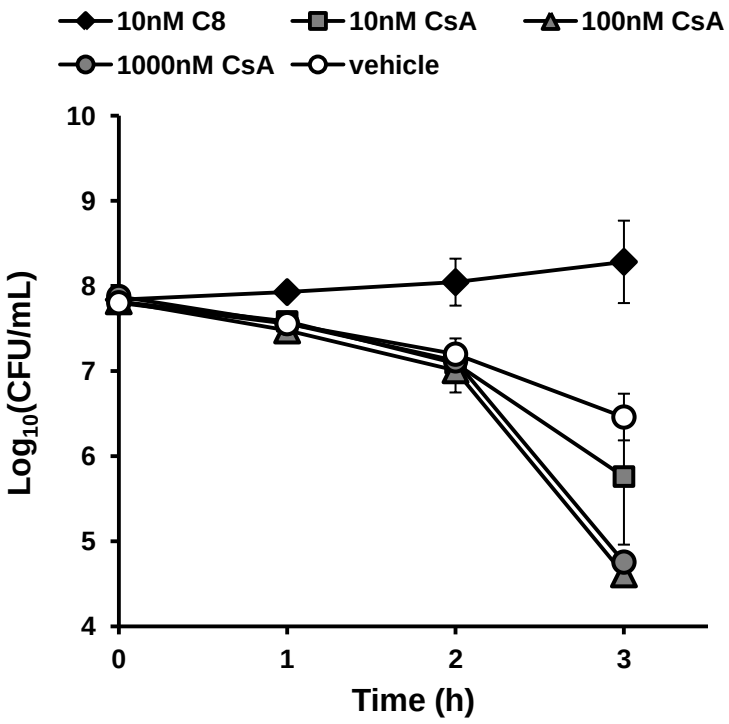

Figure S6. **Effect of high concentrations of CsA on resistance of NZ131 to 2 mg mL^{-1} lysozyme**. Cells were pre-treated with the drug for one hour at the concentrations indicated before lysozyme challenge.

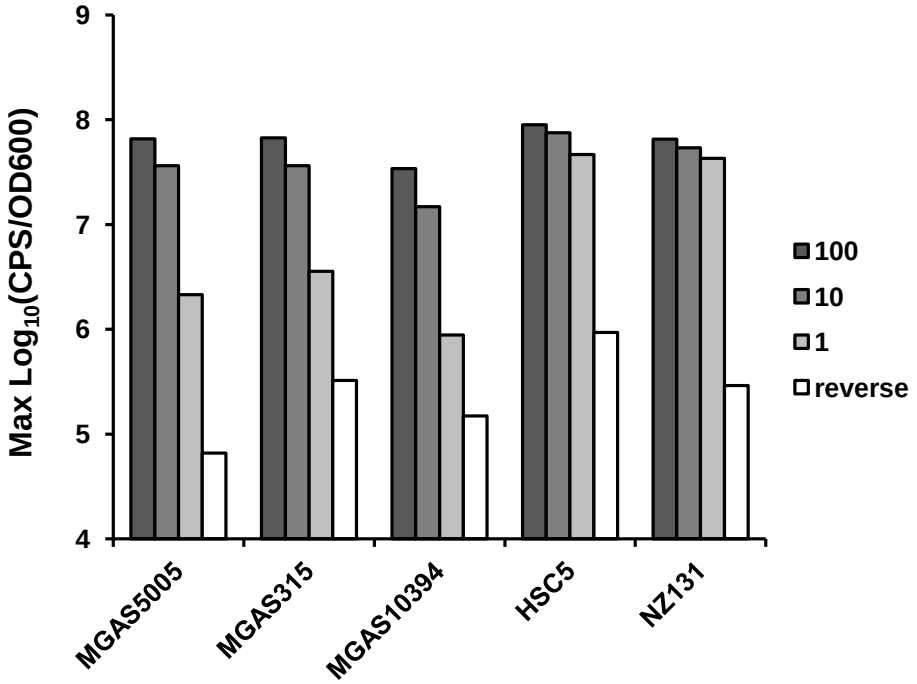

Figure S7. Reporter induction in wild-type GAS strains in response to synthetic peptide. Maximum P_{shp3}-*lux* reporter induction from different GAS serotypes exposed to 100, 10, or 1 nM synthetic SHP3-C8 or 100 nM reverse peptide.

Figure S8. Reporter activity and lysozyme resistance of wild-type GAS grown in metal-deplete vs. replete CxCDM. Wild-type GAS strains were grown in deplete or replete CxCDM, and growth (top panels) and luciferase activity of a multi-copy P_{shp3}-*lux* reporter (pJC229; middle panels) were monitored. After pre-growth in these conditions, cells were back-diluted into 10 (MGAS5005), 20 (MGAS10394 and HSC5), or 50 (MGAS315) mg mL^{-1} lysozyme. At each time point, an aliquot was removed, diluted and plated to enumerate CFUs (bottom panels).

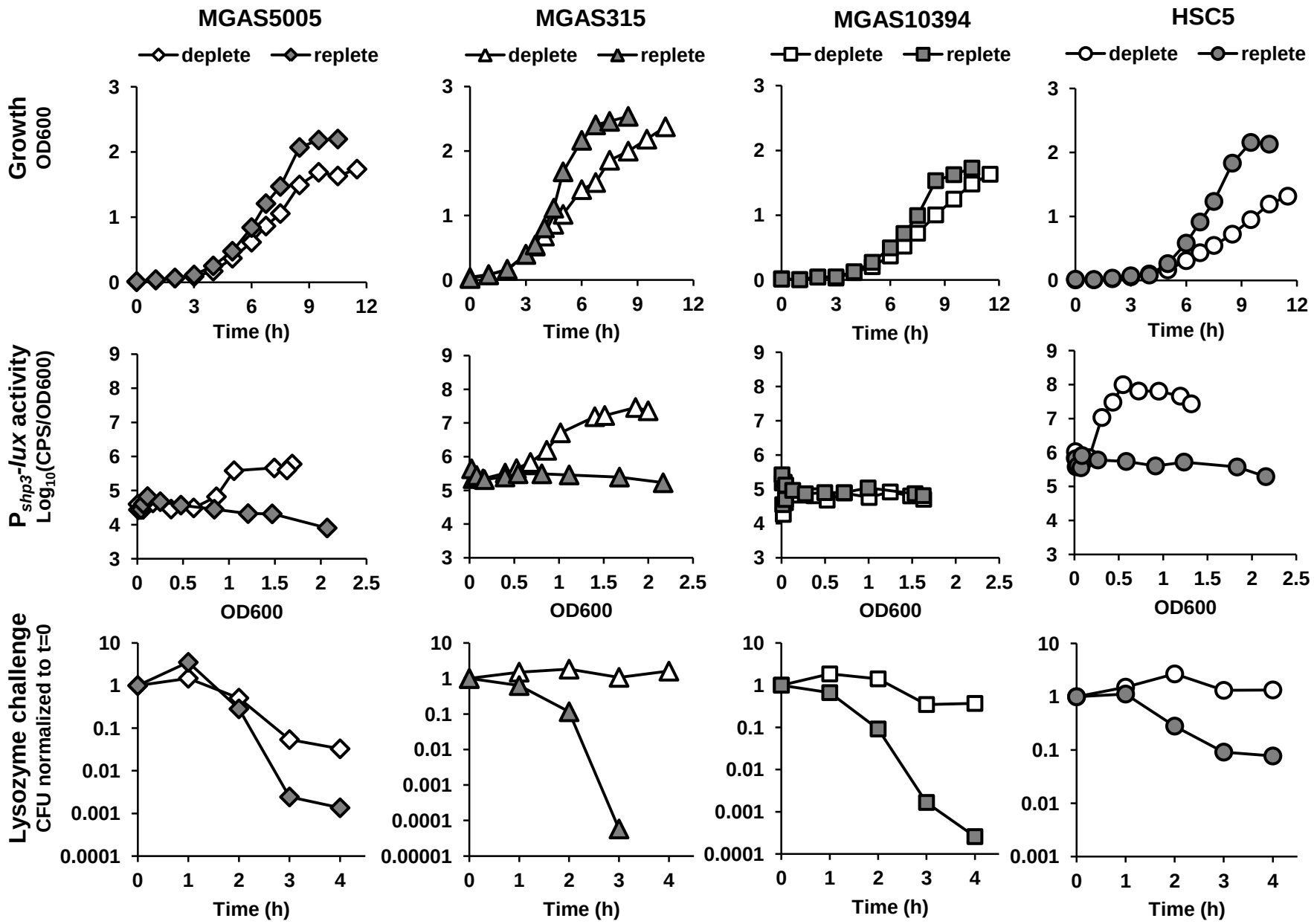

Figure S9. Reporter activity and lysozyme resistance of wild-type GAS grown in mannose or glucose. Wild-type GAS strains were grown in CDM containing 1% glucose or 1% mannose, and growth (top panels) and luciferase activity of a multi-copy P_{shp3}-*lux* reporter (pJC229; middle panels) were monitored. After pre-growth in these conditions, cells were back-diluted into 10 (MGAS5005), 20 (MGAS10394 and HSC5), or 50 (MGAS315) mg mL^{-1} lysozyme. At each time point, an aliquot was removed, diluted and plated to enumerate CFUs (bottom panels).

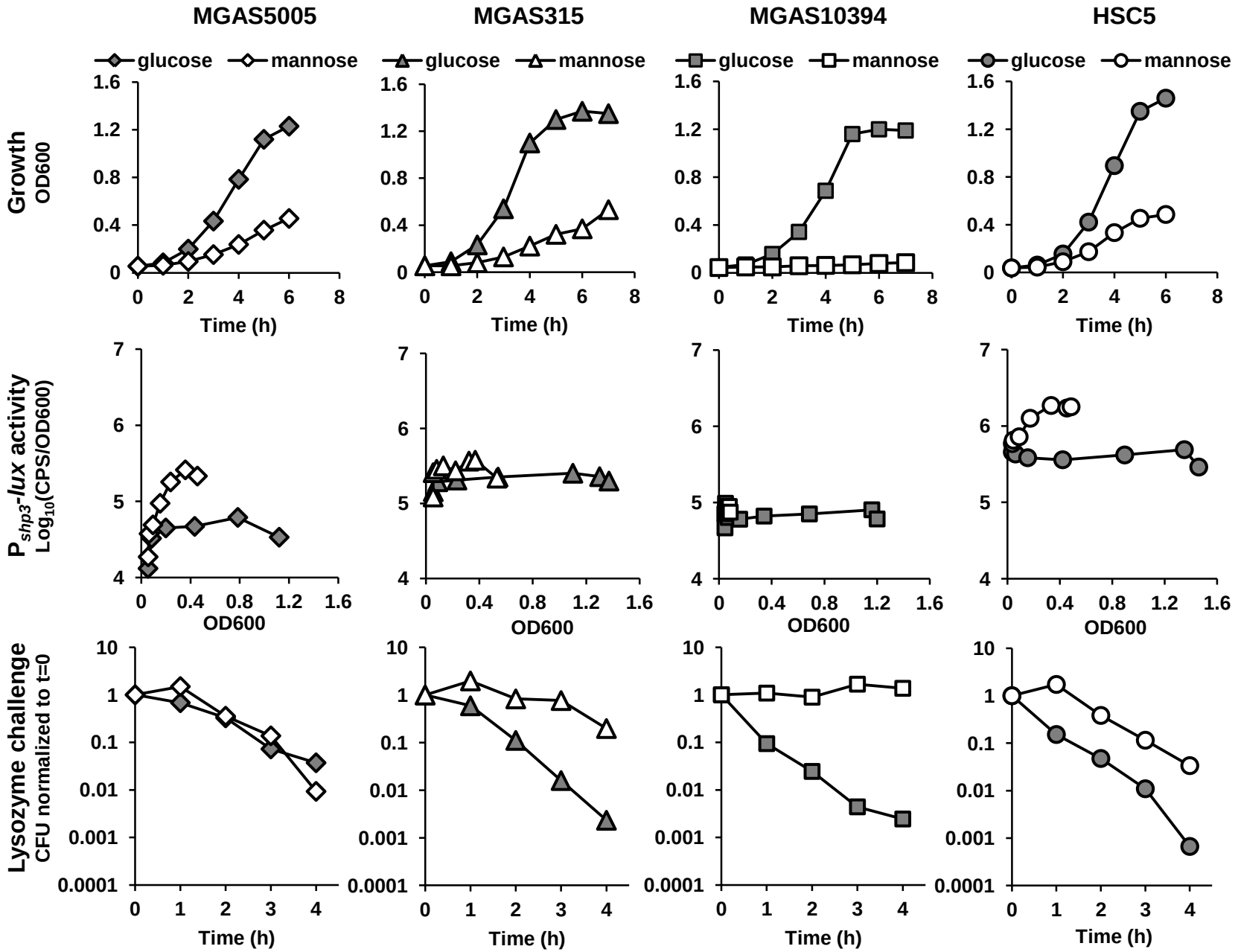

Table S1. Effect of SHP3-C8 treatment on MICs of some antibiotics

	+C8	+reverse
streptonigrin (μM)		
WT	5	5
Δ*rgg3*	5	5
Δ*mtsR*	1.25	1.25
cycloserine (mg/mL)		
WT	0.5	0.25
Δ*rgg2*	0.25	0.25
Δ*rgg3*	0.5	0.5
shp_{GGG}	0.5	0.25
erythromycin (μg/mL)		
WT	0.031	0.063
Δ*rgg2*	0.063	0.063
Δ*rgg3*	0.031	0.031
shp_{GGG}	0.031	0.063
kanamycin (μg/mL)		
WT	15.63	250
Δ*rgg2*	125	125
Δ*rgg3*	<7.81	31.25
shp_{GGG}	15.63	250
paraquat (mM)		
WT	50	>100
Δ*rgg2*	>100	>100
Δ*rgg3*	50	100
shp_{GGG}	100	>100
spectinomycin (μg/mL)		
WT	31.25	125
Δ*rgg2*	125	125
Δ*rgg3*	31.25	62.5
shp_{GGG}	31.25	125
streptomycin (μg/mL)		
WT	15.63	250
Δ*rgg2*	125	250
Δ*rgg3*	7.81	15.63
shp_{GGG}	15.63	250

Table S2. GAS growth on different carbon sources

Carbon source	Growth[a]	$P_{shp\text{-}luxAB}$ induction[b]
Carbohydrates		
N-Acetyl-D-Glucosamine	++	-
D-Fructose	++	-
α-D-Glucose	++	+/-
D-Glucosamine	++	-
Maltose	++	-
Maltotriose	++	-
Sucrose	++	-
D-Trehalose	++	-
N-Acetyl-β-D-Mannosamine	+	-
D-Cellobiose	+	-
Lactulose	+	-
D-Mannose	+	++
3-*O*-β-D-Galacto-pyranosyl-D-Arabinose	+/-	-
D-Glucose-6-Phosphate	+/-	-
α-D-Lactose	+/-	-
α-Methyl-D-Galactoside	+/-	-
β-Methyl-D-Glucoside	+/-	-
Amino acids and Amines		
Phenylethyl- amine	++	-
D-Alanine	+	-
L-Alanine	+	-
L-Glutamic Acid	+	-
L-Serine	+	-
L-Threonine	+	-
L-Asparagine	+/-	-
L-Aspartic Acid	+/-	-
L-Glutamine	+/-	-
Glycyl-L-Aspartic Acid	+/-	-
Glycyl-L-Proline	+/-	-
L-Proline	+/-	-
D-Threonine	+/-	-
Tyramine	+/-	-
Nucleic Bases		
Adenosine	+	-
Inosine	+	-
2-Deoxy Adenosine	+/-	-
Uridine	+/-	-
Organic Acids and Alcohols		
2-Aminoethanol	+	-
Dulcitol	+	-
Formic Acid	+	-
L-Galactonic Acid-γ-Lactone	+	-
D-Gluconic Acid	+	-
α-Hydroxy Butyric Acid	+	-
α-Hydroxy Glutaric Acid-γ-Lactone	+	-
L-Lactic Acid	+	-

Mono Methyl Succinate	+	-
D-Malic Acid	+	-
L-Malic Acid	+	-
Mucic Acid	+	-
Propionic Acid	+	-
Acetic Acid	+/-	-
Adonitol	+/-	-
D-Galactonic Acid-γ-Lactone	+/-	-
Glycerol	+/-	-
D,L-α-Glycerol-Phosphate	+/-	-
D,L-Malic Acid	+/-	-
D-Mannitol	+/-	-
m-Hydroxy Phenyl Acetic Acid	+/-	-
p-Hydroxy Phenyl Acetic Acid	+/-	-
Succinic Acid	+/-	-
m-Tartaric Acid	+/-	-
Tricarballylic Acid	+/-	-

[a]Symbols: ++ Growth of more than ten fold over initial level; + Growth of more than six fold and lower than ten fold over initial level; +/- Growth of over four fold and lower than six fold over initial level.

[b]Symbols: ++ Promoter induction of over 200-fold; + Promoter induction of over 50-fold times and lower than 200-fold; +/- Promoter induction of over 20-fold times and lower than 50-fold.

Table S3. Strains and plasmids used in this study

Strain/plasmid	Description	Reference
***S. pyogenes* strains**		
NZ131	Wild-type M49 isolate	(Simon & Ferretti, 1991, McShan *et al.*, 2008)
Δ*rgg2*	NZ131 Δ*rgg2*; unmarked	(Chang et al., 2011)
Δ*rgg3*	NZ131 Δ*rgg3*::*cat*; Cm^R	(Chang et al., 2011)
shp_{GGG}	NZ131 $shp2_{GGG}shp3_{GGG}$; unmarked	(Cook et al., 2013)
Δ*mtsR*	NZ131Δ*mtsR*::*aphA3*; Kan^R	(Bates et al., 2005)
Δ*mtsR*Δ*rgg2*	NZ131Δ*mtsR*::*aphA3* Δ*rgg2*; Kan^R	This study, (Chang et al., 2011)
Δ*mtsR*Δ*rgg3*	NZ131Δ*mtsR*::*aphA3* Δ*rgg3*::*cat*; Cm^R Kan^R	This study, (Chang et al., 2011)
manM	NZ131 *spy49_1355*::pJJ216; Kan^R	This study
ptsC	NZ131 *spy49_0835*::pJJ217; Kan^R	This study
agaW	NZ131 *spy49_0526c*::pJJ215; Kan^R	This study
HSC5	Wild-type M14 isolate	(Port et al., 2013)
MGAS5005	Wild-type M1 isolate with natural *covS* mutation	(Hoe et al., 1999, Sumby et al., 2006)
MGAS315	Wild-type M3 isolate	(Beres et al., 2002)
MGAS10394	Wild-type M6 isolate; Erm^R	(Banks et al., 2004)
***P. aeruginosa* PA14**	Human clinical isolate	(Rahme et al., 1995)
Plasmids		
p7INT	Shuttle-suicide vector that integrates at streptococcal bacteriophage T12 *attB* site; Erm^R	(McShan et al., 1998)
pJC188	524 bp DNA fragment containing the *rgg3* promoter fused to *luxAB* and cloned into p7INT; Erm^R	This study
pJC190	1046 bp DNA fragment containing the *rgg2* promoter fused to *luxAB* and cloned into p7INT; Erm^R	This study
pJC219	384 bp DNA fragment containing the *shp3* promoter fused to *luxAB* and cloned into p7INT; Erm^R	(LaSarre et al., 2013b)

pJC229	384 bp DNA fragment containing the *shp3* promoter fused to *luxAB* and cloned into pLZ12-Sp; $Spec^R$	This study
pJC303	125 bp DNA fragment containing the *recA* promoter cloned into pLZ12-Sp; $Spec^R$	This study
pJJ180	Promoterless *luxAB* genes cloned into p7INT, Erm^R	This study
pJJ215	482 bp internal fragment of *agaW* gene cloned into pUC-Km for insertional disruption, Kan^R	This study
pJJ216	517 bp internal fragment of *manM* gene cloned into pUC-Km for insertional disruption, Kan^R	This study
pJJ217	430 bp internal fragment of *ptsC* gene cloned into pUC-Kan for insertional disruption, Kan^R	This study
pJJ244	345 bp DNA fragment containing the *ptsA* promoter cloned into pJJ180 upstream of *luxAB*, Erm^R	This study
pJJ252	460 bp DNA fragment containing the *manL* promoter cloned into pJJ180 upstream of *luxAB*, Erm^R	This study
pJJ251	*ptsABCD* operon cloned into pJC303 downstream of the P_{recA} promoter, $Spec^R$	This study
pLZ12-Sp	Shuttle vector encoding spectinomycin resistance; pWV01 origin, $Spec^R$	(Husmann et al., 1995)
pUC-Km	Vector with Gram-negative pMB1 origin containing the *aphA3* cassette, Kan^R	(Menard et al., 1993)

Table S4. Primers used in this study

Primer	Nucleotide sequence (5' to 3')	Description
JC148	CATGgaattcGTTATCTCTGCTACTCTGTC	Sense primer to clone *rgg3* promoter region; EcoRI site
JC149	CATGgaattcATGTAGTTGTTGTTAGTTTAGCC	Sense primer to clone *rgg2* promoter region; EcoRI site
JC159	CAAATATTTCCAAACTTCATATTTTCCCACTTTCCCAACA	Antisense primer to fuse *rgg3* promoter region to *luxAB*
JC161	CAAATATTTCCAAACTTCATTTTTCCCACTTTCACAACAAAAA	Antisense primer to fuse *rgg2* promoter region to *luxAB*
JC415	CATGgaattcAAAGAGGAGAAAGTATTGGC	Sense primer to clone *recA* promoter; EcoRI site
JC417	CATGgcggccgcCCCTTACCAAAATCTTTTTCAATAT	Antisense primer to clone *recA* promoter; NotI site
JJ222	CATGgtcgacGCAACAACCTTTACCATTAT	Sense primer to clone internal fragment of *ptsC* gene into pUC-Kan; SalI site
JJ223	CATGgaattcCTGGTAATTTCAAATAAGCTG	Antisense primer to clone internal fragment of *ptsC* gene into pUC-Km; EcoRI site
JJ224	CATGgtcgacGTCATCCTTGGTGGTACA	Sense primer to clone internal fragment of *manM* gene into pUC-Km; SalI site
JJ225	CATGgaattcATAGCTGCAAGAGCAAAAC	Antisense primer to clone internal fragment of *manM* gene into pUC-Km; EcoRI site
JJ226	CATGgtcgacAGCTATCTCCGAATTAGCTT	Sense primer internal fragment of *agaW* gene into pUC-Km; SalI site
JJ227	CATGgaattcCCAATCAGTACAAAAGGAAT	Antisense primer to internal fragment of *agaW* gene into pUC-Km; EcoRI site
JJ276	CATGgtcgacTGGAACTGGTTATGCTATGA	Sense primer to clone *manL* promoter; SalI site
JJ257	CATGgcggccgcCTCCTTTAAAATATTTTTCGTTG	Antisense primer to clone *manL* promoter; NotI site
JJ254	CATGgtcgacTTATCGTGATGGCTATGG	Sense primer to clone *ptsA* promoter; SalI site
JJ255	CATGgcggccgcCTATTAACAAAAACCGCTGA	Antisense primer to *ptsA* promoter; NotI site
JJ273	CATGgcggccgcATGAAACGAAAATTTCTCATT	Sense primer to clone *ptsABCD* ORFs into pJC303; NotI site
JJ274	CATGagatctCTTACCTTTATTGCTAGGAGAT	Antisense primer to clone *ptsABCD* ORFs into pJC303; BglII site