Supplementary Figures and Tables

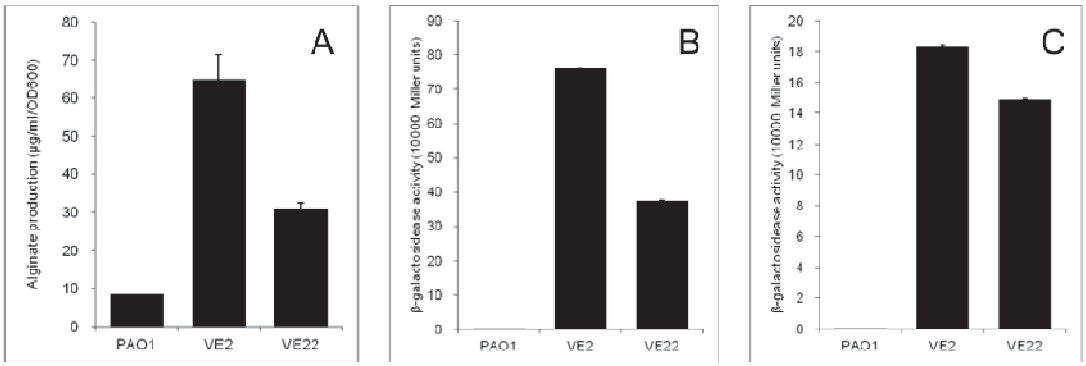

Figure S1. Alginate production and promoter activity of P_{algU} and P_{algD} in PAO1, PAO1-VE2 (*mucE* overexpressed) and PAO1-VE22 (*cupB5* overexpressed). Strains were streaked on PIA plates and cultured overnight at 37°C. For alginate assay, cells were grown on PIA plates without antibiotics. For β-galactosidase assay, carbencillin (300 μg ml^{-1}) was added to the medium to retain the plasmid. (A) Alginate production was measured after overnight culture. (B) Measurement of the activity of the *algU* promoter using pLP170-P_{algU} in different strains. The P_{algU} promoter was inserted into a pLP170 vector containing the promoterless *lacZ* gene. The P_{algU}-*lacZ* fusion in pLP170 was transferred into the respective strains via triparental conjugation. β-galactosidase activity was measured using the Miller assay as described in Experimental Procedures. (C) Measurement of activity of the *algD* promoter in different strains containing pLP170-P_{algD}.

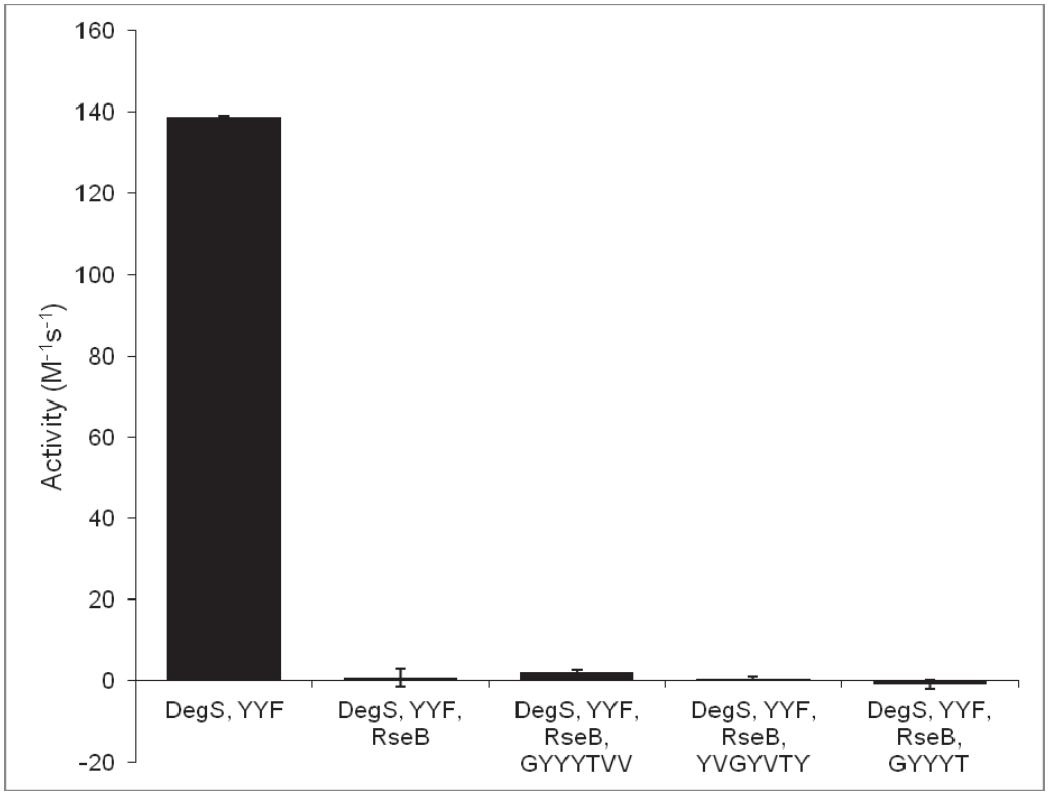

Figure S2. CupB5 peptides do not relieve RseB inhibition of RseA cleavage by DegS. Rates of cleavage of *E. coli* $RseA^{peri}$ (20 μM) by DegS (0.5 μM trimer) and YYF peptide (20 μM). When present, the concentration of RseB dimer was 25 μM and the concentrations of CupB5 peptides were 480 μM.

Figure S3. The CupB5 GYYYTVV motif is found in many orthologs in sequenced strains of *P. aeruginosa*. A total of 22 putative *Pseudomonas* orthologs of CupB5 was obtained from www.pseudomonas.com. Homology was compared using the ClustalW algorithm of MacVector software. Shown is a small portion of the CupB5 amino-acid sequence containing the GYYYTVV motif when present. PA: *P. aeruginosa*; Protegens: *P. protegens*; Pdentrifi: *P. dentrificans*; and Psyringae: *P. syringae pv. Phaseolicola*. *P. aeruginosa* PA7 has two copies of *cupB5* in its genome, *P. protegens* CHA0 has four copies, and strain *P. protegens* Pf-5 has two copies.

Supplementary Table S1. Truncations of CupB5 to identify the signal that activates alginate production.

Strains	C-terminal	Phenotype
HA-*cupB5*(1018)-His -PAO1	-NIWH_6	Mucoid
HA-*cupB5*(1015)-His -PAO1	-DYGH_6	Mucoid
HA-*cupB5*(585aa)-His -PAO1	-GSGH_6	Mucoid
HA-*cupB5*(531aa)-His-PAO1	-GPAH_6	Mucoid
HA-*cupB5*(520aa)-His-PAO1	-SYYH_6	Mucoid
HA-*cupB5*(510aa)-His-PAO1	-YVLH_6	Mucoid
HA-*cupB5*(507aa)-His-PAO1	-DGLH_6	Mucoid
HA-*cupB5*(504aa)-His-PAO1	-KNLH_6	Mucoid
HA-*cupB5*(497aa)-His-PAO1	-AQLH_6	Mucoid
HA-*cupB5*(491aa)-His-PAO1	-TVVH_6	Mucoid
HA-*cupB5*(490aa)-His-PAO1	-YTVH_6	Non-mucoid
HA-*cupB5*(489aa)-His-PAO1	-YYTH_6	Non-mucoid
HA-*cupB5*(488aa)-His-PAO1	-YYYH_6	Non-mucoid
HA-*cupB5*(483aa)-His-PAO1	-YVSH_6	Non-mucoid
HA-*cupB5*(404aa)-His-PAO1	-VNWH_6	Non-mucoid
HA-*cupB5*(353aa)-His-PAO1	-GTWH_6	Non-mucoid
HA-*cupB5*(231aa)-His-PAO1	-LNFH_6	Non-mucoid
HA-*cupB5*(176aa)-His-PAO1	-YRFH_6	Non-mucoid
HA-*cupB5*(144aa)-His-PAO1	-VLFH_6	Non-mucoid
HA-*cupB5*(135aa)-His-PAO1	-QVFH_6	Non-mucoid
HA-*cupB5*(132aa)-His-PAO1	-ANGH_6	Non-mucoid

Supplementary Table S2. Strains and plasmids used in this study

Strains and plasmids	Phenotype and genotype	Source or reference
***P. aeruginosa* strains**		
PAO1	Non-mucoid, prototroph	P. Phibbs[a]
PAO1-VE2	PAO1 TA flanked *aacC1* (Gm^R) inserted upstream of *mucE* (PA4033), mucoid	(Qiu *et al.*, 2007)
PAO1-VE22	PAO1 TA flanked *aacC1* (Gm^R) inserted upstream of *cupB5* (PA4082), mucoid	This study
PA14	Non-mucoid, prototroph	F. Ausubel[b]
FRD2	Non-mucoid, derived from *mucA*22 strain FRD1	(Olson and Ohman, 1992)
CF3715	Non-mucoid, clinical strain	D. Speert[c]
CF4009	Non-mucoid, clinical strain	D. Speert[c]
CF2	Non-mucoid, *mucA* mutant, *algU* wild type, derived from clinical mucoid strain	(Yin *et al.*, 2013a)
CF14	Non-mucoid, *mucA* mutant, *algU* mutant, derived from clinical mucoid strain	(Yin *et al.*, 2013b)
CF17	Non-mucoid, *mucA* mutant, *algU* wild type, derived from clinical mucoid strain	(Yin *et al.*, 2013b)
CF4349	Non-mucoid, *mucA* mutant, *algU* wild type, derived from clinical mucoid strain	(Yin *et al.*, 2013b)
PAO1△*algW*	PAO1 *algW*::tet^R, nonmucoid	(Qiu *et al.*, 2007)
PAO1△*mucP*	PAO1 *mucP*::tet^R, nonmucoid	(Qiu *et al.*, 2007)
PAO1△*clpX*	PAO1 *clpX* ::Gm^R	(Qiu *et al.*, 2008b)
PAO1△*clpP*	PAO1 *clpP* ::Gm^R	(Qiu *et al.*, 2008b)
PAO1△*algU*	PAO1 with in-frame deletion of *algU*	(Qiu *et al.*, 2007)
PAO1△*algD*	PAO1 with in-frame deletion of *algD*	This study
***E. coli* strains**		
TOP10	DH5α derivative	Invitrogen
SM10/ l pir	*thi thr leu tonA lacY supE recA* :: RP4-2-Tc :: Mu lpirR6K Km^R	Laboratory strain
Plasmids		
pFAC	Mini-himarI mariner transposon with a selectable marker Gm^R Ap^R	(Wong and Mekalanos, 2000)

pRK2013	Km ^{R} *Tra Mob ColE1*	(Figurski and Helinski, 1979)
pHERD 20T	pUCP20T P_{lac} replaced by fragment of *araC*–P_{BAD} cassette	(Qiu *et al.*, 2008a)
pLP170	8.3-kb, lacZ, ApR, multiple cloning site	Passador Lab[d]
PHERD 20T-HA-*cupB5*-His	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (585	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (531	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (520	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (510	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (507	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (504	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (497	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (491	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (490	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (489	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (488	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (483	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (404	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (353	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (231	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (176	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (144	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (135	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
PHERD 20T-HA-*cupB5* (132	*cupB5* (PA4082) from PAO1 in pHERD20T EcoRI/HindIII	This study
pEX100-Δ*algW*	A 1.4-kb *algW*-flanked fragment with in-frame deletion of *algW* in pEX100 NotI	(Qiu *et al.*, 2007)
pLP170-P_{algW}	Promoter of *algW* (PA4446) from PAO1 in pLP170 EcoRI/HindIII	(T. Ryan Withers *et al.*, 2013)

pLP170-P_{algU}	Promoter of *algU* (PA0762) from PAO1 in pLP170 EcoRI/HindIII	(T. Ryan Withers *et al.*, 2013)
pLP170-P_{algD}	Promoter of *algD* (PA3540) from PAO1 in pLP170 EcoRI/HindIII	(T. Ryan Withers *et al.*, 2013)

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University of British Columbia, CA; [d]University of Rochester, USA.

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