SUPPLEMENTARY INFORMATION

The anti-sigma factor MucA is required for viability in *Pseudomonas aeruginosa*

Melissa C. Schofield, Daniela Rodriguez, Amanda A. Kidman, Erin K Cassin, Lia A. Michaels, Elizabeth A. Campbell, Peter A. Jorth and Boo Shan Tseng

SUPPLEMENTARY METHODS

Media, antibiotics, and antibodies. Bacteria were grown in liquid LB (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) and LSLB (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl). Semi-solid media was prepared by adding 1.5% Bacto agar to LB or 1.0% Noble agar to VBMM (Vogel & Bonner, 1956). Pseudomonas isolation agar (PIA; Hardy Diagnostics), Pseudomonas isolation broth (PIB; US Biological), and synthetic cystic fibrosis medium (SCFM, prepared as previously described (Palmer *et al.*, 2007)) were also used. For experiments involving induction of the *araC*-P_{araBAD} promoter, L-arabinose was used at the indicated concentrations; the *rhaSR*-P_{rhaBAD} promoter, L-rhamnose at 0.05%. Antibiotics were used at the following concentrations: for *E. coli*, 10 mg/L gentamicin, 50 mg/L kanamycin, 50 mg/L carbenicillin, 100 mg/L ampicillin, 10 mg/L tetracycline; for *P. aeruginosa*, 60 mg/L gentamicin for chromosomally integrated strains, 50 mg/L gentamicin for plasmid-borne strains, 300 mg/L carbenicillin, and 100 mg/L tetracycline. Antibodies against RpoD (ThermoFisher) were used at 1/1000 and RNAP (ThermoFisher) at 1/10,000.

Calculation of growth rate. Bacteria were grown overnight on LB plates (unless otherwise indicated) at 37˚C. Single colonies were taken from plates to cultures in appropriate liquid media, and grown with shaking at 250rpm at 37°C until stationary phase. These cultures were then used to seed the cells into a 96 well plate using the indicated medium at OD_{600} of 0.001. Using a Synergy Hybrid HTX Microplate Reader (Bio-Tek Instruments), the OD_{600} was measured every 5 min for 16 h at 37°C with shaking. To calculate growth rates, the slope of exponential growth phase of the growth curve was determined. For normalized growth rates, the rates were normalized to the positive control of the respective experiment.

Construction of strains. Sequenced strains of *P. aeruginosa* were obtained from the Pseudomonas Genome Database (www.pseudomonas.com)(Winsor *et al.*, 2016). Bacterial genomic DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen), and PCR fragments were purified using the Wizard SV Gel and PCR Clean-up System (Promega). To delete genes, allelic exchange was used with the corresponding plasmid containing the deletion allele, as previously described (Hmelo *et al.*, 2015). For miniTn7 and miniCTX, the alleles were chromosomally integrated, as previously described (Choi & Schweizer, 2006, Hoang *et al.*, 2000).

To construct deletion alleles, the regions flanking the gene of interest were produced by PCR of PAO1 gDNA using the respective UpF/UpR and DownF/DownR primer pairs. These fragments were then connected by SOE PCR and inserted into pDONRPEX18Gm using GateWay cloning (Invitrogen) to produce deletion vectors, which were confirmed via Sanger sequencing.

The miniTn7-based plasmid containing the full-length *mucA* allele under its native promoter (pBT399) was constructed via PCR of PAO1 gDNA using OBT603/604B and OBT605B/606B. These fragments were then connected by SOE PCR and inserted into pDONRPUC18T-miniTn7T2-Gm using GateWay cloning. To create the *mucA* truncation series, QuikChange site directed mutagenesis (Agilent Technologies) of pBT399 was used to engineer plasmids with alleles of the desired *mucA* length (pBT406, pBT407, pBT408, pBT410, pBT413, pBT414, pMS009, pMS023, pMS024). To construct *mucA* single amino acid substitutions, the same methods were used with pBT408.

A miniTn7-based plasmid containing the full-length *algU* allele under its native promoter (pAAK014) was constructed via PCR of PAO1 gDNA using OAK12/OAK2. To construct *algU* single amino acid substitutions, PCR of PAO1 gDNA using OAK12 with the respective UpR

primer (OAK20, OAK22, OAK26, or OAK29) and OAK2 with the respective DownR primer (OAK21, OAK23, OAK27, or OAK30) were performed. These fragments were then connected by SOE PCR and inserted into pDONRPUC18T-miniTn7T2-Gm using GateWay cloning.

To construct the strains used in the yeast two-hybrid assays, the open reading frame (ORF) of *algU*, a shortened allele of *mucA* (encoding the first 75 residues), and mutations of *mucA* (*mucA* 1-75 encoding the D15A, E22A, or R42A substitutions) were amplified by PCR and recombined into pDONR221 P1-P2 using BP clonase (Invitrogen). These plasmids were then recombined with the pEXP22 or pEXP32 vectors, resulting in either the Gal4 activation domain-based prey vectors (pAAK001, pAAK003) or the DNA binding domain-based bait vectors (pAAK002, pAAK004, pAAK033, pAAK034, pAAK042). Plasmids were transformed into *Saccharomyces cerevisiae* MaV203 using the S.c. Easy Comp Transformation Kit (ThermoFisher).

A miniTn7-based plasmid with *mucA* under the control of a rhamnose-inducible promoter (pLM7) was constructed via PCR of pBT399 with OLM24/25, followed by restriction digest with HindIII and ApaI (New England Biolabs) and ligation of this PCR product into pJM220.

A miniTn7-based plasmid with *algU* under the control of an arabinose-inducible promoter was made by first inserting *algU* into a GateWay compatible vector using the PCR product of OCT1/2 with PAO1 gDNA. An LR reaction with this vector, pJJH187, and pUC18-miniTn7T2-Gm-GW was performed, resulting in a pDEST construct (pCT2). The *algU* single amino acid substitution at K57A under the control of an arabinose-inducible promoter was constructed via QuikChange on the pCT2 plasmid using OAK22/OAK23 (pMS050).

A miniCTX-based plasmid with *rpoD* under the control of an arabinose-inducible promoter was first made by inserting *rpoD* into a GateWay compatible vector using the PCR product of OMS182/216 with PAO1 gDNA. An LR reaction with this vector, pJJH187, and pDONRminiCTX2T2.1Gm-GW was performed, resulting in pDEST construct (pMS037).

To construct the *algD* reporter strains, a plasmid was constructed containing *gfpmut3* under the control of the *algD* promoter. The promoter is the same as that used by Damron and associates (Damron *et al.*, 2009). pBT435 was constructed via PCR of PAO1 gDNA using OBT846/847 and of pBT212 using OBT342/848. These fragments were then connected by SOE PCR and inserted into pDONR221 P1-P2 using GateWay cloning. An LR reaction was then performed to recombine this plasmid into pUCP22T2-GW, resulting in the final reporter (pBT435). For the vector control plasmid (pBT336), pBT212, pBT244, and pUCP22T2-GW were recombined using Gateway cloning. These plasmids were then transformed into the various strains via electroporation (Choi *et al.*, 2006).

Analysis of whole genome sequencing. *P. aeruginosa* genomes were sequenced using an Illumina NextSeq platform at the Microbial Genome Sequencing Center (Pittsburgh, PA). Sequencing reads were aligned to the appropriate reference genomes (Winsor *et al.*, 2016) for PAO1, PA103, and PAK using Breseq (Deatherage & Barrick, 2014). Mutations detected in Δ*mucA* strains were compared to their respective wild-type parent strains to identify suppressor mutations.

Construction of AlgU-holoenzyme model. The model of AlgU-holoenzyme with promoter DNA was made by superimposing σ^E Region 2 and σ^E Region 4 from the crystal structure of the *E. coli* σ^E with the anti-σ factor RseA (PDB 1OR7)(Campbell *et al.*, 2003) onto the respective domains of σ^{70} from an *E. coli* σ^{70}-holoenzyme crystal structure (PDB 4LJZ)(Bae *et al.*, 2013).

The promoter DNA is a hybrid of DNAs taken from superimpositions of structures of *E. coli* σ^E Region 2 bound to its -10 element (PDB 4LUP)(Campagne *et al.*, 2014) and σ^E Region 4 bound to the -35 element (PDB 2H27)(Lane & Darst, 2006). The remaining promoter region was modeled from the *M. tuberculo*sis RNAP/open complex structure (PDB 6EDT)(Boyaci *et al.*, 2019).

Semi-quantitative Western blots. Western blot quantification was performed using AzureSpot software (Azure BioSystems). All signals were background subtracted. The RpoD signal was then normalized to that of RNAP for the same sample. To determine the relative level, this normalized signal was divided by that of the parental strain within the same replicate.

Table S1. Eliminating alginate biosynthesis does not alleviate *mucA* essentiality.

PAO1	Number of isolates resolved to	
	WT	Δ*mucA*
WT	168	0*
Δ*algD*	144	0*
Δ*algB*	158	0*
Δ*algR*	153	0*
Δ*amrZ*	131	0*
Δ*algB* Δ*algR* Δ*amrZ*	133	0*

* $p < 0.0001$, Fischer's exact test

Table S2. Description of changes found in *algU* of PAO1 Δ*mucA att*Tn7::P_{rhaBAD}-*mucA* revertants that can grow in the absence of *mucA* expression.

	Isolate	Genomic Change to *algU*[†]	Effect on AlgU[‡]
Multi-base pair deletions			
	#1-10	831242-831394 del	No product made
	#2-9	831298-831491 del	No product made
	#2-7	831467-831473 del	I56fs X97
	#2-11	831469-831502 del	K57fs X88
	#2-6	831595-831715 del	V99fs X107
Single base pair deletions			
	#2-14	831467 del	I56fs X99
	#1-1	831510 del	Y72fs X99
	#2-10	831538 del	I80fs X99
Nonsense mutations			
	#2-1	831433 C>T	Q45X
	#2-8	831521 G>A	W74X
Multi-base pair insertions			
	#1-9	831427-831435 ins GACGCCCAG	D43-A44 ins DAQ
	#1-8	831430-831438 ins GCCCAGGAA	A44-Q45 ins AQE
	#1-11	831436-831444 ins GAAGCCCAG	E46-A47 ins EAQ
	#2-4	831436-831444 ins GAAGCCCAG	E46-A47 ins EAQ
	#1-5	831445-831456 ins GACGTAGCCCAG	D49-V50 ins DVAQ
	#1-6	831451-831459 ins GCCCAGGAA	A51-Q52 ins AQE
Missense mutations			
	#1-3	831353 A>G	D18G
	#2-12	831362 C>T	A21V
	#2-3	831386 A>G	Y29C
	#2-2	831439 G>A	A47T
	#1-2	831446 A>G	D49G
	#2-13	831446 A>G	D49G
	#1-7	831476 A>G	Y59C
	#2-5	831541 A>G	N81D
	#1-4	831820 C>G	R174G

[†] genomic location based on the Pseudomonas Genome Database (Winsor *et al.*, 2016) for PAO1; bp, base pair; del, deletion; ins, insertion.
[‡] fs, frameshift; ins, insertion; X, stop codon.

Table S3. Statistical differences in growth rate among groups under conditions of *algU* induction.

Medium: LB	Strain	*att*Tn7::P_{araBAD}-*algU*				Δ*algU* *att*Tn7::P_{araBAD}-*algU*				Δ*algU* Δ*mucA* *att*Tn7::P_{araBAD}-*algU*			
Strain	Ara	0	0.1	0.25	1	0	0.1	0.25	1	0	0.1	0.25	1
*att*Tn7:: P_{araBAD}-*algU*	0		*	*	*								
	0.1				*								
	0.25											*	
	1								*				*
Δ*algU* *att*Tn7:: P_{araBAD}-*algU*	0							*	*				
	0.1							*	*		*		
	0.25								*			*	
	1												
Δ*algU* Δ*mucA* *att*Tn7:: P_{araBAD}-*algU*	0										*	*	*
	0.1											*	*
	0.25												
	1												

Medium: PIB	Strain	*att*Tn7:: P_{araBAD}-*algU*		Δ*algU* *att*Tn7:: P_{araBAD}-*algU*		Δ*algU* Δ*mucA* *att*Tn7:: P_{araBAD}-*algU*	
Strain	Ara	0	1	0	1	0	1
*att*Tn7:: P_{araBAD}-*algU*	0		*				
	1						
Δ*algU* *att*Tn7:: P_{araBAD}-*algU*	0				*		
	1						
Δ*algU* Δ*mucA* *att*Tn7:: P_{araBAD}-*algU*	0						*
	1						

Medium: SCFM	Strain	*att*Tn7:: P_{araBAD}-*algU*		Δ*algU* *att*Tn7:: P_{araBAD}-*algU*		Δ*algU* Δ*mucA* *att*Tn7:: P_{araBAD}-*algU*	
Strain	Ara	0	1	0	1	0	1
*att*Tn7:: P_{araBAD}-*algU*	0		*				
	1						
Δ*algU* *att*Tn7:: P_{araBAD}-*algU*	0				*		
	1						
Δ*algU* Δ*mucA* *att*Tn7:: P_{araBAD}-*algU*	0						*
	1						

Medium: VBMM	Strain	*att*Tn7:: P_{araBAD}-*algU*		Δ*algU* *att*Tn7:: P_{araBAD}-*algU*		Δ*algU* Δ*mucA* *att*Tn7:: P_{araBAD}-*algU*	
Strain	Ara	0	1	0	1	0	1
*att*Tn7:: P_{araBAD}-*algU*	0		*			*	
	1						*
Δ*algU* *att*Tn7:: P_{araBAD}-*algU*	0				*		
	1						*
Δ*algU* Δ*mucA* *att*Tn7:: P_{araBAD}-*algU*	0						*
	1						

Significance was determined via a two-way ANOVA with post-hoc Bonferroni. Ara, concentration of arabinose (%). Asterisk, $p < 0.05$. Open box, no statistical difference.

Table S4. Statistical differences in growth rate among groups overexpressing *algU* and/or *rpoD*.

	Strain	Δ*algU*Δ*mucA* P_{BAD}-*rpoD*		Δ*algU*Δ*mucA* P_{BAD}-*algU*^{WT}		Δ*algU*Δ*mucA* P_{BAD}-*algU*^{WT} P_{BAD}-*rpoD*		Δ*algU*Δ*mucA* P_{BAD}-*algU*^{K57A}		Δ*algU*Δ*mucA* P_{BAD}-*algU*^{K57A} P_{BAD}-*rpoD*	
Strain	Ara	0	2	0	2	0	2	0	2	0	2
Δ*algU*Δ*mucA*	0										
	2				*				*		
Δ*algU*Δ*mucA* P_{BAD}-*rpoD*	0										
	2				*				*		
Δ*algU*Δ*mucA* P_{BAD}-*algU*^{WT}	0				*						
	2						*		*		*
Δ*algU*Δ*mucA* P_{BAD}-*algU*^{WT} P_{BAD}-*rpoD*	0						*				
	2										
Δ*algU*Δ*mucA* P_{BAD}-*algU*^{K57A}	0								*		
	2										
Δ*algU*Δ*mucA* P_{BAD}-*algU*^{K57A} P_{BAD}-*rpoD*	0										*
	2										

Significance was determined via a two-way ANOVA with post-hoc Bonferroni. Ara, concentration of arabinose (%). Asterisk, $p < 0.05$. Open box, no statistical difference.

Table S5. Bacterial strains used in this study.

Strains	Relevant characteristics	Source
Escherichia coli		
NEB5α	For cloning; *fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80Δ (lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	NEB
ccdB Survival2 T1R	F⁻ *mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araΔ139 Δ(ara-leu)7697 galU galK rpsL endA1 nupG fhuA::IS2*, Sm^r	Invitrogen
S17.1 (λ_{pir})	For conjugation; F⁻ RP4-2-Tc::Mu *aphA*::Tn7 *recA* λ_{pir} lysogen, Sm^r, Tc^r	(Kristensen *et al.*, 1995)
Pseudomonas aeruginosa		
PAO1	Wild-type PAO1	(Holloway, 1955)
PAO1 Δ*mucA*	PAO1 containing an unmarked deletion of *mucA*	(Pritchett *et al.*, 2015)
PA103	Wild-type PA103	(Frank *et al.*, 1994)
PA103 Δ*mucA*	PA103 containing nonpolar deletion of *mucA*	(Intile *et al.*, 2014)
PAK	Wild-type PAK	(Takeya & Amako, 1966)
PAK	*att*CTX::lacP1 Δlac1-lac2	(Fulcher *et al.*, 2010)
PAK Δ*mucA*	Δ*mucA att*CTX::lacP1 Δlac1-lac2	(Jones *et al.*, 2010)
PA14	Wild-type PA14	(Rahme *et al.*, 1995)
CF127	Mucoid CF isolate	(Wolfgang *et al.*, 2003)
CF18	Non-mucoid CF isolate	(Wolfgang *et al.*, 2003)
CF27	Rugose CF isolate	(Wolfgang *et al.*, 2003)
X13273	Blood isolate	(Wolfgang *et al.*, 2003)
X24509	UTI isolate	(Wolfgang *et al.*, 2003)
MSH10	Water isolate	(Wolfgang *et al.*, 2003)
E2	Tomato plant isolate	(Wolfgang *et al.*, 2003)
BTPa156	PAO1 containing an in-frame deletion of *algD*	(Tseng *et al.*, 2013)
BTPa669	PAO1 containing an in-frame deletion of *algB*	This study
BTPa671	PAO1 containing an in-frame deletion of *amrZ*	This study
BTPa675	PAO1 containing an in-frame deletion of *algR*	This study
BTPa685	PAO1 containing an in-frame deletions of *algB, amrZ,* and *algR*	This study
BTPa391	PAO1 Δ*algU* Δ*mucA*	This study
BTPa355	PAO1 Δ*mucA att*Tn7::P_{algU}-*mucA*(1-194)-10His	This study
BTPa628	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-194)-10His	This study
BTPa715	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-143)-10His	This study
BTPa620	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-110)-10His	This study
BTPa622	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-75)-10His	This study

BTPa601	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-62)-10His	This study
BTPa603	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-50)-10His	This study
BTPa605	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-40)-10His	This study
BTPa606	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-24)-10His	This study
BTPa673	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(51-194)-10His	This study
BTPa832	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-75) D15A-10His	This study
BTPa844	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-75) E22A-10His	This study
BTPa835	PAO1 Δ*algD att*Tn7::P_{algU}-*mucA*(1-75) R42A-10His	This study
BTPa643	PAO1 Δ*mucA* Δ*algD att*Tn7::P_{algU}-*mucA*(1-194)-10His	This study
BTPa635	PAO1 Δ*mucA* Δ*algD att*Tn7::P_{algU}-*mucA*(1-110)-10His	This study
BTPa762	PAO1 Δ*mucA* Δ*algD att*Tn7::P_{algU}-*mucA*(1-143)-10His	This study
BTPa637	PAO1 Δ*mucA* Δ*algD att*Tn7::P_{algU}-*mucA*(1-75)-10His	This study
BTPa639	PAO1 Δ*mucA* Δ*algD att*Tn7::P_{algU}-*mucA*(1-62)-10His	This study
BTPa641	PAO1 Δ*mucA* Δ*algD att*Tn7::P_{algU}-*mucA*(1-50)-10His	This study
BTPa548	PAO1 Δ*mucA* Δ*algD att*Tn7::P_{algU}-*mucA*(1-75) E22A-10His	This study
BTPa812	PAO1 *att*Tn7::P_{algU}-*mucA*(1-155)-10His	This study
BTPa816	PAO1 Δ*mucA att*Tn7::P_{algU}-*mucA*(1-155)-10His	This study
BTPa382	PAO1 Δ*algU*	This study
BTPa740	PAO1 Δ*algU att*Tn7::P_{algU}-*algU*	This study
BTPa748	PAO1 Δ*algU att*Tn7::P_{algU}-*algU* K57A	This study
BTPa754	PAO1 Δ*algU att*Tn7::P_{algU}-*algU* N81A	This study
BTPa846	PAO1 Δ*algU att*Tn7::P_{algU}-*algU* E46G	This study
BTPa757	PAO1 Δ*algU att*Tn7::P_{algU}-*algU* A58T	This study
BTPa763	PAO1 Δ*algU* Δ*mucA att*Tn7::P_{algU}-*algU* K57A	This study
BTPa764	PAO1 Δ*algU* Δ*mucA att*Tn7::P_{algU}-*algU* N81A	This study
BTPa849	PAO1 Δ*algU* Δ*mucA att*Tn7::P_{algU}-*algU* E46G	This study
BTPa837	PAO1 Δ*algU* Δ*mucA att*Tn7::P_{algU}-*algU* A58T	This study
BTPa616	PAO1 *att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His	This study
BTPa624	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His	This study
BTPa646	PAO1 *att*Tn7::P_{araBAD}-*algU*-10His::Gm	This study
BTPa527	PAO1 Δ*algU att*Tn7::P_{araBAD}-*algU*::Gm	This study
BTPa529	PAO1 Δ*algU* Δ*mucA att*Tn7::P_{araBAD}-*algU*::Gm	This study
BTPa853	PAO1 Δ*algU* Δ*mucA att*CTX2::P_{araBAD}-*rpoD*	This study
BTPa855	PAO1 Δ*algU* Δ*mucA att*CTX2::P_{araBAD}-*rpoD att*Tn7::P_{BADara}-*algU*::Gm	This study
BTPa859	PAO1 Δ*algU* Δ*mucA att*Tn7::P_{araBAD}-*algU* K57A::Gm	This study
BTPa861	PAO1 Δ*algU* Δ*mucA att*CTX2::P_{araBAD}-*rpoD att*Tn7::P_{BADara}-*algU* K57A::Gm	This study
BTPa149	PAO1 pBT336 (vector control)	This study
BTPa550	PAO1 pBT435	This study
BTPa559	PAO1 Δ*algU* pBT435	This study
BTPa552	PAO1 Δ*algU att*Tn7::P_{algU}-*algU* pBT435	This study
BTPa553	PAO1 Δ*algU att*Tn7::P_{algU}-*algU* K57A pBT435	This study
BTPa554	PAO1 Δ*algU att*Tn7::P_{algU}-*algU* N81A pBT435	This study
BTPa555	PAO1 Δ*algU att*Tn7::P_{algU}-*algU* E46G pBT435	This study
BTPa556	PAO1 Δ*algU att*Tn7::P_{algU}-*algU* A58T pBT435	This study
BTPa557	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His pBT435	This study
MSPa82	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-10	This study
MSPa83	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-2	This study

MSPa84	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-3	This study
MSPa85	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-4	This study
MSPa86	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-5	This study
MSPa87	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-6	This study
MSPa88	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-7	This study
MSPa90	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-8	This study
MSPa91	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-9	This study
MSPa92	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-10	This study
MSPa93	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #1-11	This study
MSPa94	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-1	This study
MSPa95	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-2	This study
MSPa96	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-3	This study
MSPa98	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-4	This study
MSPa100	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-5	This study
MSPa101	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-6	This study
MSPa102	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-7	This study
MSPa103	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-8	This study
MSPa106	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-9	This study
MSPa107	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-10	This study
MSPa109	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-11	This study
MSPa111	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-12	This study
MSPa113	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-13	This study
MSPa114	PAO1 Δ*mucA att*Tn7::*rhaSR*-P_{rhaBAD}-*mucA*-10His revertant; #2-14	This study
MSPa130	MSPa82 pBT435	This study
MSPa131	MSPa83 pBT435	This study
MSPa132	MSPa84 pBT435	This study
MSPa133	MSPa85 pBT435	This study
MSPa134	MSPa86 pBT435	This study
MSPa135	MSPa87 pBT435	This study
MSPa136	MSPa88 pBT435	This study
MSPa137	MSPa90 pBT435	This study
MSPa138	MSPa91 pBT435	This study
MSPa139	MSPa92 pBT435	This study
MSPa140	MSPa93 pBT435	This study
MSPa141	MSPa94 pBT435	This study
MSPa142	MSPa95 pBT435	This study
MSPa143	MSPa96 pBT435	This study
MSPa144	MSPa98 pBT435	This study
MSPa145	MSPa100 pBT435	This study
MSPa146	MSPa101 pBT435	This study
MSPa147	MSPa102 pBT435	This study
MSPa148	MSPa103 pBT435	This study
MSPa149	MSPa106 pBT435	This study
MSPa150	MSPa107 pBT435	This study
MSPa151	MSPa109 pBT435	This study
MSPa152	MSPa111 pBT435	This study
MSPa153	MSPa113 pBT435	This study

MSPa154	MSPa114 pBT435	This study
Saccharomyces cerevisiae		
MaV203	*MATα, leu2-3,112, trp1-901, his3Δ200, ade2-101, gal4Δ, gal80Δ, SPAL10::URA3, GAL1::lacZ, HIS3::HIS3@LYS2, can1R, cyh2R*	Invitrogen
BTSc1-4	MaV203 containing pEXP32-Krev1 and pEXP22-RalGDS-WT	This study
BTSc9-12	MaV203 containing pEXP32-Krev1 and pEXP22-RalGDS-m2	This study
BTSc13-16	MaV203 containing pDEST32 and pDEST22	This study
BTSc20,41-44	MaV203 containing pAAK004 and pAAK001	This study
BTSc21-24	MaV203 containing pAAK002 and pAAK003	This study
BTSc25-28	MaV203 containing pAAK002 and pDEST22	This study
BTSc29-32	MaV203 containing pAAK004 and pDEST22	This study
BTSc33-36	MaV203 containing pDEST32 and pAAK003	This study
BTSc37-40	MaV203 containing pDEST32 and pAAK001	This study
BTSc68-71	MaV203 containing pAAK033 and pAAK001	This study
BTSc76-79	MaV203 containing pAAK034 and pAAK001	This study
BTSc131-134	MaV203 containing pAAK042 and pAAK001	This study

Table S6. Plasmids used in this study.

Plasmids	Relevant characteristics	Source
pDONR221 P5-P2	GateWay-compatible vector with attP5 and attP2 recombination sites and *ccdB*; Kn^r, Cm^r	Invitrogen
pDONRPEX18Gm	pEX18-based, GateWay-compatible suicide vector (Accession No. KM880128) with *att*P1 and *att*P2 recombination sites and *ccdB*; Gm^r, Cm^r	(Hmelo *et al.*, 2015)
pUC18-miniTn7T2-Gm-GW	GateWay-compatible miniTn7 vector with *att*R1 and *att*R2 recombination sites and *ccdB*; Ap^r, Gm^r, Cm^r	(Zhao *et al.*, 2013)
pDONRPUC18T-miniTn7T2-Gm	GateWay-compatible miniTn7 vector with *att*P1 and *att*P2 recombination sites and *ccdB*; Ap^r, Gm^r, Cm^r	(Tseng *et al.*, 2018)
pDONR-miniCTX2T2.1	GateWay-compatible miniCTX2 vector with *att*P1 and *att*P2 recombination sites and *ccdB*; Tc^r	Joe J. Harrison
pUCP22T2-GW	GateWay-compatible pUCP22 vector with attR1 and attR2 recombination sites and *ccdB*; Ap^r, Gm^r, Cm^r	(Almblad *et al.*, 2015)
pTNS2	T7 transposase expression vector; Ap^r	(Choi & Schweizer, 2006)
pFLP2	Plasmid that expresses Flp recombinase; Ap^r	(Choi & Schweizer, 2006)
pJJH187	pDONR221P1P5r-based Gateway-compatible vector encoding the *araC* repressor protein and the P_{araBAD} promoter; Kn^r	(Khakimova *et al.*, 2013)
pJM220	pUC18T-miniTn7T based vector encoding *rhaSR* and the P_{rhaBAD} promoter; Gm^r	(Meisner & Goldberg, 2016)
pBX39	pEX18Ap with an in-frame deletion of *amrZ*; Ap^r	(Xu & Wozniak, 2015)
pDEST22	Gal4 activation domain-based prey vector for Yeast Two-Hybrid system; Ap^r, Cm^r	Invitrogen
pDEST32	Gal4 DNA binding domain-based bait vector for Yeast Two-Hybrid system; Gm^r, Cm^r	Invitrogen
pDEST22-Krev1	Two-hybrid control plasmid containing full-length rat Krev1; Ap^r	Invitrogen
pDEST32-RalGDS-WT	Two-hybrid control plasmid containing wild-type RalGDS domain; Gm^r	Invitrogen
pDEST32-RalGDS-m2	Two-hybrid control plasmid containing mutated RalGDS domain; Gm^r	Invitrogen
pBT212	GateWay compatible plasmid containing *gfpmut3* flanked by attR5 and attL1 recombination sites; Kn^r	(Armbruster *et al.*, 2019)
pBT244	GateWay-compatible plasmid containing a null promoter flanked by attL2 and attL5 recombination sites; Kn^r	(Tseng *et al.*, 2013)
pBT336	pUCP22T2.1 containing *gfpmut3* driven by a null promoter; Ap^r, Gm^r	This study
pBT342	pDONRPEX18Gm with an in-frame deletion allele of *algD*; Gm^r	(Tseng *et al.*, 2013)
pBT396	pDONRPEX18Gm with an in-frame deletion allele of *mucA*; Gm^r	This study
pBT399	pUC18-miniTn7T2 with an allele encoding all 194 aa of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pBT406	pUC18-miniTn7T2 with an allele encoding the first 50 aa of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pBT407	pUC18-miniTn7T2 with an allele encoding the first 110 aa of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pBT408	pUC18-miniTn7T2 with an allele encoding the first 75 aa of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study

pBT410	pUC18-miniTn7T2 with an allele encoding the first 62 aa of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pBT411	pDONRPEX18Gm with an in-frame deletion allele of *algU*; Gm^r	This study
pBT412	pDONRPEX18Gm with an in-frame deletion allele of *mucA*, for use in a Δ*algU* background; Gm^r	This study
pBT413	pUC18-miniTn7T2 with an allele encoding the first 40 aa of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pBT414	pUC18-miniTn7T2 with an allele encoding the first 24 aa of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pBT435	pUCP22T2.1 containing *gfpmut3* driven by the *algD* promoter; Ap^r, Gm^r	This study
pMS009	pUC18-miniTn7T2 with an allele encoding the aa 51-195 of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pMS014	pDONRPEX18Gm with an in-frame deletion allele of *algB*; Gm^r	This study
pMS015	pDONRPEX18Gm with an in-frame deletion allele of *algR*; Gm^r	This study
pMS023	pUC18-miniTn7T2 with an allele encoding the first 143 aa of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pMS024	pUC18-miniTn7T2 with an allele encoding the first 155 aa of *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pMS034	pUC18-miniTn7T2 with an allele encoding an R42A substitution in *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pMS035	pUC18-miniTn7T2 with an allele encoding a D15A substitution in *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pMS037	miniCTX2T2.1-GW with an allele encoding *rpoD* driven by an arabinose-inducible promoter; Tc^r	This study
pMS039	pUC18-miniTn7T2 with an allele encoding an E22A substitution in *mucA*, driven by its native promoter; Ap^r, Gm^r	This study
pMS041	pUC18-miniTn7T2 with an allele encoding an E46G substitution in *algU*, driven by its native promoter; Ap^r, Gm^r	This study
pMS050	pUC18-miniTn7T2 with an allele encoding an K57A substitution in *algU*, driven by an arabinose-inducible promoter; Ap^r, Gm^r	This study
pAAK001	pDEST22 based expression plasmid encoding AlgU fused to the Gal4 activating domain	This study
pAAK002	pDEST32 based expression plasmid encoding AlgU fused to the Gal4 DNA binding domain	This study
pAAK003	pDEST22 based expression plasmid encoding the first 75 aa of MucA fused to the Gal4 activating domain	This study
pAAK004	pDEST32 based expression plasmid encoding the first 75 aa of MucA fused to the Gal4 activating domain	This study
pAAK014	pUC18-miniTn7T2 with an allele encoding full length *algU*, driven by its native promoter; Ap^r, Gm^r	This study
pAAK018	pUC18-miniTn7T2 with an allele encoding an N81A substitution in *algU*, driven by its native promoter; Ap^r, Gm^r	This study
pAAK020	pUC18-miniTn7T2 with an allele encoding an K57A substitution in *algU*, driven by its native promoter; Ap^r, Gm^r	This study
pAAK021	pUC18-miniTn7T2 with an allele encoding an A58T substitution in *algU*, driven by its native promoter; Ap^r, Gm^r	This study
pAAK033	pDEST32 based expression plasmid encoding the first 75 aa of MucA with an R42A substitution fused to the Gal4 activating domain	This study

pAAK034	pDEST32 based expression plasmid encoding the first 75 aa of MucA with an D15A substitution fused to the Gal4 activating domain	This study
pAAK042	pDEST32 based expression plasmid encoding the first 75 aa of MucA with an E22A substitution fused to the Gal4 activating domain	This study
pCT2	pUC18-miniTn7T2 with an allele encoding AlgU, driven by an arabinose-inducible promoter; Ap^r, Gm^r	This study
pLM7	pUC18T-miniTn7T with an allele encoding MucA, driven by a rhamnose-inducible promoter; Gm^r	This study

Table S7. Oligonucleotides used in this study.

Primer	Sequence
OBT401 (*algD* SeqF)	AGCCCTTGTGGCGAATAG
OBT402 (*algD* SeqR)	GCTTGTGCCACTCGCTC
OMS103 (*algR* SeqF)	CTCGAGGCTGGCGTAGGTG
OMS104 (*algR* UpF)	ggggacaagtttgtacaaaaaagcaggctcaGCAGATGGTGTAGAGGCCGAG
OMS105 (*algR* UpR)	AGGCGTCTGATGCATCAGCTC
OMS106 (*algR* DownF)	gagctgatgcatcagacgcctCATAAGCTCAGGCTTCCTGCATG
OMS107 (*algR* DownR)	ggggaccactttgtacaagaaagctgggtaCAACTGGACTGGCAGGTGCAC
OMS108 (*algR* SeqR)	GCTGGACCTGTCCGACCTGTTC
OMS109 (*algB* SeqF)	GGTAGCGAACCGGCCTGTGTC
OMS110 (*algB* UpF)	ggggacaagtttgtacaaaaaagcaggctcaCCGCATGCCAGCCTTTCTG
OMS111 (*algB* UpR)	CAGCAGGATGCGCCCCTG
OMS112 (*algB* DownF)	caggggcgcatcctgctgCAGTACGGCCTATGAGCATGC
OMS113 (*algB* DownR)	ggggaccactttgtacaagaaagctgggt*att*CGCTGAAGCCGTCGTTGTC
OMS114 (*algB* SeqR)	CTTCGCTGATGCCGGCCAG
OMS115 (*amrZ* SeqF)	GATGCTGCCGCCGTTGTC
OMS116 (*amrZ* SeqR)	CCAGCCTACG*ATT*CGCCTC
OBT597 (*mucA* UpF)	ggggacaagtttgtacaaaaaagcaggctcaCAGAGGATGCGGAGTTCTTCGAG
OBT598 (*mucA* UpR)	ctcctcagcggttttccaggctCTGCAGGGCTTCACGACTCATAG
OBT599 (*mucA* DownF)	ctatgagtcgtgaagccctgcagAGCCTGGAAAACCGCTGAGGAG
OBT600 (*mucA* DownR)	ggggaccactttgtacaagaaagctgggtaCTGGGCATCGGCCAGTTGGTC
OBT601 (*mucA* SeqF)	GCCATCAACACCGCGAAGAAC
OBT602 (*mucA* SeqR)	TCGTACCAGGAAGCCAGCTG
OBT603 (*algU* promoter UpF)	ggggacaagtttgtacaaaaaagcaggctcaCGCTGCCGATGACCAGTACATC
OBT604B (*algU* promoter UpR)	cttctcgcaacaaaggctgcagTTGCTGATCCTGTTCCTGGGTTAGC
OBT605B (*mucA* DownF)	gctaacccaggaacaggatcagcaaCTGCAGCCTTTGTTGCGAGAAG
OBT606B (*mucA* DownR)	GGGGACCACTTTGTACAAGAAAGCTGGGT*Attatt*agtggtggtgatggtgatgatggtggtgatggctgctgccGTCTCTCCTCAGCGGTTTTCCAG
OBT620 (MucA 1-194 aa)	CAGCCTGGAAAACCGCGGCAGCAGCCATCAC
OBT621 (MucA 1-194 aa)	GTGATGGCTGCTGCCGCGGTTTTCCAGGCTG
OBT622 (MucA 1-50 aa)	GGCGCGGTCCGTCATGGGCAGCAGCCATCAC
OBT623 (MucA 1-50 aa)	GTGATGGCTGCTGCCCATGACGGACCGCGCC
OBT624 (MucA 1-110 aa)	GTACAACCAGAACGACGGCAGCAGCCATCAC
OBT625 (MucA 1-110 aa)	GTGATGGCTGCTGCCGTCGTTCTGGTTGTAC
OBT673 (MucA 1-75 aa)	GACGAGGCCGCTCCGGGCAGCAGCCATCAC
OBT674 (MucA 1-75 aa)	GTGATGGCTGCTGCCCGGAGCGGCCTCGTC
OBT679 (MucA 1-62 aa)	GAAGCTGGATATCGCTGGCAGCAGCCATCAC
OBT680 (MucA 1-62 aa)	GTGATGGCTGCTGCCAGCGATATCCAGCTTC
OBT681 (*algU* UpF)	ggggacaagtttgtacaaaaaagcaggctcgGTGCTGGAACTTTCTTAGACGCATC
OBT682 (*algU* UpR)	gtgtcaggcttctcgcaacaaaggCTGATCCTGTTCCTGGGTTAGCATG
OBT683 (*algU* DownF)	catgctaacccaggaacaggatcagCCTTTGTTGCGAGAAGCCTGACAC
OBT684 (*algU* DownR)	ggggaccactttgtacaagaaagctgggtaTGGTCCCCTGTTGCGCCATTTG
OBT685 (*algU* SeqF)	ATGAGCTGCGGGCCTGTCATC
OBT686 (*algU* SeqR)	GGACGAGGAGTTGGTGATCACCTG

OBT687 (*mucA* UpF for Δ*algU*)	ggggacaagtttgtacaaaaaagcaggctcaGTTCCAAAGCAGGATGCCTGAAGAC
OBT690 (MucA 1-40 aa)	CTGCGTTCCACCTGGGGCAGCAGCCATCAC
OBT691 (MucA 1-40 aa)	GTGATGGCTGCTGCCCCAGGTGGAACGCAG
OBT694 (MucA 1-24 aa)	GAACTCGAGTTGCGGGGCAGCAGCCATCAC
OBT695 (MucA 1-24 aa)	GTGATGGCTGCTGCCCCGCAACTCGAGTTC
OMS077 (MucA 51-195 aa)	gctaacccaggaacaggatcagcaaCACCGCGAGCCTACCCTG
OMS078 (MucA 51-195 aa)	GGGGACCACTTTGTACAAGAAAGCTGGGT*Attatt*agtggtggtgatggtgatgatggtggtgatggctgctgccGCGGTTTTCCAGGCTGGCTG
OMS118 (*mucA* UpF)	CAGAGGATGCGGAGTTCTTCGAG
OMS119 (*mucA* DownR)	CTGGGCATCGGCCAGTTGGTC
OMS132 (MucA 1-143 aa)	CTACAGCGAAGAGCAGGGGGGCAGCAGCCATCAC
OMS133 (MucA 1-143 aa)	GTGATGGCTGCTGCCCCCCTGCTCTTCGCTGTAG
OMS158 (MucA 1-155 aa)	GGGGACCACTTTGTACAAGAAAGCTGGGT*Attatt*agtggtggtgatggtgatgatggtggtgatggctgctgccATCGCTGGACGAGGAGTTGGT
OMS173 (MucA R42A)	GAGCTGCGTTCCACCTGGTCGGCCTACCAGTTGGCGCGGTCCGTC
OMS174 (MucA R42A)	GACGGACCGCGCCAACTGGTAGGCCGACCAGGTGGAACGCAGCTC
OMS175 (MucA D15A)	CAGGAAACTCTGTCCGCTGTGATGGCCAACGAAGCGGATGAACTCGAG
OMS176 (MucA D15A)	CTCGAGTTCATCCGCTTCGTTGGCCATCACAGCGGACAGAGTTTCCTG
OMS214 (MucA E22A)	GAAGCGGATGAACTCGCCTTGCGGCGGGTGCTC
OMS215 (MucA E22A)	GAGCACCCGCCGCAAGGCGAGTTCATCCGCTTC
OMS218 (AlgU E46G)	GCCCTGGGCGTCGTGCACGAACCGCAC
OMS182 (*rpoD* DownR)	ggggaccactttgtacaagaaagctgggtagc*att*tgccgctgtgTCACTCGTCGAGGAAGGAGCGAAG
OMS216 (*rpoD* UpF)	ggggacaactttgtatacaaaagttgcgaaagaggaggt*att*aagcATGTCCGGAAAAGCGCAACAGCA
OAK2 (*algU* DownR)	GGGGACCACTTTGTACAAGAAAGCTGGGT*Attatt*aAGCGTAATCTGGAACATCGTATGGGTAgctgctgccGGCTTCTCGCAACAAAGGCTG
OAK12 (*algU* promoter UpF)	ggggacaagtttgtacaaaaaagcaggctcaCCTTGCTGAGGACGGCGATG
OAK20 (AlgU N81A UpR)	GTTCTTCGCGGTGGCGATGGCGATCCGATAC
OAK21 (AlgU N81A DownF)	GTATCGGATCGCCATCGCCACCGCGAAGAAC
OAK22 (AlgU K57A UpR)	AGCGCACGGTATGCGGCGATGAAGGCTTC
OAK23 (AlgU K57A DownF)	GAAGCCTTCATCGCCGCATACCGTGCGCT
OAK26 (AlgU A58T UpR)	GAGCGCACGGTAGGTCTTGATGAAGGCTTC
OAK27 (AlgU A58T DownF)	GAAGCCTTCATCAAGACCTACCGTGCGCTC
OAK29 (AlgU E46G DownF)	CTACGTCCTGGGCGCCCTGGGCGTCGTG
OAK30 (AlgU E46G UpR)	CACGACGCCCAGGGCGCCCAGGACGTAG
OCT1 (AlgU UpF)	GGGGACAACTTTGTATACAAAAGTTGCGaaagaggaggt*att*aagcATGCTAACCCAGGAACAGGATCAG
OCT2 (AlgU DownR)	GGGGACCACTTTGTACAAGAAAGCTGGGT*Attatt*agtggtggtgatggtgatgatggtggtgatggctgctgccGGCTTCTCGCAACAAAGGCTG
OLM24 (MucA Fwd)	TACAGTaagcttGCCTTTGTTGCGAGAAGCCTGAC
OLM25 (MucA Rev)	TGCTTAgggcccGCCTGCAGGTCGACTCTAGAG
OBT342 (GFP DownR)	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGTTACTTGTACAGCTCGTCCATGCC
OBT846 (*algD* promoter UpF)	ggggACCACTTTGTACAAGAAAGCTGGGTaCTGATGAAGCTTTCCGACAAGCTC
OBT847 (*algD* promoter UpR)	cagtgaaaagttcttctcctttacgcatCGC*ATT*CACCTCG*ATT*GTTTGTCG
OBT848 (GFP DownF)	ATGCGTAAAGGAGAAGAACTTTTCACTG

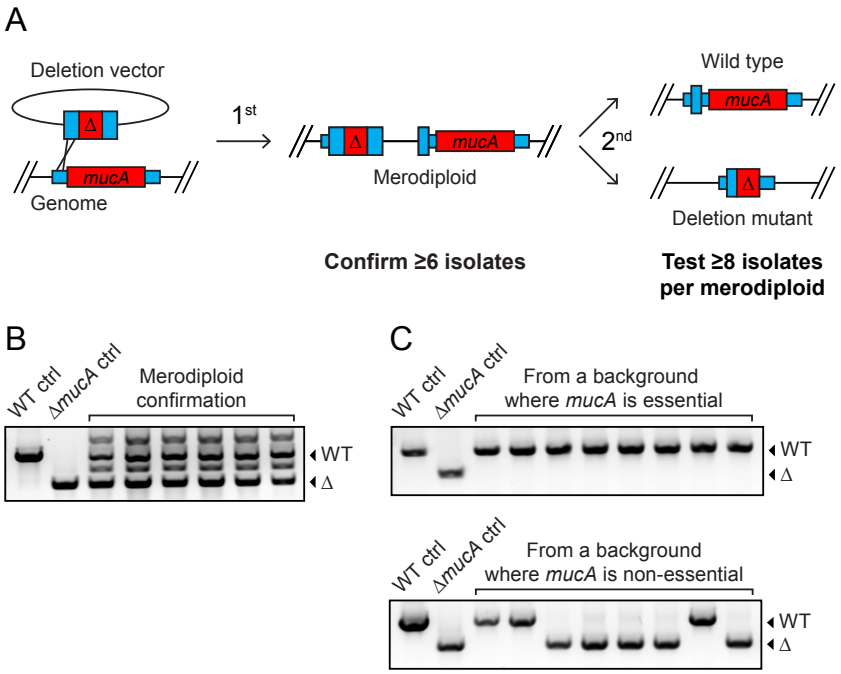

Figure S1. Allelic exchange assay.
(A) Schematic of assay. To delete the endogenous *mucA* (short red box with *mucA*) from the genome, the deletion vector pBT396, which contains an allele of *mucA* that is missing >95% of the coding region (tall red box with delta), was introduced into *P. aeruginosa* via conjugation. Regions of homology (tall blue boxes) to the genome (short blue boxes) approximately 400 base pairs in length flank the deletion allele and allow for recombination into the genome (X) to create a merodiploid. This first recombination event (1st) can be selected for using antibiotics due to integration of the vector backbone, which contains a gentamicin resistance marker. At least six merodiploids were confirmed via PCR to ensure the genome contained both the deletion and endogenous *mucA* allele. Merodiploids can then undergo a second recombination event (2nd) that will lead to the loss of one of the two alleles, resolving to either the endogenous or deletion allele, which results in a cell that is either wild-type (top) or a deletion mutant (bottom), respectively. For each of the six confirmed merodiploids, we counter-selected for the second recombination event via the loss of *sacB*, a vector backbone marker. Using PCR, eight isolates per merodiploid were tested to determine which allele each isolate resolved to. If a gene is non-essential, we expect to observe both wild type or deletion mutants. However, if a gene is essential, we expect to isolate only those cells that resolved to wild type, as the cells cannot survive with the deletion allele. If we are unable to delete *mucA* from the first replicate of this experiment, we perform two additional biological replicates, for a minimum total of 125 colonies screened. If all isolates resolve to wild-type, we deem *mucA* to be essential in that strain background ($p < 0.0001$, Fisher's exact test). **(B)** Representative image of merodiploid confirmation. The PCR products corresponding to the endogenous allele and the deletion allele are indicated with an arrow labelled "WT" and "Δ," respectively. Positive (WT ctrl; PAO1) and negative (Δ*mucA* ctrl; BTPa355) controls are included. PCR products from six representative merodiploids are shown. **(C)** Representative image of PCR products from isolates after the second recombination. Image is labeled as in (B). Top, the PCR products of 8 isolates from a strain in which *mucA* was deemed essential. Bottom, the PCR products of 8 isolates from a strain in which *mucA* was not essential.

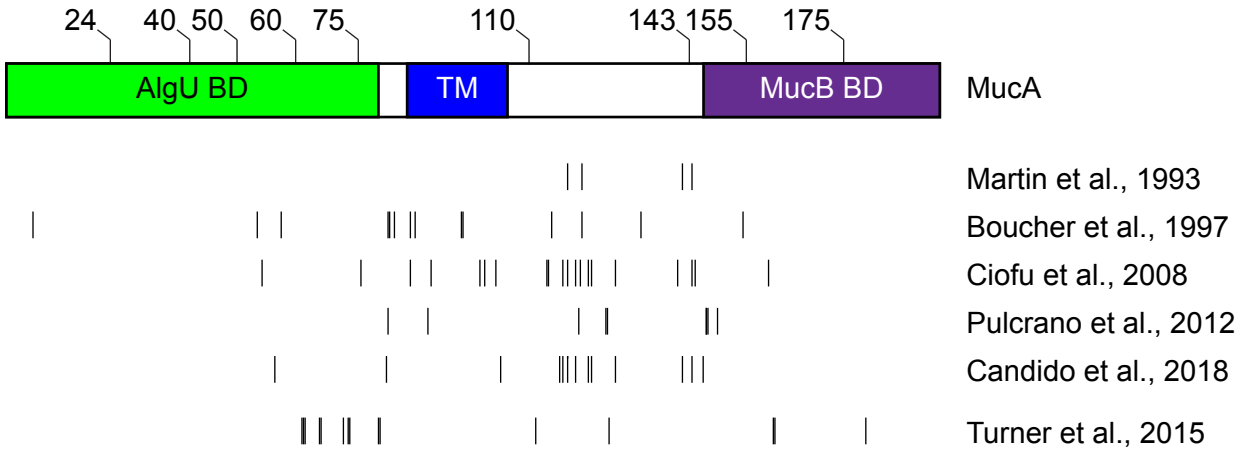

Figure S2. Published mutations in *mucA*.
Top, schematic of MucA with the regions indicated encoding for the AlgU binding domain (green, AlgU BD), the transmembrane domain (blue, TM), and the MucB binding domain (purple, MucB BD). Hash marks on top indicate the residue number in MucA. Bottom, sites of *mucA* mutations that lead to the production of a truncated protein in published isolates. Each line represents one or more individual isolates described in the references on the right. The mutations for the upper five references (Boucher *et al.*, 1997, Candido Cacador *et al.*, 2018, Ciofu *et al.*, 2008, Martin *et al.*, 1993, Pulcrano *et al.*, 2012) are in cystic fibrosis clinical isolates of *P. aeruginosa*, while the mutations in the last reference (Turner *et al.*, 2015) are random transposon insertions in the laboratory PAO1 and PA14 strains.

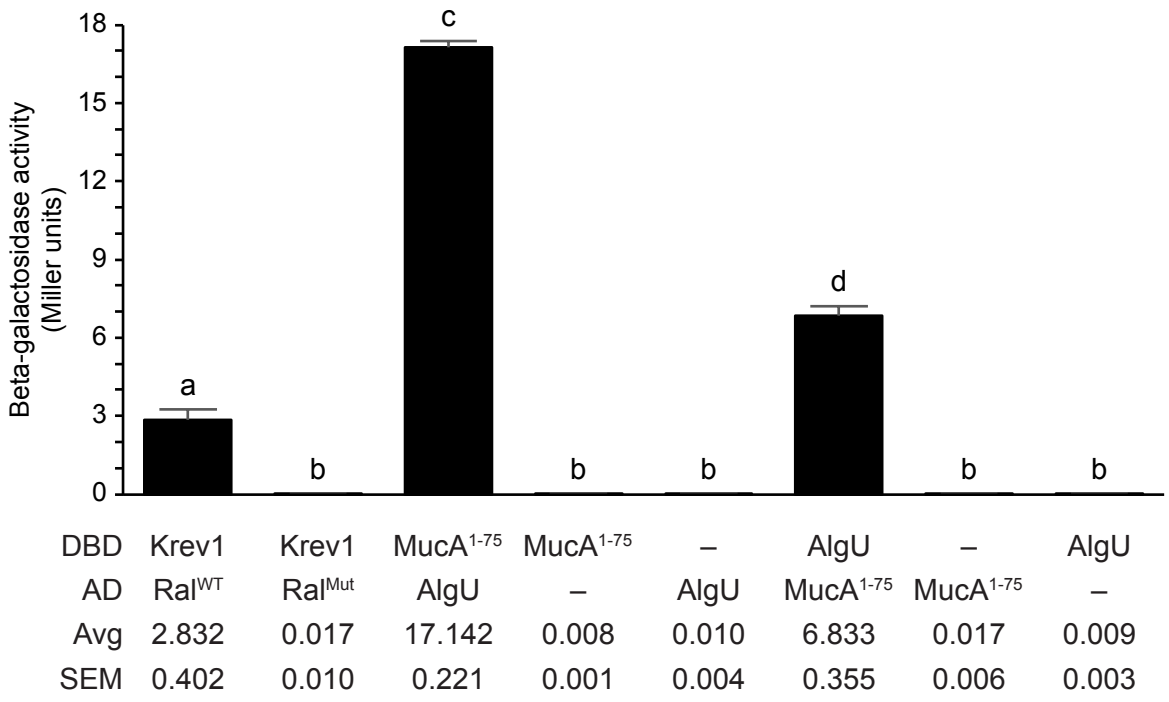

Figure S3. MucA aa 1-75 and AlgU interact via yeast two-hybrid assay.
The indicated proteins were fused to the Gal4 DNA binding domain (DBD; "bait") or the Gal4 activation domain (AD; "prey"). Interaction of the bait and prey proteins drive the expression of *lacZ*. Beta-galactosidase activity (in Miller units) was used as a proxy for the protein interaction strength. The average (Avg) and standard error of the mean (SEM) of each bar are indicated (N=3). As positive and negative controls, Krev1 is known to interact with wild-type RalGDS (Ral^{WT}), but not the mutant RalGDS (Ral^{Mut}) via yeast two-hybrid. AlgU, construct encoding full-length wild-type AlgU; $MucA^{1-75}$, construct encoding only the first 75 residues of wild-type MucA; –, no fusion protein included; error bars, SEM (N=3); letters, statistical groups with the different letters representing statistically different groups ($p < 0.01$; biological triplicate with technical quadruplicates; ANOVA with post-hoc Tukey HSD).

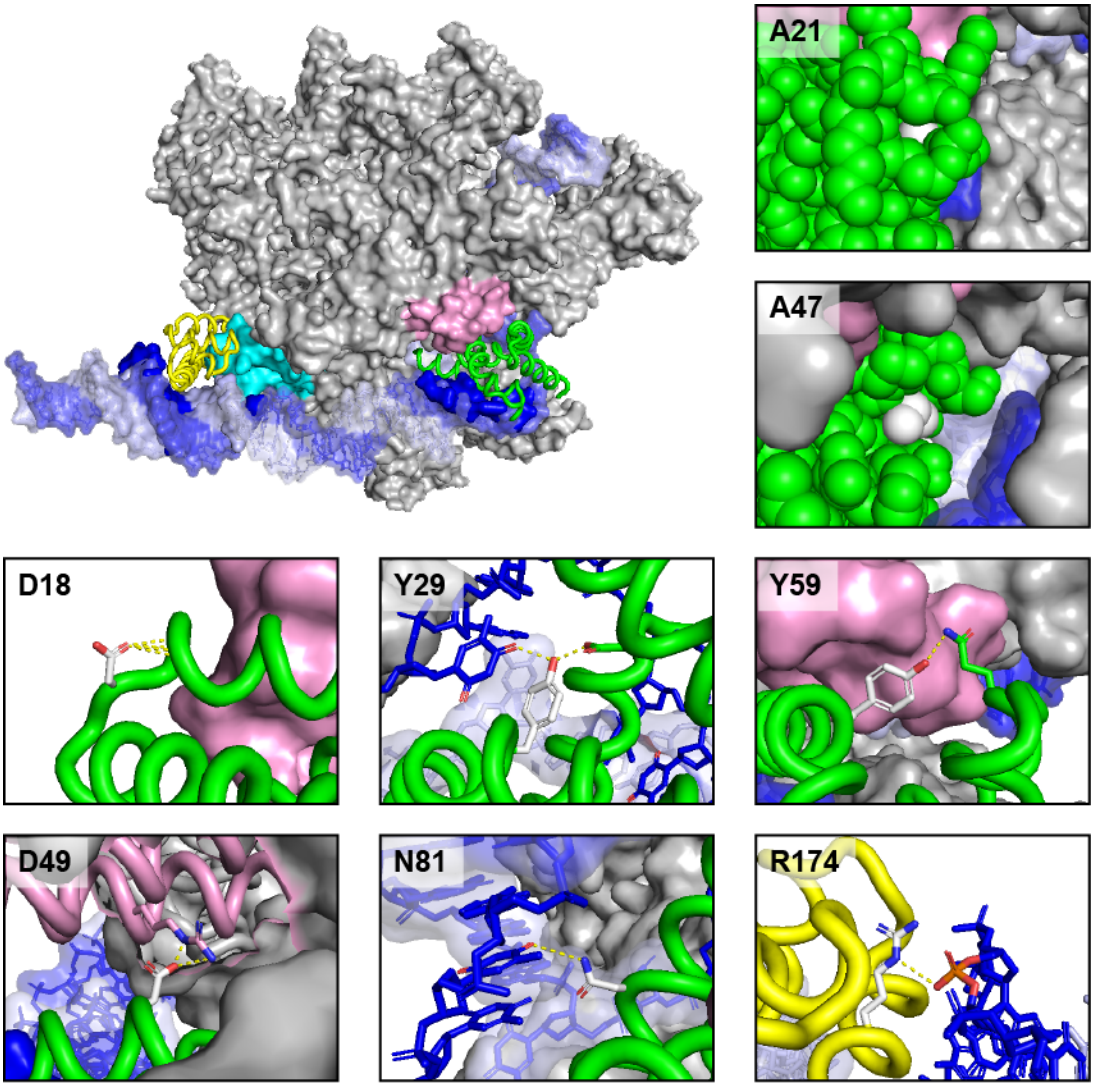

Figure S4. Location of affected AlgU residues in revertants that can grow in the absence of MucA.

Model of the RNAP holoenzyme containing σ^E bound to the promoter. Gray, RNAP core; cyan, β flap; pink, β' coiled coil; green, σ^E Region 2; yellow, σ^E Region 4; blue/light blue, promoter element. Insets, location of residues that are substituted in the revertants with missense *algU* mutations. The side chain of the affected residue is in white. Substitution of A21 and A47 (V in model) likely affect protein packing and folding of AlgU. Substitution of D18, Y29, and Y59 would reduce predicted intra- and inter-molecular hydrogen bonding and likely affect AlgU folding. Substitution of D49, N81, and R174 would likely affect the interaction of AlgU with the RNAP core or the promoter. Dashed yellow lines, hydrogen bonds; red atoms, oxygen; blue atoms, nitrogen; orange, phosphorus.

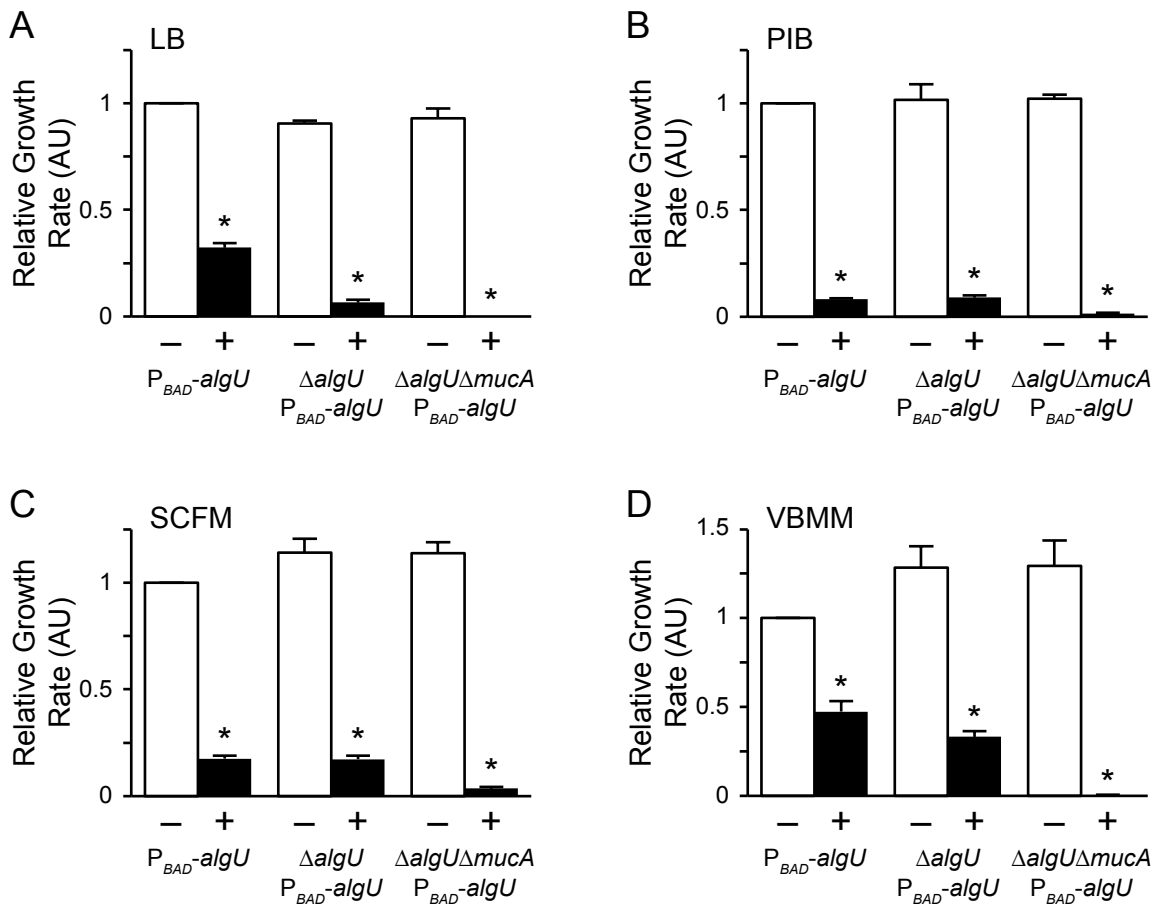

Figure S5. Overexpression of *algU* in the absence of *mucA* causes a growth defect in various media.

Growth rate of indicated strains grown in (A) LB, (B) PIB, (C) SCFM, and (D) VBMM with (+) or without (–) 1% arabinose (relative to PAO1 *att*Tn7::P_{araBAD}-*algU* grown in the absence of arabinose). Error bars, SEM (N=3). Asterisk, statistically different from the same strain grown in the absence of arabinose ($p < 0.01$, N = 3, two-way ANOVA with post-hoc Bonferroni). See Table S3 for full statistical comparisons. LB contains tryptone and yeast extract, both of which provide protein hydrolates, as the main carbon source. The main carbon source of PIB is also protein hydrolates, but from pancreatic gelatin digest. While PIB also contains glycerol, which can serve as a carbon source, it is not a preferred carbon source for *Pseudomonas aeruginosa*. SCFM is a defined medium that contains amino acids as the primary carbon source, and mimics the nutrients found in the CF lung environment. VBMM is a minimal medium that contains citrate as the sole carbon source.

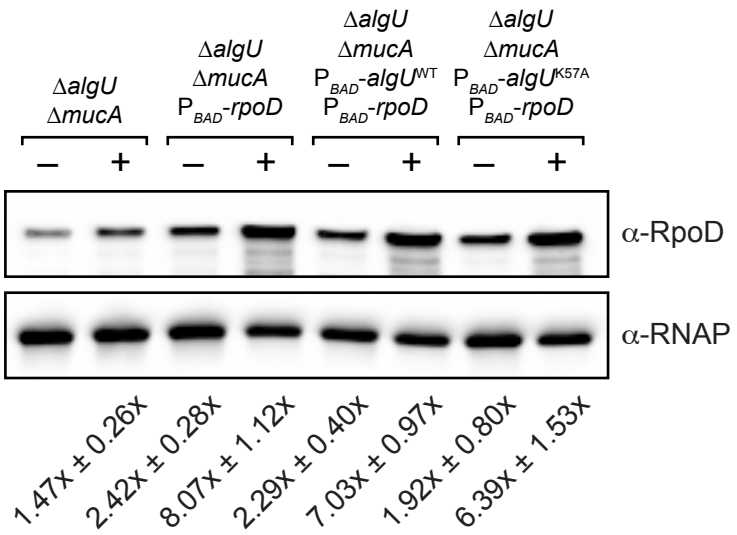

Figure S6. RpoD production in strains with an ectopic inducible *rpoD* allele.
Expression of *rpoD* was inferred via semi-quantitative Western blot in the indicated strains (top). RNAP was used as a loading control (bottom). Strains were grown in LB with (+) or without (–) 2% arabinose. The relative amount of RpoD is indicated, where 1.00x represents RpoD levels in the parental strain without arabinose (± SD; N=3). While the strains produce significantly more RpoD in the presence of arabinose relative to the same strain in the absence of arabinose ($p < 0.01$), there is no statistical difference in the amount of RpoD produced from the induced strains containing an ectopic *rpoD* allele (two-way ANOVA with post-hoc Bonferroni).

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