

## SUPPORTING INFORMATION

**Table S1.** The RocA2 regulon: microarray data.

PA number	Gene / Alternate gene name	Predicted function	Fold-change (Overexpression <i>rocS2</i> : WT/ $\Delta$ <i>rocA2</i> )
PA3045	<i>rocA2</i>	two-component response regulator	17.93
PA2275	<i>yahK</i> ;	probable alcohol dehydrogenase (Zn-dependent)	2.869
PA0248	<i>pobR</i> ;	probable transcriptional regulator	2.552
PA3788		hypothetical protein	2.243
PA3411		hypothetical protein	2.116
PA4182		hypothetical protein	2.111
PA1899	<i>phzA2</i>	probable phenazine biosynthesis protein	2.096
PA0707	<i>toxR</i>	transcriptional regulator ToxR	2.046
PA3593		probable acyl-CoA dehydrogenase	1.961
PA4224	<i>pchG</i>	pyochelin biosynthetic protein PchG	1.855
PA0510	<i>nirE</i> ;	probable uroporphyrin-III c-methyltransferase	1.835
PA2413	<i>pvdH</i>	L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase, PvdH	1.82
PA1426		hypothetical protein	1.736
PA1569		probable major facilitator superfamily (MFS) transporter	1.723
PA0698		hypothetical protein	1.714
PA1404		hypothetical protein	1.71
PA2358		hypothetical protein	1.702
PA1334		probable oxidoreductase	1.686
PA5541	<i>pyrQ</i>	dihydroorotase	1.682
PA0642		hypothetical protein	1.667
PA2418		hypothetical protein	1.662
PA2440		hypothetical protein	1.637
PA4938	<i>purA</i>	adenylosuccinate synthetase	1.621
PA2680		probable quinone oxidoreductase	1.597
PA0195	<i>pntAA</i>	putative NAD(P) transhydrogenase, subunit alpha part 1	1.586
PA0268		probable transcriptional regulator	1.585
PA2683	<i>tdcB</i> ;	probable serine/threonine dehydratase, degradative	1.585
PA5013	<i>ilvE</i>	branched-chain amino acid transferase	1.573
PA4159	<i>fepB</i>	ferrienterobactin-binding periplasmic protein precursor FepB	1.571
PA4121		conserved hypothetical protein	1.571
PA0339		hypothetical protein	1.566
PA2289		conserved hypothetical protein	1.558
PA5297	<i>poxB</i>	pyruvate dehydrogenase (cytochrome)	1.554
PA1854		conserved hypothetical protein	1.549
PA4183		hypothetical protein	1.544
PA4651		probable pili assembly chaperone	1.54
PA2665	<i>ygaA</i> ;	probable transcriptional regulator	1.539
PA2431		hypothetical protein	1.537
PA3723	<i>yqjM</i> ;	probable FMN oxidoreductase	1.528

PA3307		hypothetical protein	1.515
PA5390		probable peptidic bond hydrolase	1.514
PA0723	coaB	coat protein B of bacteriophage Pfl)	1.514
PA0636		hypothetical protein	1.511
PA5342		probable transcriptional regulator	1.51
PA0203		probable binding protein component of ABC transporter	-389.5
PA0424	mexR	multidrug resistance operon repressor MexR	-23.76
PA0426	mexB	Resistance-Nodulation-Cell Division (RND) multidrug efflux transporter MexB	-8.42
PA0427	oprM	Major intrinsic multiple antibiotic resistance efflux outer membrane protein OprM precursor	-8.229
PA0425	mexA	Resistance-Nodulation-Cell Division (RND) multidrug efflux membrane fusion protein MexA precursor	-8.05
PA1087	flgL	flagellar hook-associated protein type 3 FlgL	-6.988
PA0207		probable transcriptional regulator	-4.138
PA0202		probable amidase	-3.862
PA1892		hypothetical protein	-2.829
PA2412		conserved hypothetical protein	-2.359
PA2310		hypothetical protein	-2.338
PA4507		hypothetical protein	-2.013
PA4506	dppF ;	probable ATP-binding component of ABC dipeptide transporter	-1.962
PA2135		probable transporter	-1.924
PA2670		hypothetical protein	-1.889
PA5053	hslV	heat shock protein HslV	-1.828
PA5256	dsbH	disulfide bond formation protein	-1.813
PA2130	cupA3	usher CupA3	-1.806
PA1664		hypothetical protein	-1.798
PA1699	pcr1 ;	conserved hypothetical protein in type III secretion	-1.778
PA0717		hypothetical protein of bacteriophage Pfl	-1.765
PA5191		hypothetical protein	-1.759
PA0719		hypothetical protein of bacteriophage Pfl	-1.727
PA3406	hasD	transport protein HasD	-1.699
PA1893		hypothetical protein	-1.686
PA3842	orf1 ;	probable chaperone	-1.659
PA0044	exoT	exoenzyme T	-1.651
PA0213	mdeG ;	hypothetical protein	-1.624
PA0996	pqsA	probable coenzyme A ligase	-1.623
PA1708	popB	translocator protein PopB	-1.61
PA2891	atuF	geranyl-CoA carboxylase, alpha-subunit (biotin-containing)	-1.604
PA1706	pcrV	type III secretion protein PcrV	-1.599
PA3126	ibpA	heat-shock protein IbpA	-1.594
PA5450	wzt	ABC subunit of A-band LPS efflux transporter	-1.569
PA4120		probable transcriptional regulator	-1.565
PA2595		conserved hypothetical protein	-1.559
PA2303		hypothetical protein	-1.557
PA4188		conserved hypothetical protein	-1.556
PA4500		probable binding protein component of ABC transporter	-1.54
PA1991		probable iron-containing alcohol dehydrogenase	-1.516

<b>PA0186</b>	atsR ;	probable binding protein component of ABC transporter	-1.511
<b>PA2653</b>	yuiF ;	probable transporter	-1.507

---

**Table S2.** Oligonucleotides used in this study

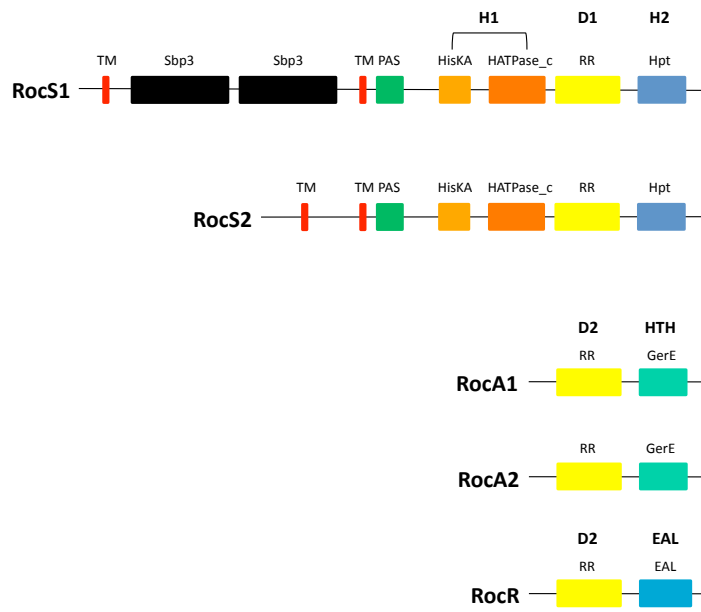
Oligonucleotide name	Oligonucleotide sequence*
<i>rocA2</i> deletion	
UA3045	AACGAGCTCCGTGGCTCGGTCAATGAAGA
LA3045	CGCGGATCCGCTCATTGTCTGATTCCTT
UB3045	CGCGGATCCATCTAAGGGCCGGAACCGAA
LB3045	GCTCTAGACCAGTCGGAATCGGTCAACA
<i>cupC1</i> promoter	
PromC1up	AGATCGGACCAGATAGAGGT
PromC1down	CCGAGGCTTACGTCGATGGTC
<i>cupB1</i> promoter	
PromB1up	AGGGTTCTCCTCGTCGTGGG
PromB1down	TTGATATCGCACGCGACTTCG
Bacterial two-hybrid	
rocS2H2up	GCTCTAGAGGGCGACCTCTTTCGGACGCGA
rocS2H2down	GGGGTACCGGAAGGGCTCATACCGCAGC
rocA2D2up	GCTCTAGAAAATCAGACAATGAGCCGTA
rocA2D2down	GGGGTACCTCAAACCGAGCTGGGAAAGTAACTG
gacSHDHup	GCTCTAGACAGGATCAAGTCCGAGTTCC
gacSHDHdown	GGGGTACCTGCGCATGGTCAGAGTTCGC
gacAD2up	AAAACCTGCAGCGTGATTAAGGTGCTGGTGGTCGACG
gacAD2down	CGGAATTCTATTAGGCCGAAGACCTGGCGAATCGCC
trpOD2up	GCCTCTAGAGCGCATGAGTAAGGTGCTGATCGTCG
trpOD2down	GGGGTACCTCAATGGTTGATGCTGCGCAACGCCCGGT
qPCR	
mexAup	TGAACGGCATCATCCTCAAG
mexAdown	TGGTAGTCGGCCTCGTAGGT
mexRup	CGCCCGACGTCCATGTAT
mexRdown	CGGGTGATCAGTGCCTTGT
16SrRNAup	CGCCGTAAACGATGTCGACTA
16SrRNA down	TTAACCTTGCGGCCGTA

\*Oligonucleotides are presented in the orientation 5'-3'.

**Fig. S1.** Domain organization of paralogous Roc proteins of *P. aeruginosa*. This representation is based on predictions of Protein families' database of alignments (Pfam). Pfam accession for each domain is as follows: Sbp3 (PF00497), PAS (PF00989, PF08448), HisKA (PF00512), HATPase\_c (PF02518), RR (PF00072), Hpt (PF01627), GerE (PF00196) and EAL (PF00563). The sensor kinases RocS1 and RocS2 domains named H1, D1 and H2 refer to the HisKA/HATPase\_c, RR and Hpt domains, respectively. For the response regulators, the RR and GerE are referred to as the D2 and helix-turn-helix (HTH) domains, respectively (Kulasekara *et al.*, 2005). TM indicates the location of the transmembrane segment of the sensor kinases. RocS1 and RocS2 are paralogous proteins with 45% identity, 63% similarity and RocA1 and RocA2 are also paralogous proteins with 59% identity, 73% similarity. The RocR response regulator is also shown with a D2 domain and an EAL-containing domain with phosphodiesterase activity.

**Fig. S2.** Activity of the *cupB* promoter in different *P. aeruginosa* strains. The strains tested are the wild type PAK, the *rocA2* mutant (PAK $\Delta$ *rocA2*), the *rocA1* mutant (PAK $\Delta$ *rocA1*), and the *rocA1/rocA2* mutant, all carrying the transcriptional *cupB-lacZ* fusion on the chromosome at the *attB* site. Where indicated the strain contains a plasmid expressing the *rocS1* gene (pMMB67-*rocS1*), or empty vector (pMMB67EH). All data presented are the average of biological duplicates and error bars indicate one standard deviation of the mean.  $\beta$ -galactosidase activity is expressed in Miller units.

**Figure S1**



**Figure S2**

