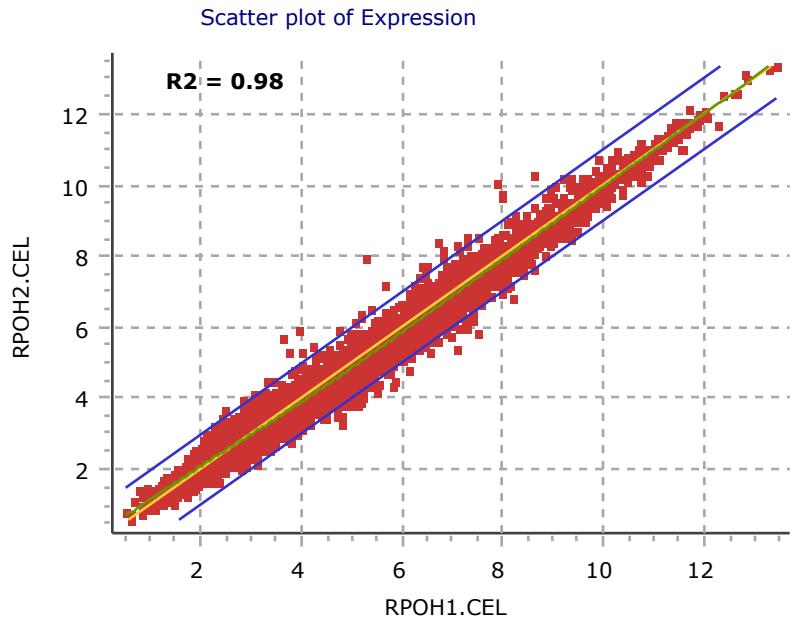
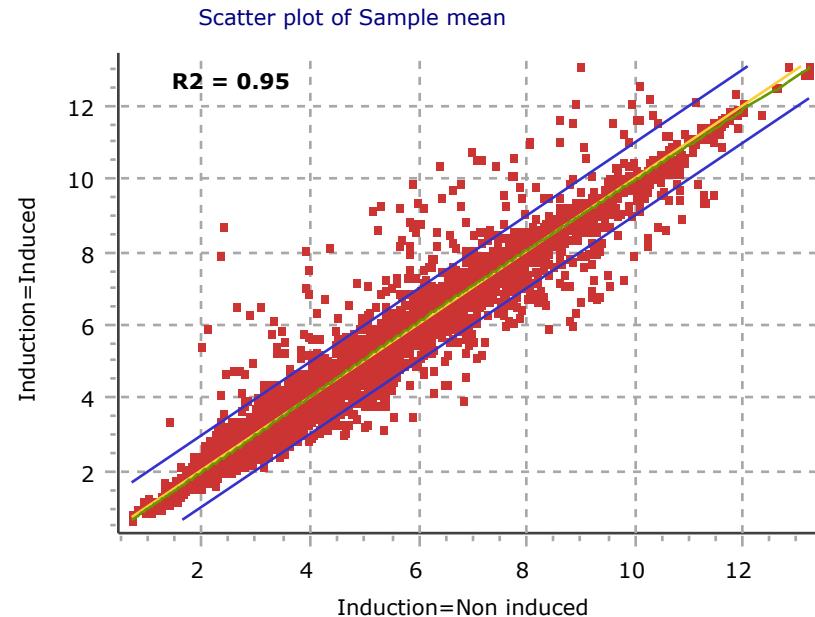


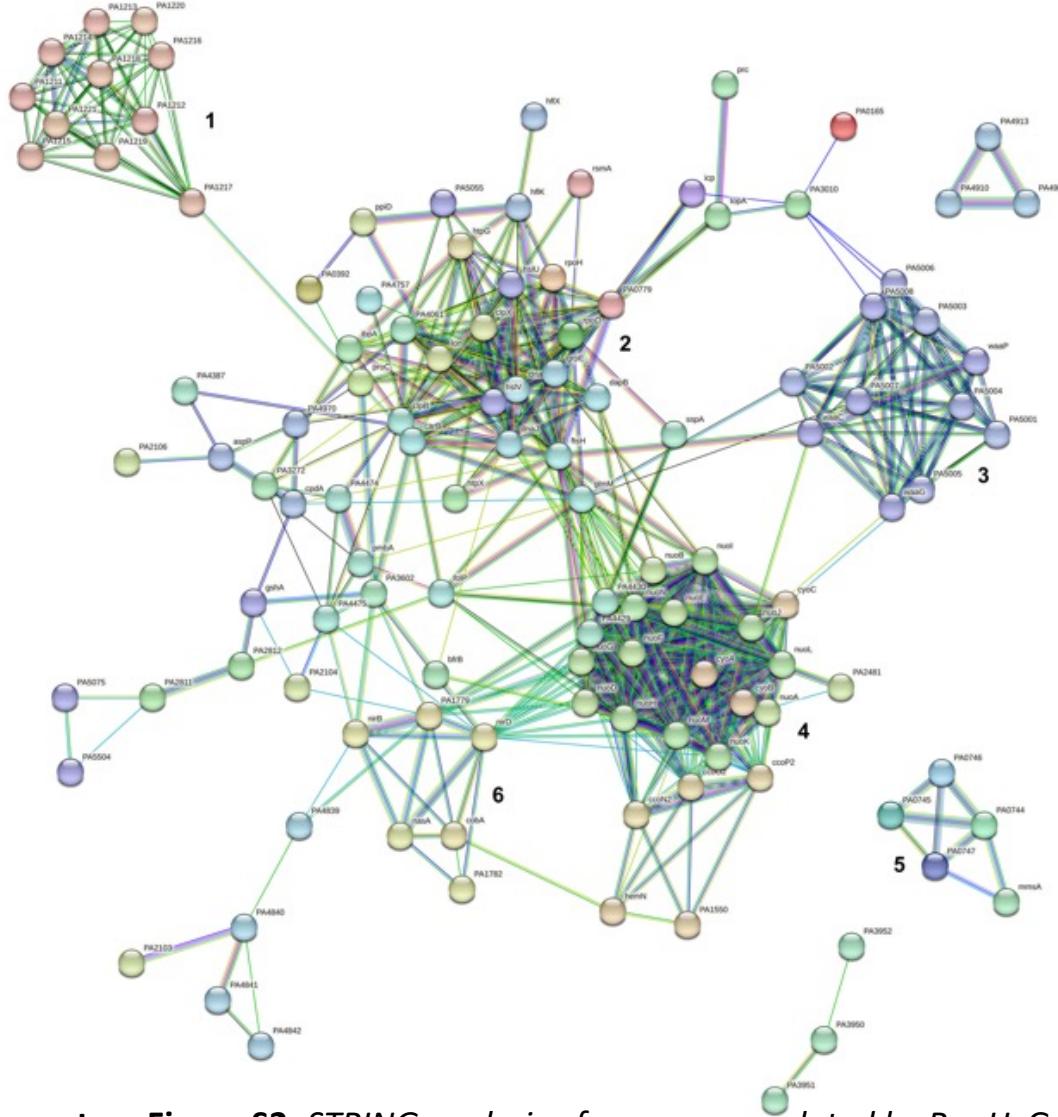
S1A.



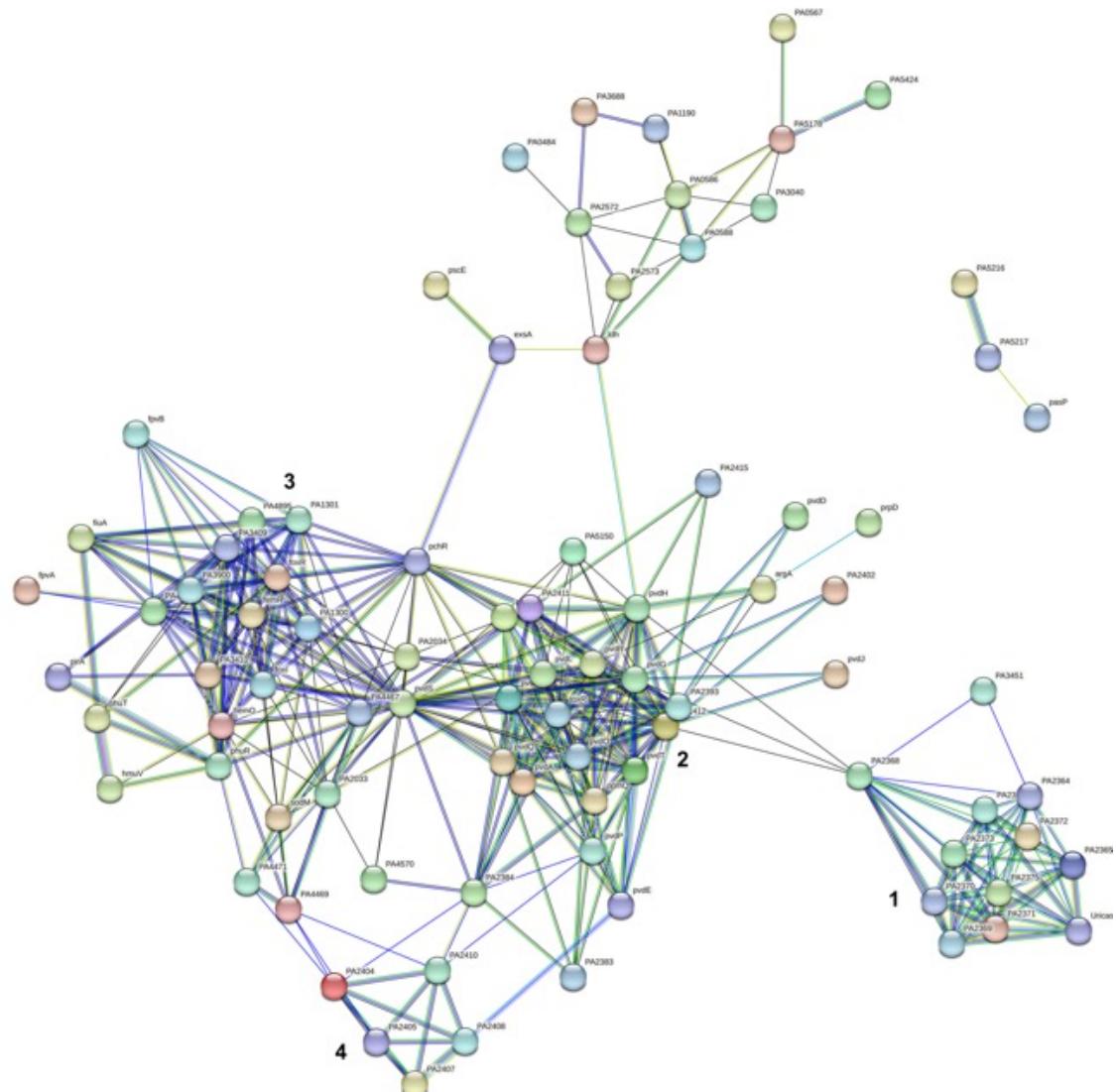
S1B.



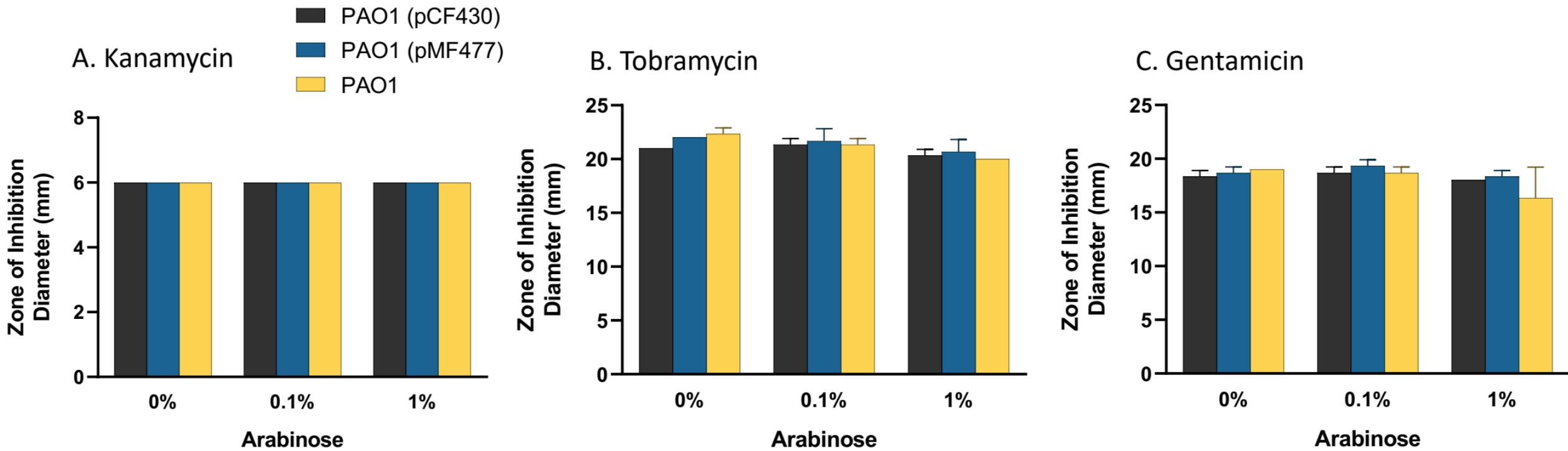
**Figure S1.** Scatter plots of *P. aeruginosa*  $\Delta rpoH$  ( $P_{BAD}$ -*rpoH*) transcriptome expression data. (A) Independent biological replicates showed highly reproducible gene expression values;  $R^2 = 0.98$  in the example above. (B) Arabinose induction of *rpoH* resulted in 258 genes with significantly different expression (at  $p < 0.05$  and  $>$  two-fold change) compared to the non-induced controls. Three independent biological replicates were performed.



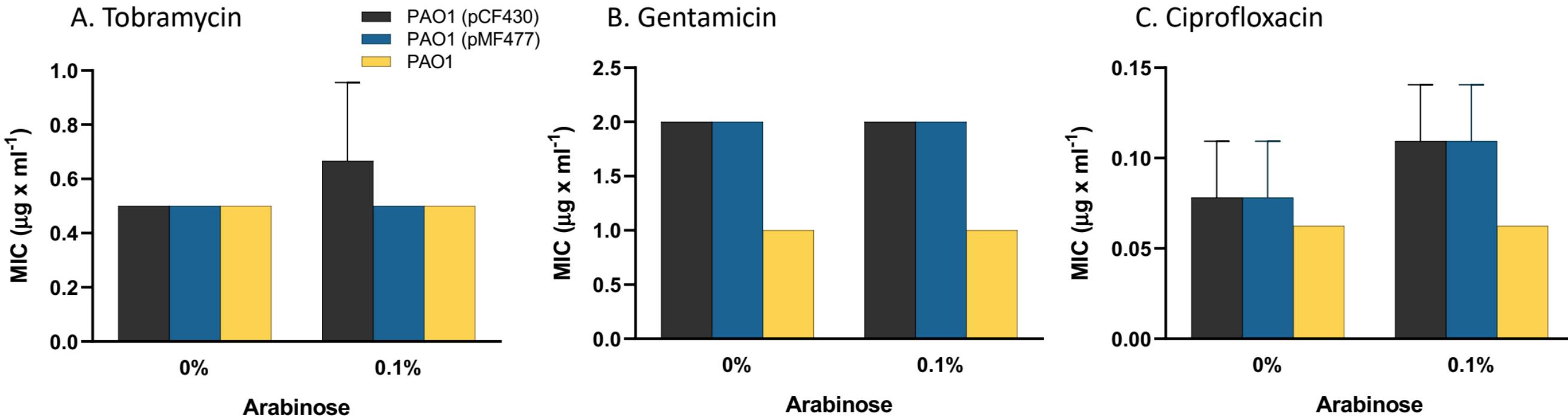
**Supplementary Figure S2.** STRING analysis of genes upregulated by RpoH. Genes with no functional connections, and those connected only to one partner, are not shown for clarity. General functional assignments of labeled clusters are as follows: 1) Non-ribosomal peptide antibiotic biosynthesis; 2) Chaperones and proteases; 3) Cell wall and membrane synthesis and modification; 4) NADH dehydrogenase complex *nuo*; 5) Isomerases / dehydrogenases involved in redox reactions; 6) Assimilatory nitrate reduction.



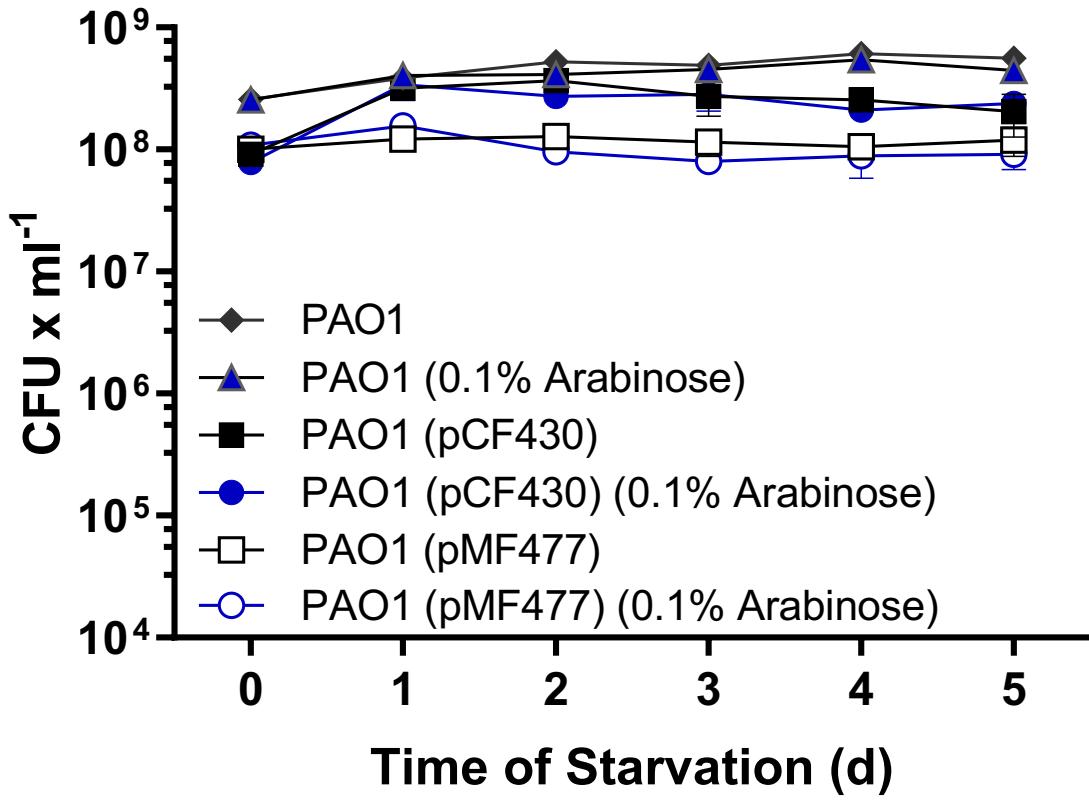
**Supplementary Figure S3.** STRING analysis of genes downregulated by RpoH. Genes with no functional connections, and those connected only to one partner, are not shown for clarity. General functional assignments of labeled clusters are as follows: 1) Members of type VI secretion system and uncharacterized; 2) Pyoverdine synthesis and export; 3) Multiple sigma factors; 4) Iron binding and reduction, *fpvGHJKCDEF* operon.



**Figure S4.** Antibiotic sensitivities of *P. aeruginosa* PAO1, *P. aeruginosa* PAO1 (pCF430), and *P. aeruginosa* PAO1 (pMF477) assayed by the disk diffusion assay, to determine the effect of plasmids used in this study on antibiotic tolerance of *P. aeruginosa*. The results show the mean and standard deviation of three independent biological replicates. The results show no effects on the zone of clearing around the aminoglycoside disks due to the plasmids used in this study.



**Figure S5.** Antibiotic sensitivities of *P. aeruginosa* PAO1, *P. aeruginosa* PAO1 (pCF430), and *P. aeruginosa* PAO1 (pMF477) assayed as minimum inhibitory concentration (MIC), to determine the effect of plasmids used in this study on antibiotic tolerance of *P. aeruginosa*. The results show the mean and standard deviation of three independent biological replicates. The results show that the vector control and the plasmid containing *rpoH* (pMF477) resulted in an increased MIC to gentamicin, but no significant effect on the MIC to tobramycin or ciprofloxacin.



**Figure S6.** Effect of nutrient limitation on the viability of *P. aeruginosa* and *P. aeruginosa* (pCF430) and *P. aeruginosa* (pMF477), to determine the effect of the plasmids used in this study on survival of *P. aeruginosa* during starvation. Following growth to early stationary phase, cells were incubated in phosphate buffered saline with or without arabinose as indicated, for up to 5 days. The viability of the cells was determined as colony forming units. The results show no effect of the plasmids on the survival of *P. aeruginosa* during starvation, in the presence or absence of arabinose.

**Table S1.** Primers used in this study.

**Gene amplification primers**

RpoH Nhe 5'	CAGCTAGCGAGTTCTGTGAATCGGAGGGATTG
RpoH Hind 3'	GCAAGCTTGCAGGGTGCAGTTTCGCG

**Allelic exchange primers**

RpoH HindIII 5' Up	aaGCTTCGTCTCGGCCTGACCATC
RpoH T7 Up R Acc	ctatagtggatcgatttaggtaccGCGAATCCTCCGATTACGAAAC.
RpoH HindIII 3' Down	aagcTTGCGGATAGACCCAGGCCTCGC
RpoH T7 Down F Acc	ggtacctaatacgactcaactatTCGCCGCGCTCGCGAAAACCGC.

**RT-qPCR primers**

acpLeftRT-PCR2	ACTCGCGTGAAAGGAAGAAG	Williamson, 2012
acpRightRT-PCR2	CGACGGTGTCAAGGGAGT	
PA4463-For (hpf)	GCCATTTCGACAAGATCACC	Williamson, 2012
PA4463-Rev (hpf)	GCTGCCGTTCGAGATACTTC	
PA3126-For (IbpA)	CTGTTCCGTATTCCGTAGG	Williamson, 2012
PA3126-Rev (IbpA)	GGCGATAACGATGCGATACT	
RpoH_F	CCGAACGCCTTTCTATCAG	
RpoH_R	TTGGCGATATGAACAACGAA	
GroES_1F	GAGGAAGAGACCAAGACCGC	
GroES_1R	GTCCAGTACACGACCGGTAC	
PA1216_F	GACGACACCCTGGAGATCAT	
PA1216_R	GCTGGTGGAGAGGAAGAACAA	
WapP_F	AGCCGCTGGTGTATTCATC	
WapP_R	GTGGTACTTGGCACCTTGT	

**Table S2.** Genes significantly downregulated in PAO1Δ*rpoH* ( $P_{BAD}$ -*rpoH*) by > 2-fold change and  $p < 0.05$  following a 15-minute induction of *rpoH*.

Gene	Function	Fold change	p-value
PA0035	Tryptophan synthase alpha chain	-2.2	$1.3 \times 10^{-2}$
PA0179	Two-component response regulator	-2.0	$2.6 \times 10^{-2}$
PA0423	Antibiotic sequester PasP	-2.4	$2.0 \times 10^{-3}$
PA0460	hypothetical protein	-2.0	$1.1 \times 10^{-2}$
PA0470	Ferrichrome receptor FiuA	-2.1	$2.3 \times 10^{-2}$
PA0484	Similar to glycine cleavage system transcriptional repressor	-2.6	$1.3 \times 10^{-3}$
PA0567	Conserved hypothetical protein	-2.4	$3.6 \times 10^{-3}$
PA0586	Conserved hypothetical protein	-2.2	$3.5 \times 10^{-2}$
PA0587	Contains a von Willebrand domain	-2.3	$4.2 \times 10^{-2}$
PA0588	Possible response regulator	-2.1	$1.1 \times 10^{-3}$
PA0672	Heme oxygenase	-2.7	$1.7 \times 10^{-2}$
PA0707	Transcriptional regulator ToxR	-3.4	$3.1 \times 10^{-3}$
PA0792	Propionate catabolic protein PrpD	-2.4	$1.8 \times 10^{-3}$
PA0798	Phospholipid methyltransferase	-2.1	$1.4 \times 10^{-2}$
PA0931	Ferric enterobactin receptor PirA	-2.2	$2.1 \times 10^{-2}$
PA1041	Outer membrane protein precursor	-3.3	$4.7 \times 10^{-4}$
PA1134	Hypothetical protein	-4.4	$1.9 \times 10^{-2}$
PA1245	Alkaline protease secretion protein AprX	-2.6	$7.4 \times 10^{-4}$
PA1246	Alkaline protease secretion protein AprD	-2.3	$1.2 \times 10^{-2}$
PA1300	ECF sigma factor HxuI	-2.6	$1.6 \times 10^{-2}$
PA1301	Anti-sigma factor HxuR	-2.1	$1.2 \times 10^{-2}$
PA1650	Transporter	-2.3	$1.4 \times 10^{-3}$
PA1713	Transcriptional regulator ExsA	-5.2	$2.8 \times 10^{-3}$
PA1745	Hypothetical protein	-2.0	$1.8 \times 10^{-2}$
PA1753	ATP binding/hydrolysis	-2.1	$1.5 \times 10^{-2}$
PA1860	SAM-dependent methyltransferase	-2.8	$1.7 \times 10^{-3}$
PA1871	LasA protease precursor	-2.0	$1.4 \times 10^{-2}$
PA1888	Putative esterase/lipase	-3.1	$4.0 \times 10^{-2}$
PA1911	Sigma factor regulator, FemR	-2.7	$2.4 \times 10^{-2}$
PA2033	Siderophore-interacting FAD oxydoreductase	-3.1	$5.8 \times 10^{-3}$
PA2034	SAM-dependent methyltransferase	-3.8	$6.7 \times 10^{-3}$
PA2062	Pyridoxal-phosphate dependent enzyme	-2.5	$2.2 \times 10^{-2}$
PA2174	Hypothetical protein	-2.6	$3.4 \times 10^{-2}$
PA2204	Putative binding component of ABC transporter	-3.0	$2.7 \times 10^{-2}$
PA2259	Transcriptional regulator PtxS	-2.0	$3.5 \times 10^{-4}$
PA2364	Lip3, a membrane bound component of the type VI secretion system	-2.9	$2.2 \times 10^{-3}$
PA2365	HsiB3, type VI secretion system	-4.2	$8.9 \times 10^{-3}$

PA2366	HsiC3, type VI secretion system	-4.1	$4.3 \times 10^{-3}$
PA2367	Hcp3, type VI secretion system	-4.3	$3.1 \times 10^{-2}$
PA2368	HsiF3, type VI secretion system	-4.8	$6.9 \times 10^{-3}$
PA2369	HsiG3, type VI secretion system	-2.1	$2.2 \times 10^{-2}$
PA2370	HsiH3, type VI secretion system	-2.2	$1.8 \times 10^{-2}$
PA2371	ClpA/B-type protease	-2.4	$1.1 \times 10^{-2}$
PA2372	Hypothetical protein	-3.4	$1.5 \times 10^{-2}$
PA2373	VgrG3, Phage base V domain-containing protein, type 6SS	-2.5	$2.5 \times 10^{-2}$
PA2375	Hypothetical protein	-2.3	$4.6 \times 10^{-2}$
PA2383	Transcriptional regulator	-2.8	$4.8 \times 10^{-3}$
PA2384	Fur-like DNA binding domain	-3.8	$8.0 \times 10^{-4}$
PA2385	3-oxo-C12-homoserine lactone acylase PvdQ	-5.1	$1.4 \times 10^{-3}$
PA2386	L-ornithine N5-oxygenase	-3.7	$1.6 \times 10^{-3}$
PA2389	PvdR	-3.9	$7.0 \times 10^{-3}$
PA2390	PvdT	-5.5	$3.1 \times 10^{-4}$
PA2391	Outer membrane protein precursor	-3.1	$2.9 \times 10^{-2}$
PA2392	PvdP	-5.5	$2.6 \times 10^{-4}$
PA2393	Putative dipeptidase	-5.8	$1.2 \times 10^{-2}$
PA2394	PvdN	-5.8	$2.3 \times 10^{-3}$
PA2395	PvdO	-5.5	$1.3 \times 10^{-3}$
PA2396	Pyoverdine synthetase F	-3.8	$2.5 \times 10^{-4}$
PA2397	Pyoverdine biosynthesis protein PvdE	-4.8	$2.9 \times 10^{-2}$
PA2398	Ferripyoverdine receptor	-3.6	$4.3 \times 10^{-2}$
PA2399	Pyoverdine synthetase D	-2.2	$1.4 \times 10^{-2}$
PA2400	PvdJ, a nonribosomal peptide synthetase condensation module	-2.4	$4.4 \times 10^{-3}$
PA2401	PvdJ, fused with previous	-2.9	$8.0 \times 10^{-3}$
PA2402	Pyoverdine peptide synthetase	-4.4	$1.6 \times 10^{-3}$
PA2404	FpvH	-2.4	$2.6 \times 10^{-3}$
PA2405	FpvJ	-2.8	$5.9 \times 10^{-3}$
PA2407	FpvC	-2.3	$4.3 \times 10^{-3}$
PA2408	FpvD	-3.2	$5.8 \times 10^{-4}$
PA2409	FpvE	-2.1	$9.6 \times 10^{-3}$
PA2410	FpvF	-2.9	$1.4 \times 10^{-3}$
PA2411	Thioesterase	-3.7	$7.4 \times 10^{-4}$
PA2412	MbtH-like, a nonribosomal peptide synthetase partner domain	-3.3	$2.8 \times 10^{-3}$
PA2413	L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase, PvdH	-6.3	$8.0 \times 10^{-4}$
PA2415	Hypothetical protein	-2.6	$9.1 \times 10^{-3}$
PA2424	PvdL	-7.1	$7.5 \times 10^{-4}$
PA2425	PvdG	-7.5	$4.7 \times 10^{-4}$
PA2427	hypothetical protein	-5.0	$9.2 \times 10^{-4}$
PA2451	Putative esterase	-4.6	$5.4 \times 10^{-5}$
PA2452	Putative esterase	-4.2	$1.4 \times 10^{-2}$

PA2467	Anti-sigma factor FoxR	-2.6	$1.2 \times 10^{-3}$
PA2468	ECF sigma factor FoxI	-2.1	$4.1 \times 10^{-2}$
PA2572	Two-component response regulator	-2.1	$2.9 \times 10^{-3}$
PA2573	Chemotaxis transducer	-2.7	$8.6 \times 10^{-6}$
PA2746	Conserved hypothetical protein	-2.9	$1.5 \times 10^{-2}$
PA2786	With GAF domain, putative photosensitive histidine kinase	-3.0	$5.0 \times 10^{-2}$
PA2937	Hypothetical protein	-2.0	$1.2 \times 10^{-2}$
PA3040	Hypothetical protein	-2.1	$1.8 \times 10^{-5}$
PA3409	HasS, putative anti-sigma factor	-3.0	$1.7 \times 10^{-3}$
PA3418	Leucine dehydrogenase	-2.1	$2.5 \times 10^{-2}$
PA3451	Hypothetical protein	-2.1	$3.3 \times 10^{-2}$
PA3688	Hypothetical protein	-2.1	$2.5 \times 10^{-2}$
PA3691	DUF4398 domain-containing protein	-2.2	$2.2 \times 10^{-2}$
PA3692	Lipotoxin F, LptF	-2.3	$1.5 \times 10^{-2}$
PA3712	Hypothetical protein	-2.8	$7.0 \times 10^{-4}$
PA3866	Pyocin S4	-2.6	$3.2 \times 10^{-2}$
PA3900	FecR, anti-sigma regulator	-2.4	$1.1 \times 10^{-2}$
PA4168	Second ferric pyoverdine receptor FpvB	-3.3	$9.5 \times 10^{-4}$
PA4227	Transcriptional regulator PchR	-2.4	$2.6 \times 10^{-2}$
PA4296	Two-component response regulator, PprB	-2.0	$2.0 \times 10^{-3}$
PA4390	Putative outer membrane porin	-2.8	$8.7 \times 10^{-3}$
PA4467	Putative metal transporter	-5.8	$1.2 \times 10^{-3}$
PA4468	Superoxide dismutase	-3.7	$4.4 \times 10^{-3}$
PA4469	A TPR-repeat protein	-3.2	$2.4 \times 10^{-2}$
PA4471	Hypothetical protein	-4.2	$4.5 \times 10^{-2}$
PA4570	Hypothetical protein	-3.7	$3.7 \times 10^{-3}$
PA4607	Hypothetical protein	-2.5	$1.2 \times 10^{-2}$
PA4614	Conductance mechanosensitive channel	-2.1	$3.2 \times 10^{-2}$
PA4710	Heme/Hemoglobin uptake outer membrane receptor PhuR precursor	-5.3	$1.5 \times 10^{-3}$
PA4895	Transmembrane sensor	-2.2	$3.0 \times 10^{-2}$
PA4896	Sigma-70 factor, ECF subfamily	-2.8	$2.1 \times 10^{-2}$
PA5150	Short-chain dehydrogenase	-2.3	$8.1 \times 10^{-3}$
PA5173	Carbamate kinase	-2.3	$9.5 \times 10^{-4}$
PA5178	Putative potassium binding protein	-2.2	$9.3 \times 10^{-3}$
PA5424	Hypothetical protein	-2.4	$2.4 \times 10^{-3}$
PA5528	Hypothetical protein	-2.5	$7.6 \times 10^{-3}$