Supplemental Material

Physiological framework for the regulation of quorum sensing-dependent public goods in *Pseudomonas aeruginosa*

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Table S1

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Supplemental Material References

Table S1. Primers used in this study.

Construct or gene	Primer name	Primer sequence (5'-3')[a]
Plasmid		
mini-CTX-*pepB'-lacZ*	PA2939-*Eco*RI-F	N_6GAATTCGGAGGACGTCGTTTTCATGG
	PA2939-*Bam*HI-R	N_6GGATCCGAGACTCCGTTCCTTGTGAG
mini-CTX-*lasB'-lacZ*	PA3724-*Hind*III-F	N_6AAGCTTGGCCTACAA GCTCGACGTCA
	PA3724-*Eco*RI-R	N_6GAATTCCTTCTTCATCTTTTCAGTTCTCC
mini-CTX-*phzA1'-lacZ*	PA4210-*Xho*I-F	N_6CTCGAGCCAGAGCCTTTTCCTGCGTA
	PA4210-*Bam*HI-R	N_6GGATCCCTCGCGGCATCGGTTATTC
Real-time qPCR		
pepB	PA2939-qPCR-F	CGGAAGCGCAACAGTTCAC
	PA2939-qPCR-R	CAACGGCGATTTGCAGATC
lasB	PA3724-qPCR-F	CCAGGCCAAGAGCCTGAAG
	PA3724-qPCR-R	CGGATCACCAGTTCCACTTTG
phzA1	PA4210-qPCR-F	CCACTACATCCATTCCTTCGAACT
	PA4210-qPCR-R	AATTTCTGCATCGGGTTCATG
rhlA	PA3479-qPCR-F	GGCGCGAAAGTCTGTTGGT
	PA3479-qPCR-R	CCAACGCGCTCGACATG
lasR	*lasR*-qPCR-F	AGCCGGGAGAAGGAAGTGTT
	lasR-qPCR-R	GAGCAGTTGCAGATAACCGATATC
rhlR	*rhlR*-qPCR-F	ACCGCGAGATCCTGCAATG
	rhlR-qPCR-R	TCAGGATGATGGCGATTTCC
lasI	*lasI*-qPCR-F	GCCCCTACATGCTGAAGAACA
	lasI-qPCR-R	CGAGCAAGGCGCTTCCT
rhlI	*rhlI*-qPCR-F	GCAGCTGGCGATGAAGATATT
	rhlI-qPCR-R	TGGCGCCCAGGTACCA

[a]Restriction sites are underlined.

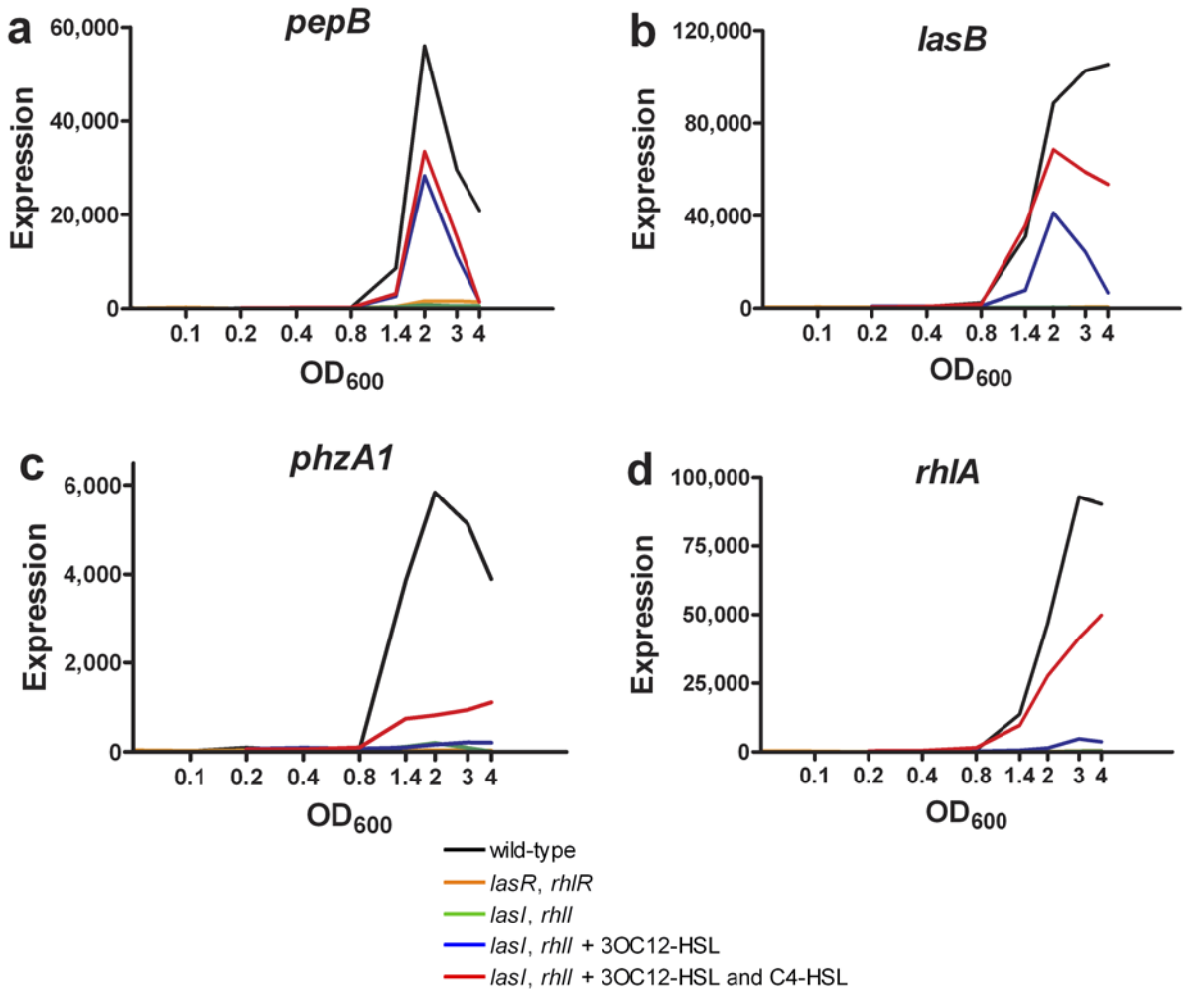

Figure S1. QS-controlled gene expression in complex medium. Graphical representation of microarray expression data from Schuster *et al.*(1) for QS-controlled genes investigated in this study. Expression of **(a)** *pepB*, **(b)** *lasB*, **(c)** *phzA1*, and **(d)** *rhlA* in the *P. aeruginosa* PAO1 wild-type (black line), an isogenic *lasR*, *rhlR* receptor mutant (orange line), and a non-isogenic *lasI*, *rhlI* signal generation mutant without added acyl-HSL (green line), with 3OC12-HSL (blue line), and with C4-HSL and 3OC12-HSL (red line). Strains were cultured in LB medium from early exponential to stationary phase and transcript levels were determined at the indicated culture densities (OD_{600}). An $OD_{600} \geq 1.4$ signifies stationary phase. The values on the *y*-axis represent transcript abundance as determined by the array software. The absence of green and yellow lines in some panels indicates baseline gene expression too low to be visible.

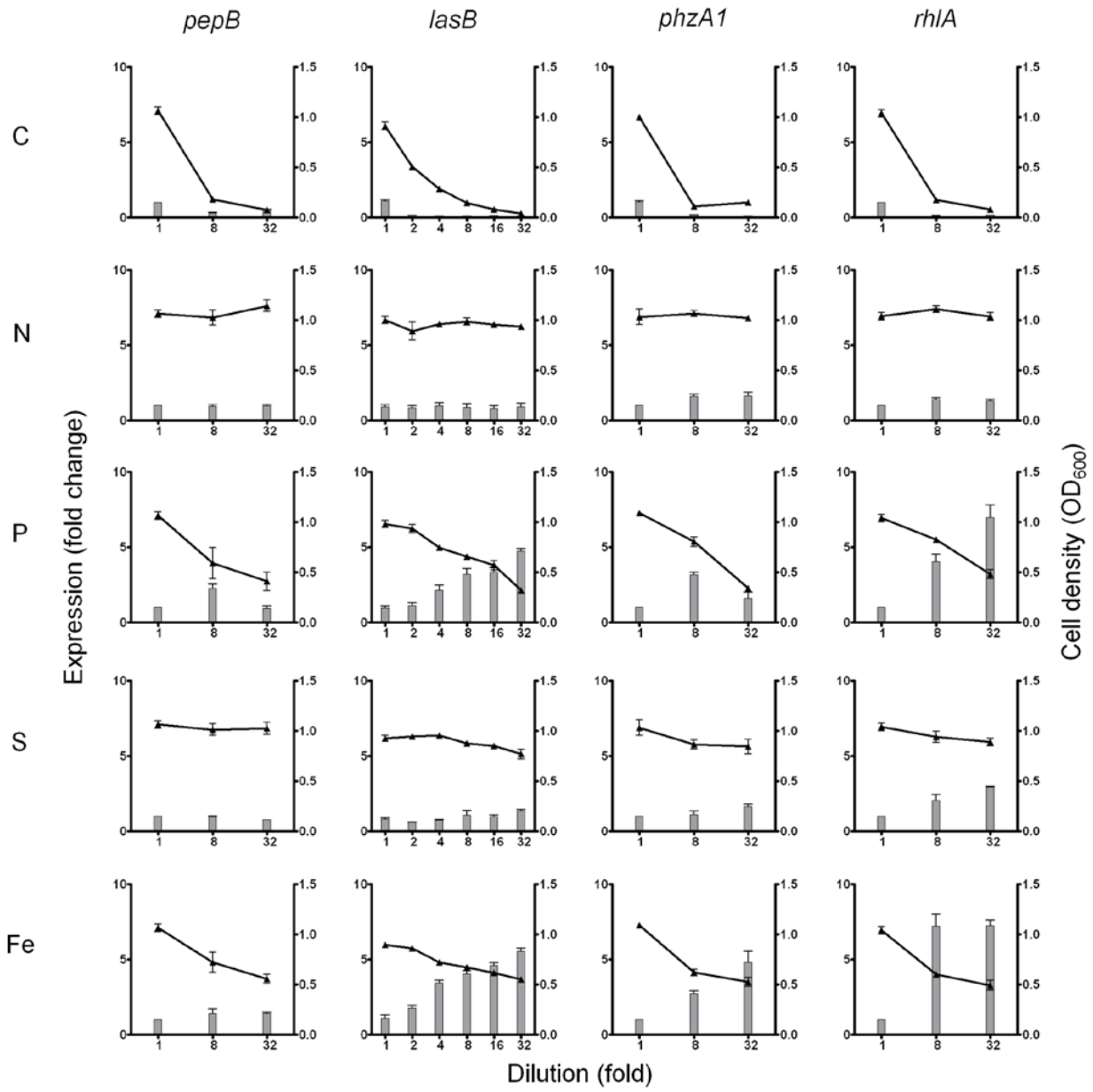

Figure S2. Nutrient dilution in glutamate minimal medium. Progressive dilution of carbon (C), nitrogen (N), phosphorous (P), sulfur (S), and iron (Fe) was carried out in MOPS minimal medium batch cultures with glutamate as the sole C-source and with *P. aeruginosa pepB*, *lasB*, *phzA1*, and *rhlA* reporter strains. Bars indicate the fold change in β-galactosidase expression compared to undiluted medium (left *y*-axis). Triangles indicate culture density (OD_{600}; right *y*-axis). Fold change values shown in graphs are means from three independent biological replicates, normalized to OD_{600}. Error bars indicate standard deviations of the mean.

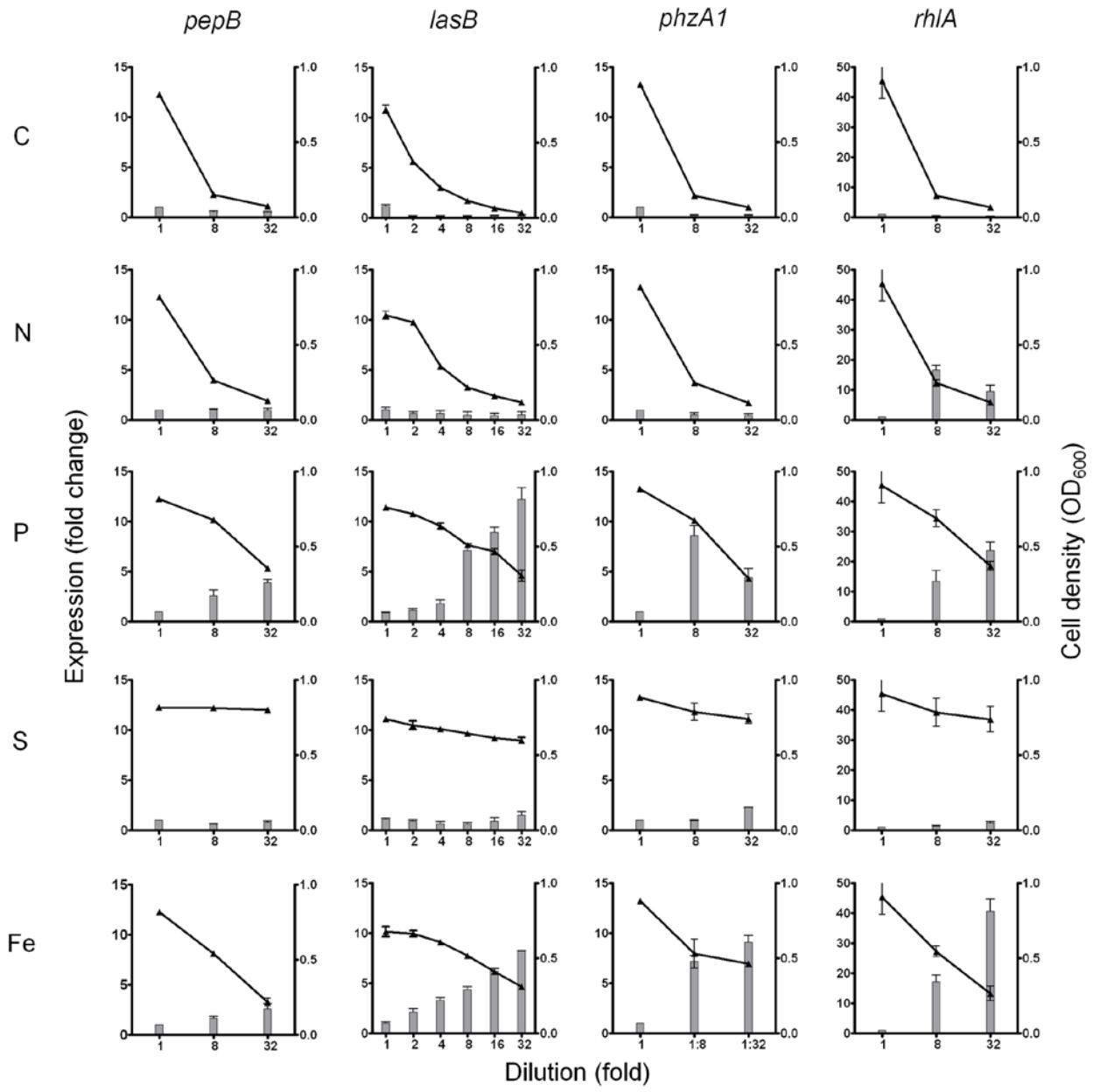

Figure S3. Nutrient dilution in succinate minimal medium. Progressive dilution of C, N, P, S, and Fe was carried out in MOPS minimal medium batch cultures with succinate as the sole carbon source and with *P. aeruginosa pepB*, *lasB*, *phzA1*, and *rhlA* reporter strains. Bars indicate the fold change in β-galactosidase expression compared to undiluted medium (left *y*-axis). Triangles indicate culture density (OD_{600}; right *y*-axis). Fold change values shown in graphs are means from three independent biological replicates, normalized to OD_{600}. Error bars indicate standard deviations of the mean.

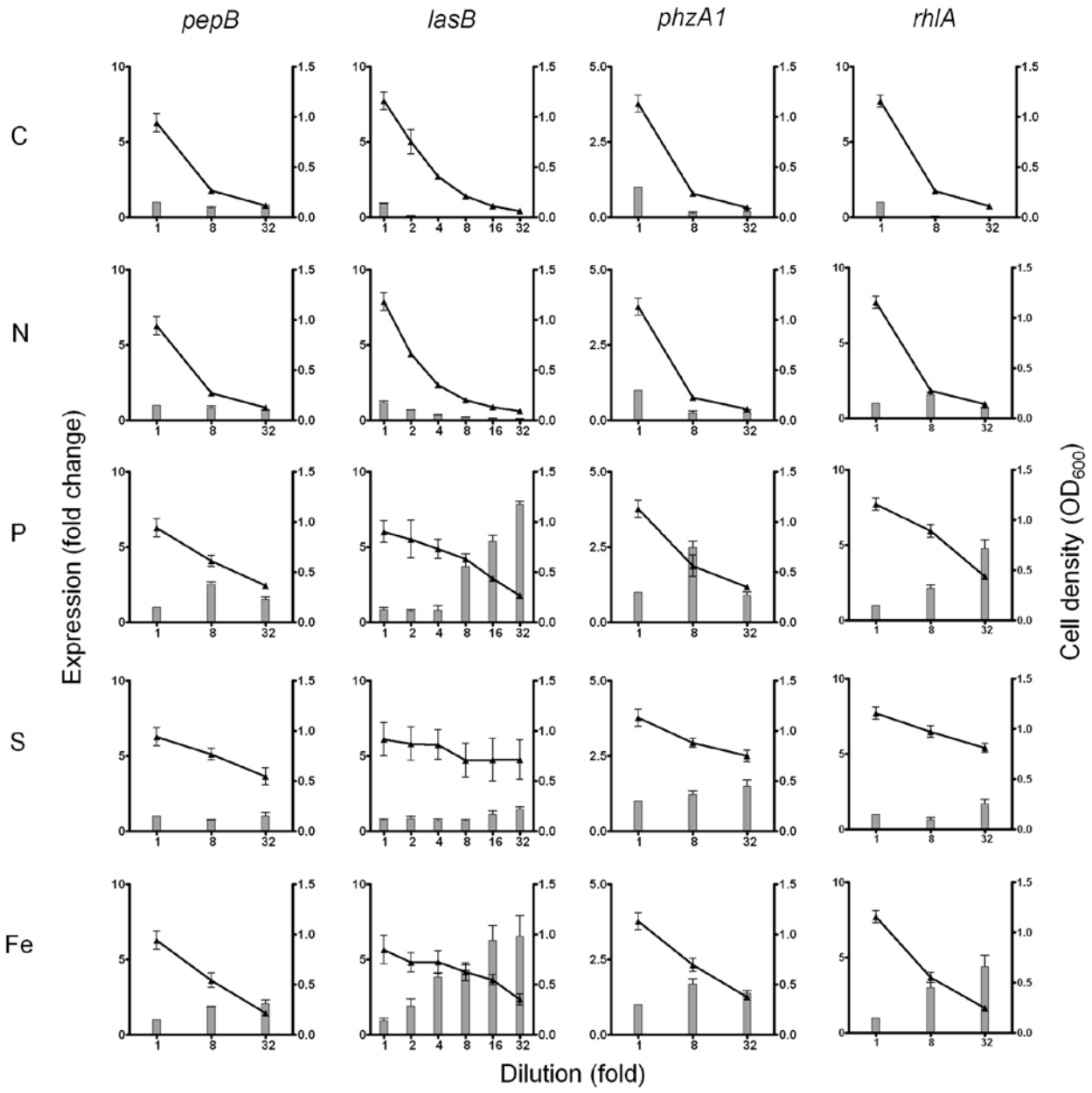

Figure S4. Nutrient dilution in glucose minimal medium. Progressive dilution of C, N, P, S, and Fe was carried out in MOPS minimal medium batch cultures with glucose as the sole C-source and with *P. aeruginosa pepB, lasB, phzA1*, and *rhlA* reporter strains. Bars indicate the fold change in β-galactosidase expression compared to undiluted medium (left *y*-axis). Triangles indicate culture density (OD_{600}; right *y*-axis). Fold change values shown in graphs are means from three independent biological replicates, normalized to OD_{600}. Error bars indicate standard deviations of the mean.

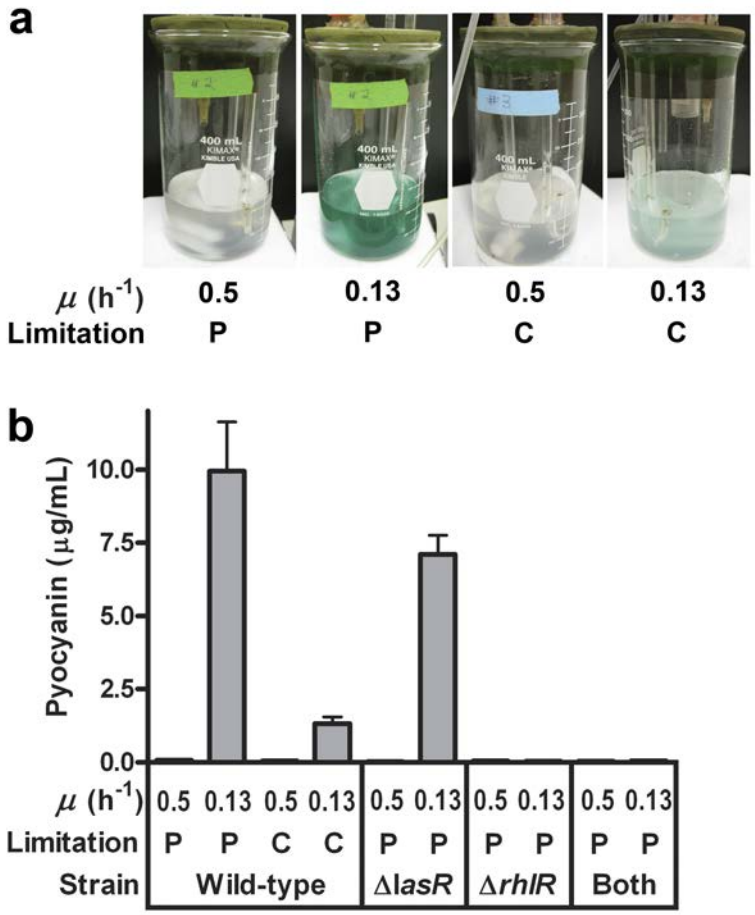

Figure S5. Influence of growth rate and limiting nutrients on pyocyanin production in chemostat culture. *P. aeruginosa* was grown in P-limited or C-limited glutamate minimal medium. **(a)** Images of *P. aeruginosa* wild-type cultures. The blue-green pigmentation is characteristic of pyocyanin, a secreted, redox-active, phenazine antibiotic. **(b)** Quantitation of pyocyanin production of the *P. aeruginosa* wild-type, a *lasR* mutant, a *rhlR* mutant, and a *lasR rhlR* double mutant (indicated as "both"). Pyocyanin concentrations are the means of three independent biological replicates. Error bars indicate standard deviations of the mean.

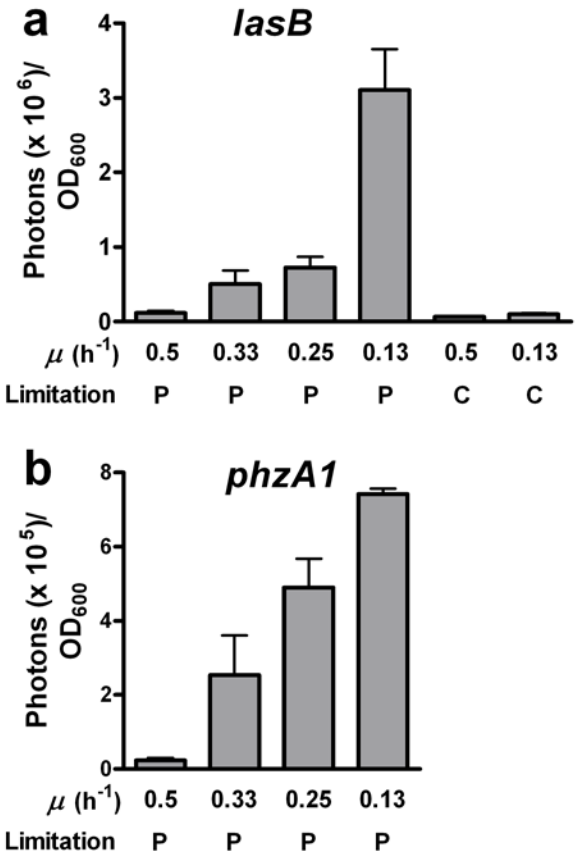

Figure S6. QS-controlled gene expression during chemostat culture measured by *lacZ* reporter fusions. Expression of **(a)** *lasB* and **(b)** *phzA1* in *P. aeruginosa* reporter strains during P-limited or C-limited growth in glutamate minimal medium, as indicated. Bars indicate β-galactosidase expression (in photons) normalized to culture density (OD_{600}). Values shown in the graph are the means of three independent biological experiments. Error bars indicate standard deviations of the means of three independent biological replicates.

SUPPLEMENTAL MATERIAL REFERENCES

1. **Schuster M, Lohstroh CP, Ogi T, Greenberg EP.** 2003. Identification, timing and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: A transcriptome analysis. J. Bacteriol. **185:**2066-2079.