Supporting Information

Contributions of the Heme Coordinating Ligands of the *Pseudomonas aeruginosa* Outer Membrane Receptor HasR to Extracellular Heme Sensing and Transport

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Table S1. Strains and plasmids used in this study.

Strains	Relevant genotype or description	Source of Reference
E. coli		
S17-1-λ-pir	pro thi hsdR_ Tpr Smr; chromosome::RP4–2 Tc::Mu-Km::Tn7/λpir	(1)
BL21 (DE3)	F^- ompT hsdSB (rB^- mB^-) gal dcm (DE3)	Stratagene
OneShot Top10	F- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ *lac*X74 *rec*A1 *ara*D139 Δ(*araleu*)7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	Invitrogen
P. aeruginosa		
PAO1	Wild type	(2)
H221R	Chromosomal in frame *hasR* mutation in PAO1	This study
H624A	Chromosomal in frame *hasR* mutation in PAO1	This study
I694G	Chromosomal in frame *hasR* mutation in PAO1	This study
H624Y	Chromosomal in frame *hasR* mutation in PAO1	This study
Plasmids		
pCR 2.1-TOPO TA	Amp^R, KanR; Subcloning vector	Invitrogen
pEX18Tc	Tc^R; allelic replacement vector	(3)
pEX18Tc-H221R	Tc^R; *hasR* mutant allele cloned into pEX18Tc plasmid by Gibson assembly	This study
pEX18Tc-H624A	Tc^R; *hasR* mutant allele cloned into pEX18Tc plasmid by Gibson assembly	This study
pEX18Tc-I694G	Tc^R; *hasR* mutant allele cloned into pEX18Tc plasmid by Gibson assembly	This study
pEX18Tc-H624Y	Tc^R; *hasR* mutant allele cloned into pEX18Tc plasmid by Gibson assembly	This study
cytochrome b_5-PET11a	Expression vector	(4)

Table S2. Oligonucleotide primers and probes used in this study.

Name	Sequence	Reference
RT-qPCR Primers and probes		
qPCR-*hasR* probe	5'-/FAM/CTG GCC TAC GGG CAG CTC TCC TA/3BHQ_1/-3'	
qPCR-*hasR* F	5'-CGT GGC GTC GAG TAC CAG-3'	
qPCR-*hasR* R	5'-GGT CTT CGA ACA GAA GTC GTT G-3'	
qPCR-*hasAp* probe	5'-/56-FAM/TCG ACC CGA GCC TGT/3BHQ_1/-3'	
qPCR-*hasAp* F	5'-ATC GAC GCG CTG CTG AA-3'	
qPCR-*hasAp* R	5'-TGG TCG AAG GTG GAG TTG ATC-3'	
qPCR-*phuR* probe	5'-/56-FAM/CTA CGC GCA GAC CCA CCG CAA C/3BHQ_1/-3'	
qPCR-*phuR* F	5'-TGA CCA ACG ACT TCT TCA GC-3'	
qPCR-*phuR* R	5'-CTT TAC GAT GTC CGG ATC GAC-3'	
qPCR-PA*16S* F	5'-GGT GGT TCA GCA AGT TGG ATG TG-3'	(5)
qPCR-PA*16S* R	5'-CCA GGT GGT CGC CTT CGC-3'	(5)
Allelic Exchange		
HasR upstream	5'-GCG GTA CCT CGC TTC CGA GAG C-3'	This study
HasR downstream	5'-GCT CTA GAC AAC GAG TGG TGA ATG-3'	This study
Quickchange mutagenesis primers		
H221R-F	5'-GAA CTA CCA GCA GAG CGG CCG CCA GCA GCG CAA C-3'	This study
H221R-R	5'-GTT GCG CTG CTG GCG GCC GCT CTG CTG GTA GTT C-3'	This study
H624A-F	5'-GAT CAC CGG CCG CCC CGC CGG CGG CGG CGC GGA AAA C-3'	This study
H624A-R	5'-GTT TTC CGC GCC GCC GCC GGC GGG GCG GCC GGT GAT C-3'	This study
I694G-F	5'-GTA CGG CAT GGC CGG GGG CGG CAA CAG CGC CTA C-3'	This study
I694G-R	5'-GTA GGC GCT GTT GCC GCC CCC GGC CAT GCC GTA C-3'	This study
H624Y-F	5'-GAT CAC CGG CCG CCC CTA CGG CGG CGG CGC GGA AAA C-3'	This study
H624Y-R	5'-GTT TTC CGC GCC GCC GCC GTA GGG GCG GCC GGT GAT C-3'	This study

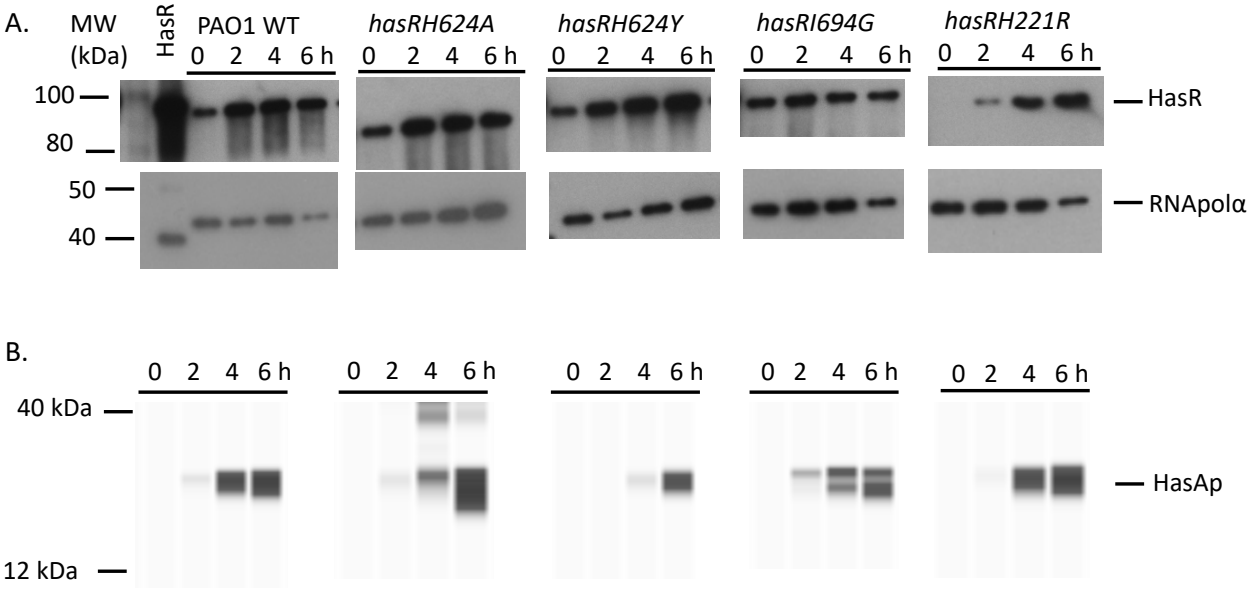

Figure S1. Relative HasR and HasAp protein levels for the *hasR* allelic strains in iron-deplete conditions. *A*. Representative Western blot of HasR for the WT and *hasR* allelic strains following growth in M9 minimal media. Total protein (5 μg) was loaded in each well. The RNA polymerase α subunit was used as a loading control. The RNA polymerase α panel for PAO1 WT and *hasRI694G* strain are the same as shown in Fig 4C as they represent the same set of biological samples from replicates collected in iron-deplete conditions. *B*. Representative Wes digital images of HasAp for the PAO1 WT and *hasR* allelic strains following growth in M9 minimal media. 4 μl extracellular supernatant was loaded and run on the automated Wes as described in the Experimental Procedures.

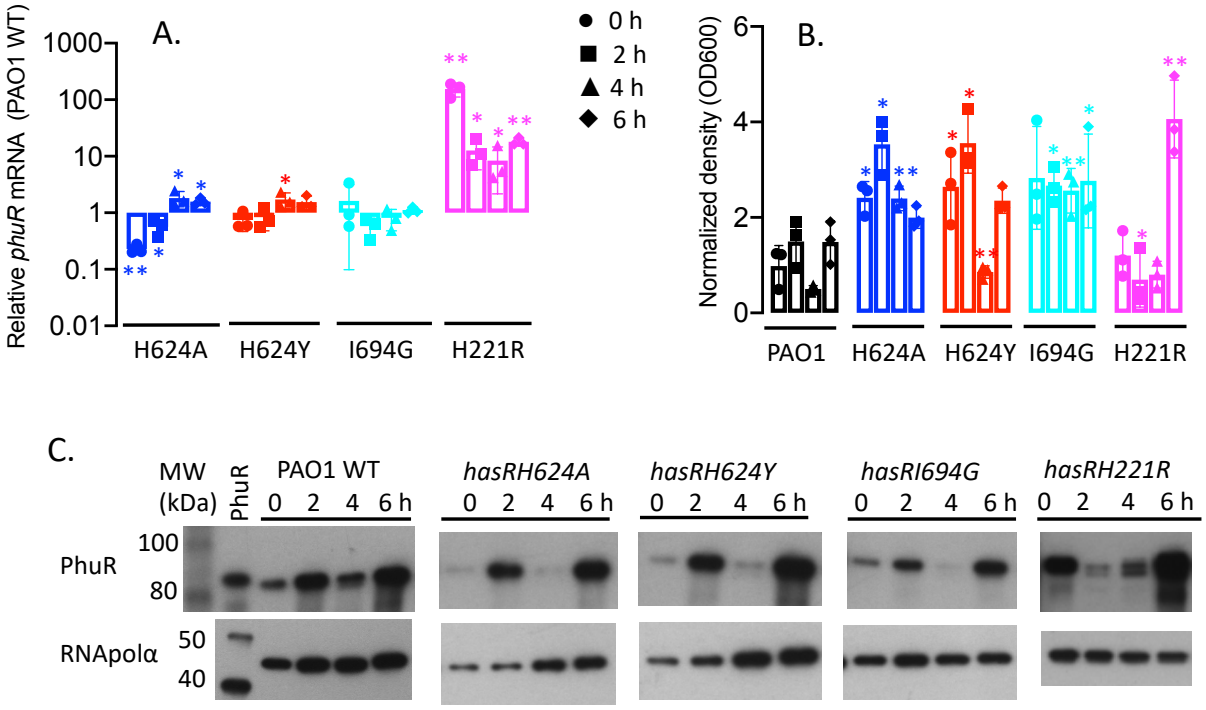

Figure S2. Relative *phuR* mRNA and protein levels for the *hasR* allelic strains in heme supplemented conditions. *A*. mRNA isolated at 0, 2, 4, and 6 h following growth in M9 minimal media supplemented with 1μM heme. mRNA values represent the mean from three biological experiments each performed in triplicate and normalized to PAO1 at the same timepoint. *Error bars* represent the standard deviation from three independent experiments performed in triplicate. The indicated *p* values were normalized to mRNA levels of PAO1 WT at the same timepoint, where *, $p < 0.05$; **, $p < 0.005$. *B.* Normalized density ($n = 3$) was performed on Western blots for three separate biological replicates. The indicated *p* values were normalized to PAO1 at the same timepoint where *, $p < 0.05$; *p,* < 0.005. *C.* Representative Western blot of PAO1 WT and the *hasR* allelic strains. Total protein (5 μg) was loaded in each well. The RNA polymerase α subunit was used as a loading control. The RNA polymerase α panel for PAO1 WT, *hasR624A,* and *hasRH221R* strain are the same as shown in Fig 8C as they represent the same set of biological samples from replicates collected in heme supplemented conditions.

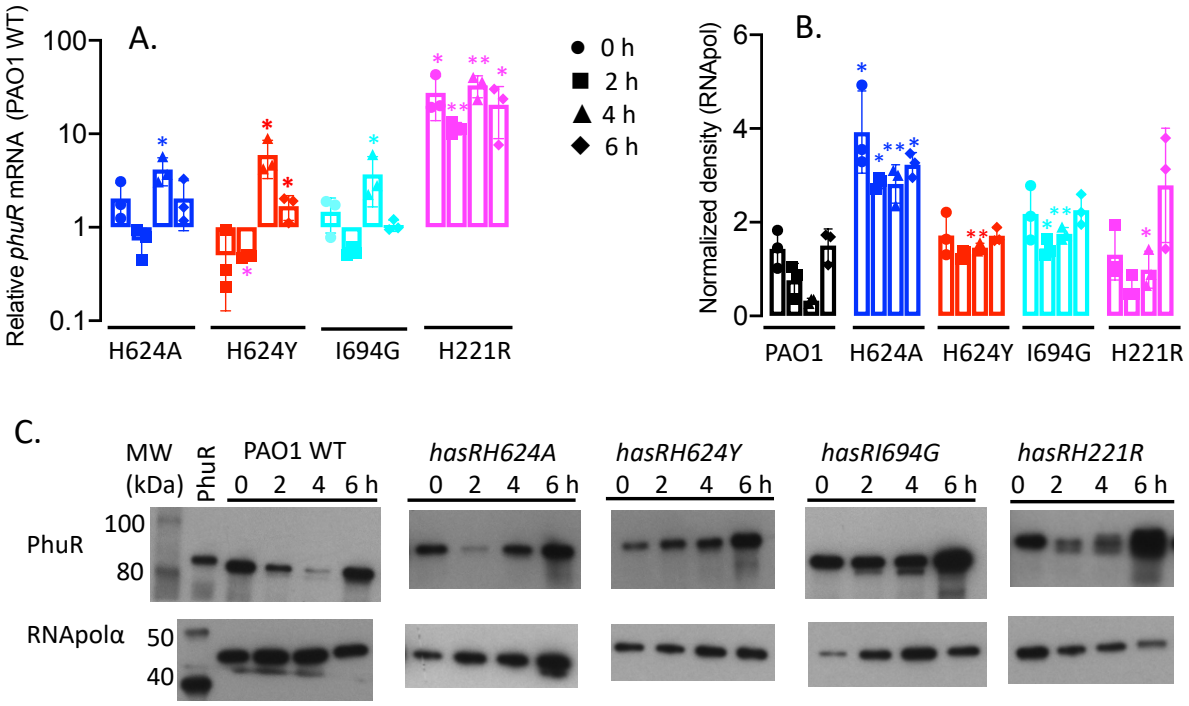

Figure S3. Relative *phuR* mRNA and protein levels for the *hasR* allelic strains in holo-HasAp supplemented conditions. *A*. mRNA isolated at 0, 2, 4, and 6 h following growth in M9 minimal media supplemented with 1μM holo-HasAp. mRNA values represent the mean from three biological experiments each performed in triplicate and normalized to PAO1 at the same timepoint. *Error bars* represent the standard deviation from three independent experiments performed in triplicate. The indicated *p* values were normalized to mRNA levels of PAO1 WT at the same timepoint, where *, $p < 0.05$; **, $p < 0.005$. *B.* Normalized density ($n = 3$) was performed on Western blots for three separate biological replicates. The indicated *p* values were normalized to PAO1 WT at the same timepoint where *, $p < 0.05$; *p,* < 0.005. *C.* Representative Western blot of PAO1 WT and the *hasR* allelic strains. Total protein (5 μg) was loaded in each well. The RNA polymerase α subunit was used as a loading control. The RNA polymerase α panels are the same as shown in Fig 10C as they represent the same set of biological samples from replicates collected in holo-HasAp supplemented conditions.

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