Table S1

Bacterial strains	Description	Reference
Escherichia coli		
Top10	*E. coli* laboratory cloning strain	Invitrogen
Top10+pBAD-*sodA*-TEV-His	*E. coli* strain for expression of rSodA	This study
Streptococcus pyogenes		
5448	*S. pyogenes* invasive M1T1 strain	(1)
5448Δ*pmtA*	*S. pyogenes* 5448Δ*pmtA::aphA-3* deletion mutant	(2)
5448Δ*pmtA::pmtA*	*S. pyogenes* 5448 *pmtA* complemented strain	(2)
5448Δ*perR*	*S. pyogenes* 5448Δ*perR::aad9* deletion mutant	(3)
5448Δ*perR::perR*	*S. pyogenes* 5448Δ*perR* complemented strain	(3)
5448Δ*mtsR*	*S. pyogenes* 5448Δ*mtsR::aad9* deletion mutant	This study
5448Δ*mtsR::mtsR*	*S. pyogenes* 5448Δ*mtsR* complemented strain	This study
5448Δ*mtsABC*	*S. pyogenes* 5448Δ*mtsABC::aphA-3*	This study
5448Δ*mtsABC::mtsABC*	*S. pyogenes* 5448Δ*mtsABC* complemented strain	This study
5448Δ*sodA*	*S. pyogenes* 5448Δ*sodA::aphA-3* deletion mutant	This study
5448Δ*sodA::sodA*	*S. pyogenes* 5448Δ*sodA* complemented strain	This study

Plasmids	Description	Reference
pJRS233	Temperature sensitive shuttle plasmid::Em^R	(4)
pLZ12-TS	Temperature sensitive shuttle plasmid::$Spec^R$	This study
pUC4ΩKm2	Template for Km^R	(5)
pUCSpec	Template for $Spec^R$	(6)
pBAD-Myc-His-A	Template plasmid for generation of pBAD-TEV-His	
pBAD-TEV-His		This study
pBAD-*sodA*-TEV-His	pBAD-TEV-His vector with *sodA* inserted at *Nco*I and *Apa*I site	This study
pJRS233-*mtsR*-KO	pJRS233 + *mtsR* knockout construct inserted at the *Xho*I and *Bam*HI site	This study
pJRS233-*mtsR*-comp	pJRS233 + *mtsR* complement construct inserted at the *Xho*I and *Bam*HI site	This study
pJRS233-*mtsABC*-KO	pJRS233 + *mtsABC* knockout construct inserted at the *Xho*I and *Pst*I site	This study
pJRS233-*mtsABC*-comp	pJRS233 + *mtsABC* complement construct inserted at the *Xho*I and *Pst*I site	This study
pLZ12-TS-*sodA*-KO	pLZ12-TS + *sodA* KO construct	This study
pJRS233-*sodA*-comp	pJRS233 + *sodA* complement construct	This study

Table S2

Primer	Sequence (5'-3')	
Deletion mutation and reverse complementation constructs		
pBAD-site-F	GGGAATTCGAAGCTTGGGCCCGAGGATCTGTACTTTCAGAGCGTCGACCATCAT CATCATCA	
pBAD-site-R	ATATGGTACCAGCTGCAGATCTCGA	
sodA-prot-F	CCCGGG**CCATGG**CTATTATTTTACCAGAACTTCC	***Nco*I**
sodA-prot-R	CGCGCG**GGGCCC**TTTAGCGGCTTGGTAAAGTTCT	***Apa*I**
sodA-KO-1	TGGTGTTTATGGCTCTCTTGGTCGTCAGACTGATGGGCCCTGATGCGGCTAGAG ATTTGATTCATGATT	*pLZ-5*'
sodA-KO -2	CAAAGTTGGCGTATAACATACGCGTATGGAAGTTCTGG	*aphA-3* 5'
sodA-KO -3	TACTGGATGAATTGTTTTAGACTTTACCAAGCCGCTAAATAAGAAAAGG	*aphA-3* 3'
sodA-KO -4	TACGAACTGGCACAGATGGTCATAACCTGAAGGAAGATCTCTGCATCCATAACA GCTTGCTTAGC	*pLZ-*3'
sodA-comp-F	CGGGCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATTGATGCGGCTAGAG ATTTGATTCATGATT	*pJRS-5*'
sodA-comp-R	CTCTAGAACTAGTGGATCCCCCGGGCTGCAGGAATTCGATTCTGCATCCATAAC AGCTTGCTTAGC	*pJRS-*3'
pJRS233-F	ATCGAATTCCTGCAGCCCGG	
pJRS233-R	ATCAAGCTTATCGATACCGTCGACC	
km-sodA-F	TACCAGAACTTCCATACGCGTATGTTATACGCCAACTTTGAAAACAACTTTGAA AAAGC	*aphA-3* 5'
km-sodA-R	ATTTAGCGGCTTGGTAAAGTCTAAAACAATTCATCCAGTAAAATATAATATTTT ATTTTCTCCCAAT	*aphA-3* 3'
mtsR-KO-1	CCCGG**CTCGAG**CCATCAGACACGGCAAAGTA	***Xho*I**
mtsR-KO-2	GATATGATCTTTCATTTCCATAAAACTAAAGTCTTCTTTATTAGGCGTCAT	*aad9* 5'
mtsR-KO-3	GGAAATATTCATTCTAATTGGTAATCAGCTTTATGTCACAGCCCTTTAA	*aad9* 3'
mtsR-KO-4	CGCGCG**GGATCC**GAATTGCAATCACTTGACCAAA	***Bam*HI**
spec-F	CTTTAGTTTTATGGAAATGAAAGATCATATC	
spec-R	CTGATTACCAATTAGAATGAATATTTCC	
mtsABC-KO-1	CCCGGG**CTCGAG**TCGTTTGGCCTTTCTCTTATT	***Xho*I**
mtsABC-KO-2	CAAAGTTGGCGTATAACATACAACATCTTCTGGTAATGGTTC	*aphA-3* 5'
mtsABC-KO-3	TTACTGGATGAATTGTTTTAGTATTGATTACGCCAGCAGCGACA	*aphA-3* 3'
mtsABC-KO-4	CCCGGG**CTGCAG**CCTTATCAGATAATAGGCTAAATACC	***Pst*I**
km-F	TATGTTATACGCCAACTTTG	
km-R	CTAAAACAATTCATCCAGTAA	
pLZ-12-F	AGATCTTCCTTCAGGTTATG	
pLZ-12-R	GGGCCCATCAGTCTGACG	
Gene expression studies		
pmtA-qRT-F	GAAAAGCAAAACCGCCACCT	
pmtA-qRT-R	GGGCACATGGTGAAGCTACT	
sodA-qRT-F	CCTGAACCAAAACGTCCAGT	
sodA-qRT-R	CACCTAAACCATGCGCTTTT	
mtsA-qRT-F	CAAACAATTGATTGCAAAGGATCC	
mtsA-qRT-R	GTCTAGTTTTTCCAATTTAGCCACA	
dpr-qRT-F	ATGCGTGGTCCAGGTTTCTT	
dpr-qRT-R	AATAAGGCGCTCCGCCAATA	
siaA-qRT-F	CACTTCGGTTGCTGTGGTTG	
siaA-qRT-R	ATGGGTAAACCCACACGCTT	
shp-qRT-F	TGGAAGCTTTCAGGCAGTTGA	
shp-qRT-R	TTCAACAAACATGCTGCCTCG	
shr-qRT-F	GAAAGCAACAAAGACGCGGT	
shr-qRT-R	ACAGTCGATGAGTATCGGCG	
ahpF-qRT-F	TTAATGGCCGAAGTGGAGGC	
ahpF-qRT-R	CCATTTGGCCCCAAGAGCTA	
gyrA-RT-F	GAAGTGATCCCTGGACCTGA	
gyrA-RT-R	CCCGACCTGTTTGAGTTGTT	

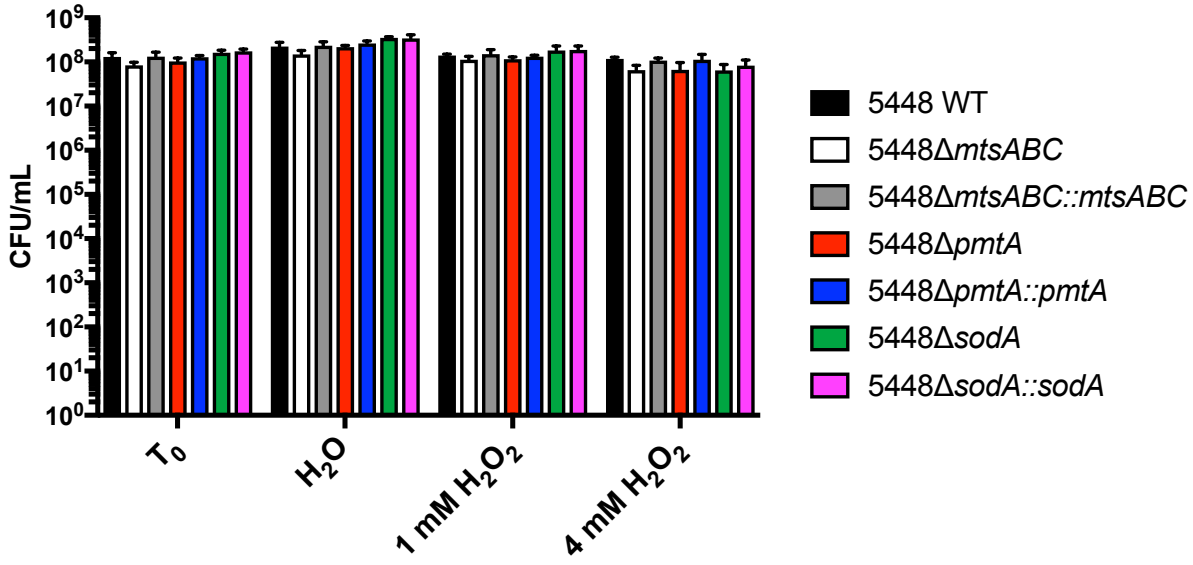

Figure S1 - Viability of GAS strains following hydrogen peroxide challenge. Strains 5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC::mtsABC*, 5448Δ*pmtA*, 5448Δ*pmtA::pmtA*, 5448Δ*sodA* and 5448Δ*sodA::sodA* were grown in THY to mid exponential phase (OD_{600} = 0.6) and challenged with either H_2O, 1 mM H_2O_2 or 4 mM H_2O_2 and cell viability assessed by plating on THY agar after 30 min challenge.

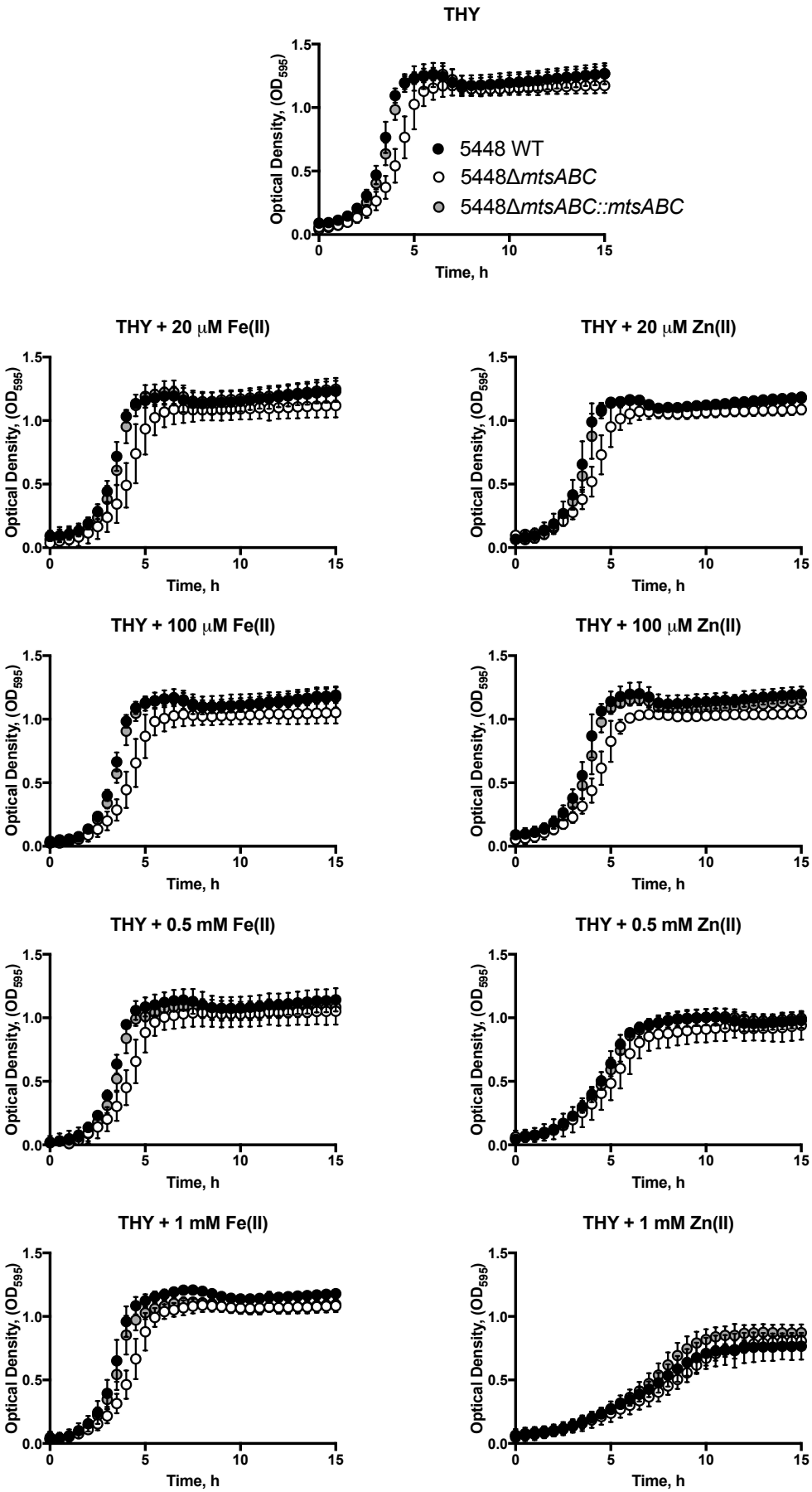
THY
Optical Density, (OD595)
Time, h
5448 WT
5448ΔmtsABC
5448ΔmtsABC::mtsABC
THY + 20 μM Fe(II)
THY + 20 μM Zn(II)
THY + 100 μM Fe(II)
THY + 100 μM Zn(II)
THY + 0.5 mM Fe(II)
THY + 0.5 mM Zn(II)
THY + 1 mM Fe(II)
THY + 1 mM Zn(II)

Figure S2 - Growth curve analysis of 5448 WT, 5448Δ*mtsABC* and 5448Δ*mtsABC::mtsABC* in the presence of Fe(II) or Zn(II).

Growth curve analysis of 5448 WT, 5448Δ*mtsABC* and 5448Δ*mtsABC::mtsABC* in the presence of Fe(II) or Zn(II). Overnight cultures of 5448 WT (black circles), 5448Δ*mtsABC* (open circles) and 5448Δ*mtsABC::mtsABC* (grey circles) were diluted to OD_{600} = 0.05 into THY broth alone or THY supplemented with 20 μM, 100 μM, 0.5 mM or 1 mM Fe(II) or Zn(II). Growth was monitored at 37°C by optical density recording at 595 nm (OD_{595}). Graphs represent mean ± standard deviation of 3 independent biological replicates

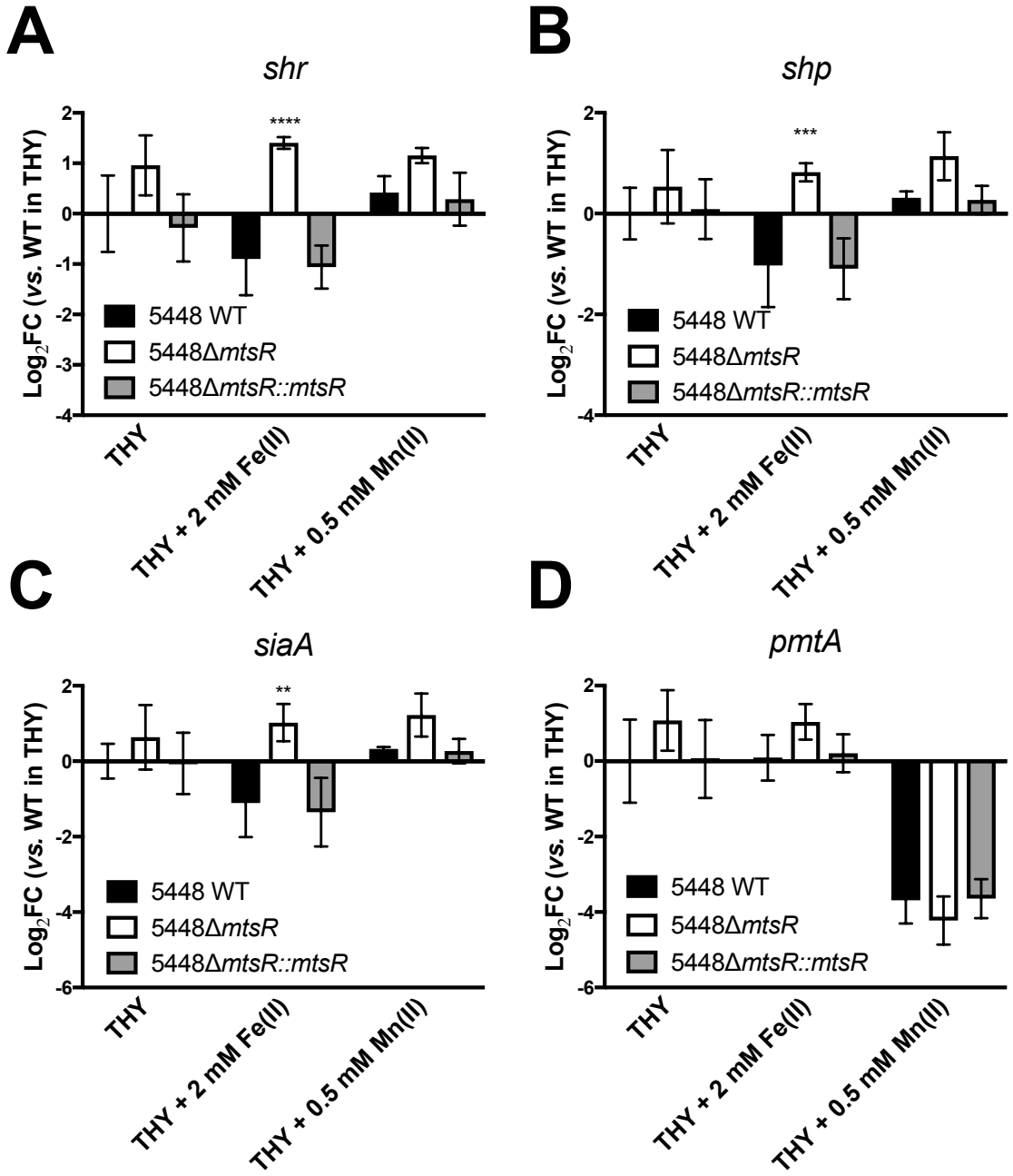

Figure S3 - Gene expression analysis of MtsR-regulated genes in 5448 WT, 5448Δ*mtsR* and 5448Δ*mtsR::mtsR*

5448 WT, 5448Δ*mtsR*, and 5448Δ*mtsR::mtsR* were grown at 37°C to mid-exponential phase (OD_{600} = 0.6-0.8) in either THY alone, THY + 2 mM Fe(II) or 0.5 mM Mn(II) and gene expression of *shp* (**A**), *shr* (**B**), *siaA* (**C**), and *pmtA* (**D**) analysed by qPCR using *gyrA* as the reference gene. Data represents the mean ± standard deviation of 3 independent biological

replicates (2-way ANOVA used comparing all to control of that strain ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

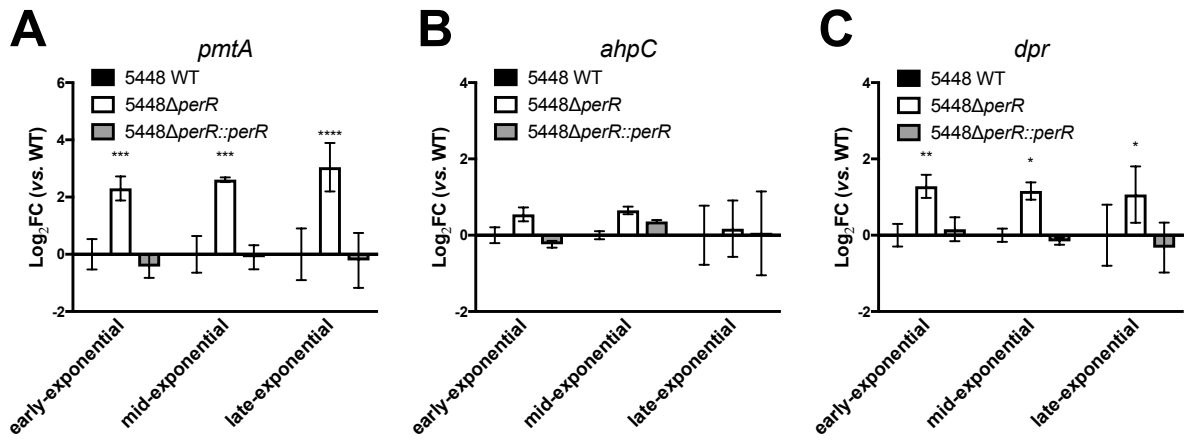

Figure S4 - Gene expression analysis of PerR-regulated genes in 5448 WT, 5448Δ*perR* and 5448Δ*perR::perR* at various phases of growth

5448 WT, 5448Δ*perR*, and 5448Δ*perR::perR* were grown in THY at 37°C and harvested at OD_{600} = 0.3 (early), OD_{600} = 0.6 (mid), or OD_{600} = 0.8 (late) -exponential phase. Gene expression of *pmtA* (**A**), *ahpC* (**B**) and *dpr* (**C**) was analysed by qPCR using *gyrA* as the reference gene. Data represents the mean ± standard deviation of 3 independent biological replicates (2-way ANOVA used comparing all to control of that strain * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Figure S4D in main manuscript.

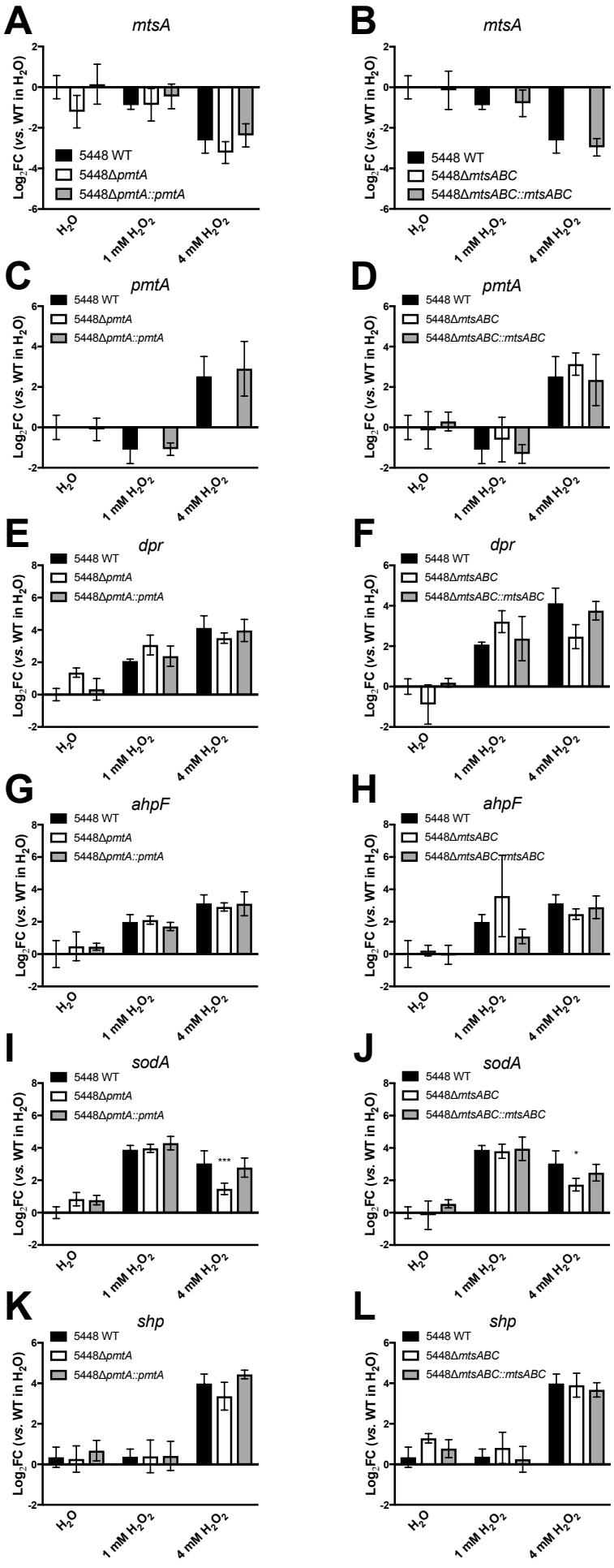
A
mtsA
B
mtsA
C
pmtA
D
pmtA
E
dpr
F
dpr
G
ahpF
H
ahpF
I
sodA
J
sodA
K
shp
L
shp
Log2FC (vs. WT in H2O)
5448 WT
5448ΔpmtA
5448ΔpmtA::pmtA
5448ΔmtsABC
5448ΔmtsABC::mtsABC
H2O
1 mM H2O2
4 mM H2O2
***
*

Figure S5 - Gene expression analysis during hydrogen peroxide stress

5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC::mtsABC*, 5448Δ*pmtA* and 5448Δ*pmtA::pmtA* were grown at 37°C to mid-exponential phase (OD_{600} = 0.6-0.8) in THY and challenged with either sterile H_2O, 1 mM H_2O_2 or 4 mM H_2O_2 and gene expression analysed by qPCR using *gyrA* as the reference gene. Data represents the mean ± standard deviation of 3 independent biological replicates. 2-way ANOVA used comparing strains to 5448 WT of that treatment (* $P < 0.05$, ** $P < 0.001$). Figure S5K in main manuscript.

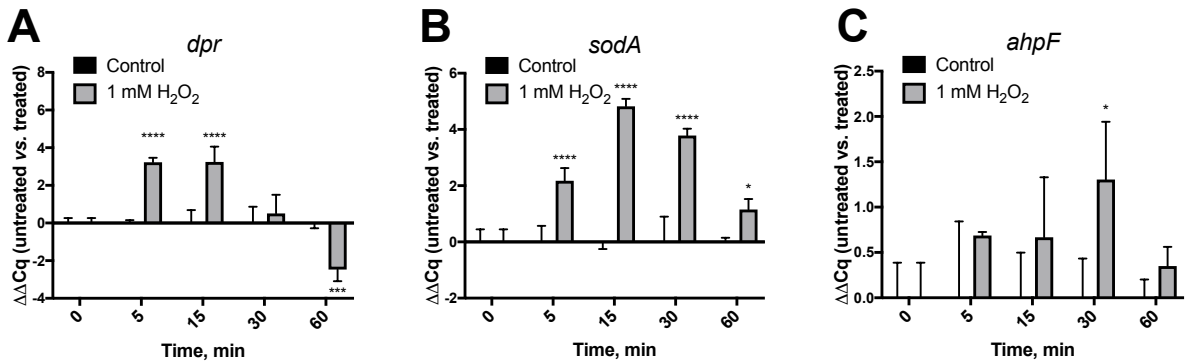

Figure S6 – Gene expression timecourse analysis during hydrogen peroxide stress

5448 WT was grown at 37°C to mid-exponential phase (OD_{600} = 0.6-0.8) in THY and challenged with either sterile H_2O (control) (black bars) or 1 mM H_2O_2 (grey bars) and gene expression analysed at t = 0, 5, 15, 30 or 60 min by qPCR using *gyrA* as the reference gene. Data represents the mean ± standard deviation of 3 independent biological replicates. Figure S6D in main manuscript.

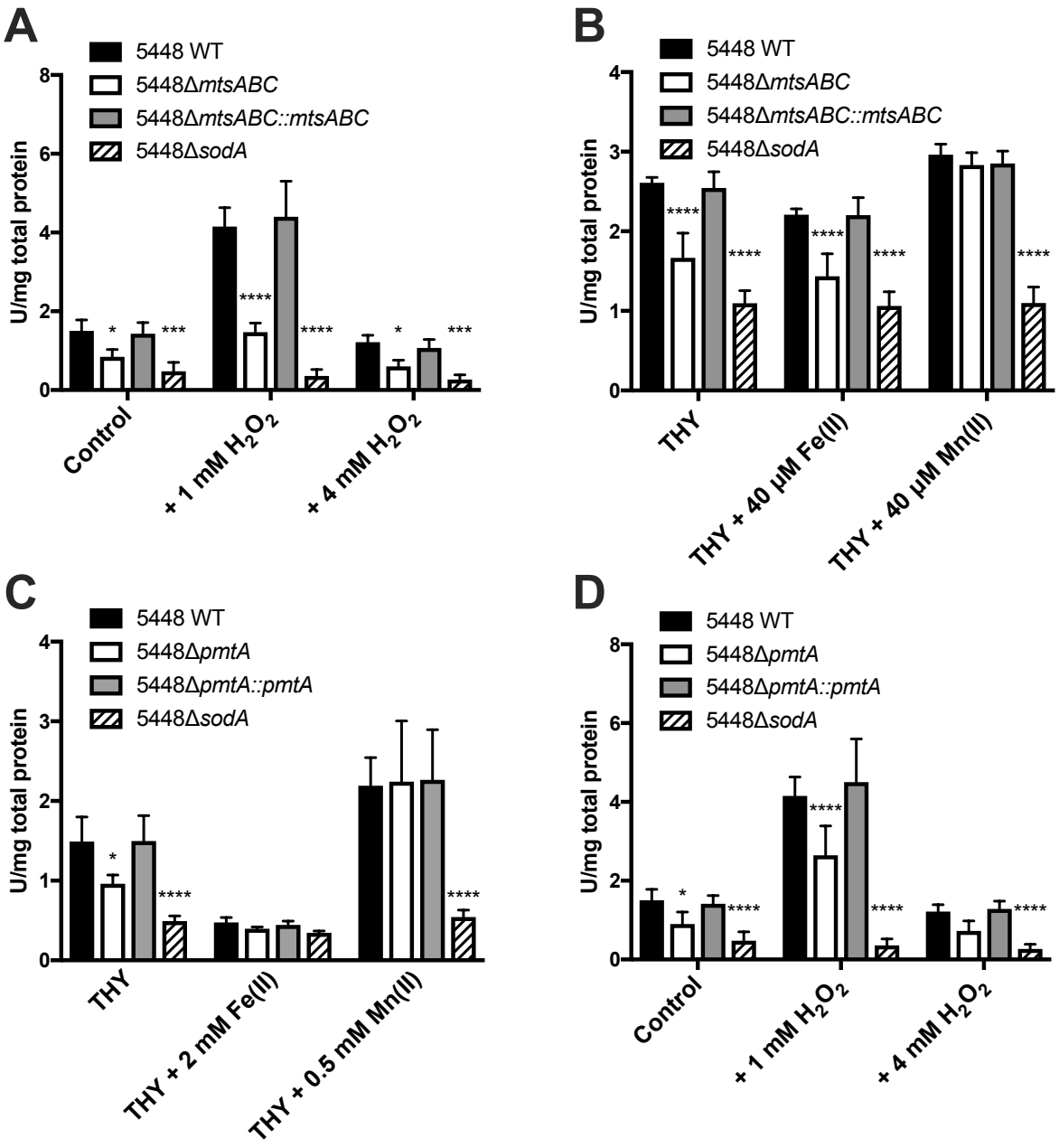

Figure S7 - Superoxide dismutase activity

5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC::mtsABC* and 5448Δ*sodA* (**A**) or 5448 WT, 5448Δ*pmtA*, 5448Δ*pmtA::pmtA* and 5448Δ*sodA* (**D**) were grown in THY at 37°C to mid-exponential phase (OD_{600} 0.6 – 0.8), challenged with either H_2O, 1 mM H_2O_2 or 4 mM H_2O_2 cells harvested and SOD activity analysed. 5448 WT, 5448Δ*mtsABC*, 5448Δ*mtsABC::mtsABC* and 5448Δ*sodA* were grown in THY, THY + 40 µM Fe(II), or THY + 40 µM Mn(II) to mid-exponential growth phase (OD_{600} 0.6 – 0.8), cells harvested and SOD activity analysed (**B**).

5448 WT, 5448Δ*pmtA*, 5448Δ*pmtA::pmtA* and 5448Δ*sodA* were grown in THY, THY + 2 mM Fe(II) or THY + 0.5 mM Mn(II) to mid-exponential growth phase (OD_{600} 0.6 – 0.8), cells harvested and SOD activity analysed (**C**) (2-way ANOVA of strains compared to 5448 WT of that condition, * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$).

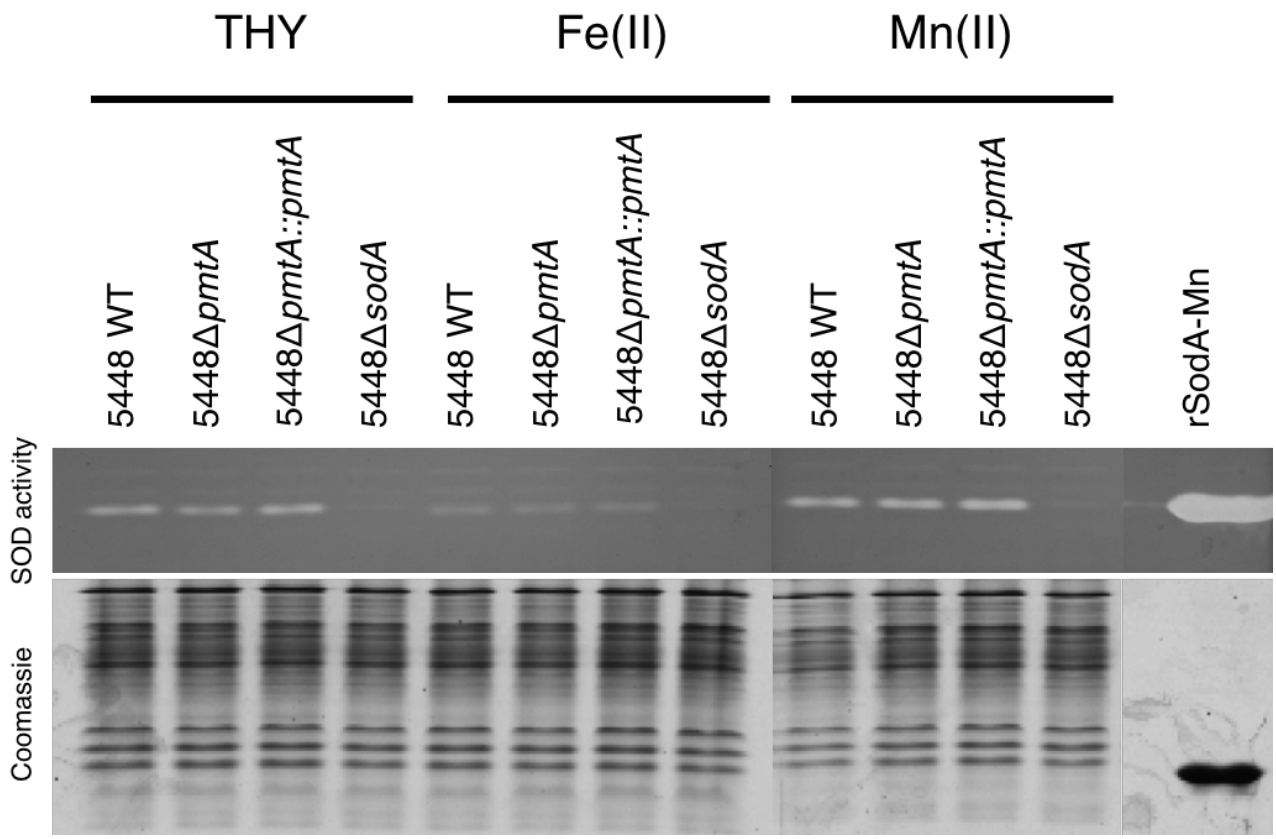

Figure S8 – SOD in-gel activity assay and coomassie gel

5448 WT, 5448Δ*pmtA* and 5448Δ*pmtA::pmtA* were grown in THY, THY + 2 mM Fe(II) or THY + 0.5 mM Mn(II) to mid-exponential growth phase (OD_{600} 0.6 – 0.8), cells harvested and mechanically lysed. 40 μg protein was loaded into native gels and in-gel SOD activity analysed (top) or stained with coomassie for total protein (bottom).

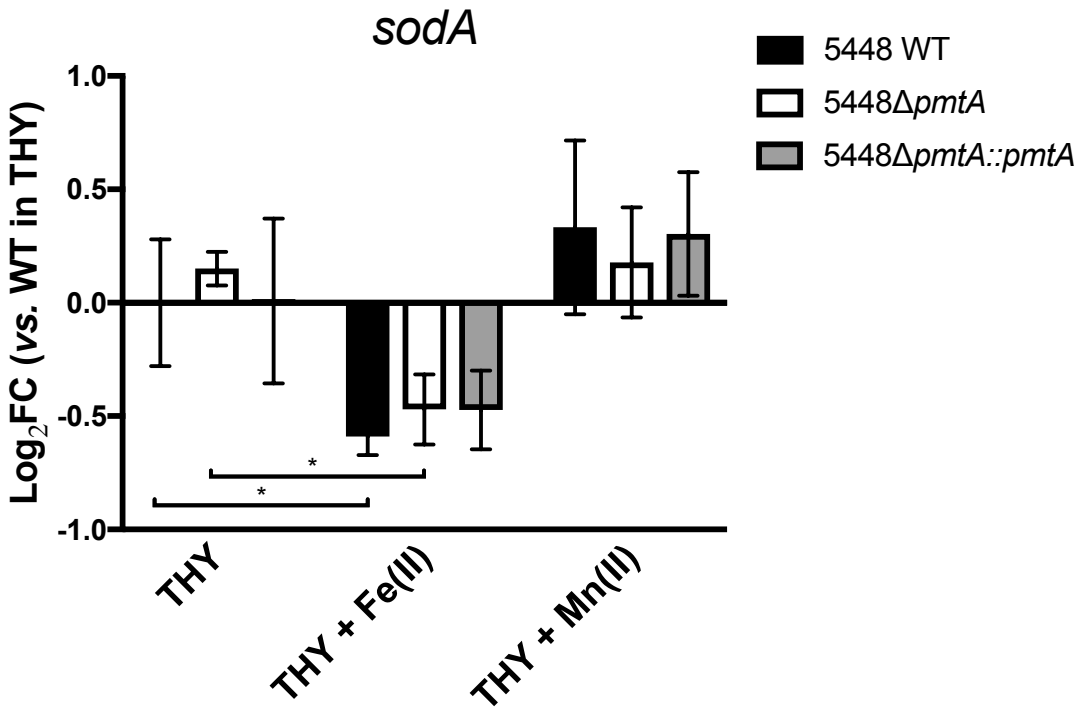

Figure S9 – Gene expression analysis of *sodA*

Gene expression analysis of *sodA*. 5448 WT, 5448Δ*pmtA* and 5448Δ*pmtA::pmtA* were grown at 37°C to mid-exponential phase (OD_{600} = 0.6-0.8) in either THY alone, THY + 2 mM Fe(II) or 0.5 mM Mn(II) and gene expression of *sodA* analysed by qPCR using *gyrA* as the reference gene. Data represents the mean ± standard deviation of 3 independent biological replicates.

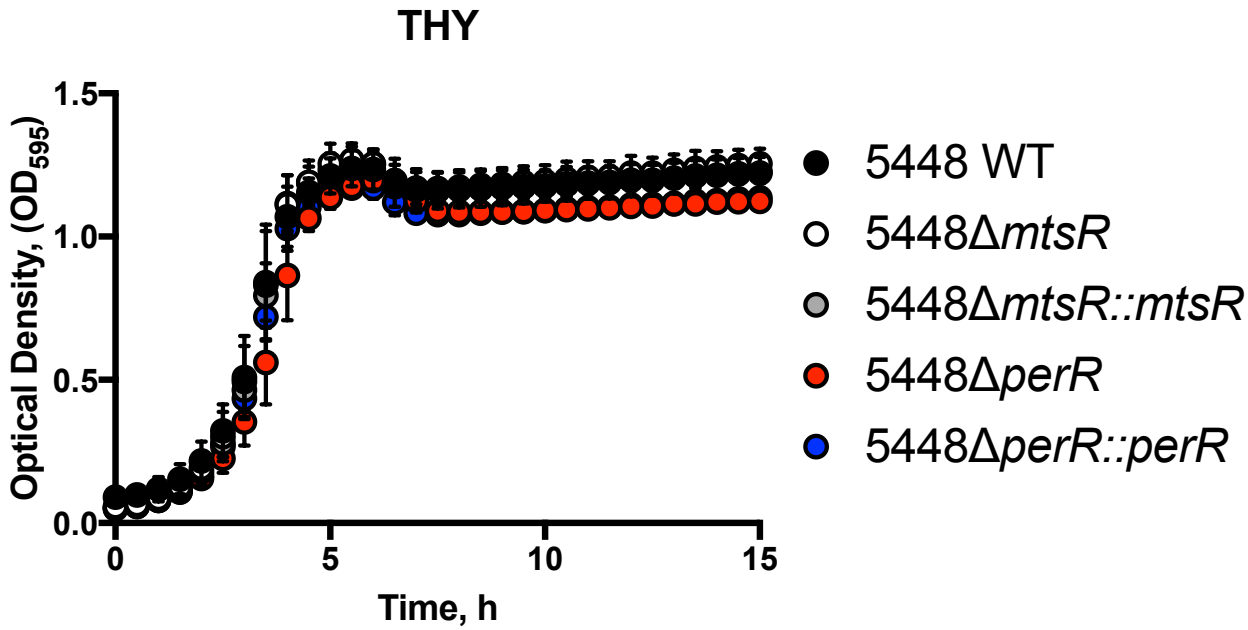

Figure S10 - Growth of strains in THY for streptonigrin control

5448 WT (black circles), 5448Δ*mtsR* (white circles), 5448Δ*mtsR::mtsR* (grey circles), 5448Δ*perR* (red circles) and 5448Δ*perR::perR* (blue circles) were diluted to OD_{600} = 0.05 into THY broth. Growth was monitored at 37°C by optical density recording at 595 nm (OD_{595}). Graphs represent mean ± standard deviation of 3 independent biological replicates.

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