Table S1 Gene accession numbers for phylogenetic analysis

Organism	**Accession number**	**Reference**
Streptococcus pyogenes **PmtA**	AAZ51785.1	(1)
Streptococcus agalactiae	KLL21255.1	
Streptococcus canis	WP_003044256.1	
Streptococcus dysgalactiae	WP_046159323.1	
Streptococcus iniae	WP_003101088.1	
Streptococcus mutans	WP_002304112.1	
Streptococcus pneumoniae	CVM74562.1	
Streptococcus porcinus	WP_003083554.1	
Streptococcus suis	WP_029187041.1	
Streptococcus thermophilus	WP_049555285.1	
Enterococcus faecalis	WP_034863010.1	
Enterococcus faecium	WP_058138288.1	
Listeria monocytogenes **FrvA**	WP_014929421.1	(2-4)
Bacillus subtilis **PfeT**	CUB56232.1	(4, 5)
Mycobacterium smegmatis **CtpD**		(6)
Sulfitobacter sp. NAS 14.1 **sCoaT**	ZP_00964573.1	(7)
Cupriavidus metallidurans **CzcP**	WP_011514822.1	(8)
Mycobacterium tuberculosis **CtpD**	WP_057125145.1	(9)
Mycobacterium tuberculosis **CtpJ**	WP_031727628.1	(9)
Chlamydia trachomatis	CRH88065.1	

Table S2 List of strains and plasmids used in this study

Bacterial strains	Description	Reference
Escherichia coli		
MC1061	*E. coli* laboratory cloning strain	(10)
Top10	*E. coli* laboratory cloning strain	
Streptococcus pyogenes		
5448	*S. pyogenes* invasive M1T1 strain	(11)
5448Δ*pmtA*	*S. pyogenes* 5448Δ*pmtA::km*^{R} deletion mutant	This study
5448Δ*pmtA::pmtA*	*S. pyogenes* 5448 *pmtA* complemented strain	This study
5448Δ*perR*	*S. pyogenes* 5448Δ*perR::spec*^{R} deletion mutant	(12)
5448Δ*perR::perR*	*S. pyogenes* 5448Δ*perR* complemented strain	(12)

Plasmids	Description	Reference
pHY304	Temperature sensitive shuttle plasmid::Em^R	(13)
pJRS233	Temperature sensitive shuttle plasmid::Em^R	(14)
pUC4ΩKm2	Template for *Km*^{R}	(15)
pHY304-*pmtA*-KO	pHY304 + *pmtA* knockout construct inserted at the *Xho*I and *Bam*HI site	This study
pJRS233-*pmtA*	pJRS233 + *pmtA* complement construct inserted at the *Xho*I and *Bam*HI site	This study

Table S3 List of primers used in this study

Primer	Sequence (5'-3')	
Deletion mutation and reverse complementation constructs		
pmtA-KO-1	CCCGG**CTCGAG**TTGATAGCGACAAGGTTCTTTTT	***Xho*I**
pmtA-KO-2	CAAAGTTGGCGTATAACATACCGATAGCTGCCAAAATCAT	*Km*^{R} 5'
pmtA-KO-3	TTACTGGATGAATTGTTTTAGAGAACGAACCGCCAATTATG	*Km*^{R} 3'
pmtA-KO-4	GGGCCC**GGATCC**CTTTCATAAGTCTTACTATTATTATAAACAC	***Bam*HI**
km-F	TATGTTATACGCCAACTTTG	
km-R	CTAAAACAATTCATCCAGTAA	
Gene expression studies		
pmtA-RT-F	GAAAAGCAAAACCGCCACCT	
pmtA-RT-R	GGGCACATGGTGAAGCTACT	
gyrA-RT-F	GAAGTGATCCCTGGACCTGA	
gyrA-RT-R	CCCGACCTGTTTGAGTTGTT	

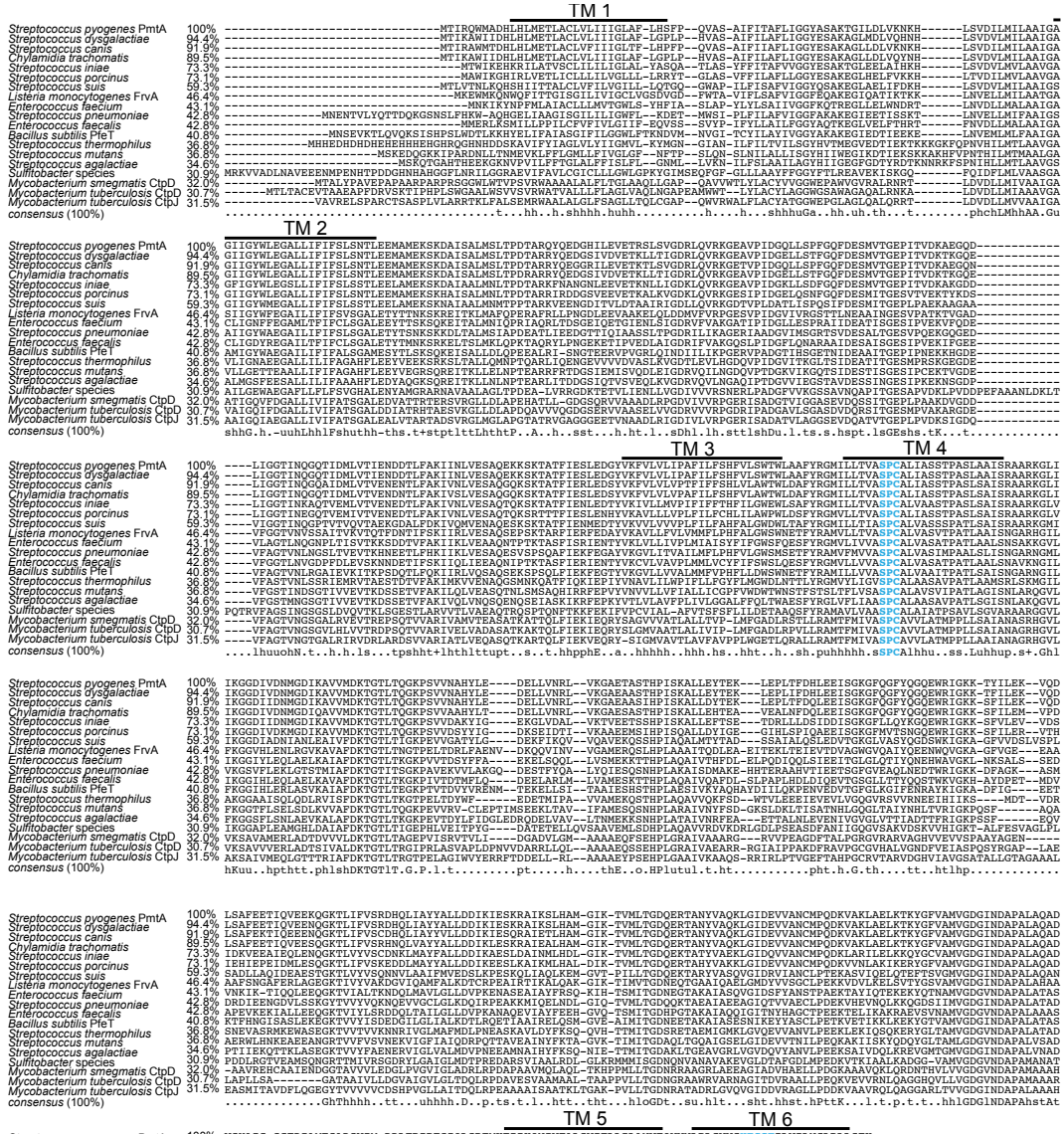

Figure S1: Multiple sequence alignment of *S. pyogenes* PmtA and homologous $P_{1\text{-}B4}$-type ATPase family sequences. Relative location of six core purported transmembrane helices based on the coordinates from *Bacillus subtilis* PfeT transmembrane profiles (reference). Key conserved motifs within TM helices 4 and 6 are highlighted in blue and the essential serine within TM 6 is highlighted in red. Consensus patterns based on discriminating equivalence class at a 100% threshold are

indicated under the alignment. Percent amino acid identity relative to *S. pyogenes* PmtA (TM 1-6) are indicated next to the species name.

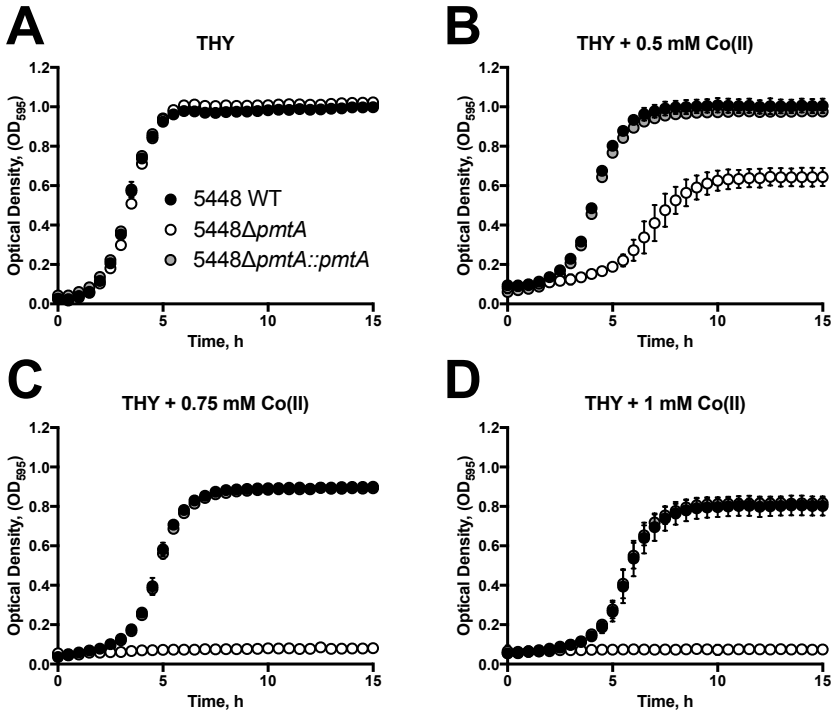

Figure S2: Growth analysis in the presence of Co(II). Overnight cultures of 5448 WT (black circles), 5448Δ*pmtA* (open circles) and 5448Δ*pmtA::pmtA* (grey circles) were diluted to OD_{600} = 0.05 into THY broth alone (A) or THY broth with 0.5 mM (B), 0.75 mM (C) or 1 mM (D) Co(II). Growth was monitored at 37°C by optical density recording at 595 nm (OD_{595}). Graphs represent mean ± standard deviation of 3 independent biological replicates.

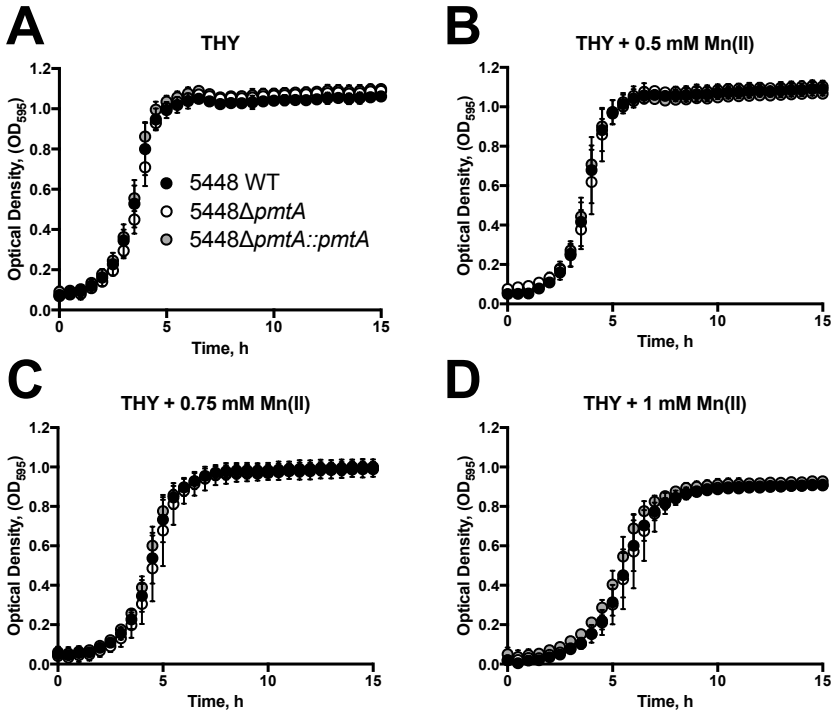

Figure S3: Growth analysis in the presence of Mn(II). Overnight cultures of 5448 WT (black circles), 5448Δ*pmtA* (open circles) and 5448Δ*pmtA::pmtA* (grey circles) were diluted to OD_{600} = 0.05 into THY broth alone (A) or THY broth with 0.5 mM (B), 0.75 mM (C) or 1 mM (D) Mn(II). Growth was monitored at 37°C by optical density recording at 595 nm (OD_{595}). Graphs represent mean ± standard deviation of 3 independent biological replicates.

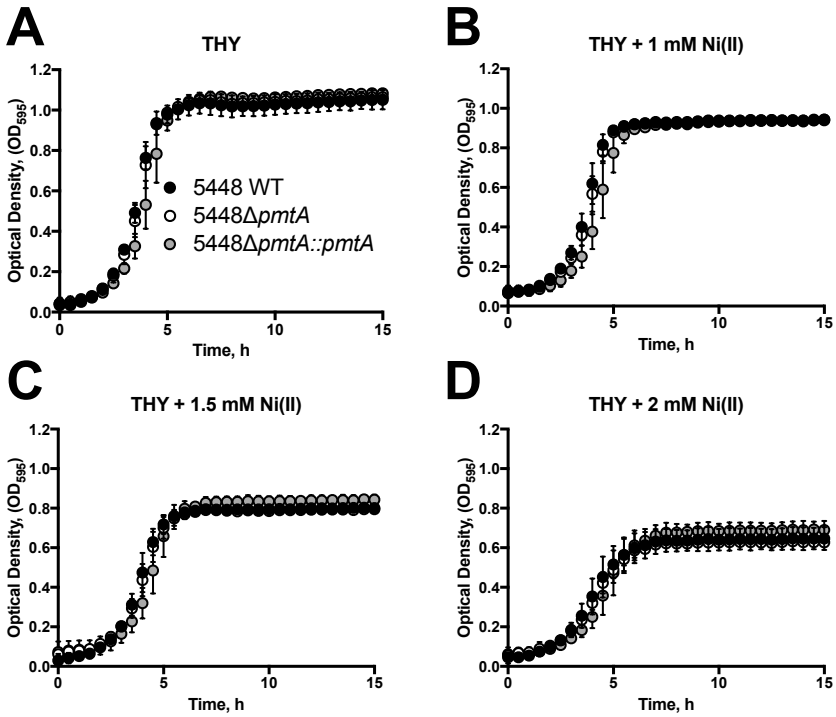

Figure S4: Growth analysis in the presence of Ni(II). Overnight cultures of 5448 WT (black circles), 5448Δ*pmtA* (open circles) and 5448Δ*pmtA::pmtA* (grey circles) were diluted to OD_{600} = 0.05 into THY broth alone (A) or THY broth with 1 mM (B), 1.5 mM (C) or 2 mM (D) Ni(II). Growth was monitored at 37°C by optical density recording at 595 nm (OD_{595}). Graphs represent mean ± standard deviation of 3 independent biological replicates.

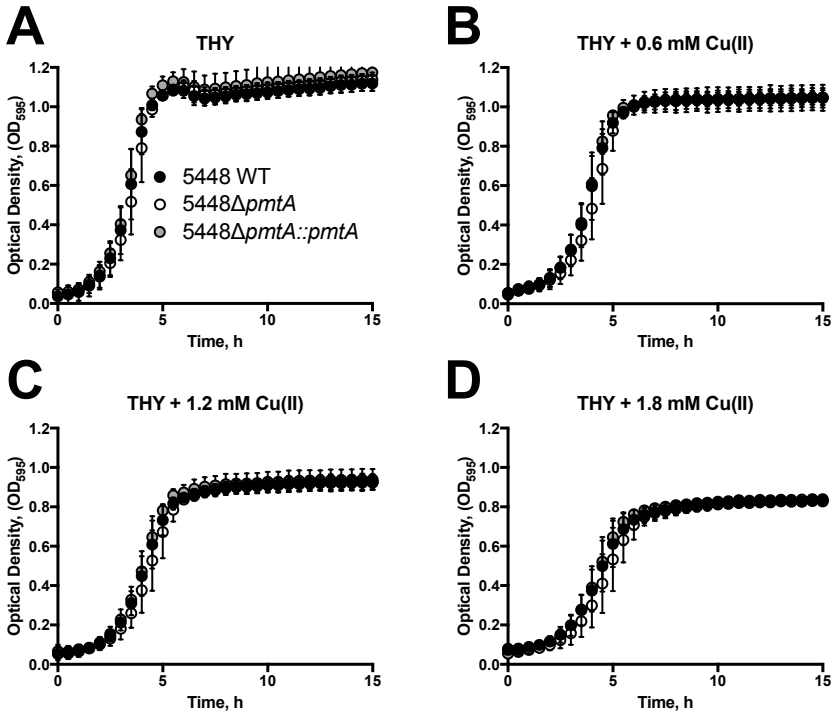

Figure S5: Growth analysis in the presence of Cu(II). Overnight cultures of 5448 WT (black circles), 5448Δ*pmtA* (open circles) and 5448Δ*pmtA::pmtA* (grey circles) were diluted to OD_{600} = 0.05 into THY broth alone (A) or THY broth with 0.6 mM (B), 1.2 mM (C) or 1.8 mM (D) Cu(II). Growth was monitored at 37°C by optical density recording at 595 nm (OD_{595}). Graphs represent mean ± standard deviation of 3 independent biological replicates.

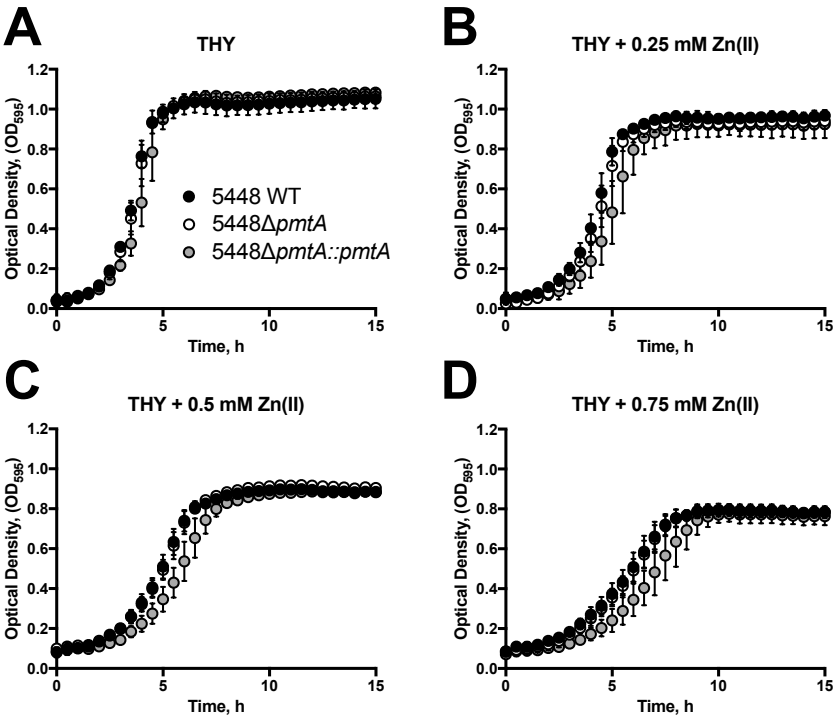

Figure S6: Growth analysis in the presence of Zn(II). Overnight cultures of 5448 WT (black circles), 5448Δ*pmtA* (open circles) and 5448Δ*pmtA::pmtA* (grey circles) were diluted to OD_{600} = 0.05 into THY broth alone (A) or THY broth with 0.25 mM (B), 0.5 mM (C) or 0.75 mM (D) Zn(II). Growth was monitored at 37°C by optical density recording at 595 nm (OD_{595}). Graphs represent mean ± standard deviation of 3 independent biological replicates.

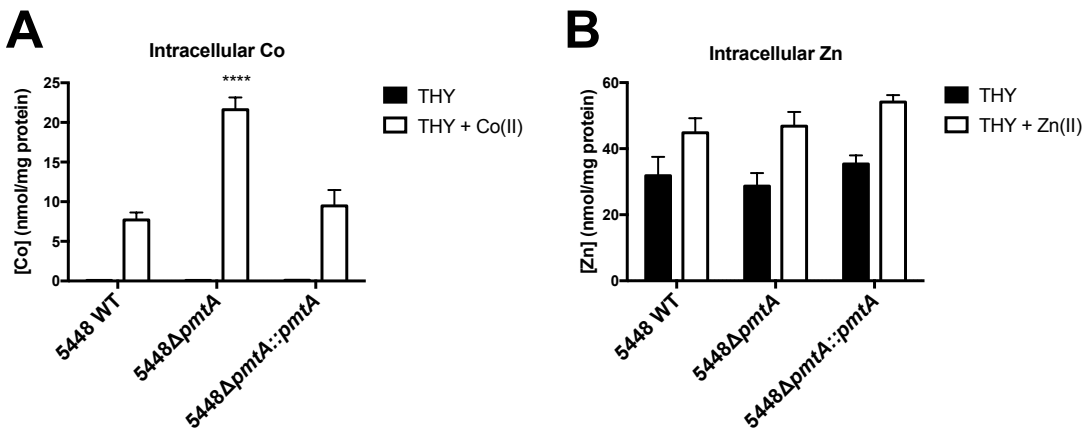

Figure S7: Intracellular metal accumulation of Co and Zn. Mid-exponential cultures (OD_{600} 0.6-0.8) of 5448 WT, 5448Δ*pmtA* and 5448Δ*pmtA::pmtA* were challenged with either sterile water (black bars) or 0.50 mM Co(II) (A) or 1 mM Zn(II) (B) (white bars) for 1 h at 37°C. Cells were analyzed by inductively-coupled plasma mass spectrometry (ICP-MS). Total metal content was normalized to protein content of the sample. Graph represents mean + standard deviation of 3 independent biological experiments (2-way ANOVA used comparing all to 5448 WT of that condition, **** $P < 0.0001$)

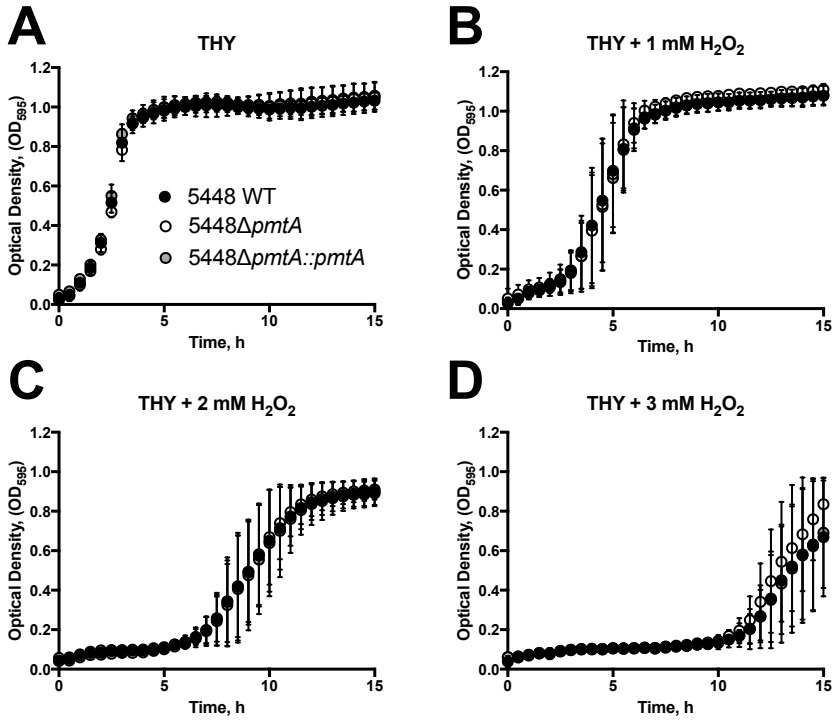

Figure S8: Growth curve analysis of strains 5448 WT (black circles), 5448Δ*pmtA* (open circles) and 5448Δ*pmtA::pmtA* (grey circles) in hydrogen peroxide. Strains were grown in THY to mid-exponential phase (OD 0.6-0.8) and diluted to OD_{600} = 0.05 in THY broth (A) or THY broth containing 1 mM (B), 2 mM (C) or 3 mM (D) H_2O_2. 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.

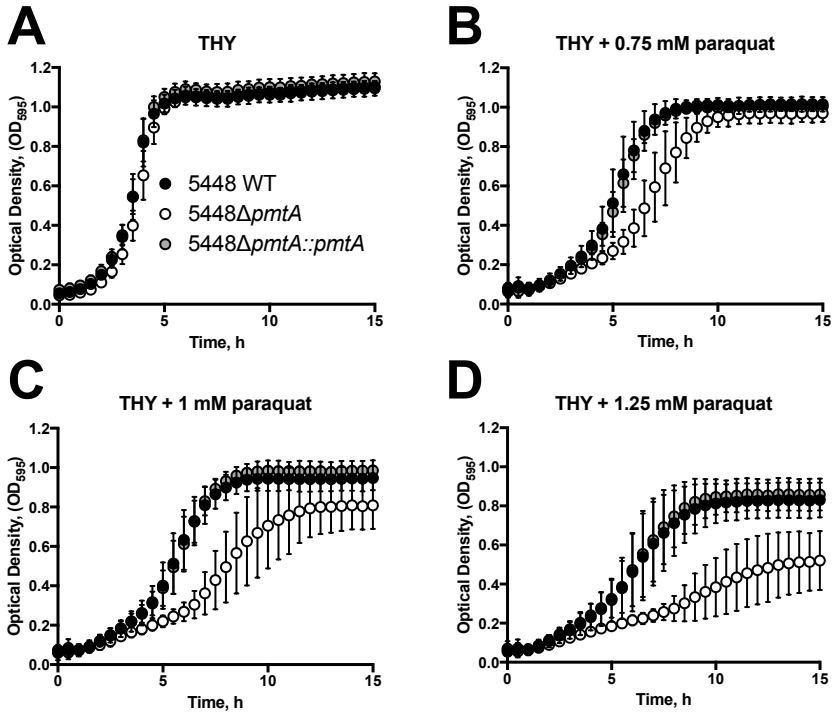

Figure S9: Growth analysis in the presence of the superoxide generator, paraquat. Overnight cultures of 5448 WT (black circles), 5448Δ*pmtA* (open circles) and 5448Δ*pmtA::pmtA* (grey circles) were diluted to OD_{600} = 0.05 THY broth alone (A) or THY broth with 0.75 mM (B), 1 mM (C) or 1.25 mM (D) paraquat. 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.

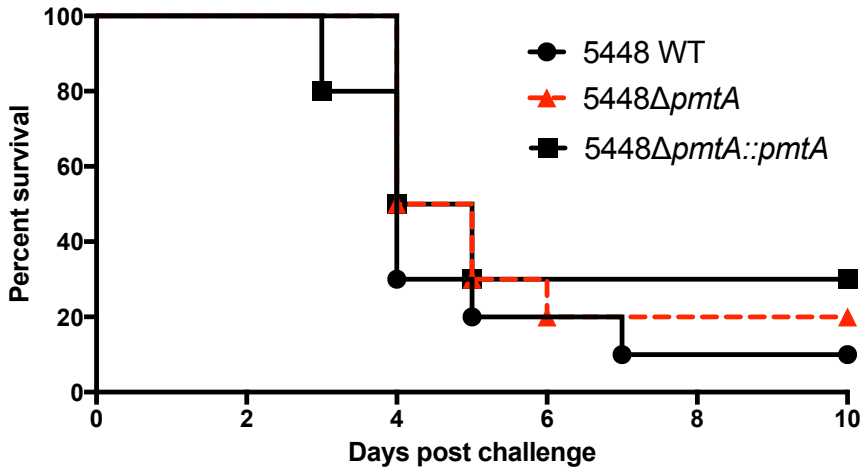

Figure S10: Virulence of 5448Δ*pmtA* in murine model of infection. Survival of mice after subcutaneous challenge of transgenic humanized plasminogen *AlbPLG1* C57BL/J6 mice with 5448 WT (black circles), 5448Δ*pmtA* (red triangles) and 5448Δ*pmtA::pmtA* (black squares). Infecting dose 2 x 10^8 colony-forming units (CFU). Mantel-cox log rank test was performed comparing 5448Δ*pmtA* to 5448 WT ($P = 0.5162$) and 5448Δ*pmtA::pmtA* ($P = 0.9723$) (Graphpad Prism 7)

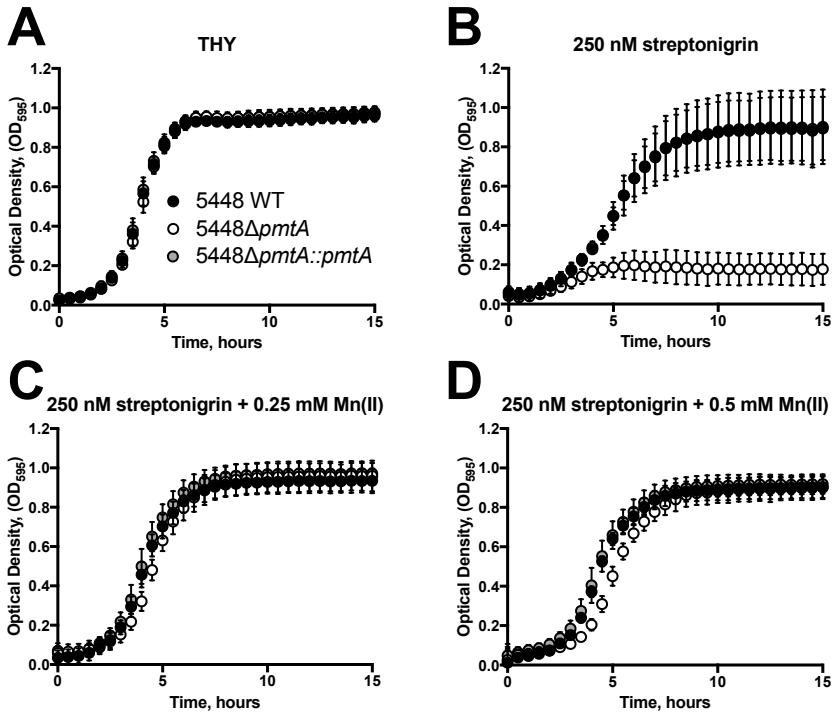

Figure S11: Growth curve analysis of streptonigrin rescue by Mn(II). Overnight cultures of strains 5448 WT (black circles), 5448Δ*pmtA* (open circles) and 5448Δ*pmtA::pmtA* (grey circles) were diluted to OD_{600} = 0.05 in THY broth (A) or THY broth with 250 nM streptonigrin (B), 250 nM streptonigrin + 0.25 mM Mn(II) (C) or 250 nM streptonigrin + 0.5 mM Mn(II) (D). 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.

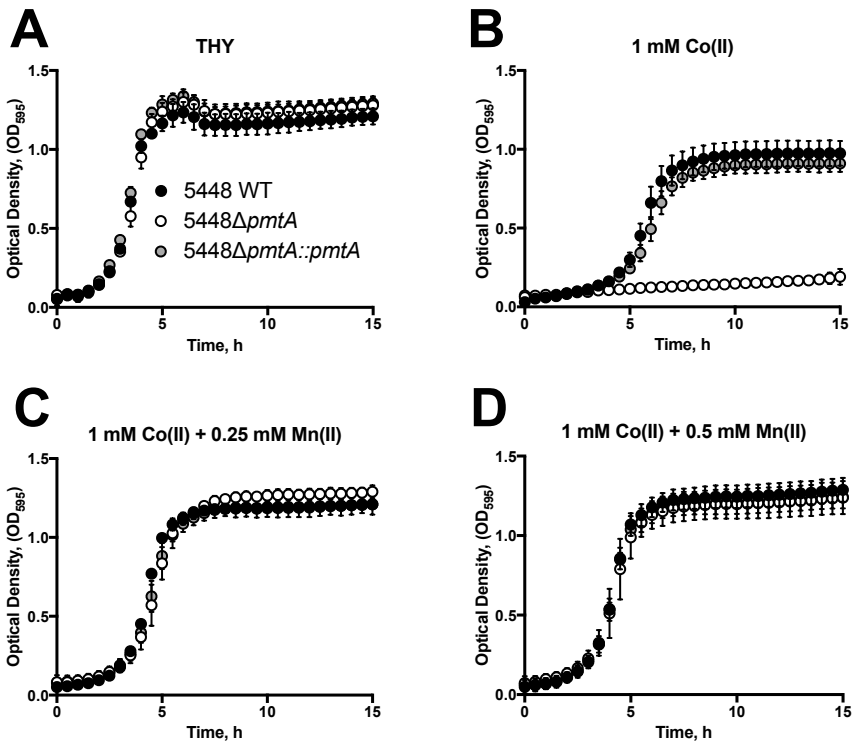

Figure S12: Growth curve analysis of Co(II) rescue by Mn(II). Overnight cultures of strains 5448 WT (black circles), 5448Δ*pmtA* (open circles) and 5448Δ*pmtA::pmtA* (grey circles) were diluted to OD_{600} = 0.05 in THY broth (A) or THY broth with 1 mM Co(II) (B), 1 mM Co(II) + 0.25 mM Mn(II) (C) or 1 mM Co(II) + 0.5 mM Mn(II) (D). 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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