Supplementary Table S1. Genes that are upregulated during iron toxicity.

Locus tag	Spy Number[a]	Fold-change[b]	Annotation[c]
Iron efflux			
pmtA	SpyM3_1093	17.6	Iron efflux pump
Arginine and serine catabolism			
arcD	SpyM3_1193	3.0	Arginine/ornithine antiporter
arcB	SpyM3_1194	3.48	Ornithine carbamoyl transferase
SpyM3_1195	SpyM3_1195	3.42	N-acetyl transferase
argR	SpyM3_1198	2.95	Arginine repressor
sdhA	SpyM3_1841	2.01	L-serine dehydratase α subunit
sdhB	SpyM3_1842	2.10	L-serine dehydratase β subunit
Carbohydrate metabolism			
SpyM3_0576	SpyM3_0576	2.34	Uncharacterized membrane protein
apbA	SpyM3_0577	2.30	Dehydropantoate reductase
fruR	SpyM3_0578	2.41	DeoR family transcription regulator
fruK	SpyM3_0579	2.25	Phosphofructokinase
fruA	SpyM3_0580	2.0	Fructose PTS subunit IIC
manX	SpyM3_0741	3.34	Mannose/Fructose PTS subunit IIA
agaB	SpyM3_0742	3.30	Mannose/Fructose PTS subunit IIB
manY	SpyM3_0743	3.30	Mannose/Fructose PTS subunit IIC
manZ	SpyM3_0744	3.51	Mannose/Fructose PTS subunit IID
manL	SpyM3_1511	2.01	Mannose-specific PTS component IIAB
pmi/manA	SpyM3_1566	2.00	Mannose-6-phosphate isomerase
Enzymes			
adh1	SpyM3_0037	4.51	Zn-free alcohol dehydrogenase
pfl	SpyM3_1596	10.5	Pyruvate formate lyase
nrdD	SpyM3_1794	2.31	Anaerobic ribonucleoside triphosphate reductase
Hypothetical proteins			
M3_0112	SpyM3_0112	3.00	Hypothetical protein

M3_0746	SpyM3_0746	2.10	Hypothetical protein
M3_0747	SpyM3_0747	2.20	Hypothetical protein
M3_1763	SpyM3_1763	2.43	Hypothetical protein
M3_1092	SpyM3_1092	3.20	Hypothetical protein

Supplementary Table S2. Genes that are downregulated during iron toxicity

Locus tag	**Spy Number**[a]	**Fold-change**[b]	**Annotation**[c]
Fatty acid biosynthesis			
accA	SpyM3_1517	3.10	Acetyl-CoA carboxylase α subunit
accB	SpyM3_1521	3.50	Acetyl-CoA carboxylase biotin carboxyl carrier protein subunit
accC	SpyM3_1519	3.20	Acetyl-CoA carboxylase biotin carboxylase subunit
accD	SpyM3_1518	3.12	Acetyl-CoA carboxylase β subunit
fabZ	SpyM3_1520	3.38	β-hydroxy myristoyl ACP dehydratase
fabF	SpyM3_1522	3.33	3-oxoacyl ACP synthase III
fabG	SpyM3_1523	4.17	β-ketoacyl ACP reductase
fabD	SpyM3_1524	4.33	ACP S-malonyl transferase
fabK	SpyM3_1525	4.84	Enoyl ACP reductase II
acpP	SpyM3_1526		Acyl carrier protein P
fabH	SpyM3_1527	3.40	3-oxoacyl ACP synthase III
fabT	SpyM3_1528	3.40	MarR-family regulator
phaB	SpyM3_1529	3.60	Enoyl-CoA hydratase
Iron-sulfur cluster assembly			
sufC	SpyM3_0208	1.90	Iron-sulfur cluster assembly ATPase
sufD	SpyM3_0209	2.10	Iron-sulfur cluster assembly protein
sufS	SpyM3_0210	2.10	Iron-sulfur cluster assembly – cysteine desulphurase
sufB	SpyM3_0212	2.20	Iron-sulfur cluster assembly protein
IscU	SpyM3_0211	2.20	Iron-sulfur cluster assembly scaffold protein
Amino acid metabolism and transport			
braB	SpyM3_0236	3.80	Branched amino acid transport system – carrier protein
sstT	SpyM3_0237	2.90	Serine/Threonine transporter SstT

glnP	SpyM3_0997	3.75	Glutamine binding domain
glnQ	SpyM3_0998	3.20	Glutamine binding ATPase
glnA	SpyM3_1621	2.35	Glutamine synthetase
glnR	SpyM3_1622	2.20	Glutamine repressor
atmA	SpyM3_0232	2.60	Methionine ABC transporter – solute binding protein
atmE	SpyM3_0235	2.0	Methionine ABC transporter permease
Metal transport			
mtsA	SpyM3_0318	3.65	Manganese transporter – substrate - binding protein
mtsB	SpyM3_0319	3.3	Manganese transporter – ATP binding subunit
mtsC	SpyM3_0320	2.96	Manganese transporter – permease
adcB	SpyM3_0071	2.0	Zinc transporter - permease
DNA metabolism			
nrdF.2	SpyM3_0301	2.4	Ribunucleoside diphosphate Reductase
nrdI.2	SpyM3_0302	2.2	Ribonucleotide reductase
nrdE.2	SpyM3_0303	2.1	Ribonucleotide reductase
Miscellaneous			
opuABC	SpyM3_0144	2.22	Glycine/betaine ABC transporter – permease
opuAA	SpyM3_0143	2.36	Glycine/betaine ABC transporter – ATP binding subunit
sodM	SpyM3_1071	2.7	Superoxide dismutase
csrA	SpyM3_0739	3.23	Methionine sulfoxide reductase B
cfa	SpyM3_0905	2.5	cAMP factor
spI	SpyM3_0583	2.2	Signal Peptidase I
mscL	SpyM3_0517	2.8	Putative mechano-sensitive channel
Hypothetical			
M3_0629	SpyM3_0629	4.6	Hypothetical protein
M3_0630	SpyM3_0630	3.2	Hypothetical protein
M3_0631	SpyM3_0631	2.2	Hypothetical protein
M3_1665	SpyM3_1665	2.2	Hypothetical protein

M3_1666	SpyM3_1666	2.74	Hypothetical protein
M3_1667	SpyM3_1667	2.36	Hypothetical protein
M3_0101	SpyM3_0101	2.1	Hypothetical protein
M3_0103	SpyM3_0103	2.23	Hypothetical protein
M3_0272	SpyM3_0272	2.5	Hypothetical protein
M3_0775	SpyM3_0775	2.32	Hypothetical protein
M3_1022	SpyM3_1022	2.2	Hypothetical protein
M3_0813	SpyM3_0813	2.17	Hypothetical protein
M3_1362	SpyM3_1362	2.17	Hypothetical protein
M3_0740	SpyM3_0740	2.1	Hypothetical protein
M3_0164	SpyM3_0164	2.0	Hypothetical protein
M3_0077	SpyM3_0077	4.1	Hypothetical protein

[a] Spy number according to the reference genome MGAS315

[b] Comparison between WT GAS grown in the presence and absence of 1 mM $FeSO_4$

[c] Annotation according to the reference genome MGAS315

Supplementary Table S3. Bacterial strains and plasmids used in this study.

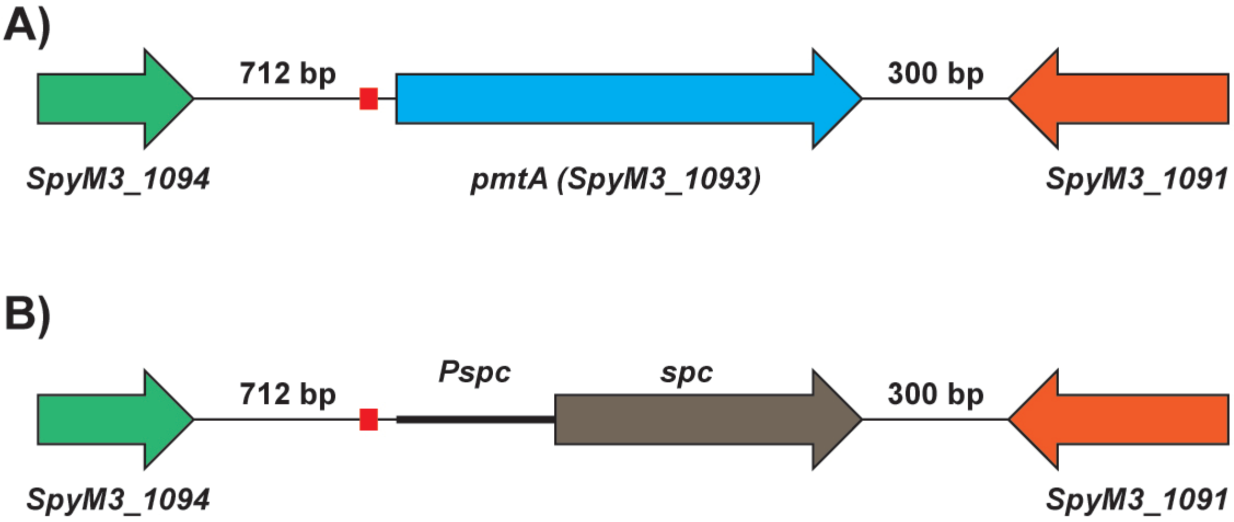
Strain or plasmid	Description	Reference
Strains		
MGAS10870	Invasive isolate, serotype M3	(Beres *et al.*, 2010)
MGAS10870Δ*pmtA*	MGAS10870Δ*pmtA*::*aad9*	This study
MGAS10870:*pDC*	MGAS10870 with empty vector, Cm^{+}	(Shelburne III *et al.*, 2011)
Δ*pmtA*:*pDC*	MGAS10870Δ*pmtA*::*aad9*, empty vector, Cm^{+}	This study
Δ*pmtA*:*pDC-pmtA*	MGAS10870Δ*pmtA*::*aad9*, *pmtA*, Cm^{+}	This study
Δ*perR*	MGAS10870Δ*perR*::*aad9*	(Makthal *et al.*, 2013)
Δ*perR*:*pDC*	MGAS10870Δ*perR*::*aad9*, empty vector, Cm^{+}	
Δ*perR-pDC:perR*	MGAS10870Δ*perR*::*aad9*, *perR*$^{+}$, Cm^{+}	(Makthal *et al.*, 2013)
Plasmids		
	Low-copy number plasmid capable of replication in GAS and *Escherichia coli*,	

pDC123	Cm$^+$	(Li *et al.*, 1997)
pDC-pmtA	*pDC123* with *pmtA* gene fused with its native promoter, Cm$^+$	This study
pDC:perR	*pDC123* with entire *perR* gene plus promoter, Cm$^+$	
pSL60-1	Vector containing *aad9* gene encoding spectinomycin resistance	(Lukomski *et al.*, 2000)

Supplementary Table S4. Primers and probes used in this study

Primer	**Sequence (5’-3’)**	**Target**
***pmtA*-A**	AATGGAGAAATTGTGACAG	5’ primer for 5’ region of *pmtA*
***pmtA*-B**	GTTATAGTTATTATAACATGTATTTTTTAGTTCCCTCGCAATTAAAAGT	3’ primer for 5’ region of *pmtA* with *spc* sequence overlap
***pmtA*-C**	CTATTTAAATAACAGATTAAAAAAATTATAATTTAGAAACTTGCTTGAAAACTTG	5’ primer for 5’ region of *pmtA* with *spc* sequence overlap
***pmtA*-D**	TTGTGCATTTTGATCAAGC	3’ primer for 3’ region of *pmtA*
***pmtA*-E**	TCTAGAAAAATCTTGTTGC	5’ primer for 5’ region of *pmtA*
***pmtA*-F**	TCAAAGCTTAGACGATTTTCGTG	3’ primer for 3’ region of *pmtA*
***pmtA*-G**	TACTATCTTCAGATAACACG	5’ primer for 5’ region of *pmtA*
***pmtA*-H**	TACTACTTAGACTTTGTCAG	3’ primer for 3’ region of *pmtA*
***pmtA*-spcF**	ACTTTTAATTGCGAGGGAACTAAAAAATACATGTTATAATAACTATAAC	5’ primer for *spc* along with *pmtA* overlap sequence
***pmtA*-spcR**	CAAGTTTTCAAGCAAGTTTCTAAATTATAATTTTTTTAATCTGTTATTTAAATAG	3’ primer for *spc* along with *pmtA* overlap sequence
***pDC-pmtA*-F**	TTCAAAAGCTGATAAGTCAGGCACCATCTC	5’ *pmtA* primer for complementation
***pDC-pmtA* - R**	TTAGAGATGGTGCCTGACTTATCAGCTTTTGAAG	3’ *pmtAB* primer for full length *pmtA* complementation
***pmtA* qRT 5’**	AGCTATCGGTGCTGGAATTATC	5’ primer for *pmtA* qRT-PCR
***pmtA* qRT 3’**	GGCCATTTCTTCAAGCGTATTG	3’ primer for *pmtA* qRT-PCR
***pmtA* probe**	AAATAAGCAGAGCACCCTCCAGCC	Probe for *pmtA* qRT-PCR
***tufA* qRT 5’**	CAACTCGTCACTATGCGCACAT	5’ primer for *tufA* qRT-PCR
***tufA* qRT 3’**	GAGCGGCACCAGTGATCAT	3’ primer for *tufA* qRT-PCR
***tufA* probe**	CTCCAGGACACGCGGACTACGTTAAAAA	Probe for *tufA* qRT-PCR

Supplementary figure S1



Supplementary figure S1. Schematic diagram showing the genetic context of *pmtA* in GAS genome (A) and insertional inactivation of *pmtA* by spectinomycin cassette (B). The coding region of *pmtA* and genes flanking on either side of *pmtA* are shown in block arrows, whereas the intergenic region that separates *pmtA* from its adjacent genes are marked as thin lines. Spy identification number of each gene is indicated below. The numbers above the line indicate the number of bases that separate *pmtA* and its flanking coding regions. The PerR binding site, *per* box, located upstream of *pmtA* gene is marked as red box. The spectinomycin resistance marker along with its native promoter used for the insertional inactivation of *pmtA* is shown in grey (bottom panel).

Supplementary figure S2

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FrvA_Lmo          --------------------MKEWMKQNWQFITTGISGILIVIGCLVGSNVGDFWTAVIF
PfeT_Bsu          MNEQVIVQRDPHEPLKTDKREKNWA-QHAELIAALVSGALILAGWLLSG--YQVLSIILF
S. pyogenes       ------------------MTIRQWMADHLHLMETLACLVLIIIGLAFLHS-FPQVASAIF
S. agalactiae     MSKQ-----T----GA---HTHEEKGKNVPVILFFTGLALFFISLFLGN--MLLVKNILF
S. mutans         MSKE-----D----QGKKIPARDNLLTNMEVKLFFLGMLLFIVGLGFNF--TPSLQNSLN
S. pneumoniae     MNENTVLYQT-TDQKGSNSLFHKWA-QHGELIAAGISGILILIGWFLKD--ETMWSIPLF
S. suis           ------------------MTLVTNLKQHSHIITTALCLVFILVGILLLQT-GQGWAPILF
S. dysgalactiae   ------------------MTIKAWIIDHLHLMETLACLVLIIIGLAFLGP-LPHVASAIF
E.  faecalis      -----MCYSEF----EKEGFMMERL-KSMILLPPILCFVFIVLGIIFEQV-SSSVYPIFY
                                                .        ::. .  .           :

FrvA_Lmo          LSAFVIGGFEQAKEGIQATIKTKK------LNVELLMILAATGASIIGYWFEGAILIFIF
PfeT_Bsu          LLAFVIGGFAKAKEGIEETLESKT------LNVELLMIFAAIGSALIGYWAEGAILIFIF
S. pyogenes       ITAFLIGGYASAKTGILDLVKNKH------LSVDILMILAAIGAGIIGYWLEGALLIFIF
S. agalactiae     SLAAILAGYHIIGEGFGDTYRDTKNNRKFSPNIHLLMTLAAVGSALMGSFEESALLILIF
S. mutans         ILALLISGYHIIWEGIKDTIEKSKKAKHFVPNTHILMTMAALGAVLLGETTEAALLIFIF
S. pneumoniae     ILAFVIGGFAKAKEGIEETISSKT------LNVELLMIFAAIGSAIIGYWAEGAILIFIF
S. suis           ISAFVIGGYQSAKEGLAELIFDKH------LSVDVLMILAAIGSGIIGYWMEGALLIFIF
S. dysgalactiae   ILAFLIGGYESAKAGLMDLVQHNH------LSVDVLMILAAIGAGIIGYWLEGALLIFIF
E. faecalis       LLAILFGGYAQTKEGLVELFTHRT------FNVDLLMALAAIGACLIGDYREGAILTFIF
                    * ::.*:     *:               . .:** :** *: ::*   *.*:* :**

FrvA_Lmo          SVSGALETYTTNKSKREITKLMAFQPERAFRLLPNGDLEEVAAKELQLDDMVFVRPGESV
PfeT_Bsu          SLSGALETYTMNKSSRDLTSLMQLEPEEATLMV-NGETKRVPVSDLQAGDMIVIKPGERV
S. pyogenes       SLSNTLEEMAMEKSKDAISALMSLTPDTARQYQEDGHILEVETRSLSVGDRLQVRKGEAV
S. agalactiae     AAAHFLEDYAQGKSQREITKLLNLNPTEARLITDDGSIQTVSVEQLKVGDRVQVLNGAQI
S. mutans         AGAHFLEEYVEGRSQREITKLLELNPTEARRFRTDGSIEMISVQDLEIGDRVQILNGDQV
S. pneumoniae     SLSGALETYTSNKSKKDLTALMSIAPDEATLIEEDGTTIQIAASSLTPGDRILIKAGERI
S. suis           SLSSTLEELAMEKSKNAIAALMNMTPPTARKVEENGDITVLDTAAIRIGDLLQVRKGDTV
S. dysgalactiae   SLSNTLEEMAMEKSKDAISALMSLTPDTARRYQEDGSIVDVETKLLTIGDRLQVRKGEAV
E. faecalis       CLSGALETYTMNKSRKELTSLMKLQPKTAQRYLPNGEKETIPVEDLAIGDRIFVAKGQSL
                  . :  **  .  :*   :: *: : *  *     :*    : .  :  .* : :  *  :

FrvA_Lmo          PIDGVIVRGSTTLNEAAINGESVPATKTVGADVFGGTVNVSSAITVKVTQTFDNTIFSKI
PfeT_Bsu          AADGIIESGSTSLDESALTGESMPVEKNTGDTVFTGTVNRNGSLTVRVTKANEDSLFRKI
S. pyogenes       PIDGQLLSPFGQFDESMVTGEPITVDKAEGQDLIGGTINQGQTIDMLVTIENDDTLFAKI
S. agalactiae     PTDGVVIEGSTAVDESSINGESIPKEKNSGDPVFGSTMNGSGTIVVEVTKDSSETVFAKI
S. mutans         PTDGKVIKGQTSIDESTISGESIPCEKTVGDEVFGSTINDSGTIVVEVTKDSSETVFAKI
S. pneumoniae     AADGVIMSGRTSVDESALTGESVPQEKGQGEDVFAGTVNLNGSLTVEVTKHNEETLFHKI
S. suis           PLDATLISPQSIFDESMITGEPLPAEKAAGAAVIGGTINQGPTVTVQVTAEKGDALFDKI
S. dysgalactiae   PIDGELLSTFGQFDESMVTGEPITVDKTKGQELIGGTINQGQTIDMLVTIENDDTLFAKI
E. faecalis       PIDGFLQNARAAIDESAISGESIPVEKIFGEEVFGGTLNVGDPFDLEVSKNNDETIFSKI
                    *. :      .:*: :.** :   *  *  :: .*:* .  . : *:    :::* **

FrvA_Lmo          IRLVESAQSEPSKTARFIERFEDAYVKAVLLFVLVMMFLPHFALGWSWNETFYRAMVLLT
PfeT_Bsu          IKLVESAQNSVSPAQAFIERFENAYVKGVLIAVALLLFVPHFALGWSWSETFYRAMVFMV
S. pyogenes       INLVESAQEKKSKTATFIESLEDGYVKFVLVLIPAFILFSHFVLSWTWLAAFYRGMILLT
S. agalactiae     VQLVNQSQENQSEIASKIKRFEPKYVTLVLAVFPLIVLGGALFFQLTWAESFYRGLVFLI
S. mutans         LQLVEASQTNLSMSAQHIRRFEPVYVNVVLLVFIALLICGPFVWDWTWNSTFSTSLTFLV
S. pneumoniae     IKLVESAQESVSPSQAFIEKFEGAYVKGVLITVAILMFLPHFVLGWSMSETFYRAMVFMV
S. suis           VQMVENAQESKSKTATFIENMEDTYVKVVLVVVPLFILFAHFALGWDWLTAFYRGMILLT
S. dysgalactiae   INLVESAQEKKSKTATFIESLEDGYVKFVLVLIPAFILFSHFVLSWTWLAAFYRGMILLT
E. faecalis       IQLIEEAQNIPTKTASFIERIENTYVKCVLVAVPLMMLVCYFIFSWSLQESFYRGMVLLV
                  :.::: :*   :     *. :*  **. **  .  :::   :        :*  .: ::
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FrvA_Lmo         VASPCALVASVTPATLAAISNGARHGILFKGGVHLENLRGVKAVAFDKTGTLTNGTPELT
PfeT_Bsu         VASPCALVASIMPAALSLISNGARNGMLVKGSVFLEQLGSVQMIAFDKTGTVTKGQPAVE
S. pyogenes      VASPCALIASSTPASLAAISRAARKGLIIKGGDIVDNMGDIKAVVMDKTGTLTQGKPSVV
S. agalactiae    AASPCALAASAVPATLSGISNLAKQGVLFKGGSFLSNLAEVKALAFDKTGTLTKGKPEVT
S. mutans        SASPCALAVSVIPATLAGISNLARQGVLFKGGTFLSELSDLKVVAFDKTGTLTQGKPEVV
S. pneumoniae    VASPCALVASIMPAALSLISNGARNGMLVKGSVFLEKLGTSTMIAFDKTGTITSGKPAVE
S. suis          IASPCALVASSSPATLSAISRAARKGMIIKGGDIADNIANLEAIVFDKTGTLTIGKPEVV
S. dysgalactiae  VASPCALIASSTPASLAAISRAARKGLIIKGGDIVDNMGDIKAVVMDKTGTLTQGKPSVV
E. faecalis      VASPCALVASATPATLAALSNAVKNGILIKGGIHLEQLAELKAVAFDKTGTLTKGKPIVT
                  ****** .*  **:*: :*. .::*::.**.   .::     :.:*****:* * * :

FrvA_Lmo         DRLFAENVDK--QQVINVVGAMERQSLHPLAAAITQDLEAEITEKLTEIEVTDVAGWGVQ
PfeT_Bsu         TIRIAEGFSE--AEVLEAVYAIETQSSHPLAQAITAYAESRGVNQSGYISIEETSGFGVM
S. pyogenes      NAHYLEDELL--V--NRLVKGAEAASTHPISKALLEYTEAV--EALNFDQLEEIAGKGFQ
S. agalactiae    DYLFIDGLEDRQDELVAVLTNMEKKSNHPLATAIVNRFEAETTA--LNLEVENIVGVGLV
S. mutans        RVCLEPTI-MSEEKLTAVIFAMESQSNHPLARAIVNYFSDGKSLDKLTISATNHLGQGLT
S. pneumoniae    KVVLAKGQDE--STFYQALYQIESQSNHPLAKAISDMAKEHHTERAAHVTIEETSGFGVE
S. suis          GATYLGDEKF--I--KQVVQAVEKQSSHPIAQALMTYTADS--SAIALQSLEDVTGKGLV
S. dysgalactiae  NAHYLEDELL--V--NRLVKGAEAASTHPISKALLEYTEKL--EPLIFDHLEEISGKGFQ
E. faecalis      DTMFLQDEEL--AR--LMLVAMESKTTHPLAQAIVQAFDLSLPAPLHDLDIQEVTGSGLL
                                   :   *  : **:: *:                  :  * *.

FrvA_Lmo         AIYQEENWQVGKAGFVGKEAA---AAFSNGAFERLAGEGKTIVYVAKDGVIQAMFALKDT
PfeT_Bsu         AEVSGAKWKVGKAGFIGEEMA---AQFMKQTASDVIQSGHTIVFVKKDDQIAGCIALKDQ
S. pyogenes      GLYQGQEWRIGKKSFILEMVPDL--SAFEETIQVEESQGKTLIFVSRDNQLVAYYALLDD
S. agalactiae    TTIADTTFRIGKPSSFEQV-----PTIIEKQTTKLASEGKTVVYFAENERVIGLVALMDV
S. mutans        AIYNHLTVRIGKPQSFAQA-----AERWLHNKEAEEANGRTVVFVSVNEKVIGFIAIQDR
S. pneumoniae    AQLNEDTWRIGKKDFAGKASM---DRDIEENGDVLSSKGYTVVYVQKNQEVVGCLGLKDQ
S. suis          ASYQGDSWKIGKAGFVVDSLVSPLSADLLAQIDEAESTGKTLVYVSQNNVLAAIFMVEDS
S. dysgalactiae  GFYQGQEWRIGKKTFILEKVQDL--SAFEETIQVEENQGKTLIFVSRDHQLIAYYALLDD
E. faecalis      TTYQQSTWKVGKHAYDPETMDV--APEVKEKIALLEEQGKTVIYLSRDDQLTAILGLLDV
                         ::**     .                    * *:::.  :  : .   : *

FrvA_Lmo         CRPEAIRTIKALQAKGIKTIMVTGDNEQTGAAIQAELGMDYVVSGCLPEKKVDVLKELSV
PfeT_Bsu         IRPEAKEVMEELNRLGIKTAMLTGDHEDTAQAIAKEAGMTTVVAECLPDQKVNEIKRLKE
S. pyogenes      IKLESKRAIEALHAMGIKTVMLTGDQERTANYVAQKLGIDEVVANCMPQDKVAKLAELKT
S. agalactiae    PNEEAMNAIHYFKSQNIETTMITGDAKLTGEAVGRLVGVDQVYANVLPEEKSAIVDQLKR
S. mutans        PQTTAVEAINYFKTAGVKTIMITGDAQLTGQAIGSELGIDEVVTNILPEQKAKIISKYQD
S. pneumoniae    IRPEAKKMIQELNDLGIQTVMLTGDQQKTAEAIAEEAGIQTVVAECLPDEKVHEVNQLKK
S. suis          LKPESKQLIAQLKEMGVTPILLTGDQEKTARYVASQVGIDRVIANCLPTEKASVIQELQT
S. dysgalactiae  IKIESKRAIKSLHAMGIKTVMLTGDQERTANYVAQKLGIDEVVANCMPQDKVAKLAELKT
E. faecalis      PKANAQEVIAYFEEHGVQTSMITGDHPGTAKAIAQQIGITNYHAGCTPEEKTELIKAKRA
                  .  : . :  :.  .:   ::***   *.  :    *:    :   * .*   :

FrvA_Lmo         TYGSVAMVGDGINDAPALAHASVGIAMGEGTDIAMETADVVLMKNDLEKIPYAYNLSERL
PfeT_Bsu         EFGTIAMVGDGINDAPALKAADVGIAMGGGTDVALETADMVLMKNDLKKLVNMCRLSRKM
S. pyogenes      KYGFVAMVGDGINDAPALAQADVSYAIGSGTDIAMESADSVIMD-DLTRIPFSIQLSRKM
S. agalactiae    EVGMTGMVGDGINDAPALVNADIGVAMGDGTDIAIDVADVVVMKNDLSKLGYAHRVSKRL
S. mutans        QYGLTAMLGDGVNDAPALVSADIGIAMGDGTDVAIETADVVLMRNDLTKLVKAHKLSKRL
S. pneumoniae    QGDSIIMVGDGINDAPALATADVGVAMGGGTDVALETADLILMKNNLNNLTKMIRLSRKM
S. suis          EFTSVGMVGDGINDAPALAQANVSYAMGSGTDIAMESADIVLME-DLTRIPYSIRLSKKM
S. dysgalactiae  KYGFVAMVGDGINDAPALAQADVSYAIGSGTDIAMESADSVIMD-DLTRIPFSIQLSRKM
E. faecalis      EVSVNAMVGDGVNDAPALAAASIGIAMGKGTDIAMDVADLVLVKNDLSKLVYSHKLSLKL
                       *:***:******  *.:. *:* ***:*:: ** :::  :* .:    .:* ::

FrvA_Lmo         HWITWQNICFAIAVILVLITANVFQLINLPFGVVGHEGSTILVILNGLRLLRSNRKK---
PfeT_Bsu         NRIIKQNIVFSLAVICLLICANFLQAMELPFGVIGHEGSTILVILNGLRLLK--------
S. pyogenes      KTIIKQNIVFALSVITLLILANVFQVVNLPLGVVGHEGSTILVILNGLRLLSFK------
S. agalactiae    NKIVQQNIIFSMLVVATLIILNFLGIANIAFSVLIHEGSTLVVIFNGLRLLVNTK-----
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S. mutans        KNIIRQNILFALLVIMMLISFTFFGHLSVVASVALHEGSTLIVLLNSLRLLMNKN-----
S. pneumoniae    NRIIKQNIIFSLTVICLLICANFLQVLDLPFGVIGHEGSTILVILNGLRLLRAS------
S. suis          RGIIKQNIIFALSVIALLIISNLFQSINLPLGVVGHEGSTILVILNGLRLLYFK------
S. dysgalactiae  KTIIKQNIVFALSVITLLILANVFQVVNLPLGVVGHEGSTILVILNGLRLLSFK------
E. faecalis      RKIIKQNIIFSVSVICLLVLSNFIQILNLPIGVVGHEGSTILVILNGLRLLRGVDAVPNL
                 . *  *** *:: *:  *:  ..:   .:  .*  *****::*::*.****

FrvA_Lmo         --------------
PfeT_Bsu         --------------
S. pyogenes      --------------
S. agalactiae    --------------
S. mutans        --------------
S. pneumoniae    --------------
S. suis          --------------
S. dysgalactiae  --------------
E. faecalis      RAAKETKQTIDIDA
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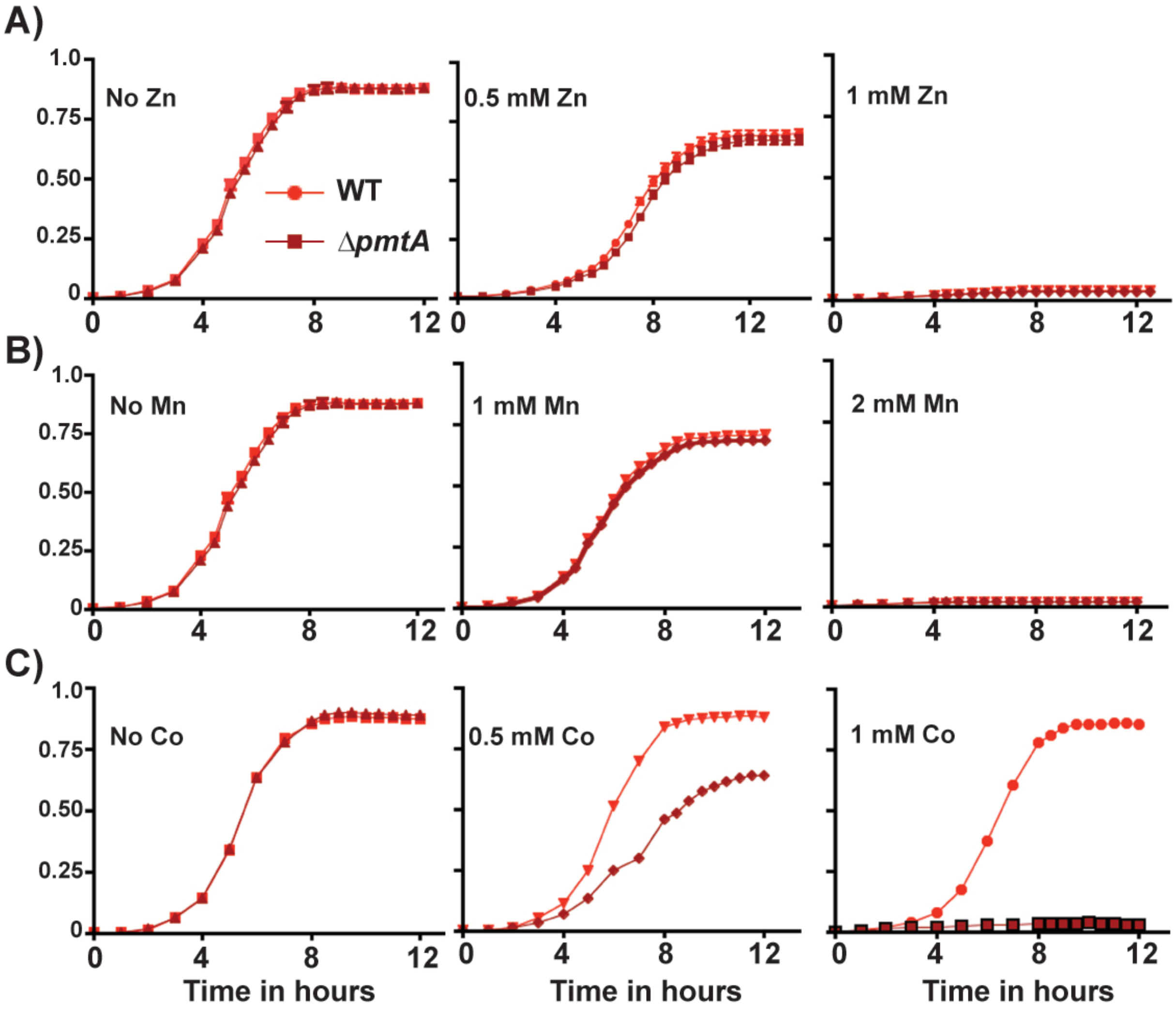
Supplementary figure S2. Amino acid sequence alignment of PmtA and its orthologs from the genus *Streptococcus*. The highly conserved S-P-C motif from the predicted TM-4 helix and H-E-G-S-T motif from the predicted TM-6 helix are highlighted and colored red.

Supplementary figure S3



Supplementary figure S3. Growth kinetics of the WT GAS and the isogenic Δ*pmtA* mutant strains in THY medium supplemented with increasing concentrations of indicated metals. Three biological replicates were grown and the graph represents mean ± standard deviation.