Supplemental Material

TABLE S1. Oligonucleotides used in this study.

Primer	Nucleotide sequence (5'-3')[a]
mgr1314fwRT	ATGGTTTCGCGTATTGAACAGC
mgr1314revRT	CTATTTGTCGTCGGAATCACCG
mgr1314up_fw	CAATTGCGCATGACCGAATTCGCCCCG
mgr1314up_rev	GCGGCCGCAATACGCGAAACCATGGC
mgr1314do_fw	ACCGGTTTGATTCCGACGACAAATAG
mgr1314do_rev	ACCGGTCCAGTTCGGAAAATACCTTC
mgr1314fw	GAGCTCAAGACCGCTTACAACATG
mgr1314rev	GAGCTCCTATTTGTCGTCGGAATCAC
mgr1314_del_fw	CAATTACGCCGCGCATCG
mgr1314_del_rev	AGCCGCCTCGACCTCGTT
mgr1314_probe_fw	TCGCCCCCAATTACGCCGCG
mgr1314 _probe_rev	CGCTCGGCGCCTCTTCATAGCGG
PfhuFfw	GGGCCCGATTTCATCTCTTTCATT
PfhuFrev	CCATGGTCGGGATAGTAATCTAAATGATAAT
Pmms16fw	CTCGAGGAGCCTCTCCATTAAACAATG
Pmms16rev	CATATGCTTGAATTCCTCCAACCGGGGGTATG
PrplKfw	AAGCTTGGCATCAAGGTTTCGGAAG
PrplKrev	CATATGTTTACCCTACCTCTGGTCG
Pmgr4079fw	CATATGCTGGCCCTCACCCGCTGA
Pmgr4079rev	CTCGAGGCCGCCAGCTTCTGTCCT
PmamDCrev	CATATGCTGATCTCCGGCAAGTGTATG
PmamDCfw	CTCGAGCAATGACCACCACCACCTTA AAC
PmamABfw	CTCGAGATGGCGCAAAGATGTGACGT C
PmamABrev	CATATGTCCCGTCACAATTCACCTCC
amb1009fw	CATATGATTTCTCGAATCGAACAACG
amb1009rev	GAGCTCTCAGTCGGCGGTTTCGTC
amb4460fw	CATATGATTTCACGCATCGAGCAAC
amb4460rev	GAGCTCCTATTTCTCTTCCGGTTTATCA
Ecfurfw	CATATGACTGATAACAATACCGCC
Ecfurrev	GAGCTCTTATTTGCCTTCGTGCGC

[a] Underlining indicates restriction site used for cloning purposes

TABLE S2. Identified Fur-like proteins of *M. gryphiswaldense* and similarity (%) to homologues. Locus tags refer to *M. magneticum* AMB-1 (Amb prefix), *M. magnetotacticum* MS-1 (Magn prefix), *Magnetococcus sp.* MC-1 (Mmc1 prefix), *Desulfovibrio magneticus* RS-1 (DMR prefix) and to an uncultured MTB (fos002_0290).

Protein	Similarity to *Ec*Fur	Best hit outside MTB	Similarity to MTB homologues	
		Locus tag / organism / similarity	Locus tag	Similarity
Mgr1314	50%	Rru_A3767 / *Rhodospirillum rubrum* ATCC 11170 / 80 %	Amb4460	91%
			Amb1009	86%
			Magn03009848	91%
			Magn03007092	86 %
			fos002_0290	64%
			Mmc1_0894	44%
			DMR_43000	47%
			DMR_04370	43%
			DMR_19790	41%
Mgr1305	33%	*rpsP* / alpha proteobacterium BAL199 / 52 %	-	-
Mgr1399	37%	RC1_2944 / *Rhodospirillum centenum* SW / 76 %	Amb4306	89 %
			Magn03009205	90%
Mgr3480	34%	Atu0153 / *Agrobacterium tumefaciens* str. C58 / 65 %	Amb1662	62%
Mgr3335	30%	MELB17_01720 / *Marinobacter sp.* ELB17 / 59 %	Amb2309	75%
			Magn03007851	68%
			Mmc1_3182	50%
			DMR_26810	33%

TABLE S3. Similarity (%) between *E. coli*, *M. gryphiswaldense* (locus tags with Mgr prefix) and *M. magneticum* (locus tags with Amb prefix) Fur and Fur-like proteins. Numbers in brackets indicate similarity to the Fur domain of Amb4306.

No.	Locus tag	% similarity											
		1	2	3	4	5	6	7	8	9	10	11	12
1	*Ec*Fur	100											
2	Mgr1314	49.7	100										
3	Mgr1305	33.3	28.4	100									
4	Mgr1399	37.2	37.1	53.1	100								
5	Mgr3480	34.2	34.7	66.9	63.8	100							
6	Mgr3335	29.5	33.1	26.3	23.1	24.5	100						
7	Amb1009	52.3	86	33.3	38.9	37.1	31.6	100					
8	Amb4460	51.7	90.9	30.4	34.4	38.9	38.9	87.4	100				
9	Amb1662	36.2	35.4	55.4	52.4	61.6	32.7	38.6	37.2	100			
10	Amb4306	19.9 (39.9)	19.5 (38.9)	26.9 (51.4)	47.6 (88.5)	32.5 (67.2)	13.1 (24.4)	19.6 (40.1)	17.1 (38.2)	26.2 (51.8)	100 (47.6)		
11	Amb2309	35.5	29.4	26.7	27.3	28	75.2	30	30.6	32.1	11.6 (25.5)	100	
12	*Ec*Zur						37.4					39.1	100

TABLE S4. Expression levels of MAI proteins identified by LC-MS/MS

Locus tag	Protein identification	Molecular Weight	no. of TMDs TMHMM / Sosui	No. of peptide ions				Relative expression level in RU-1	
				WT		RU-1		RU-1 –Fe / WT -Fe	RU-1 +Fe / WT +Fe
				-Fe	+Fe	-Fe	+Fe		
MGR4022	TPR-like protein	50 kDa	0	10	n.d.	11	11	1.10	unique
MGR4041	conserved hypothetical protein	9 kDa	0	n.d.	n.d.	11	n.d.	unique	n.d.
MGR4072	MmsF	14 kDa	3 / 2	17	15	12	13	-1.42	-1.15
MGR4073	Mms6	13 kDa	1	17	56	11	24	-1.55	-2.33
MGR4076	MamF	12 kDa	3	16	14	15	15	-1.07	1.07
MGR4077	MamD	30 kDa	1 / 0	37	19	31	19	-1.19	1.00
MGR4078	MamC	12 kDa	2	170	188	224	174	1.32	-1.08
MGR4091	MamE	78 kDa	1	75	51	82	52	1.09	1.02
MGR4092	MamJ	44 kDa	0	19	18	17	18	-1.12	1.00
MGR4093	MamK	39 kDa	0	31	27	25	20	-1.24	-1.35
MGR4095	MamM	34 kDa	3 / 6	15	12	14	17	-1.07	1.42
MGR4097	MamO	65 kDa	8 / 10	34	37	36	34	1.06	-1.09
MGR4099	MamA	24 kDa	0	26	26	23	27	-1.13	1.04
MGR4100	MamQ	30 kDa	1	16	16	16	15	1.00	-1.07
MGR4101	MamR	8 kDa	0	23	20	23	22	1.00	1.10
MGR4102	MamB	32 kDa	3 / 4	12	13	11	14	-1.09	1.08
MGR4103	MamS	19 kDa	1 / 2	16	19	14	17	-1.14	-1.12
MGR4104	MamT	19 kDa	1	15	13	14	15	-1.07	1.15
MGR4109	HlyB (Type I secretion system ATPase)	81 kDa	5 / 6	n.d.	n.d.	11	11	unique	unique
MGR4115	hypothetical protein	12 kDa	0	n.d.	13	13	n.d.	unique	unique (WT)
MGR4147	ftsZ like protein	34 kDa	0	12	11	13	13	1.08	1.18
MGR4150	MamY	41 kDa	1 / 2	17	16	17	14	1.00	-1.14
MGR4191	Adenine phosphoribosyltransferase	20 kDa	0	10	12	10	11	1.00	-1.09

TABLE S5. Putative Fur-box sequences identified by similarity to predicted Fur-box sequences of *M. magneticum* (1) and their relative position to the startcodon of differentially expressed genes in the *fur* mutant.

Locus tag	Protein identification / function	Putative Fur-box sequence
MGR0081	**TonB-dependent outer membrane receptor**	**GTTGAGAGTGCGACGCATT**
MGR0236	**bacterial extracellular solute-binding protein**	**CATGCAAATCACTCGCATA**
MGR0237	PAS:GGDEF	
MGR0662	hypothetical protein	AAAGCGAGCTTCCCGCTGG
MGR0705	conserved hypothetical protein	TTTGATAGTGATTTGCGTT
MGR0706	conserved hypothetical protein	
MGR0707	conserved hypothetical protein	
MGR1021	periplasmic trypsin-like serine protease	-
MGR1446	**Feo B2**	**GGTGATAACCAATCGCGAC**
MGR1447	**Feo A2**	
MGR1593	transcriptional regulator ArsR family	GATGGCAAGGGGTTTTTAT
MGR4109	HlyB type I secretion system ATPase	TTTGACGCGTGTATGCATC
MGR0698	CydA cytochrome d ubiquinol oxidase subunit 1	GGTGCGGGGCAGTCGCCAG
ABL14106	**Feo B1**	**ATCGCAACTCATTCGCAAT**

[a] Proteins with putative relation to iron metabolism are shown in bold.

Figure S1. Confirmation of *fur* deletion by Southern blot analysis of NcoI-digested WT (lane 1) and putative *fur* mutant (lane 2-7) genomic DNA. The blot was hybridized with a probe overlapping the NcoI restriction site. Digestion of WT should result in a 208 bp band and a 1362 bp fragment, whereas digestion of genomic DNA from *fur*$^{-}$ clones should result in a fragment of 208 bp. Lane M, molecular weight marker.

Figure S2. Similarity tree of α-proteobacterial and *E. coli* Fur-like proteins. Sequences are designated by locus tag accession numbers. Fur-like proteins of MTB are shown in bold. Proteins that have been characterized experimentally are underlined. MgFur is boxed.

Figure S3. Intracellular iron content of WT, RU-1 and RU-1 transcomplemented with pBBR1MCS-2fur grown in FSM under microaerobic conditions. Values are given as means ± SD of three independent replicates.

Figure S4. Growth yield of WT and RU-1 after 24 h growth at 30 °C at different concentrations of iron (A), manganese (B), and) paraquat (C). Values are given as means ± SD of three independent replicates.

Figure S5. Whole-cell transmission Mössbauer spectra of (A) WT, (B) RU-1 +Fe and (C) RU-1 –Fe. Precultures of (A) and (B) were grown in iron replete media. Precultures of (C) were kept in low iron media. Subspectrum 1 corresponds to a ferritin-like component characterized by a temperature-dependent doublet exhibiting an unusually low isomer shift for an oxo mineral; species 2 shows an isomer shift and quadrupole splitting typical for high spin ferrous iron in an octahedral oxygen ligand sphere. Subspectra (3) and (4) represent two magnetically split sextets attributed to magnetite A and magnetite B, respectively.

Figure S6. FURTA assay with *E. coli* H1717 carrying (1) pGEM, (2) pGEMPfhuF, (3) pGEMPmgr4079, (4) pGEMPrplK, (5) pGEMPmms16, (6) pGEMPmamDC and (7) pGEMPmamAB streaked on (A) iron-depleted MacConkey (100 µM 2,2-dipyridyl) and (B) iron-replete MacConkey (30 µM $FeCl_3$)

Figure S7. Coomassie silver blue stained 2D-gels of soluble protein fractions obtained from A) WT and B) RU-1 after growth under iron-deplete conditions

Figure S8. Putative Fur-box consensus sequence of *M. gryphiswaldense* Fur regulated genes *feoAB1, feoAB2, mgr0081 and mgr0236* derived from homology search with Fur-box sequences of *M. magneticum* (1).

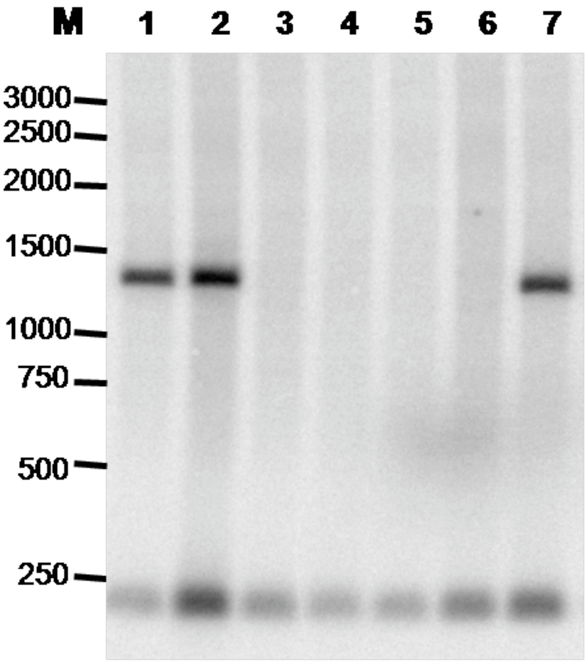
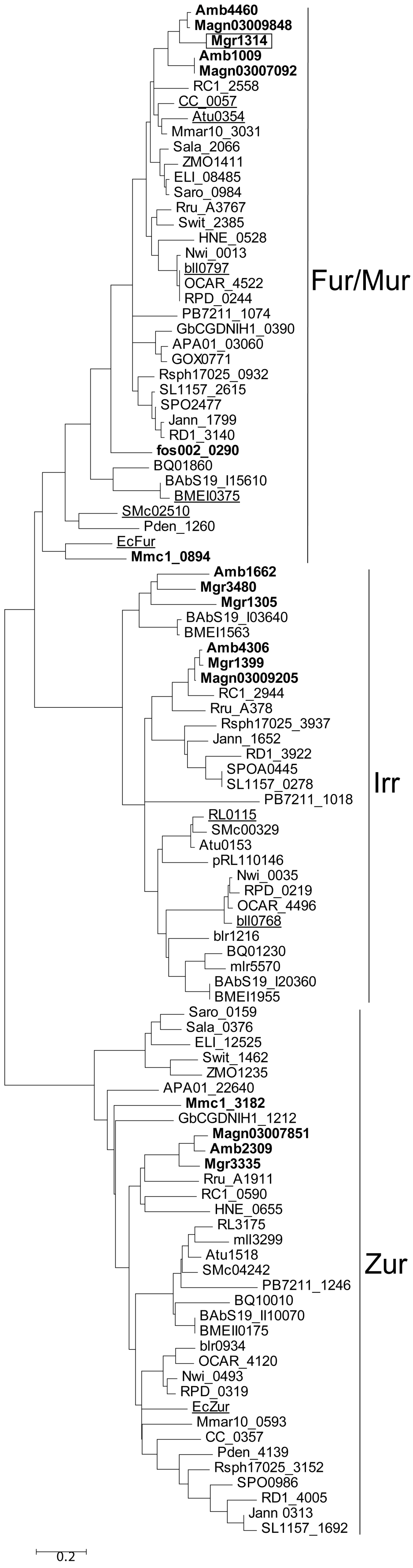


Figure S1.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EcFur
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Fur/Mur
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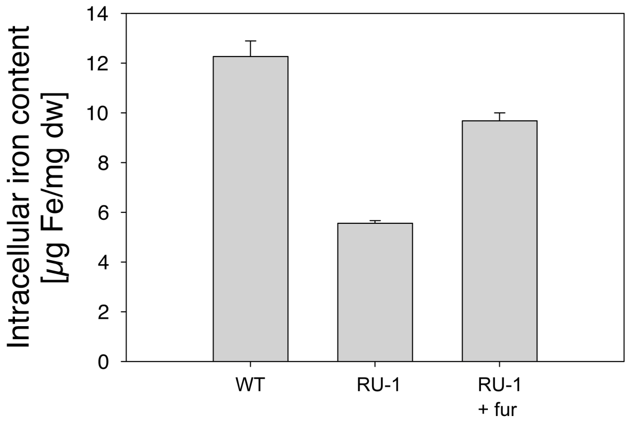
Figure S2.


Intracellular iron content [µg Fe/mg dw]
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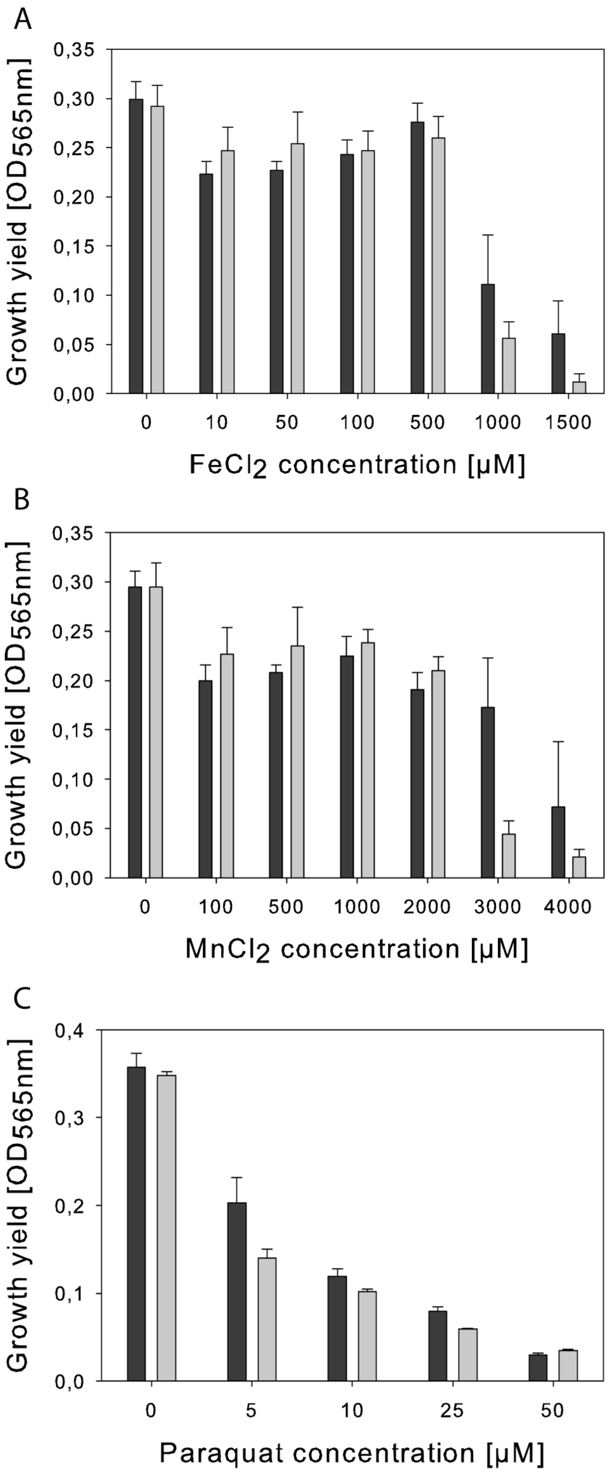
2
4
6
8
10
12
14
WT
RU-1
RU-1 + fur

Figure S3.


A
Growth yield [OD565nm]
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0,05
0,10
0,15
0,20
0,25
0,30
0,35
0
10
50
100
500
1000
1500
FeCl2 concentration [µM]
B
Growth yield [OD565nm]
0,00
0,05
0,10
0,15
0,20
0,25
0,30
0,35
0
100
500
1000
2000
3000
4000
MnCl2 concentration [µM]
C
Growth yield [OD565nm]
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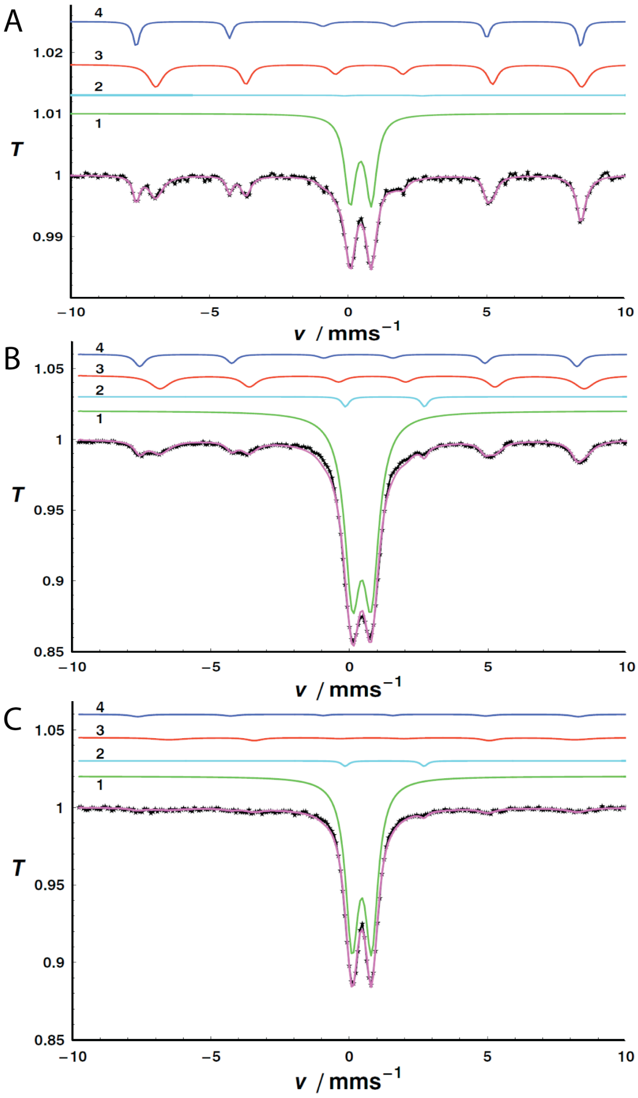
0,1
0,2
0,3
0,4
0
5
10
25
50
Paraquat concentration [µM]

Figure S4.



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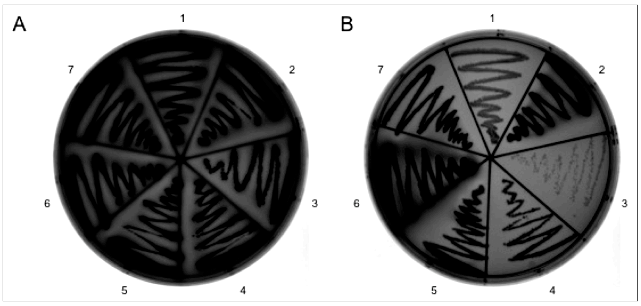
51 Figure S5.



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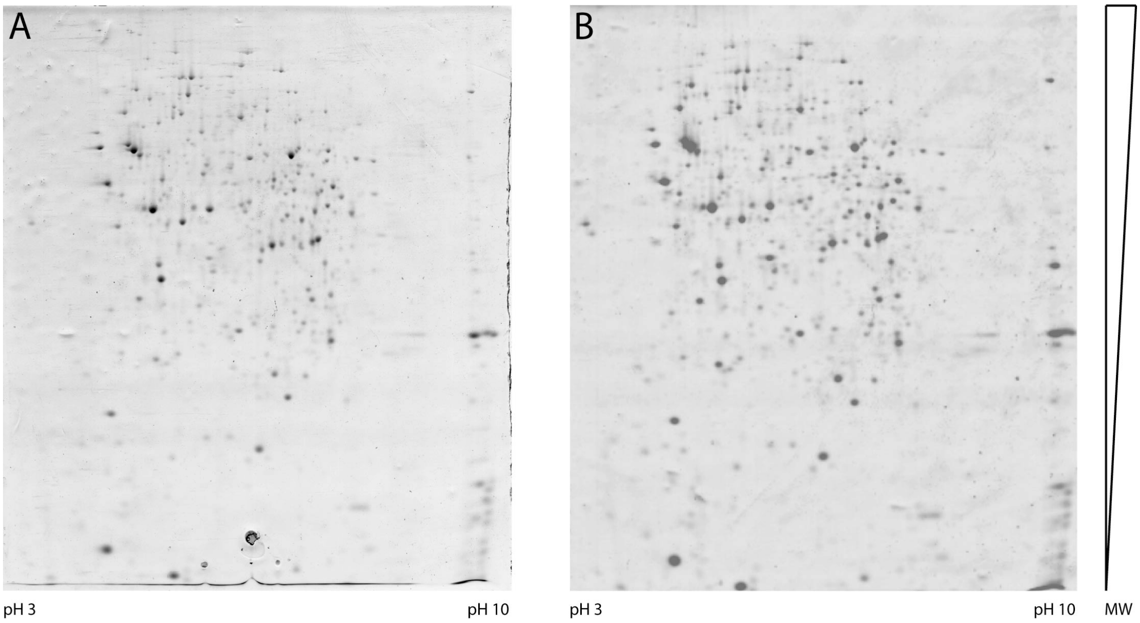

Figure S7.

Figure S8.

References

1. **Rodionov, D., M. Gelfand, J. Todd, A. Curson, and A. Johnston.** 2006. Computational Reconstruction of Iron- and Manganese-Responsive Transcriptional Networks in *α*-Proteobacteria. PLoS Comput. Biol. **2:**1568-1585.