Supporting Information

spxA2, encoding a regulator of stress resistance in *Bacillus anthracis,* is controlled by SaiR, a new member of the Rrf2 protein family

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Tables S1-S4

Figs. S1-S6

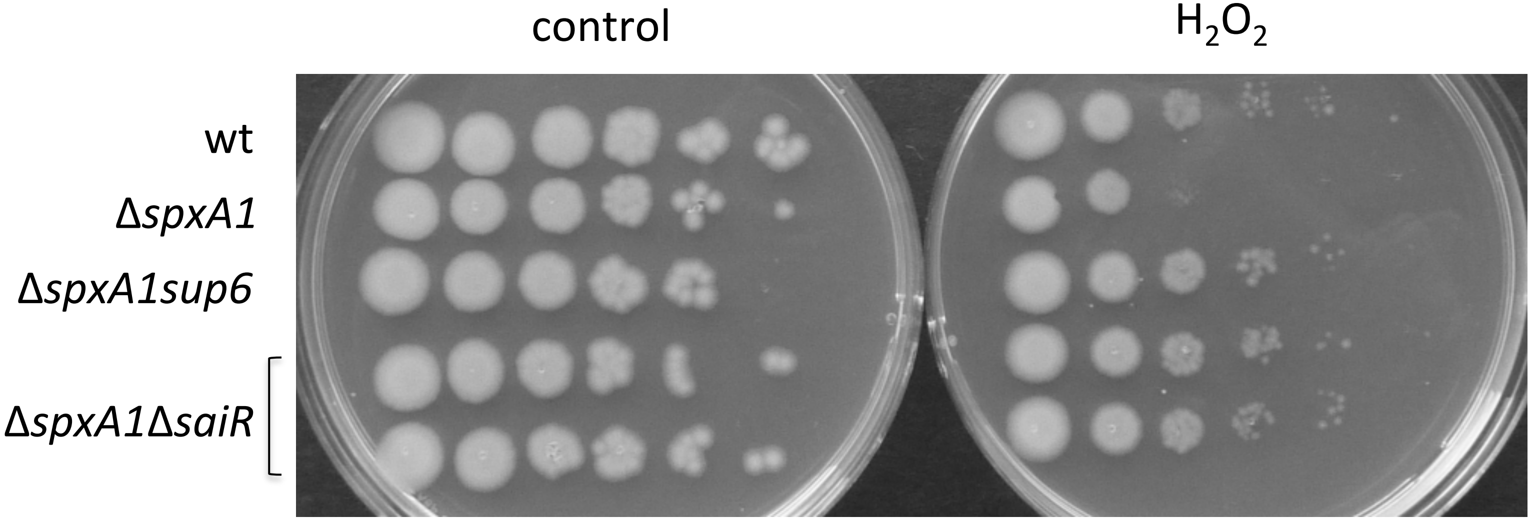
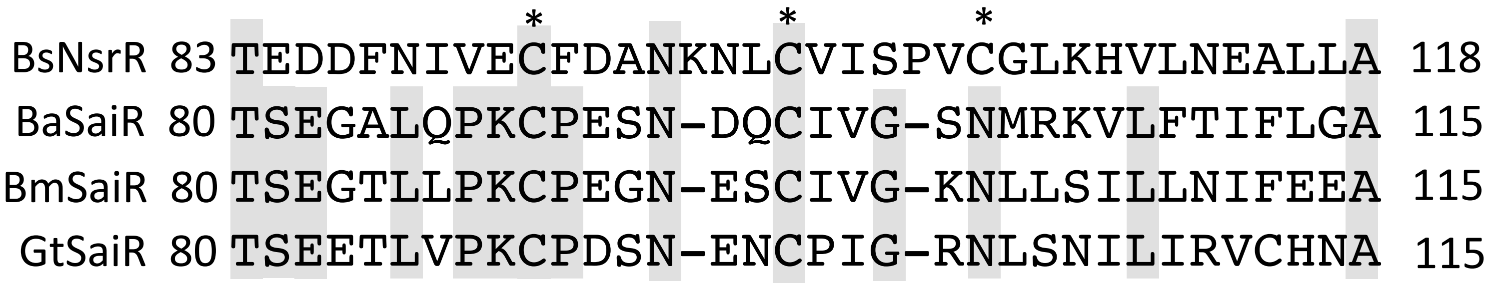


Fig. S1. Hydrogen peroxide sensitivity of the Δ*spxA1* mutant is restored by mutations in *saiR*. *B. anthracis* strains were grown in LB until OD_{600} reached ca. 1.0. Five µl of 10-fold serial dilutions of cells (left to right) were spotted onto LB agar with and without 0.44 mM H_2O_2. Strains: wt, 7702; Δ*spxA1*, ORB8170; Δ*spxA1sup6*, ORB8492; Δ*spxA1*Δ*saiR*, ORB8606. The results are representatives of three experiments.



Bs: *Bacillus subtilis*
Ba: *Bacillus anthracis*
Bm: *Bacillus methanolicus*
Gt: *Geobacillus thermoglucosidasius*

Fig. S2. Amino acid alignment of effector-binding regions of SaiR orthologs with *Bacillus subtilis* NsrR. Numbers indicate amino acid residues of each protein. Shaded are conserved amino acids and the asterisks show three cysteine residues that are ligands of the 4Fe-4S cluster in NsrR.

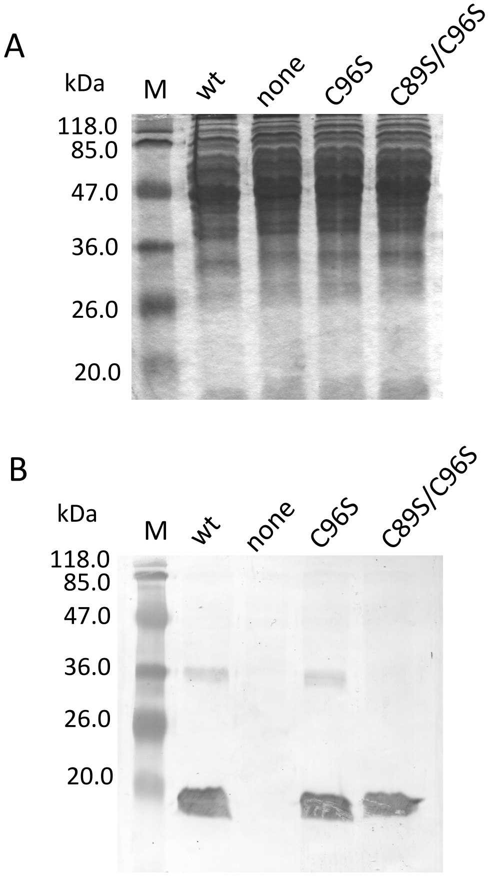


Fig. S3. SaiR cysteine mutant proteins are produced in *B. subtilis*. The wild-type and mutant SaiR proteins were induced with 1 mM IPTG for 1 h in *B. subtilis* (at OD$_{600}$ around 0.5). Each lysate contained 20 µg of total protein was loaded on SDS-15% polyacrylamide gels.
A. Stained gel with Coomassie brilliant blue.
B. Western analysis using anti-His tag monoclonal antibody (GenScript).
Lanes: wt, wild-type *saiR-his*$_6$ (ORB8985); none, with the empty vector (ORB8824); C96S, C96S *saiR-his*$_6$ (ORB9047); C89S C96S, C89S C96S *saiR-his*$_6$ (ORB9064). The higher molecular-weight protein that cross-reacts with the antibody is likely a SaiR dimer caused by intermolecular disulfide bond formation.

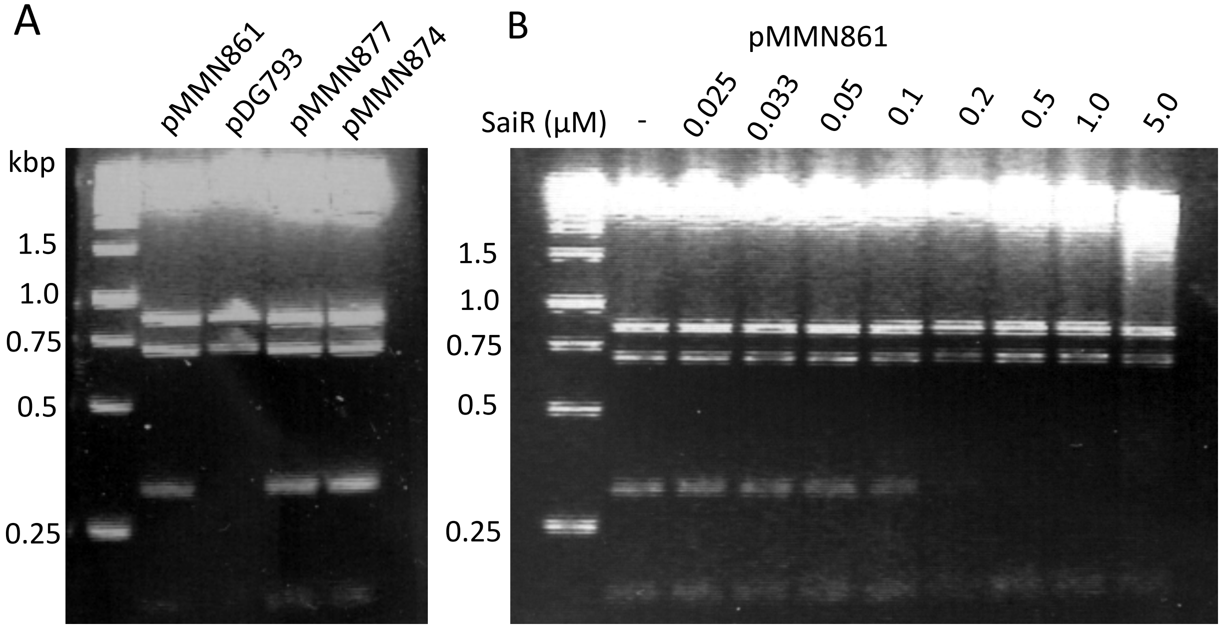


Fig. S4. SaiR protects *Dra*I cleavage of the *spxA2* DNA but not other *Dra*I recognition sites in the PDG793 vector plasmid.

A. 300 ng of each plasmid was digested with 10 units of *Dra*I in 10 μl reaction at 37°C for 1 h. After inactivation of the enzyme at 65°C for 20 min, the reaction was run on 2% agarose to separate the digested fragments. pMMN861, 874, and 877 are pDG793 carrying *spxA2*(-50/+31), *spxA2*(-50/+174), and *spxA2*(-50/+31: A-17C, G-20T) DNA.

B. pMMN861 was digested with *Dra*I as described above in the absence and presence of increasing concentrations of SaiR.

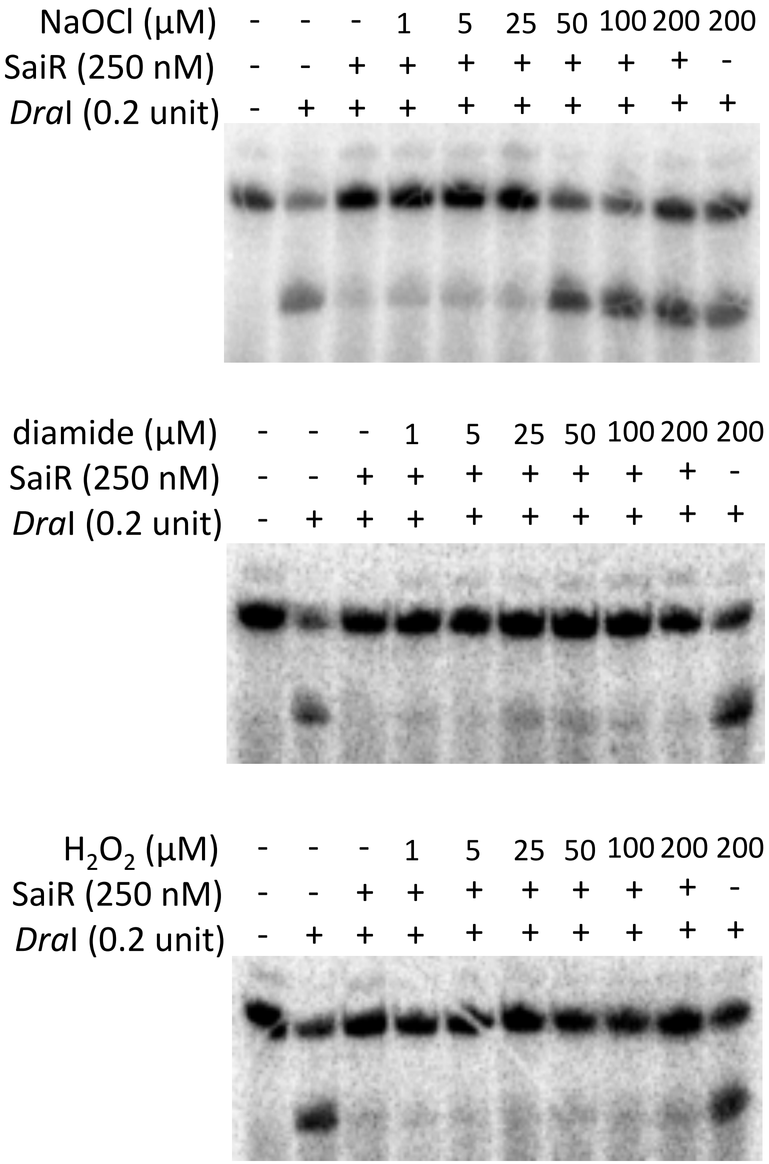


Fig. S5A. NaOCl affects binding of SaiR to *spxA2* DNA carrying SaiR box-1. The *Dra*I-digestion protection assay using the wild-type probe was carried out as described in Fig. 5, except that SaiR was treated with increasing concentrations of NaOCl, diamide, or H_2O_2 at room temperature for 5 min before addition of the radioactive wild-type *spxA2* DNA probe, followed by *DraI* digestion.

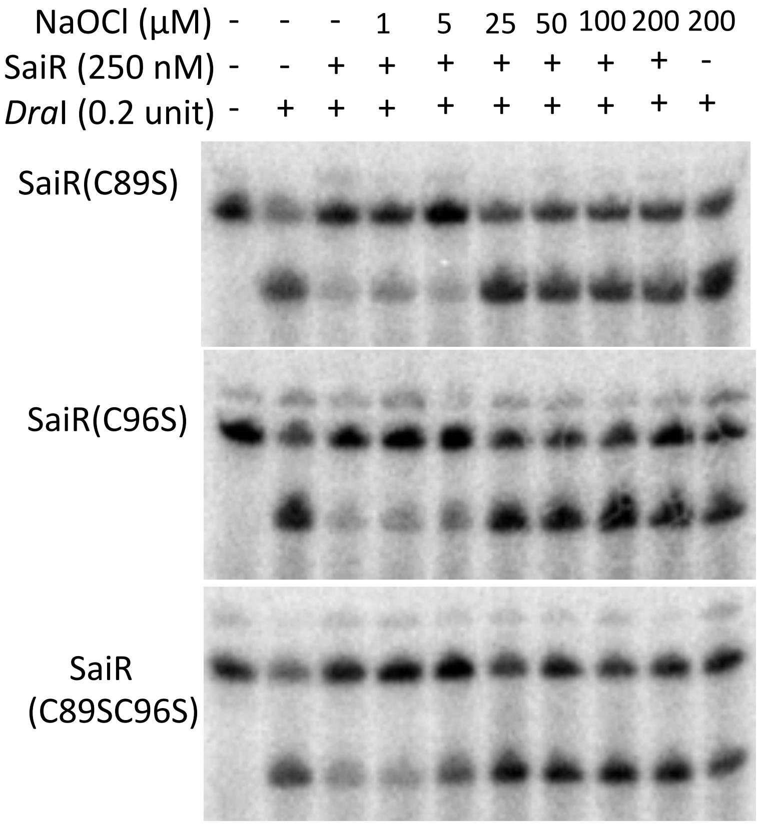


Fig. S5B. SaiR proteins with cysteine substitutions respond to NaOCl. The protection assay was performed with the wild-type *spxA2* probe as described in Fig. S5A.

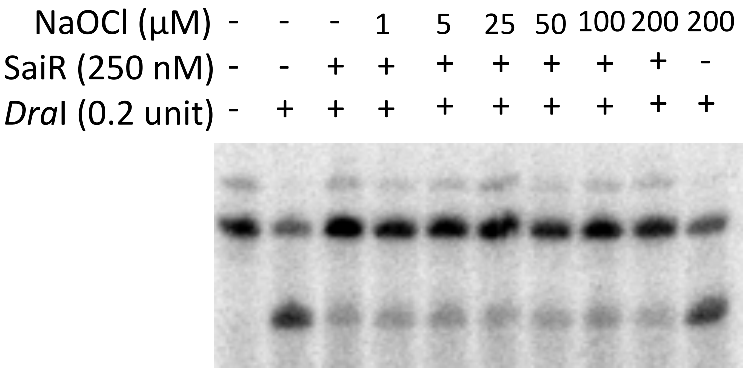
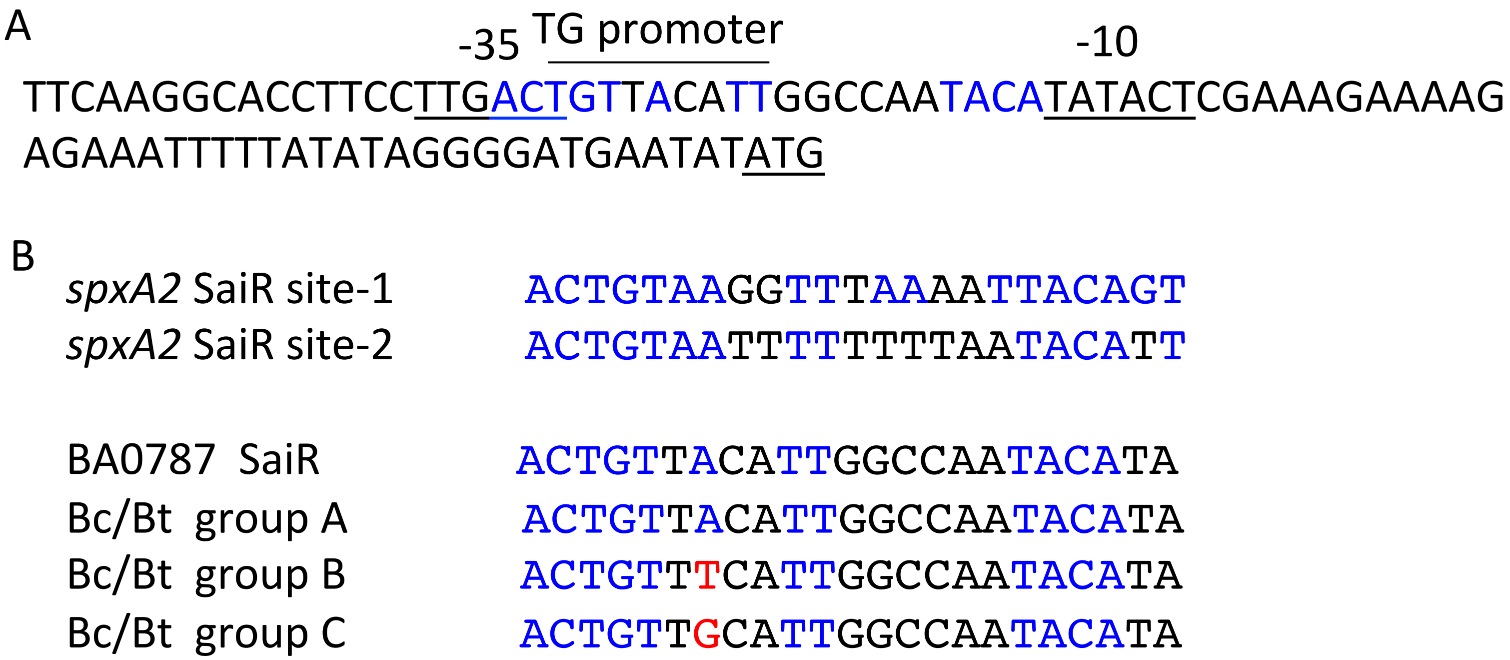


Fig. S5C. Preformed SaiR-DNA complex is resistant to NaOCl. The wild-type *spxA2* probe was incubated with SaiR at room temperature for 15 min and the reaction was treated with increasing concentrations of NaOCl for 5 min before *Dra*I digestion.



A

-35 TG promoter -10

TTCAAGGCACCTTCCTTGACTGTTACATTGGCCAATACATATACTCGAAAGAAAAG
AGAAATTTTTATATAGGGGATGAATATATG

B

spxA2 SaiR site-1	ACTGTAAGGTTTAAAATTACAGT
spxA2 SaiR site-2	ACTGTAATTTTTTTTAATACATT
BA0787 SaiR	ACTGTTACATTGGCCAATACATA
Bc/Bt group A	ACTGTTACATTGGCCAATACATA
Bc/Bt group B	ACTGTTTCATTGGCCAATACATA
Bc/Bt group C	ACTGTTGCATTGGCCAATACATA

Fig. S6. A possible SaiR site is present around the core promoter of BA0787. (A) The nucleotide sequence of the promoter region of BA0787. The ATG translational start site is underlined. Putative -10 and -35 elements are underlined and possible TG (extended) promoter is marked. (B) Comparison of the possible SaiR-binding site in BA0787 with the two sites in the *spxA2* promoter. Conserved sequences are shown in blue. Blast search showed that *spxA2* SaiR site-1 and site-2 in *B. cereus* and *B. thuringiensis* have perfect matches with those in *B. anthracis*. The regions corresponding to BA0787 are classified into three groups. Four *B. cereus* and one *B. thuringiensis* strains belong to group A, six *B. cereus* and four *B. thuringiensis* strains belong to group B, and two *B. cereus* and three *B. thuringiensis* strains belong to group C.

Table S1 Strains used in the study

Strain	**Relevant genotype**	**Construction**
B. anthracis		
7702	Sterne	(Cataldi *et al.*, 1990)
7702 SR1	streptomycin resistant	(Barendt *et al.*, 2013)
ORB8170	Δ*spxA1*	(Barendt et al., 2013)
ORB8285	Δ*spxA1* Δ*spxA2*	(Barendt et al., 2013)
ORB8398	Δ*spxA1* streptomycin resistant	(Barendt et al., 2013)
ORB8404	Δ*spxA1* ICE*Bs1*-P*spank*(*hy*)-*spxA1DD* streptomycin resistant	(Barendt et al., 2013)
ORB8438	Δ*spxA2*	(Barendt et al., 2013)
ORB8476	Δ*spxA1 saiR*(C95A) *cya*(G2265T)	*spxA1* suppressor
ORB8477	Δ*spxA1 saiR*(T83A)	*spxA1* suppressor
ORB8478	Δ*spxA1 saiR*(C86T) BAS3859 (C2237T)	*spxA1* suppressor
ORB8479	Δ*spxA1 saiR*(G179T)	*spxA1* suppressor
ORB8480	Δ*spxA1 saiR*(G221A)	*spxA1* suppressor
ORB8492	Δ*spxA1 saiR*(ΔT144)	*spxA1* suppressor
ORB8493	Δ*spxA1 saiR*(+T145)	*spxA1* suppressor
ORB8494	Δ*spxA1 saiR*(G32A)	*spxA1* suppressor
ORB8495	Δ*spxA1 saiR*(ΔACCCA104-108)	*spxA1* suppressor
ORB8496	Δ*spxA1 saiR*(ΔT201 Δ204)	*spxA1* suppressor
ORB8497	Δ*spxA1 saiR*(G181A)	*spxA1* suppressor
ORB8564	Δ*spxA1* Δ*spxA2* Δ*saiR*	pMMN839 (Δ*saiR*) x ORB8285
ORB8571	Δ*saiR*	pMMN839 (Δ*saiR*) x 7702
ORB8606	Δ*spxA1* Δ*saiR*	pMMN839 (Δ*saiR*) x ORB8170
ORB8611	Δ*spxA1* Δ*saiR* streptomycin resistant	Spontaneous isolate from ORB8606
ORB8723	Δ*spxA1* Δ*saiR* ICE*Bs1*-P*spank*(*hy*)-*saiR*(C89S) streptomycin resistant	ICE*Bs1*::pMMN851 [*saiR*(C89S)] x ORB8611
ORB8724	Δ*spxA1* Δ*saiR* ICE*Bs1*-P*spank*(*hy*)-*saiR*(C96S) streptomycin resistant	ICE*Bs1*::pMMN852 [*saiR*(C96S)] x ORB8611
ORB8725	Δ*spxA1* Δ*saiR* ICE*Bs1*-P*spank(hy)-saiR*(wt) streptomycin resistant	ICE*Bs1*::pMMN850 [*saiR*(wt)] x ORB8611
B. subtilis		
JH642 and its derivatives		
JH642	parent	James Hoch
JMA475	*cgeD*::P*spank*(*hy*)-*rapI*	(Auchtung *et al.*, 2005)
ORB8713	ICE*Bs1*-P*spank*(*hy*)-*saiR*(C89S)	pMMN851 x JH642
ORB8714	ICE*Bs1*-P*spank*(*hy*)-*saiR*(C96S)	pMMN852 x JH642
ORB8715	ICE*Bs1*-P*spank*(*hy*)-*saiR*(wt)	pMMN850 x JH642
ORB8718	*cgeD*::P*spank*(*hy*)-*rapI ICEBs1-Pspankhy-saiR(C89S)*	JMA475 x ORB8713
ORB8719	*cgeD*::P*spank*(*hy*)-*rapI* ICEBs1-P*spankhy*-*saiR*(C96S)	JMA475 x ORB8714
ORB8720	*cgeD*::P*spank*(*hy*)-*rapI* ICEBs1-P*spankhy*-*saiR*(wt)	JMA475 x ORB8715
168 and its derivatives		
168	parent	(Kunst *et al.*, 1997)

ORB8812	*amyE*::P*spank*(*hy*)-*saiR*	pSS9 x 168
ORB8820	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	ORB8605 x ORB8812
ORB8821	*amyE::pDR111*	pDR111 x 168
ORB8822	*amyE*::P*spank*(*hy*)-*saiR*(C89S)	pSS10 x 168
ORB8823	*amyE*::P*spank*(*hy*)-*saiR*(C96S)	pSS11 x 168
ORB8824	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::*pDR111*	ORB8605 x ORB8821
ORB8825	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::P*spank*(*hy*)-*saiR*(C89S)	ORB8605 x ORB8822
ORB8826	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::P*spank*(*hy*)-*saiR*(C96S)	ORB8605 x ORB8823
ORB8835	*thrC*::*spxA2*(-50/+31)-*lacZ*	pMMN861 x 168
ORB8839	*thrC*::*spxA2*(-50/+31)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN861 x ORB8812
ORB8845	*thrC*::*trxB-lacZ*	ORB6824 x 168
ORB8846	*thrC*::*trxB-lacZ amyE*::P*spank*(*hy*)-*saiR*	ORB6824 x ORB8812
ORB8855	*thrC*::*spxA2*(-100/+31)-*lacZ*	pMMN865 x 168
ORB8859	*thrC*::*spxA2*(-100/+31)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN865 x ORB8812
ORB8863	*thrC*::*spxA2*(-50/+4)-*lacZ*	pMMN869 x 168
ORB8864	*thrC*::*spxA2*(-38/+31)-*lacZ*	pMMN870 x 168
ORB8865	*thrC*::*spxA2*(-50/+4)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN869 x ORB8812
ORB8866	*thrC*::*spxA2*(-35/+31)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN870 x ORB8812
ORB8884	*thrC*::*spxA2*(-247/+268)-*lacZ*	pSB20 x 168
ORB8889	*thrC*::*spxA2*(-100/-39)-*lacZ*	pMMN871 x 168
ORB8890	*thrC*::*spxA2*(-100/-39)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN871 x ORB8812
ORB8900	*thrC*::*spxA2*(-100/+268)-*lacZ*	pMMN872 x 168
ORB8901	*thrC*::*spxA2*(-247/+31)-*lacZ*	pMMN873 x 168
ORB8902	*thrC*::*spxA2*(-100/+268)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN872 x ORB8812
ORB8903	*thrC*::*spxA2*(-247/+31)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN873 x ORB8812
ORB8910	*thrC*::*spxA2*(-50/+174)-*lacZ*	pMMN874 x 168
ORB8911	*thrC*::*spxA2*(-50/+174)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN874 x ORB8812
ORB8922	*thrC*::*spxA2*(-50/+174:T160G)-*lacZ*	pMMN875 x 168
ORB8923	*thrC*::*spxA2*(-50/+174:G142T, A145C)-*lacZ*	pMMN876 x 168
ORB8924	*thrC*::*spxA2*(-50/+174:T160G)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN875 x ORB8812
ORB8827	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::pDR111	ORB8821 x ORB8605
ORB8925	*thrC*::*spxA2*(-50/+174:G142T, A145C)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN876 x ORB8812
ORB8930	*thrC*::*spxA2*(-50/+31:A-17C, G-20T)-*lacZ*	pMMN877 x 168
ORB8931	*thrC*::*spxA2*(-50/+174:A-17C, G-20T)-*lacZ*	pMMN878 x 168
ORB8932	*thrC*::*spxA2*(-50/+31:A-17C, G-20T)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN877 x ORB8812
ORB8933	*thrC*::*spxA2*(-50/+174:A-17C, G-20T)-*lacZ amyE*::P*spank*(*hy*)-*saiR*	pMMN878 x ORB8812
ORB8945	*thrC*::*spxA2*(-50/+174:A-17C, G-20T, G142T, A145C)-*lacZ*	pMMN879 x 168
ORB8946	*thrC*::*spxA2*(-50/+174:A-17C, G-20T, G142T, A145C)-*lacZ* *amyE*::P*spank(hy)-saiR*	pMMN879 x ORB8812
ORB8985	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::P*spank*(*hy*)-*saiRhis6*	pMMN880 x ORB8884
ORB9046	*thrC*::*spxA2*(-247/+268)-*lacZ spx*::*neo*	ORB3834 x ORB8884
ORB9047	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::P*spank*(*hy*)-*saiR*(C96S)*his6*	pYA1 x ORB8884

ORB9049	*thrC*::*spxA2*(-247/+268)-*lacZ spx*::*neo amyE*::pDR111	pDR111 x ORB9046
ORB9050	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::P*spank*(*hy*)-*saiR spx*::*neo*	pSS9 x ORB9046
ORB9055	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::P*spank*(*hy*)-*saiR*(C89S C96S)	pYA2 x ORB8884
ORB9064	*thrC*::*spxA2*(-247/+268)-*lacZ amyE*::P*spank*(*hy*)-*saiR*(C89S C96S) *his6*	pYA3 x ORB8884

References

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Cataldi, A., Labruyere, E., and Mock, M. (1990) Construction and characterization of a protective antigen-deficient *Bacillus anthracis* strain. *Mol Microbiol* **4**: 1111-1117.

Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V.*, et al.* (1997) The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* **390**: 249-256.

Table S2. Plasmids used in this study

Plasmid name	**Feature**	**Source**
pDG793	*thrC* integration vector carrying promoter-less *lacZ*	(Guérout-Fleury *et al.*, 1996)
pDR111	*amyE* integration vector carrying IPTG-inducible P*spank*(*hy*) promoter	(Britton *et al.*, 2002)
pET23a	expression vector for C-terminal 6xHis-tagged protein	Novagen
pJMA402	cloning vector for ICEBs1 conjugation	A. D. Grossman
pRP1028	Plasmid for allelic exchange in *B. anthracis*	S. Stibitz
pMMN839	pRP1028 carrying Δ*saiR*	this study
pMMN850	pJMA402 carrying *saiR*(wt)	this study
pMMN851	pJMA402 carrying *saiR*(C89S)	this study
pMMN852	pJMA402 carrying *saiR*(C96S)	this study
pMMN856	pET23a carrying *saiR*(wt)his_6	this study
pMMN857	pET23a carrying *saiR*(C89S)his_6	this study
pMMN858	pET23a carrying *saiR*(C96S)his_6	this study
pMMN881	pET23a carrying *saiR*(C89S C96S)his_6	this study
pMMN861	pDG793 carrying *spxA2*(-50/+31) promoter	this study
pMMN865	pDG793 carrying *spxA2*(-100/+31) promoter	this study
pMMN869	pDG793 carrying *spxA2*(-50/+4) promoter	this study
pMMN870	pDG793 carrying *spxA2*(-38/+31) promoter	this study
pMMN871	pDG793 carrying *spxA2*(-100/-39) promoter	this study
pMMN872	pDG793 carrying *spxA2*(-100/+268) promoter	this study
pMMN873	pDG793 carrying *spxA2*(-247/+31) promoter	this study
pMMN874	pDG793 carrying *spxA2*(-50/+174) promoter	this study
pMMN875	pDG793 carrying *spxA2*(-50/+174:T190G) promoter	this study
pMMN876	pDG793 carrying *spxA2*(-50/+174:G142T, A145C) promoter	this study
pMMN877	pDG793 carrying *spxA2*(-50/+31: A-17C, G-20T) promoter	this study
pMMN878	pDG793 carrying *spxA2*(-50/+174:A-17C, G-20T) promoter	this study
pMMN879	pDG793 carrying *spxA2*(-50/+174:A-17C, G-20T, G142T, A145C) promoter	this study
pMMN880	pDR111 carrying *saiR*his_6	this study
pSB20	pDG793 carrying *spxA2*(-247/+268) promoter	this study
pSS9	pDR111 carrying *saiR*(wt)	this study
pSS10	pDR111 carrying *saiR*(C89S)	this study
pSS11	pDR111 carrying *saiR*(C96S)	this study
pYA1	pDR111 carrying *saiR*(C96S)his_6	this study
pYA2	pDR111 carrying saiR(C89S C96S)	this study
pYA3	pDR111 carrying saiR(C89S C96S)his_6	this study

References

Britton, R.A., Eichenberger, P., Gonzalez-Pastor, J.E., Fawcett, P., Monson, R., Losick, R., and Grossman, A.D. (2002) Genome-wide analysis of the stationary-phase sigma factor (sigma-H) regulon of *Bacillus subtilis*. *J Bacteriol* **184**: 4881-4890.

Guérout-Fleury, A.M., Frandsen, N., and Stragier, P. (1996) Plasmids for ectopic integration in *Bacillus subtilis*. *Gene* **180**: 57-61.

Table S3 oligonucleotides used in this study

Primer	**Sequence (5′ to 3′)***	**Usage**
oMN13-588	GCTAACCTATCTGTAATATTTATGGCTA	sequence for BAS3200
oMN13-589	GTATTTTCCATTACATTTCAATAGTAAGA	sequence for BAS3200
oMN13-590	TTCTTGATTGATAAGCAAAGTAAAGTCGCTGCTCATTGT	construction of ΔBAS3200
oMN13-591	CGACTTTACTTTGCTTATCAATCAAGAACGTTAAGCGGCT	construction of ΔBAS3200
oMN13-592	gcaggatccACGTAGCGTTCGAACATTTTTAAA	construction of ΔBAS3200
oMN13-593	ggaatgcatAACAATTGTTATCAGAGGTGTGCA	construction of ΔBAS3200
oMN13-620	TGCAATCGCTGTTACTCGTC	BA0787 RT-qPCR
oMN13-621	CGCTTGTAGTGCAGGTTGAA	BA0787 RT-qPCR
oMN13-627	GTTActcgagACGTTCTTGATTGATAAGAT	construction of pMMN856, 857, 858, and 881
oMN13-638	TATCGTTTGTTGAAATGTATTAAAAA	construction of pMMN874 and 878
oMN13-640	gaccgaattccAAGACTTAACATTTGATTAAT	construction of oMMN861, 869, 874, 875, 876, and 877
oMN13-641	attggatccAGATGATGACAACTCACTTCTT	construction of pMMN861, 865, 870, and 873
oMN13-647	gactgaattcATTTGATTAATAGAACTGTAAGGT	construction of pMMN870
oMN13-648	gaaggatccTATACTGTAATTTTAAACCTTACA	construction of pMMN869
oMN13-649	gaatgaattcAATTGGTCAGCATAACACTATGGG	construction of pMMN871 and 872
oMN13-652	gaaggatccTTAAGTCTTGGATTAATTAATCAT	construction of pMMN871
oMN14-659	gaccgaattccAAGACTTAACATTTGATTAATAGAACTTTACGGTTTA	construction of pMMN877, 878, and 879
oMN14-660	TATCGTTTGTTGAACTGTATTAAAAA	construction of pMMN875, 876, and 879
oMN14-661	TATCGTTTGTTGAAATGTATTAAAAAAAAGTAAAGTTTA	construction of pMMN879
oMN14-662	TGAAAGGAGGAAGCAGGAAA	BA3454 RT-qPCR
oMN14-663	AAAGACTCCATTGTTTCTTCACTTG	BA3454 RT-qPCR
oMN14-664	gcgaagcttAGGAGGAAAACAATGAGCAGCGACTTTACTTTG	construction of pMMN880
oMN14-665	tcggcatgcTCAGTGGTGGTGGTGGTGCTCGAGACGTTC	construction of pMMN880, pYA1, and 3
oMN14-666	ATAGAACTGTAAGGTTTAAAATTACAGTATAGA	construction of SaiR site 1 (wt) for restriction enzyme protection assay
oMN14-667	TCTATACTGTAATTTTAAACCTTACAGTTCTAT	construction of SaiR site 1 (wt) for restriction enzyme protection assay
oMN14-668	ATAGAACTTTAAGGTTTAAAATTACAGTATAGA	construction of SaiR site 1 (G-20T) for restriction enzyme protection assay
oMN14-669	TCTATACTGTAATTTTAAACCTTAAAGTTCTAT	construction of SaiR site 1 (G-20T) for restriction enzyme protection assay
oMN14-670	ATAGAACTGTAAGGTTAAAATTACAGTATAGA	construction of SaiR site 1 (Δ-12T) for restriction enzyme protection assay
oMN14-671	TCTATACTGTAATTTTAACCTTACAGTTCTAT	construction of SaiR site 1 (Δ-12T) for restriction enzyme protection assay
oMN14-674	AAGCATGCAAAGAACCGAGT	*spxA2* leader RT-qPCR
oMN14-675	TTTTCGACATGAAGCACAGC	*spxA2* leader RT-qPCR
oSB80	taggggaattcCCGATGTAGAACTGCACTAGAAAG	construction of pSB20 and pMMN873
oSB81	taggcggatccCGCTTTTCGACATGAAGCACAGCTTGC	construction of pSB20 and pMMN872
oSB103	taggcctgcagAATTGGTCAGCATAACACTATGGGGAC	construction of pMMN865
oSH07F	tgcgaagcttAGGAGGAAAACAATGAGCAGC	construction of pSS9, 10, 11, pMMN856, 857, 858, 881, and pYA2
oSH08R	tcggcatgcTTAACGTTCTTGATTGATAAGATCAAC	construction of pSS9, 10, 11, and pYA2
oSH09F	TCAAATGATCAGAGTATTGTAGGTTCA	construction of pSS11, pMMN881, and pYA2
oSH010R	TGAACCTACAATACTCTGATCATTTGA	construction of pSS11, pMMN881, and pYA2
oSH11R	ATTTGATTCTGGGCTCTTAGGCTGCAACG	construction of pSS10
oSH12F	CGTTGCAGCCTAAGAGCCCAGAATCAAAT	construction of pSS10
793up	TTTTAAGATAATGCACTATCAACACACTC	*in vitro* transcription template
3455F1	AAAGGAGGAAACTGAAGGTGA	BA3455 RT-qPCR

3455R1	TCCACACCGTATCTTTGCTT	BA3455 RT-qPCR
rpoBF	GAGCCGTTTGATAACCGTGT	*rpoB* RT-qPCR
rpoBR	ACCTCCATCTCACCAAAACG	*rpoB* RT-qPCR

*lowercase letters show sequences to generate restriction enzyme recognition sites for cloning purpose.

Table S4. Genes regulated by the *saiR* mutation

A. Genes upregulated both in the wild-type and *spxA1* backgrounds

Locus	GeneN	GeneS	7702 vs 8571			Median	Mean	8170 vs 8606			Median	Mean
BA3452	hypothetical protein		5.62	4.51	6.08	5.62	5.41	6.46	5.48	5.40	5.48	5.78
BA3454	hypothetical protein		4.70	4.91	7.43	4.91	5.68	4.51	4.87	4.21	4.51	4.53
BA3455	hypothetical protein		3.70	5.47	4.55	4.55	4.58	3.47	4.38	3.49	3.49	3.78
BA3456	transcription regulator	*spxA2*	3.20	3.96	6.04	3.96	4.40	3.60	3.85	3.71	3.71	3.72
BA5693	major facilitator family transporter		1.92	1.70	1.98	1.92	1.87	NaN	NaN	NaN		
BA4024	dihydroorotate dehydrogenase, electron transfer subunit	*pyrK*	1.85	1.80	1.99	1.85	1.88	2.35	1.81	1.72	1.81	1.96
BA4028	aspartate carbamoyltransferase	*pyrB*	1.82	1.76	1.62	1.76	1.73	3.03	2.32	3.86	3.03	3.07
BA4023	dihydroorotate oxidase	*pyrD*	1.65	1.82	1.73	1.73	1.73	1.89	1.77	1.65	1.77	1.77
BA4025	carbamoyl-phosphate synthase, large subunit	*carB*	1.47	1.62	1.64	1.62	1.58	1.93	1.51	1.97	1.93	1.80
BA4021	orotate phosphoribosyltransferase	*pyrE*	1.61	1.57	1.56	1.57	1.58	1.82	1.71	1.91	1.82	1.81
BA4027	dihydroorotase	*pyrC*	1.51	1.65	1.52	1.52	1.56	2.62	2.38	2.25	2.38	2.42
BA4022	orotidine 5-phosphate decarboxylase	*pyrF*	1.33	1.47	1.59	1.47	1.46	1.47	0.87	1.24	1.24	1.19
BA4026	carbamoyl-phosphate synthase, small subunit	*carA*	1.64	1.39	1.36	1.39	1.46	2.00	1.97	1.77	1.97	1.91
BA0871	LPXTG-motif cell wall anchor domain protein		1.52	1.33	1.25	1.33	1.37	1.30	0.97	1.49	1.30	1.25
BA3406	ABC transporter, ATP-binding protein		1.16	1.46	1.28	1.28	1.30	NaN	NaN	NaN		
BA4029	uracil permease	*uraA*	1.16	1.23	1.24	1.23	1.21	1.45	2.56	1.44	1.45	1.81
BA5475	nucleoside transporter, NupC family		0.89	1.09	1.25	1.09	1.08	0.17	0.23	0.34	0.23	0.25
BA0610	L-lactate permease	*lldP-1*	1.21	0.97	1.00	1.00	1.06	1.42	1.23	1.10	1.23	1.25

B. Genes upregulated only in the *spxA1* background

Locus	GeneN	GeneS	8170 vs 8606			Median	Mean
BA0885	S-layer protein Sap	*sap*	4.54	4.63	4.51	4.54	4.56
BA0887	S-layer protein EA1	*eag*	4.82	3.34	3.83	3.83	4.00
BA0787	major facilitator family transporter		3.79	2.05	3.95	3.79	3.27
BA0800	ABC transporter, permease protein, putative		4.80	2.79	3.38	3.38	3.66
BA0956	hypothetical protein		2.46	1.18	2.02	2.02	1.88
BA3524	conserved hypothetical protein		1.57	1.90	2.06	1.90	1.84
BA0556	conserved hypothetical protein		1.48	1.85	1.98	1.85	1.77
BA0535	potassium channel protein, putative		2.03	1.66	NaN	1.84	1.84
BA3438	alcohol dehydrogenase, zinc-containing		3.50	1.77	1.83	1.83	2.36
BA2029	hypothetical protein		1.80	1.50	2.11	1.80	1.81
BA2390	ABC transporter, permease protein, putative		2.96	1.50	1.78	1.78	2.08
BA0918	hypothetical protein		0.70	2.19	1.75	1.75	1.55
BA4875	universal stress protein family		1.72	1.92	1.30	1.72	1.65
BA2289	aldehyde dehydrogenase family protein		1.69	1.65	1.64	1.65	1.66
BA1317	ykgG family protein		1.60	1.64	1.64	1.64	1.63
BA1316	iron-sulfur cluster-binding protein		1.68	1.63	1.55	1.63	1.62
BA3465	metallo-beta-lactamase family protein		1.55	1.62	2.04	1.62	1.74
BA4668	ABC transporter, permease protein		1.61	1.53	1.79	1.61	1.64
BA1951	conserved hypothetical protein		1.63	1.40	1.58	1.58	1.54
BA1127	S-layer protein, putative		1.46	1.53	1.54	1.53	1.51
BA3324	RNA polymerase sigma-70 factor, ECF subfamily		1.50	1.48	1.52	1.50	1.50
BA3322	conserved domain protein		1.46	1.36	1.48	1.46	1.43
BA3466	conserved hypothetical protein		NaN	1.09	1.81	1.45	1.45
BA2389	ABC transporter, ATP-binding protein		1.57	1.40	1.32	1.40	1.43
BA3655	oxidoreductase, Gfo/Idh/MocA family		1.30	1.45	1.22	1.30	1.32
BA3529	hypothetical protein		1.19	1.29	1.43	1.29	1.30
BA0196	oxidoreductase, aldo/keto reductase family		1.30	1.16	1.28	1.28	1.25
BA4669	ABC transporter, ATP-binding protein		1.24	1.23	0.87	1.23	1.12
BA4935	hypothetical protein		1.13	1.19	1.23	1.19	1.18
BA5648	uracil-DNA glycosylase	*ung*	NaN	1.08	1.28	1.18	1.18
BA2902	CAAX amino terminal protease family protein		1.40	1.16	1.17	1.17	1.24
BA3433	glucose-6-phosphate 1-dehydrogenase	*zwf*	1.14	1.16	1.48	1.16	1.26
BA3525	conserved hypothetical protein		0.78	1.19	1.15	1.15	1.04
BA2269	membrane protein, putative		1.66	0.90	1.14	1.14	1.23
BA0197	pyrroline-5-carboxylate reductase, putative		1.22	1.05	1.13	1.13	1.13
BA3323	conserved hypothetical protein		1.11	1.12	1.09	1.11	1.11
BA3947	tRNA pseudouridine synthase B	*truB*	1.14	1.09	0.92	1.09	1.05
BA5238	PAP2 family protein		2.25	1.16	1.01	1.07	1.47
BA1122	hypothetical protein		1.06	1.37	1.03	1.06	1.16

C. Genes downregulated more in the *spxA1* background

Locus	GeneN	GeneS	7702 vs 8571			Median	Mean
BA5072	hypothetical protein		-2.79	-2.75	-2.40	-2.75	-2.64
BXA0072	hypothetical protein		-2.61	-2.33	-2.40	-2.40	-2.45
BA4370	hypothetical protein		-2.16	-2.00	-2.04	-2.04	-2.07
BA0422	conserved hypothetical protein		-2.00	-1.89	-2.19	-2.00	-2.03
BA4490	ribosomal protein L33	*rpmG-2*	-1.89	-2.35	-2.00	-2.00	-2.08
BA2666	hypothetical protein		-1.99	-1.91	-1.94	-1.94	-1.94

BA1182	conserved hypothetical protein		-2.09	-1.88	-1.89	-1.89	-1.95
BA1284	hypothetical protein		-1.86	-1.80	-1.95	-1.86	-1.87
BA1741	hypothetical protein		-1.77	-1.83	-1.72	-1.77	-1.77
BA0034	transition state transcriptional regulatory protein AbrB	*abrB*	-1.83	-1.76	-1.72	-1.76	-1.77
BA3752	conserved hypothetical protein		-1.61	-1.75	-1.79	-1.75	-1.72
BA1137	hypothetical protein		-1.37	-1.74	-1.77	-1.74	-1.62
BA3426	conserved hypothetical protein		-1.72	-1.72	-2.14	-1.72	-1.86
BA4617	hypothetical protein		-1.66	-1.69	-1.69	-1.69	-1.68
BA0406	conserved hypothetical protein		-1.77	-1.51	-1.66	-1.66	-1.65
BA5626	4-oxalocrotonate tautomerase		-1.81	-1.59	-1.65	-1.65	-1.68
BA4473	conserved domain protein		-1.83	-1.62	-1.60	-1.62	-1.68
BA3860	copper-ion-binding protein		-1.38	-1.56	-1.72	-1.56	-1.55
BA1363	conserved domain protein		-1.95	-1.49	-1.51	-1.51	-1.65
BA2262	heat shock protein, Hsp20 family		-1.59	-1.45	-1.40	-1.45	-1.48
BA2282	lemA domain protein		-1.40	-1.43	-1.65	-1.43	-1.49
BA1461	conserved domain protein		-1.45	-1.36	-1.42	-1.42	-1.41
BA1607	conserved hypothetical protein		-1.30	-1.43	-1.40	-1.40	-1.38
BA3411	conserved hypothetical protein		-1.46	-1.40	-1.38	-1.40	-1.41
BA4779	conserved hypothetical protein		-1.34	-1.39	-1.42	-1.39	-1.38
BA1247	hypothetical protein		-1.66	-1.34	-1.38	-1.38	-1.46
BA1579	conserved hypothetical protein		-1.23	-1.38	-1.37	-1.37	-1.33
BA4163	conserved hypothetical protein		-1.37	-1.35	-1.54	-1.37	-1.42
BA1897	conserved hypothetical protein		-1.48	-1.31	-1.37	-1.37	-1.39
BA2166	hypothetical protein		-1.55	-0.81	-1.36	-1.36	-1.24
BA2363	transcriptional regulator, ArsR family		-1.33	-1.28	-1.32	-1.32	-1.31
BA4109	prophage LambdaBa02, fosfomycin resistance protein	*fosB-2*	-1.44	-1.31	-1.14	-1.31	-1.30
BA5738	ribosomal protein L34	*rpmH*	-1.37	-1.25	-1.31	-1.31	-1.31
BA0851	hypothetical protein		-1.39	-1.23	-1.30	-1.30	-1.31
BA4265	hypothetical protein		-1.20	-1.29	-2.01	-1.29	-1.50
BA3713	hut operon transcriptional activator	*hutP*	-1.13	-1.37	-1.29	-1.29	-1.27
BA4550	conserved hypothetical protein		-1.19	-1.36	-1.28	-1.28	-1.28
BA4329	conserved hypothetical protein		-1.57	-1.28	-1.23	-1.28	-1.36
BA1621	hypothetical protein		-1.53	-1.20	-1.28	-1.28	-1.34
BA0488	hypothetical protein		-1.15	-1.35	-1.28	-1.28	-1.26
BA0817	hypothetical protein		-1.14	-1.34	-1.26	-1.26	-1.25
BA1135	cold shock protein CspA	*cspA-1*	-1.17	-1.31	-1.24	-1.24	-1.24
BA4060	acetyltransferase, GNAT family		-1.14	-1.24	-1.27	-1.24	-1.22
BA5007	hypothetical protein		-1.13	-1.39	-1.24	-1.24	-1.25
BA1782	transposase, IS605 family, OrfA		-1.19	-1.26	-1.24	-1.24	-1.23
BA4769	spore coat protein C, putative		-1.25	-1.23	-1.19	-1.23	-1.22
BA5255	conserved hypothetical protein TIGR00106		-1.20	-1.22	-1.44	-1.22	-1.29
BA0816	conserved hypothetical protein		-1.12	-1.32	-1.22	-1.22	-1.22
BA0775	conserved hypothetical protein		-1.22	-1.19	-1.24	-1.22	-1.21
BA4321	conserved hypothetical protein		-1.20	-1.22	-1.14	-1.20	-1.19
BA2876	hypothetical protein		-1.42	-1.16	-1.19	-1.19	-1.26
BA2302	conserved hypothetical protein		-1.19	-1.13	-1.30	-1.19	-1.21
BA3858	DNA-binding protein HU	*hup-3*	-1.19	-1.22	-1.17	-1.19	-1.19
BA4167	hypothetical protein		-1.19	-1.21	-0.93	-1.19	-1.11
BA4242	hypothetical protein		-1.16	-1.06	-1.21	-1.16	-1.14
BXA0057	hypothetical protein		-1.15	-1.12	-1.24	-1.15	-1.17
BA5632	conserved hypothetical protein		-1.14	-1.20	-1.13	-1.14	-1.16
BA5316	SET domain protein		-1.13	-1.12	-1.17	-1.13	-1.14
BA2013	general stress protein	*dps*	-1.05	-1.12	-1.16	-1.12	-1.11

D. Genes downregulated more in the *spxA2* background

Locus	GeneN	GeneS	8170 vs 8606			Median	Mean
BA0161	gluconate operon transcriptional repressor	*gntR*	-3.80	-4.04	-3.70	-3.80	-3.85
BA0163	gluconate permease	*gntP-1*	-3.75	-3.19	-3.43	-3.43	-3.46
BA1581	spore coat protein D, putative		-2.62	-3.26	-2.70	-2.70	-2.86
BA0162	gluconate kinase, authentic point mutation		-2.41	-2.97	-3.17	-2.97	-2.85
BA4266	hypothetical protein		-2.37	-2.52	-1.94	-2.37	-2.27
BXA0137	hypothetical protein,		-1.63	-1.65	-1.51	-1.63	-1.60
BA0164	6-phosphogluconate dehydrogenase, decarboxylating	*yqjI*	-1.32	-1.28	-1.32	-1.32	-1.31
BA1237	hypothetical protein		-1.31	-1.60	-1.90	-1.60	-1.60
BXA0124	S-layer protein,		-1.29	-1.88	-1.86	-1.86	-1.68
BA5301	sodium/alanine symporter family protein		-1.23	-1.04	-1.27	-1.23	-1.18

***Green shading and negative values represent genes downregulated in mutant strains compared to wild type. Red shading and positive values represent genes upregulated in mutant strains compared to wild type. NaN represents genes where no signal was present in the wild-type or mutant samples. Values are log2 changes. Columns G/L and H/M represent the median and mean value across 3 biological replicate experiements.**