Supplemental Figures
Xue et al.

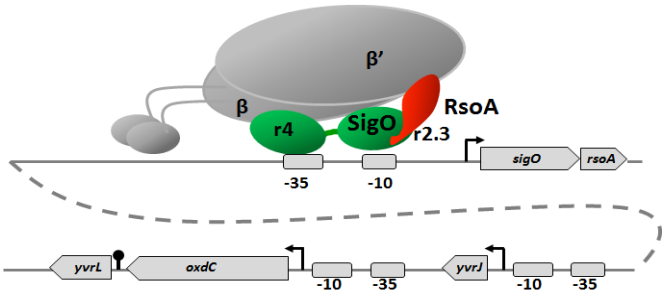

Fig. S1. Model of SigO-RsoA transcription complex. RsoA is situated proximal to promoter -10 element based on region 2.3 interaction with amino terminus of SigO (this work) and the β' clamp helices (MacLellan *et al.*, 2009). The SigO-RsoA complex autoregulates its own expression and the expression of *yvrJ* and the *oxdC-yvrL* operon. An intergenic stem-loop structure attenuates expression of YvrL relative to OxdC (MacLellan *et al.*, 2008).

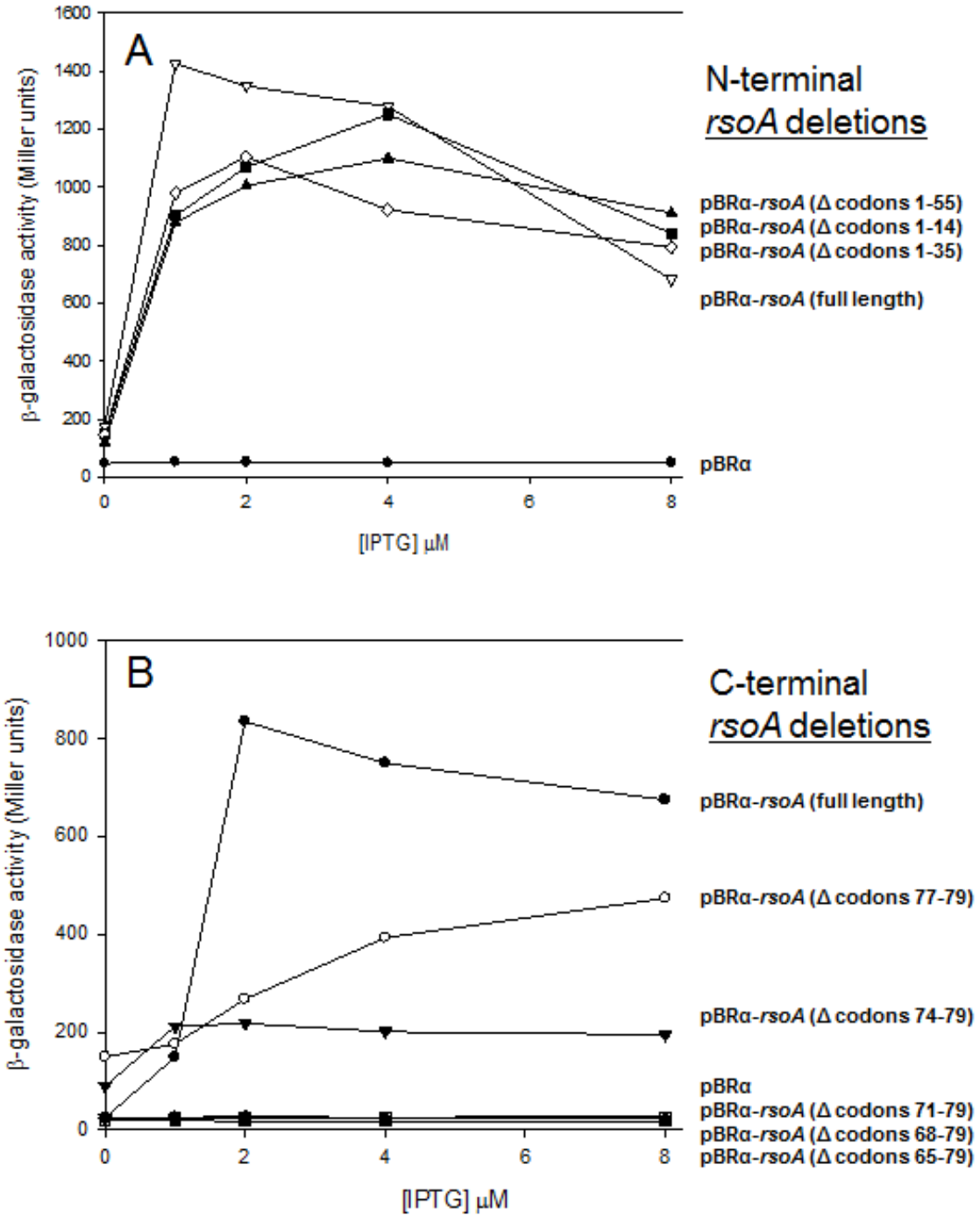

Fig. S2. Alternative BACTH assay of A) 14, 35, and 55 amino acid RsoA N-terminal deletion derivatives and B) 3, 6, 9, 12, and 15 amino acid RsoA C-terminal deletion derivatives using λcI-SigO fusions and RpoA (α subunit)-RsoA fusions in host strain *E. coli* FW Kan O_L2–62 lac. Details of assay methodology are as described in Dove and Hochschild (2004) and MacLellan et al., (2009).

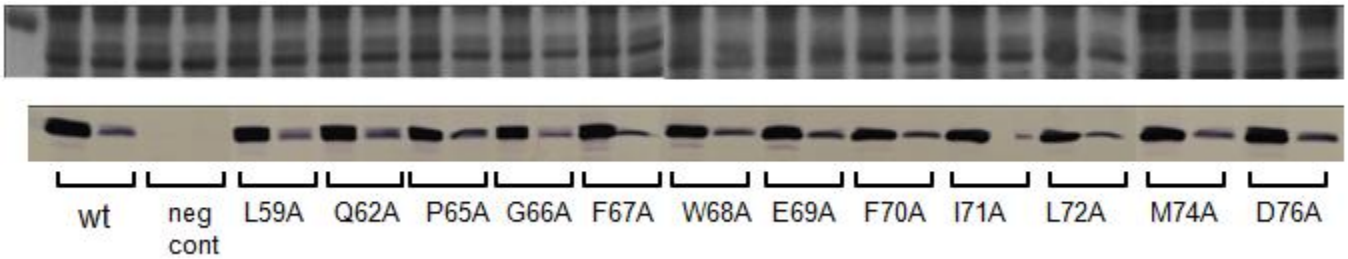

Fig.S3. wt and region 2.3 alanine mutant RsoA accumulation and solubility in CyaA+ strain for strains used in Fig.4. After cell lysis, crude (uncentrifuged) and clarified (centrifuged) lysate was run (lanes 1 and 2 for each isolate, respectively) to examine accumulation of total and soluble RsoA in cells via Western immunoblotting (lower panel). Coomassie stained proteins (upper panel) act as loading control. This image is a composite picture produced from several gels run during same experiment.

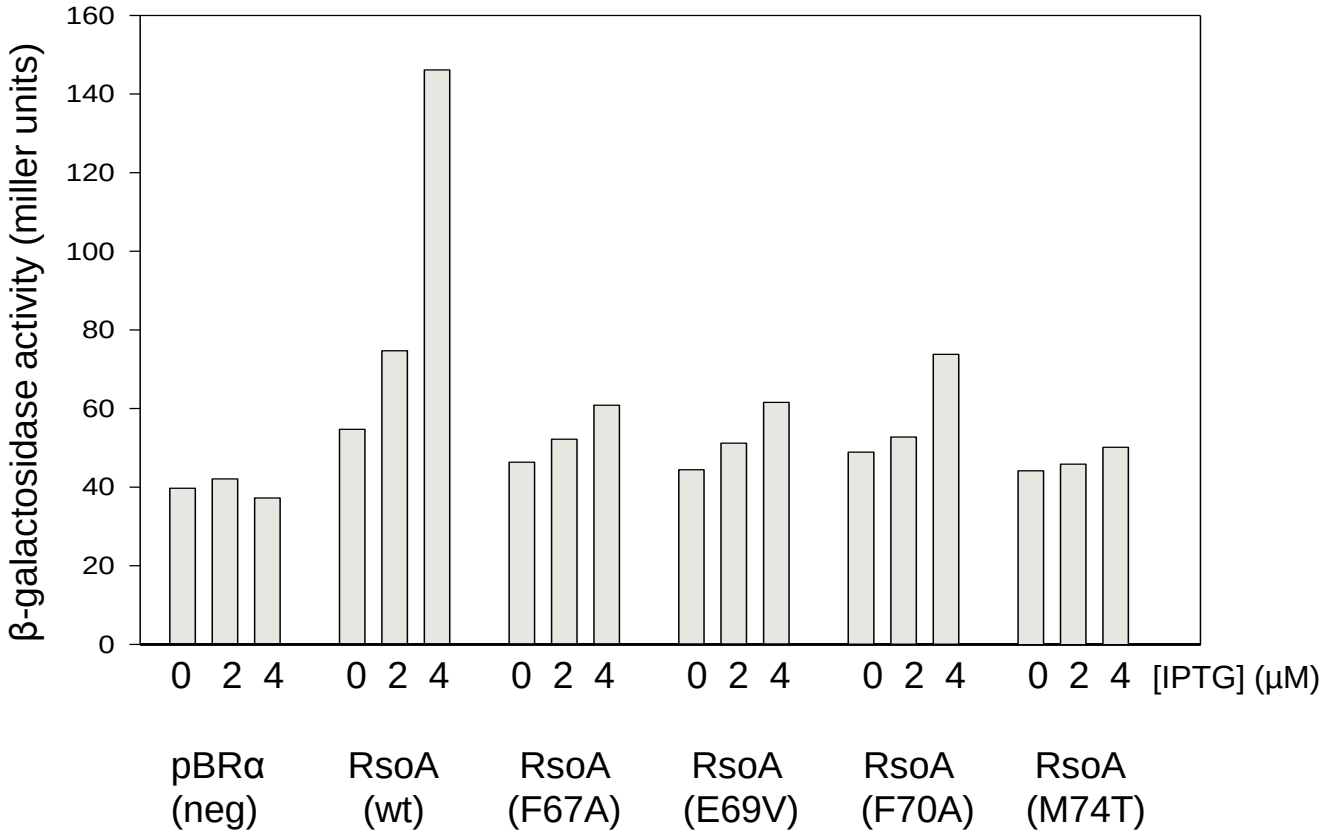

Fig. S4. Activity of key RsoA mutants interacting with β' subunit N-terminus (amino acids 1-303). λcI-RpoC (β' subunit) fusion and RpoA (α subunit)-RsoA fusions were tested in host strain *E. coli* FW Kan O_L2–62 lac. Details of assay methodology are as described in Dove and Hochschild (2004) and MacLellan et al., (2009).

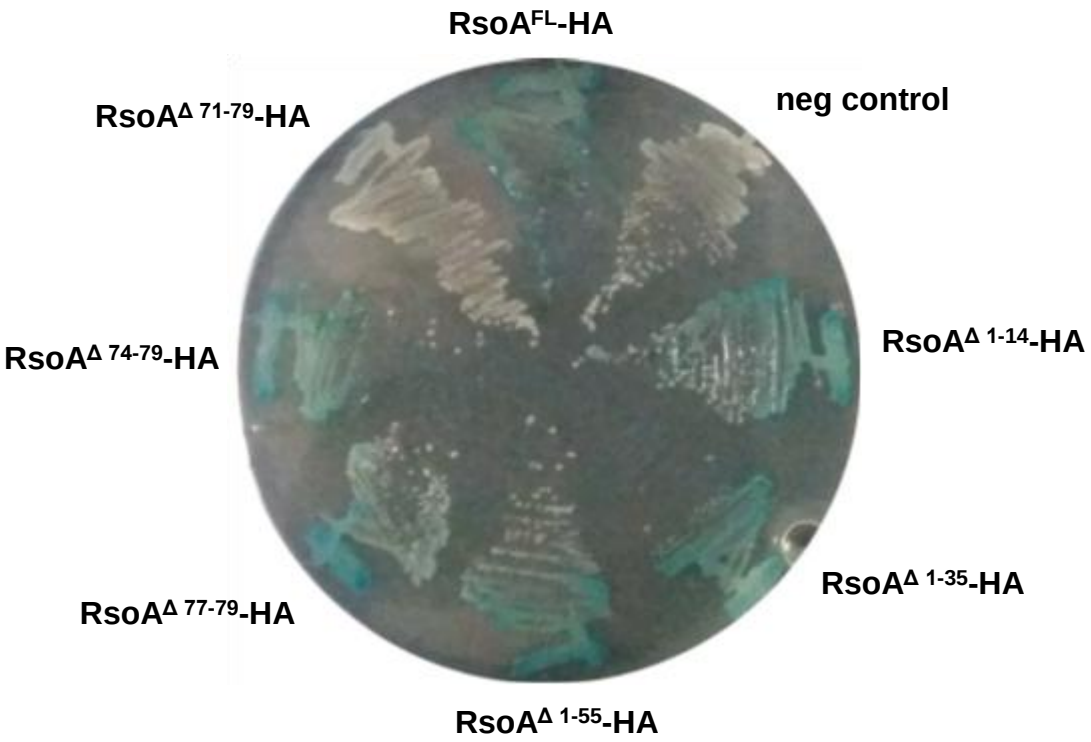

Fig. S5. Interaction of epitope-tagged RsoA derivatives with SigO-FLAG. Each strain expresses a CyaA^{T18}-SigO^{Nterm}-FLAG fusion protein in *E. coli* BTH101. RsoA derivatives expressed as CyaA^{T25} fusions with C-terminal haemagluttinen (HA) epitope tags. RsoA derivatives tested include 79 amino acid full-length RsoA (FL) and three N-terminal deletions (deleted amino acids 1-14, 1-35, and 1-55) and three C-terminal deletions (deleted amino acids 77-79, 74-79, and 71-79). Negative control strain expresses a CyaA^{T18}-SigO^{Nterm}-FLAG fusion protein but carries non-recombinant plasmid pKT25.

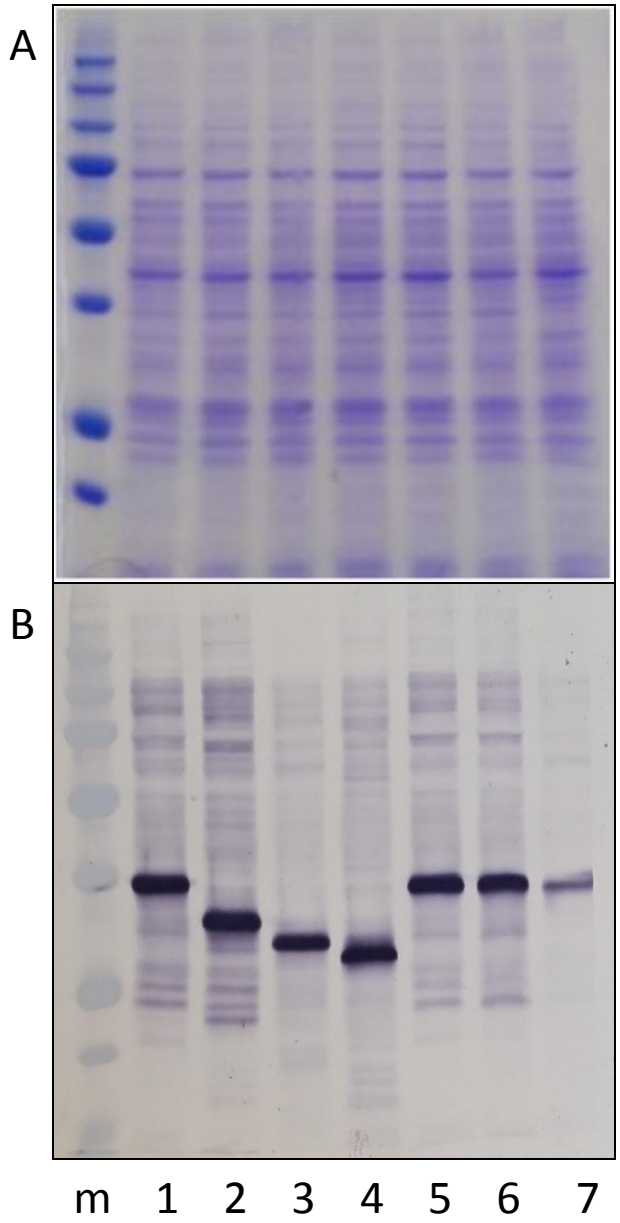

Fig. S6. Accumulation of CyaA^{T25}-RsoA-HA fusion proteins in *E. coli.* A) Coomassie-stained gel (loading control). B) Immunoblot using anti-HA antibodies. Lanes: m, molecular weight marker; 1, full length RsoA (79 amino acids); 2, Δ amino acids 1-14; 3, Δ amino acids 1-35; 4, Δ amino acids 1-55; 5, Δ amino acids 77-79; 6, Δ amino acids 74-79; 7, Δ amino acids 71-79.

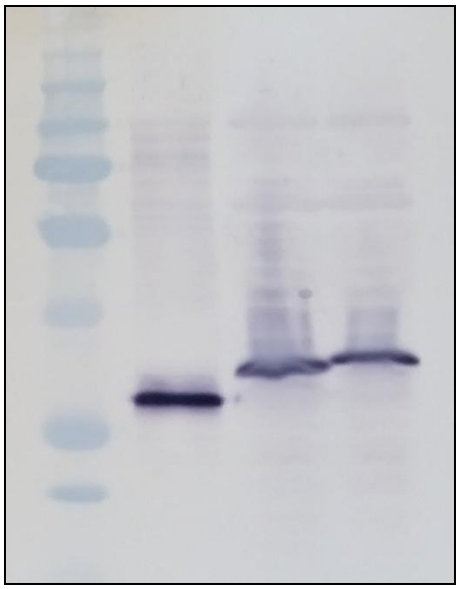

Fig. S7. Mutation of RsoA F67 results in aberrant mobility of CyaA-RsoA$^{2.3}$-HA during SDS-PAGE. Immunoblot using anti-HA antibodies. Lanes: m, molecular weight marker; wt, CyaA-RsoA$^{2.3}$-HA; and the F67A and F67S derivatives of CyaA-RsoA$^{2.3}$-HA.

Fig. S8. Multiple sequence alignment of *B. subtilis* RsoA (top line) and 44 predicted orthologous amino acid sequences. Asterisks indicate 100% conservation. Regions of similarity to σ^{70} regions 2.2 and 2.3 indicated and delineated as for Fig. 1 alignment. Positions of RsoA residues F67 and F70 as indicated.

References cited in Supplemental Information

MacLellan, S. R., Wecke, T. & Helmann, J. D. (2008). A previously unidentified sigma factor and two accessory proteins regulate oxalate decarboxylase expression in *Bacillus subtilis*. Mol Microbiol 69, 954–967.

Dove, S. L. & Hochschild, A. (2004). A bacterial two-hybrid system based on transcription activation. Methods Mol Biol 261, 231–46.

MacLellan, S. R., Guariglia-Oropeza, V., Gaballa, A. & Helmann, J. D. (2009). A two-subunit bacterial sigma-factor activates transcription in *Bacillus subtilis*. Proc Natl Acad Sci U S A 106, 21323–21328.

Table S1. Bacterial strains used in this study.

Strains	Parental Strains/characteristics	Source
E. coli strains		
E. coli DH5α	F^- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(r_K^- m_K^+), λ–	Novagen
E. coli BL21 (DE3) pLysS	F^- ompT gal dcm lon $hsdS_B(r_B^-$ $m_B^-)$ λ(DE3) pLysS(cm^R)	Novagen
E. coli BL21 (DE3) pLysE	F^- ompT gal dcm lon $hsdS_B(r_B^-$ $m_B^-)$ λ(DE3) pLysE(cm^R)	Novagen
E. coli BTH101	F^- cya-99 araD139 galE15 galK16 rpsL1 (Str^R) hsdR2 mcrA1 mcrB1	Euromedex
NB357	BTH101 (pXM10 x pXM12)	this work
NB358	BTH101 (pXM10 x pSAD1)	this work
NB359	BTH101 (pXM10 x pSAD2)	this work
NB360	BTH101 (pXM10 x pSAD3)	this work
NB361	BTH101 (pXM10 x pSAD4)	this work
NB362	BTH101 (pXM10 x pSAD5)	this work
NB363	BTH101 (pXM10 x pSAD6)	this work
NB364	BTH101 (pXM10 x pSAD7)	this work
NB624	BTH101 (pUT18C x pKT25)	this work
NBXOO1	BTH101 (pXM10 x pXM41)	this work
NBXOO2	BTH101 (pXM10 x pXM14)	this work
NBXOO3	BTH101 (pXM10 x pXM15)	this work
NBXOO4	BTH101 (pXM10 x pXM16)	this work
NBXOO5	BTH101 (pXM10 x pXM17)	this work
NBXOO6	BTH101 (pXM10 x pXM18)	this work
NBXOO7	BTH101 (pXM10 x pXM19)	this work
NBXOO8	BTH101 (pXM10 x pXM20)	this work
NBXOO9	BTH101 (pXM10 x pXM21)	this work
NBXO10	BTH101 (pXM10 x pXM22)	this work
NBX011	BTH101 (pXM10 x pXM23)	this work
NBXO12	BTH101 (pXM10 x pXM24)	this work
NBXO13	BTH101 (pXM10 x pXM55)	this work
NBXO14	BTH101 (pXM10 x pXM25)	this work
NBXO15	BTH101 (pXM10 x pXM56)	this work
NBXO16	BTH101 (pXM10 x pXM26)	this work
NBXO17	BTH101 (pXM10 x pXM57)	this work
NBXO18	BTH101 (pXM10 x pXM58)	this work
NBXO19	BTH101 (pXM10 x pXM59)	this work
NBXO20	BTH101 (pXM10 x pXM13)	this work
NBXO21	BTH101 (pXM10 x pXM28)	this work
NBXO22	BTH101 (pXM10 x pXM29)	this work
NBXO23	BTH101 (pXM10 x pXM30)	this work
NBXO24	BTH101 (pXM10 x pXM31)	this work
NBXO25	BTH101 (pXM10 x pXM32)	this work
NBXO26	BTH101 (pXM10 x pXM33)	this work
NBXO27	BTH101 (pXM10 x pXM34)	this work

NBXO28	BTH101 (pXM10 x pXM35)	this work
NBXO29	BTH101 (pXM10 x pXM36)	this work
NBXO30	BTH101 (pXM10 x pXM60)	this work
NBXO31	BTH101 (pXM10 x pXM61)	this work
NBXO32	BTH101 (pXM10 x pXM62)	this work
NBXO32	BTH101 (pXM10 x pAB1)	this work
NBXO32	BTH101 (pXM10 x pAB2)	this work
NBXO32	BTH101 (pXM10 x pAB3)	this work
NBXO32	BTH101 (pXM10 x pAB4)	this work
NB1116	BTH101 (pXM10 x pSRM15)	this work
NB1117	BTH101 (pXM10 x pSRM16)	this work
NB1118	BTH101 (pXM10 x pSRM17)	this work
NB1119	BTH101 (pXM10 x pSRM18)	this work
NB1120	BTH101 (pXM10 x pSRM19)	this work
NB1121	BTH101 (pXM10 x pSRM20)	this work
NB1122	BTH101 (pXM10 x pSRM22)	this work
B. subtilis strains		
CU1065	W168 trpC2 attSPβ	lab collection
HB7734	(CU1065) Δ*sigO-rsoA* (pSM002) P_{xylA}-*sigO*, (pSM004) P_{oxdC}-*lacZ* fusion	1
NB485	(HB7734) pSM019, ectopic expression of wt RsoA	this work
NB486	(HB7734) pXM1, ectopic expression of RsoA (L59A)	this work
NB487	(HB7734) pXM2, ectopic expression of RsoA (Q62A)	this work
NB488	(HB7734) pSM194, ectopic expression of RsoA (P65A)	this work
NB489	(HB7734) pSM195, ectopic expression of RsoA (G66A)	this work
NB490	(HB7734) pSM048, ectopic expression of RsoA (F67A)	this work
NB491	(HB7734) pSM049, ectopic expression of RsoA (W68A)	this work
NB492	(HB7734) pSAD46, ectopic expression of RsoA (E69A)	this work
NB493	(HB7734) pSM051, ectopic expression of RsoA (F70A)	this work
NB494	(HB7734) pSAD43, ectopic expression of RsoA (I71N)	this work
NB495	(HB7734) pXM3, ectopic expression of RsoA (L72A)	this work
NB496	(HB7734) pXM37, ectopic expression of RsoA (M74A)	this work
NB497	(HB7734) pXM38, ectopic expression of RsoA (V75A)	this work
NB498	(HB7734) pXM39, ectopic expression of RsoA (D76A)	this work
NB540	(HB7734) pSM016, neg control	this work
NB544	(HB7734) pSAD50, ectopic expression of RsoA (E69V)	this work
NB545	(HB7734) pSAD47, ectopic expression of RsoA (M74T)	this work
NB546	(HB7734) pSAD48, ectopic expression of RsoA (I71N)	this work
NB552	(HB7734) pSAD49, ectopic expression of RsoA (F67S)	this work

Table S2. Plasmids used in this study.

Plasmids	**Parental/characteristics**	**Source**
pET-DUET	dual MCS, IPTG inducible	Novagen
pUT18C	BACTH *cyaA* (T18), Kn^R	Euromedex
pKT25	BACTH *cyaA* (T25), Kn^R	Euromedex
pAX01	xylose inducible expression vector, *lacA* integration	2
pSWEET	xylose inducible expression vector, *amyE* integration	3
pDG1663	lacZ reporter, *thrC* integration	4
pSM002	(pSWEET) *sigO*	5
pSM004	(pDG1663) P*oxdC-lacZ* fusion	5
pSM016	Kn^r derivative of pAX01	5
pSM019	(pSM016) *rsoA*	5
pSM048	(pSM019) $rsoA^{FL}$ (F67A)	5
pSM049	(pSM019) $rsoA^{FL}$ (W68A)	5
pSM051	(pSM019) $rsoA^{FL}$ (F70A)	5
pSM194	(pSM019) $rsoA^{FL}$ (P65A)	5
pSM195	(pSM019) $rsoA^{FL}$ (G66A)	5
pBRα	BACTH α subunit fusion	6
pACλcI	BACTH λcI fusion	6
pSAD1	(pKT25) *rsoA* Δ codons 77-79	this work
pSAD2	(pKT25) *rsoA* Δ codons 74-79	this work
pSAD3	(pKT25) *rsoA* Δ codons 71-79	this work
pSAD4	(pKT25) *rsoA* Δ codons 68-79	this work
pSAD5	(pKT25) *rsoA* Δ codons 1-14	this work
pSAD6	(pKT25) *rsoA* Δ codons 1-34	this work
pSAD7	(pKT25) *rsoA* Δ codons 1-56	this work
pSAD46	(pSM019) $rsoA^{FL}$ (E69A)	this work
pSAD47	(pSM019) $rsoA^{FL}$ (M74T)	this work
pSAD48	(pSM019) $rsoA^{FL}$ (I71N)	this work
pSAD49	(pSM019) $rsoA^{FL}$ (F67S)	this work
pSAD50	(pSM019) $rsoA^{FL}$ (E69V)	this work
pSAD51	(pSM019) $rsoA^{FL}$ (F70A)	this work
pXM1	(pSM019) $rsoA^{FL}$ (L59A)	this work
pXM2	(pSM019) $rsoA^{FL}$ (Q62A)	this work
pXM3	(pSM019) $rsoA^{FL}$ (L72A)	this work
pXM10	(pUT18C) *sigO*	this work
pXM12	(pKT25) $rsoA^{FL}$	this work
pXM13	(pKT25) $rsoA^{2.3}$	this work
pXM14	(pKT25) $rsoA^{FL}$ (R41A)	this work
pXM15	(pKT25) $rsoA^{FL}$ (E42A)	this work
pXM16	(pKT25) $rsoA^{FL}$ (D33A)	this work
pXM17	(pKT25) $rsoA^{FL}$ (L44A)	this work
pXM18	(pKT25) $rsoA^{FL}$ (E47A)	this work
pXM19	(pKT25) $rsoA^{FL}$ (L59A)	this work

pXM20	(pKT25) *rsoA*^{FL} (Q62A)	this work
PXM21	(pKT25) *rsoA*^{FL} (P65A)	this work
pXM22	(pKT25) *rsoA*^{FL} (G66A)	this work
pXM23	(pKT25) *rsoA*^{FL} (F67A)	this work
pXM24	(pKT25) *rsoA*^{FL} (W68A)	this work
pXM25	(pKT25) *rsoA*^{FL} (F70A)	this work
pXM26	(pKT25) *rsoA*^{FL} (L72A)	this work
pXM27	(pKT25) *rsoA*$^{2.3}$ (L59A)	this work
pXM28	(pKT25) *rsoA*$^{2.3}$ (Q62A)	this work
pXM29	(pKT25) *rsoA*$^{2.3}$ (P65A)	this work
pXM30	(pKT25) *rsoA*$^{2.3}$ (G66A)	this work
pXM31	(pKT25) *rsoA*$^{2.3}$ (F67A)	this work
pXM32	(pKT25) *rsoA*$^{2.3}$ (W68A)	this work
pXM33	(pKT25) *rsoA*$^{2.3}$ (E69A)	this work
pXM34	(pKT25) *rsoA*$^{2.3}$ (F70A)	this work
pXM35	(pKT25) *rsoA*$^{2.3}$ (I71A)	this work
pXM36	(pKT25) *rsoA*$^{2.3}$ (L72A)	this work
pXM37	(pSM019) *rsoA*^{FL} (M74A)	this work
pXM38	(pSM019) *rsoA*^{FL} (V75A)	this work
pXM39	(pSM019) *rsoA*^{FL} (D76A)	this work
pXM40	(pUT18C) *sigO*-FLAG	this work
pXM41	(pKT25) *rsoA*^{FL} -HA	this work
pXM46	(pKT25) *rsoA*^{FL} -HA (F67A)	this work
pTS19	(pKT25) *rsoA*^{FL} -HA (F67S)	this work
pTS20	(pKT25) *rsoA*^{FL} -HA (E69V)	this work
pXM55	(pKT25) *rsoA*^{FL} (E69A)	this work
pXM56	(pKT25) *rsoA*^{FL} (I71A)	this work
pXM57	(pKT25) *rsoA*^{FL} (M74A)	this work
pXM58	(pKT25) *rsoA*^{FL} (V75A)	this work
pXM59	(pKT25) *rsoA*^{FL} (D76A)	this work
PXM60	(pKT25) *rsoA*$^{2.3}$ (M74A)	this work
pXM61	(pKT25) *rsoA*$^{2.3}$ (V75A)	this work
pXM62	(pKT25) *rsoA*$^{2.3}$ (D76A)	this work
pSRM15	(pKT25) *rsoA* polyGLY-Ala^{56}-Asp^{76} (wt)	this work
pSRM16	(pKT25) *rsoA* polyGLY-Leu^{59}-Asp^{76} (wt)	this work
pSRM17	(pKT25) *rsoA* polyGLY-Gln^{62}-Asp^{76} (wt)	this work
pSRM18	(pKT25) *rsoA* polyGLY-Pro^{65}-Asp^{76} (wt)	this work
pSRM19	(pKT25) *rsoA* polyGLY-Phe^{67}-Asp^{76} (wt)	this work
pSRM20	(pKT25) *rsoA* polyGLY-Trp^{68}-Asp^{76} (wt)	this work
pSRM22	(pKT25) *rsoA* polyGLY-A^{56}-Asp^{76} (F67A)	this work
pSRM23	(pUT18C) *sigO*^{Nterm}-FLAG	this work
pSRM24	(pKT25) *rsoA*^{FL} (wt)-HA	this work
pSRM25	(pKT25) *rsoA*^{FL} (F67A)-HA	this work
pSRM26	(pKT25) *rsoA*^{FL} (E69V)-HA	this work
pSRM27	(pKT25) *rsoA*^{FL} (F70A)-HA	this work
pSRM28	(pKT25) *rsoA*^{FL} (M74T)-HA	this work

pLEC2	(pKT25) *rsoA* -HA (Δ codons 1-14)	this work
pLEC2	(pKT25) *rsoA* -HA (Δ codons 1-34)	this work
pLEC2	(pKT25) *rsoA* -HA (Δ codons 1-56)	this work
pEF2	(pKT25) *rsoA* -HA (Δ codons 77-79)	this work
pEF3	(pKT25) *rsoA* -HA (Δ codons 74-79)	this work
pEF4	(pKT25) *rsoA* -HA (Δ codons 71-79)	this work
pSRM29	(pBRα)-*rsoA* (wt)	this work
pSRM30	(pBRα)-*rsoA* (F67A)	this work
pSRM31	(pBRα)-*rsoA* (E69V)	this work
pSRM32	(pBRα)-*rsoA* (F70A)	this work
pSRM33	(pBRα)-*rsoA* (M74T)	this work
pSRM34	(pAC λcI)-*rpoC* (codons 1–303)	this work
pSM186	(pBRα) *rsoA* -HA (Δ codons 1-14)	this work
pSM187	(pBRα) *rsoA* -HA (Δ codons 1-34)	this work
pSM188	(pBRα) *rsoA* -HA (Δ codons 1-56)	this work
pSM189	(pBRα) *rsoA* -HA (Δ codons 77-79)	this work
pSM189	(pBRα) *rsoA* -HA (Δ codons 74-79)	this work
pSM189	(pBRα) *rsoA* -HA (Δ codons 71-79)	this work

References

1. MacLellan SR, Wecke T, Helmann JD (2008) A previously unidentified sigma factor and two accessory proteins regulate oxalate decarboxylase expression in Bacillus subtilis. Mol Microbiol 69:954–967.
2. Hartl B, Wehrl W, Wiegert T, Homuth G, SchumannW(2001) Development of a new integration site within the Bacillus subtilis chromosome and construction of compatible expressioncassettes. J Bacteriol 183:2696–2699.
3. Bhavsar AP, Zhao X, Brown ED. (2001) Development and characterization of a xylose-dependent system for expression of cloned genes in Bacillus subtilis: Conditional complementation of a teichoic acid mutant. Appl Environ Microbiol 67:403–410.
4. Guerout-Fleury AM, Frandsen N, Stragier P (1996) Plasmids for ectopic integration in Bacillus subtilis. Gene 180:57–61.
5. MacLellan SR, Guariglia-Oropeza V, Gaballa A, Helmann JD (2009) A two-subunit bacterial sigma-factor activates transcription in Bacillus subtilis. Proc Natl Acad Sci U S A. 106:21323-8.
6. Dove SL, Hochschild A. (2004). A bacterial two-hybrid system based on transcription activation. Methods Mol Biol 261:231–46.