

Supporting Information for

**Analysis of the role of *Bacillus subtilis*  $\sigma^M$  in  $\beta$ -lactam resistance reveals  
an essential role for c-di-AMP in peptidoglycan homeostasis**

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Figs. S1 to S5

**Table S1.** Oligos used in this study

| #    | Name                         | Sequence <sup>1</sup>                            |
|------|------------------------------|--|
| 5244 | yybT-for(xbaI)               | GAG <u>TCTAGA</u> CATGGTGGGGAGTGATAGAAATGC       |
| 5245 | yybT-rev(bglII)              | GAGAGATCTTCATCTCTGTACGCCTCCCT                    |
| 5258 | yybT(1-303aa)<br>rev (bglII) | GAGAGATCTTTAGCCGTTTGGCAGCTTAATG                  |
| 5249 | disA for(xmaI)               | GAG <u>CCCGGG</u> TACTTCATTAGGAGGATAATAGATG      |
| 5250 | disA rev(ClaI)               | GAGATCGATTCATAAGGTTTTAACCGAAATCA                 |
| 5252 | ybbP for(XmaI)               | GAG <u>CCCGGG</u> AATCTTGGAGGACGAGGAAATG         |
| 5253 | ybbP rev(ClaI)               | GAGATCGATAGCGGTTGTTTAAGAATTTATCCA                |
| 5255 | yojJ for(xmaI)               | GAG <u>CCCGGG</u> TTCGTGAAAAGTTGGAAATTTAAACAGGAG |
| 5256 | yojJ rev(ClaI)               | GAGATCGATTGTCTCATGATAGGATTCTTAATCAG              |
| 5293 | yybT D420A<br>up-rev         | TGTGTAGCAACGATCACAAGCAGTGT                       |
| 5294 | yybT D420A<br>do-for         | ACACTGCTTGTGATCGTTGCTACACATAAGCCGTCACTCGT        |
| 5584 | ybbP-rev-GSP3                | GAACAAGCACGATGACTACA                             |
| 5585 | ybbP-rev-GSP4                | TACCAAACAAGGAGAATATCA                            |

<sup>1</sup> The endonuclease digestion sites are underlined.

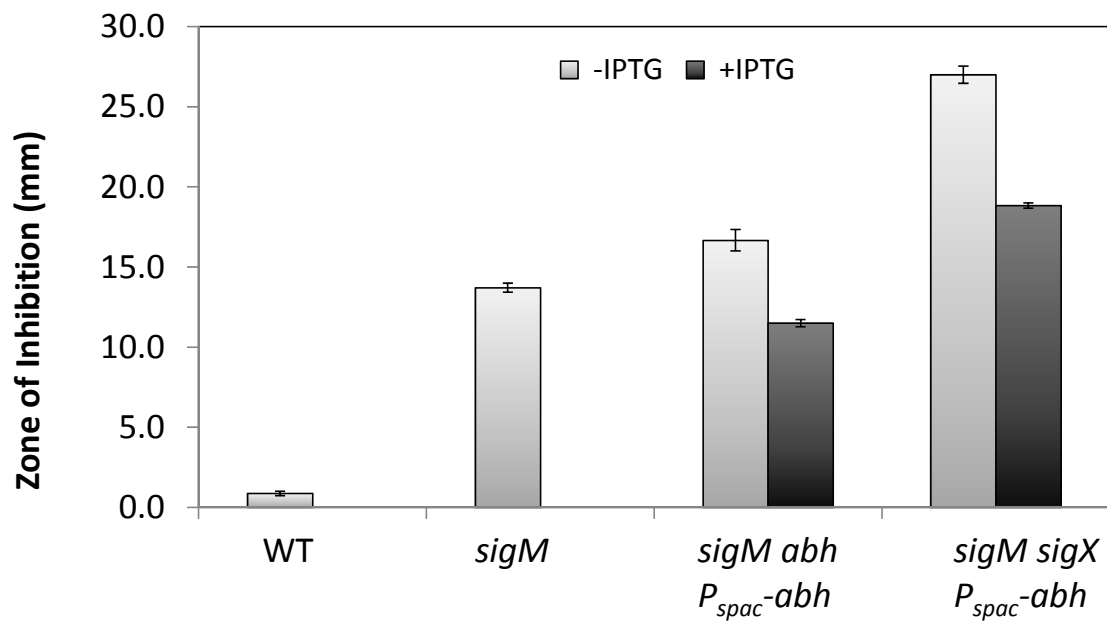
**Table S2.** Strains used in the supporting information.

| <b>Strain</b> | <b>Genotype</b>   | <b>Reference/ Construction</b> |
|---------------|---|--------------------------------|
| JH642         | <i>trpC2 pheA1</i>  | Lab strain                     |
| PY79          | SPβ-cured prototroph strain   | Lab strain                     |
| BZH73         | JH642 <i>abh::kan amyE::P<sub>spac</sub>-abh cat</i><br><i>thrC::sunA'-lacZ spc</i> | (Strauch <i>et al.</i> , 2007) |
| HB10158       | 168 <i>amyE::P<sub>spac</sub>-abh cat</i>   | chrDNA of BZH73 --> 168        |
| HB10159       | 168 <i>sigM::tet amyE::P<sub>spac</sub>-abh cat</i>                                 | chrDNA of HB10158 --> HB10016  |
| HB15808       | 168 <i>sigM::kan abh::spc</i>   | chrDNA of HB10131 --> HB10216  |
| HB15809       | 168 <i>sigM::tet abh::spc amyE:: P<sub>spac</sub>-abh cat</i>                       | chrDNA of HB10131 --> HB10159  |
| HB15810       | 168 <i>sigM::tet sigX::kan amyE:: P<sub>spac</sub>-abh cat</i>                      | chrDNA of HB10103 --> HB10159  |
| ORB4271       | JH642 <i>amyE:: P<sub>spank(hy)</sub>-spx spc</i>                                   | (Nakano <i>et al.</i> , 2003)  |
| ORB4342       | JH642 <i>amyE: P<sub>spank(hy)</sub>-spx<sup>DD</sup> spc</i>                       | (Nakano <i>et al.</i> , 2003)  |
| HB10392       | 168 <i>amyE:: P<sub>spank(hy)</sub>-spx spc</i>                                     | chrDNA of ORB4271 --> 168      |
| HB10393       | 168 <i>amyE:: P<sub>spank(hy)</sub>-spx<sup>DD</sup> spc</i>                        | chrDNA of ORB4342 --> 168      |
| HB10394       | 168 <i>sigM::kan amyE:: P<sub>spank(hy)</sub>-spx spc</i>                           | chrDNA of ORB4271 --> HB10216  |
| HB10395       | 168 <i>sigM::kan amyE: P<sub>spank(hy)</sub>-spx<sup>DD</sup> spc</i>               | chrDNA of ORB4342 --> HB10216  |
| HB15817       | 168 <i>sigM::kan spx::mIs amyE:: P<sub>spank(hy)</sub>-spx spc</i>                  | chrDNA of HB10348 --> HB10394  |
| HB15818       | 168 <i>sigM::kan spx::mIs amyE: P<sub>spank(hy)</sub>-spx<sup>DD</sup> spc</i>      | chrDNA of HB10348 --> HB10395  |
| HB15821       | 168 <i>sigM::tet sigX::kan amyE:: P<sub>spank(hy)</sub>-spx spc</i>                 | chrDNA of HB10392 --> HB10113  |
| HB15822       | 168 <i>sigM::tet sigX::kan amyE: P<sub>spank(hy)</sub>-spx<sup>DD</sup> spc</i>     | chrDNA of HB10393 --> HB10113  |
| HB10372       | 168 <i>sigM::kan disA::spc</i>  | chrDNA of HB10216 --> HB10353  |
| HB10375       | 168 <i>sigM::kan ybbP::tet</i>  | chrDNA of HB10216 --> HB10355  |
| HB10390       | 168 <i>sigM::kan ybbP::tet sigX::spc</i>  | chrDNA of HB7007 --> HB10375   |

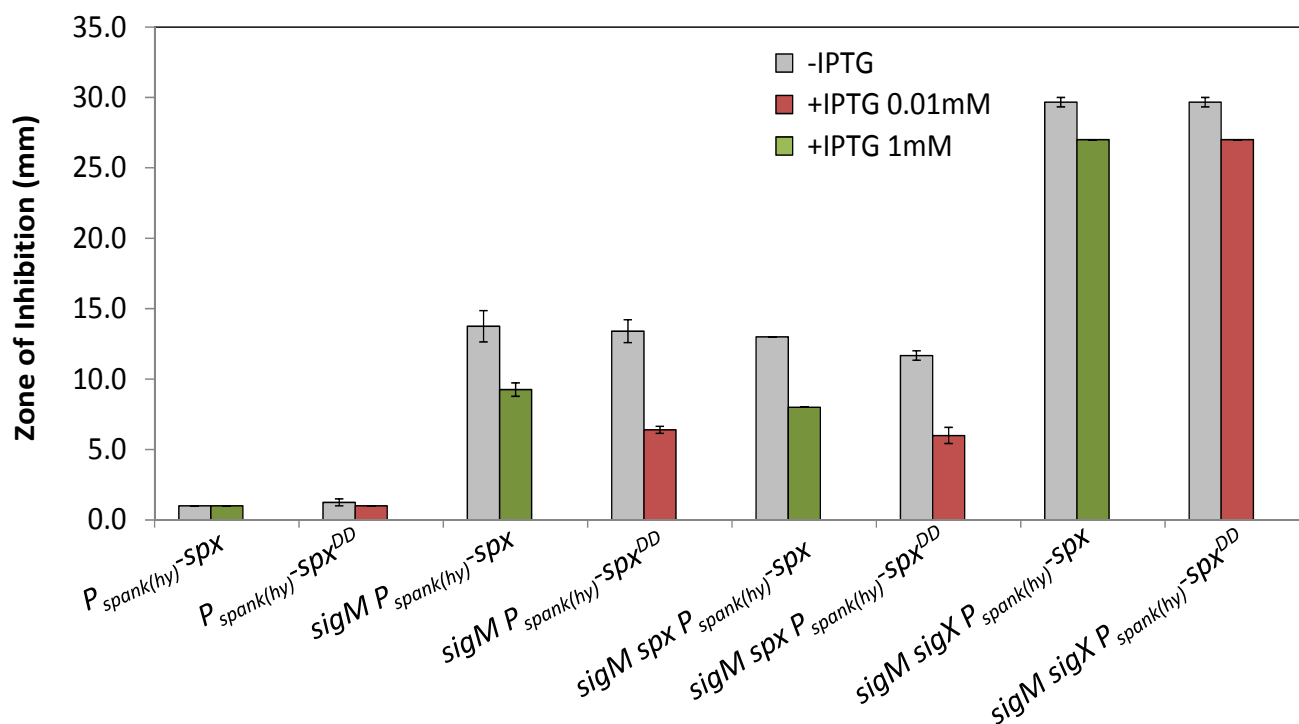
|         |                                |                             |
|---------|--------------------------------|-----------------------------|
| RL2774  | PY79 <i>clpC::tet</i>          | (Chai <i>et al.</i> , 2010) |
| RL2173  | PY79 <i>clpX::spc</i>          | Win Chai                    |
| HB15839 | 168 <i>clpC::tet</i>           | chrDNA RL2774 --> 168       |
| HB15840 | 168 <i>sigM::kan clpC::tet</i> | chrDNA RL2774 --> HB10216   |
| HB15841 | 168 <i>clpX::spc</i>           | chrDNA RL2173-->168         |
| HB15842 | 168 <i>sigM::kan clpX::spc</i> | chrDNA RL2173--> HB10216    |

**Table S3. MIC values of strain 168 and its derivative mutants.**

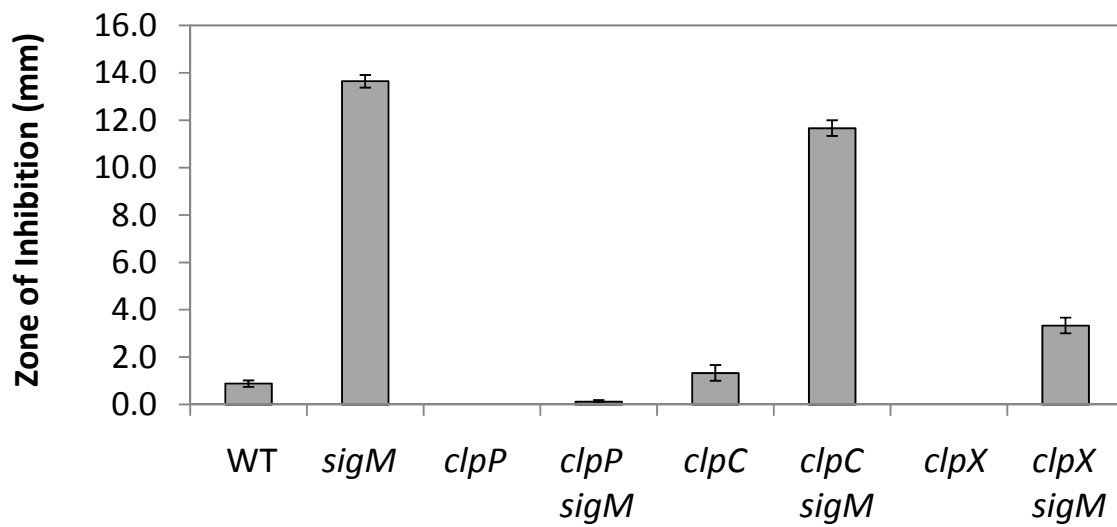
| Strain # | Genotype                           | MIC (CEF, µg/ml) |
|----------|------------------------------------|------------------|
| 168      | WT                                 | 4                |
| HB10216  | <i>sigM::kan</i>                   | 0.06             |
| HB10113  | <i>sigM::tet sigX::kan</i>         | 0.03             |
| HB10131  | <i>abh::spc</i>                    | 2                |
| HB10328  | <i>spx::spc</i>                    | 3                |
| HB15808  | <i>sigM::kan abh::spc</i>          | 0.03             |
| HB10329  | <i>sigM::kan spx::mls</i>          | 0.06             |
| HB15811  | <i>sigM::kan abh::spc spx::mls</i> | 0.03             |
| HB10353  | <i>disA::spc</i>                   | 3                |
| HB10334  | <i>ybbP::tet</i>                   | 1                |
| HB10335  | <i>yojJ::kan</i>                   | 4                |



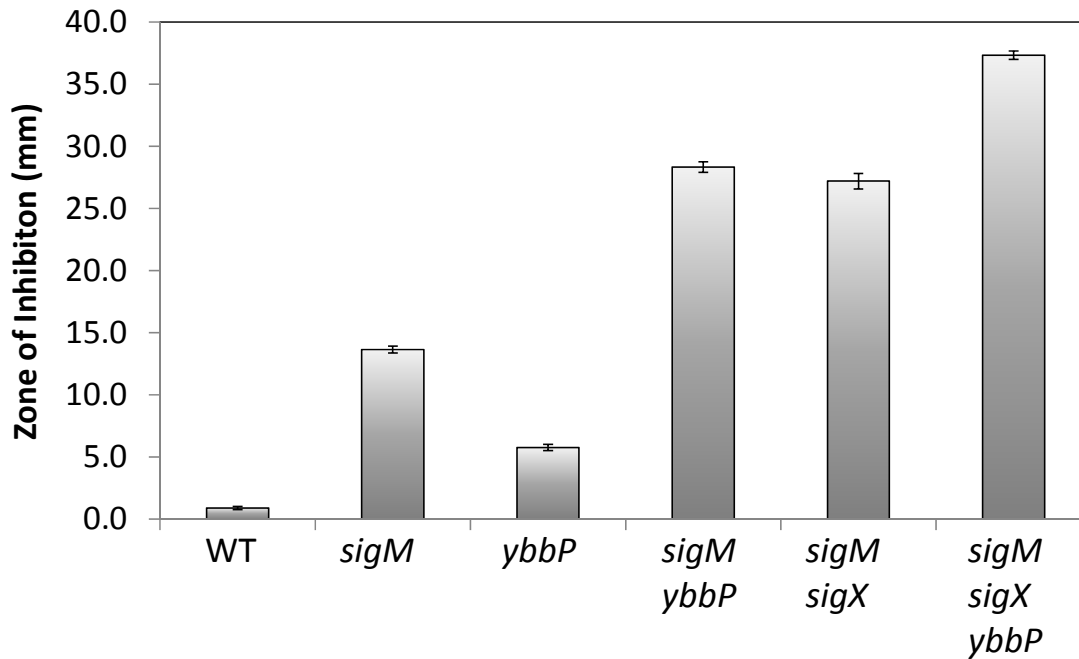
**Fig.S1.** Complementation test of *abh*. Disk diffusion tests were performed with 6  $\mu$ g CEF. Averages and SE based on three biological replicates and two independent experiments are shown. 1 mM IPTG was added at where indicated.



**Fig.S2.** Complementation test of *spx*. Disk diffusion tests were performed with 6  $\mu$ g CEF. Averages and SE based on three biological replicates and two independent experiments are shown. 1 mM IPTG was used to induce  $P_{spank(hy)}-spx$ , and 0.01 mM IPTG were used to induce  $P_{spank(hy)}-spx^{DD}$ . Note that Spx is a substrate of ClpXP protease, and thereby it can not be accumulated to a high level even in the presence of 1 mM IPTG.  $Spx^{DD}$  can not be degraded by ClpXP, but inducing  $P_{spank(hy)}-spx^{DD}$  with more than 0.01 mM IPTG is lethal.

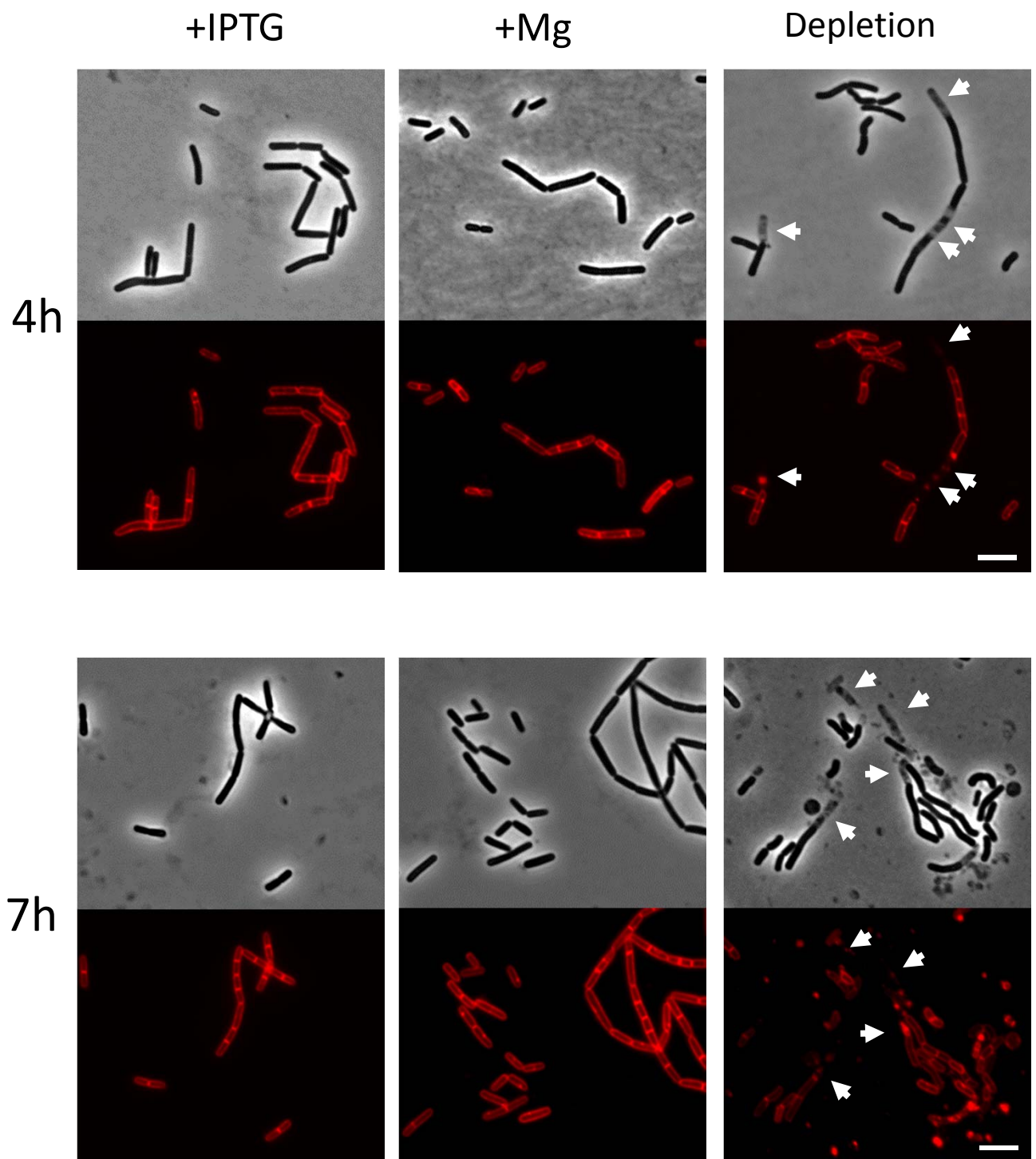


**Fig.S3.** Disk diffusion tests with *clpP*, *clpC* and *clpX* mutant in the backgrounds of strain WT and *sigM* mutant and 6  $\mu$ g CEF. Averages and SE based on three biological replicates and two independent experiments are shown.



**Fig.S4.** *ybbP* is additive to *sigM* and *sigX* in CEF susceptibility. Disk diffusion tests were performed with 6  $\mu$ g CEF. Averages and SE based on three biological replicates and two independent experiments are shown.





**Fig.S5.** Cells observed using phase contrast and fluorescence microscopy. Cells were grown in MH medium supplemented with 1 mM IPTG to mid-log phase, washed, resuspended in fresh MH medium alone, or supplemented with 1 mM IPTG, or with 10 mM  $\text{MgSO}_4$ , and returned to 37°C incubation with vigorous shaking in a Bioscreen incubator. Cells were examined at three time points 2 h (cell morphology was indistinguishable from 4 h; data not shown), 4 h and 7 h. Cell membrane was stained with FM4-64, and colored in red. Arrows indicate lysed cells. Size bar is 10  $\mu\text{m}$ . The corresponding growth curves are shown in Fig. 7.

## Reference

- Chai, Y., R. Kolter & R. Losick, (2010) Reversal of an epigenetic switch governing cell chaining in *Bacillus subtilis* by protein instability. *Mol Microbiol* **78**: 218-229.
- Nakano, S., E. Kuster-Schock, A. D. Grossman & P. Zuber, (2003) Spx-dependent global transcriptional control is induced by thiol-specific oxidative stress in *Bacillus subtilis*. *Proc Natl Acad Sci U S A* **100**: 13603-13608.
- Strauch, M. A., B. G. Bobay, J. Cavanagh, F. Yao, A. Wilson & Y. Le Breton, (2007) Abh and AbrB control of *Bacillus subtilis* antimicrobial gene expression. *J Bacteriol* **189**: 7720-7732.