Supplemental Information – Manetsberger J, Ghosh A, Hall EA and Christie G.

Table S1 Orthologues of proteins encoded in the *B. megaterium* QM B1551 genome that are either present or involved in the formation of the *B. subtilis* crust or *B. cereus* family exosporium

B. subtilis[a]	*B. cereus* family[b]	*B. megaterium*[c]
CgeA	-	-
CotG	-	-
CotV	+[d]	-
CotW	+[d]	BMQ_pBM60030
CotX	+[d]	BMQ_pBM60028 BMQ_pBM60029
CotY	CotY	-
CotZ	ExsY	-
-	*Alr* (alanine racemase)	BMQ_0226
-	BclA/BclB	BMQ_pBM50077 BMQ_pBM50081
-	BetA	-
-	ExsFA (BxpB)/ExsFB	BMQ_pBM60048
-	ExsA[e]	BMQ_4649 (SafA)
-	ExsB	-
-	ExsC	-
-	ExsFB	-
-	ExsH	-
-	ExsK	-
-	ExsM	-

[a] Crust proteins defined by (1, 2).
[b] Exosporium proteins defined by (3).
[c] Orthologues identified by BLAST searches against the *B. megaterium QM B1551* genome using *B. subtilis* and/or *B. cereus* family proteins as query sequences.
[d] Absent in *B. anthracis* (4).
[e] ExsA is orthologous to *B. subtilis* SafA, which is not a component of the spore crust.

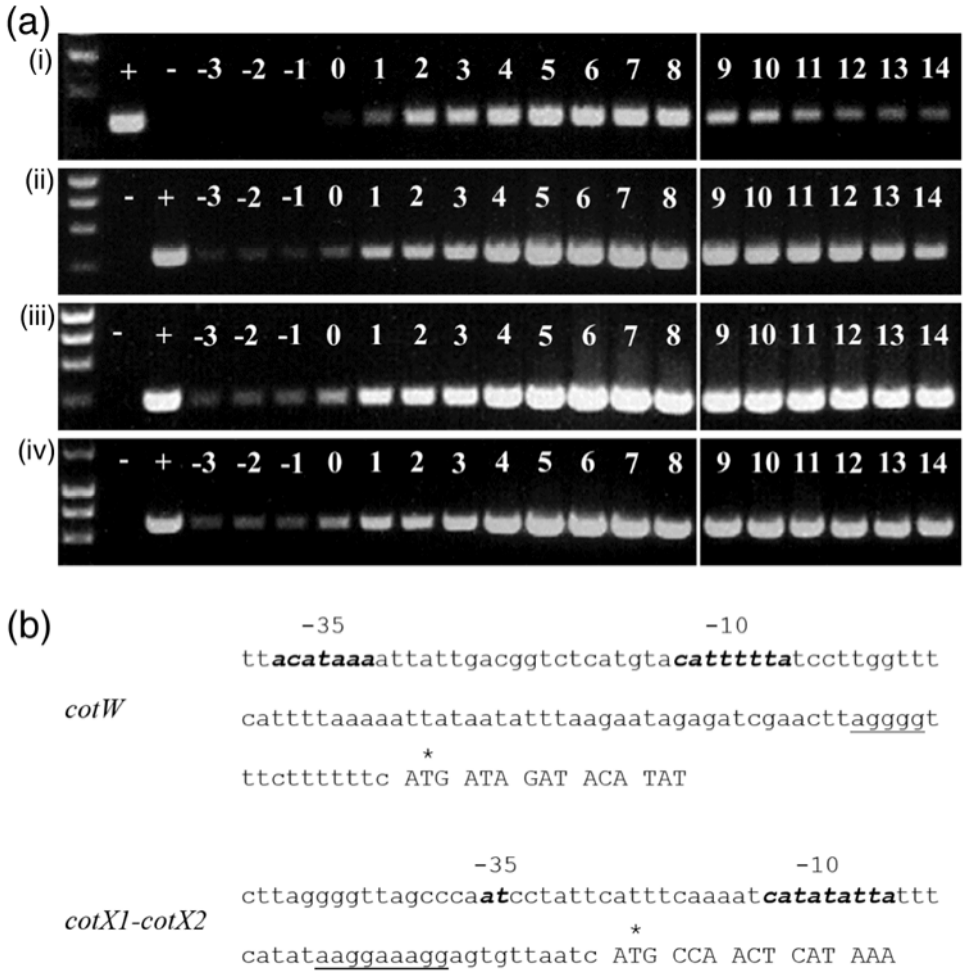

Figure S1 (a) Transcription of (i) *cotW*, (ii) *cotX1* and (iii) *cotX2* during sporulation of *B. megaterium* QM B1551. RT-PCR derived products obtained using primers that span the putative *cotX1-cotX2* operon are shown in (iv). RT-PCR was conducted on samples collected at hourly intervals preceding and during sporulation. Key: – and +, RT-PCR controls; numbers denote time (h) preceding and post entry to stationary phase (0).

(b) Putative upstream promoter sequences for *cotW* and *cotX1-cotX*2. Predicted -35 and -10 regions are italicised and in bold, and potential Shine-Dalgarno sequences underlined. Predicted start codons are marked with an asterisk.

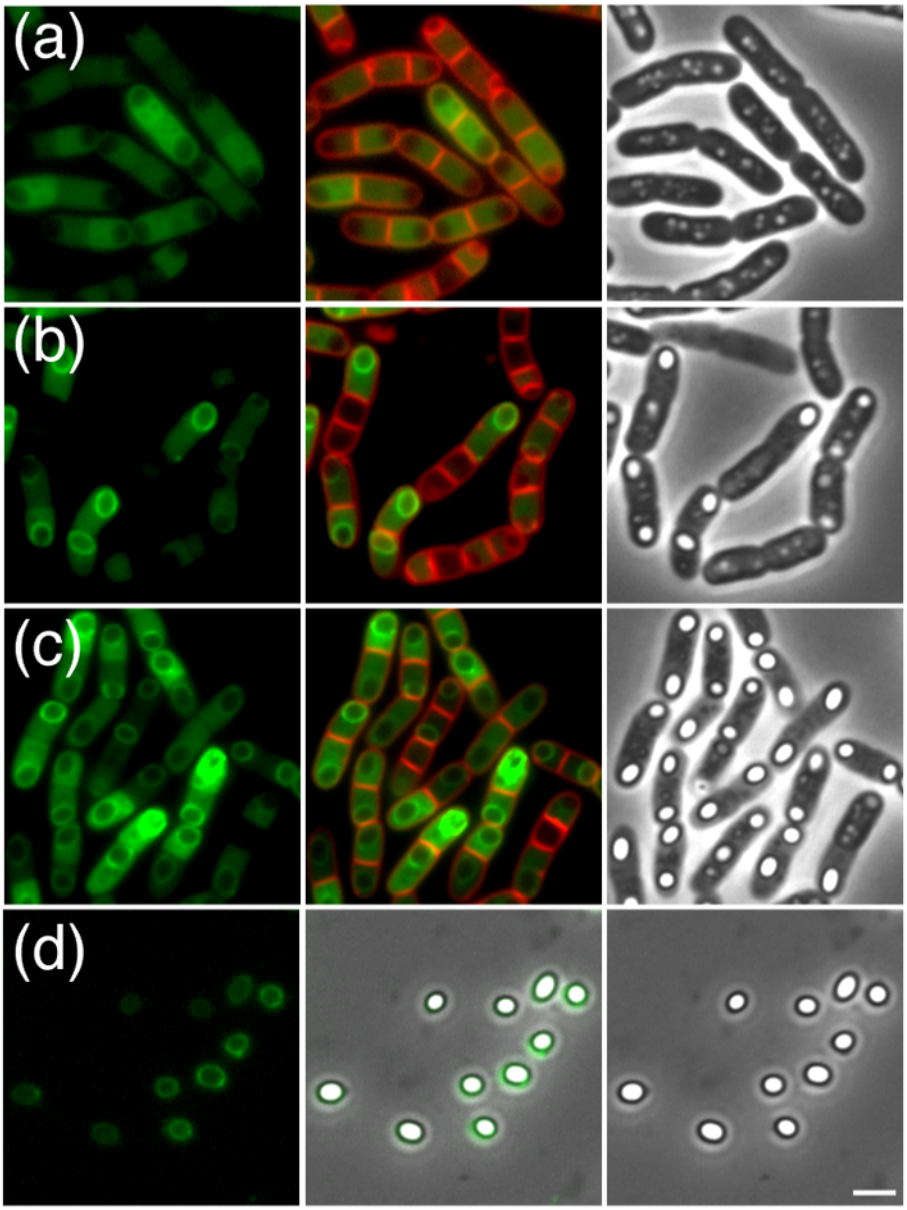

Figure S2 Fluorescence and phase contrast micrographs of sporulating *B. megaterium* QM B1551 *cotW-gfp* cells. The columns show (from left to right) GFP-associated fluorescence, FM4-64 stains overlaid with GFP, and phase contrast images respectively. Rows (a), (b), (c) and (d) denote images taken 3, 5, 8 and 24 hours after entry to sporulation. The scale bar represents 2.5 μm.

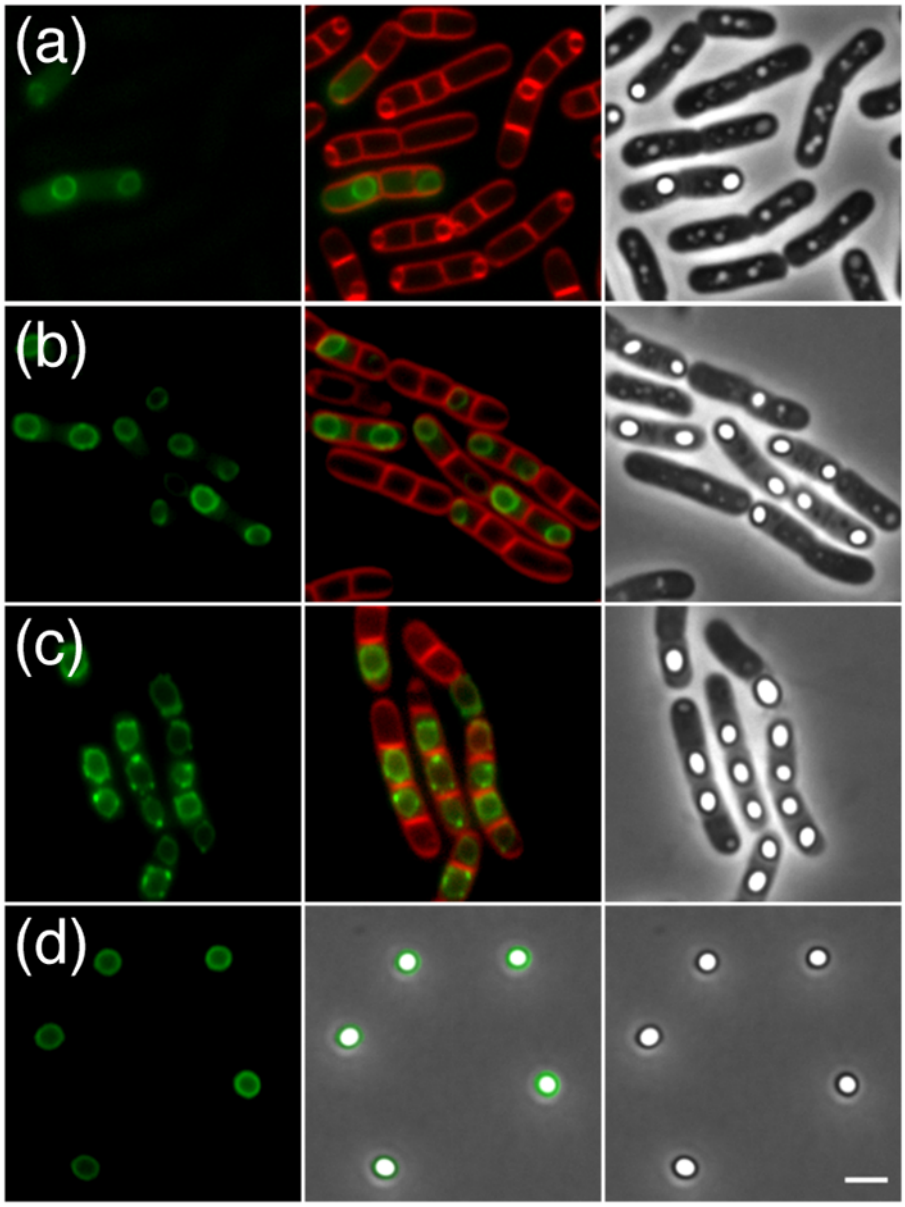

Figure S3 Fluorescence and phase contrast micrographs of sporulating *B. megaterium* QM B1551 *gfp-cotX1* cells. The columns show (from left to right) GFP-associated fluorescence, FM4-64 stains overlaid with GFP, and phase contrast images respectively. Rows (a), (b), (c) and (d) denote images taken 3, 5, 8 and 24 hours after entry to sporulation. The scale bar represents 2.5 µm.

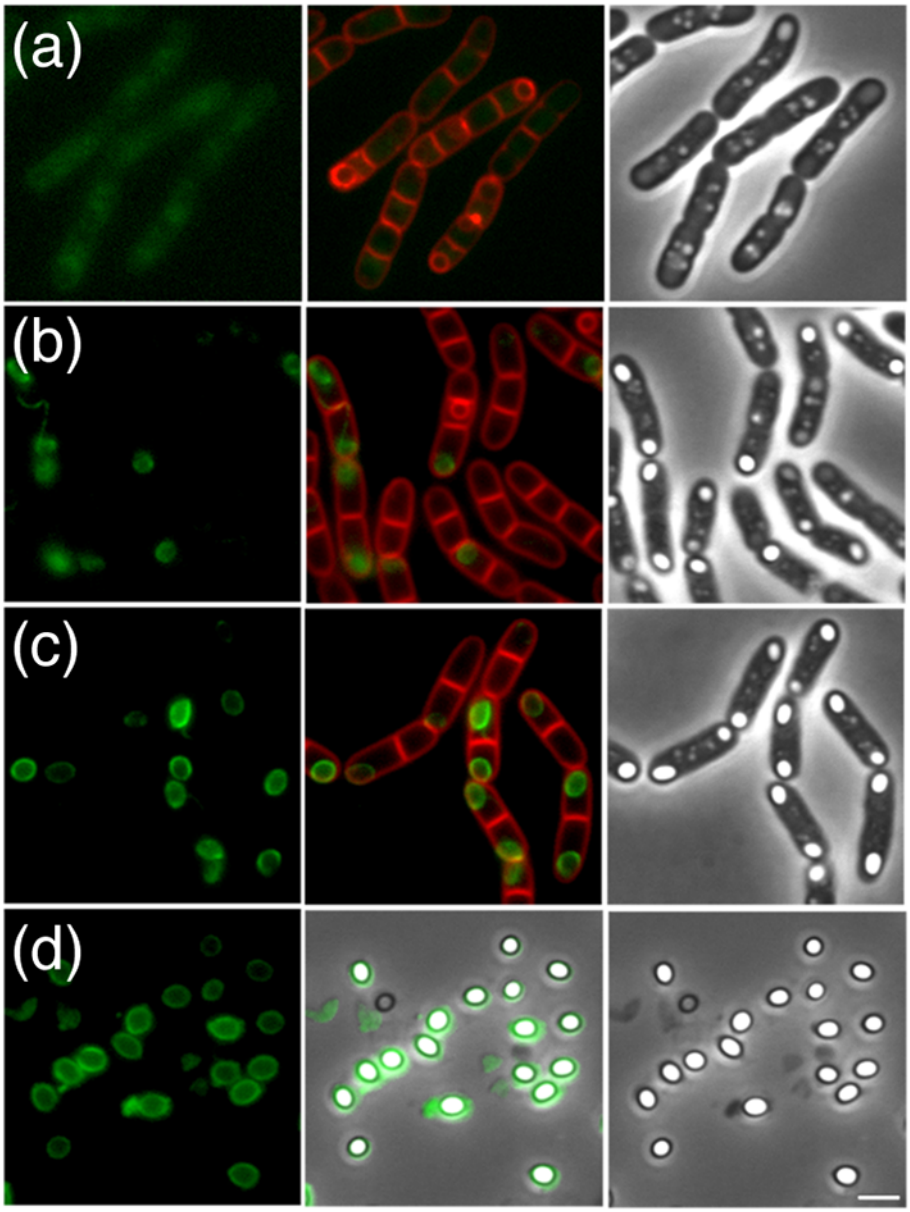

Figure S4 Fluorescence and phase contrast micrographs of sporulating *B. megaterium* QM B1551 *cotX2-gfp* cells. The columns show (from left to right) GFP-associated fluorescence, FM4-64 stains overlaid with GFP, and phase contrast images respectively. Rows (a), (b), (c) and (d) denote images taken 3, 5, 8 and 24 hours after entry to sporulation. The scale bar represents 2.5 μm.

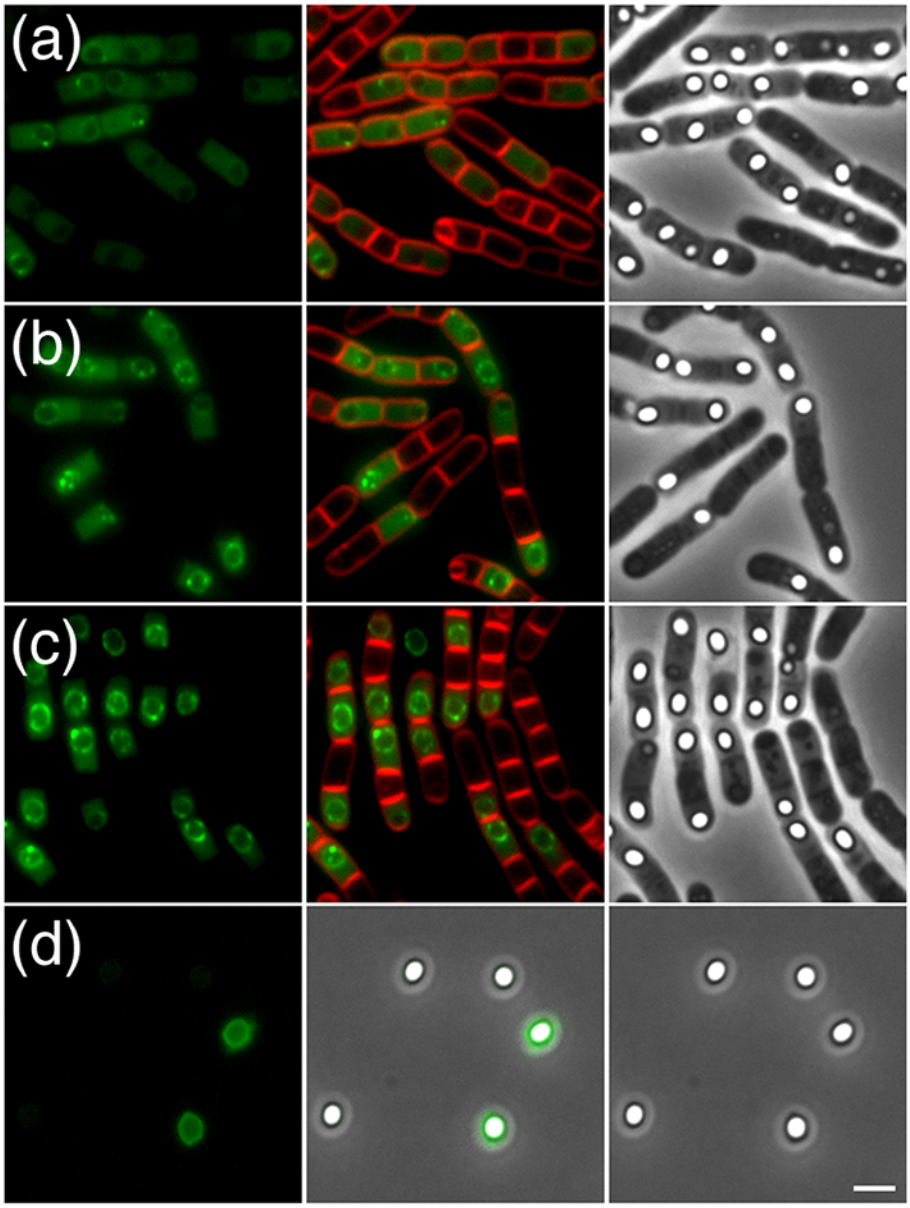

Figure S5 Fluorescence and phase contrast micrographs of sporulating *B. megaterium* PV361 *gfp-cotX1*cells. The columns show (from left to right) GFP-associated fluorescence, FM4-64 stains overlaid with GFP, and phase contrast images respectively. Rows (a), (b), (c) and (d) denote images taken 4, 6, 8 and 24 hours after entry to sporulation. The scale bar represents 2.5 μm.

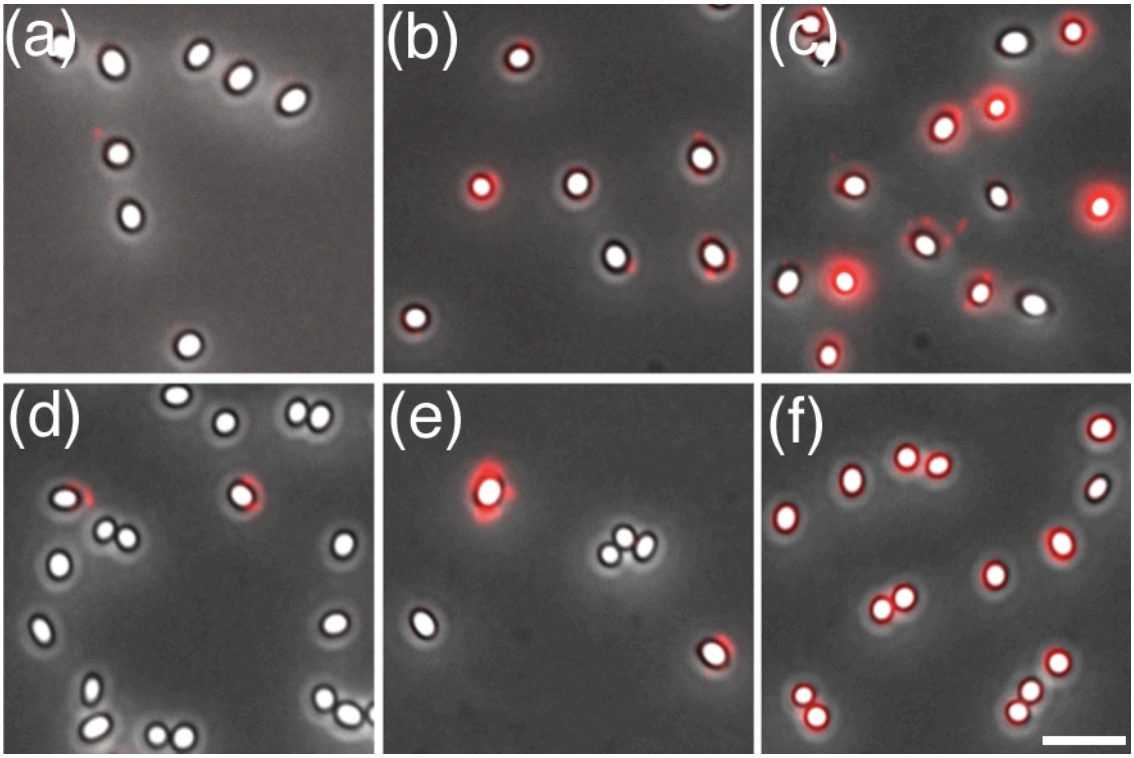

Figure S6 Immuno-detection of CotW-GFP and CotX-GFP fusion proteins in spores of *B. megaterium* QM B1551 carrying plasmid-borne copies of (a) *cotW-gfp*, (b) *gfp-cotX1* and (c) *cotW-cotX1-cotX2-gfp*. Phase contrast images overlaid with fluorescence micrographs are also shown for spores of the PV361 strain complemented with plasmid-borne (d) *cotW-gfp*, (e) *gfp-cotX1*, and (f) *cotW-cotX1-cotX2-gfp*. The scale bar denotes 5 μm.

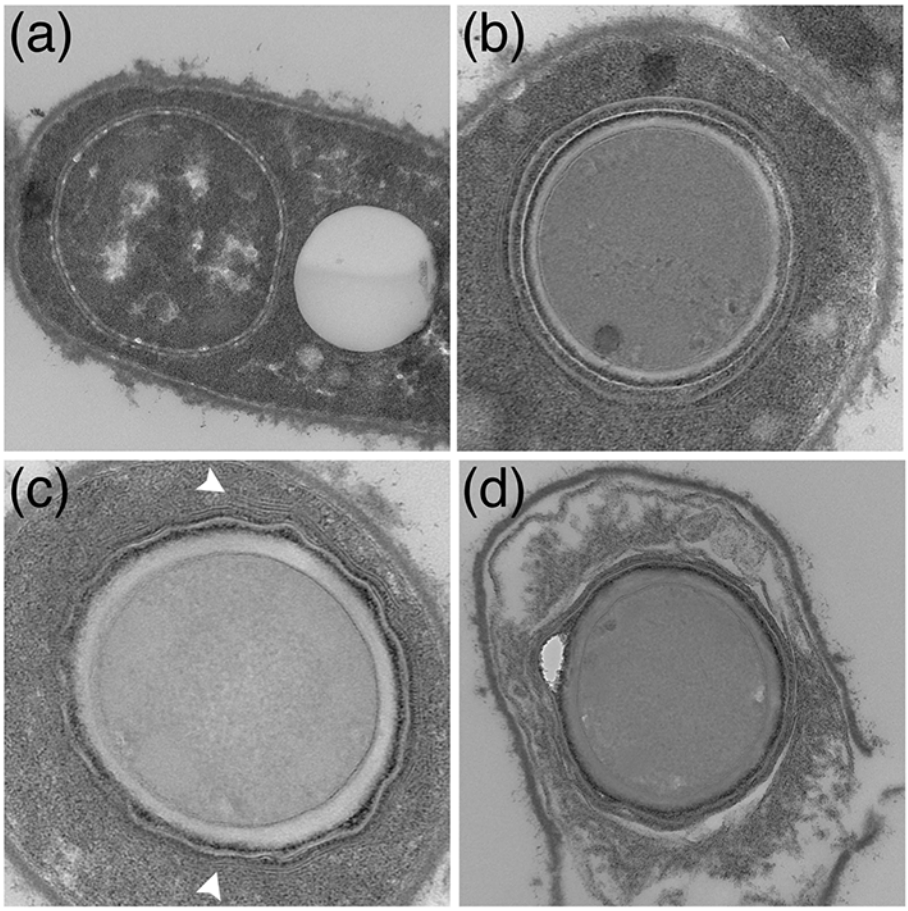

Figure S7 Thin section transmission electron micrographs of sporulating *B. megaterium* PV361 pHT315:*cotW cotX1 cotX2* cells. Cells were imaged at (a) 4 (b) 6 (c) 9, and (d) 24 hours after entry to sporulation. The white arrows in (c) are pointing to concentric rings that form outer coat or exosporium material. Granular exosporium-like material is evident in (d), which shows a mother cell undergoing lysis.

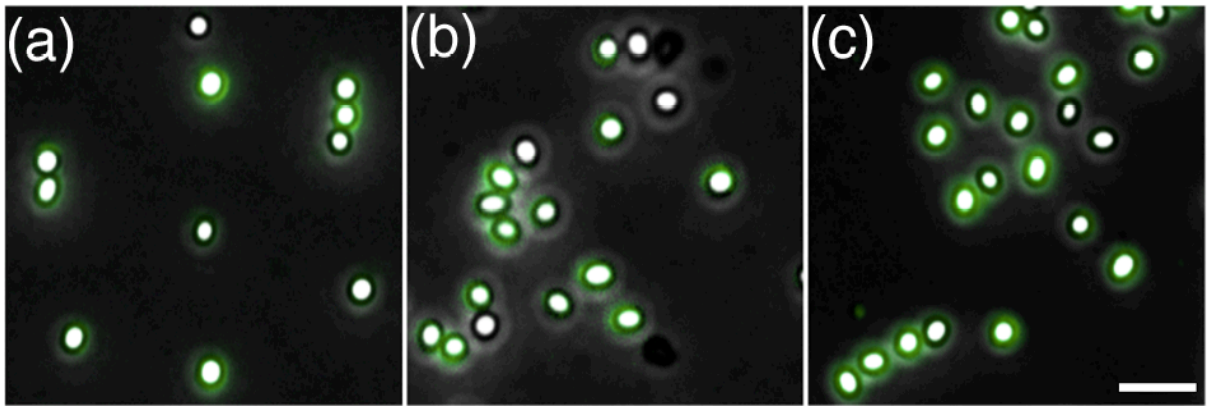

Figure S8 Assessing the stability of the rudimentary exosporium layer in response to shear stress and sonication. The images show fluorescence/phase contrast over-laid images of PV361 *cotW-cotX1-cotX2-gfp* spores following (a) no treatment, (b) two passages through a cell-disrupter instrument operating at 20 kPsi, and (c) seven x 1 min rounds of sonication. Spores were resuspended in 4 ml ice-cold deionised water at an OD_{600} of ~70 prior to cell disruption. Similarly, spores were resuspended in ice-cold deionised water at an OD_{600} of ~10 for sonication and cooled in ice-slurry between sonication cycles. The size bar denotes 5 μm.

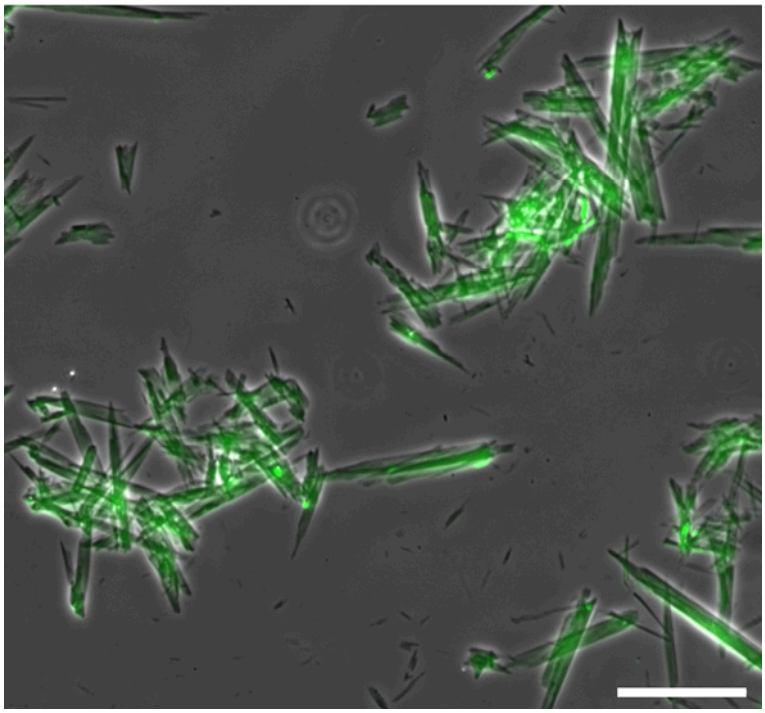

Figure S9 Fluorescence-phase microscopy overlay of thioflavin-T stained recombinant *B. megaterium* CotW protein. The size bar denotes 100 μm.

References

1. Imamura D, Kuwana R, Takamatsu H, Watabe K. 2011. Proteins involved in formation of the outermost layer of *Bacillus subtilis* spores. J Bacteriol 193:4075-4080.

2. McKenney PT, Driks A, Eskandarian HA, Grabowski P, Guberman J, Wang KH, Gitai Z, Eichenberger P. 2010. A distance-weighted interaction map reveals a previously uncharacterized layer of the *Bacillus subtilis* spore coat. Curr Biol 20:934-938.

3. Stewart GC. 2015. The exosporium layer of bacterial spores: a connection to the environment and the infected host. Microbiol Mol Biol Rev 79:437-457.

4. Henriques AO, Moran CP. 2007. Structure, assembly, and function of the spore surface layers. Annu Rev Microbiol 61:555-588.