Supplemental information associated with 'Proteins encoded by the *gerP* operon are localised to the inner coat in *Bacillus cereus* spores and are dependent on GerPA and SafA for assembly' by Ghosh *et al*. 2017

Table S1 Amino acid sequence identity of *B. cereus* 14579 GerP proteins with orthologues present in other *Bacillus* species

Protein	**Locus tag**	**Identity (%) with GerP from:**		
		B. subtilis	*B. megaterium*	*B. anthracis*
GerPA	BC1145	53	52	98
GerPB	BC1144	45	51	98
GerPC	BC1143	35	28	95
GerPD	BC1142	55	62	96
GerPE	BC1141	30	32	90
GerPF	BC1140	42	42	100

Table S2 Amino acid sequence identity between predicted *B. cereus* 14579 GerPF orthologues

Protein	**Locus tag**	**Sequence identity (%)**		
		GerPF1	**GerPF2**	**GerPF3**
GerPF	BC1140	100	94	49
GerPF2	BC2276	94	100	52
GerPF3	BC4794	49	52	100

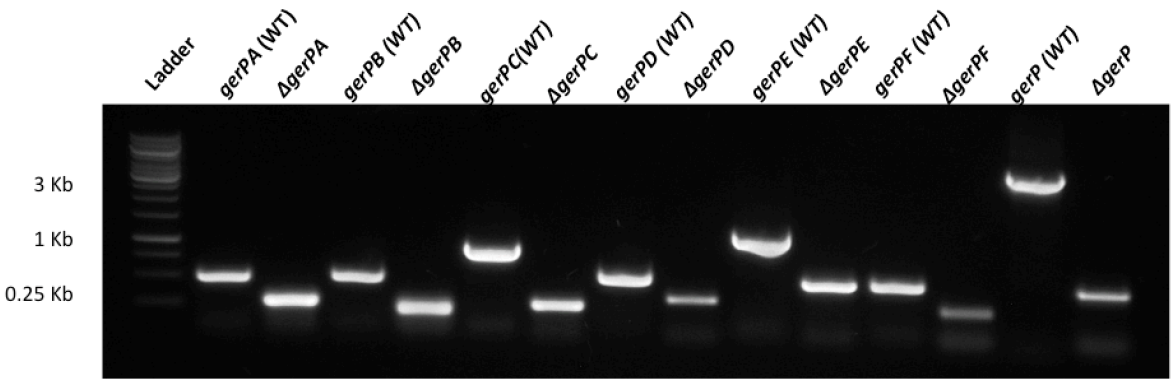


Figure S1 Agarose gel showing the products of diagnostic PCR reactions used to characterise the various *B. cereus* GerP null mutant strains. Primers employed in these reactions flanked the various *gerP* genes, hence in all cases PCR products associated with mutant strains are smaller than the equivalent reaction conducted with wild type DNA.

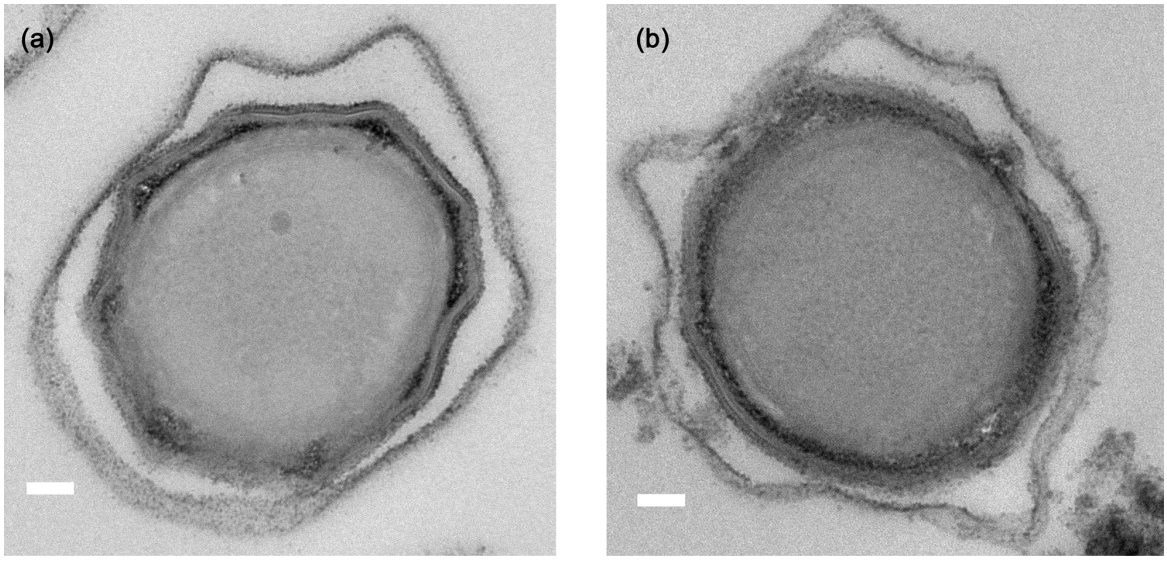
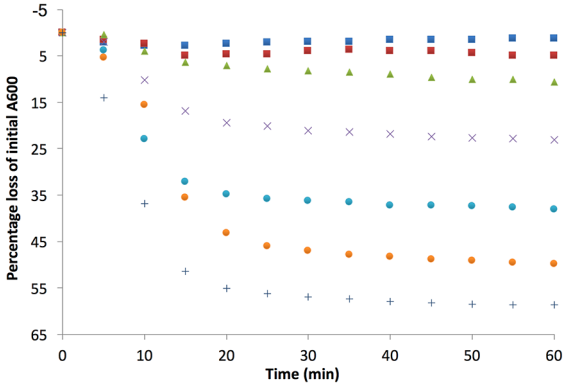


Figure S2 Thin section TEM micrographs of spores of (a) wild type *B. cereus* 14579, and (b) *B. cereus* 14579 Δ*gerP*. The marker represents 100 nm.

(a) wild type spore germination in alanine



(b) *gerP* spore germination in alanine

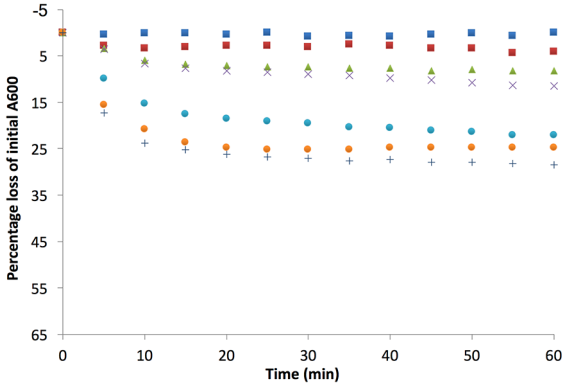
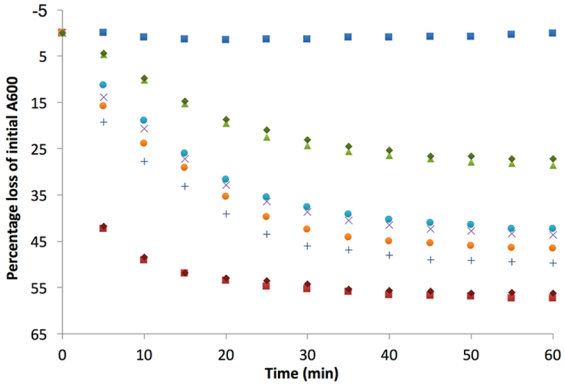
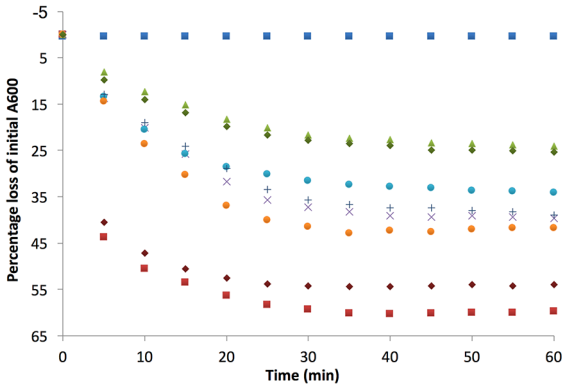


Figure S3 Germination of *B. cereus* 14579 wild type and *gerP* null spores in L-alanine. Spores were incubated in Tris-HCl buffer supplemented with the designated concentration of alanine and absorbance at 600 nm (A600) recorded as described in the Materials and Methods. Key – buffer (dark blue squares); 10 mM alanine (red squares); 20 mM alanine (olive triangles); 30 mM alanine (purple x); 50 mM alanine (light blue circles); 75 mM alanine (orange circles); 100 mM alanine (blue +).

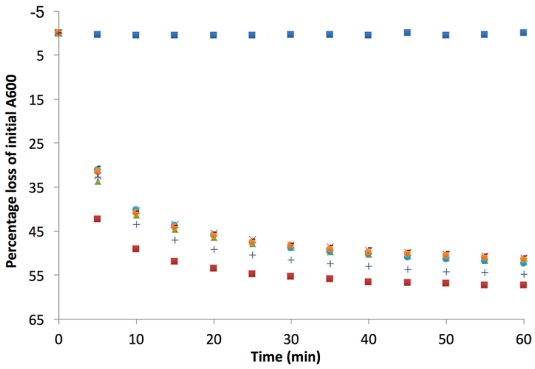
(a) *gerP* null mutants in 100 mM alanine



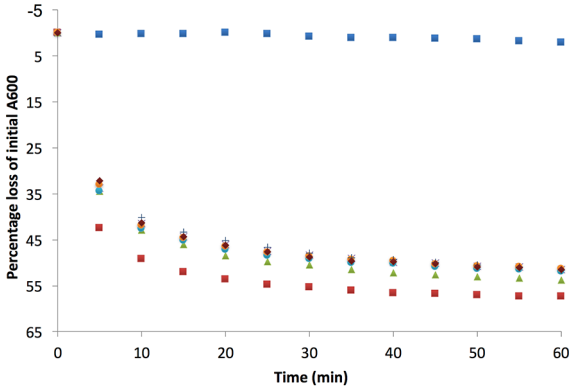
(b) *gerP* null mutants in 10 mM inosine



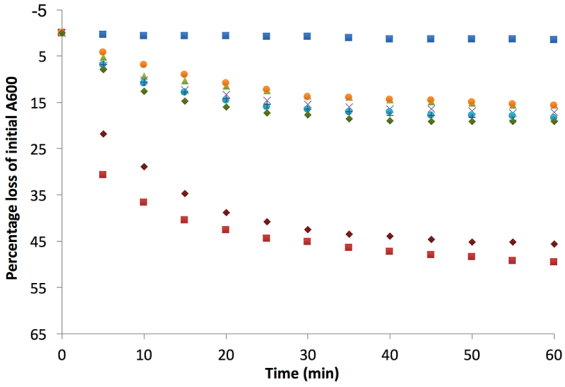
(c) *gerP* complemented strains in 100 mM alanine



(d) *gerP-gfp* complemented strains in 100 mM alanine



(e) *gerP* null mutants in 50 mM CaDPA



(f) *gerP* null mutants in 1 mM dodecylamine

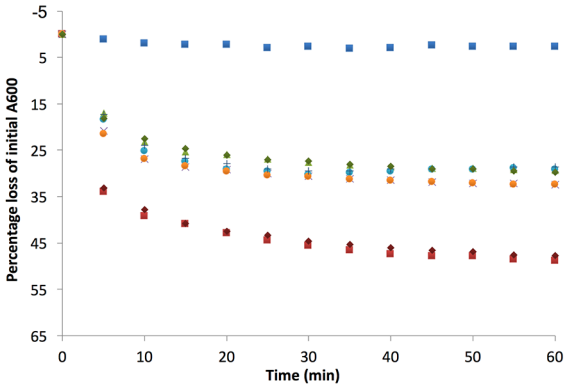


Figure S4 Germination of *B. cereus* 14579 *gerP* spores in nutrient and non-nutrient germinants. Spores were incubated in Tris-HCl buffer supplemented with the designated germinant compound and absorbance at 600 nm (A600) recorded as described in the Materials and Methods. Key – wild type in buffer (dark blue squares); wild type in germinant (red squares); *gerP* (green diamonds); *gerPA* (olive triangles); *gerPB* (purple x); *gerPC* (light blue circles); *gerPD* (orange circles); *gerPE* (blue +), *gerPF* (dark red diamonds). In (c) symbols refer to individual *gerP* null strains complemented with the corresponding gene; in (d), symbols refer to the *gerP* null strain complemented with *gerP* containing single *gerP-gfp* fusions.

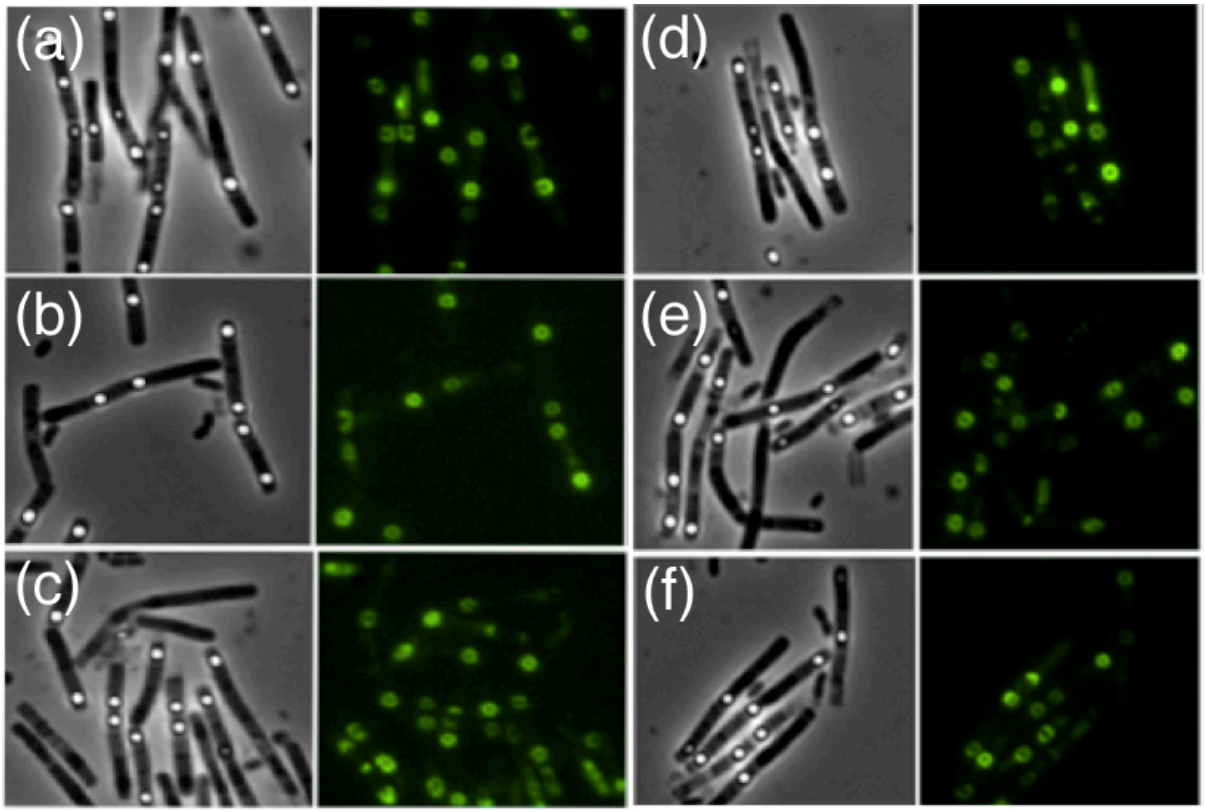


Figure S5 Phase contrast and fluorescence microscopy analysis of sporulating *B. cereus gerPB* cells complemented with plasmid borne copies of (a) *gerPA-gfp*, (b) *gerPB-gfp*, (c) *gerPC-gfp*, (d) *gerPD-gfp*, (e) *gerPE-gfp*, and (f) *gerPF-gfp*. The various genes were placed under control of the native *gerP* operon regulatory sequences. Fluorescence associated with the various GFP fusions indicates that the GerP proteins can localise in the absence of GerPB. Similar observations were made in other strains that were null for individual *gerP* genes, except in the *gerPA* background (see manuscript Figure 5).

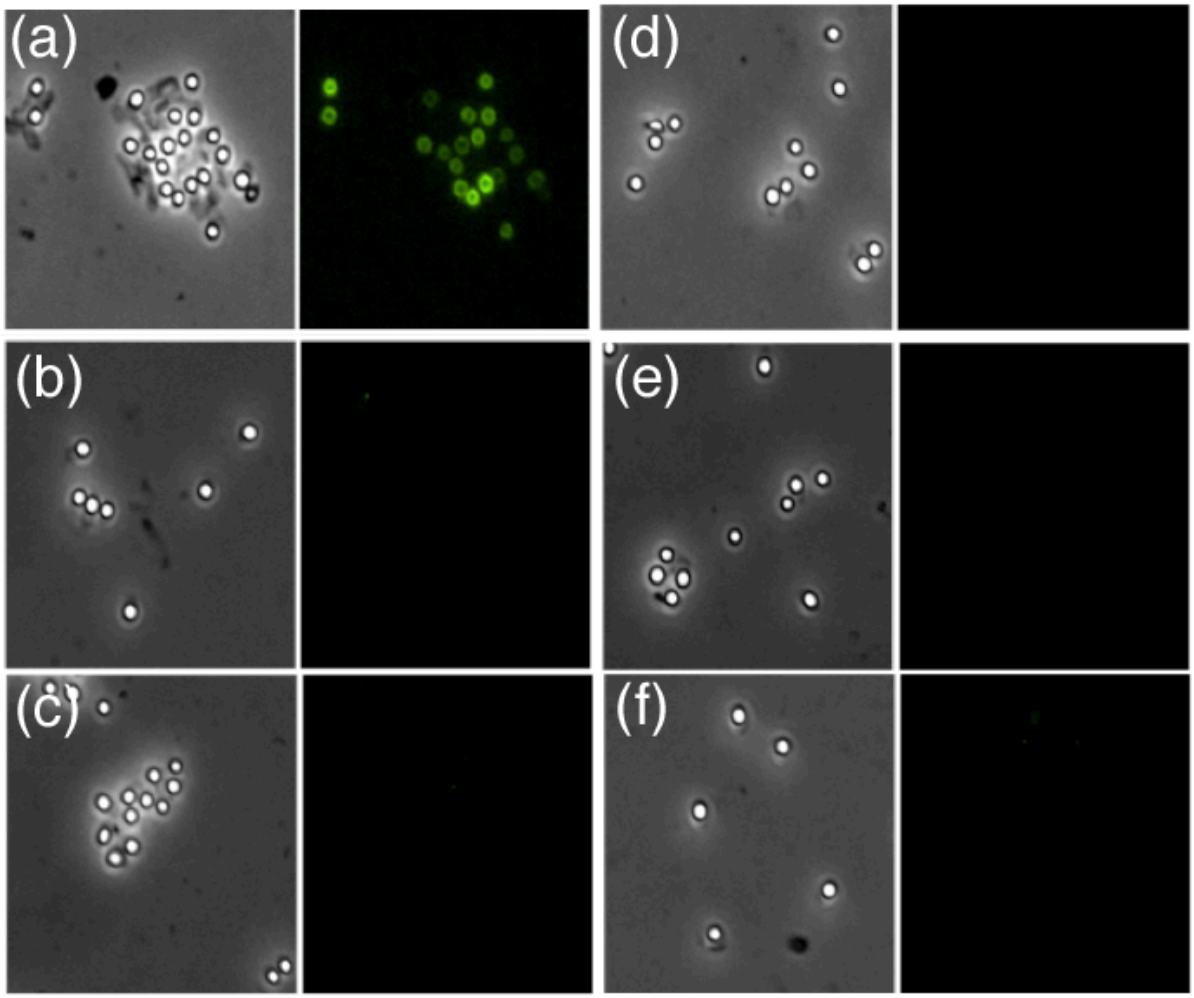


Figure S6 Phase contrast and fluorescence microscopy analysis of *B. cereus gerP* null spores complemented with plasmid borne copies of (a) *gerPA-gfp*, (b) *gerPB-gfp*, (c) *gerPC-gfp*, (d) *gerPD-gfp*, (e) *gerPE-gfp*, and (f) *gerPF-gfp*. The various genes were placed under control of the native *gerP* operon regulatory sequences. Fluorescence associated with GerPA-GFP indicates that this protein can localise in the absence of the remaining GerP proteins.