SUPPLEMENTAL INFORMATION

Degradation of a morphogenetic protein as a quality control mechanism to remove unfit cells from a population of differentiating bacteria

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Supplemental Inventory

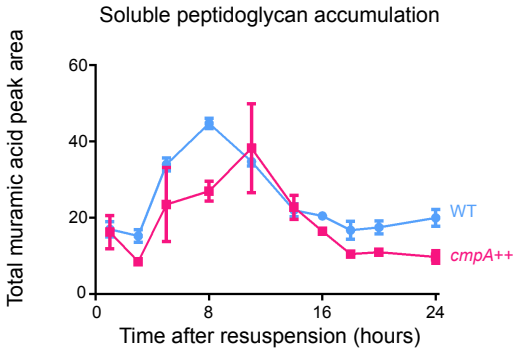
Soluble peptidoglycan accumulation
Total muramic acid peak area
60
40
20
0
0
8
16
24
Time after resuspension (hours)
WT
cmpA++

Figure S1

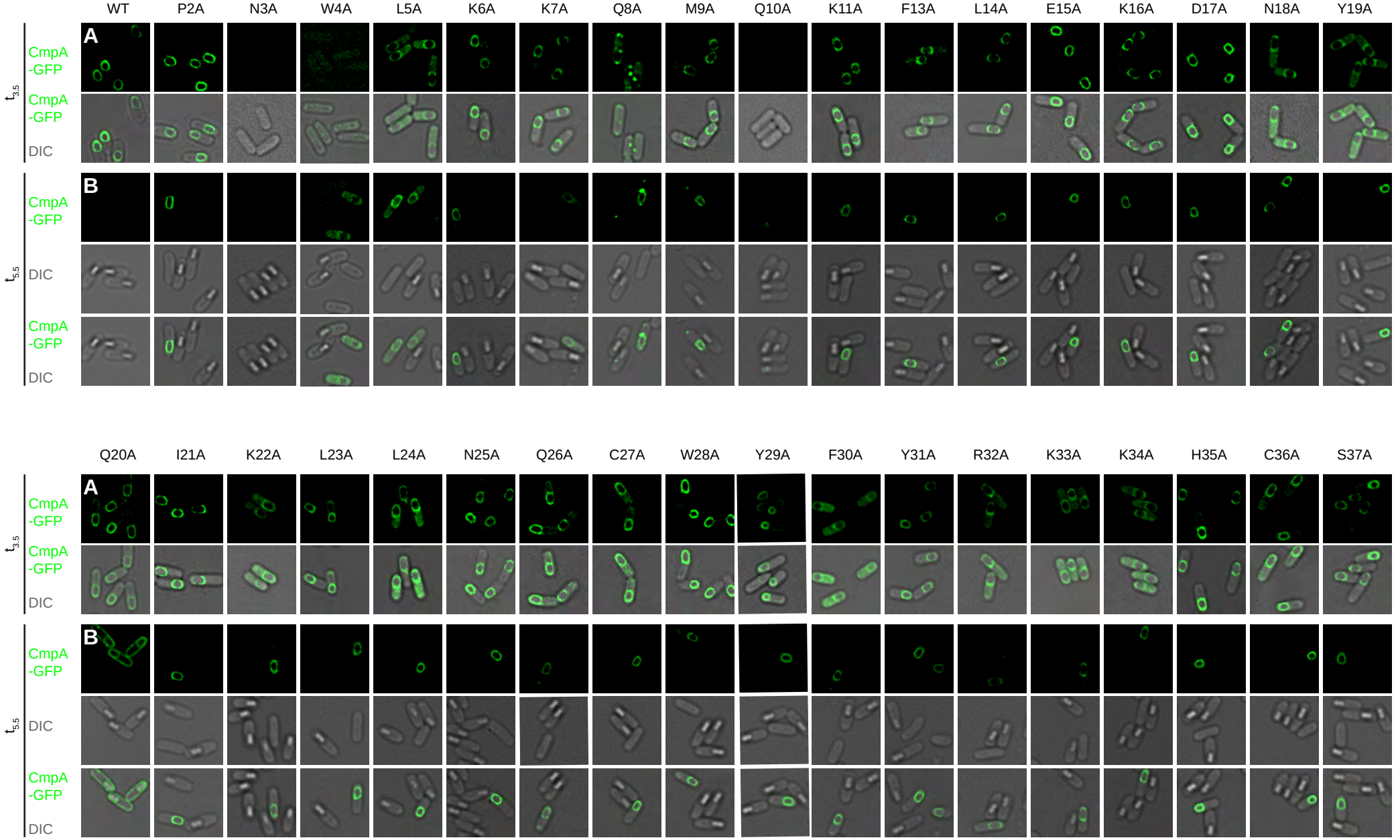
WT
P2A
N3A
W4A
L5A
K6A
K7A
Q8A
M9A
Q10A
K11A
F13A
L14A
E15A
K16A
D17A
N18A
Y19A
A
B
CmpA -GFP
DIC
$t_{3.5}$
$t_{5.5}$
Q20A
I21A
K22A
L23A
L24A
N25A
Q26A
C27A
W28A
Y29A
F30A
Y31A
R32A
K33A
K34A
H35A
C36A
S37A

Figure S2

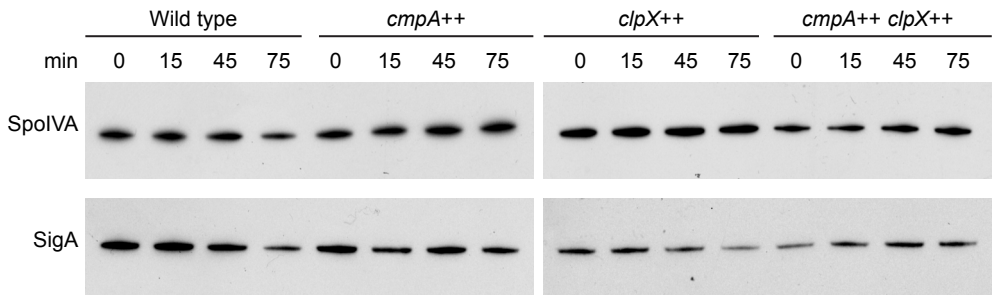
Wild type
cmpA++
clpX++
cmpA++ clpX++
min
0 15 45 75 0 15 45 75 0 15 45 75 0 15 45 75
SpoIVA
SigA

Figure S3

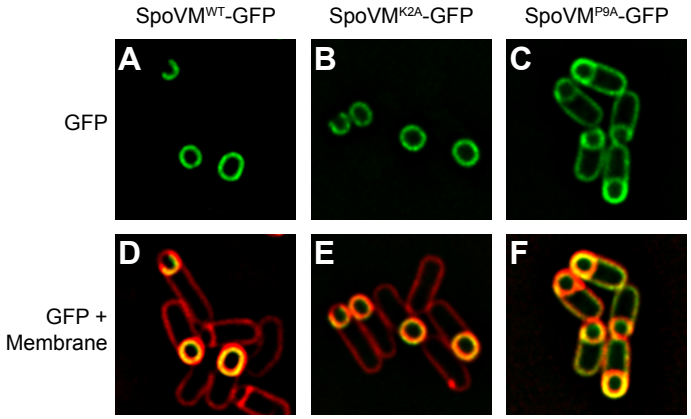
SpoVM^WT-GFP
SpoVM^K2A-GFP
SpoVM^P9A-GFP
A
B
C
GFP
D
E
F
GFP +
Membrane

Figure S4

SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1. Overexpression of *cmpA* has no effect on soluble peptidoglycan precursors. Accumulation of soluble peptidoglycan precursors during sporulation in wild type (●; PY79) and *cmpA* overexpressing (■; IT478) cells. Symbols represent mean values obtained from three independent measurements; error bars represent standard error of the mean.

Figure S2, related to Figure 3. Localization and persistence of CmpA-GFP mutants. (A) Localization of CmpA-GFP or indicated CmpA-GFP variants 3.5 h after induction of sporulation. Top row: GFP fluorescence; bottom row: overlay, GFP and DIC. (B) Localization of CmpA-GFP or indicated CmpA-GFP variants 5.5 h after induction of sporulation. Top row: GFP fluorescence; middle row: DIC; bottom row: overlay, GFP and DIC.

Figure S3, related to Figure 5. Overexpression of *cmpA*, *clpX* or both together are not sufficient to induce degradation of SpoIVA at an early time point (3.5 h after induction of sporulation). Immunoblot of cell lysate harvested from wild type (PY79), *cmpA* overexpressing (cmpA++; SE191), *clpX* overexpressing (clpX++; IT479) or *cmpA* and *clpX* overexpressing cells (cmpA++ clpX++; IT481). Overexpression was induced at t_3 of sporulation by addition of 1 mM IPTG. Spectinomycin (200ug ml^{-1}) was added at $t_{3.5}$ to arrest translation and cells were harvested at 0, 15, 45 and 75 minutes after addition of spectinomycin.

Figure S4, related to Figure 6. $SpoVM^{K2A}$-GFP localizes to the forespore. (A-C) Localization of SpoVM-GFP (A; CVO1195), $SpoVM^{K2A}$-GFP (B; KRC1) and $SpoVM^{P9A}$-GFP (C; CVO1395). (D-F) Overlay of GFP and membrane stain in (A-C), respectively.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Strain construction

Construction of integration plasmids pSE18 and pSE24, for integration of IPTG-inducible *cmpA* ($P_{hyperspank}$-*cmpA*) and *cmpA-gfp* ($P_{hyperspank}$-*cmpA-gfp*), respectively, at *amyE* have been previously described (Ebmeier et al., 2012). To integrate $P_{hyperspank}$-*cmpA* at the *thrC* locus, *cmpA* was subcloned from pSE18 into the integration vector pDP150 (Kearns et al., 2005) to create pIT43. To construct constitutively expressing *cmpA* (P_{sigA}-*cmpA*), *lacI* expression in pSE18 and pIT43 was abolished by deleting the immediate upstream region and start codon of *lacI* in addition to introducing a stop codon using Quikchange site-directed mutagenesis (Agilent Technologies) to create pIT59 and pIT66. $P_{hyperspank}$-*cmpA-gfp* was subcloned from pSE24 using EcoRI and BamHI into the integration vector pSac-cm (Middleton and Hofmeister, 2004) for integration at *sacA* to create pIT80. *clpX* at *thrC* was created by PCR-amplifying the *clpX* ORF, 260 nucleotides upstream and 20 nucleotides downstream with primers ‘5'BsaI clpX’ (aaaaggtctcgaattcgcgcgaatcattcgtgcctt) and ‘3'BamHI clpX’ (cgacggatcctcaggaggtttgtgcttatctt). The PCR fragment was then cloned into the EcoRI and BamHI sites in the integration vector pDG1664 (Guerout-Fleury et al., 1996) to create pIT89. $P_{hyperspank}$-*clpX* at *thrC* and $P_{hyperspank}$-*clpX-His*$_6$ at *amyE* were constructed by PCR-amplifying the *clpX* ORF and introducing a ribosomal binding site (RBS) as well as the restriction sites NheI and SphI using primers ‘5' NheI RBS clpX’ (aaaagctagctaaggaggaatgagcctatgtttaaatttaacgagga) and ‘3' SphI clpX’ (aaaagcatgcctcctgagtgttaccac) or ‘3' SphI 6xHisclpX’ (aaaagcatgcttagtggtgatggtgatgatgtgcagatgttttatcttg) to add a His_6 tag. The PCR fragments were then digested with NheI and SphI and *clpX* was cloned into pDP 150 to create pIT60 while *clpX-His*$_6$ *was* cloned into pDR111 to create pIT76. $P_{hyperspank}$-*clpX*$^{\Delta 1-53}$ at *amyE* was constructed by PCR-amplifying the *clpX* ORF starting at the nucleotides that encode for amino acid 54 and introducing a RBS, start codon and restriction sites NheI and SphI using the primers ‘5' NheI

RBS N-term t-clpX' (aaaagctagctaaggaggaatgagcctatggaagaagtagaattt) and '3' SphI clpX'. The PCR fragment was digested with NheI and SphI and cloned into pDR111.

Spore purification by density gradient centrifugation

Cells were allowed to sporulate in DSM for greater than 96 hours. Cells were pelleted, washed with water and resuspended in 20% metrizoic acid (Sigma S4506). Resuspended cells were then added to the top of a step gradient of five different metrizoic acid concentrations (70%, 60%, 50%, 40% and 30%) and centrifuged at 40,000 x g for 60 min at 4°C (Fukushima et al., 2004). Spores were found in the middle layers and were collected, washed with water and resuspended in water of the original volume. The total number of viable spores per ml was then determined by serial dilution, plating on LB agar, and counting colony forming units (cfu). The total number of heat resistant spores per ml was determined by submerging the spores in an 80°C water bath for 20 minutes followed by serial dilution and quantification of cfu/ml as described above.

DPA and peptidoglycan harvesting

5 ml of sporulating cell culture was harvested at each time point and the cell pellet was resuspended in 500 µl distilled water. Cells were boiled for 20 min to release DPA into the water. The amount of DPA was determined by a colorimetric assay, in which changes in absorption at OD_{440} caused by complex formation between ferrous iron and DPA was measured (Janssen et al., 1958; Nicholson and Setlow, 1990). Peptidoglycan was harvested and analyzed as previously described (Meador-Parton and Popham, 2000; Vasudevan et al., 2007). Briefly, 1ml of sporulating cell culture was pelleted, washed, acid hydrolyzed in 6N HCl and subjected to amino acid/sugar analysis.

Isolation of spontaneous suppressors

Mutant strains were grown in 30 ml of DSM for at least 24 h at 37°C in order to sporulate and accumulate spontaneous mutations. The culture was then incubated at 80°C to kill cells which did not sporulate successfully. The 30 ml culture was re-inoculated into 300 ml of fresh DSM, allowed to germinate, grow and sporulate for at least 24 h at 37°C. The procedure was repeated with re-inoculation of 30 ml of heat-killed culture until an increase in sporulation efficiency was observed (usually three rounds). Candidate spontaneous suppressors were collected and characterized. Suppressor mutations were mapped by linkage analysis and whole genome sequencing.

SUPPLEMENTAL REFERENCES

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Table S1, related to Figure 1. Effect of cortex hydrolase deletion on sporulation efficiency of *cmpA* overexpressing cells (as measured by heat resistance). Standard deviation from mean is reported in parentheses (n=3). "++" indicates overproduction.

Strain[s]	CmpA	*cwlJ sleB*	Sporulation Efficiency (relative to WT)
A	WT	WT	1
B	++	WT	5.7×10^{-3} (6.1×10^{-4})
C	WT	Δ	0.01 (0.003)
D	++	Δ	1.2×10^{-5} (9.5×10^{-6})

[a] Strain A: PY79; B: IT504; C: IT517; D: IT575. Genotypes are listed in Table S8.

Table S2, related to Figure 1. Sporulation efficiencies of mother cell hydrolase mutants with and without overproduction of CmpA. Standard deviation from mean is reported in parentheses (n=3).

Strain[a]	Mutation	CmpA	Sporulation Efficiency (heat resistance)
A	WT		1
B	-	++	0.006 (0.002)
C	Δ*cwlC*		1.1 (0.4)
D	Δ*cwlC*	++	0.005 (0.001)
E	Δ*cwlH*		1.6 (0.2)
F	Δ*cwlH*	++	0.01 (0.007)
G	Δ*lytC*		0.43 (0.07)
H	Δ*lytC*	++	0.004 (0.002)
I	Δ*lytC* Δ*cwlC*		0.94 (0.11)
J	Δ*lytC* Δ*cwlC*	++	.05 (.007)

[a] Strain A: PY79; B: IT478; C:IT957; D: IT959; E:IT958; F:IT960; G:IT962; H: IT963; I: KR640; J: KR639. Genotypes are listed in Table S8.

Table S3, related to Figure 2. Sporulation efficiencies (as measured by heat resistance) of additional *spoVM*^{I15A} suppressors. Standard deviation from mean is reported in parentheses (n=3).

Strain[a]	*spoVM*	Suppressor	Sporulation Efficiency (relative to WT)
A	WT	-	1
B	*spoVM*^{I15A}	-	5×10^{-6} (1.7×10^{-6})
C	*spoVM*^{I15A}	*spoIVA*^{E423G}	0.49 (0.16)
D	*spoVM*^{I15A}	spoIVA^{L424F}	0.22 (0.11)

[a] Strain A: PY79; B: KR322; C: SE249; D: IT89; Genotypes are listed in Table S8.

Table S4, related to Figure 3. Sporulation efficiencies (as measured by heat resistance) of *B. subtilis* strains overproducing CmpA and harboring various alleles of *clpX* and *clpP*. Standard deviation from mean is reported in parentheses (n=3). "++" indicates overproduction.

Strain[a]	CmpA	Suppressor	Sporulation Efficiency (relative to WT)
A	WT	-	1
B	++	-	0.006 (0.004)
C	++	*clpX*^{D21Y}	0.39 (0.28)
D	++	*clpX*^{I34M}	0.62 (0.33)
E	++	*clpX*^{E44G}	0.62 (0.31)
F	++	*clpP*^{D187N}	0.35 (0.14)

[a] Strain A: PY79; B: IT504; C: IT525; D: IT367; E: IT342; F: IT531. Genotypes are listed in Table S8.

Table S5, related to Figure 3. Sporulation efficiencies (as measured by heat resistance) of CmpA-overproducing *B. subtilis* strains harboring various alleles of *clpX*. "++" indicates overproduction. Standard deviation from mean is reported in parentheses (n=3)

Strain[a]	CmpA	ClpX	Sporulation Efficiency (relative to WT)
A	WT	WT	1
B	WT	Δ	4.5×10^{-6} (2×10^{-6})
C	++	WT	0.006 (0.003)
D	++	Δ	7.1×10^{-6} (3×10^{-6})
E	WT	ClpX ++	0.79 (0.06)
F	Δ	ClpX ++	0.83 (0.17)
G	++	ClpX ++	7.6×10^{-5} (4×10^{-5})
H	++	$ClpX^{\Delta 1-53}$ ++	0.26 (0.12)
I	++	$ClpX^{F270W}$ ++	0.61 (0.13)

[a] Strain A: PY79; B: IT482; C: SE191; D: IT483; E: IT966; F: IT967; G: IT545; H: IT571 I: IT616. Genotypes are listed in Table S8.

Table S6, related to Figure 3. Mean sporulation efficiencies (as measured by heat resistance) of strains harboring *VM*^{I15A} and various alleles of *cmpA*. Standard deviation from mean (n≥3) is reported in parentheses. Genotypes are listed in Table S8.

Strain	*spoVM*	*cmpA*	*amyE*	*thrC*	Sporulation Efficiency (relative to WT)
PY79	WT	WT	--	--	1
KR322	Δ	WT	*spoVM*^{I15A}	--	1.2×10^{-6}
SE181	Δ	Δ	*spoVM*^{I15A}	--	.15
IT139	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{P2A}	.18 (.071)
CW13	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{N3A}	3.9×10^{-3} (3.4×10^{-3})
IT141	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{W4A}	.24 (.13)
IT165	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{L5A}	.12 (.050)
CW16	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{K6A}	8.9×10^{-4} (9.6×10^{-4})
CW31	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{K7A}	5.7×10^{-3} (2.3×10^{-3})
CW139	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{Q8A}	.019 (.011)
CW28	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{M9A}	.12 (.071)
CW34	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{Q10A}	.13 (.090)
CW37	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{K11A}	.027 (.023)
CW53	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{F13A}	.33 (.16)
CW56	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{L14A}	2.7×10^{-4} (1.0×10^{-4})
CW163	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{E15A}	3.7×10^{-4} (7.8×10^{-5})
CW125	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{K16A}	.038 (.019)
CW103	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{D17A}	.08 (.07)
CW166.1	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{N18A}	1.7×10^{-6} (1.4×10^{-6})
CW131	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{Y19A}	5.7×10^{-6} (2.3×10^{-6})
CW142	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{Q20A}	6.1×10^{-3} (7.7×10^{-3})
IT166	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{I21A}	.11 (.18)
IT179	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{K22A}	.018 (.031)
IT168	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{L23A}	.045 (.75)

IT147	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{L24A}	.16 (.13)
IT169	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{N25A}	.049 (.036)
CW128	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{Q26A}	2.2×10^{-4} (3.1×10^{-4})
CW169.1	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{C27A}	8.4×10^{-6} (3.1×10^{-6})
CW172	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{W28A}	.093 (.049)
CW79	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{Y29A}	1.6×10^{-3} (1.4×10^{-3})
CW82	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{F30A}	5.9×10^{-6} (5.5×10^{-6})
CW107	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{Y31A}	.09 (.01)
CW145	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{R32A}	3.7×10^{-3} (1.6×10^{-3})
CW148	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{K33A}	4.6×10^{-3} (1.7×10^{-3})
CW151	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{K34A}	.031 (.015)
CW175	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{H35A}	1.8×10^{-3} (6.8×10^{-4})
CW182	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{C36A}	2.3×10^{-4} (1.4×10^{-4})
CW185	Δ	Δ	*spoVM*^{I15A}	*cmpA*^{S37A}	1.5×10^{-3} (7.2×10^{-4})

Table S7, related to Figure 6. Fraction of heat resistant spores of *spoVM* and *spoIVA* mutants in the presence and absence of CmpA. Standard deviation from mean is reported in parentheses (n=3).

Strain[a]	*spoVM* or *spoIVA* allele	*cmpA*	Viable Spores (CFU/ml)	Heat Resistant Spores (CFU/ml)	Fraction Heat Resistant Spores (heat resistant / viable)	% Heat Resistant Spores Relative to WT
A	WT	-	8.6×10^7 (6×10^7)	4.1×10^7 (4×10^7)	0.44 (0.17)	1
B	$spoVM^{I15A}$		347 (141)	273 (116)	0.79 (0.19)	9×10^{-4} (7×10^{-4})
C	$spoVM^{I15A}$	Δ	6.1×10^6 (4×10^6)	3.0×10^6 (2×10^6)	0.49 (0.13)	7 (2)
D	$spoIVA^{T70A\text{-}T71A}$		25500 (36665)	21125 (28940)	0.85 (0.14)	4.8×10^{-2} (4×10^{-2})
E	$spoIVA^{T70A\text{-}T71A}$	Δ	4.0×10^6 (2×10^6)	4.0×10^6 (2×10^6)	0.74 (0.18)	6 (0.8)

[a]Strain A: PY79; B: KR322; C:SE181; D: JPC221; E: IT882. Genotypes are listed in Table S8. CFU: colony forming units; WT: wild type.