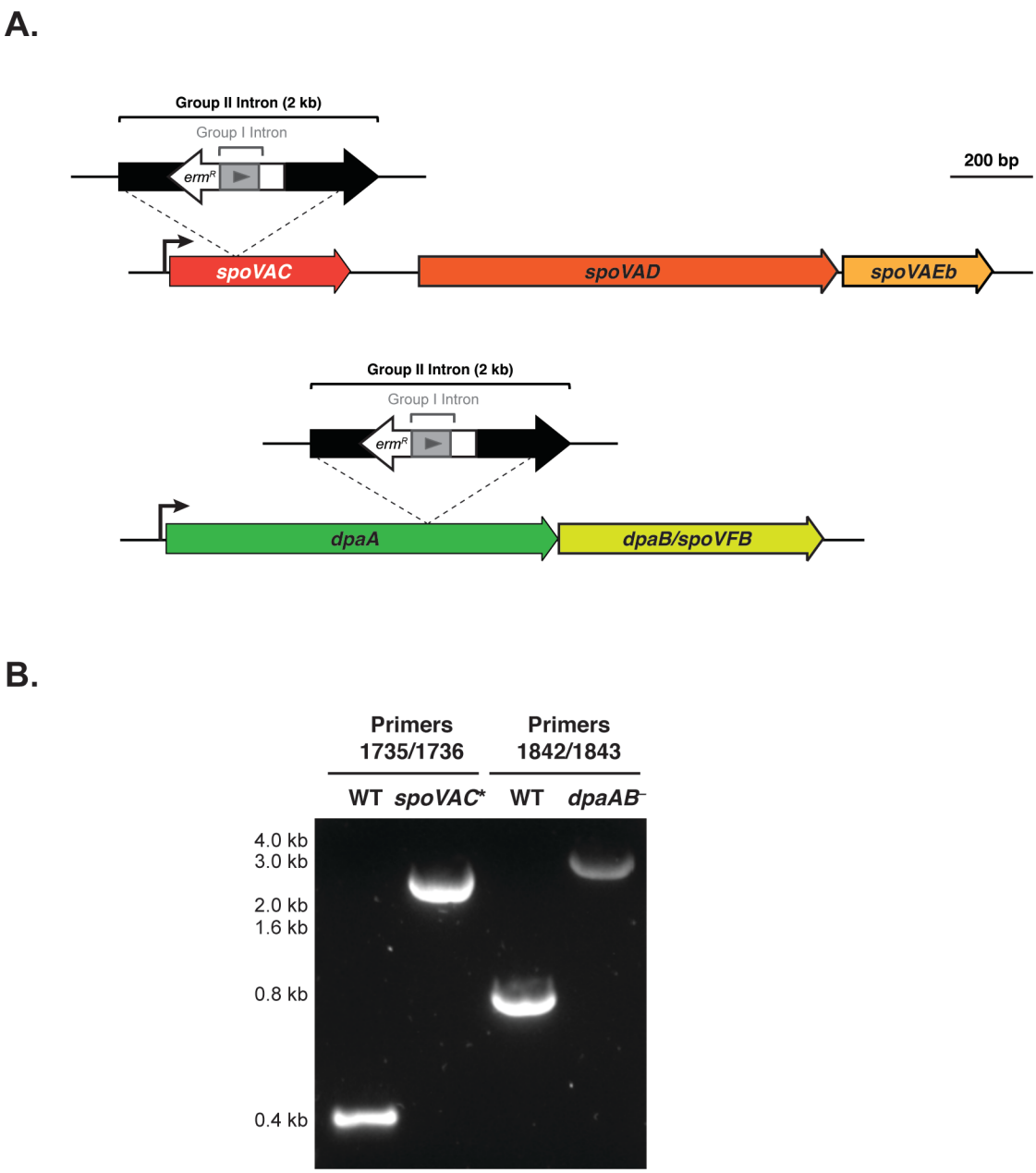


FIG S1. Generation of *spoVAC** and *dpaAB*⁻ strains using Targetron gene disruption. (A) Schematic of the Group II Intron system (32) used for insertional mutagenesis of *spoVAC* (177 bp) and *dpaA* (666 bp). Bent arrows indicated the promoters mapped by RNA-Seq (51). (B)

Colony PCR of wildtype, *spoVAC**, and *dpaAB*$^{-}$ strains using primers that flank the gene of interest. The Group II Intron is ~2 kb.

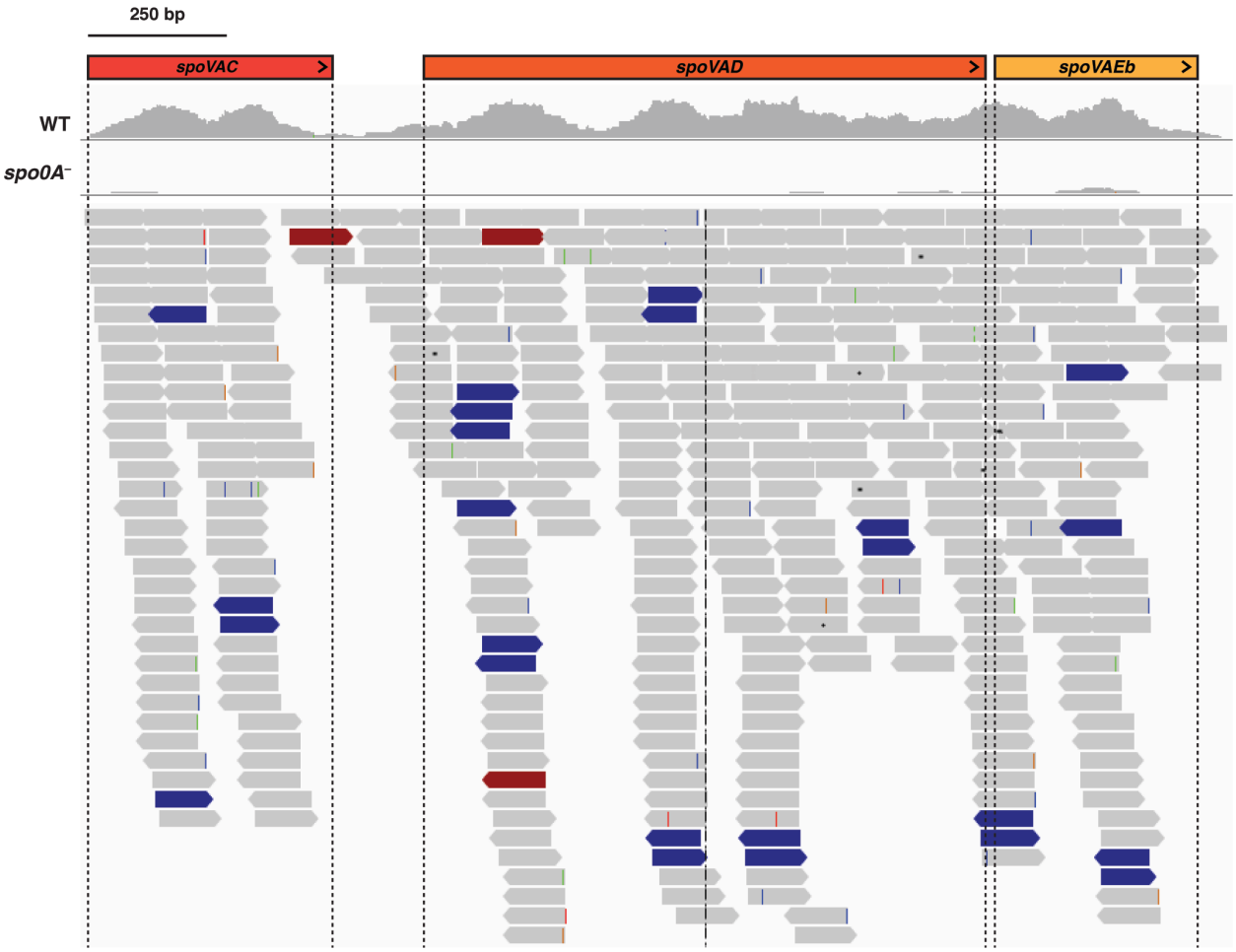


FIG S2. Representative image of Integrated Genome Viewer software (52) used to visualize of *spoVAC-VAD-VAEb* RNA-Seq data from (40). Histograms of RNA sequence reads obtained in wild type and *spo0A*$^{-}$ are shown in grey. The direction of transcription is indicated by the angled bracket. The reads mapped to this locus are shown. A 166 bp intergenic region separates *spoVAC* and *spoVAD* genes.

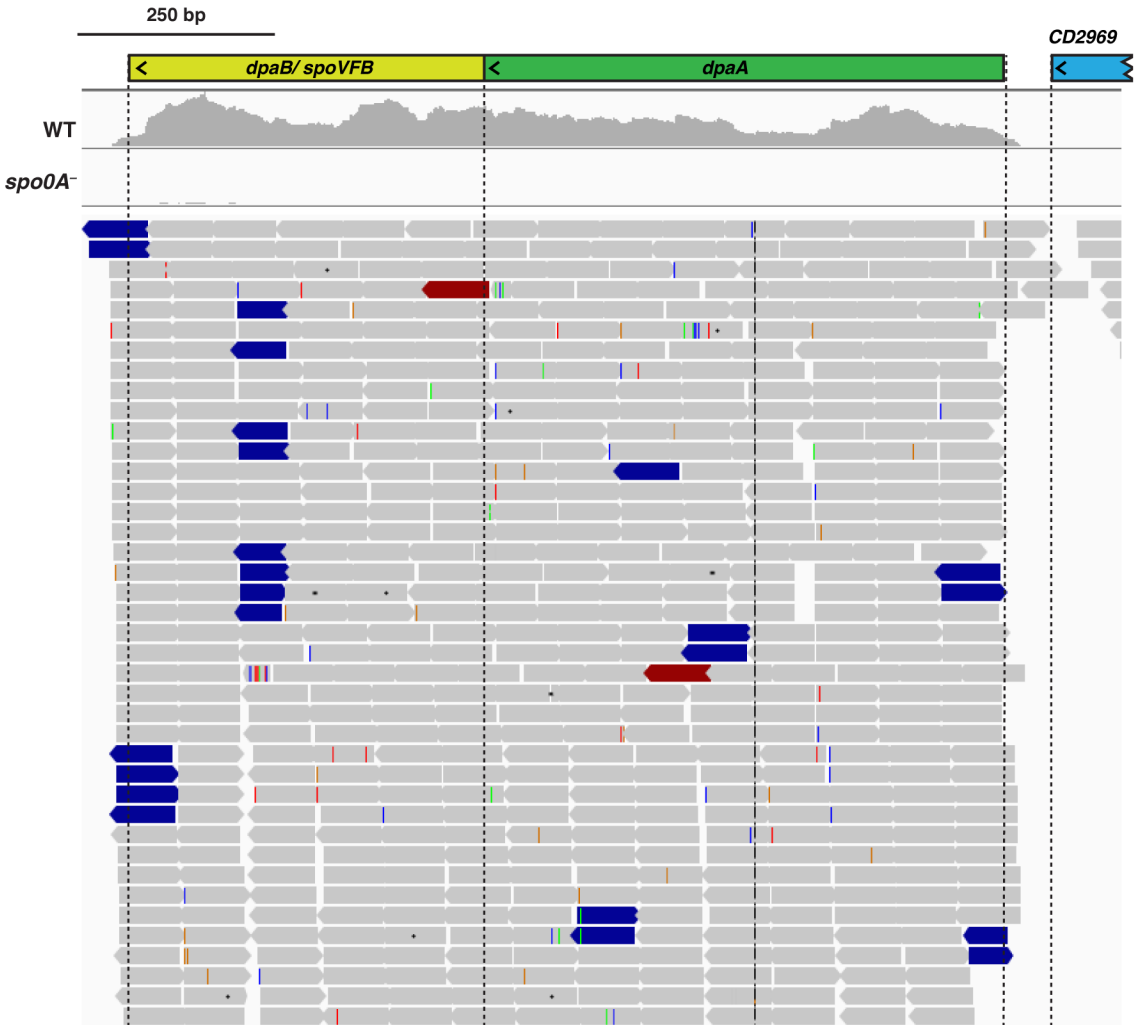


FIG S3. Representative image of Integrated Genome Viewer software (52) used to visualize of *dpaAB* RNA-Seq data from (40). Histograms of RNA sequence reads obtained in wild type and *spo0A*⁻ are shown in grey. The direction of transcription is indicated by the angled bracket. The reads mapped to this locus are shown.

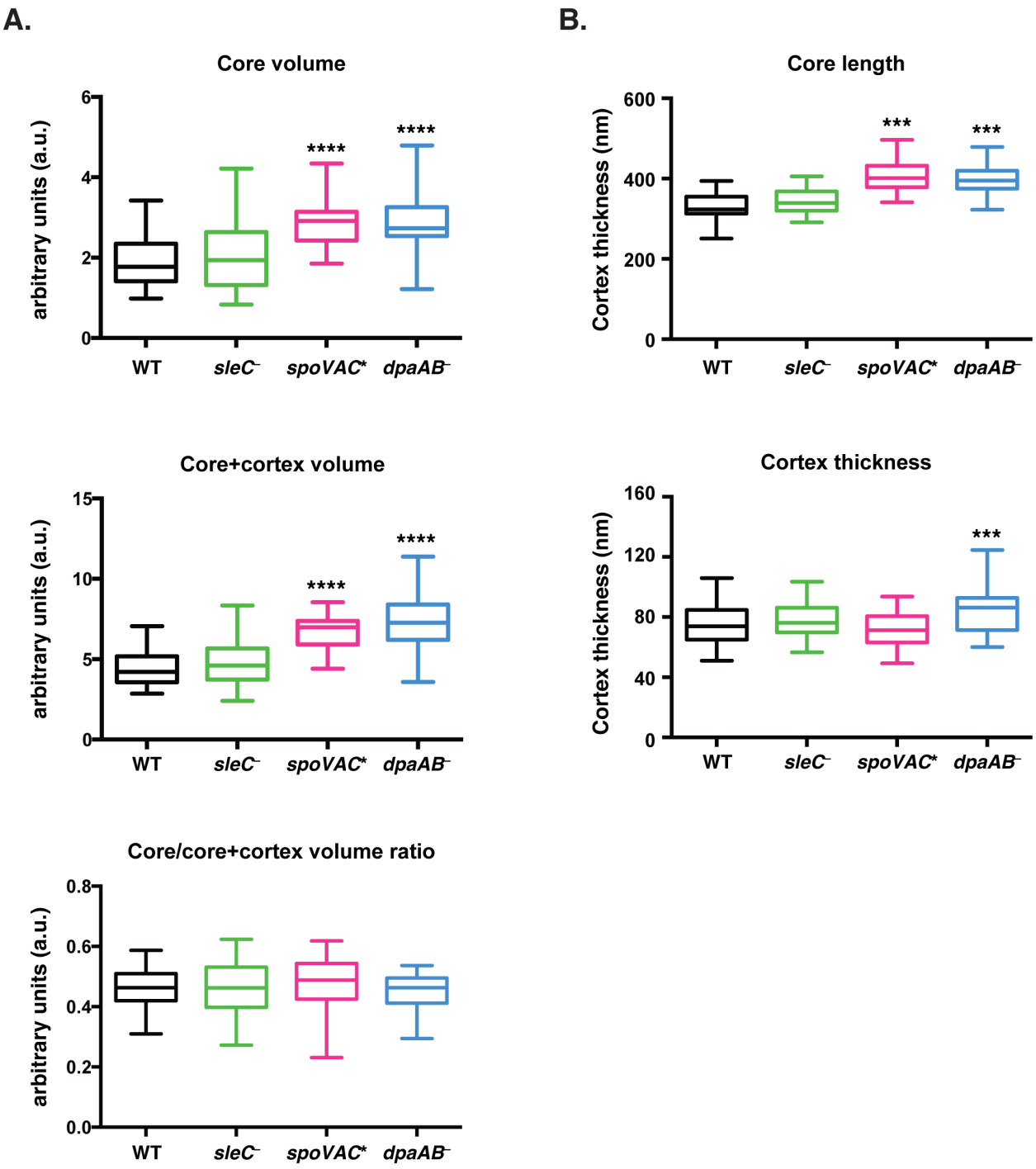


FIG S4. Transmission electron microscopy (TEM) analyses of wildtype, *sleC*⁻, *spoVAC**, and *dpaAB*⁻ spores. (A) Box and whiskers plot of core and core+cortex volumes determined from measurements of the average length and width of longitudinal sections of spores. The volume was estimated as described by Beaman *et al*. (44). These measurements derive from TEM

analyses of a minimum of 35 spores from the images used in Fig. 4. (B) Box and whiskers plot of cortex thickness and core length of *spoVAC** and *dpaAB*$^{-}$ spores. Measurements derive from TEM analyses of a minimum of 50 spores from the images used in Fig. 4. Statistical significance was determined using ANOVA and Tukey's test (*** $p < 0.001$, **** $p < 0.0001$).

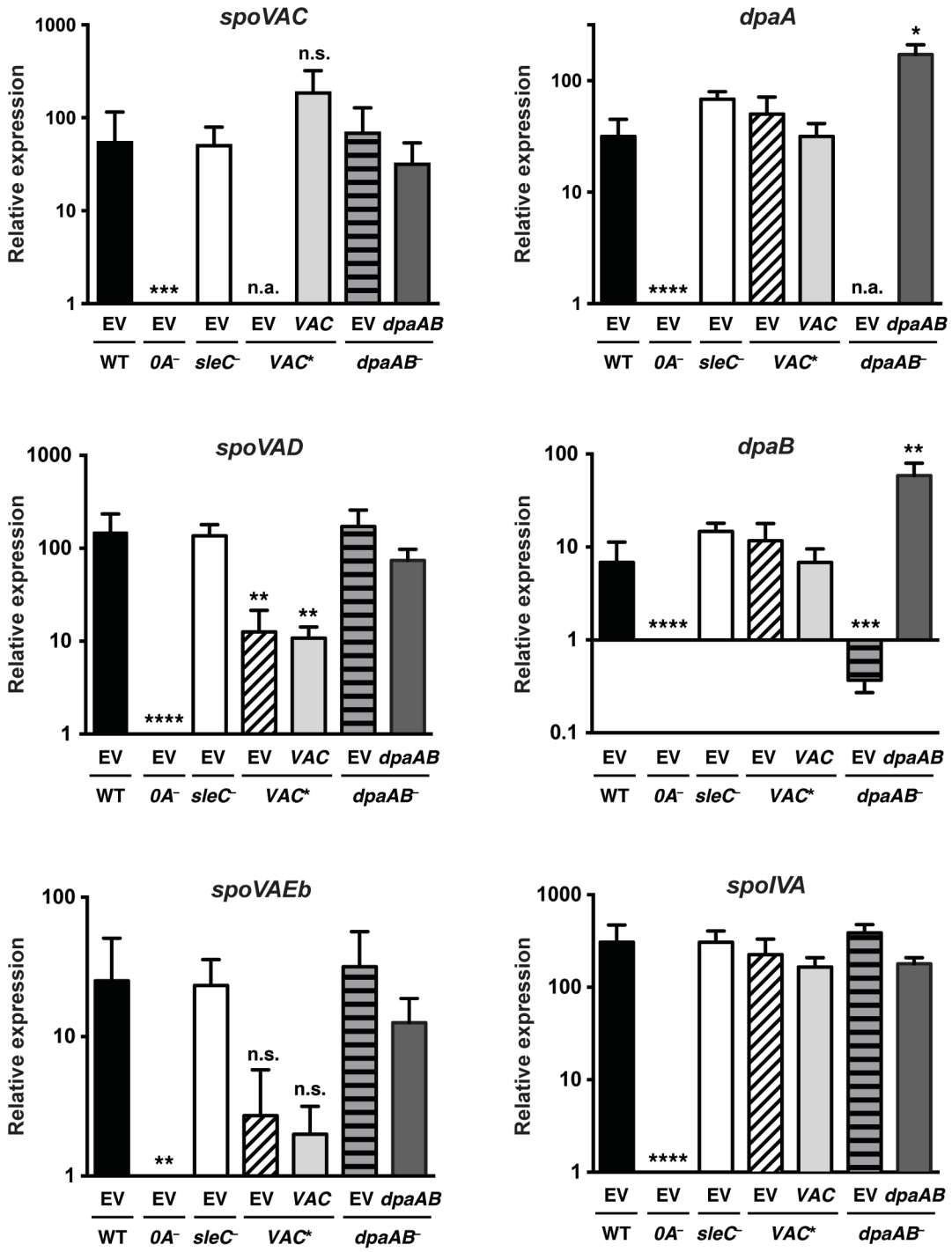


FIG S5. qRT-PCR analyses of *spoVA* and *dpaAB* transcript levels. RNA was isolated from sporulating cells of wildtype carrying empty vector (WT/EV), *spoVAC** or *dpaAB*⁻ carrying either empty vector or the indicated complementation constructs. Transcript levels were

normalized to the housekeeping gene *rpoB* using the standard curve method and are shown relative to the *spo0A*$^{-}$ strain. Data represents the average of three biological replicates. Error bars indicate the standard error of the mean. n.a. indicates not applicable, since the region amplified spans the disrupted gene.

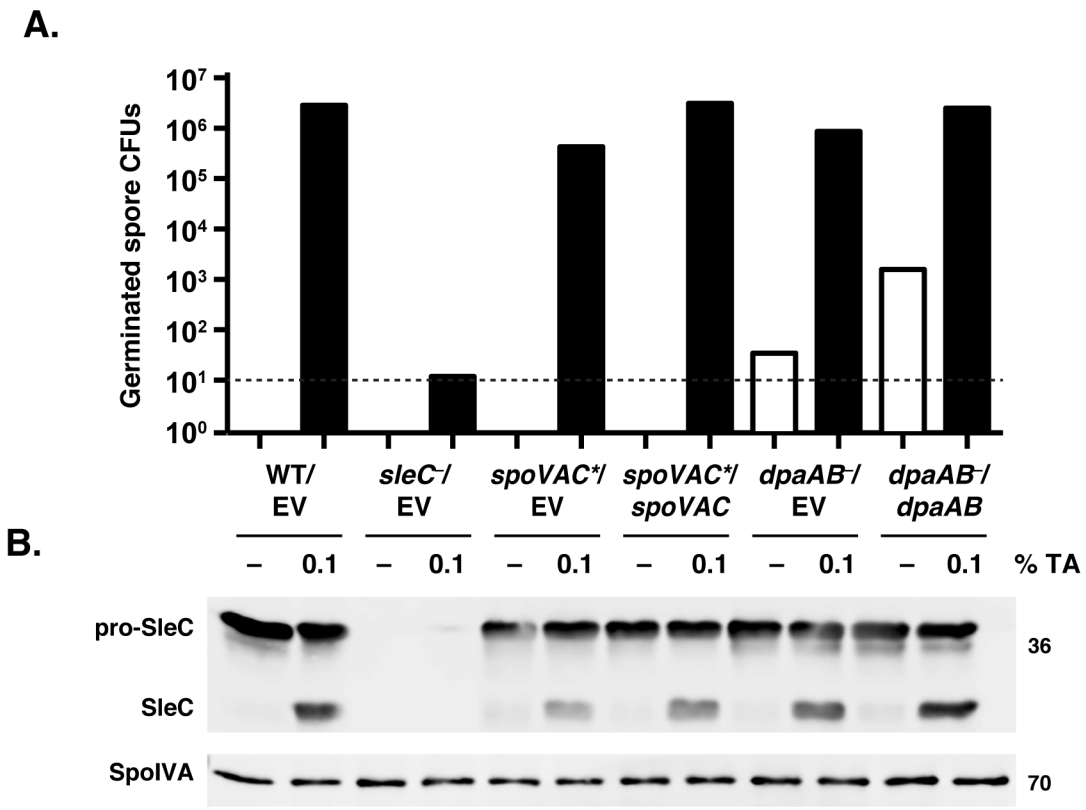


FIG S6. SleC cleavage and germination of untreated *spoVAC** and *dpaAB*⁻ spores. (A) Germination of indicated strains of purified spores in response to either 0% or 1% taurocholate exposure for 20 min. Samples were plated on BHIS to measure the number of spores that germinated during this incubation. Statistical significance was evaluated using ANOVA and Tukey's test (**** $p < 0.0001$). n.s. = no statistical significance. (B) Western blot analyses of samples from one representative replicate of the *in vitro* germination assay shown above. The zymogen pro-SleC is processed by CspB in response to taurocholate addition (12).

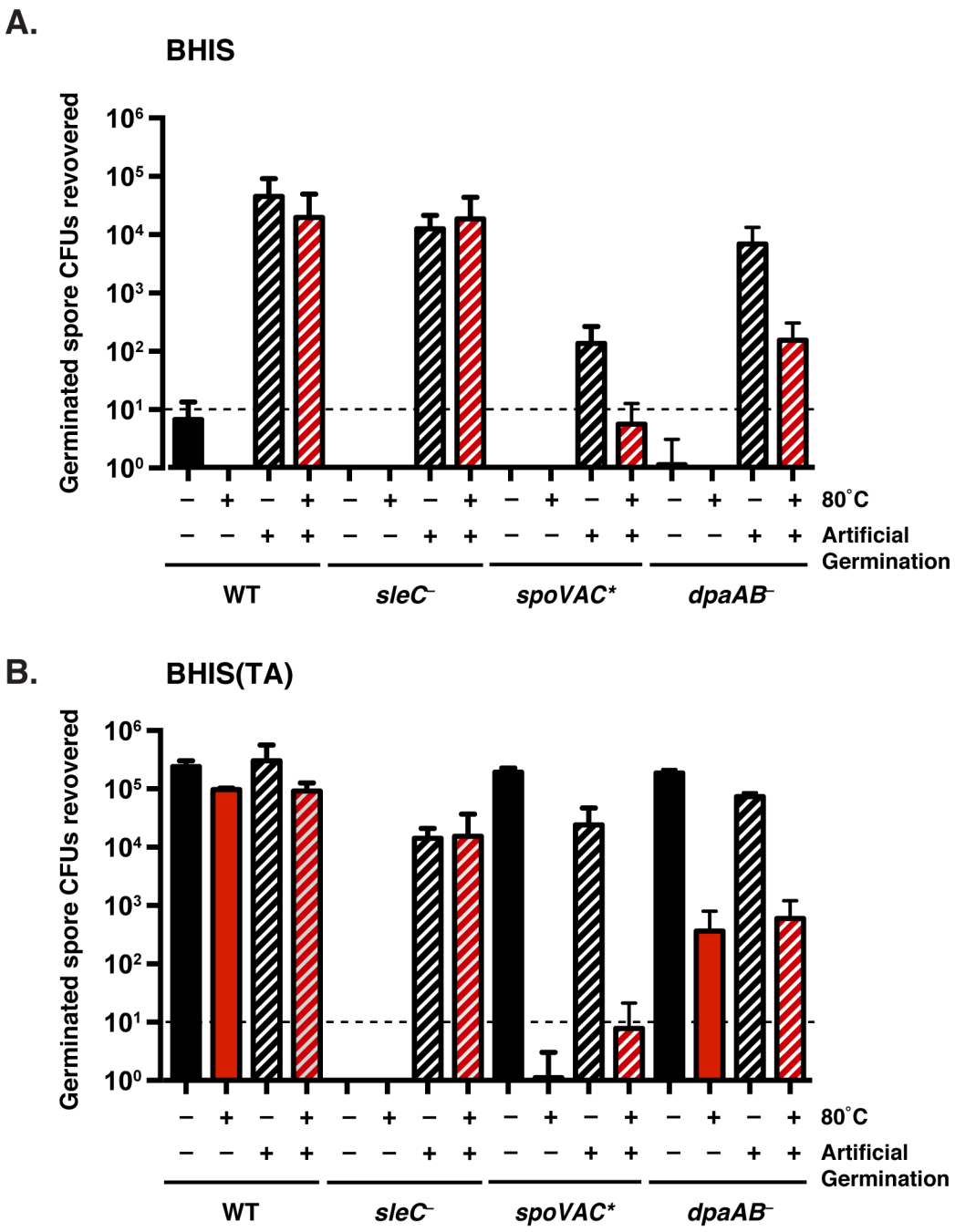


FIG S7. Artificial germination of either unheated or heat-treated wildtype, *spoVAC**, and *dpaAB*⁻ spores. Spores were either unheated or heated at 80˚C for 15 min prior to their incubation with thioglycollate and lysozyme (artificial germination) as previously described (13). The spores were serially diluted and plated on either (A) BHIS or (B) BHIS containing 0.1% taurocholate.

Untreated samples were exposed to the same heating and spinning procedures as the artificial germination samples. Data represents the average of 3 biological replicates.

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

| Primer | Name | Sequence |
|---|---|---|
| 532 | 3’ Universal EBS | CGAAATTAGAAACTTGCGTTCAGTAAAC |
| 1714 | 5' IBS1 *spoVAC* 264 | AAAAAAGCTTATAATTATCCTTAGGTTCCATAATTGTGCGCCCAGATAGGGTG |
| 1715 | 3' EBS1d *spoVAC* 264 | CAGATTGTACAAATGTGGTGATAACAGATAAGTCATAATTCCTAACTTACCTTTCTTTGT |
| 1716 | 5' EBS2 *spoVAC* 264 | TGAACGCAAGTTTCTAATTTCGATTGAACCTCGATAGAGGAAAGTGTCT |
| 1735 | 5' *spoVAC* 22 bp | TATGTAGACCAAATAAGCCCAAAACC |
| 1736 | 3' *spoVAC* 407 bp | CCACATAAAATAGAAGAACCTATCCC |
| 1814 | 5' IBS1 *dpaA* 423 | AAAAAAGCTTATAATTATCCTTAGCTATCGCAGAAGTGCGCCCAGATAGGGTG |
| 1815 | 3' EBS1d *dpaA* 423 | CAGATTGTACAAATGTGGTGATAACAGATAAGTCGCAGAAGCTAACTTACCTTTCTTTGT |
| 1816 | 5' EBS2 *dpaA* 423 | TGAACGCAAGTTTCTAATTTCGATTATAGCTCGATAGAGGAAAGTGTCT |
| 1820 | 5' *dpaA* (22) for qRT | ACTGTTATTGGGGGAGACCTGC |
| 1821 | 3' *dpaA* (151) for qRT | GGAACTTTTGGATAGTGCTTCAGC |
| 1842 | 5' NdeI *dpaA* | AAAACATATGTCAAATCAATACGATATAA |
| 1843 | 3' XhoI *dpaA* No Stop | AAAACTCGAGATCCCTTCTTTCATTTATAATATTTT |
| 1855 | 5' NotI *spoVAC* upstream | AATGCGGCCGCGGAAAAATAGATTTTTTCTTTATTAGGC |
| 1856 | 3' XhoI *spoVAC* +Stop Codon | AATCTCGAGCAATTAGTTACTTTAGTAGCTATTTTCAG |
| 1891 | 5’ NotI *dpaA* 373 bp upstream | AATAGCGGCCGCGTGATGACATTATAAATATTGGAGG |
| 1892 | 3’ XhoI *dpaB* over stop | AAGCTCGAGCTATTGATGTGGACTTTTTATAACAGGC |
| 1931 | 5' *spoVAE* Start | ATGTTAATGGACTATGTAAGAGTATTTATTGTAG |
| 1932 | 3' *spoVAE* Stop | TTATGGTTTTGCTTTTGGAGTAAATACTACTGAC |
| 2030 | 5' *spoVAD* 471bp | TCAAAAACCAATGACAGCCCAG |
| 2031 | 3' *spoVAD* 832bp | AACACCCACATCCACTTCCACC |
| 2034 | 5' *spoVFB* 335bp | TGGCAGCAAAAGGTCATC |
| 2035 | 3' *spoVFB* 386bp | GCTTAGAGCATCATTCGTTG |

Restriction sites are underlined.

**Table S2. $A_{270}$ measurement of Total DPA in *spoVAC** and *dpaAB*$^-$ spores**

| | WT | *spoVAC*$^-$ | *dpaA*$^-$ | *sleC*$^-$ |
|---|---|---|---|---|
| Untreated Biol. Rep 1.1 | 0.018 | 0.199 | 1.360 | 0.013 |
| Untreated Biol. Rep 1.2 | 0.018 | 0.398 | 1.200 | 0.013 |
| Untreated Biol. Rep 1.3 | 0.023 | 0.429 | 1.205 | 0.015 |
| Untreated Biol. Rep 2.1 | 0.009 | 0.064 | 0.051 | 0.071 |
| Untreated Biol. Rep 2.2 | 0.009 | 0.010 | 0.008 | 0.018 |
| Boiled Biol. Rep 1.1 | 0.202 | 0.253 | 1.420 | 0.205 |
| Boiled Biol. Rep 1.2 | 0.181 | 0.477 | 1.299 | 0.177 |
| Boiled Biol. Rep 1.3 | 0.176 | 0.471 | 1.260 | 0.186 |
| Boiled Biol. Rep 2.1 | 0.287 | 0.196 | 0.135 | 0.347 |
| Boiled Biol. Rep 1.1 | 0.293 | 0.105 | 0.089 | 0.322 |
| $\Delta A_{270}$ Biol. Rep 1.1 | 0.184 | 0.054 | 0.060 | 0.192 |
| $\Delta A_{270}$ Biol. Rep 1.2 | 0.163 | 0.079 | 0.099 | 0.164 |
| $\Delta A_{270}$ Biol. Rep 1.3 | 0.153 | 0.042 | 0.055 | 0.171 |
| $\Delta A_{270}$ Biol. Rep 2.1 | 0.278 | 0.132 | 0.084 | 0.276 |
| $\Delta A_{270}$ Biol. Rep 1.1 | 0.284 | 0.095 | 0.081 | 0.304 |

Data represents the $A_{270}$ values measured for three Biological Replicate (Biol. Rep) for spore preparation 1.X (spores were ~two months old) and two biological replicates of spore preparation 2.X (spores were one week old).