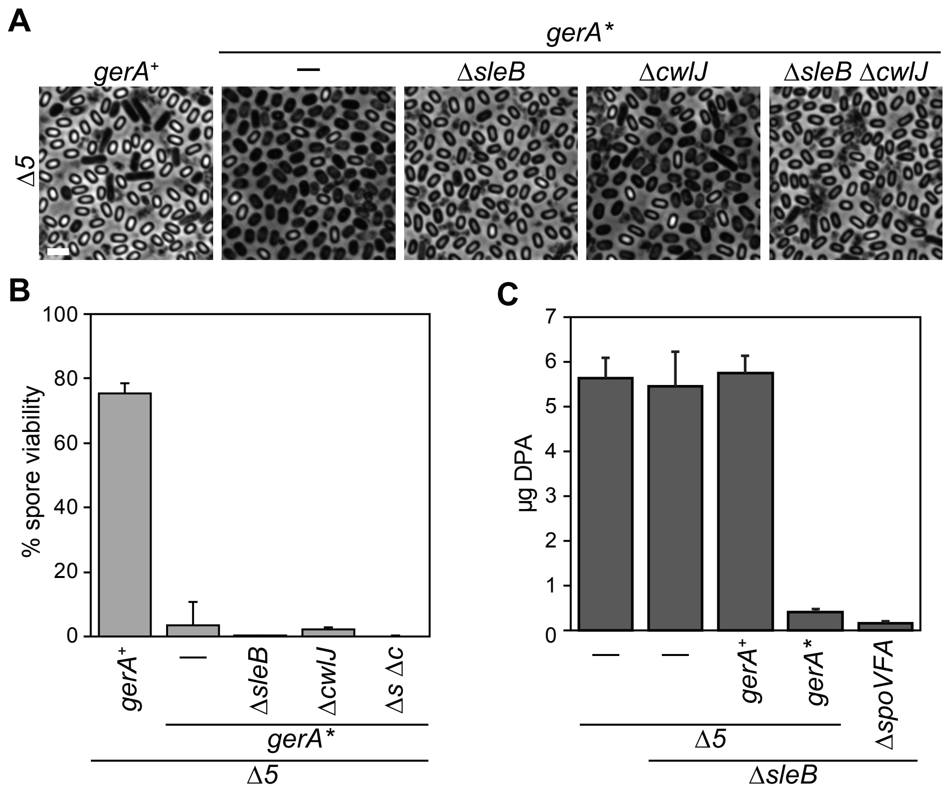
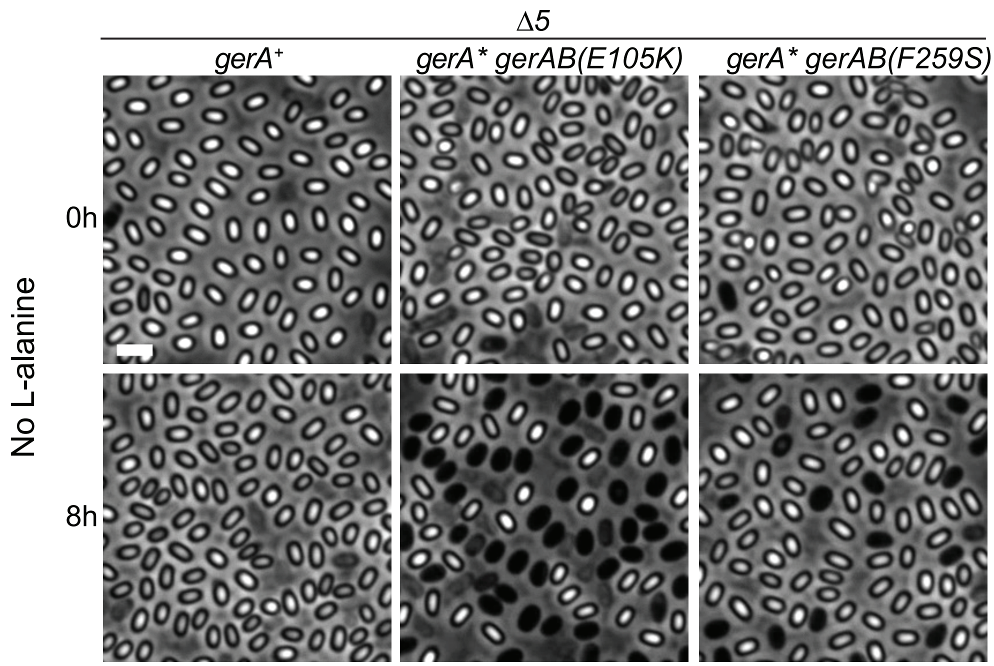
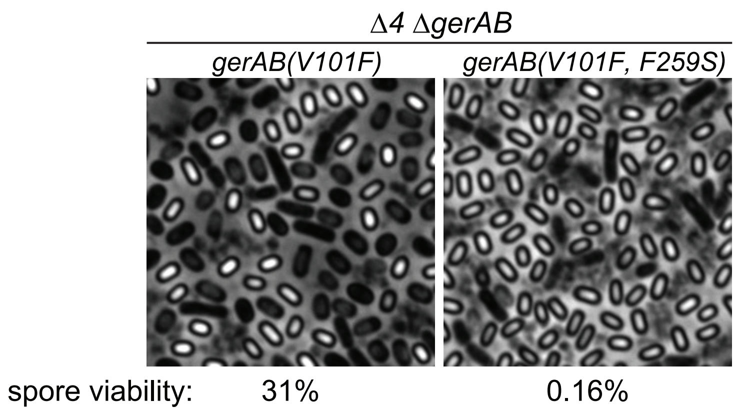


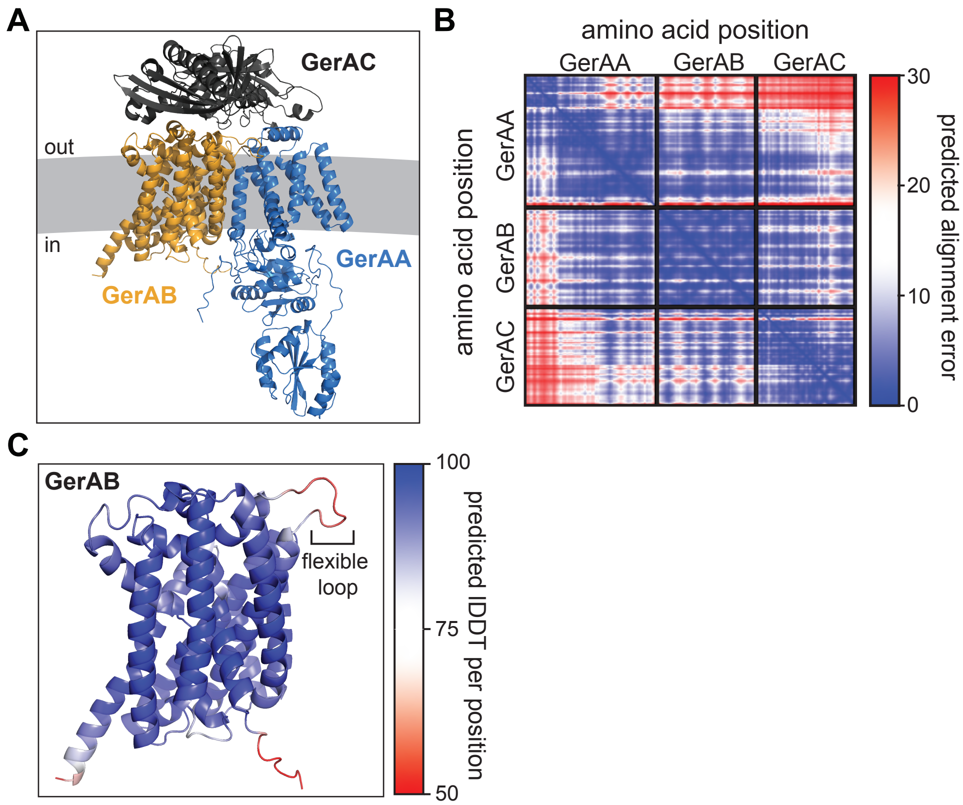
Supplemental Figure 1: GerA* triggers DPA release and SleB activation when it is the sole germinant receptor. (A) Phase-contrast micrographs of cultures sporulated by nutrient exhaustion. Δ*5* – Δ*gerA* Δ*gerBB* Δ*gerKB* Δ*yndE* Δ*yfkT*. Experiments were performed in biological triplicate; representative images are shown. Scale bar is 2μm. (B) Sporulated cultures in (A) were heat treated (80°C for 20 min), and serial dilutions were plated on LB to assess heat-resistant colony forming units. Wild type spore viability (3.3x10^8 CFU/ml) was set to 100%. Δ*s* Δ*c* – Δ*sleB* Δ*cwlJ*. Error bars indicate standard deviation, n=3. (C) Phase-grey and -bright spores were purified from sporulated cultures in (A) using lysozyme and SDS. Spores were boiled to release DPA. DPA was then quantified by measuring fluorescence when combined with a solution containing $TbCl_3$ compared to standards. Values are reported as micrograms of DPA released from 1ml of purified spores adjusted to OD_{600}=1. Error bars indicate standard deviation, n=4.



Supplemental Figure 2: Constitutive germination of spores harboring *gerAA(P326S)* and *gerAB(E105K)* or *gerAB(F259S)*. Phase-contrast micrographs of purified spores before and after agitation in buffer for 8 h at 37°C. Images correspond to the plots in Figures 3C and 3D. Representative images of three biological triplicates are shown. Scale bar is 2μm.



Supplemental Figure 3: Epistatic analysis of *gerAB(F259S)* and *gerAB(V101F)*. Cultures were sporulated by nutrient exhaustion. Representative phase-contrast micrographs of three biological replicates are shown. Scale bar is 2µm. Cultures were heat-treated (80°C for 20 min) then serially diluted and plated on LB to assess heat-resistant colony forming units. *gerAB*⁺ spore viability (3.7 x10^8 CFU/ml) was set to 100%.



Supplemental Figure 4: Structural prediction of GerA complex. (A) Alphafold2-predicted structures of GerAA, GerAB, and GerAC in complex, situated in the inner spore membrane (grey). (B) Predicted alignment error of all residues against all residues. Low error (blue) corresponds to well-defined relative domain positions. (C) Predicted IDDT per position mapped onto the predicted GerAB structure. Higher pIDDT (blue) corresponds to a more confident prediction.

Supplemental Table 1. Suppressors of *gerAA-P326S*

GerAA		GerAB		GerAC	
Alteration	n	Alteration	n	Alteration	n
P126L	1	E105K	1	S28I	1
M165I	1	R107Q	1	V119F	1
A236S	1	R107W	3	S342P	2
M263I	1	V163E	1	T368L	1
S265P	1	W253L	1	T368R	1
L293V	1	**F259S**	**1**		
S294P	1	**F259L**	**2**		
A299V*	**1**	**G266D**	**2**		
S302T*	**1**	**G266S**	**6**		
A313T	**3**	**I267R**	**1**		
A313V	**2**				
T315M	**2**				
S317L	**1**				
A318T	**1**				
A318V	**3**				
E321G	**4**				
E321A	**1**				
E321V	**1**				
P324L	**1**				
S326P	**6**				
T337I	1				
Q366H	1				
V369I	1				
E370A	1				
A371V	1				
L378F	3				
A386T	8				
T391I	1				
F401V	1				
R402Q	3				
S408P	3				
Total	58		19		6

Bold indicates alteration is within the extracellular loops discussed in the manuscript

*Residues 299 and 302 of GerAA are variable across laboratory strains. See reference 26 for further details

Supplemental Table 2. *Bacillus subtilis* strains used in this study

Strain	Genotype	Source	Figure
168	*trpC2*	Zeigler *et al.*, 2008	1, 2, 3, S1
BJA153a	*ycgO::gerAA-gerAB-gerAC (spec)*	This study	1
BJA134b	*ycgO::gerAA(P326S)-gerAB-gerAC (spec)*	This study	1
BJA567	*ycgO::gerAA(P326S)-gerAB-gerAC (spec) ΔsleB::lox72*	This study	1
BJA148a	*ycgO::gerAA(P326S)-gerAB-gerAC (spec) ΔcwlJ::lox72*	This study	1
BJA568	*ycgO::gerAA(P326S)-gerAB-gerAC (spec) ΔsleB::lox72 ΔcwlJ::lox72*	This study	1
BDR3487	*ΔsleB::lox72*	Amon *et al.,* 2020	1, S1
BDR4199	*ΔsleB::lox72 ΔspoVFA:lox72*	This study	1, S1
BLA176	*ΔgerA::cat ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::kan*	Artzi, *et al.* 2021	2
BLA197	*ΔgerA::cat ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAA-gerAB-gerAC (spec)*	Artzi, *et al.* 2021	2, 3, S1, S2
BJA177a	*ΔgerA::cat ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAA(P326S)-gerAB-gerAC (spec)*	This study	2, S1
BJA185f	*ΔgerA::cat ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAA(A313T, P326S)-gerAB-gerAC (spec)*	This study	2
BJA185k	*ΔgerA::cat ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAA(P326S, A386T)-gerAB-gerAC (spec)*	This study	2
BJA561	*ΔgerA::cat ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAA(P326S)-gerAB(E105K)-gerAC (spec)*	This study	2, 3, S2
BJA185d	*ΔgerA::cat ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAA(P326S)-gerAB(F259S)-gerAC (spec)*	This study	2, 3, S2
BJA235	*ΔgerA::cat ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAA(P326S)-gerAB-gerAC(S28I) (spec)*	This study	2
BJA236	*ΔgerA::cat ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAA(P326S)-gerAB-gerAC(S342P) (spec)*	This study	2
BLA174	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::kan*	Artzi, *et al.* 2021	3
BLA178	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB*	Artzi, *et al.* 2021	3, 4
BJA278	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(E105K)*	This study	3
BJA279	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(R107Q)*	This study	3
BJA280	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(R107W)*	This study	3
BJA281	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(W253L)*	This study	3
BJA282	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(F259S)*	This study	3, 4
BJA283	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(G266D)*	This study	3
BJA284	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(G266S)*	This study	3
BJA285	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(I267R)*	This study	3
BJA569	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(V101F)*	This study	S3
BJA570	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(T287L)*	This study	4
BJA571	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(V101F, F259S)*	This study	S3
BJA572	*ΔgerAB::lox72 ΔgerBB::lox72 ΔgerKB::lox72 ΔyfkT::lox72 ΔyndE::lox72 ycgO::gerAB(F259S,T287L)*	This study	4

BJA317a	*∆gerA::cat ∆gerBB::lox72 ∆gerKB::lox72 ∆yfkT::lox72 ∆yndE::lox72 ycgO::gerAA(P326S)-gerAB-gerAC (spec) ∆sleB::erm*	This study	S1
BJA318a	*∆gerA::cat ∆gerBB::lox72 ∆gerKB::lox72 ∆yfkT::lox72 ∆yndE::lox72 ycgO::gerAA(P326S)-gerAB-gerAC (spec) ∆cwlJ::erm*	This study	S1
BJA319a	*∆gerA::cat ∆gerBB::lox72 ∆gerKB::lox72 ∆yfkT::lox72 ∆yndE::lox72 ycgO::gerAA-gerAB-gerAC (spec) ∆sleB::erm*	This study	S1
BJA568	*∆gerA::cat ∆gerBB::lox72 ∆gerKB::lox72 ∆yfkT::lox72 ∆yndE::lox72 ycgO::gerAA(P326S)-gerAB-gerAC (spec) ∆sleB::lox72 ∆cwlJ::lox72*	This study	S1
BJA287a	*∆gerA::cat ∆gerBB::lox72 ∆gerKB::lox72 ∆yfkT::lox72 ∆yndE::lox72 ycgO::kan ∆sleB::erm*	This study	S1

Supplemental Table 3. Plasmids used in this study

Plasmid	Description	Source
pLA25	*ycgO::gerAA-gerAB-gerAC (spec)*	Artzi, *et al*. 2021
pJA39	*ycgO::gerAA(P326S)-gerAB-gerAC (spec)*	This study
pLA13	*ycgO::gerAB (spec)*	Artzi, *et al*. 2021
pJA048	*ycgO::gerAB(E105K) (spec)*	This study
pJA049	*ycgO::gerAB(R107Q) (spec)*	This study
pJA050	*ycgO::gerAB(R107W) (spec)*	This study
pJA051	*ycgO::gerAB(W253L) (spec)*	This study
pJA052	*ycgO::gerAB(F259S) (spec)*	This study
pJA053	*ycgO::gerAB(G266D) (spec)*	This study
pJA054	*ycgO::gerAB(G266S) (spec)*	This study
pJA055	*ycgO::gerAB(I267R) (spec)*	This study
pLA129	*ycgO::gerAB(V101F) (spec)*	Artzi, et al. 2021
pJA078	*ycgO::gerAB(V101F, F259S) (spec)*	This study
pLA125	*ycgO::gerAB(T287L) (spec)*	Artzi, et al. 2021
pJA079	*ycgO::gerAB(F259S, T287L) (spec)*	This study

Supplemental Table 4. Oligonucleotides used in this study

Oligonucleotide	Sequence
oLA111	ggcACTAGTatcctttgaatatttgtattttggatttg
oLA108	ggcGGATCCctggatgtcagtgaccggacg
oJA105	ggcgaacagggaaaacgtgccgttctctccgatatttgaagccctgctgatg
oJA106	catcagcagggcttcaaatatcggagagaacggcacgttttccctgttcgcc
oJA152	TCCTCGGCGTAGCCAGCTTCAAGACACGGGCAATGGCTGA
oJA153	TCAGCCATTGCCCGTGTCTTGAAGCTGGCTACGCCGAGGA
oJA154	GCGTAGCCAGCTTCGAGACATGGGCAATGGCTGAAATGGTGA
oJA155	TCACCATTTCAGCCATTGCCCATGTCTCGAAGCTGGCTACGC
oJA156	CGTAGCCAGCTTCGAGACACAGGCAATGGCTGAAATGGTGA
oJA157	TCACCATTTCAGCCATTGCCTGTGTCTCGAAGCTGGCTACG
oJA158	CGAGGTGAAAACGCTGATTTTGCCGACTATTTCTCTCTTTC
oJA159	GAAAGAGAGAAATAGTCGGCAAAATCAGCGTTTTCACCTCG
oJA160	TTGGCCGACTATTTCTCTCTCTCAGTCCTTTGAGCTTAAAG
oJA161	CTTTAAGCTCAAAGGACTGAGAGAGAGAAATAGTCGGCCAA
oJA162	TCAGTCCTTTGAGCTTAAAGACATATTTATTGAACGGTTTG
oJA163	CAAACCGTTCAATAAATATGTCTTTAAGCTCAAAGGACTGA
oJA164	TTCAGTCCTTTGAGCTTAAAAGCATATTTATTGAACGGTTT
oJA165	AAACCGTTCAATAAATATGCTTTTAAGCTCAAAGGACTGAA
oJA166	GTCCTTTGAGCTTAAAGGCAGATTTATTGAACGGTTTGAAT
oJA167	ATTCAAACCGTTCAATAAATCTGCCTTTAAGCTCAAAGGAC

Supplemental Methods

Plasmid construction

pLA25 [*ycgO::gerAA-gerAB-gerAC (spec)*] was generated in a two-way ligation with a SpeI-BamHI digested PCR product containing the entire *gerA* operon (oligonucleotide primers oLA111 + oLA108 using genomic DNA from *B. subtilis* 168 as template) and pCB014 similarly digested with SpeI and BamHI. pCB014 [*ycgO::spec*] is an ectopic integration vector for double crossover integrations at the *ycgO* locus (laboratory stock).

pJA039 [*ycgO::gerAA(P326S)-gerAB-gerAC (spec)*] was generated by site-directed mutagenesis of pLA25 with oligonucleotides oJA105 and oJA106.

pJA048 [*ycgO::gerAB(E105K) (spec)*] was generated by site-directed mutagenesis of pLA13 with oligonucleotides oJA152 and oJA153.

pJA049 [*ycgO::gerAB(R107Q) (spec)*] was generated by site-directed mutagenesis of pLA13 with oligonucleotides oJA156 and oJA157.

pJA050 [*ycgO::gerAB(R107W) (spec)*] was generated by site-directed mutagenesis of pLA13 with oligonucleotides oJA154 and oJA155.

pJA051 [*ycgO::gerAB(W253L) (spec)*] was generated by site-directed mutagenesis of pLA13 with oligonucleotides oJA158 and oJA159.

pJA052 [*ycgO::gerAB(F259S) (spec)*] was generated by site-directed mutagenesis of pLA13 with oligonucleotides oJA160 and oJA161.

pJA053 [*ycgO::gerAB(G266D) (spec)*] was generated by site-directed mutagenesis of pLA13 with oligonucleotides oJA162 and oJA163.

pJA054 [*ycgO::gerAB(G266S) (spec)*] was generated by site-directed mutagenesis of pLA13 with oligonucleotides oJA164 and oJA165.

pJA055 [*ycgO::gerAB(I267R) (spec)*] was generated by site-directed mutagenesis of pLA13 with oligonucleotides oJA166 and oJA167.

pJA078 [*ycgO::gerAB(V101F, F259S) (spec)*] was generated by site-directed mutagenesis of pLA129 with oligonucleotides oJA160 and oJA161.

pJA079 [*ycgO::gerAB(F259S, T287L) (spec)*] was generated by site-directed mutagenesis of pLA125 with oligonucleotides oJA160 and oJA161.