Supplementary Information for:

Identification and characterization of the *Bacillus subtilis* spore germination protein GerY

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Supplementary Methods

Strain Construction

BDR4313 (Δ*gerP*) was generated by direct transformation of *B. subtilis* 168 with an isothermal assembly product derived from 3 PCR products: (1) a PCR product containing an upstream region of *gerPA* amplified with oligonucleotide primers oFR217/oFR218 and *B. subtilis* 168 genomic DNA as template; (2) a PCR product containing the Erm cassette; (3) a PCR product containing a downstream region of *gerPF* amplified with oligonucleotide primers oFR219/oFR220 and *B. subtilis* 168 genomic DNA as template.

BDR4376 [Δ*gerY::lox72, amyE*::*gerY* (spec)] was generated by transforming BDR4328 (Δ*gerY*) with pFR57.

BDR4377 [Δ*yqeF::lox72, amyE*::*yqeF* (spec)] was generated by transforming BDR4324 (Δ*yqeF*) with pFR54.

BDR4378 [Δ*yodN::lox72, amyE*::*yodN* (spec)] was generated by transforming BDR4305 (Δ*yodN*) with pFR56.

BDR4381 [Δ*ydgB, amyE*::*ydgB* (spec)] was generated by transforming BDR4306 (Δ*ydgB*) with pFR58.

BDR4406 [*yycR*::P_{ydgB}-*optRBS-yfp* (spec)] was generated by transforming BDR2413 (WT) with pFR60.

BDR4407 [*yycR*::P_{gerY}-*optRBS-yfp* (spec)] was generated by transforming BDR2413 WT) with pFR61.

BDR4408 [*yycR*::P_{yodN}-*optRBS-yfp* (spec)] was generated by transforming BDR2413 (WT) with pFR62.

BDR4409 [*yycR*::P_{yqeF}-*optRBS-yfp* (spec)] was generated by transforming BDR2413 (WT) with pFR63.

BDR4412 [Δ*ydgB::lox72, ycgO*::*ydgB-yfp* (spec)] was generated by transforming BDR4306 (Δ*ydgB*) with pFR66.

BDR4444 [Δ*gerY::lox72, ycgO*::P_{gerY}-*optRBS-yfp*-linker-*gerY* (spec)] was generated by transforming BDR4328 (Δ*gerY*) with pFR73.

BDR4461 [*gerT* Ω *gerT-gfp* (spec)] was generated by back-crossing (2x) gDNA from CF172 (PY79 bearing the construct *gerT*Ω*gerT-gfp* (1) into BDR2413 (WT).

BDR4466 [*gerT* Ω *gerT-gfp* (spec) Δ*safA::tet*] and **BDR4470 [*gerT* Ω *gerT-gfp* (spec) Δ*cotE::cat*]** were generated by transforming BJA020a and BJA018 (2) with gDNA from BDR4461 (*gerT* Ω *gerT-gfp* (spec)], respectively.

BDR4460 [*ycgO::gerPA-yfp* (spec)] was generated by transforming BDR2413 (WT) with pFR74.

BDR4465 [*ycgO::gerPA-yfp* (spec) Δ*safA::tet*] and **BDR4469 [*ycgO::gerPA-yfp* (spec) Δ*cotE::cat*]** were generated by transforming BJA020a (Δ*safA::tet*) and BJA018 (Δ*cotE:cat*) with pFR74, respectively.

BDR4467 [Δ*gerY* + *ycgO*::P_{gerY}-*optRBS-yfp*-linker-*gerY* (spec) Δ*safA::tet*] and **BDR4471 [Δ*gerY* + *ycgO*::P_{gerY}-*optRBS-yfp*-linker-*gerY* (spec) Δ*cotE::cat*]** were generated by transforming BJA020a and BJA018 with pFR73, respectively.

Plasmids construction

pFR54 [*amyE*::*yqeF* (spec, amp)], **pFR57** [*amyE*::*gerY* (spec, amp)] and **pFR58** [*amyE*::*ydgB* (spec, amp)] were constructed in a two-way ligation with pLD30 cut with BamHI and EcoRI and BamHI-EcoRI PCR products containing the *yqeF, gerY* and *ydgB* genes, respectively. These PCR products were amplified with oligonucleotide primers oFR229/oFR230 for *yqeF,* oFR235/oFR236 for *gerY*, and oFR243/oFR244 for *ydgB* and gDNA from *B. subtilis* 168 as template. pLD30 is a double-crossover vector for ectopic integration at the *amyE* locus (Rudner lab stock).

pFR56 [*amyE*::*yodN* (spec, amp)] was constructed in a two-way ligation with a BamHI-BamHI PCR product containing the *yodN* gene (amplified with oligonucleotide primers oFR233/oFR234 and gDNA from *B. subtilis* 168 as template) and pLD30 cut with BamHI.

pFR60 [*yycR::*P_{ydgB}*-optRBS-yfp* (spec*,* amp)], **pFR61** [*yycR::*P_{gerY}*-optRBS-yfp* (spec*,* amp)], **pFR62** [*yycR::*P_{yodN}*-optRBS-yfp* (spec*,* amp)] and **pFR63** [*yycR::*P_{yqeF}*-optRBS-yfp* (spec*,* amp)] were constructed in a two-way ligation with pCB138 cut with EcoRI and HindIII and EcoRI-HindIII PCR products containing the promoters of *ydgB, gerY, yodN* and *yqeF,* respectively. These PCR products were amplified with oligonucleotide primers oFR347/oFR348 for *ydgB,* oFR349/oFR350 for *gerY*, oFR351/oFR352 for *yodN*, and oFR353/oFR354 for *yqeF* and gDNA from *B. subtilis* 168 as template. pCB138 [(*yycR::*P_{sspB}*-optRBS-yfp spec*)] is a double-crossover vector for ectopic integration at the *yycR* locus, containing the promoter of the *sspB* gene (which was removed with the EcoRI-HindIII cut) and an optRBS-*yfp* reporter (Barajas-Ornelas and Rudner, unpublished).

pFR66 [*ycgO::ydgB-yfp* (spec*,* amp)] was constructed in a three-way ligation with an EcoRI-XhoI PCR product containing the *ydgB* gene without stop codon (amplified with oligonucleotide primers oFR345/oFR346 and gDNA from *B. subtilis* 168 as template), a BamHI-XhoI fragment containing the *yfp* gene cut from pFR20 [*ycgO*::P_{spoIVF}*-spoIVFB*Δ10-*yfp* (erm, amp)] and pKM083 cut with BamHI-EcoRI. pKM83 is an ectopic integration vector for double crossover integration at the *ycgO* locus (Rudner lab stock)

pFR73 [*ycgO*::P_{gerY}*-optRBS-yfp*-linker-*gerY* (spec, amp)] was constructed in a three-way ligation with an EcoRI-XhoI PCR product containing the promoter of *gerY* fused to an *optRBS-yfp* reporter without stop codon (amplified with oligonucleotide primers oFR411/oFR349 and pFR61 as template), a XhoI-BamHI fragment containing the LEGSGSG linker fused to the *gerY* gene without start codon and pKM083 cut with EcoRI-BamHI.

pFR74 [*ycgO:: gerPA-yfp* (spec*,* amp)] was constructed in a two-way ligation with an EcoRI-XhoI PCR product containing the *gerPA* gene without stop codon (amplified with oligonucleotide primers oFR418/oFR419 and gDNA from *B. subtilis* 168 as template) and pFR66 cut with EcoRI-XhoI.

Supplemental references

1. Ferguson CC, Camp AH and Losick R. 2007. *gerT*, a newly discovered germination gene under the control of the sporulation transcription factor σ^K in *Bacillus subtilis*. J Bacteriol 189: 7681-7689

2. Amon JD, Yadav AK, Ramírez-Guadiana FH, Meeske AJ, Cava F, Rudner DZ. 2020. SwsB and SafA are required for CwlJ dependent spore germination in *Bacillus* subtilis. J Bacteriol 202:e00668-19. https://doi.org/10.1128/JB.00668-19

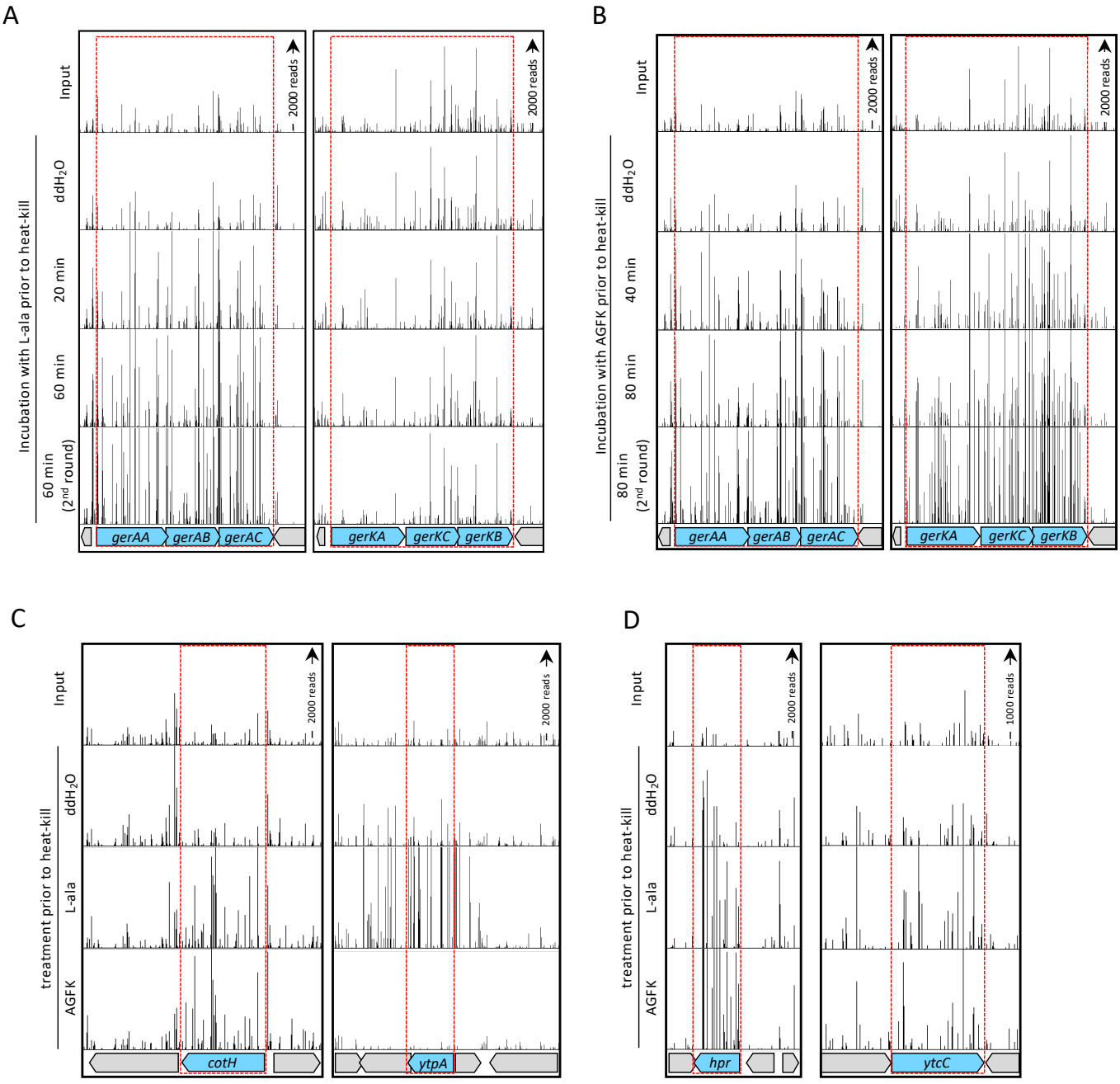


Figure S1. Transposon insertion profiles from six regions of the *B. subtilis* chromosome.
Red boxes highlight the *gerA* and *gerK* operons and *cotH, ytpA, hpr* and *ytcC.* **(A)** Transposon insertions were overrepresented in the *gerA* genes after 20min incubation with L-alanine prior to heat-kill compared to incubation with H_2O. Transposon insertions became more overrepresented after 60 min incubation with L-alanine and even more so after a second round of enrichment. By contrast, the transposon insertion profile of the *gerK* locus was unchanged under the same conditions. **(B)** Transposon insertions in the *gerK* genes were enriched when AGFK was the germinant and the incubation times were 40 and 80 min. Insertions in the *gerA* locus were also enriched in the presence of AGFK under these conditions. **(C)** Examples of genes in which transposon insertions were overrepresented after incubation with germinant but spores derived from in-frame deletions were only modestly (*cotH* and *ytpA*) or weakly (*hpr* and *ytcC*) impaired in germination as assessed by drop of optical density (**Fig. S2**). Transposon insertion profiles after the second round of enrichment are shown in panels C and D.

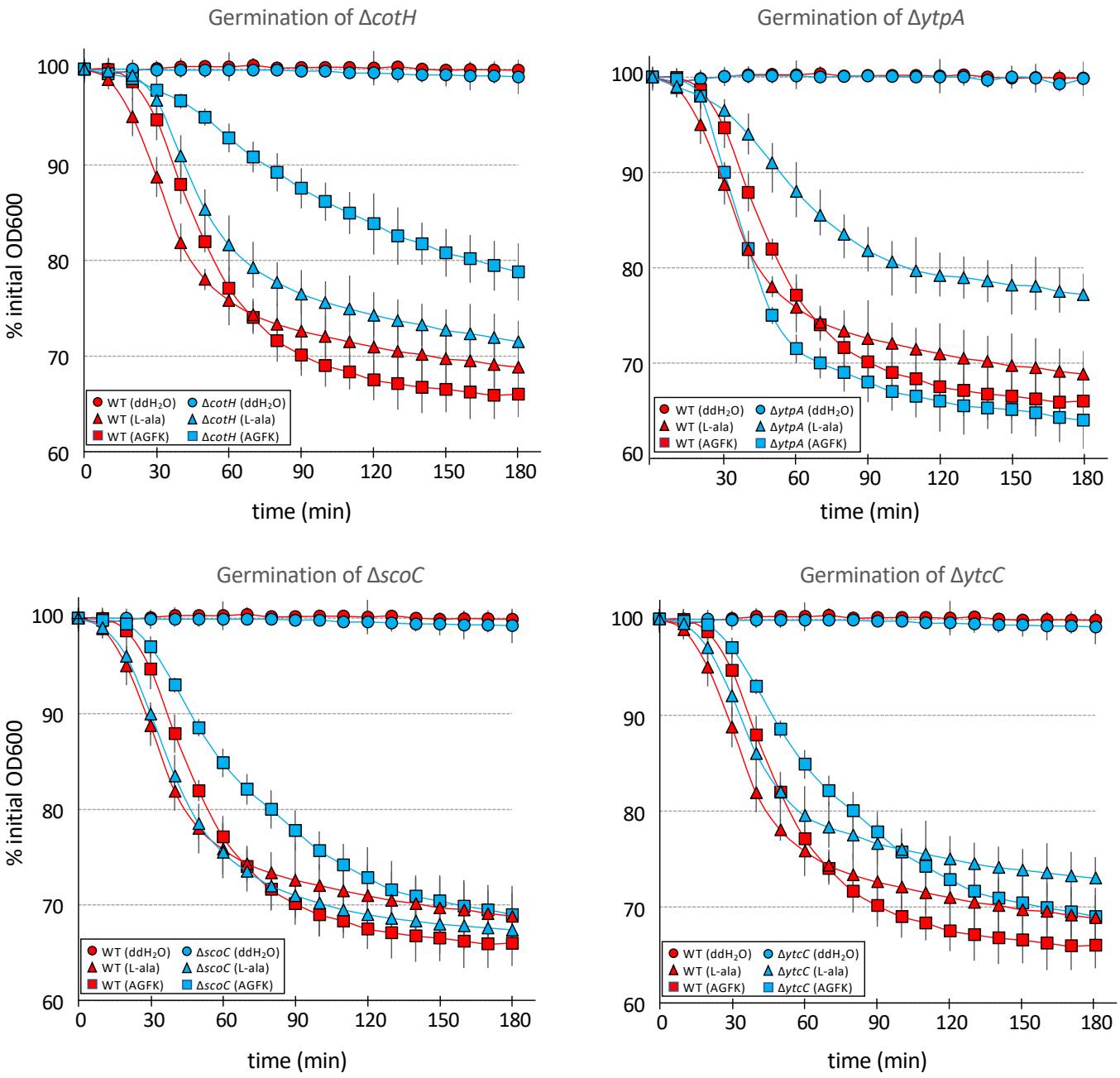


Figure S2. Germination assays for four mutants identified in the Tn-seq screen.
Germination assays of wild-type (WT), Δ*cotH*, Δ*ytpA*, Δ*scoC* and Δ*ytcC* spores in response to L-alanine or AGFK as assessed by the percent reduction in OD_{600} over time. Purified spores from the indicated strains were incubated with germinant at 37 ˚C and the drop in optical density was monitored over time. Representative data from one of three biological replicates are shown. Error bars indicate ± SD of three technical replicates.

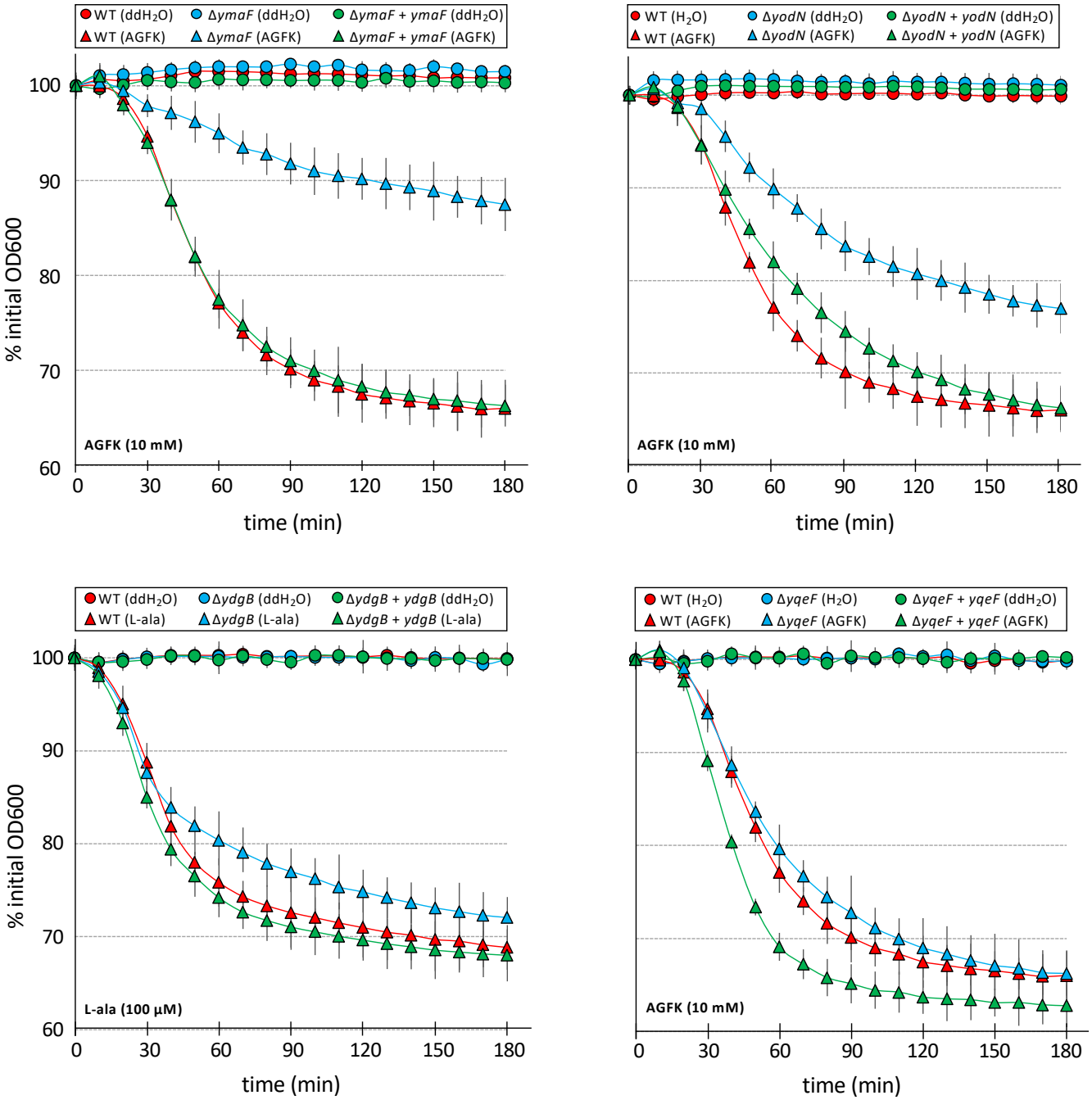
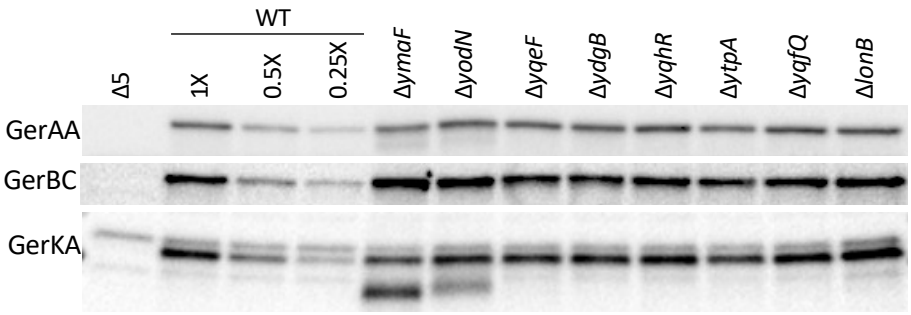


Figure S3. YmaF, YodN, YdgB and YqeF are required for efficient spore germination.
Germination of wild-type (WT), Δ*ymaF*, Δ*yodN*, Δ*ydgB* and Δ*yqeF* spores in response to L-alanine (100 µM) or to AGFK (10 mM) was assessed by the percent reduction in OD_{600} over time. Purified spores from the indicated strains were incubated with the indicated germinant at 37 ˚C and the drop in optical density was monitored over time. The germination defect of all four mutants could be complemented *in trans*. Representative data from one of three biological replicates are shown. Error bars indicate ± SD of three technical replicates.

A



B

Protein	Δ5	WT (1x)	WT (0.5x)	WT (0.25x)	Δ*ymaF*	Δ*yodN*	Δ*yqeF*	Δ*ydgB*	Δ*yqhR*	Δ*ytpA*	Δ*yqfQ*	Δ*lonB*
GerAA	0	1.0	0.5	0.2	0.9	1.2	1.2	1.2	1.0	1.2	1.0	0.8
GerBC	0	1.0	0.4	0.2	1.2	1.3	1.1	1.0	0.9	1.1	0.9	0.9
GerKA	0	1.0	0.4	0.2	0.5	0.9	1.1	1.2	1.0	1.2	1.0	0.9

Figure S4. Levels of the germinant receptors in spores remain largely unchanged in the germination defective mutants. (A) Representative immunoblots of spore lysates. Purified spores from the indicated strains were mechanically lysed, normalized based on total protein, and ~10 µg were subjected to SDS-PAGE followed by immunoblot analysis to detect of GerAA, GerBC and GerKA. The wild-type (WT) spore lysate was diluted 2-fold and 4-fold into a spore lysate from a strain lacking all 5 germinant receptors (Δ5) to maintain the same amount of total protein loaded. **(B)** Quantification of GerAA, GerBC, and GerKA using Fiji-ImageJ software is shown. In virtually all cases, the level of each germinant receptor was within ~10% of wild-type.

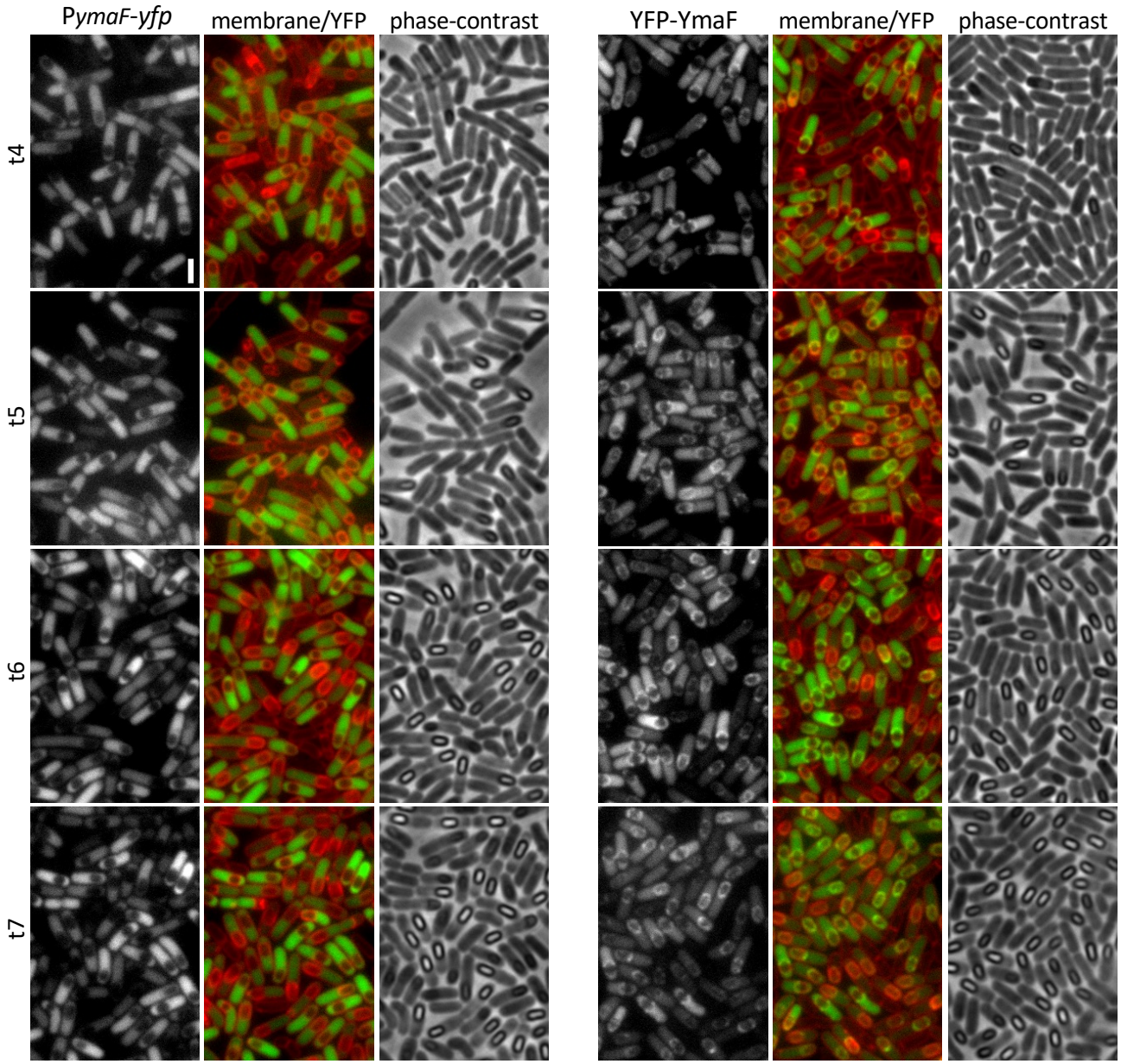


Figure S5. Expression of *ymaF*, *yodN*, *ydgB*, and *yqeF* during sporulation.
(A) Representative fluorescence and phase-contrast images of strains harboring a *yfp* fusion to the *ymaF* promoter (left) or a translational YFP-YmaF fusion (right) during a sporulation time-course. **(B)** Representative fluorescence and phase-contrast images of strains harboring either a *yfp* fusion to the *ydgB* promoter (left) or a translational YdgB-YFP fusion (right) during a sporulation time-course. **(C)** Representative fluorescence and phase-contrast images of a strain harboring a *yfp* fusion to the *yodN* promoter during a sporulation time-course. **(D)** Representative images during a full sporulation time-course of a strain harboring a *yfp* fusion to the *yqeF* promoter and a strain lacking a *yfp* reporter as control for the weak signal of the P*yqeF-yfp* fusion. The membranes (false-colored red) were stained with the fluorescent dye TMA-DPH and merged with the YFP signal (false-colored green). Scale bars indicate 2 µm.

Figure S5B
Ramírez-Guadiana et al.

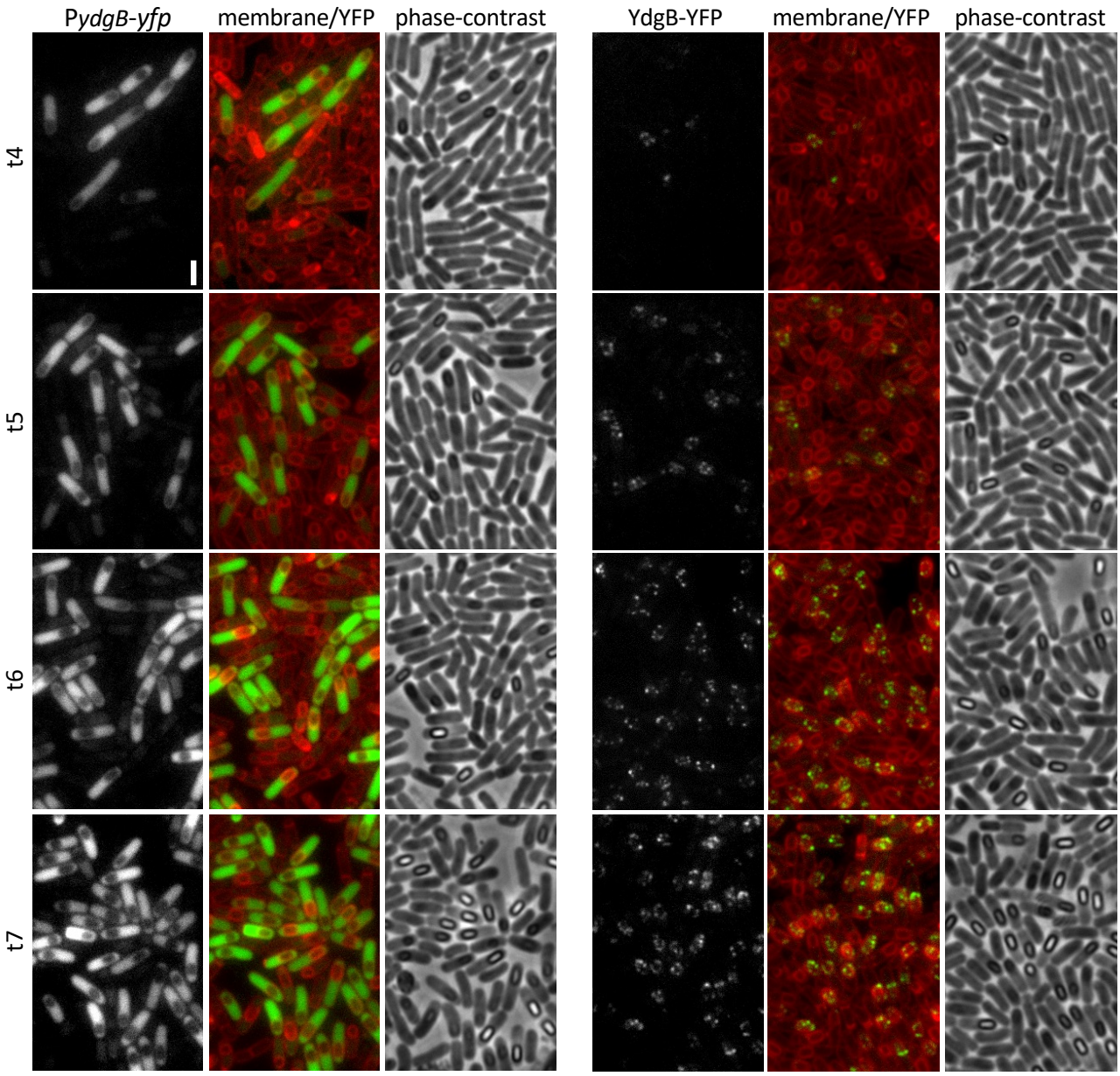


Figure S5C
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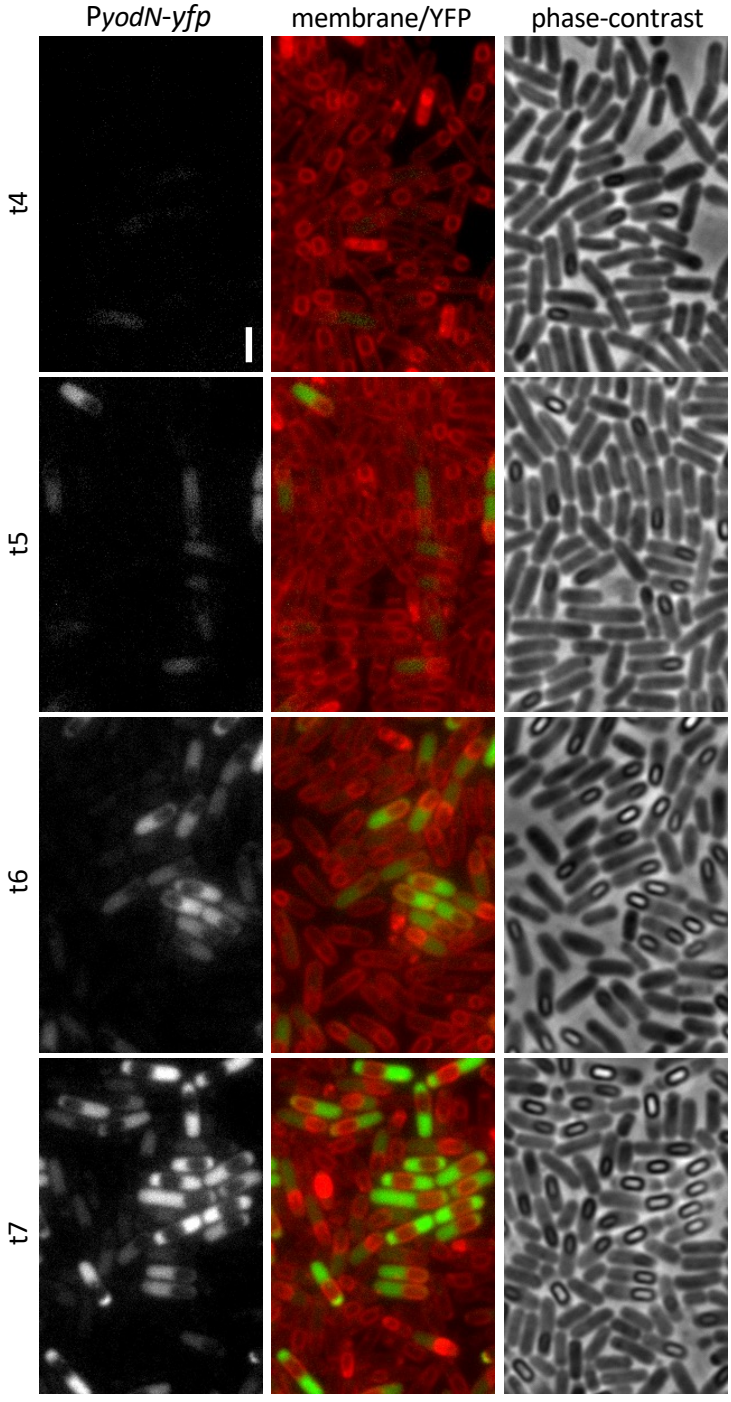

Figure S5D
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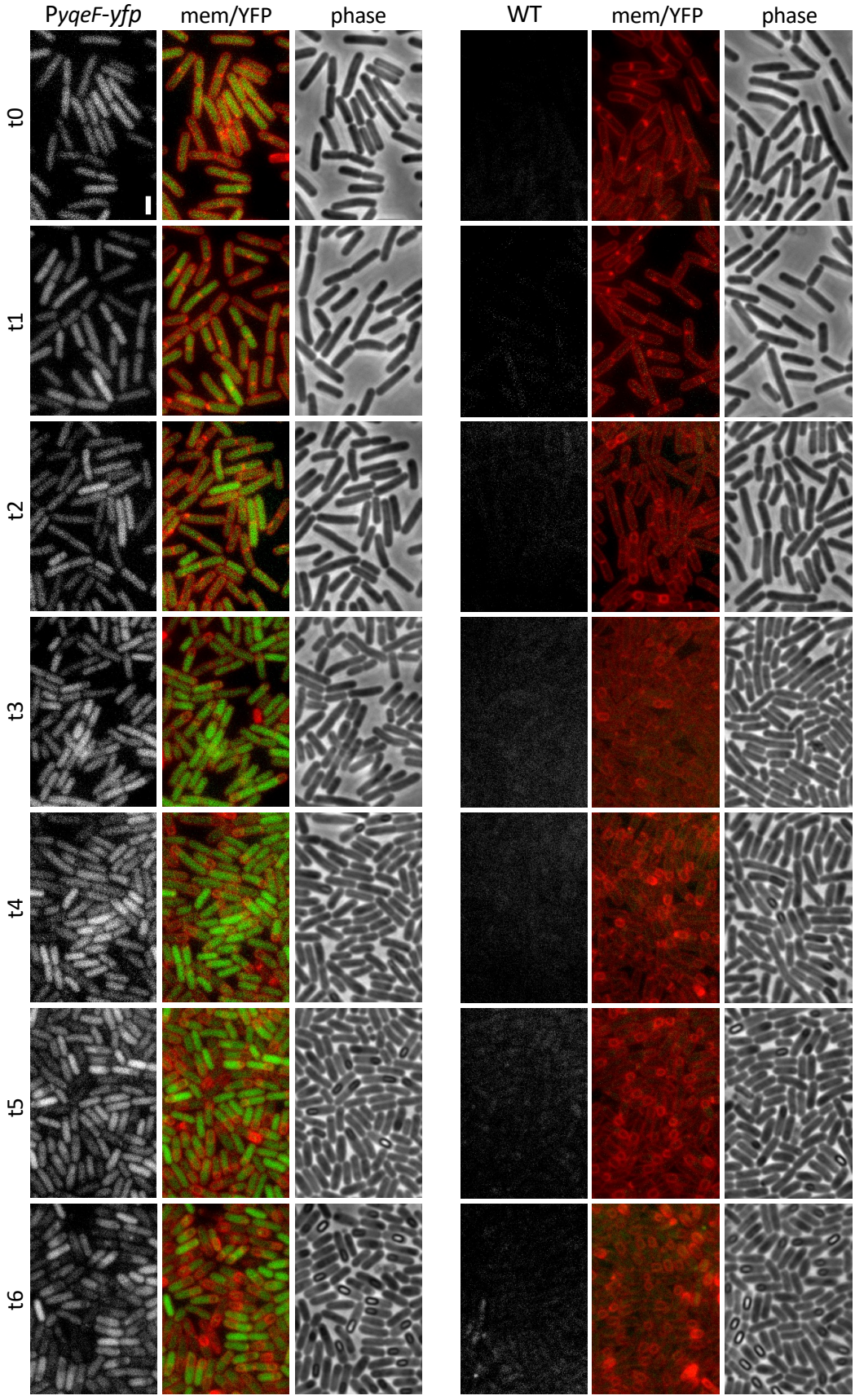

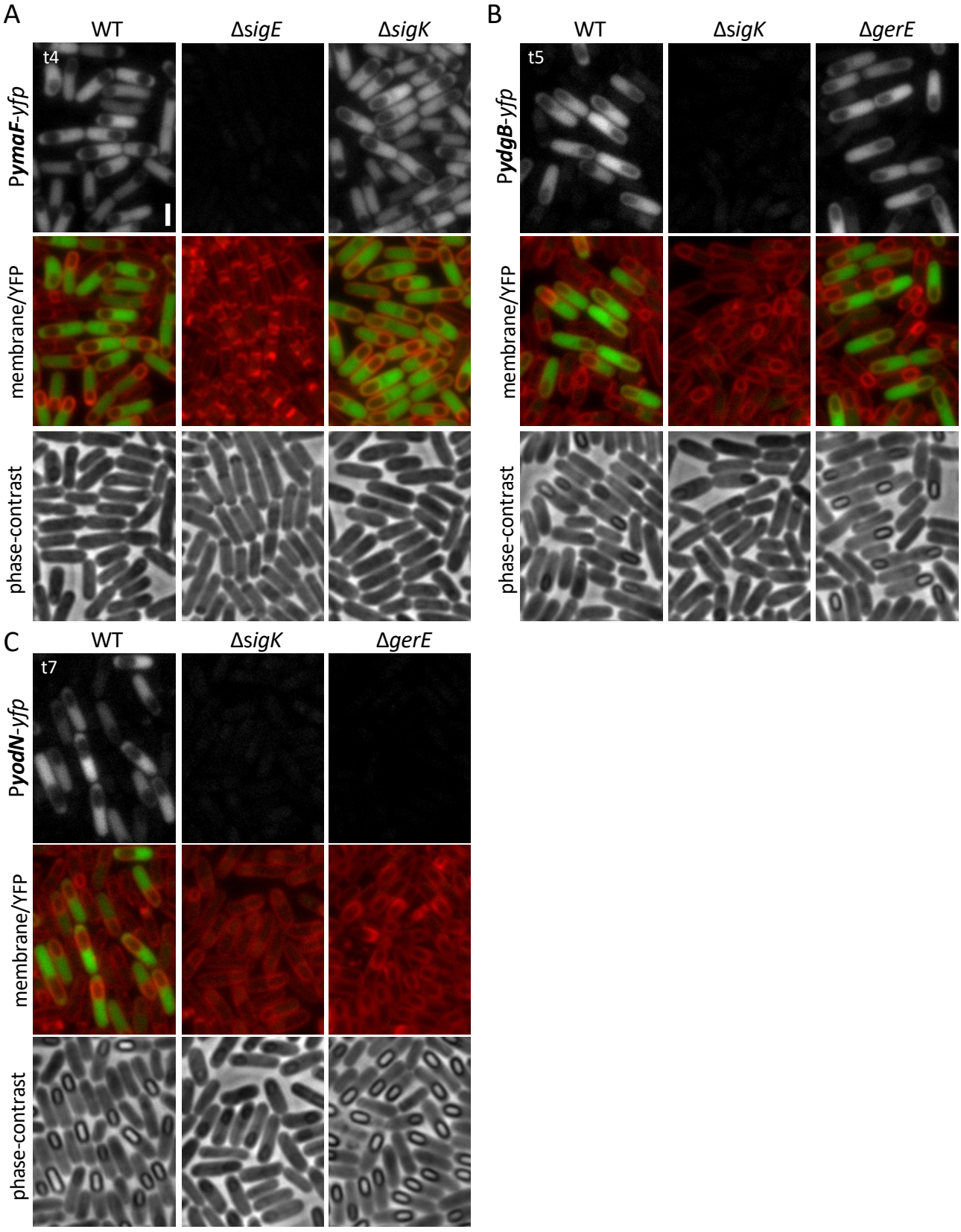

Figure S6. YmaF, YdgP and YodN are produced in the mother cell under the control of SigE, SigK, and GerE, respectively. Representative fluorescence and phase-contrast images of strains harboring *yfp* fusions to the promoters of *ymaF*, yodN and *ydgB* during at the indicated times in hours after the onset of sporulation. **(A)** Sporulating cells lacking SigE but not SigK fail to activate the P*ymaF*-YFP reporter. **(B)** Sporulating cells lacking SigK but not GerE fail to activate the P*ydgB*-YFP reporter. **(C)** Sporulating cells lacking SigK and GerE fail to activate the P*yodN*-YFP reporter. The membranes (false-colored red) were stained with the fluorescent dye TMA-DPH and merged with the YFP signal (false-colored green). Scale bars indicate 2 µm.

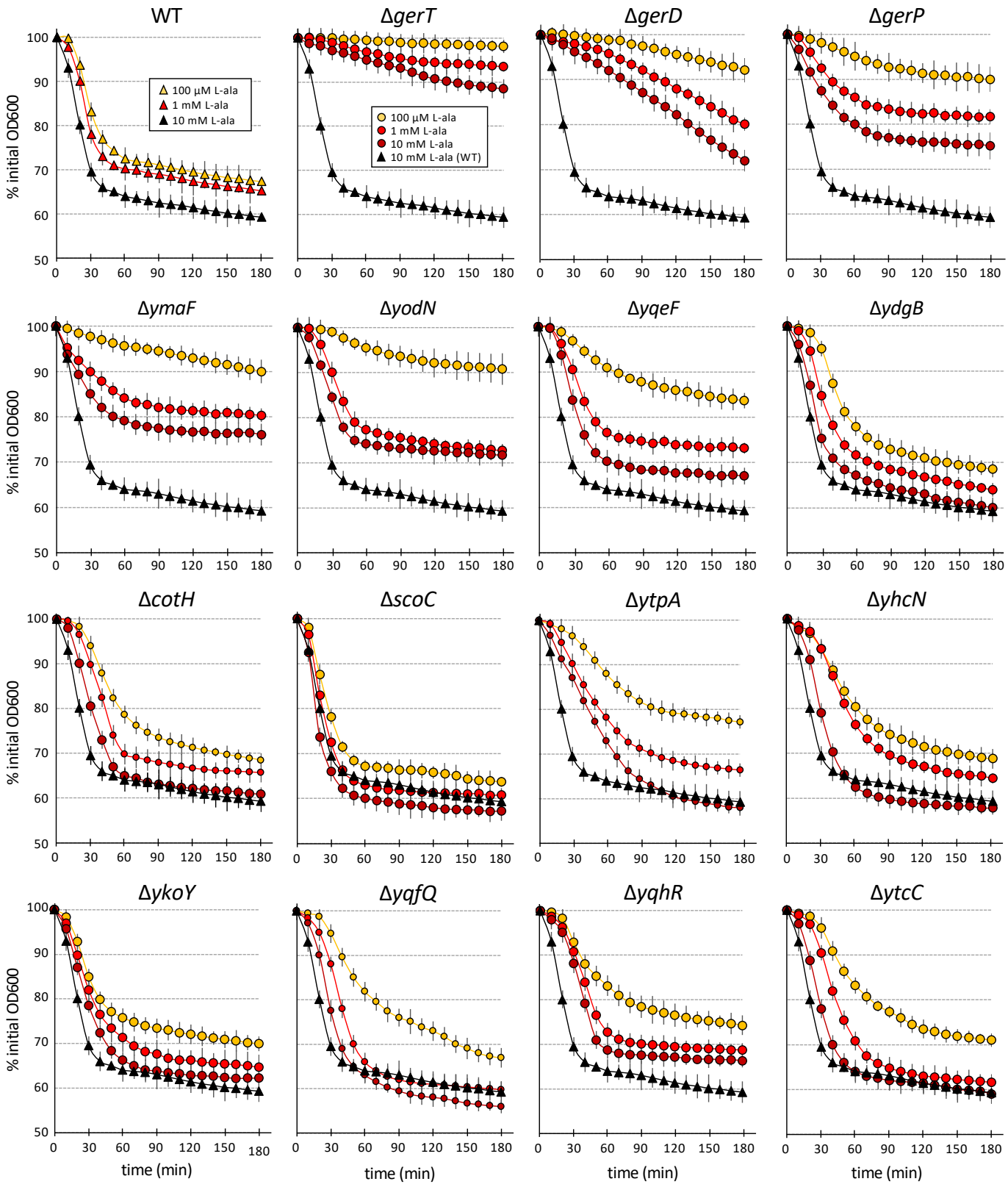

Figure S7. Germination assays of mutants identified in the Tn-seq screen with different concentrations of L-alanine. Germination of wild-type (WT) and the indicated mutant spores in response to 100 µM, 1 mM, and 10 mM L-alanine was assessed by the percent reduction in OD_{600} over time. Purified spores from the indicated strains were incubated with germinant at 37 ˚C and the drop in optical density was monitored over time. Representative data from one of three biological replicates are shown. Error bars indicate ± SD of three technical replicates.

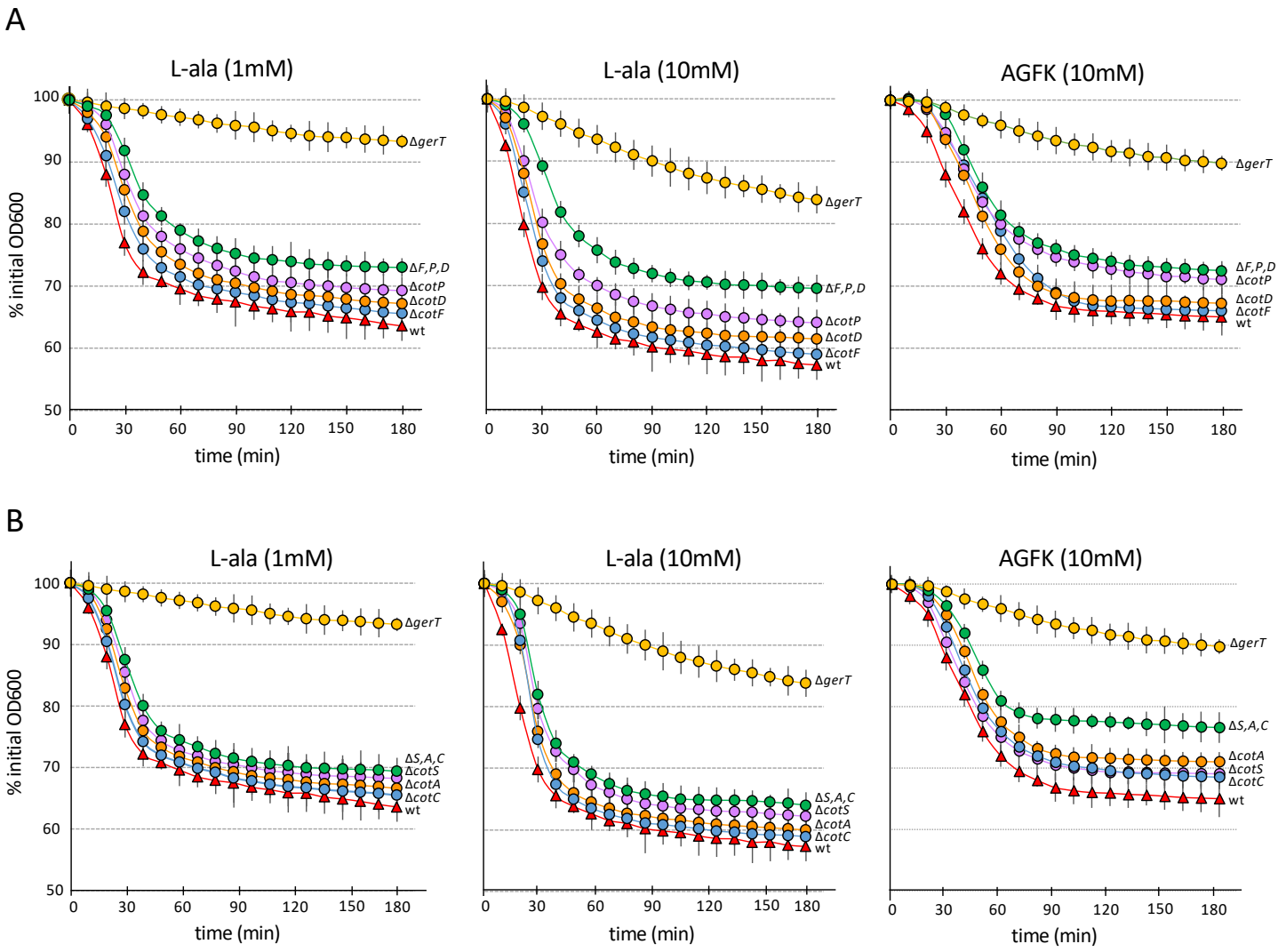

Figure S8. Germination response of inner and outer coat protein mutants to L-alanine and AGFK. Spores of wild-type, Δ*gerT,* the inner coat mutants Δ*cotP*, Δ*cotD*, Δ*cotF* (**A**) and the outer coat mutants Δ*cotS*, Δ*cotA*, Δ*cotC* (**B**) were germinated with L-alanine or AGFK and the drop in optical density was monitored over time. Representative data from one of three biological. Error bars indicate ± SD of three technical replicates.

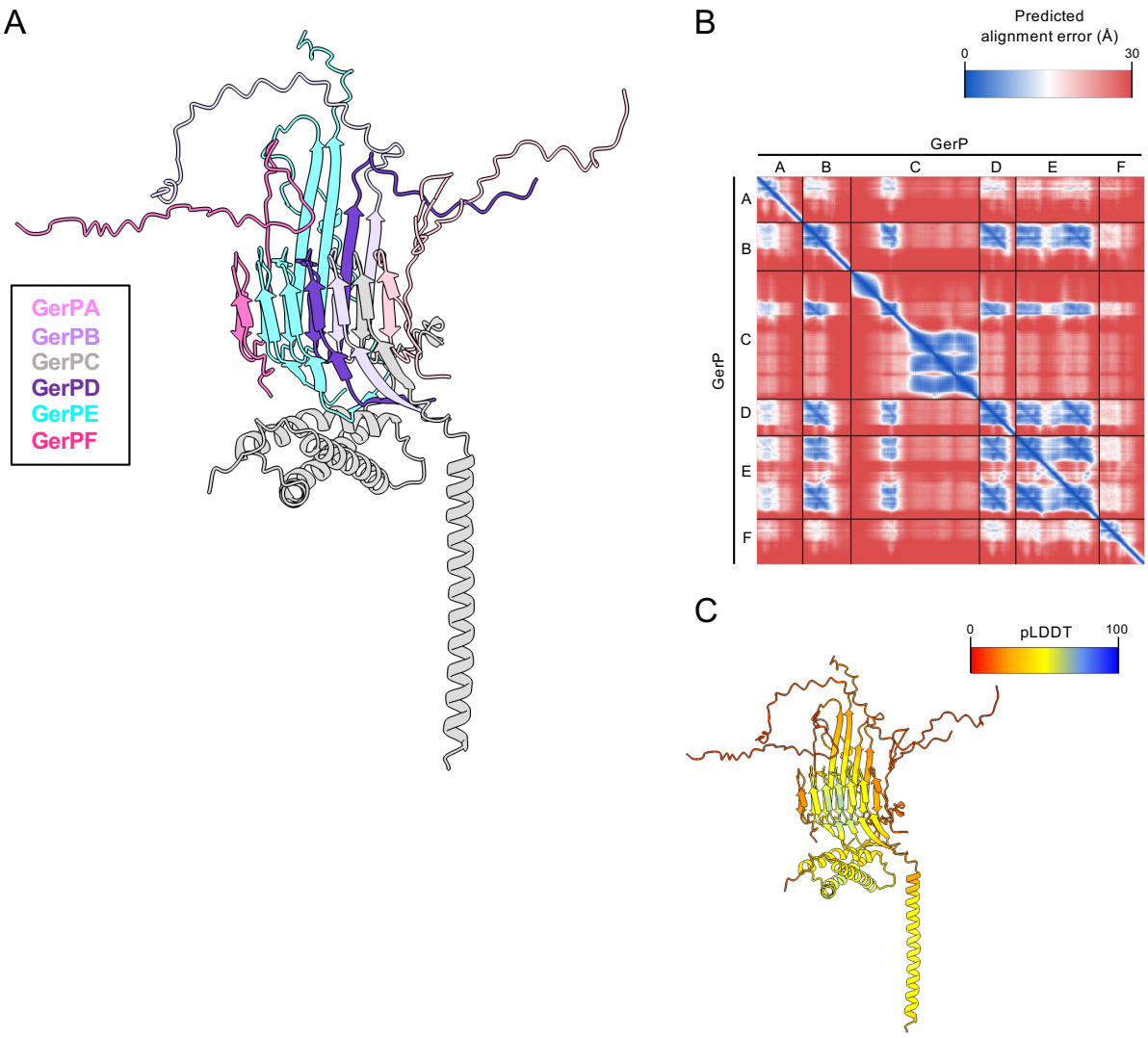

Figure S9. Alphafold2 predicts that the proteins in the GerP operon form a complex.
(A) Alphafold2 predicted model of the GerP complex. The chain of each subunit is colored as indicated. **(B)** Predicted alignment error (PAE) plot of the predicted structure shown in (A). The plot displays the alignment error predicted between each residue of the complex from N to C-terminus. The black lines indicate the start/end of the annotated GerP protein. PAE values for each subunit are the lowest (dark blue) in the region of the beta strands that make contacts in the predicted complex. **(C)** Predicted structure in (A) colored according to the predicted local distance difference test (pLDDT).

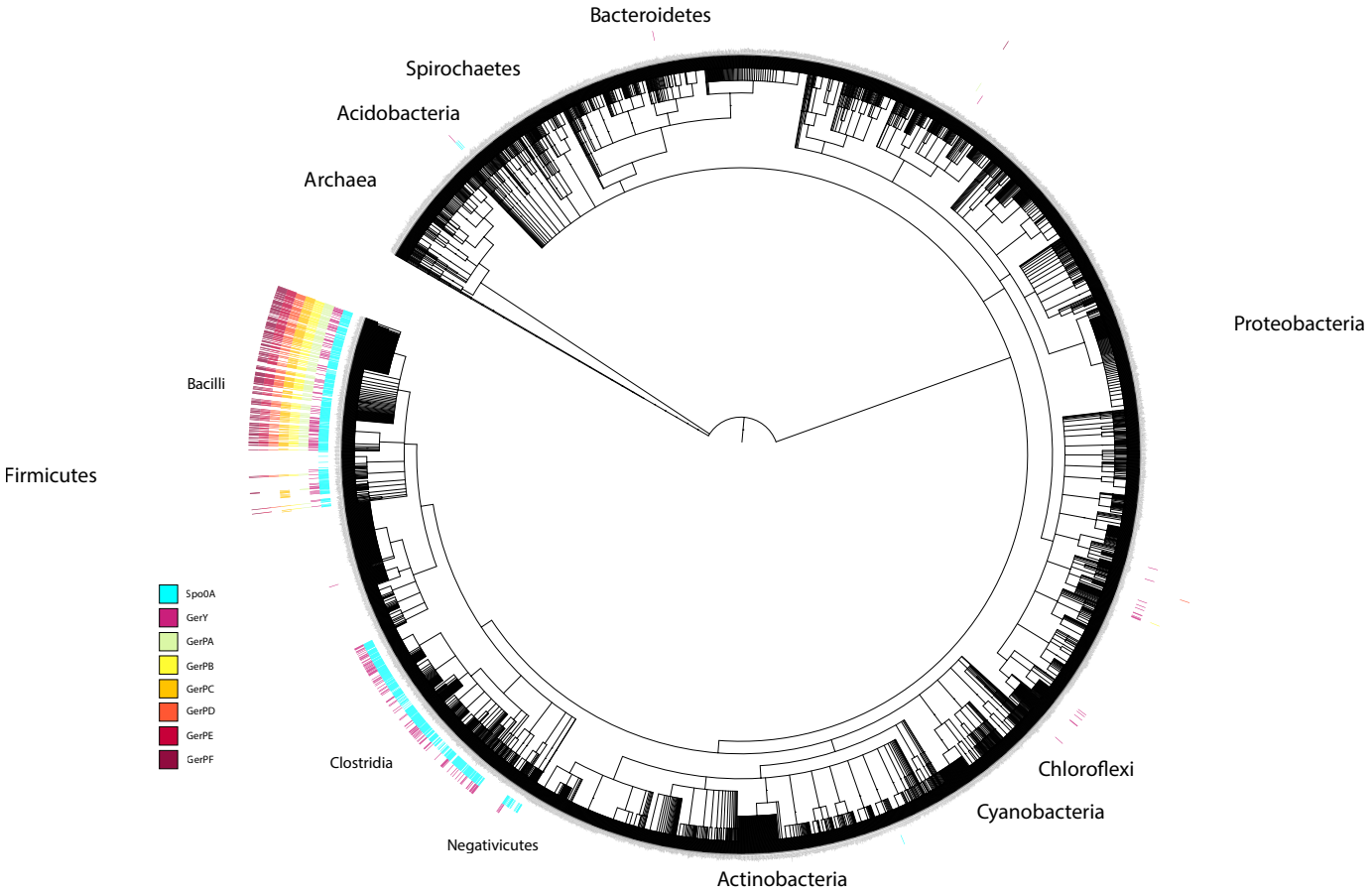

Figure S10. GerY is conserved among *Bacilli* and *Clostridia*

Phylogenetic tree showing the co-occurrence of GerY and GerP in a diverse set of 5,767 bacterial taxa. The amino acid sequences of *B. subtilis* GerY and the GerP proteins and the C-terminal domain of Spo0A (amino acids 127-247) served as queries in a PSI-BLAST search against the NCBI 'RefSeq' database with 6 iterations and an *e*-value cutoff of $1x10^{-5}$. GerT was not included in the analysis because its α-crystallin/Hps20 domain is extremely well conserved in prokaryotic and eukaryotic organisms. This analysis was performed through the Harvard Medical School Research Computing Orchestra cluster. The phylogenetic tree was constructed in PhyloT (http://phylot.biobyte.de/) and the PSI-BLAST search results were plotted against the tree. The tree was visualized and annotated using the Interactive Tree Of Life web-based tool (iTOL, v3; http://itol.embl.de).

Table S2. *Bacillus subtilis* strains used in this study.

Strain	Genotype	Source	Figures
BDR2413	Wild-type 168 (*trpC2*)	Zeigler *et al*. 2008	2, 4A, 4C, 5, S2, S3, S4, S5D, S7, and S8
BDR4328	Δ*gerY::lox72*	This work	1A, 2, 4A, 5, S3, S4, and S7
BDR4376	Δ*gerY::lox72 amyE::gerY* (*spec*)	This work	2 and S3
BDR4306	Δ*ydgB::lox72*	This work	1A, S3, S4, and S7
BDR4381	Δ*ydgB amyE::ydgB* (*spec*)	This work	2 and S3
BDR4305	Δ*yodN::lox72*	This work	1A, 2, S3, S4, and S7
BDR4378	Δ*yodN amyE::yodN* (*spec*)	This work	2 and S3
BDR4324	Δ*yqeF::lox72*	This work	1A, S3, S4, and S7
BDR4377	Δ*yqeF::lox72 amyE::yqeF* (spec)	This work	2 and S3
BDR4307	Δ*cotH::lox72*	This work	S2 and S7
BDR4323	Δ*ytpA::lox72*	This work	S2, S4, and S7
BDR4327	Δ*scoC::lox72*	This work	S2 and S7
BDR4329	Δ*ytcC::lox72*	This work	S2 and S7
BDR4347	Δ*yqhR::lox72*	This work	S4 and S7
BDR4330	Δ*yqfQ::lox72*	This work	S4 and S7
BDR4326	Δ*lonB::lox72*	This work	S4
BDR4406	*yycR*::P_{ydgB}-*optRBS-yfp* (*spec*)	This work	3A and S5B
BDR4407	*yycR*::P_{gerY}-*optRBS-yfp* (*spec*)	This work	3A and S5A
BDR4408	*yycR*::P_{yodN}-*optRBS-yfp* (*spec*)	This work	3A and S5C
BDR4409	*yycR*::P_{yqeF}-*optRBS-yfp* (*spec*)	This work	3A and S5D
BDR4444	Δ*gerY::lox72 ycgO*::P_{gerY}-*optRBS-yfp-linker-gerY* (*spec*)	This work	3B, 4B and S5A
BDR4412	Δ*ydgB ycgO*::*ydgB-yfp* (*spec*)	This work	3B and S5B
BDR4486	*yycR*::P_{gerY}-*optRBS-yfp* (*spec*) Δ*sigE*::*erm*	This work	S6
BDR4487	*yycR*::P_{gerY}-*optRBS-yfp* (*spec*) Δ*sigK*::*erm*	This work	S6
BDR4488	*yycR*::P_{ydgB}-*optRBS-yfp* (*spec*) Δ*sigK*::*erm*	This work	S6
BDR4489	*yycR*::P_{ydgB}-*optRBS-yfp* (*spec*) Δ*gerE::erm*	This work	S6
BDR4490	*yycR*::P_{yodN}-*optRBS-yfp* (*spec*) Δ*sigK*::*erm*	This work	S6
BDR4491	*yycR*::P_{yodN}-*optRBS-yfp* (*spec*) Δ*gerE::erm*	This work	S6
BDR4313	Δ*gerP::lox72*	This work	4A, 5, and S7
BDR4310	Δ*gerT::lox72*	This work	4A, 4C, 5, S7, and S8
BDR4332	Δ*gerD::lox72*	This work	1A and S7
BDR4325	Δ*yhcN::lox72*	This work	S7
BDR4343	Δ*ykoY::lox72*	This work	S7
BDR4461	*gerT*Ω*gerT-gfp* (*spec*)	This work	4B
BDR4466	*gerT*Ω*gerT-gfp* (*spec*) Δ*safA::tet*	This work	4B
BDR4470	*gerT* Ω *gerT-gfp* (*spec*) Δ*cotE::cat*	This work	4B
BDR4460	*ycgO*::*gerPA-yfp* (*spec*)	This work	4B
BDR4465	*ycgO::gerPA-yfp* (*spec*) Δ*safA::tet*	This work	4B
BDR4469	*ycgO::gerPA-yfp* (*spec*) Δ*cotE::cat*	This work	4B
BDR4467	Δ*gerY::lox72 ycgO*::P_{gerY}-*optRBS-yfp-linker-gerY* (*spec*) Δ*safA::tet*	This work	4B

BDR4471	Δ*gerY::lox72 ycgO*::P_{gerY}-*optRBS-yfp-linker-gerY* (*spec*) Δ*cotE::cat*	This work	4B
BDR4423	Δ*cotP::lox72*	This work	4C and S8
BDR4435	Δ*cotD::lox72*	This work	4C and S8
BDR4424	Δ*cotF::lox72*	This work	4C and S8
BDR4440	Δ*cotP::lox72* Δ*cotD::lox72* Δ*cotF::lox72*	This work	4C and S8
BDR4427	Δ*cotS::lox72*	This work	4C and S8
BDR4426	Δ*cotA::lox72*	This work	4C and S8
BDR4436	Δ*cotC::lox72*	This work	4C and S8
BDR4439	Δ*cotS::lox72* Δ*cotA::lox72* Δ*cotC::lox72*	This work	4C and S8
BDR4449	Δ*gerY::lox72* Δ*gerT::erm*	This work	5A
BDR4450	Δ*gerY::lox72* Δ*gerP::lox72*	This work	5A
BDR4477	Δ*cotB::lox72*	This work	5B
BDR4479	Δ*cotB::lox72* Δ*gerT::lox72*	This work	5B
BDR4480	Δ*cotB::lox72* Δ*gerP::lox72*	This work	5B
BDR4478	Δ*cotB::lox72* Δ*gerY::erm*	This work	5B

All *erm*(R) deletion mutants are derived from the *Bacillus subtilis* knock-out collection.
All unmarked mutants are in-frame deletions generated by Cre-mediated recombination and contain a *lox72* scar.

Table S3. Plasmids used in this study.

Plasmid	Description	Source
pFR54	*amyE*::*yqeF* (spec, amp)	This work
pFR56	*amyE*::*yodN* (spec, amp)	This work
pFR57	*amyE*::*gerY* (spec, amp)	This work
pFR58	*amyE*::*ydgB* (spec, amp)	This work
pFR60	*yycR*::P_{ydgB}-*optRBS-yfp* (spec, amp)	This work
pFR61	*yycR*::P_{gerY}-*optRBS-yfp* (spec, amp)	This work
pFR62	*yycR*::P_{yodN}-*optRBS-yfp* (spec, amp)	This work
pFR63	*yycR*::P_{yqeF}-*optRBS-yfp* (spec, amp)	This work
pFR66	*ycgO*::*ydgB-yfp* (spec, amp)	This work
pFR73	*ycgO*::P_{gerY}-*optRBS-yfp-linker-gerY* (spec, amp)	This work
pFR74	*ycgO*::*gerPA-yfp* (spec, amp)	This work

Table S4. List of oligonucleotide primers used in this study.

Primer	Sequence	Use / gene
oDR1049	*gagggaggaaaggcagga*	Erm cassette containing *lox71* and *lox66* sites (for isothermal assembly)
oDR1050	*cgccgtatctgtgctctc*	
oFR217	ggagacaagatcgcttgccgt	To build Δ*gerP* operon (upstream fragment for Gibson assembly)
oFR218	*tcctgcctttcctccctc*ggccggcatacgagcacatc	
oFR219	*gagagcacagatacggcg*ggaaacggataaaagcagccgct	To build Δ*gerP* operon (downstream fragment for Gibson assembly)
oFR220	gccggcagagcattatcagag	
oFR229	gccGGATCCcggataacctgtttccagtgc	To build pFR54 (*yqeF* gene)
oFR230	gccGAATTCtatatcactttggctgcgcgc	
oFR233	gccGGATCCgtgcacggcagataatagctg	To build pFR56 (*yodN* gene)
oFR234	gccGGATCCtgatggaagcactggacgga	
oFR235	gccGGATCCgtaaccgaacccgaacggtc	To build pFR57 (*gerY* gene)
oFR236	gccGAATTCgccgtcggtccgactaaaat	
oFR243	gccGGATCCctgatcctgtttgccagcttc	To build pFR58 (*ydgB* gene)
oFR244	gccGAATTCtaggcgaaagatcccctaagg	
oFR347	gccGAATTCccacgagtaatgttactca	To build pFR60 (*ydgB* promoter)
oFR348	gccAAGCTTccatctttctgtacagtac	
oFR349	gccGAATTCgtaaccgaacccgaacggt	To build pFR61 (*gerY* promoter)
oFR350	gccAAGCTTgattcgcccaatcaaatcc	
oFR351	gccGAATTCcttgggatcaatacgagtctg	To build pFR62 (*yodN* promoter)
oFR352	gccAAGCTTccgcaacatttctcatacg	
oFR353	gccGAATTCgataacctgtttccagtgc	To build pFR63 (*yqeF* promoter)
oFR354	gccAAGCTTgcctcactttaatgttatacc	
oFR345	gccGAATTCaattgagcatcactccggggc	To build pFR66 (*ydgB* gene without stop codon)
oFR346	cggCTCGAGatctaaaattggcggtggtgc	
oFR349	gccGAATTCgtaaccgaacccgaacggt	To build pFR73 (*gerY* promoter + *optRBS-yfp* without stop codon)
oFR411	cggCTCGAGgtatagttcatccatgccatg	
oFR412	cggCTCGAG**ggaagcggaagcgga**aacacattgggcctgtatcag	To build pFR73 (linker + *gerY* without start codon)
oFR413	gccGGATCCCgccgtcggtccgactaaaat	
oFR418	gccGAATTCggcggatagcggcatccttcg	To build pFR74 (*gerPA* gene without stop codon)
oFR419	cggCTCGAGcgcattggccacgatcggctg	

Homology regions for isothermal assembly are indicated in italics.
Restriction endonuclease recognition sites are indicated in capital letters.
The linker in oligonucleotide primer oFR412 is in bold.