SUPPLEMENTARY MATERIAL

The *Bacillus subtilis* germinant receptor GerA triggers premature germination in response to morphological defects during sporulation.

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- Supplemental methods.
- Figures S1 to S15.
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Supplemental Methods

Strains construction

For gene deletion mutants that were derived from the *Bacillus subtilis* knock-out collection, each deletion was confirmed by PCR using an oligonucleotide primer (oKO0) within the erythromycin resistance gene and a gene-specific primer.

BDR3311 [Δ*gerAB::erm*] (PY79) was constructed by transforming PY79 with a PCR product amplified with primers oFR1 and oFR2 and gDNA from BAM786 as template.

BDR3342 [Δ*gerAB::erm*] (3610) was constructed by transforming DK1042 with a PCR product amplified with primers oFR1 and oFR2 and gDNA from BAM786 as template.

BDR3312 [Δ*ylbJ::spec*] (PY79) was generated by transforming PY79 with gDNA from RL2532.

BDR3325 [Δ*pdaB::tet*] (PY79) was generated with gDNA from RL3678 that was back-crossed twice into PY79.

BDR3313 [Δ*gerAB::erm* Δ*ylbJ:spec*] (PY79) was constructed by transforming BDR3312 with a PCR product amplified with primers oFR1 and oFR2 and gDNA from BAM786 as template.

BDR3326 [Δ*gerAB::erm* Δ*pdaB::tet*] (PY79) was constructed by transforming and BDR3325 with a PCR product amplified with primers oFR1 and oFR2 and gDNA from BAM786 as template.

BDR3343 [Δ*pdaB::tet*] (3610) was constructed by transforming DK1042 with a PCR product amplified with primers oKO261 and oFR4 and gDNA from BDR3325 as template.

BDR3344 [Δ*ylbJ::spec*] (3610) was constructed by transforming DK1042 with a PCR product amplified with primers oKO260 and oFR3 and gDNA from BDR3312 as template.

BDR3363 [Δ*gerAB::erm* Δ*pdaB::tet*] (3610) was constructed by transforming BDR3343 with a PCR product amplified with primers oFR1 and oFR2 and gDNA from BAM786 as template.

BDR3364 [Δ*gerAB::erm* Δ*ylbJ:spec*] (3610) was constructed by transforming BDR3344 with a PCR product amplified with primers oFR1 and oFR2 and gDNA from BAM786 as template.

BDR3371 [Δ*gerA::spec*] (168) and **BDR3372** [Δ*gerA::spec* Δ*ylbJ::erm*] (168) were constructed by isothermal assembly and direct transformation into *B. subtilis* 168 and BDR3149, respectively. The assembly reaction contained three fragments: a PCR product containing sequence upstream of *gerAA* (oligonucleotide primers oFR5 and

oFR6 and 168 gDNA as a template); a PCR product containing sequence downstream of *gerAC* (oligonucleotide primers oFR7 and oFR8 and 168 gDNA as a template), and a loxP-flanked spectinomycin resistance cassette (amplified using oligonucleotide primers oAM97 and oML078 and plasmid pWX466 as template).

In-frame deletions

Erythromycin-resistance cassette removal was carried out using the Cre/lox system. Briefly, *B. subtilis* strains carrying a loxP-flanked erythromycin resistance cassette were transformed with pDR244 (a temperature-sensitive plasmid that constitutively expresses Cre recombinase marked with a spectinomycin resistance cassette). Transformants were selected on LB supplemented with spectinomycin (100 µg/mL) at 30°C, a permissive temperature for pDR244 replication. Transformants were then streaked on LB without antibiotic and incubated at 42°C, a non-permissive temperature for plasmid replication. Single colonies were re-streaked on LB, LB (spec) and LB (macrolide-lincosamide-streptogramin B, MLS [1µg/mL erythromycin plus 25µg/mL lincomycin]) agar plates and incubated 37°C. Strains that grew on LB, but not on LB (spec) or LB (MLS) had lost pDR244 and the erythromycin-resistance cassette. Markerless deletions were confirmed by PCR with oligonucleotide primers flanking the deletion and oKO-1.

Supplemental Figure Legends

Figure S1: Transposon insertions in the *gerK* and *gerA* operons are under-represented after sporulation, germination, and outgrowth, in strains in which each is the only functional germinant receptor. Transposon insertion profiles from two different regions of the genome are depicted. Mariner-based transposon libraries from the indicated strains were grown in Difco sporulation medium (DSM) until nutrient exhaustion. A sample was saved from the wild-type (WT) library at the onset of starvation (T0). The cultures were sporulated for 24 hours. Vegetative cells and mutants defective in spore formation were killed by heat treatment at 80°C for 20 minutes. The spores were germinated and outgrown on LB agar plates and pooled. The transposon insertion sites were identified by deep sequencing and mapped to the *B. subtilis* 168 reference genome. The height of each line represents the number of the sequencing reads at this position. Boxes highlight the *gerA* operon and the *gerK* operon (red). The transposon insertions are under-represented in the *gerA* operon in the library generated in the strain in which GerA is the only functional germinant receptor. Similarly, transposon insertions are under-represented in the *gerK* operon in the library generated in the strain in which GerK is the only functional germinant receptor. As expected, no insertions in the *gerKB* and *gerAB* genes were detected in the strains in which these genes were deleted. Both strains lacking germinant receptors also harbor deletions in *yndB* and *yfkB* encoding the B-subunits of minor germinant receptors. The height of each line represents the number of the sequencing reads at this position. The maximum number of reads depicted was 300 for both genomic regions.

Figure S2(A-C): Representative transposon insertion profiles from 18 different regions of the genome. Mariner-based transposon libraries from the indicated strains were grown in Difco sporulation medium (DSM) until nutrient exhaustion. A sample was saved from the wild-type (WT) library at the onset of starvation (T0). The cultures were sporulated for 24 hours. Vegetative cells and mutants defective in spore formation were killed by heat treatment at 80°C for 20 minutes. The spores were germinated and outgrown on LB agar and pooled. The transposon insertion sites were identified by deep sequencing and mapped to the *B. subtilis* 168 reference genome. The strains lacking germinant receptors also harbor deletions in *yndB* and *yfkB* encoding the B-subunits of minor germinant receptors. Transposon insertions in these loci were over-represented in the absence of a functional GerA receptor compared to wild-type and strain in which GerA was the only functional receptor. The height of each line represents the number of the sequencing reads at this position. (**A**) Boxes highlight *ymxH,* the *spoVFAB* operon, *spoVR*, *uppP*, *ccdA*, *dacB-spmAB* operon, and *spoVS* genes. The maximum number of reads depicted for the genomic regions was 100 (*spoVS*), 200 (*ccdA*, *dacB-spmAB*, *uppP*), 300 (*ymxH*, *spoVFAB*) and 500 (*spoVR*). (**B**) Boxes highlight *yqzK*, the *spoVA*(*AB*) operon, *spoVG*, the *gerP* operon, *ytaF,* and *yrbG* genes. The maximum number of reads depicted for the genomic regions was 100 (*spoVG*), 200 (*spoVA*(*AB*)*), 400* (*yrbG*), 450 (*yqzK*), 600 (*ytaF*) and 800 (*gerP* operon). (**C**) Boxes highlight *ypbH*, the *spoVIGAB* operon, the *prkA-yhbH* operon, *yfmI*, the *prpC-prkC* operon, and *rho* genes. The maximum number of reads depicted for the genomic regions was 100 (*rho*), 200 (*prpC-prkC* and *spoVIGAB*)*,* 450 (*ypbH*), 600 (*prkA-yhbH*) and 1800 (*yfmI*).

Figure S3. Cytological suppression of Δ*ylbJ* in the absence of a functional GerA receptor. (A) Representative phase-contrast images of sporulating cells during a complete sporulation time course are shown. Wild-type (wt), Δ*gerAB*, Δ*ylbJ*, and the Δ*gerAB* Δ*ylbJ* double mutant were induced to sporulate by nutrient exhaustion at 37˚C in liquid DSM. **(B)** Representative phase-contrast images of spore formation induced by nutrient exhaustion at 37˚C on DSM agar plates. Time (in hours) after the initiation of sporulation is indicated. Scale bars indicate 2μm.

Figure S4. Viability and heat resistance of Δ*ylbJ* and Δ*gerAB* Δ*ylbJ* spores. Representative phase-contrast images of spores before and after incubation in LB. Spores from Δ*ylbJ* **(A)** and Δ*gerAB* Δ*ylbJ* **(B)** mutants generated after 96h at 37˚C on DSM agar plates were washed 5 times with ddH_2O. Spores (200μL) were heat-treated (80˚C for 20min) or left untreated and then resuspended in 2mL of LB. Spores were incubated at 37˚C and at the indicated times were visualized by phase-contrast microscopy. Sporulating cells lacking *ylbJ* and a functional GerA receptor produce viable but predominantly heat-sensitive spores. Scale bar indicates 2μm.

Figure S5. Cytological suppression of Δ*ylbJ* is principally mediated by the absence of a functional GerA receptor. Representative phase-contrast images of the indicated strains sporulated for 24h at 37˚C in liquid DSM are shown. Cytological suppression was observed in all strains that lack *gerAB.* Δ4 *gerA*$^+$, Δ4 *gerB*$^+$ and Δ4 *gerK*$^+$ lack the B-subunit of four of the five germinant receptors, while Δ5 lacks the B-subunit of the five germinant receptors. Scale bar indicates 2μm.

Figure S6(A-F). Cytological suppression of 26 sporulation mutants by Δ*gerAB*. Representative phase-contrast images of the indicated mutants in the presence or absence of the *gerAB* gene are shown. All cultures were sporulated for 24h at 37˚C in liquid DSM. Partial cytological suppression was observed for all 26 mutants. Quantification of spore phenotypes for mutants Δ*gerPC,* Δ*prpC*, Δ*prkC*, Δ*rho*, Δ*spoVG*, Δ*spoVS*, Δ*yfmI,* and Δ*yrbG* in which suppression by Δ*gerAB* appeared more subtle can be found in Table S4. Scale bar indicates 2μm.

Figure S7. Cytological suppression of Δ*spoVFA,* Δ*pdaB,* Δ*spoVT and* Δ*uppP* is principally mediated by the absence of a functional GerA receptor. Representative phase-contrast images of the indicated strains sporulated for 24h at 37˚C in liquid DSM are shown. Cytological suppression was observed in all strains that lack *gerAB.* Δ4 *gerA*$^+$ and Δ4 *gerK*$^+$ strains lack the B-subunit of four of the five germinant receptors. Scale bar indicates 2μm.

Figure S8. Cytological suppression of Δ*ylbJ* and Δ*pdaB* in the absence of a functional GerA receptor in PY79 and 3610 backgrounds. Representative phase-contrast images of the indicated strains sporulated for 24h at 37˚C are shown. Strains in PY79 **(A)** and 3610 **(B)** backgrounds were induced to sporulate by nutrient exhaustion in liquid **(A)** or by resuspension **(B)**. Sporulation efficiencies are indicated above each image. Scale bar indicates 2μm.

Figure S9. GerA-dependent premature germination in a subset of wild-type sporulating cells. (A) Exponentially growing cells were spot-plated on DSM agar plates and incubated at 37˚C for 96h. Spots surrounded by 8 neighboring spots were scraped (red circles) and washed before visualization. **(B-C)** Representative phase-contrast images of the indicated strains sporulated on DSM agar prepared as described in (A). >2,000 spores were scored for each strain. The spore phenotypes were quantified as in Figure 5A. Scale bars indicate 2μm (spores) and 5mm (spots of sporulating cells).

Figure S10. GerA-dependent premature germination during sporulation on agar plates. Representative phase-contrast images of wild-type and Δ*gerAB* sporulating cells on DSM agar at 37˚C are shown. At the indicated time points (in hours), a spot from each strain was scraped and washed before visualization. Yellow carets highlight examples of prematurely germinated spores. Scale bar indicates 2μm.

Figure S11. GerA-dependent premature germination during synchronous sporulation in liquid medium. Representative phase-contrast images of wild-type and the Δ*gerAB* mutant induced to sporulate by resuspension at 37˚C are shown. Yellow carets highlight examples of prematurely germinated spores within mother cells. The percentages of phase-grey/dark spores within mother cells (intrasporangial germination) at hour 12 (T12) are indicated. >1900 sporangia were scored for each strain. Scale bar indicates 2μm.

Figure S12. Premature germination of *Bacillus cereus* sporulating cells. (A) Representative phase-contrast images of wild-type *B. cereus* sporulated on DSM agar plates at 37˚C. At the indicated time points (in hours), a spot from each strain was scraped and washed before visualization. Yellow carets highlight examples of prematurely germinated spores. **(B)** Purified spores from 96h sporulating *B. cereus* cells were heat-shocked (65˚C for 15min and then 20min on ice), resuspended in 2mL of LB and incubated at 37˚C. 20min after LB addition, germinated spores were visualized by phase-contrast microscopy. Scale bars indicate 2μm.

Figure S13. Separation of phase-bright spores and prematurely germinated phase-dark and dull phase-grey spores. Wild-type cells sporulated on DSM agar plates at 37˚C were collected after 96h, washed 3 times with ddH_2O and visualized by phase-contrast microscopy (input). After centrifugation through a 2-step (20-50%) Histodenz gradient, two fractions were obtained. The upper layer contained mainly germinated phase-dark and dull phase-grey spores, vegetative and sporulating cells. The pellet was almost exclusively dormant phase-bright spores.

Figure S14. Prematurely germinated wild-type spores are inviable or have lost resistance properties. Prematurely germinated phase-dark and dull phase-grey spores from wild-type cells that were sporulated on DSM agar were purified by density centrifugation. These spores were heat-treated (80˚C for 20min) or left untreated, resuspended in 2mL of LB and then incubated at 37˚C. At the indicated time points (in hours), an aliquot from each culture was analyzed by phase-contrast microscopy. Most

of the prematurely germinated spores failed to outgrow during the 2h incubation (untreated) and were heat sensitive. Scale bar indicates 2μm.

Figure S15. Sporulating cells lacking *alrB* do not enhance premature germination in *B. subtilis*. Representative phase-contrast images of the indicated strains sporulated for 24h at 37˚C in liquid DSM are shown. Sporulation efficiencies are indicated above each image. Scale bar indicates 2μm.

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Figure S1

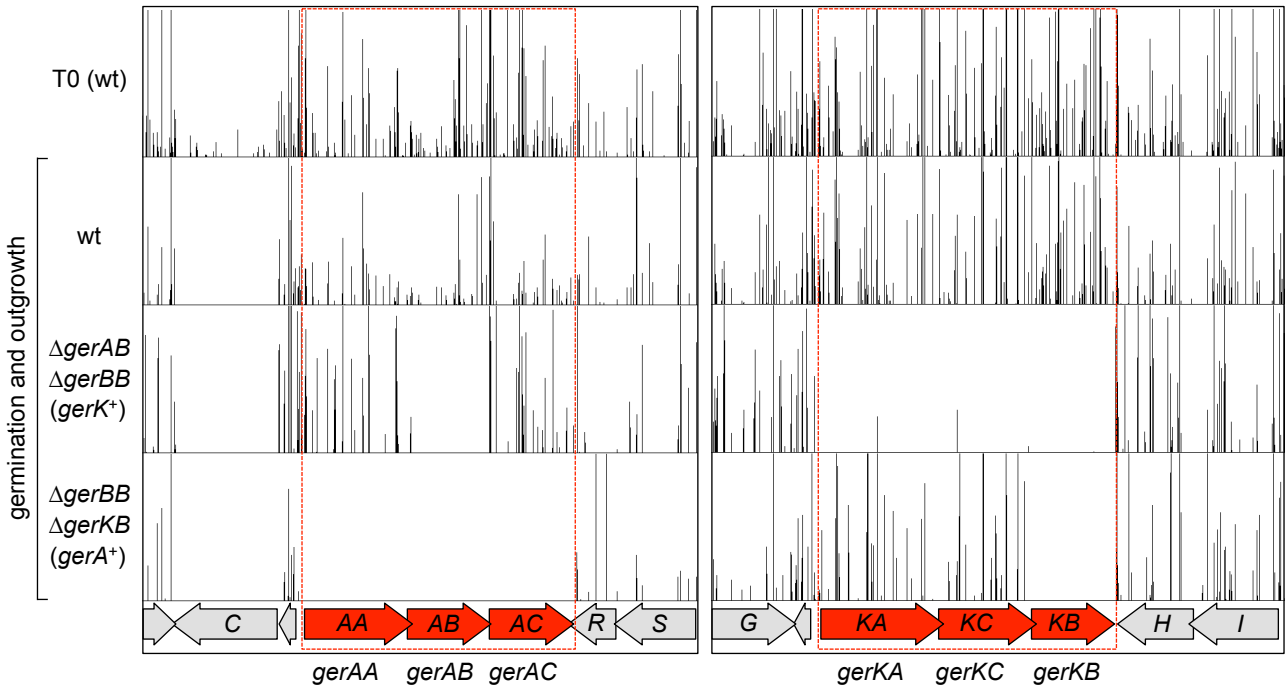

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Figure S2A

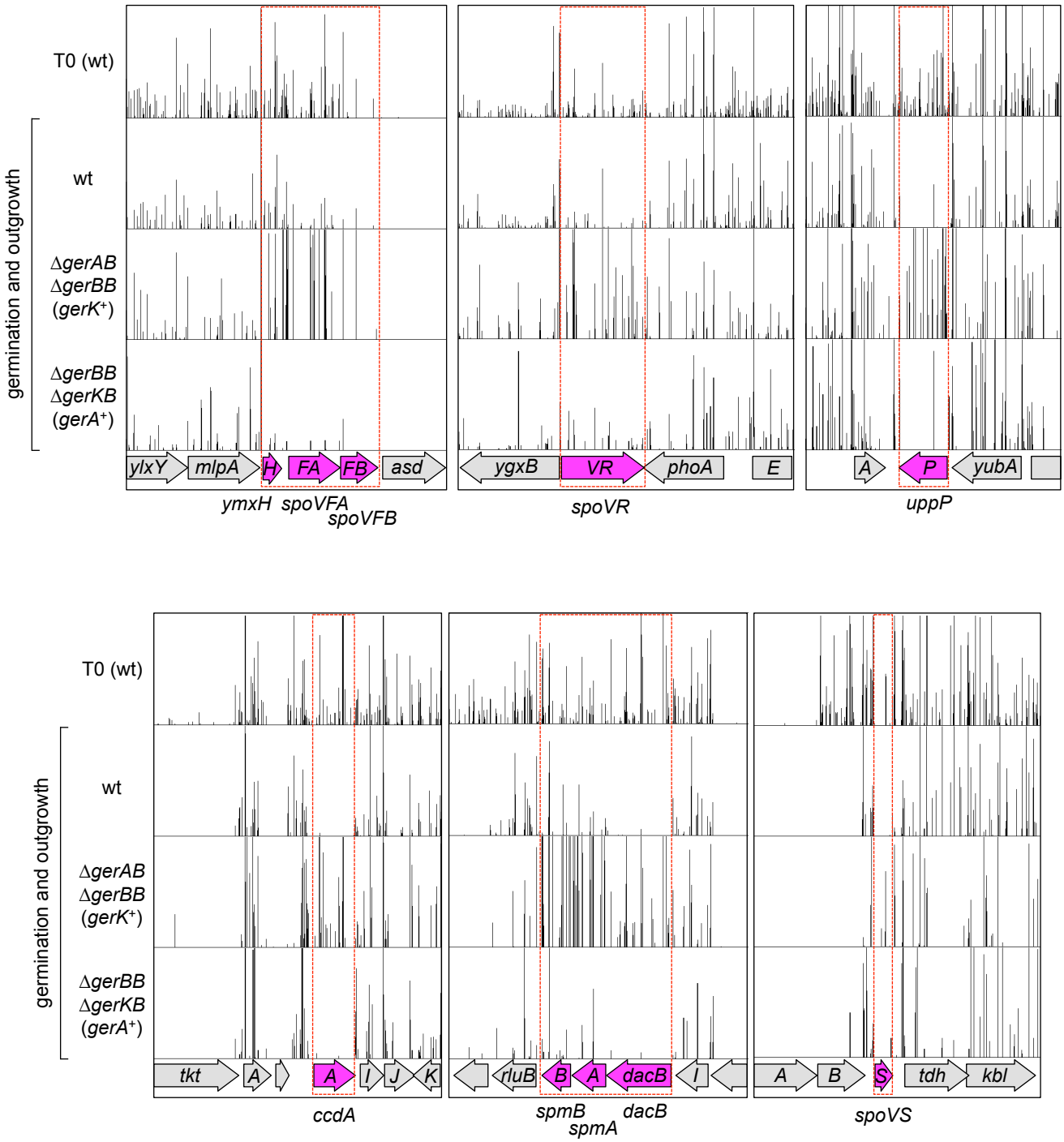

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Figure S2B

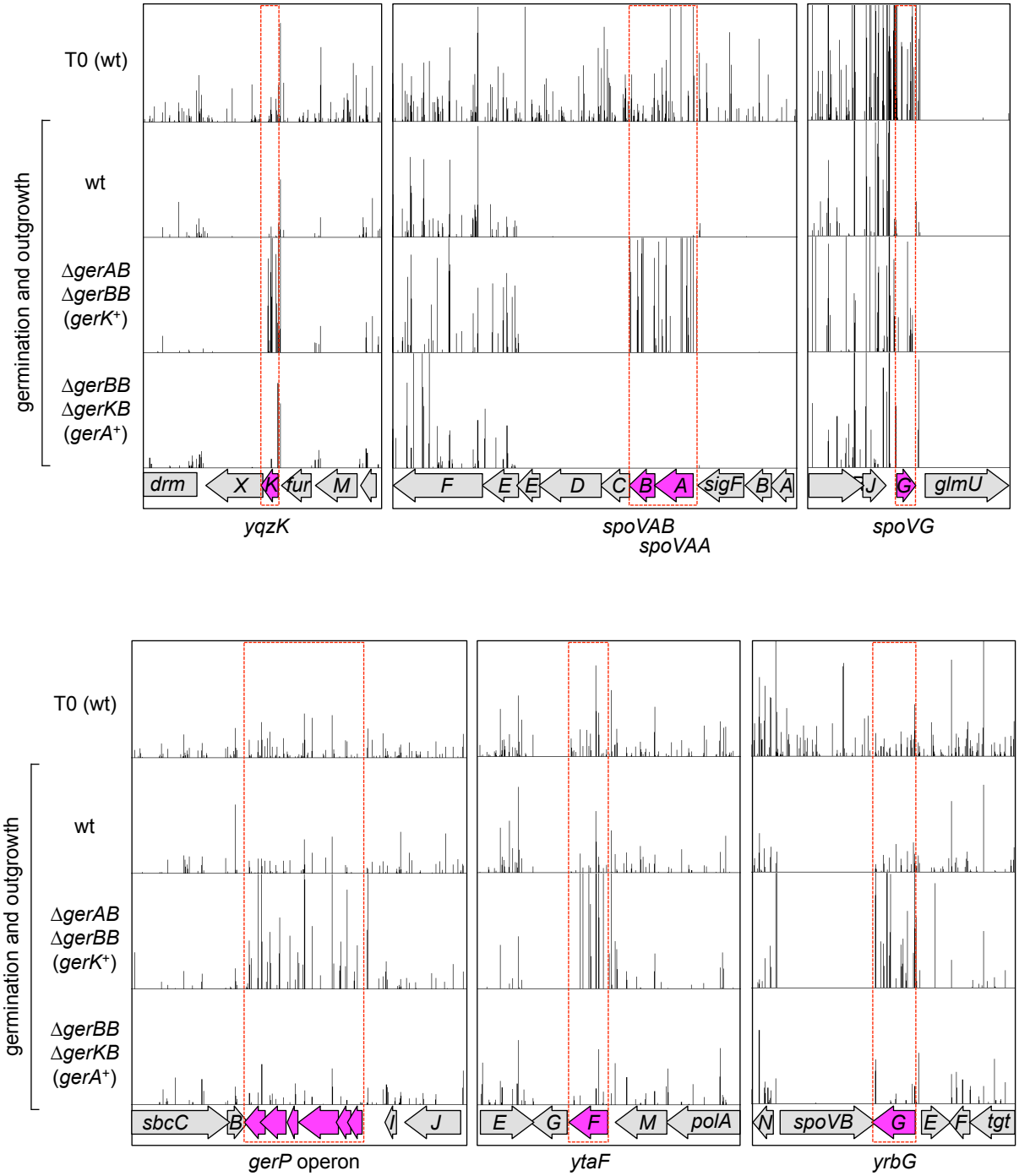

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Figure S2C

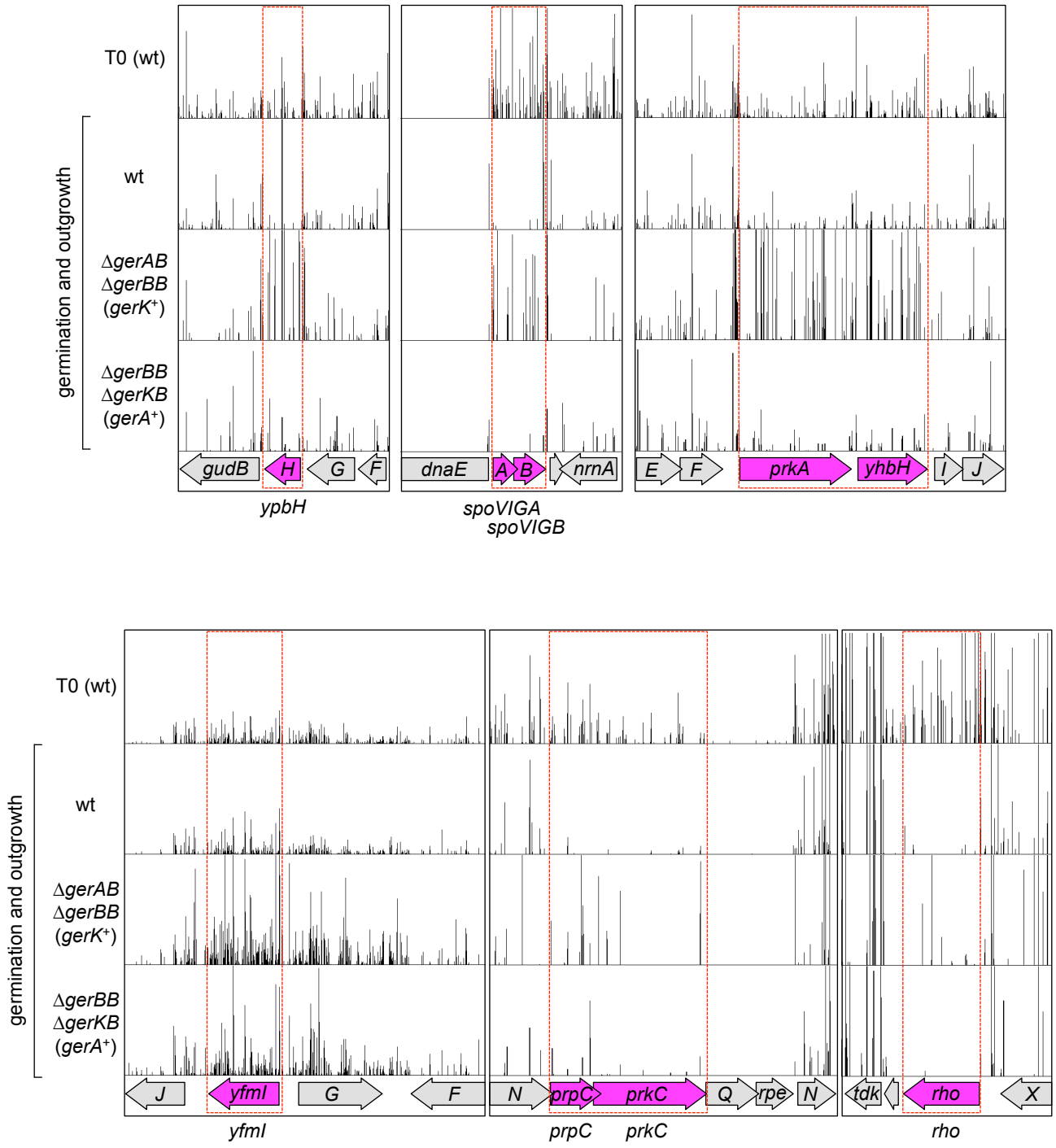

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Figure S3A

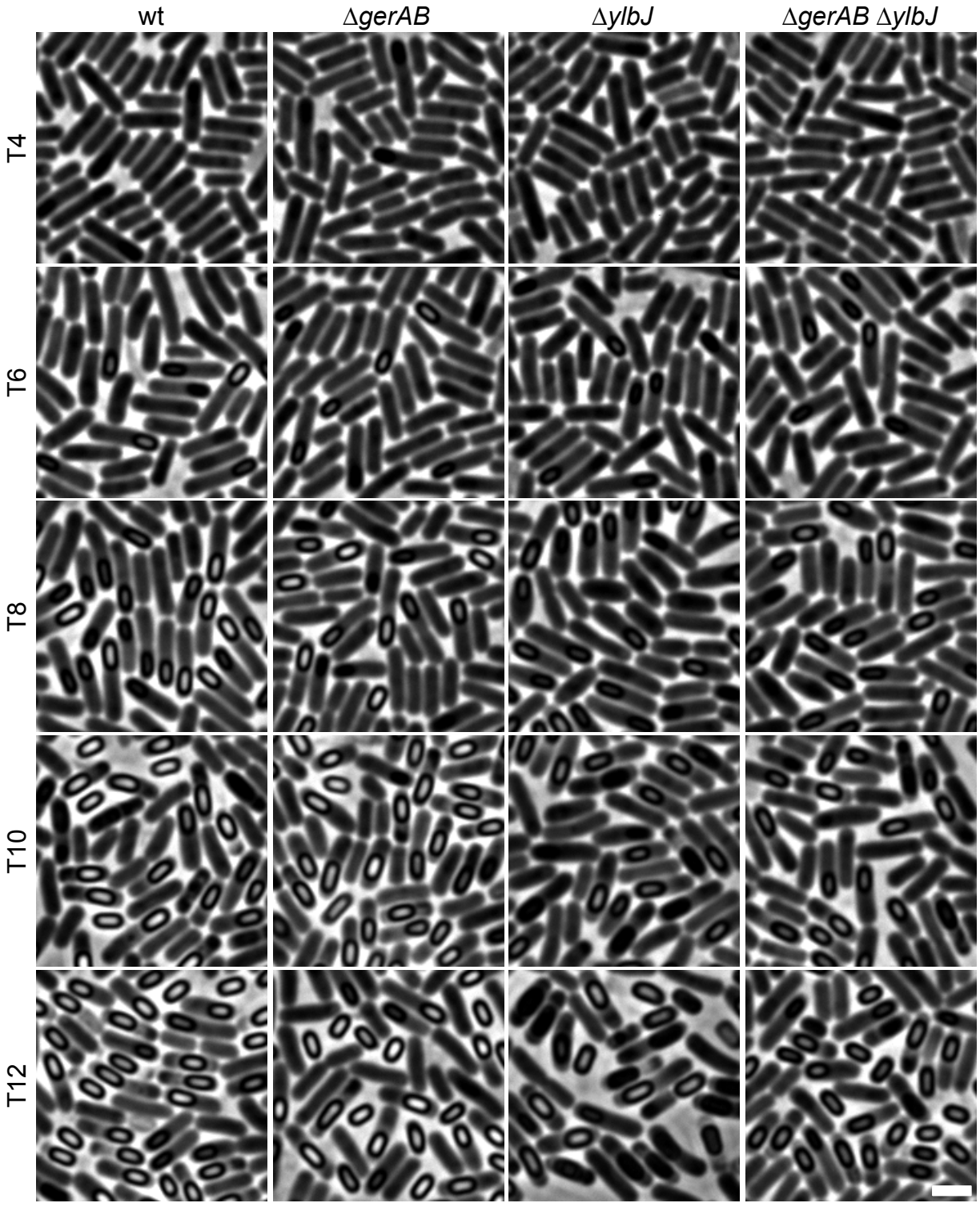

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Figure S3A (*continued*)

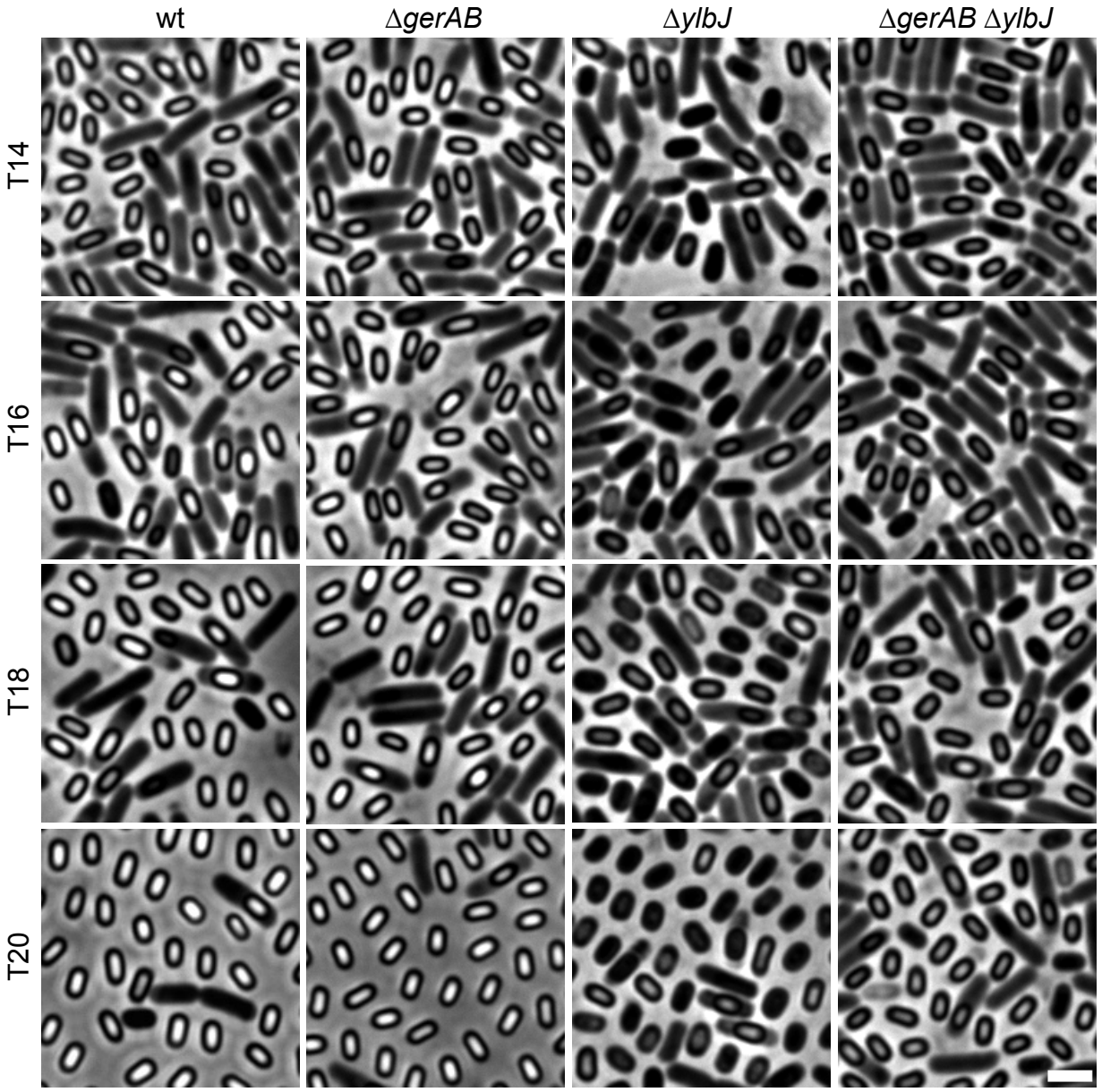

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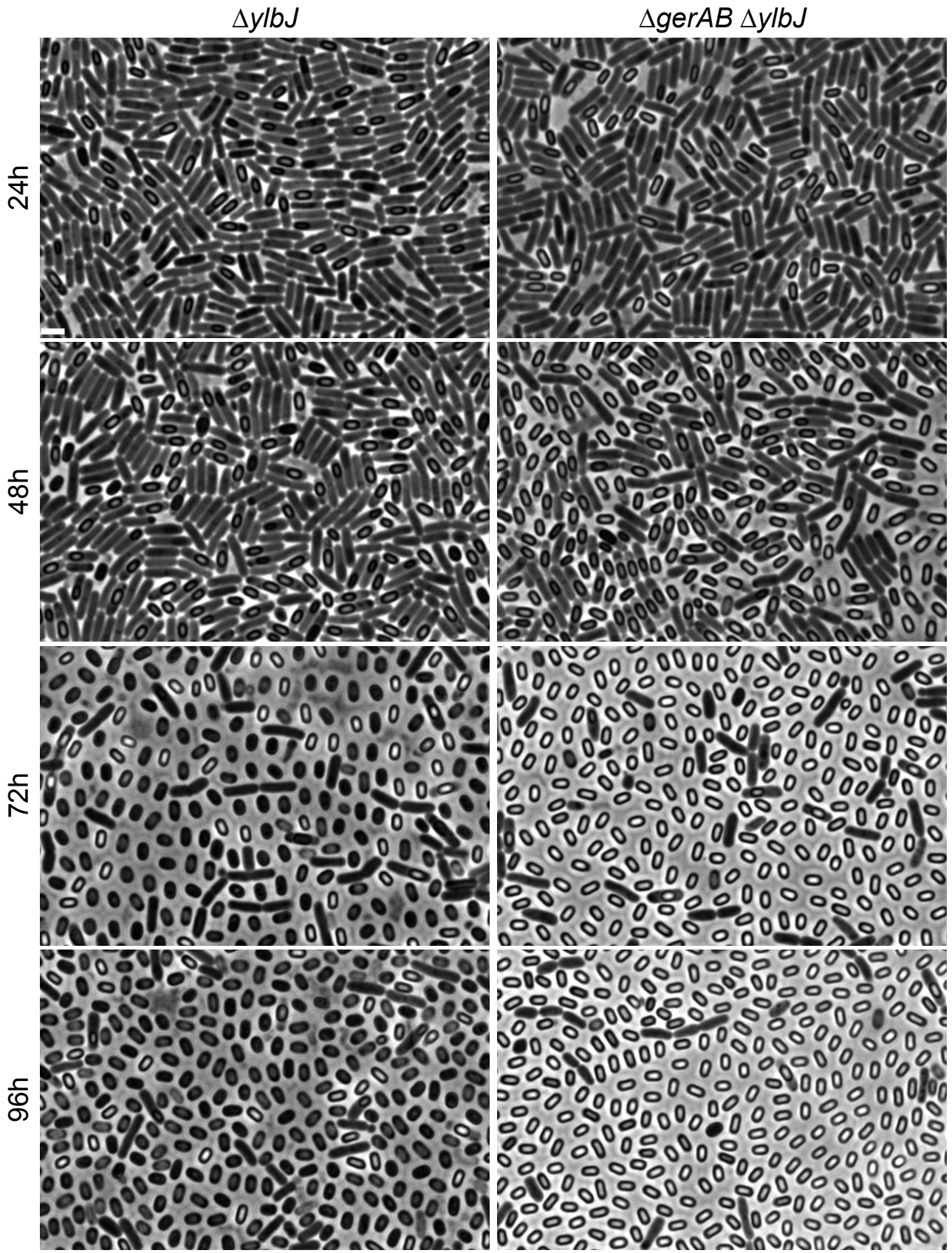
Figure S3B



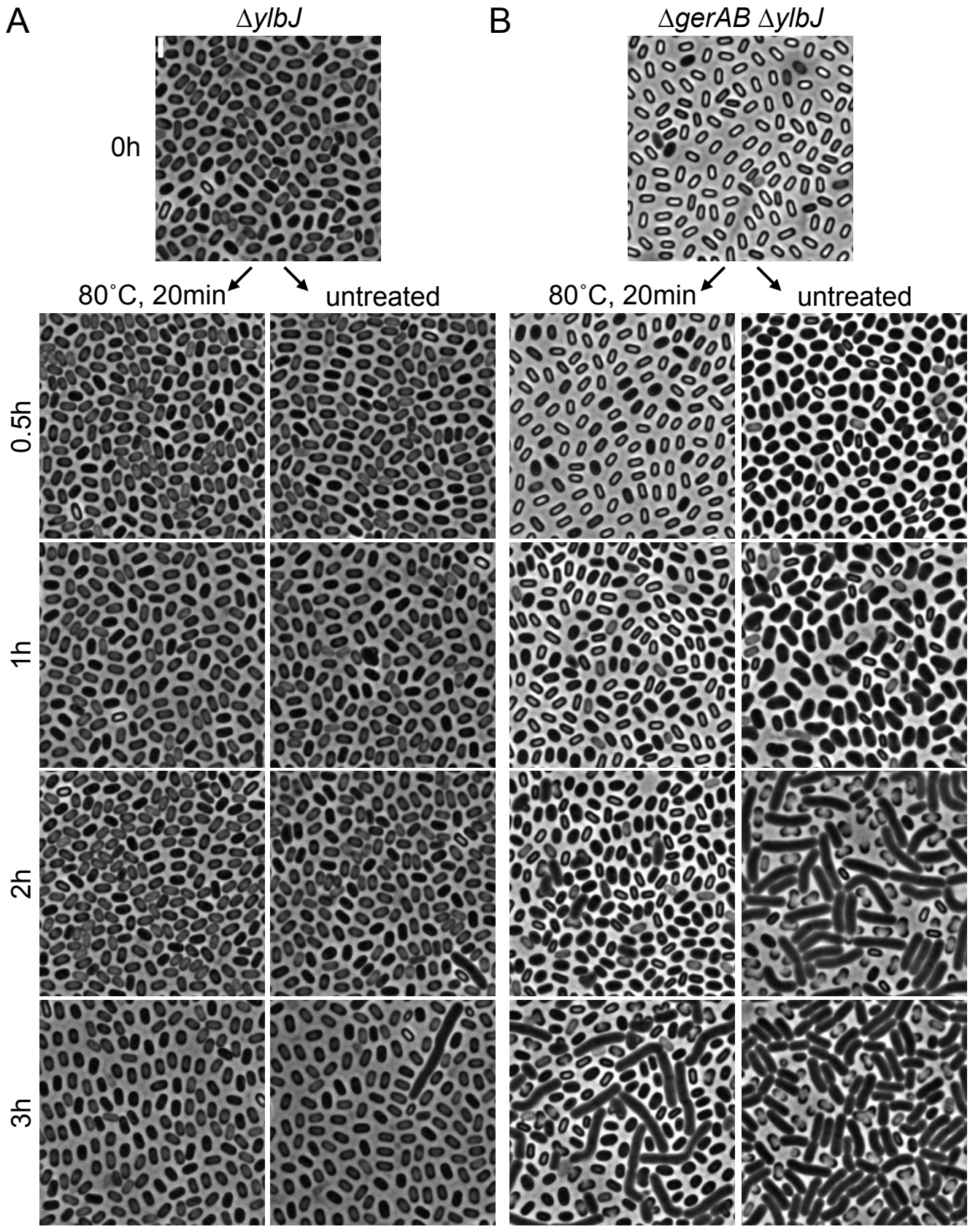

A
ΔylbJ
B
ΔgerAB ΔylbJ
0h
80˚C, 20min
untreated
80˚C, 20min
untreated
0.5h
1h
2h
3h

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Figure S5

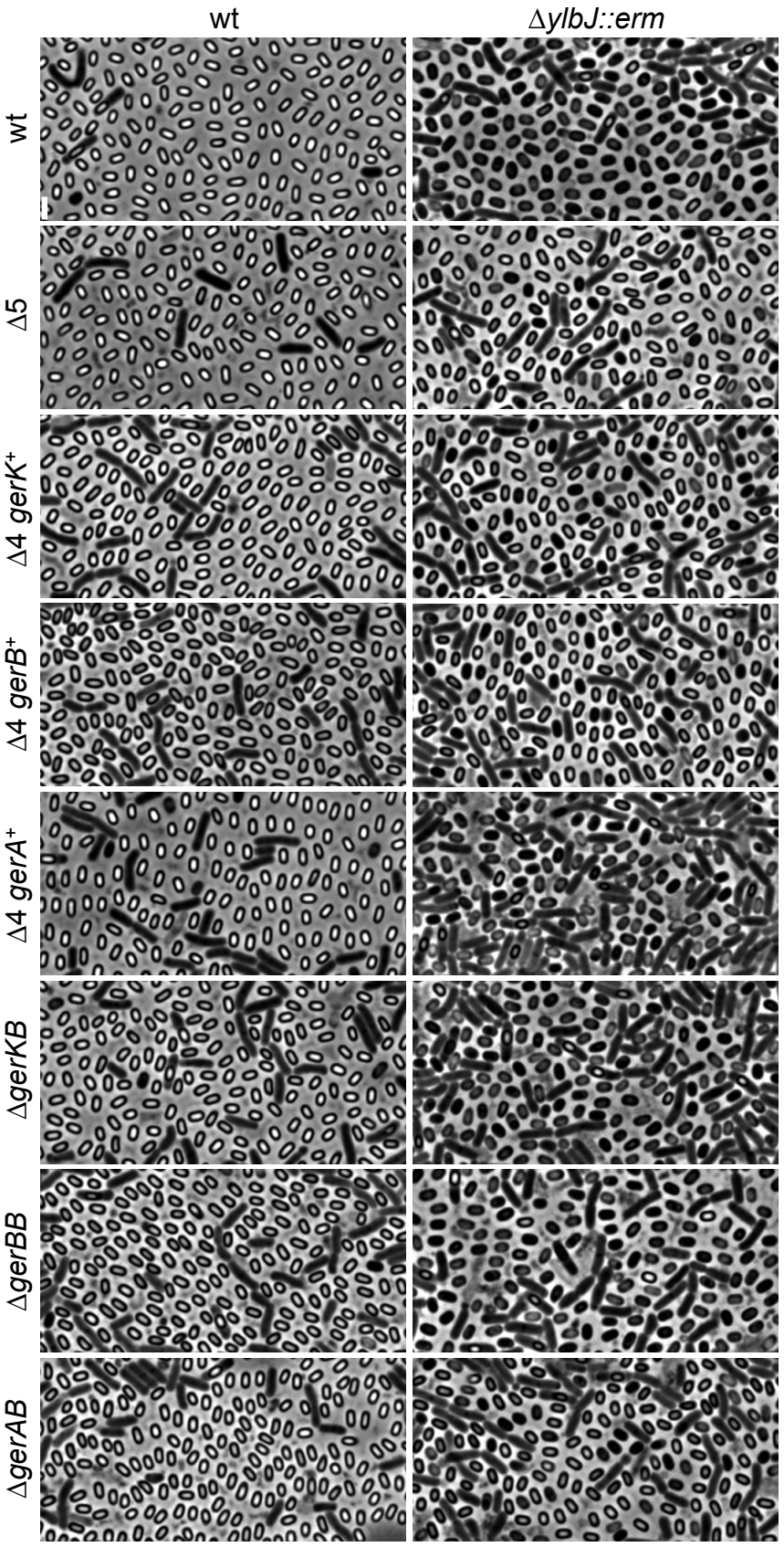

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Figure S6A

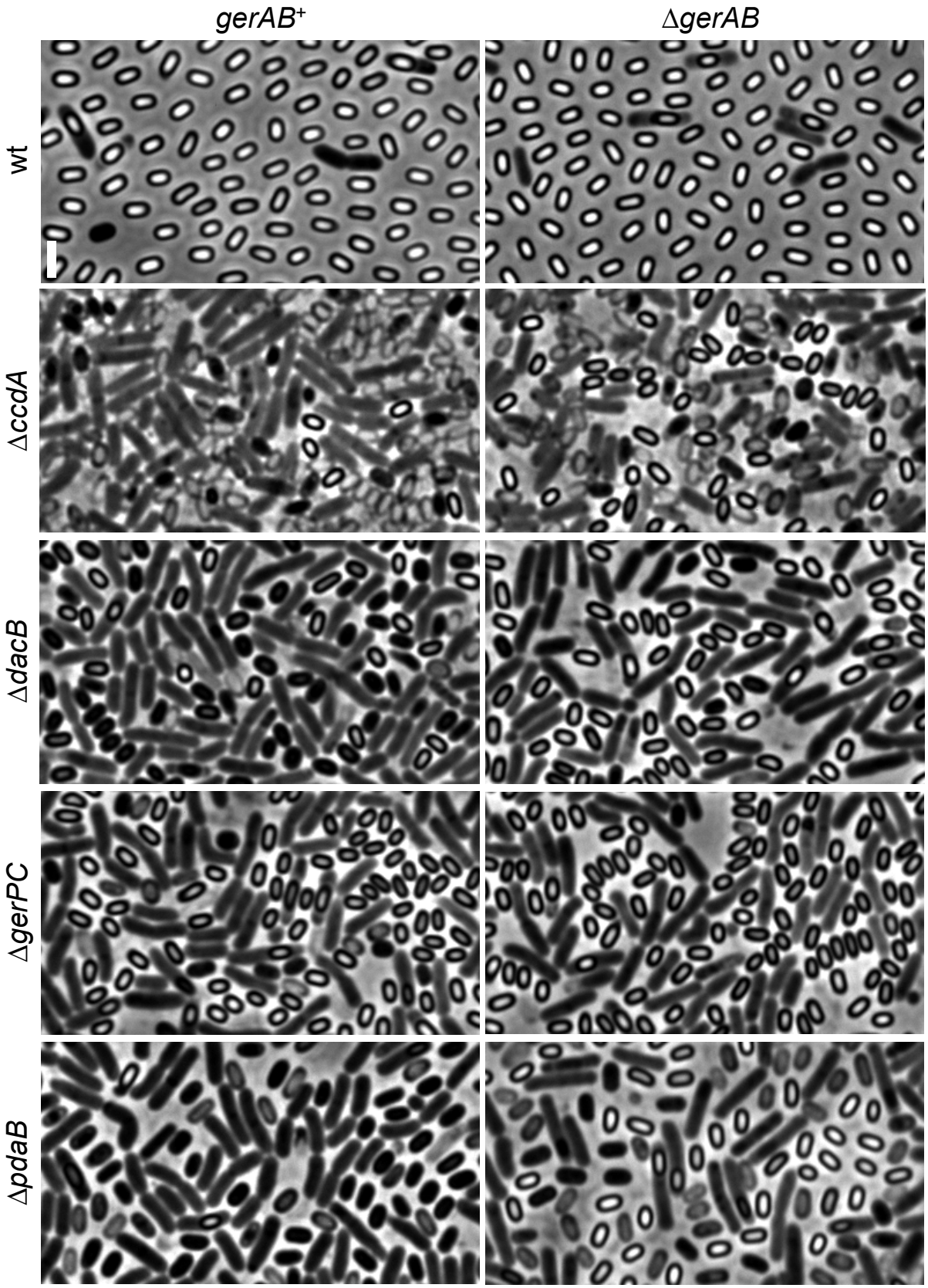

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Figure S6B

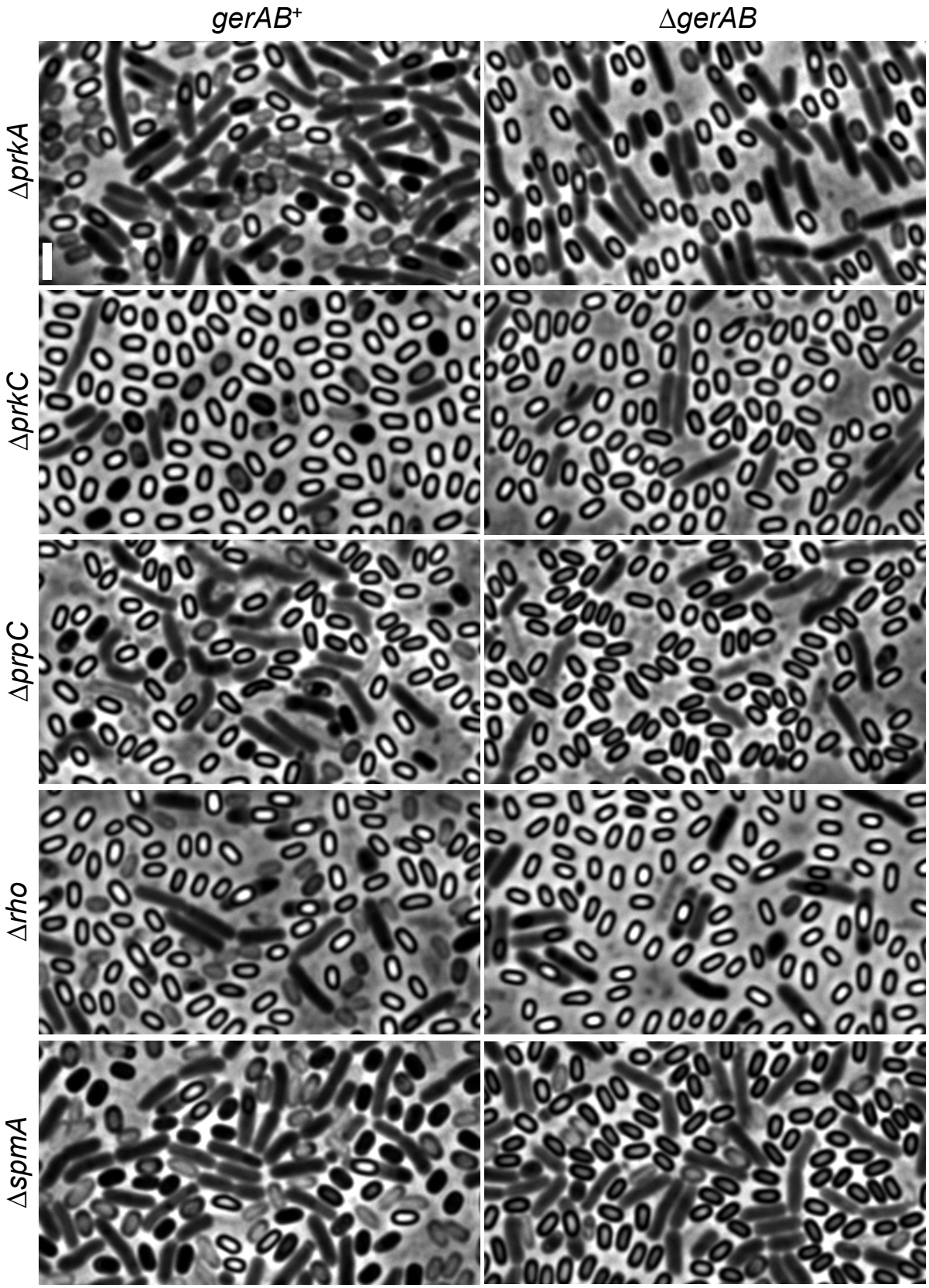

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Figure S6C

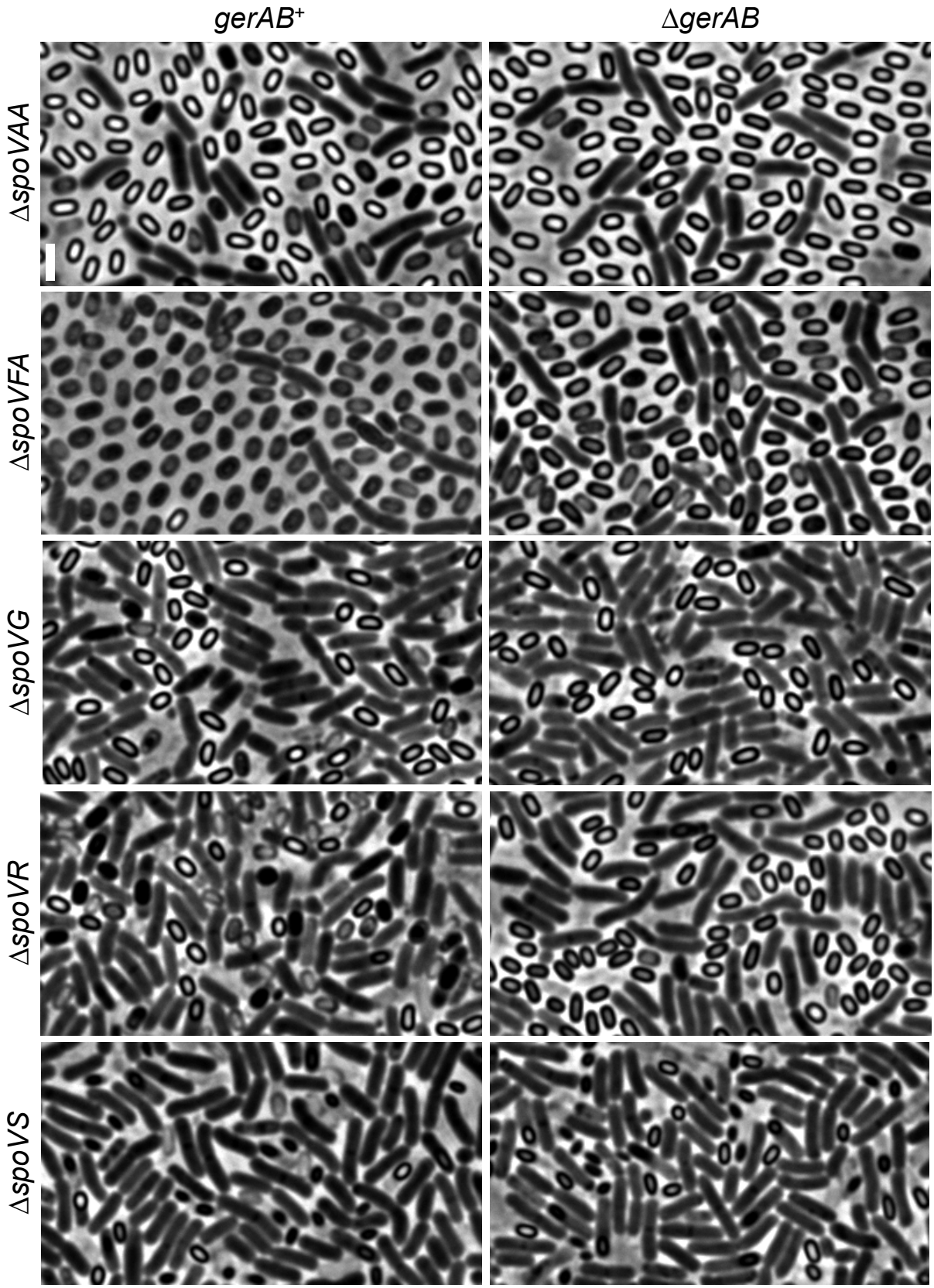

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Figure S6D

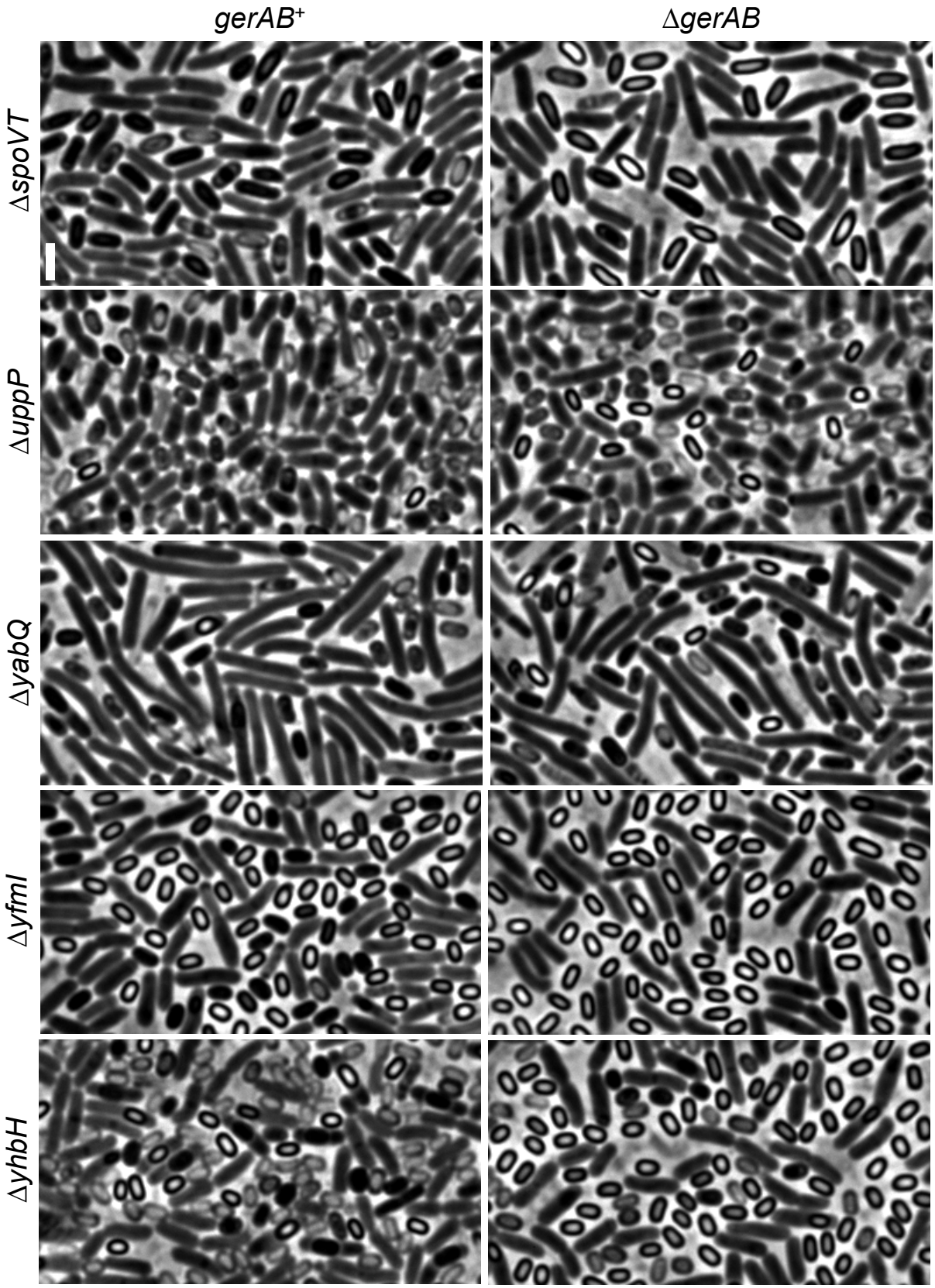

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Figure S6E

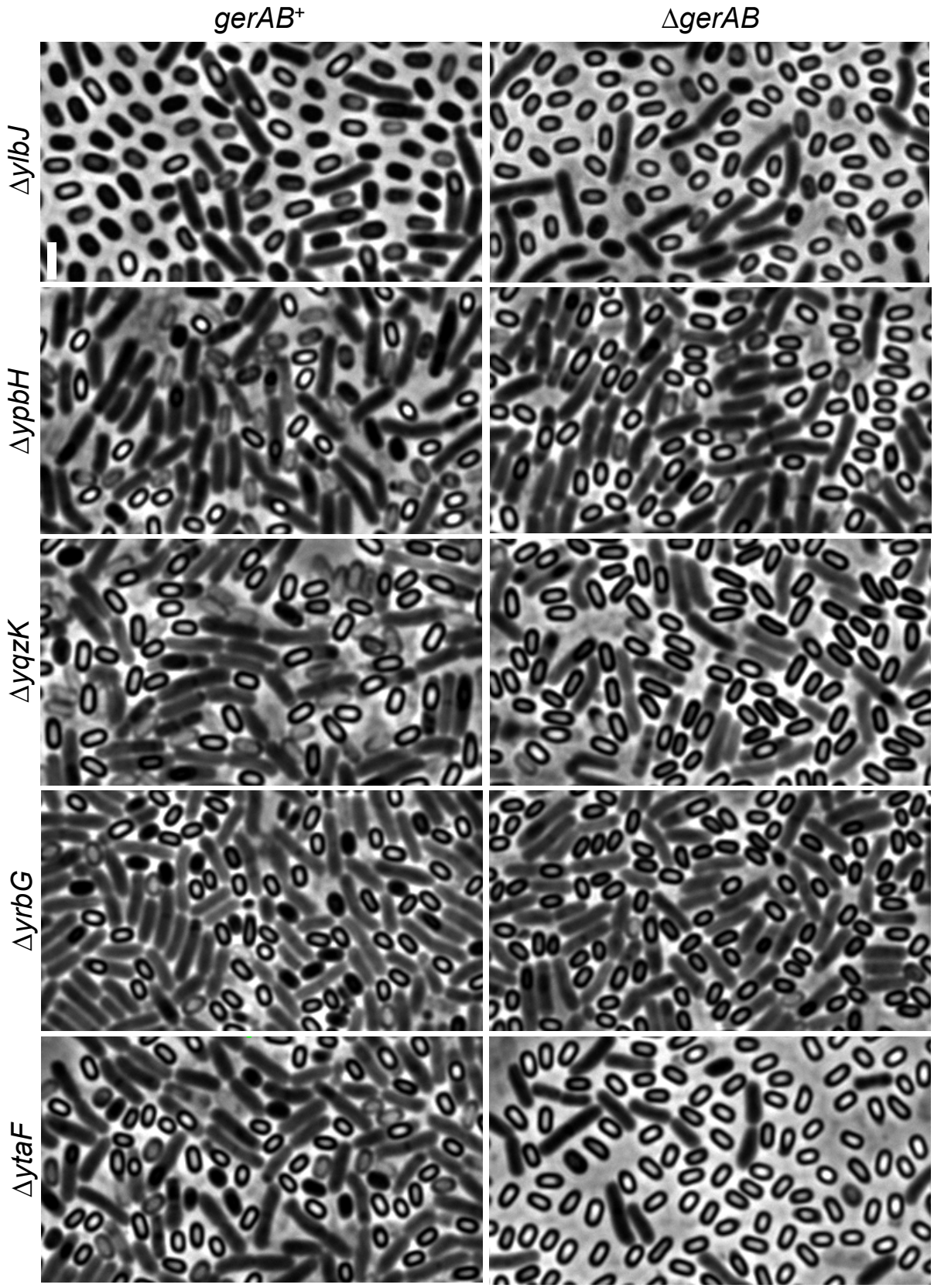

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Figure S6F

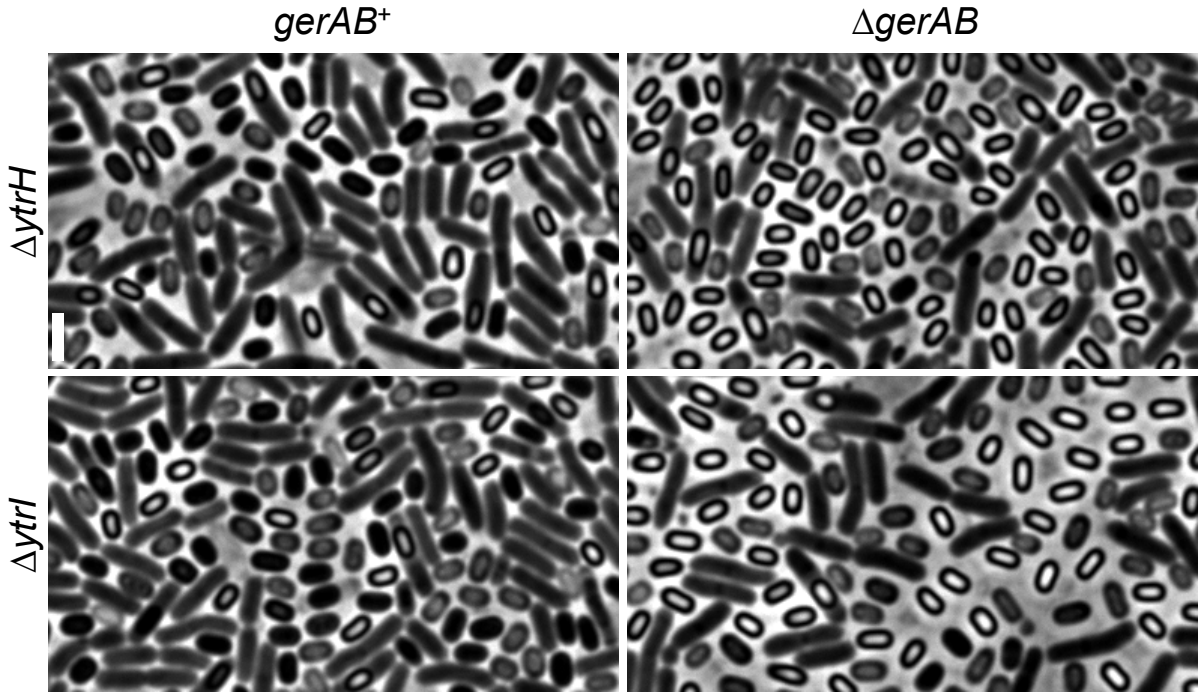

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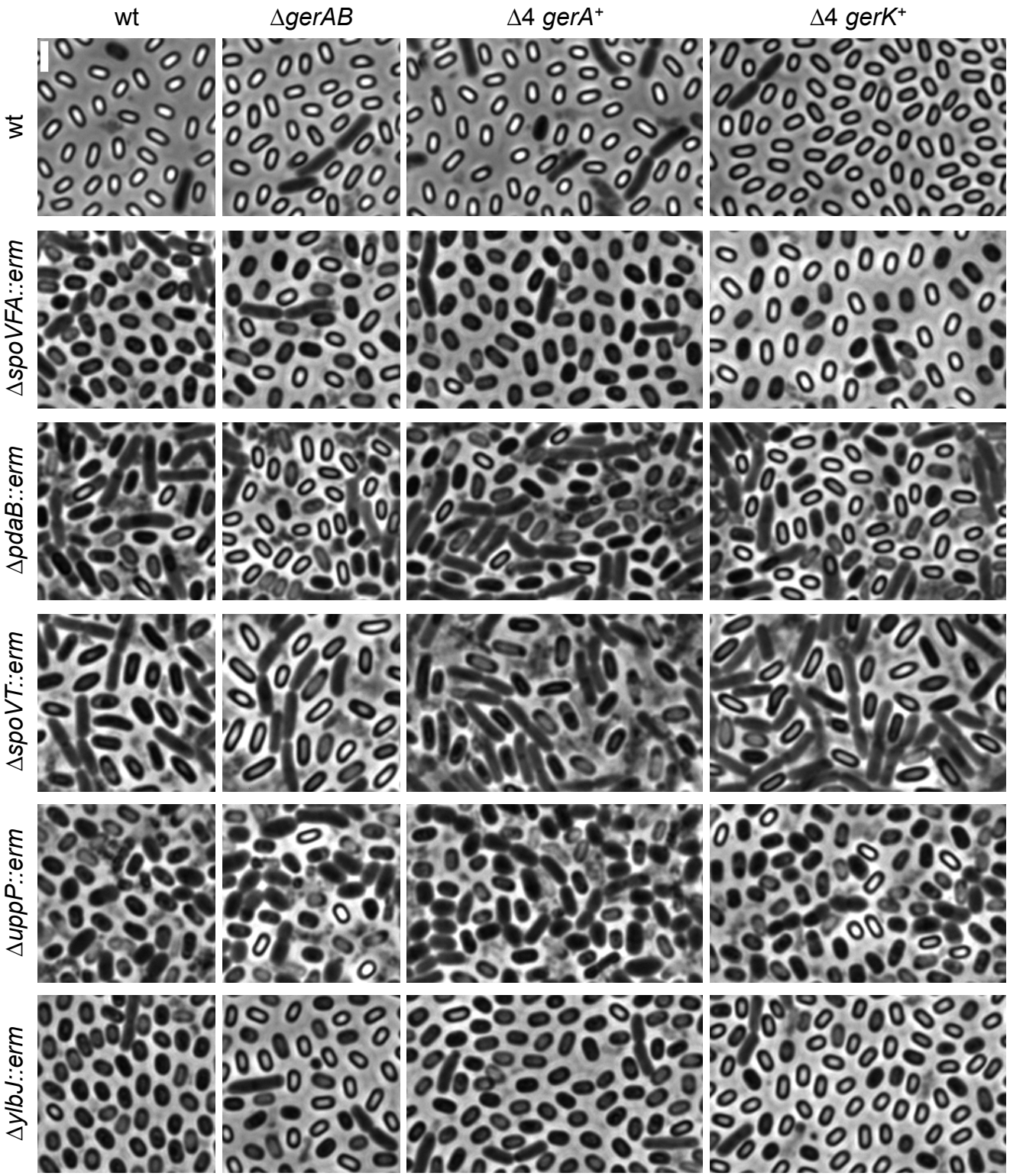
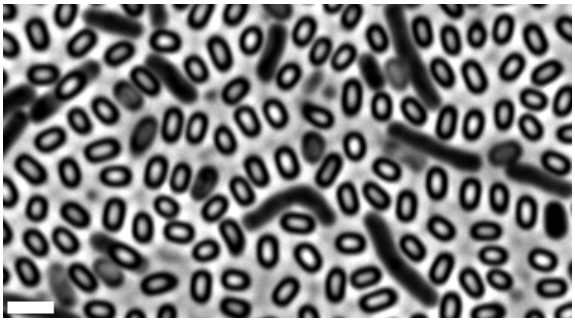
Figure S7

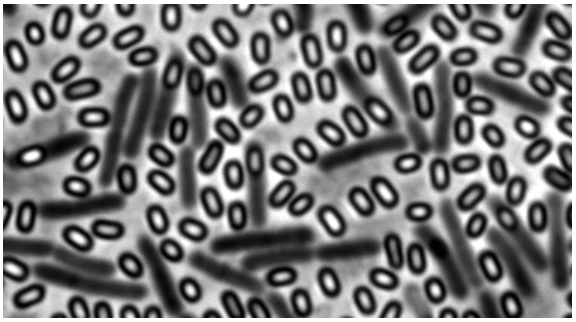


PY79 background

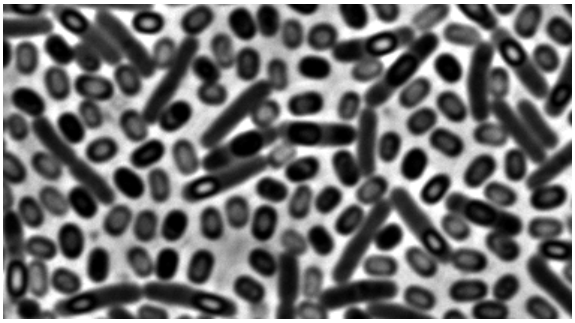
wt (100%)



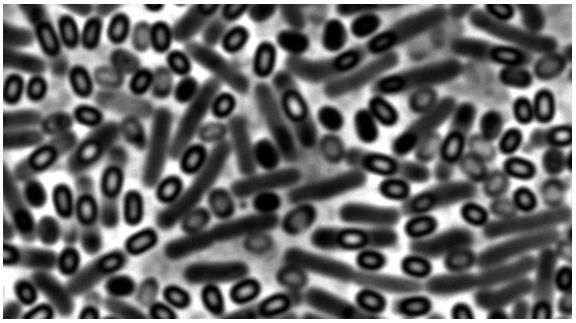
Δ*gerAB::erm* (82.2%)



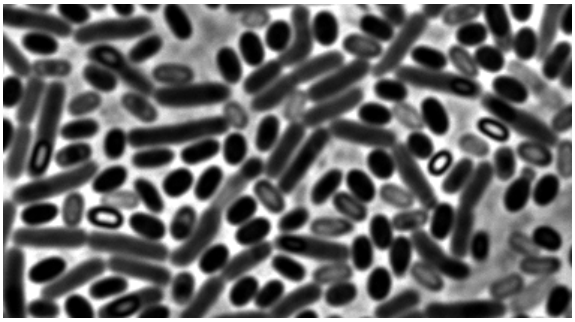
Δ*ylbJ::spec* (0.00005%)



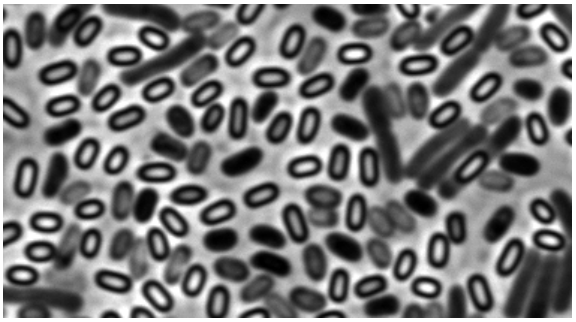
Δ*gerAB::erm* Δ*ylbJ::spec* (0.192%)



Δ*pdaB::tet* (5.2%)

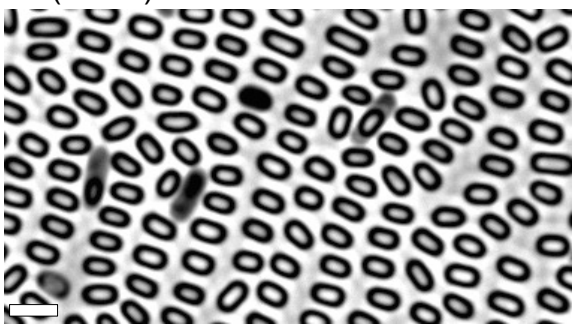


Δ*gerAB::erm* Δ*pdaB::tet* (12.7%)

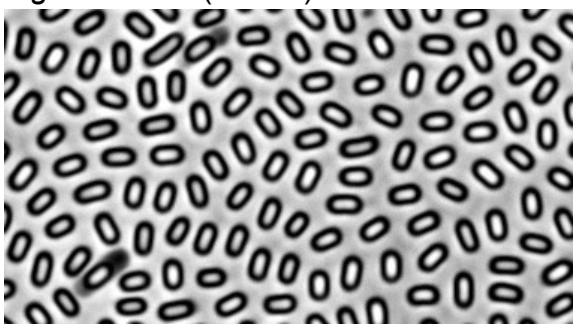


3610 background

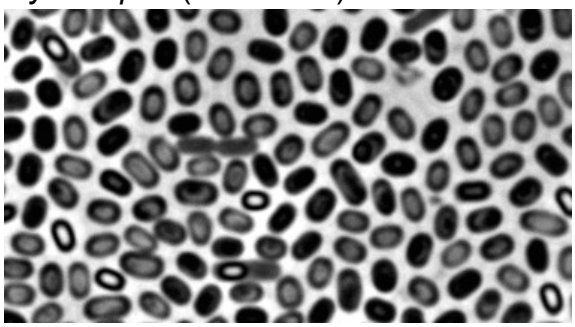
wt (100%)



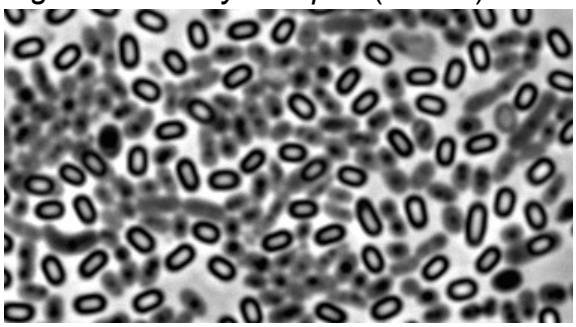
Δ*gerAB::erm* (10.3%)



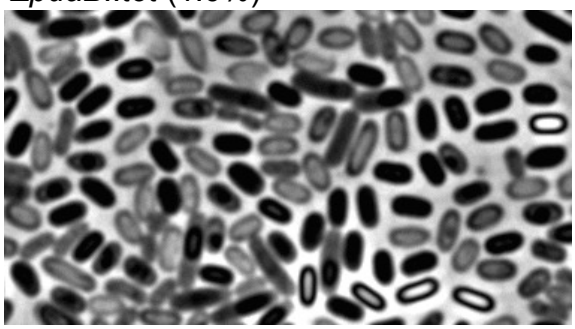
Δ*ylbJ::spec* (0.00003%)



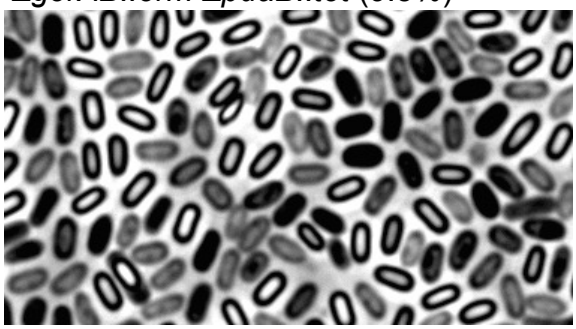
Δ*gerAB::erm* Δ*ylbJ::spec* (1.69%)



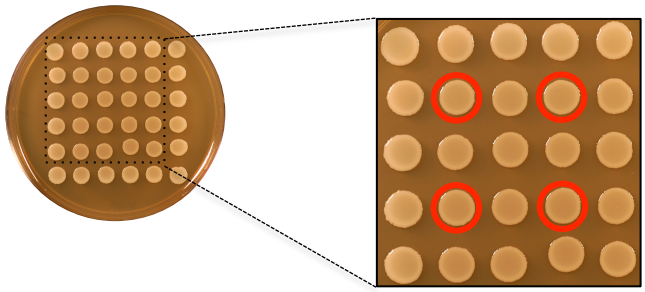
Δ*pdaB::tet* (1.0%)



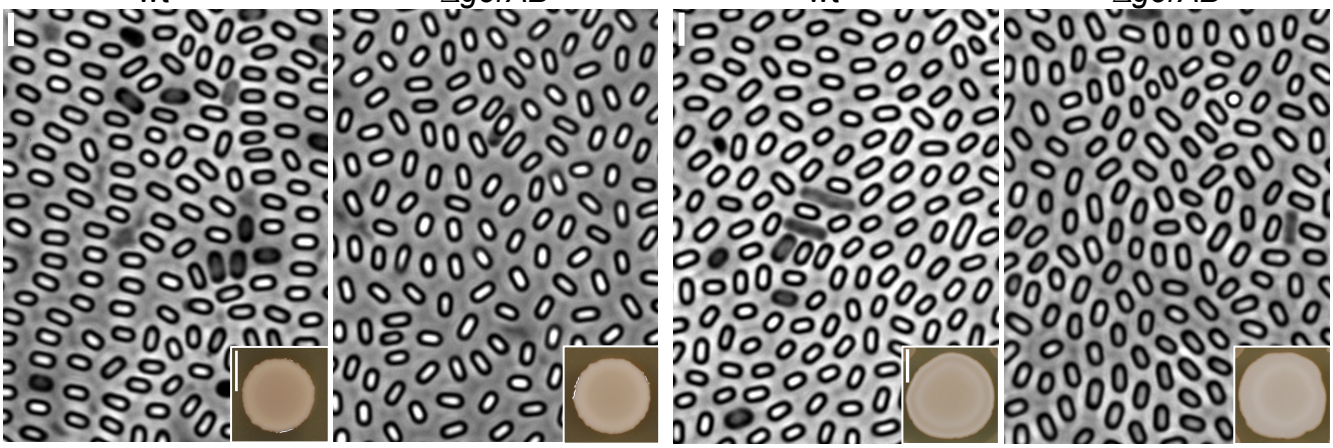
Δ*gerAB::erm* Δ*pdaB::tet* (9.5%)



A



B



	wt	ΔgerAB	wt	ΔgerAB
phase-dark:	0.9%	0%	0.1%	0%
dull phase-grey:	6.6%	0%	1.3%	0%
hollow:	0.7%	0%	0.1%	0%

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Figure S10

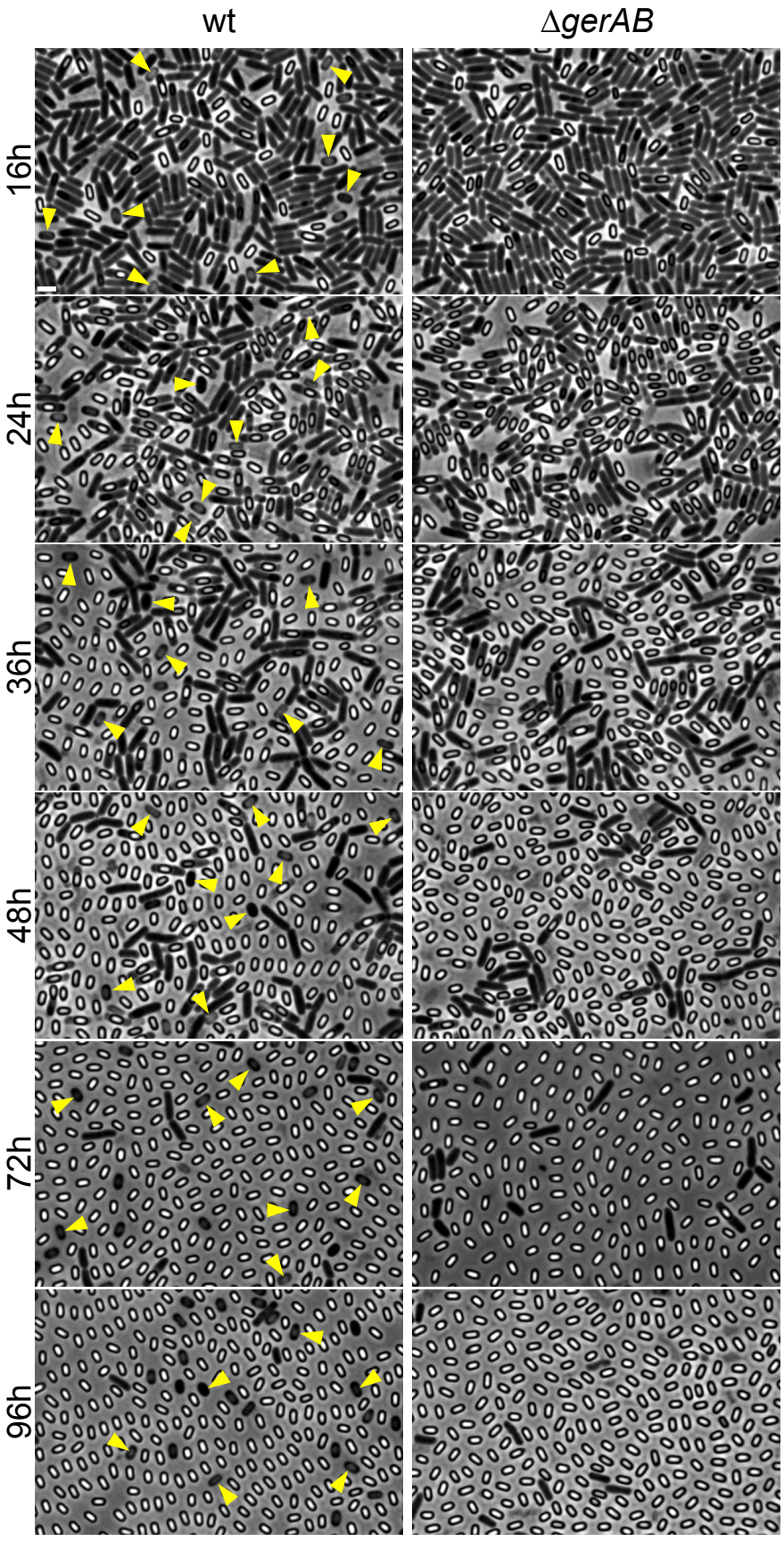

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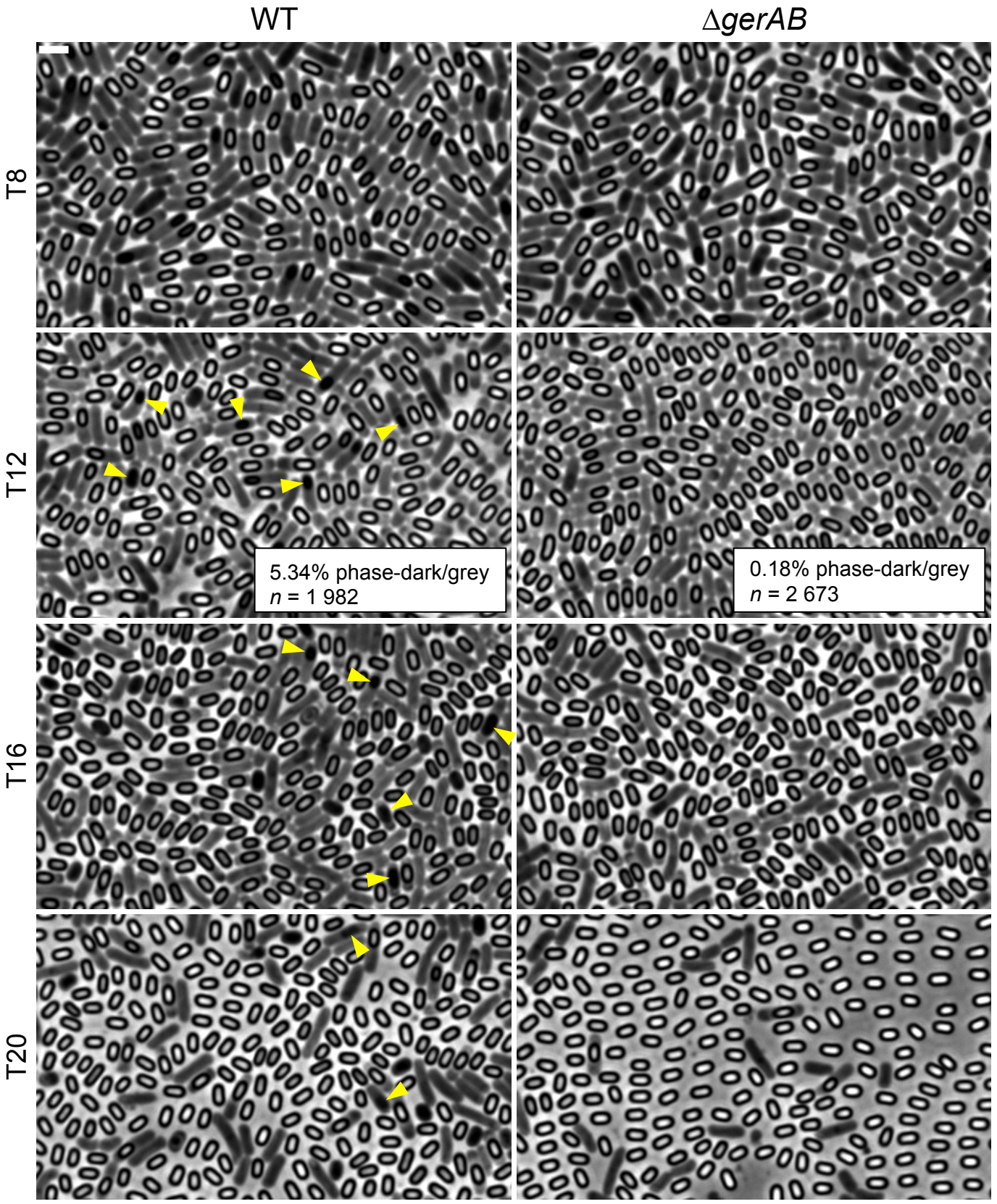
Figure S11



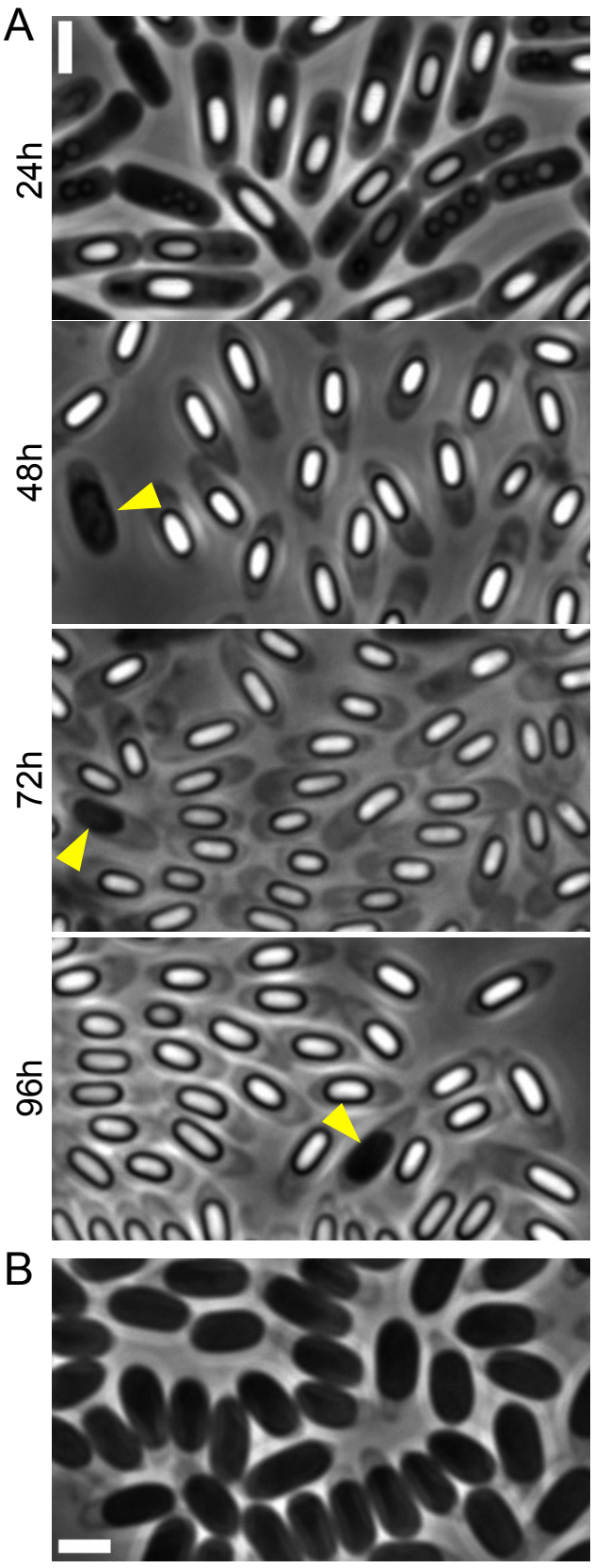

A
24h
48h
72h
96h
B

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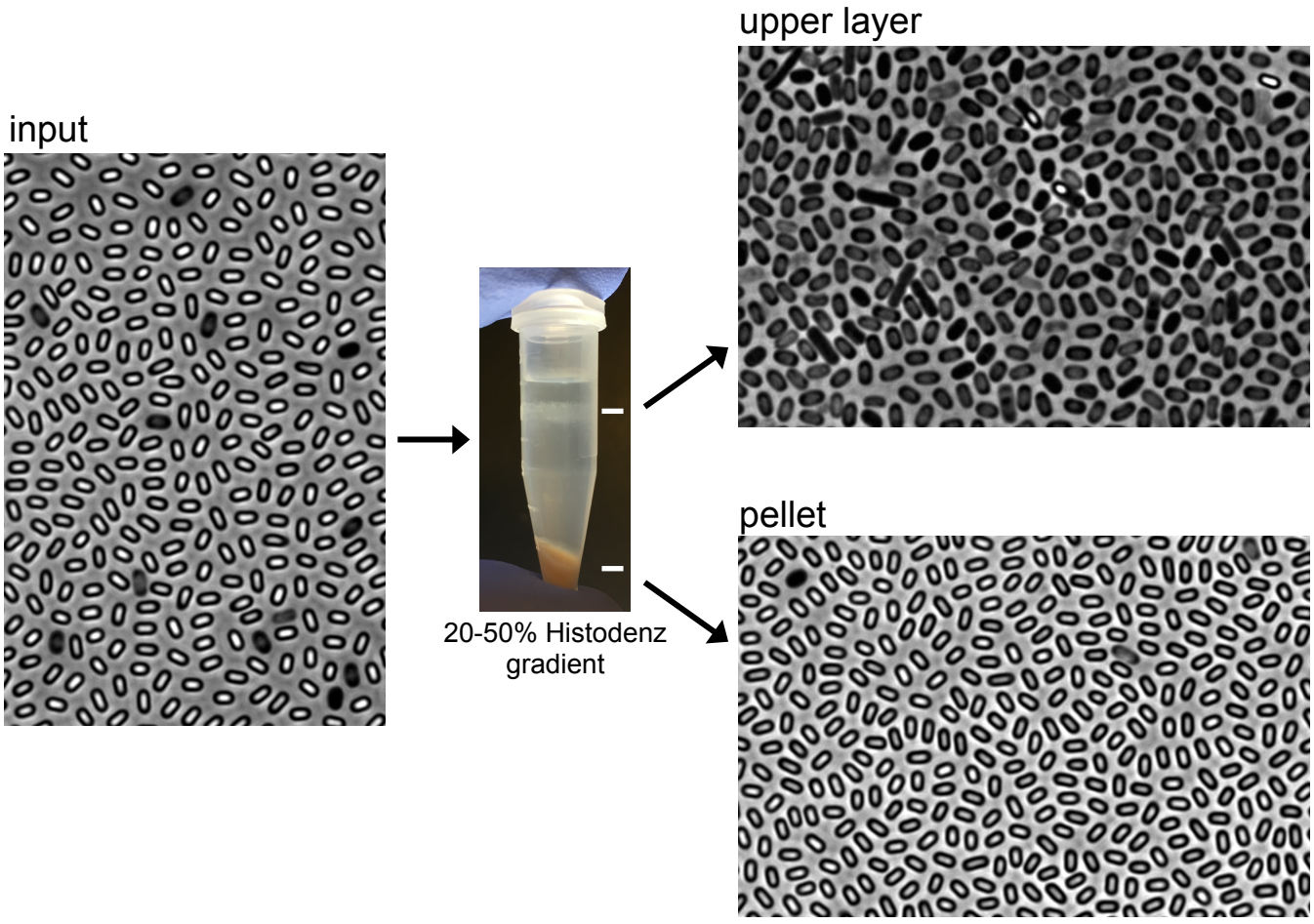
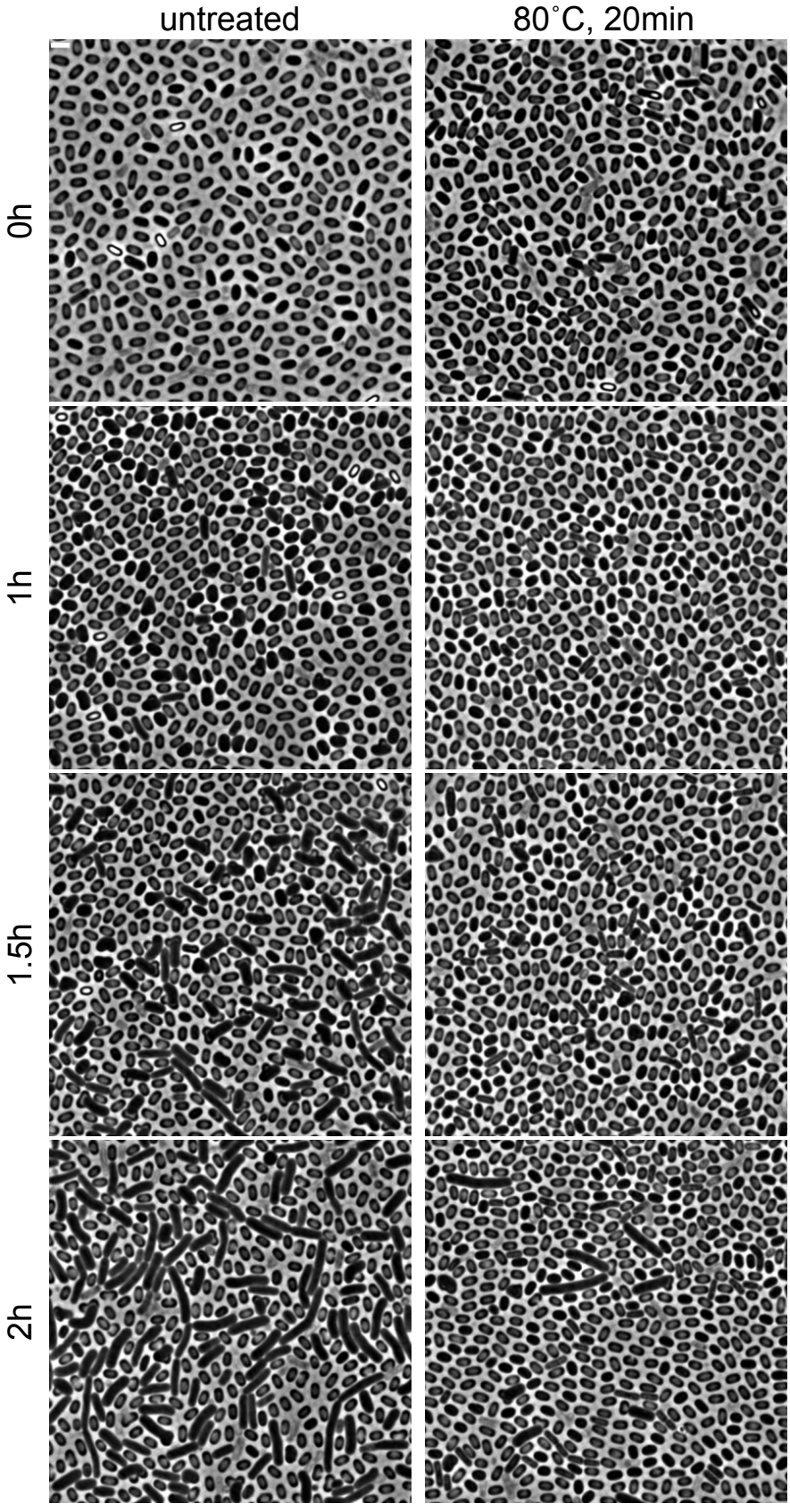
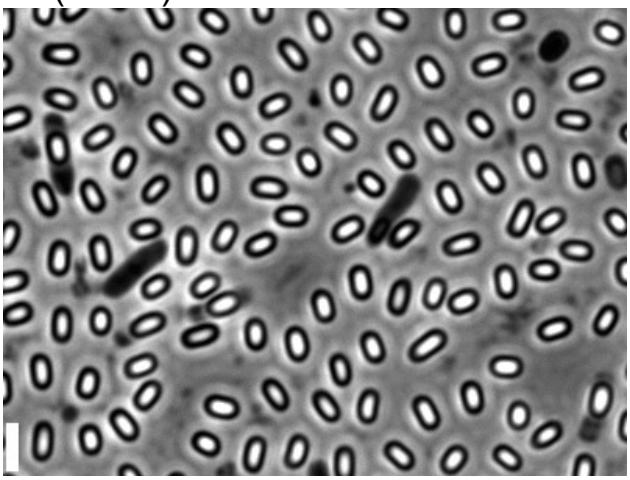
Figure S13




untreated
80˚C, 20min
0h
1h
1.5h
2h

wt (100%)



Δ*alrB* (96%)

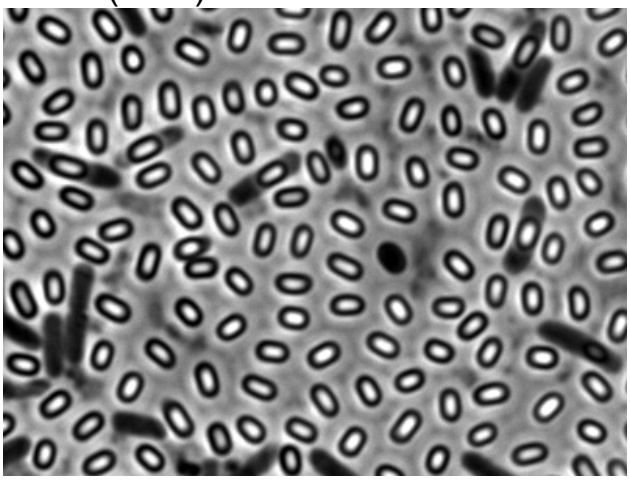


Table S1. Δ*gerA* is the principal suppressor of Δ*ylbJ*.

genotype	spores (%) [a]	fold-suppression
wt	100	-
Δ*ylbJ::erm*	3.40×10^{-5}	
Δ*gerA::spec*	87.7	1.53×10^{4}
Δ*gerA::spec* Δ*ylbJ::erm*	0.52	
Δ*gerAB*	85.6	1.72×10^{4}
Δ*gerAB* Δ*ylbJ::erm*	0.59	
Δ*gerBB*	92.2	15.5
Δ*gerBB* Δ*ylbJ::erm*	5.28×10^{-4}	
Δ*gerKB*	90.2	193.3
Δ*gerKB* Δ*ylbJ::erm*	6.57×10^{-3}	
Δ4 *gerA*$^{+}$	76.6	27.6
Δ4 *gerA*$^{+}$ Δ*ylbJ::erm*	9.40×10^{-4}	
Δ4 *gerB*$^{+}$	7.1	8.27×10^{3}
Δ4 *gerB*$^{+}$ Δ*ylbJ::erm*	0.28	
Δ4 *gerK*$^{+}$	30.6	1.94×10^{4}
Δ4 *gerK*$^{+}$ Δ*ylbJ::erm*	0.66	
Δ5	0.33	4.38×10^{3}
Δ5 Δ*ylbJ::erm*	0.15	

[a] Strains were sporulated in liquid DSM for 24h at 37˚C. Heat-resistant (80°C for 20min) colony forming units (CFU) compared to wild-type heat-resistant CFU are shown. Heat-resistant CFU were counted after 16h growth at 37˚C on agar plates for strains lacking one germinant receptor and after 96h growth for strains lacking 4 or 5 germinant receptors. Spores from latter strains germinate more slowly.

Table S2. Δ*gerAB* is required for the suppression observed by Tn-seq.

strain		spores (%)[a]	SD[b]	fold-suppression
wt	wt	100		-
	Δ*gerAB*	85.6	5.31	
	Δ4 *gerA*$^+$	78.4	3.96	
	Δ4 *gerK*$^+$	35.9	5.65	
Δ*ylbJ::erm*	wt	3.1×10^{-5}	1.3×10^{-5}	
	Δ*gerAB*	0.54	0.14	1.73×10^{4}
	Δ4 *gerA*$^+$	6.7×10^{-4}	1.6×10^{-4}	21.3
	Δ4 *gerK*$^+$	0.61	0.10	1.98×10^{4}
Δ*pdaB::erm*	wt	4.05	0.86	
	Δ*gerAB*	11.01	1.48	2.7
	Δ4 *gerA*$^+$	5.99	1.30	1.5
	Δ4 *gerK*$^+$	12.64	1.22	3.1
Δ*spoVFA::erm*	wt	2.1×10^{-3}	5.6×10^{-4}	
	Δ*gerAB*	0.45	0.13	214.5
	Δ4 *gerA*$^+$	5.5×10^{-3}	1.5×10^{-3}	2.6
	Δ4 *gerK*$^+$	0.59	0.09	279.3
Δ*spoVT::erm*	wt	0.02	0.01	
	Δ*gerAB*	0.08	0.03	3.5
	Δ4 *gerA*$^+$	0.04	0.01	1.5
	Δ4 *gerK*$^+$	0.10	0.01	4.2
Δ*uppP::erm*	wt	0.53	0.07	
	Δ*gerAB*	6.99	1.48	13.3
	Δ4 *gerA*$^+$	0.73	0.10	1.4
	Δ4 *gerK*$^+$	10.12	1.60	19.3

[a] Strains were sporulated in liquid DSM for 24h at 37˚C. Heat-resistant (80°C for 20min) colony forming units (CFU) compared to wild-type heat-resistant CFU are shown. Heat-resistant CFU were counted after 16h growth at 37˚C on agar plates for strains lacking one germinant receptor and after 96h growth for strains lacking 4 germinant receptors. Spores from the latter strains germinate more slowly.

[b] SD, standard deviation.

Table S3. Wild-type strains sporulated under different conditions exhibit GerA-dependent premature germination.

		Bacillus subtilis																		*Bacillus cereus*	
	Background	**168**										**PY79**				**3610**				**569 UM20.1**	
	strain	**wt**		**Δ*gerAB***		**Δ*gerA***		**Δ4 *gerA*⁺**		**Δ*cwlJ* Δ*sleB***		**wt**		**Δ*gerAB***		**wt**		**Δ*gerAB***		**wt**	
	spore type	number	%	number	%	number	%	number	%	number	%	number	%	number	%	number	%	number	%	number	%
DSM (liquid)	phase-bright	3695	91.94	3013	97.98	3095	97.94	2950	96.56	3003	94.1	2738	92.22	2396	98.64						
	phase-dark	64	1.59	10	0.33	12	0.39	53	1.80	0	0	48	1.75	15	0.63						
	dull phase-gray	153	4.14	29	0.94	29	0.92	48	1.57	188*	5.9*	133	4.48	13	0.54						
	hollow	107	2.66	23	0.75	24	0.76	4	0.13	0	0	50	1.68	5	0.21						
	Total	**4019**	**100**	**3076**	**100**	**3160**	**100**	**3055**	**100**	**3191**	**100**	**2969**	**100**	**2429**	**100**						
DSM (solid)	phase-bright	3614	91.91	3309	99.97			3440	94.38			2151	94.34	2203	99.91	3017	98.47	2274	100	1134	98.4
	phase-dark	31	0.86	1	0.03			27	0.78			10	0.46	1	0.05	4	0.13	0	0	15	1.3
	dull phase-gray	258	6.56	0	0.00			156	4.28			97	4.25	1	0.05	41	1.34	0	0	2	0.2
	hollow	29	0.74	0	0.00			22	0.60			22	0.96	0	0.00	2	0.07	0	0	2	0.2
	Total	**3932**	**100**	**3310**	**100**			**3645**	**100**			**2280**	**100**	**2205**	**100**	**3064**	**100**	**2274**	**100**	**1153**	**100**
Resuspension (liquid)	phase-bright	3153	95.87	3203	99.91											3197	96.79	2842	99.89		
	phase-dark	68	2.16	1	0.03											30	0.94	1	0.04		
	dull phase-gray	61	1.85	1	0.03											66	2.00	1	0.04		
	hollow	7	0.21	1	0.03											10	0.30	1	0.04		
	Total	**3289**	**100**	**3206**	**100**											**3303**	**100**	**2845**	**100**		
MSgg (solid)	phase-bright															1943	93.19	2068	98.76		
	phase-dark															32	1.65	10	0.48		
	dull phase-gray															63	3.02	9	0.43		
	hollow															47	2.25	7	0.33		
	Total															**2085**	**100**	**2094**	**100**		

*dark ring or halo around phase-grey spores

Table S4. Quantification of prematurely germinated spores and suppression by Δ*gerAB* in mutants with mild cytological defects.

strain	spore type	DSM (liquid)			
		wt		Δ*gerAB*	
		Total	%	Total	%
Δ*gerPC*	phase-bright	620	87.2	714	99.0
	phase-dark	51	7.2	2	0.3
	dull phase-gray/hollow	40	5.6	5	0.7
	total	**711**	**100**	**719**	**100**
Δ*prkC*	phase-bright	982	85.1	1158	98.4
	phase-dark	76	6.6	6	0.5
	dull phase-gray/hollow	96	8.3	13	1.1
	total	**1154**	**100**	**1177**	**100**
Δ*prpC*	phase-bright	1017	88.2	895	98.5
	phase-dark	88	7.6	4	0.4
	dull phase-gray/hollow	48	4.2	10	1.1
	total	**1153**	**100**	**909**	**100**
Δ*rho*	phase-bright	568	72.5	783	96.8
	phase-dark	41	5.2	4	0.5
	dull phase-gray/hollow	174	22.2	22	2.7
	total	**783**	**100**	**809**	**100**
Δ*spoVG*	phase-bright	526	88.0	553	96.0
	phase-dark	39	6.5	5	0.9
	dull phase-gray/hollow	33	5.5	18	3.1
	total	**598**	**100**	**576**	**100**
Δ*spoVS*	phase-bright	52	12.6	200	33.3
	phase-dark	304	73.8	381	63.5
	dull phase-gray/hollow	56	13.6	19	3.2
	total	**412**	**100**	**600**	**100**
Δ*yfmI*	phase-bright	356	63.8	813	99.4
	phase-dark	191	34.2	1	0.1
	dull phase-gray/hollow	11	2.0	4	0.5
	total	**558**	**100**	**815**	**100**
Δ*yrbG*	phase-bright	505	76.3	943	98.5
	phase-dark	90	13.6	4	0.4
	dull phase-gray/hollow	67	10.1	10	1.0
	total	**662**	**100**	**957**	**100**

Table S5. *Bacillus* strains used in this study.

Strain (***B. subtilis***)	Genotype	Source	Figure(s)
168 (BDR2413)	Wild-type (*trpC2*)	Zeigler *et al*. 2008	2, 3, 4, 5, 6, S3A, S5, S6A, S7, S9, S10, S11, S13, S14 and S15.
BAM786	Δ*gerAB::erm*	Koo *et al*. 2017	
BAM839	Δ*gerAB* Δ*gerBB* Δ*yfkT* Δ*yndE* (Δ**4** ***gerK***$^+$)	This work	S5 and S7
BAM840	Δ*gerAB* Δ*gerKB* Δ*yfkT* Δ*yndE* (Δ**4** ***gerB***$^+$)	This work	S5
BAM841	Δ*gerBB* Δ*gerKB* Δ*yfkT* Δ*yndE* (Δ**4** ***gerA***$^+$)	This work	3, S5 and S7
BAM860	Δ*gerAB* Δ*gerBB* Δ*gerKB* Δ*yfkT* Δ*yndE* (Δ***5***)	This work	S5
BDR3149	Δ*ylbJ::erm*	Koo *et al*. 2017	S5 and S7
BDR3164	Δ*gerAB* Δ*ylbJ::erm*	This work	S5 and S7
BDR3165	Δ*gerBB* Δ*ylbJ::erm*	This work	S5
BDR3166	Δ*gerKB* Δ*ylbJ::erm*	This work	S5
BDR3167	Δ4 *gerA*$^+$ Δ*ylbJ::erm*	This work	S5 and S7
BDR3168	Δ4 *gerB*$^+$ Δ*ylbJ::erm*	This work	S5
BDR3169	Δ4 *gerK*$^+$ Δ*ylbJ::erm*	This work	S5 and S7
BDR3170	Δ5 Δ*ylbJ::erm*	This work	S5
BDR3196	Δ*cwlJ* Δ*sleB*	This work	3 and 5
BDR3371	Δ*gerA::spec*	This work	
BDR3372	Δ*gerA::spec* Δ*ylbJ::erm*	This work	
BDR3143	Δ*spoVT::erm*	Koo *et al*. 2017	S7
BDR3144	Δ*spoVFA::erm*	Koo *et al*. 2017	S7
BDR3147	Δ*pdaB::erm*	Koo *et al*. 2017	S7
BDR3195	Δ*uppP::erm*	Koo *et al*. 2017	S7
BDR3176	Δ*gerAB* Δ*pdaB::erm*	This work	S7
BDR3178	Δ*gerAB* Δ*spoVFA::erm*	This work	S7
BDR3179	Δ*gerAB* Δ*spoVT::erm*	This work	S7
BDR3204	Δ*gerAB* Δ*uppP::erm*	This work	S7
BDR3298	Δ4 *gerA*$^+$ Δ*uppP::erm*	This work	S7
BDR3299	Δ4 *gerA*$^+$ Δ*spoVT::erm*	This work	S7
BDR3300	Δ4 *gerA*$^+$ Δ*spoVFA::erm*	This work	S7
BDR3310	Δ4 *gerA*$^+$ Δ*pdaB::erm*	This work	S7
BDR3528	Δ4 *gerK*$^+$ Δ*spoVT::erm*	This work	S7
BDR3529	Δ4 *gerK*$^+$ Δ*spoVFA::erm*	This work	S7
BDR3530	Δ4 *gerK*$^+$ Δ*pdaB::erm*	This work	S7
BDR3531	Δ4 *gerK*$^+$ Δ*uppP::erm*	This work	S7
BDR3158	Δ*gerAB*	This work	2, 3, 4, 5, S3A, S5, S6A, S7, S9, S10 and S11
BDR3159	Δ*gerBB*	This work	S5
BDR3160	Δ*gerKB*	This work	S5
BDR3151	Δ*spoVFA*	This work	3 and S6C
BDR3152	Δ*spoVAA*	This work	S6C

BDR3153	Δ*spmA*	This work	S6B
BDR3154	Δ*ylbJ*	This work	2, 3, S3A, S3B, S4 and S6E
BDR3155	Δ*ytrI*	This work	S6F
BDR3156	Δ*pdaB*	This work	3 and S6A
BDR3171	Δ*spoVT*	This work	3 and S6D
BDR3218	Δ*ytrH*	This work	S6F
BDR3219	Δ*dacB*	This work	S6A
BDR3220	Δ*prkA*	This work	S6B
BDR3221	Δ*yabQ*	This work	S6D
BDR3222	Δ*ytaF*	This work	S6E
BDR3223	Δ*uppP*	This work	S6D
BDR3232	Δ*yfmI*	This work	S6D
BDR3233	Δ*yrbG*	This work	S6E
BDR3291	Δ*ypbH*	This work	S6E
BDR3292	Δ*gerPC*	This work	S6A
BDR3293	Δ*rho*	This work	S6B
BDR3295	Δ*spoVR*	This work	3 and S6C
BDR3345	Δ*ccdA*	This work	S6A
BDR3346	Δ*yhbH*	This work	S6D
BDR3347	Δ*spoVG*	This work	S6C
BDR3348	Δ*spoVS*	This work	S6C
BDR3349	Δ*yqzK*	This work	S6E
BDR3350	Δ*prpC*	This work	S6B
BDR3351	Δ*prkC*	This work	S6B
BDR3205	Δ*gerAB* Δ*spoVT*	This work	3 and S6D
BDR3206	Δ*gerAB* Δ*spoVFA*	This work	3 and S6C
BDR3207	Δ*gerAB* Δ*spoVAA*	This work	S6C
BDR3208	Δ*gerAB* Δ*spmA*	This work	S6B
BDR3209	Δ*gerAB* Δ*ylbJ*	This work	2, 3, S3A, S3B, S4 and S6E
BDR3210	Δ*gerAB* Δ*ytrI*	This work	S6F
BDR3211	Δ*gerAB* Δ*pdaB*	This work	3 and S6A
BDR3225	Δ*gerAB* Δ*ytrH*	This work	S6F
BDR3226	Δ*gerAB* Δ*yfmI*	This work	S6D
BDR3227	Δ*gerAB* Δ*dacB*	This work	S6A
BDR3228	Δ*gerAB* Δ*prkA*	This work	S6B
BDR3229	Δ*gerAB* Δ*yabQ*	This work	S6D
BDR3230	Δ*gerAB* Δ*yrbG*	This work	S6E
BDR3231	Δ*gerAB* Δ*uppP*	This work	S6D
BDR3234	Δ*gerAB* Δ*ytaF*	This work	S6E
BDR3304	Δ*gerAB* Δ*spoVR*	This work	3 and S6C
BDR3305	Δ*gerAB* Δ*ypbH*	This work	S6E
BDR3307	Δ*gerAB* Δ*rho*	This work	S6B

BDR3308	Δ*gerAB* Δ*gerPC*	This work	S6A
BDR3352	Δ*gerAB* Δ*ccdA*	This work	S6A
BDR3353	Δ*gerAB* Δ*yhbH*	This work	S6D
BDR3354	Δ*gerAB* Δ*spoVG*	This work	S6C
BDR3355	Δ*gerAB* Δ*spoVS*	This work	S6C
BDR3356	Δ*gerAB* Δ*yqzK*	This work	S6E
BDR3357	Δ*gerAB* Δ*prpC*	This work	S6B
BDR3358	Δ*gerAB* Δ*prkC*	This work	S6B
BDR3316	Δ4 *gerA*$^{+}$ Δ*spoVT*	This work	3
BDR3317	Δ4 *gerA*$^{+}$ Δ*spoVFA*	This work	3
BDR3318	Δ4 *gerA*$^{+}$ Δ*pdaB*	This work	3
BDR3477	Δ4 *gerA*$^{+}$ Δ*spoVR*	This work	3
BDR3236	Δ*cwlJ* Δ*sleB* Δ*ylbJ*	This work	3
BDR3274	Δ*cwlJ* Δ*sleB* Δ*pdaB*	This work	3
BDR3275	Δ*cwlJ* Δ*sleB* Δ*spoVT*	This work	3
BDR3278	Δ*cwlJ* Δ*sleB* Δ*spoVFA*	This work	3
BDR3478	Δ*cwlJ* Δ*sleB* Δ*spoVR*	This work	3
BDR3587	Δ*alrB::erm*	Koo *et al*. 2017	S15
PY79	Prototrophic 168 derivative	Youngman *et al.,* 1983	5 and S8A
RL2532	Δ*ylbJ::spec*	Eichenberger *et al.,* 2003	
RL3678	Δ*ytvI::erm* Δ*pdaB::tet*	Silvaggi *et al.,* 2004	
BDR3311	Δ*gerAB::erm*	This work	5 and S8A
BDR3312	Δ*ylbJ::spec*	This work	S8A
BDR3313	Δ*gerAB::erm* Δ*ylbJ::spec*	This work	S8A
BDR3325	Δ*pdaB::tet*	This work	S8A
BDR3326	Δ*gerAB::erm* Δ*pdaB::tet*	This work	S8A
3610	Undomesticated strain	A gift from Dan Kearns	5, S8B and S9
DK1042	*comI*^{Q12L}	Konkol *et al.,* 2013.	
BDR3342	Δ*gerAB::erm comIQ12L*	This work	5, S8B and S9
BDR3343	Δ*pdaB::tet comIQ12L*	This work	S8B
BDR3344	Δ*ylbJ::spec comIQ12L*	This work	S8B
BDR3363	Δ*gerAB::erm* Δ*pdaB::tet comIQ12L*	This work	S8B
BDR3364	Δ*gerAB::erm* Δ*ylbJ::spec comIQ12L*	This work	S8B

Strain (*B. cereus*)	**Genotype**	**Source**	**Figure**
569 UM20.1	*trp-1 Str*^{r}	A gift from Anne Moir	S12

All unmarked mutants are in-frame deletions generated by Cre-mediated recombination and contain a *lox72* scar.

Table S6. List of oligonucleotide primers used in this study.

Primer	Sequence	Use / gene
oAM97	CACGAACGAAAATCGCCATTC	*spec* cassette (isothermal assembly)
oML078	CCATTAGAACATAGGGAGAG	*spec* cassette (isothermal assembly)
oFR1	CTTGTTTCCTTCCATCAGGG	*gerAB*
oFR2	TTCTGACCTCGTTTCCCAGC	*gerAB*
oFR3	AGGGCATGGTTGTGTATCTGC	*ylbJ*
oFR4	ACTAAACGTGCCTTTGCTCGG	*ybaN*
oFR5	TGAATGGTTTCTTTATTAGGC	*gerAA* (isothermal assembly)
oFR6	CTGAGCGAGGGAGCAGAACAATGAGGTCACCTCTTATC	*gerA::spec* (isothermal assembly)
oFR7	GTTGACCAGTGCTCCCTGTAGCAGCCGCCTAATTCAC	*gerA::spec* (isothermal assembly)
oFR8	GTTTCGCCTCAGGGTATATG	*gerAC* (isothermal assembly)
oKO260	CGCGTACGCTGCATATGTCTA	*ylbJ*
oKO261	CTAGGATTGTCAGAGGATGTC	*pdaB*
oKO-1	CGCCGTATCTGTGCTCTCTC	Confirmation of *erm* cassette removal
oKO0	CTCGTTCATAGTAGTTCCTCC	Confirmation of in-frame deletions