SUPPORTING INFORMATION

GerM is required to assemble the basal platform of the SpoIIIA-SpoIIQ transenvelope complex during sporulation in *Bacillus subtilis*

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Running Title: GerM is required to assemble the SpoIIIA-SpoIIQ complex

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SUPPLEMENTARY MATERIAL AND METHODS

TABLE S1: *Bacillus subtilis* strains used in this study

Strain	Genotype	Source
PY79	Prototrophic wild-type	Youngman *et al*., 1983
BTD23	*sacA::PspoIIIA-RBSopt-cfp-spoIIIAH (phleo)*	Doan *et al.,* 2005
BTD1541	*spoIIQ::phleo*	Doan *et al.,* 2009
BTD1609	*yycR::PsspB-rbsopt-cfp (phleo)*	Doan *et al.,* 2009
BKM1930	*sigE::erm, spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ (kan)*	Rodrigues *et al.,* 2013
BAM833*	*gerM::erm, yycR::PsspB-cfp (phleo), amyE::PspoIID-mCherry (spec), pelB::PspoIIQ-yfp (kan), lacA::PgerE-yfp (tet)*	This work
BCR46	*spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ (tet)*	Rodrigues *et al.,* 2013
BCR56	*spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ (tet), spoIIIAH::spec*	This work
BCR80	*spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ(Q168A) (kan), spoIIIAH::erm*	Rodrigues *et al.,* 2013
BCR87	*spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ(Q168A) (kan)*	Rodrigues *et al.,* 2013
BCR151	*yycR::PsspB-cfp (phleo), spoIIQ::spec*	Rodrigues *et al.,* 2013
BCR152	*ycgO::PspoIIQ-spoIIQ(Q168A) (kan), spoIIQ::phleo*	Rodrigues *et al.,* 2013
BCR163	*ycgO::PspoIIQ-spoIIQ (kan), spoIIQ::phleo*	Rodrigues *et al.,* 2013
BCR1071*	*yycR::PsspB-cfp (phleo), amyE::PspoIID-mCherry (spec), pelB::PspoIIQ-yfp (kan), lacA::PgerE-yfp (tet)*	Meeske *et al.,* 2016
BCR1189	*yycR::PsspB-cfp (phleo), spoIIIA::kan*	This work
BCR1190	*yycR::PsspB-cfp (phleo), gerM::erm*	This work
BCR1193	*amyE::PspoIIIAA-optRBS-gfp-spoIIIAG (spec)*	This work
BCR1197	*spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ (tet), spoIIIAH::spec, gerM::erm*	This work
BCR1200	*yycR::PsspB-cfp (phleo), gerM::erm, spoIIIAH::spec*	This work
BCR1211	*spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ(tet), gerM::erm*	This work
BCR1228	*spoIIIAH::erm, amyE::PspoIIIAA-gfp-spoIIIAG (spec)*	This work
BCR1233	*yycR::PsspB-cfp (phleo), spoIIIAH::spec*	This work
BCR1290	*yhdG::PgerM-yfp (cat)*	This work
BCR1296	*spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ (tet), spoIIIAH::spec, gerM::erm, yhdG::gerM (cat)*	This work
BCR1298	*spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ(tet), spoIIIAH::spec, gerM::erm, yhdG::gerM-his6 (cat)*	This work
BCR1300	*yycR::PsspB-cfp (phleo), gerM::erm, yhdG::gerM (cat)*	This work
BCR1302	*yycR::PsspB-cfp (phleo), gerM::erm, yhdG::gerM-his6 (cat)*	This work
BCR1304	*yycR::PsspB-cfp (phleo), gerM::erm, spoIIIAH::spec, yhdG::gerM (cat)*	This work
BCR1306	*yycR::PsspB-cfp (phleo), gerM::erm, spoIIIAH::spec, yhdG::gerM-his6 (cat)*	This work
BCR1313	*ycgO::spoIIQ (Q168A) (kan), spoIIQ::phleo, gerM::erm*	This work
BCR1314	*ycgO::spoIIQ (kan), spoIIQ::phleo, gerM::erm*	This work
BCR1321	*yhdG::PgerM-yfp (cat), sigE::erm*	This work
BCR1327	*sacA::PspoIIIA-cfp-spoIIIAH (phleo), gerM::erm*	This work
BCR1328	*amyE::PspoIIIAA-gfp-spoIIIAG (spec), gerM::erm*	This work
BCR1330	*yhdG::gerM-his6 (cat), gerM::erm*	This work
BCR1332	*yhdG::gerM-mCherry (cat), gerM::erm*	This work
BCR1334	*ycgO::spoIIQ (Q168A) (kan), spoIIQ::phleo, spoIIIAH::spec*	This work
BCR1335	*ycgO::spoIIQ (kan), spoIIQ::phleo, spoIIIAH::spec*	This work
BCR1339	*yhdG::gerM-his6 (cat), gerM::erm, spoIVB::spec*	This work
BCR1340	*amyE::PspoIIIAA-gfp-spoIIIAG (spec), spoIIQ::tet*	This work
BCR1343	*amyE::PspoIIIAA-gfp-spoIIIAG (spec), gerM::erm, spoIIIAH::kan*	This work
BCR1344	*yhdG::gerM-mCherry (cat), gerM::erm, spoIIIAH::spec*	This work
BCR1345	*yhdG::gerM-mCherry (cat), gerM::erm, spoIIQ::tet*	This work
BCR1346	*yhdG::gerM-mCherry (cat), gerM::erm, spoIIIA::kan*	This work
BCR1347	*yhdG::gerM-mCherry (cat), gerM::erm, spoIIP::tet*	This work
BCR1348	*yhdG::gerM-mCherry (cat), gerM::erm, spoIIQ::tet, ycgO::spoIIQ Q168A (kan)*	This work
BCR1353	*yhdG::gerM-mCherry (cat), gerM::erm, spoIIQ::tet, ycgO::spoIIQ (Q168A) (kan), spoIIIAH::spec*	This work
BCR1354	*spoIIQ::phleo, ycgO::PspoIIQ-gfp-spoIIQ (Q168A) (kan), gerM::erm*	This work
BCR1381	*yhdG::gerM-mCherry (cat), gerM::erm, spoIIP::tet, spoIID::spec*	This work
BCR1403	*yycR::PsspB-cfp (phleo), gerM::erm, yhdG::gerM-mCherry (cat)*	This work
BCR1404	*yycR::PsspB-cfp (phleo), gerM::erm, spoIIIAH::spec, yhdG::gerM-mCherry (cat)*	This work

BCR1414	*yhdG::gerM-mCherry (cat), gerM::erm, spoIID::spec*	This work
BCR1444	*sigE::erm, ycgO::PspoIIQ-gfp-spoIIQ (tet), pelB::Phyperspank-spoIID (cat), ΔspoIIQ, yrvN::Phyperspank-spoIIM (spec), ykoW::Phyperspank-spoIIP (phleo)*	This work
BCR1446	*sigE::erm, ycgO::PspoIIQ-gfp-spoIIQ (tet), pelB::Phyperspank-spoIID (cat), ΔspoIIQ, yrvN::Phyperspank-spoIIM (spec), ykoW::Phyperspank-spoIIP (phleo), amyE::Phyperspank-spoIIIAH (kan)*	This work
BCR1447	*sigE::erm, ycgO::PspoIIQ-gfp-spoIIQ (tet), pelB::Phyperspank-spoIID (cat), ΔspoIIQ, yrvN::Phyperspank-spoIIM (spec), ykoW::Phyperspank-spoIIP (phleo), amyE::Phyperspank-gerM-his6 (kan)*	This work

***** These strains are in the 168 *trpC2* wild-type background

TABLE S2: Plasmid vectors used in this study

Plasmids	Description	Source
pCR214	*amyE::PspoIIIA-optRBS-gfp(mut3)-spoIIIAG (spec)*	This work
pCR224	*yhdG::gerM (cat)*	This work
pCR225	*yhdG::gerM-his6 (cat)*	This work
pCR226	*yhdG::PgerM-yfp (cat)*	This work
pCR228	*yhdG::gerM-mCherry (cat)*	This work
pCR261	*amyE::Phyperspank-optRBS-gerM-his6 (kan)*	This work
pCR262	*amyE::Phyperspank-optRBS-spoIIIAH (kan)*	This work

TABLE S3: Oligonucleotide primers used in this study

Primers	Sequence*
oCR403	atggtagcgaccggcgctcaGGATCCttatttgtataattcgtccattccacctg
oCR431	gcgCTCGAGgccgcttgagcctccagatgatcctttgtatagttcatccatgccatg
oCR432	gcgCTCGAGatgaataaaaacggattatggaatg
oCR433	gcgGGATCCttatgaatcctcctttatttttttag
oCR471	cgcAAGCTTacagcccgggaagtcagcacaattc
oCR488	cgcGGATCCcgattgtatgtacataaacca
oCR489	gcgCTCGAGtaaggatgtttgtactattatgtatac
oCR490	gcgCTCGAGacataaggaggaactactatgagtaaaggagaagaacttttc
oCR493	cgcGGATCCttaatggtgatggtggtgatgaaaactacccgtattcacttgag
oCR498	gcgCTCGAGggatcatctggaggctcaagcggcatggtcagcaagggagaggaagat
oCR547	agcggataacaattaagcttacataaggaggaactactatgctgaaaaaaggacctgca
oCR549	agcggataacaattaagcttacataaggaggaactactatgcttaaaaaacaaaccgtt
oCR550	catgcggctagctgtcgactttatttagagggttcaaatgtga
oCR554	catgcggctagctgtcgactttaatggtgatggtggtgatg
oDR078	gccGGATCCttatttgtatagttcatccatgcc
oDR107	ggcAAGCTTacataaggaggaactactatgagtaaaggagaagaac

* Capital letters indicate restriction sites

Plasmid construction

pCR214 [*amyE::PspoIIIA-optRBS-gfp(mut3)-spoIIIAG (spec)*] was generated in a three-way ligation with a *Hind*III-*Xho*I PCR product containing *gfp(mut3)* (oligonucleotide primers oDR107 & oCR431) and a *Xho*I-*Bam*HI PCR product containing *spoIIIAG* (oligonucleotide primers oCR432 & oCR433 and PY79 genomic DNA as template) and pDT019 (*amyE::PspoIIIA-RBSspoIIIA-cfp-spoIIIAG*) [1] cut with *Hind*III and *Bam*HI.

pCR224 [*yhdG::gerM (cat)*] was generated in a two-way ligation with a *Hind*III-*Bam*HI PCR product containing the *gerM* gene (oligonucleotide primers oCR471 & oCR488 and PY79 genomic DNA as template) and pBB275 (*yhdG::cat*) cut with *Hind*III and *Bam*HI. pBB275 is an ectopic integration vector for double crossover integration at the *yhdG* locus (B. Burton and D.Z.R, unpublished).

pCR225 [*yhdG::gerM-his6 (cat)*] was generated in a two-way ligation with a *Hind*III-*Bam*HI PCR product containing the *gerM* gene with a C-terminal hexahistidine tag (oligonucleotide primers oCR471 & oCR493 and PY79 genomic DNA as template) and pBB275 (*yhdG::cat*) cut with *Hind*III and *Bam*HI.

pCR226 [*yhdG::PgerM-optRBS-yfp (cat)*] was generated in a three-way ligation with a *Hind*III-*Xho*I PCR product containing the *gerM* promoter (oligonucleotide primers oCR471 & oCR489 and PY79 genomic DNA as template), an *Xho*I-*Bam*HI PCR product containing the *yfp* gene (oligonucleotide primers oCR490 & oDR078 with pKM012 (a*myE::PspoIID-yfp*) as template) and pBB275 (*yhdG::cat*) cut with *Hind*III and *Bam*HI.

pCR228 [*yhdG::gerM-mCherry (cat)*] was generated in a three-way ligation with a *Hind*III-*Xho*I PCR product containing the *gerM* gene (oligonucleotide primers oCR471 & oCR472 with PY79 genomic DNA as template), an *Xho*I-*Bam*HI PCR product containing the *mCherry* gene (oligonucleotide primers oCR498 & oCR403 with pCR100 (a*myE::PspoIID-mCherry* (*B.subtilis* codon-optimized) as template) and pBB275 (*yhdG::cat*) cut with *Hind*III and *Bam*HI.

pCR260 [*amyE::Phyperspank (kan)*] was generated by a two-way ligation with an *Eco*RI-*Bam*HI insert from pDR11 containing the *hyperspank* promoter, multiple cloning site and *lacI* gene (*amyE::hyperspank*) and pER82 cut with *Eco*RI-*Bam*HI. pER82 (*amyE::kan*) is a double-crossover vector for ectopic integration at the *amyE* locus (E. Riley and D. Z. R. unpublished)

pCR261 [*amyE::Phyperspank-optRBS-gerM-his6 (kan)*] was generated by the double PCR technique [2]. Briefly, a PCR product containing *gerM-his6* and flanking regions for annealing to the multiple-cloning site of pCR260 (oligonucleotide primers oCR547 & oCR554 with PY79 genomic DNA as template) was used in a PCR reaction with pCR260.

pCR262 [*amyE::Phyperspank-optRBS-spoIIIAH (kan)*] was generated by the double PCR technique [2]. Briefly, a PCR product containing *spoIIIAH* with flanking regions for annealing to the multiple-cloning site of pCR260 (oligonucleotide primers oCR549 & oCR550 with PY79 genomic DNA as template) was used in a PCR reaction with pCR260.

References

1. Doan T, Morlot C, Meisner J, Serrano M, Henriques AO, et al. (2009) Novel secretion apparatus maintains spore integrity and developmental gene expression in Bacillus subtilis. PLoS Genet 5: e1000566.

2. van den Ent F, Lowe J (2006) RF cloning: a restriction-free method for inserting target genes into plasmids. J Biochem Biophys Methods 67: 67-74.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Cytological analysis of the *gerM* mutant. Representative images of wild-type (WT, BCR1071) and the Δ*gerM* mutant (BAM833) in a sporulation time course (induced by resuspension) at hours 1.75 (T1.75), 2.5 (T2), 3.5 (T3.5) and 5 (T5). Images (from left to right) are phase contrast, membrane staining with TMA-DPH, σF activity (P_{spoIIQ}-*yfp*) and σK (P_{gerE}-*yfp*), σE activity (P_{spoIID}-*mCherry*) and σG activity (P_{sspB}-*cfp*). Scale bar indicates 2 μm.

Figure S2: Complementation of the Δ*gerM* mutant with *gerM* and *gerM-his6* alleles. Representative images of sporulating cells harboring a σG-dependent reporter (P_{sspB}-*cfp*) at hour 4 after the onset of sporulation (induced by resuspension). Images are wild-type (WT, BTD1609), Δ*gerM* (BCR1190), Δ*AH* (BCR1233), the Δ*gerM* Δ*AH* double mutant (BCR1200), Δ*gerM* complemented with wild-type *gerM* (BCR1300), Δ*gerM* Δ*AH* complemented with wild-type *gerM* (BCR1304), Δ*gerM* complemented with *gerM-his6* (BCR1302), and Δ*gerM* Δ*AH* complemented with *gerM-his6* (BCR1306). Scale bar indicates 2 μm. Spore titers relative to wild-type at hour 30 are indicated on the right.

Figure S3: *gerM* transcription depends on σE. Representative images of sporulating cells containing a *gerM* promoter fusion to the gene encoding yellow fluorescent protein (*yfp)* (P_{gerM}-*yfp*) at 2.5 hours of sporulation. Images are wild-type (WT, BCR1290) and Δ*sigE* (BCR1321). Scale bar represents 2 μm.

Figure S4: *gerM* and *gerM-his6* alleles restore proper localization to GFP-Q in a Δ*AH* Δ*gerM* double mutant. Representative images of GFP-Q localization in sporulating cells at hour 2 of sporulation (induced by resuspension). Images are from Δ*AH* (BCR56), Δ*AH* Δ*gerM* (BCR1197), and Δ*AH* Δ*gerM* complemented by a wild-type copy of *gerM* (BCR1296) or *gerM-his6* (BCR1298). Scale bar represents 2 μm.

Figure S5: GerM-mCherry is functional. Representative images of sporulating cells, at hour 4 after the onset of sporulation (induced by resuspension), containing a σG-dependent reporter (P_{sspB}-*cfp*). Images are wild-type (WT, BTD1609), Δ*gerM* (BCR1190), Δ*AH* (BCR1233), the Δ*gerM* Δ*AH* double mutant (BCR1200), Δ*gerM* complemented with *gerM-mCherry* (BCR1403),

and Δ*gerM* Δ*AH* complemented with *gerM-mCherry* (BCR1404). Scale bar represents 2 μm. Spore titers relative to wild-type at hour 30 are indicated on the right.

Figure S6: GerM-mCherry localization to the septal membrane requires thinning of the septal peptidoglycan. Representative images of GerM-mCherry localization at hour 2.5 of sporulation (induced by resuspension). Images are from wild-type (BCR1332), the Δ*spoIID* Δ*spoIIP* double mutant (BCR1381), Δ*spoIIP* (BCR1347), and Δ*spoIID* (BCR1414). Enrichment of GerM-mCherry at septal bulges is highlighted (yellow carets). Scale bar represents 2 μm.

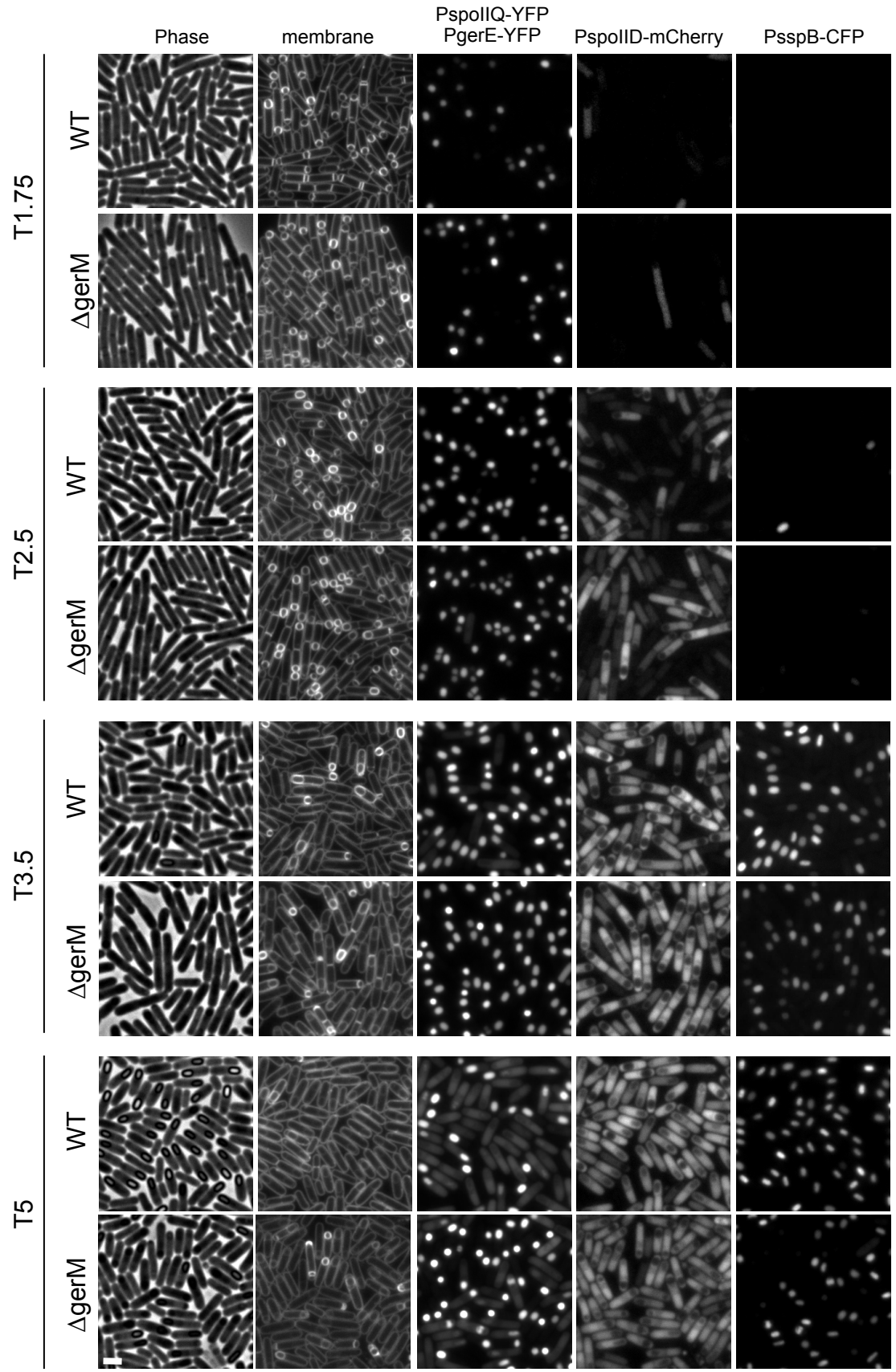
Figure S7: Quantification of septal GFP-Q fluorescence when AH or GerM is artificially produced in the absence of σE. Graphs quantifying GFP-Q fluorescence on background subtracted images using a line-scan from Metamorph image analysis software. In all cases, the sporulating cell was scanned as depicted above the graphs. The signal intensity was plotted on the Y-axis as a function of position along the sporulating cell (X-axis). **A.** Analysis of 10 sporulating cells (strain BCR1444) in which SpoIID, SpoIIP and SpoIIM were artificially produced. **B.** Analysis of 10 sporulating cells (strain BCR1446) in which SpoIID, SpoIIP, SpoIIM and SpoIIIAH were artificially produced. **C.** Analysis of 10 sporulating cells (strain BCR1447) in which SpoIID, SpoIIP and GerM were artificially produced. Images are GFP-Q (left) and merge of GFP-Q with membranes stained with TMA-DPH. Scale bar indicates 2 μm.

Figure S8: GFP-Q is mislocalized when IPTG is omitted from the experiment described in Figure 4A. Representative images of GFP-Q in sporulating cells lacking *sigE* at hour 2.5. The strains contain IPTG-inducible alleles of *spoIID*, *spoIIM* and *spoIIP* (DMP) alone (BCR1444) or together with an IPTG-inducible allele of *gerM* (BCR1447) or *AH* (BCR1446). Scale bar indicates 2 μm.

Figure S9: GerM is not required for CFP-AH localization. Representative images of CFP-AH localization in sporulating cells at hour 2.5 (induced by resuspension). Images are from wild-type (BTD23) and Δ*gerM* (BCR1327). Scale bar represents 2 μm.

FIgure S10: GerM stays behind after Q and AH are degraded. Immunoblot analysis during a 30 min time-course after the onset of sporulation (induced by resuspension). Consistent with a later role for GerM in sporulation, GerM levels stay high during late stages of sporulation, while AH and Q are degraded.

Figure S11: GerM is conserved in a subset of endospore-forming bacteria but not in the *Clostridiales*. A. Occurrence of GerM across the bacterial phylogenetic tree. Red bands indicate the presence of a GerM homologue in the indicated species. **B** and **C.** Enlargement of the boxed areas in panel A**.** The NCBI nr database was searched using the *B. subtilis* GerM amino acid sequence as the query. The BLASTp search program was used with an E-value cutoff of $1x10^{-4}$. Detected orthologs were cross-referenced with a list of 1773 diverse bacterial taxa and plotted onto a phylogenetic tree. The tree was constructed in PhyloT (http://phylot.biobyte.de) and was displayed and manually pruned in iTOL (http://itol.embl.de).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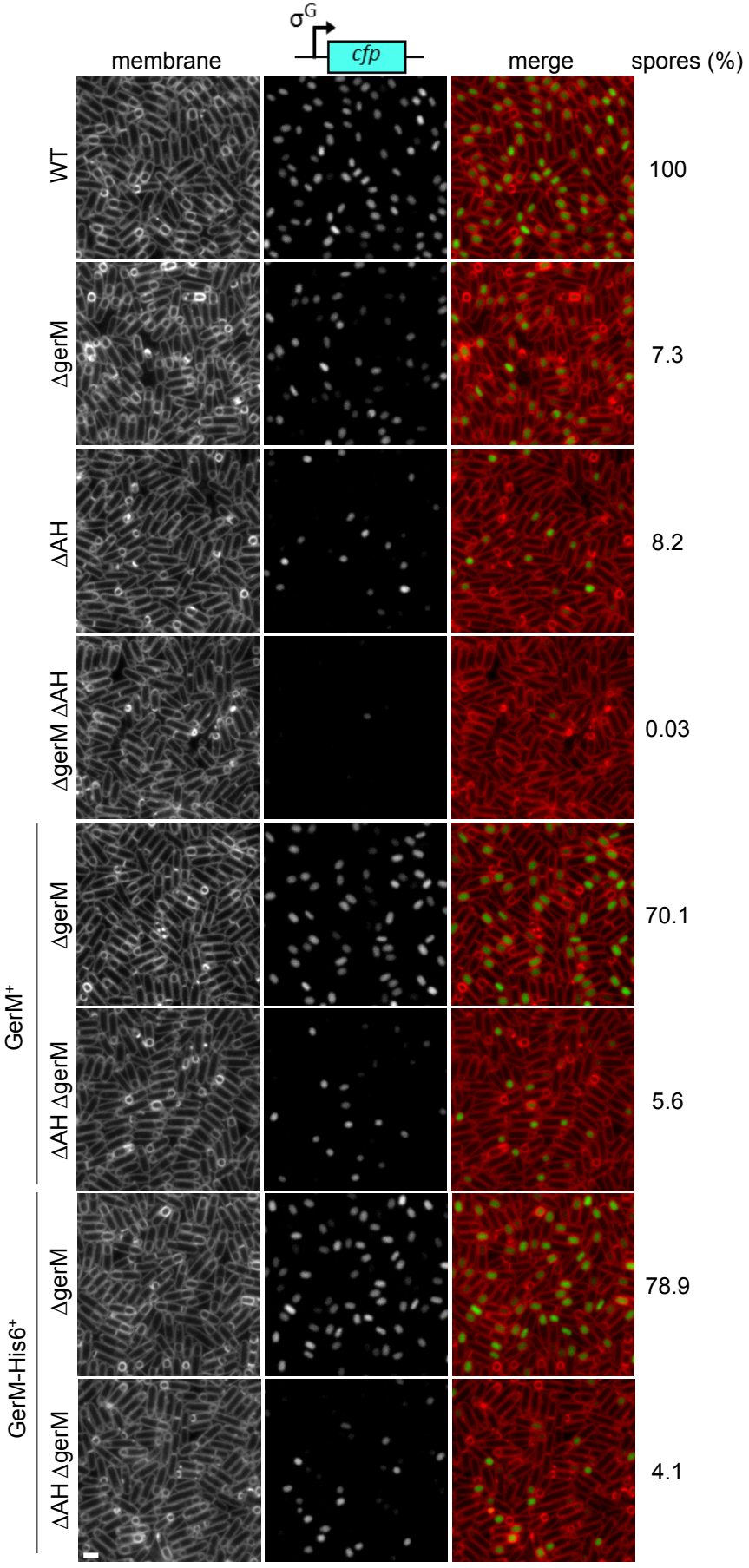
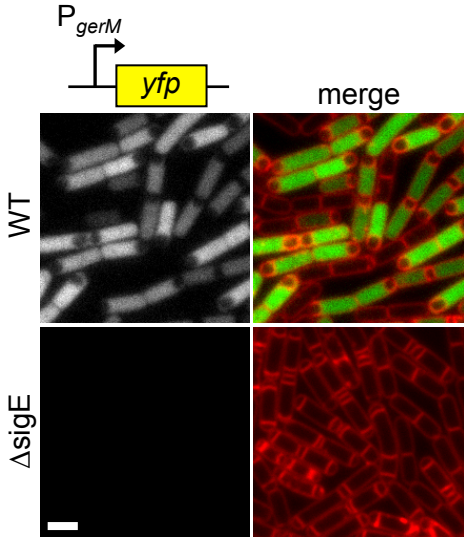
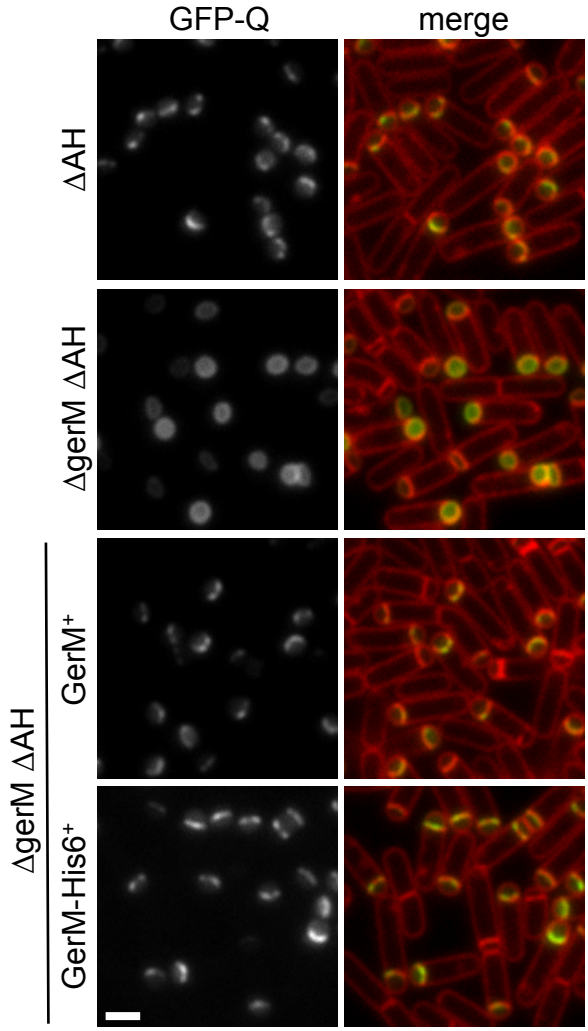
PgerE-YFP
PspoIID-mCherry
PsspB-CFP
T1.75
T2.5
T3.5
T5
WT
ΔgerM

Rodrigues et al., Fig.S2




P gerM
yfp
merge
WT
ΔsigE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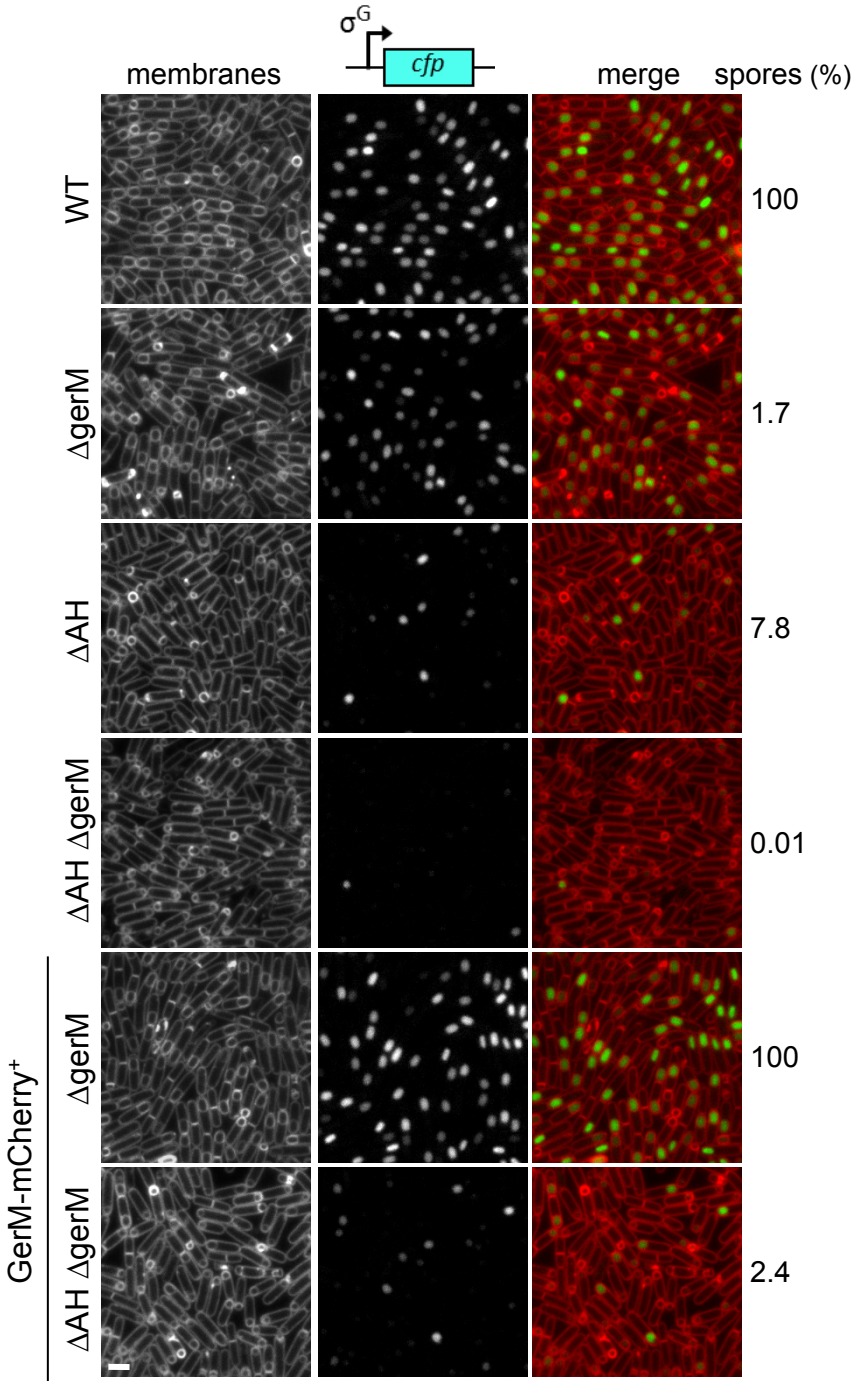
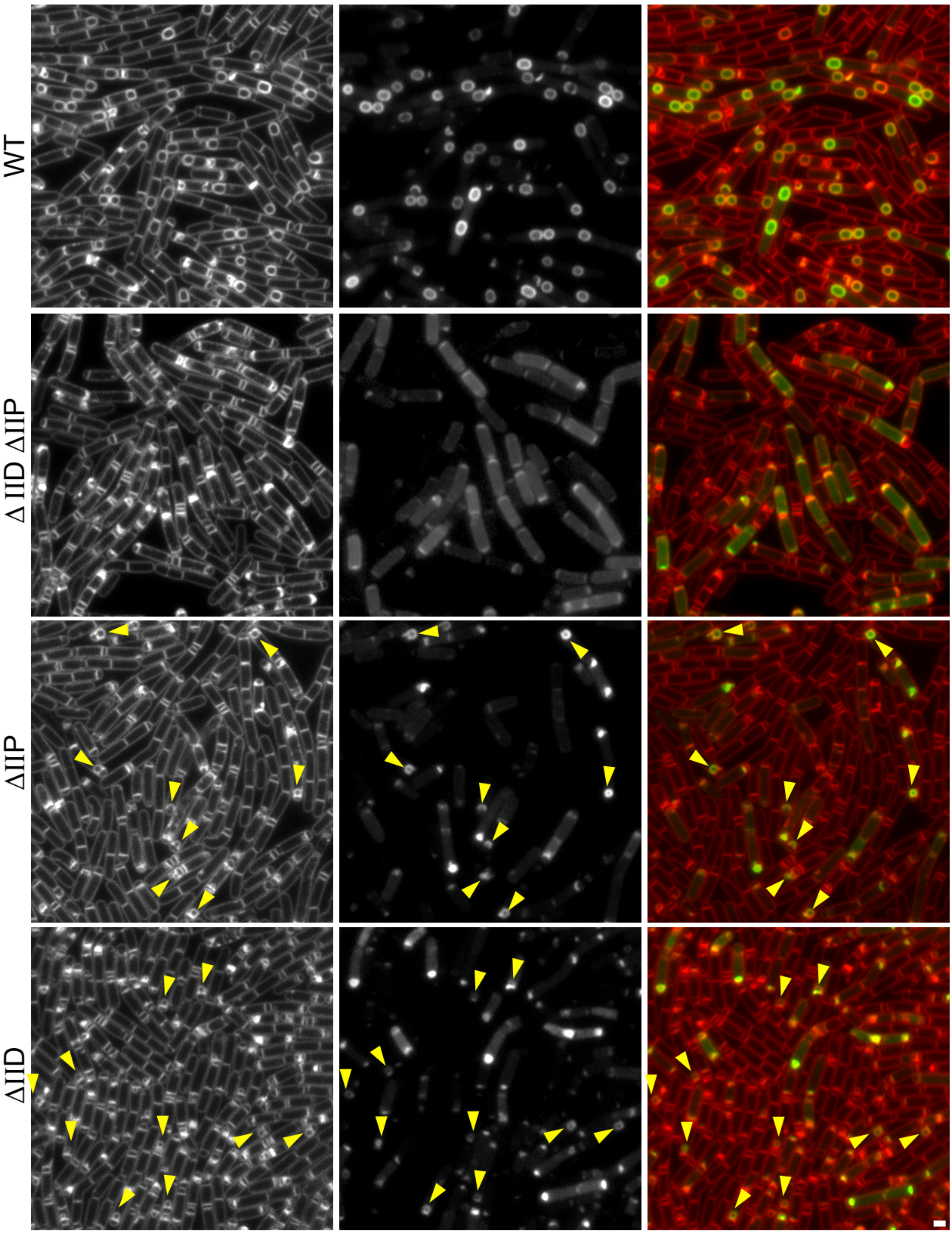
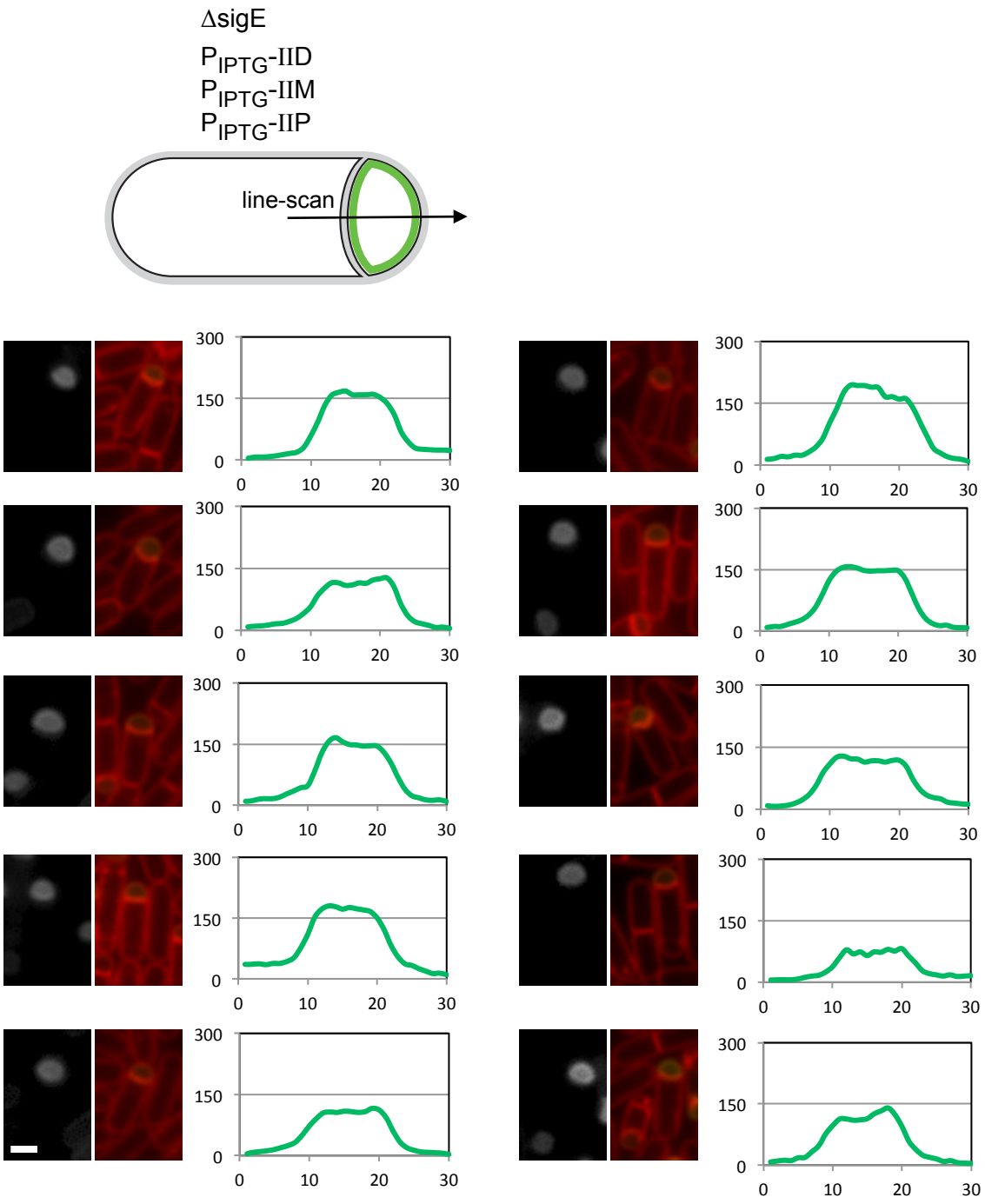
merge
ΔAH
ΔgerM ΔAH
ΔgerM ΔAH
GerM+
GerM-His6+

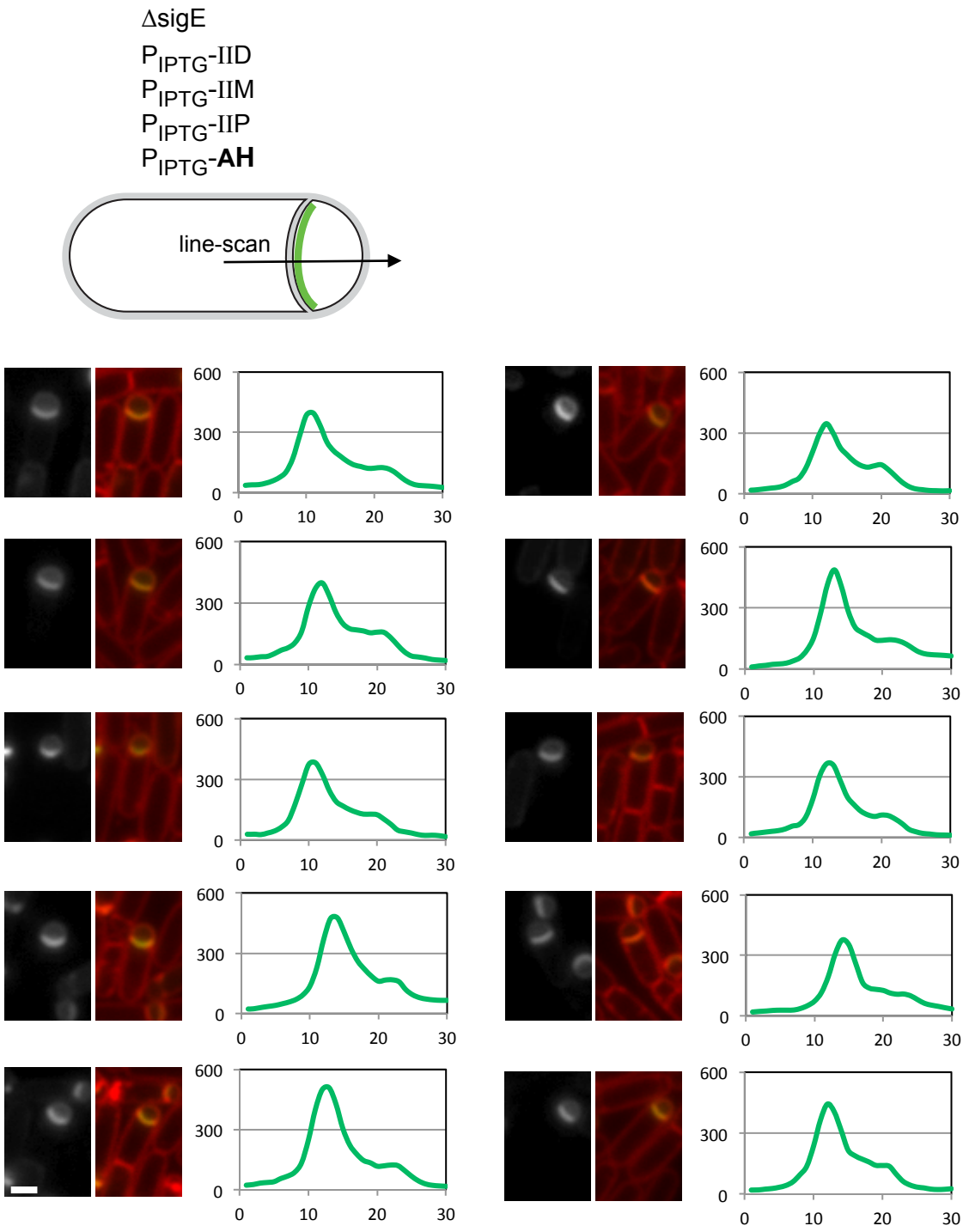

σG
cfp
membranes
merge
spores (%)
WT
ΔgerM
ΔAH
ΔAH ΔgerM
GerM-mCherry+
ΔgerM
ΔAH ΔgerM
100
1.7
7.8
0.01
100
2.4

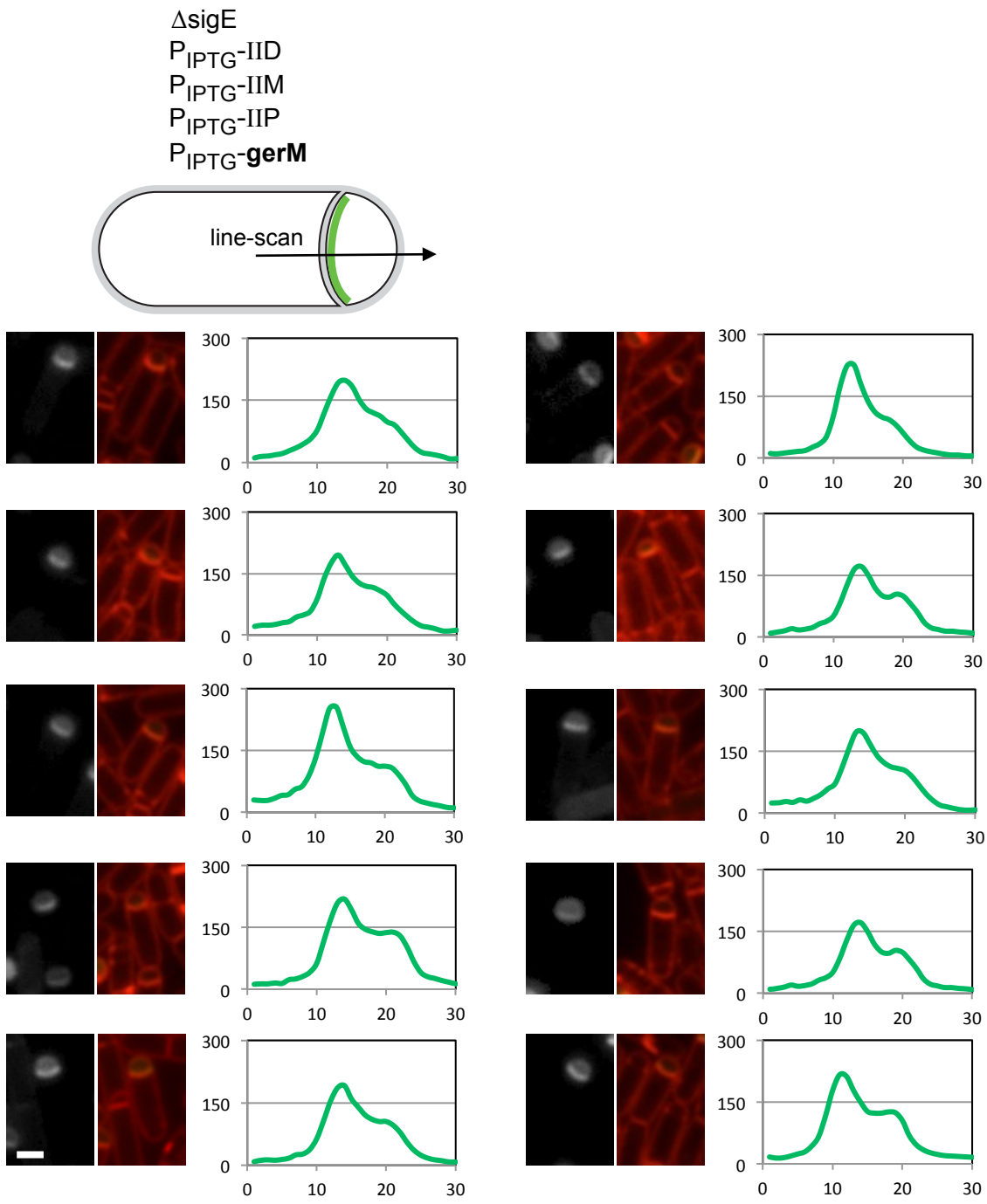
Rodrigues et al., Fig.S6




ΔsigE
P_{IPTG}-IID
P_{IPTG}-IIM
P_{IPTG}-IIP
line-scan
300
150
0
0
10
20
30

Rodrigues et al. Fig.S7B




ΔsigE
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P IPTG -IIM
P IPTG -IIP
P IPTG -gerM
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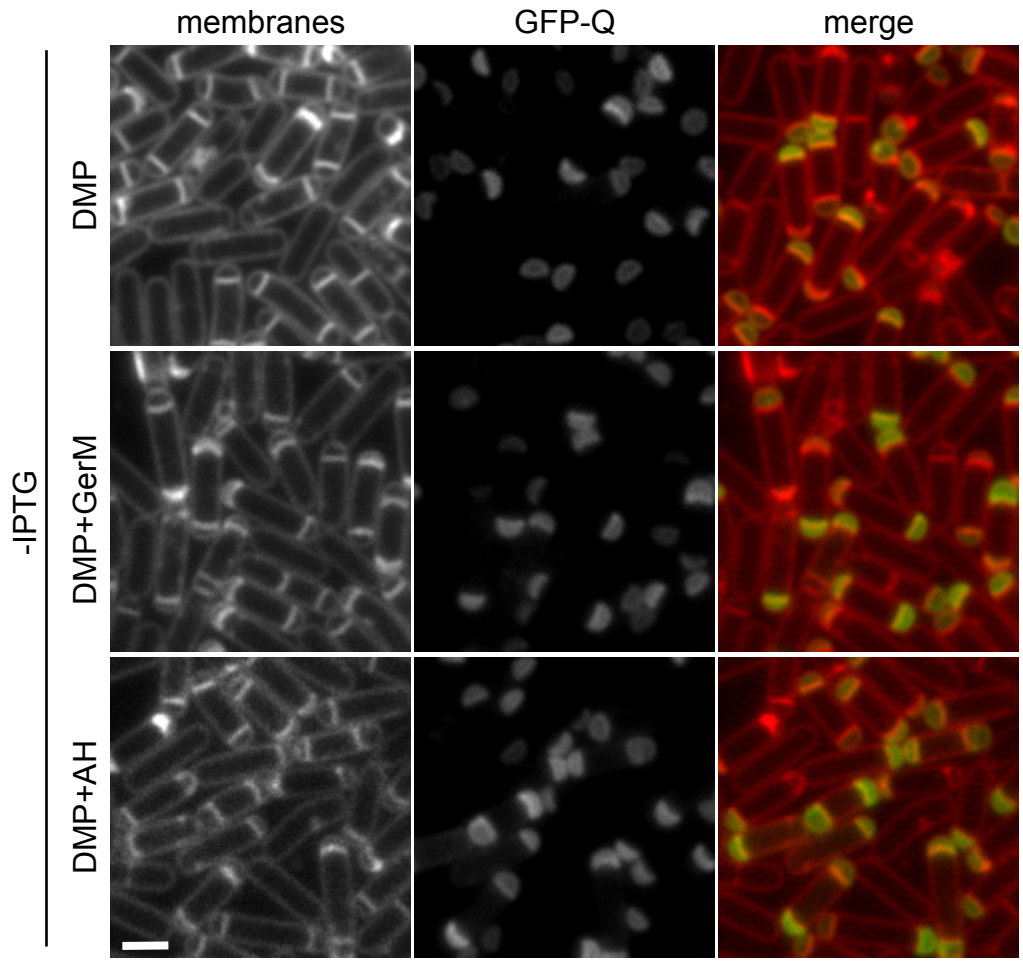
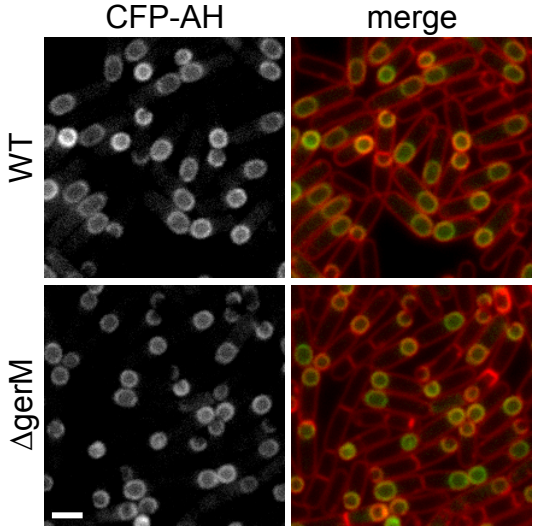
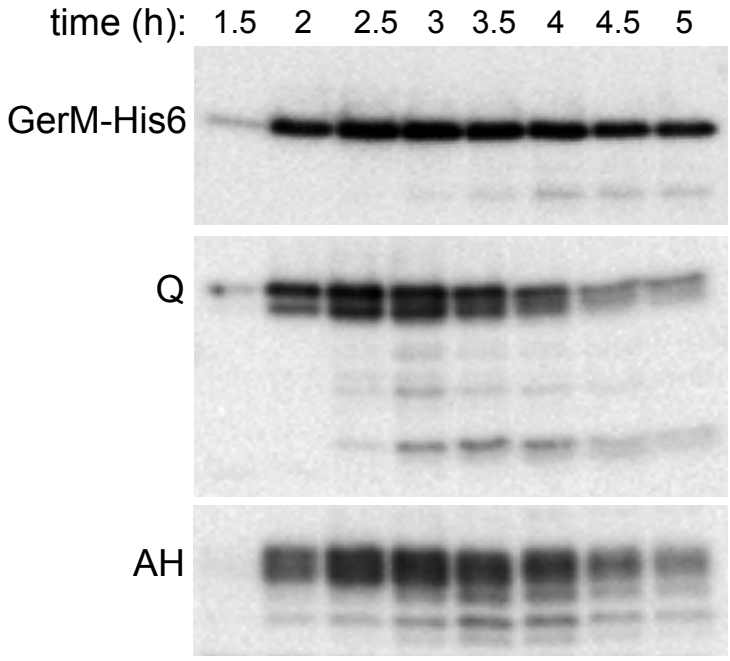
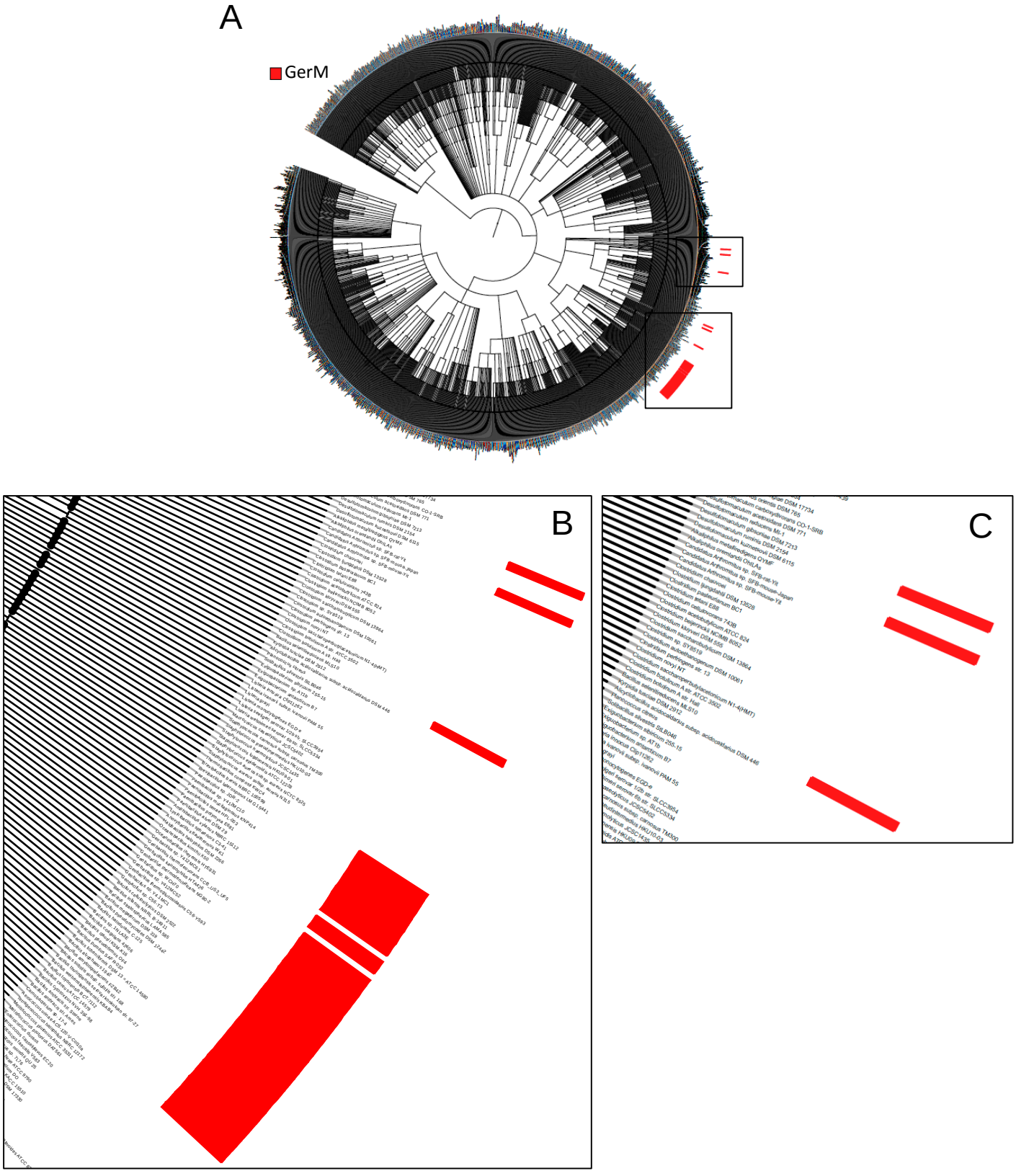
150
0
0
10
20
30


membranes
GFP-Q
merge
-IPTG
DMP
DMP+GerM
DMP+AH


CFP-AH
merge
WT
ΔgerM

Rodrigues et al., Fig.S10




A
GerM
B
C