SUPPLEMENTAL INFORMATION

Evidence that regulation of intramembrane proteolysis is mediated by substrate gating during sporulation in *Bacillus subtilis*

Fernando H. Ramírez-Guadiana, Christopher D. A. Rodrigues, Kathleen A. Marquis, Nathalie Campo, Rocío del Carmen Barajas-Ornelas, Kelly Brock, Debora S. Marks, Andrew C. Kruse, David Z. Rudner

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Supplemental Methods

S1 Methods. Plasmids and strains construction.

pKM190 [*spoIVFB-yfp* (*spec*)] (single crossover integration) was generated in a two-way ligation with an MfeI-XhoI PCR product containing the 3' end of the *spoIVFB* gene (oligonucleotide primers oDR481 + oDR482 and pDR004 (*spoIVF* operon) DNA as template) and pKL183 cut with *Eco*RI and *Xho*I. pKL183 (Lemon & Grossman 2000) is a single crossover integration vector containing the *yfp* gene.

pKM261 [*ycgO::PspoIVF-spoIVFB(E44Q)-yfp (erm)*] was generated in a three-way ligation with a *Hind*III-*Xho*I PCR product containing spoIVFB(E44Q) (oligonucleotide primers oDR106 + oDR482 and pDR019 DNA as template), a *Xho*I-*Bam*HI PCR product contain the *yfp* gene (oligonucleotide primers oDR78 + oDR79 and pKL183 as template), and pKM259 cut with *Hind*III and *Bam*HI. pKM259 [*ycgO*::P*spoIVF* (erm)] was generated in a two-way ligation with an *EcoRI*-*Hind*III fragment containing the PspoIVF promoter from pDR077 and pKM084 cut with *Eco*RI and *Hind*III. pKM084 [*ycgO::erm*] is an ectopic integration vector for double crossover integration at the ycgO locus (KAM and DZR, unpublished)

pKM266 [*spoIIIC-cfp (cat)*] (single crossover integration) was generated in a two-way ligation with an *Eco*RI-*Xho*I PCR product containing the 3' end of the spoIIIC (oligonucleotide primers oDR594 + oDR595 and pSK6 DNA as template) and pKM135 cut with *Eco*RI and *Xho*I. pKM135 is a single crossover integration vector containing the *mCFP* gene.

pKM283 [*ycgO::PspoIVF-spoIVFB-yfp (erm)*] was generated in a two-way ligation with a *Hind*III-*Xho*I PCR product containin the spoIVFB gene (oligonucleotide primers oDR106 + oDR482 and pDR004 DNA as template) and pKM261 cut with *Hind*III and *Xho*I.

pCR275 [*ycgO::Pspank-spoIVFB(E44Q)-yfp (erm)*] was generated in a two-way ligation with a *Hind*III-*Nhe*I PCR product containing *spoIVFB(E44Q)-yfp* (oligonucleotide primers oCR603 + oCR604 and pKM261 DNA as template) and pER65 cut with *Hind*III-*Nhe*I. pER65 [*ycgO::Pspank (lacI) (erm)*] is a double crossover vector, with an IPTG-inducible promoter, for ectopic integration at the *ycgO* locus (E. Riley and DZR, unpublished).

pCR276 [*ycgO::Pspank-spoIVFB-yfp (erm)*] was generated in a two-way ligation with a *Hind*III-*Nhe*I PCR product containing *spoIVFB-yfp* (oligonucleotide primers oCR603 + oCR604 and pKM283 DNA as template) and pER065 cut with *Hind*III-*Nhe*I.

pCR278 [*amyE::Pspank-pro-sigK-cfp (spec)*] was generated in a three-way ligation with a *Hind*III-*Nhe*I PCR product containing *pro-sigK* (oligonucleotide primers oCR599 + oCR600 and pDR012 (*amyE::pro-sigK (cat)*) DNA as template), an *Nhe*I-*Sph*I PCR product containing m*cfp* (oligonucleotide primers oCR601 + oCR606 and pCR034 (*sacA::PspoIIQ-cfp-spoIIQ (spec)*) DNA as template), and pDR110 cut with *Hind*III and *Sph*I. pDR110 [*amyE*::*Pspank (lacI) (spec)*] is a double crossover vector, with an IPTG-inducible promoter, for ectopic integration at the *amyE* locus (DZR, unpublished).

pCR286 [*ycgO::PspoIVF-spoIVFB(E44Q)Δ10-yfp (erm)*] was generated in a two-way ligation with *Hind*III-*Xho*I PCR product containing *spoIVFB(E44Q)Δ10* (oligonucleotide primers oDR106 + oCR619 on pKM261 DNA as template) and pKM261 cut with *Hind*III-*Xho*I.

pCR287 [*ycgO::PspoIVF-spoIVFB(E44Q)Δ66-yfp (erm)*] was generated in a two-way ligation with *Hind*III-*Xho*I PCR product containing *spoIVFB(E44Q)Δ66* (oligonucleotide primers oDR106 + oCR620 and pKM261 DNA as template) and pKM261 cut with *Hind*III-*Xho*I.

pCR288 [*ycgO::PspoIVF-spoIVFB(E44Q)Δ85-yfp (erm)*] was generated in a two-way ligation with a *Hind*III-*Xho*I PCR product containing *spoIVFB(E44Q)Δ85* (oligonucleotide primers oDR106 + oCR621 and pKM261 DNA as template and pKM261 cut with *Hind*III-*Xho*I.

pFR20 [*ycgO*::*PspoIVF-spoIVFB*Δ*10-yfp (erm)*] was generated in a two-way ligation with a *Hind*III-*Xho*I PCR product containing *spoIVFB*Δ10 (oligonucleotide primers oDR106 + oCR619 and genomic DNA from PY79 as template) and pKM261 cut with *Hind*III-*Xho*I.

pFR21 [*ycgO*::*PspoIVF-spoIVFB*Δ*66-yfp (erm)*] was generated in a two-way ligation with a *Hind*III-*Xho*I PCR product containing *spoIVFB*Δ66 (oligonucleotide primers oDR106 + oCR620 and genomic DNA from PY79 as template) and pKM261 cut with *Hind*III-*Xho*I.

pFR22 [*ycgO*::*PspoIVF-spoIVFB*Δ*85-yfp (erm)*] was generated in a two-way ligation with a *Hind*III-*Xho*I PCR product containing *spoIVFB*Δ85 (oligonucleotide primers oDR106 + oCR621 and genomic DNA from PY79 as template) and pKM261 cut with *Hind*III-*Xho*I.

pFR28 [*yvbJ::Phyperspank-spoIVFA* (*cat*)] was generated by isothermal assembly of two pieces: 1) plasmid pMS024 cut with HindIII and NheI; 2) a PCR product containing *spoIVFA* (oligonucleotide primers oFR58 + oFR59 and genomic DNA from PY79 as template). pMS024 [*yvbJ::Phyperspank (cat)*] is an ectopic integration vector harboring the P*xylA* promoter for double crossover integrations at the *yhdG* locus (M. Stanley and DZR, unpublished).

pFR29 [*yhdG::Phyperspank-bofA* (*kan*)] was generated in a two-way ligation with a *Spe*I-*Sph*I PCR product containing *bofA* (oligonucleotide primers oFR62 + oFR66 and genomic DNA from PY79 as template) and pMS036 cut with *Spe*I and *Sph*I. pMS036 [*yhdG::Phyperspank (kan)*] is an ectopic integration vector harboring the P*hyperspank* promoter for double crossover integrations at the *yhdG* locus (M. Stanley and DZR, unpublished).

pFR30 [*lacA*::*spoIVB*(S378A) (*tet*)] was generated in a two-way ligation with an *Xho*I-*Nhe*I PCR product containing *spoIVB*(S378A) (oligonucleotide primers oFR72 + oFR73 and genomic DNA from BDR1454) and pNC018 cut with *Xho*I and *Nhe*I. pNC018 [*lacA::tet*] is an ectopic integration vector for double crossover integrations at the *lacA* locus (NC and DZR, unpublished).

pFR31 [*ycgO*::*PspoIVF-spoIVFB(E44Q)∆66*-m*yfp (erm)*] was generated in a two-way ligation with a *Xho*I-*Bam*HI PCR product containing *myfp* (oligonucleotide primers oFR77 + oFR78 and plasmid DNA from pKM012 as template) and pCR287 cut with *Xho*I-*Bam*HI. pKM012 contains the m*yfp* with codons optimized for *Bacillus subtilis* (KM and DZR, unpublished).

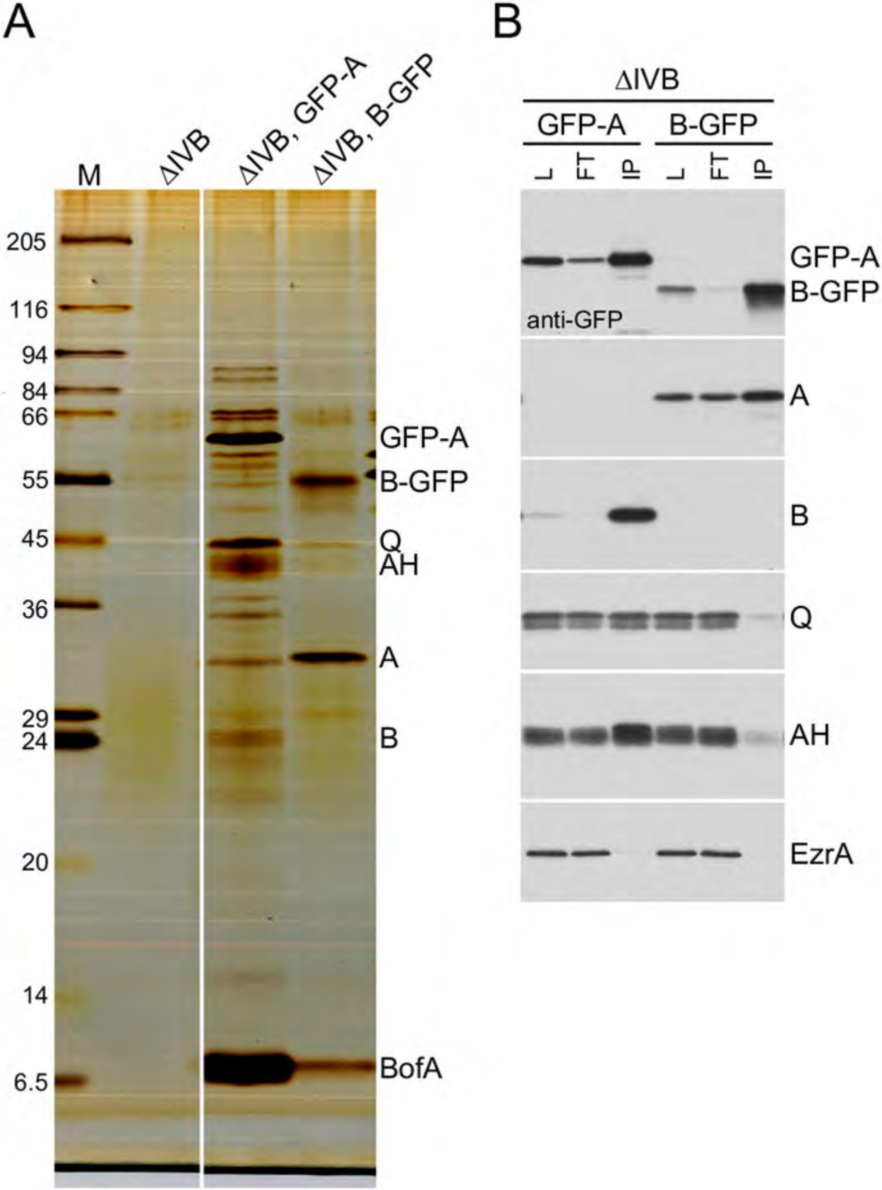
pFR32 [*ycgO*::*PspoIVF-spoIVFB∆66-myfp (erm)*] was generated in a two-way ligation with a *Xho*I-*Bam*HI PCR product containing *myfp* (oligonucleotide primers oFR77 + oFR78 and plasmid DNA from pKM012 as template) and pFR21 cut with *Xho*I-*Bam*HI.

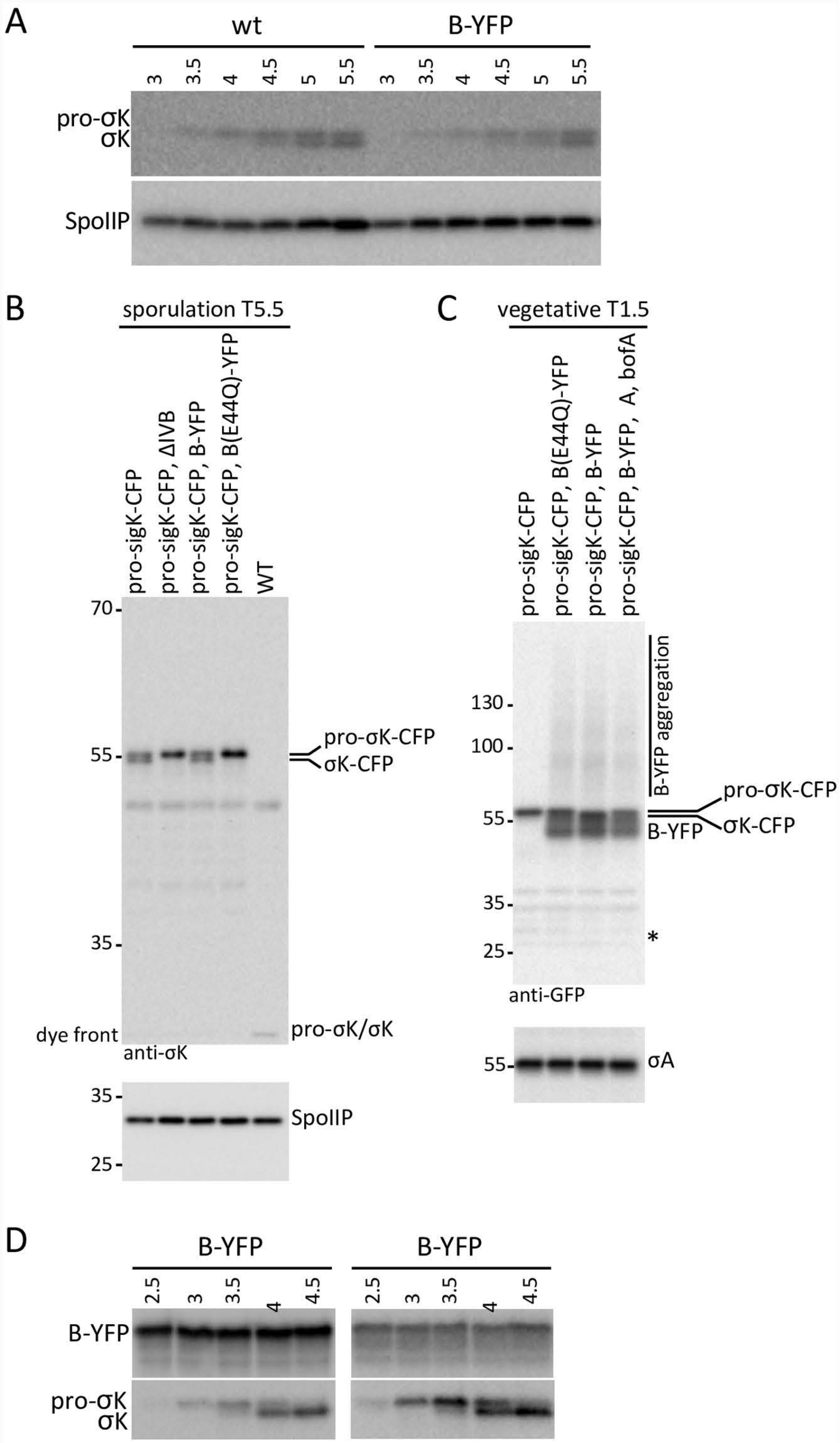
pFR36 [*ycgO*::*PspoIVF-spoIVFB∆66 (erm)*] was generated in a two-way ligation with a *Hind*III-*Bam*HI PCR product containing *spoIVFB*∆66 (oligonucleotide primers oFR83 + oFR84 and genomic DNA from PY79 as template) and pKM283 cut with *Xho*I-*Bam*HI.

pCB061 [*ycgO*::*PspoIVF-spoIVFB(F66A)-yfp (erm)*] was generated by site-directed mutagenesis oligonucleotide primers oCB038 + oCB039 and plasmid pKM283.

Strain construction:

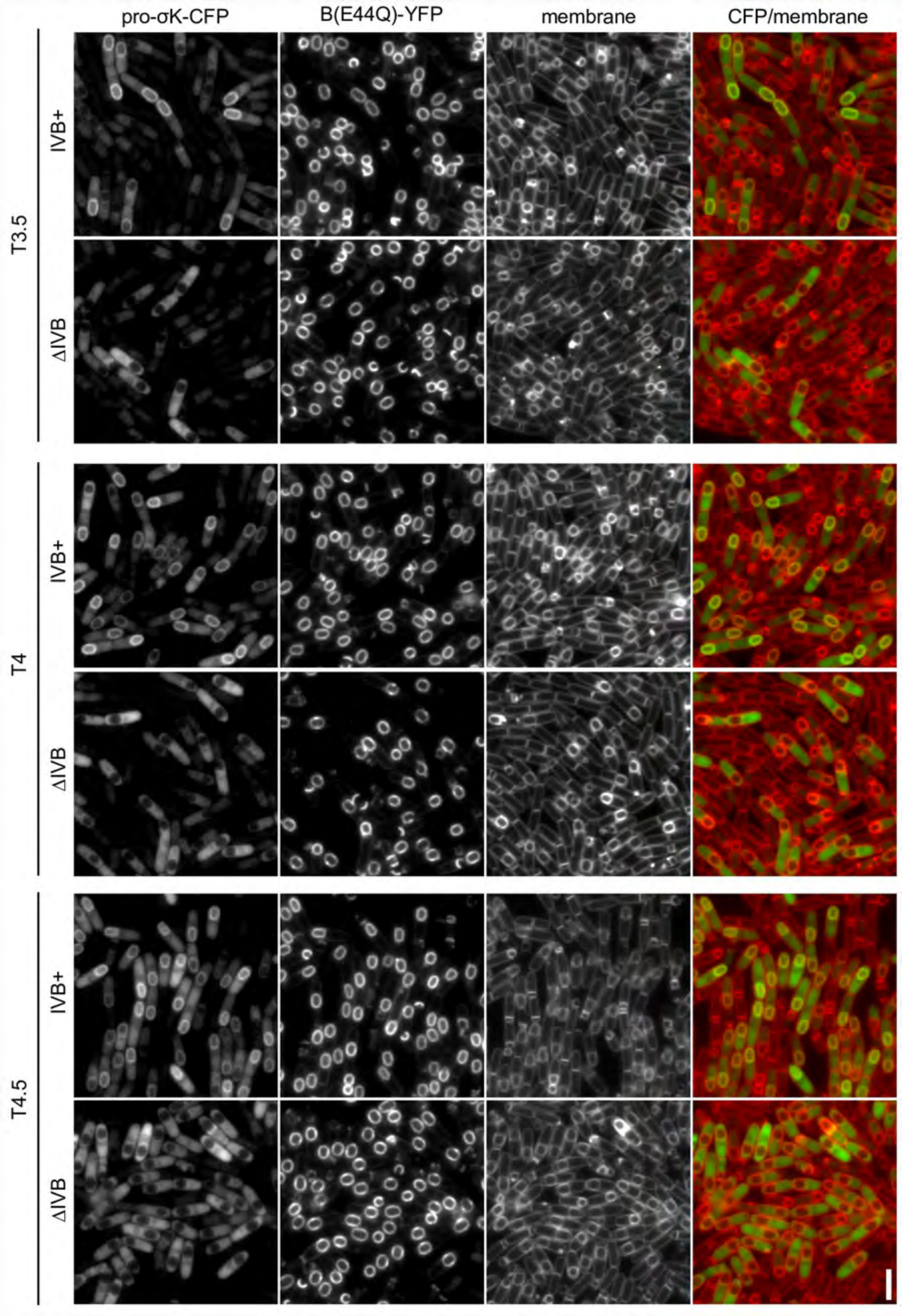
BDR3685 [∆*spoIVB::kan*] was generated by direct transformation of *B. subtilis* PY79 with an isothermal assembly product derived from 3 PCR products: 1) a PCR product containing an upstream region of *spoIVB* amplified with oligonucleotide primers oFR48 and oFR49 and *B. subtilis* PY79 genomic DNA as template; 2) a PCR product containing the Kan cassette; 3) a PCR product containing a downstream region of *spoIVB* amplified with oligonucleotide primers oFR50 and oFR51 and *B. subtilis* PY79 genomic DNA as template.


A
M
ΔIVB
ΔIVB, GFP-A
ΔIVB, B-GFP
205
116
94
84
66
55
45
36
29
24
20
14
6.5
GFP-A
B-GFP
Q
AH
A
B
BofA
B
ΔIVB
GFP-A
B-GFP
L
FT
IP
L
FT
IP
anti-GFP
GFP-A
B-GFP
A
B
Q
AH
EzrA

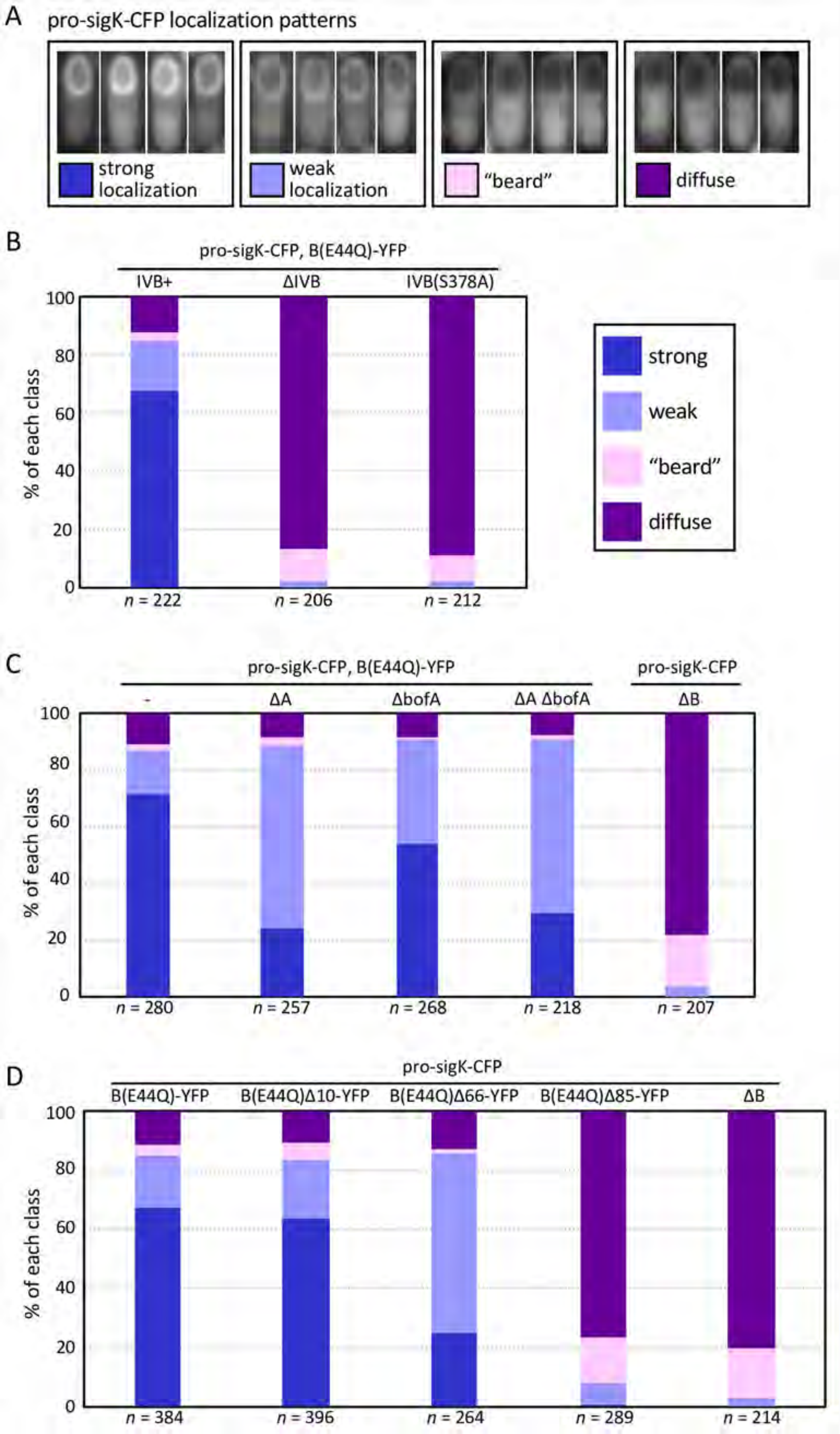


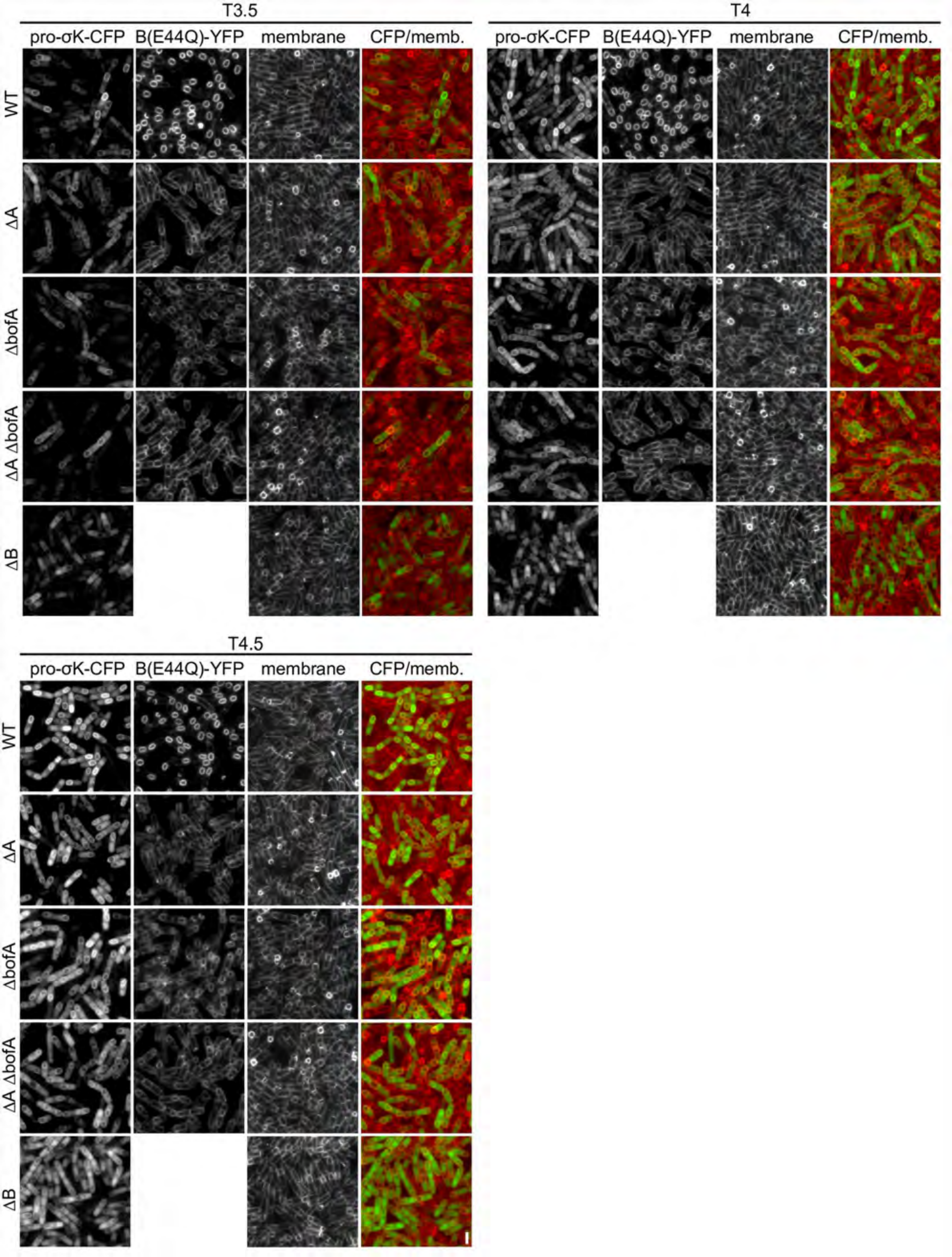
E

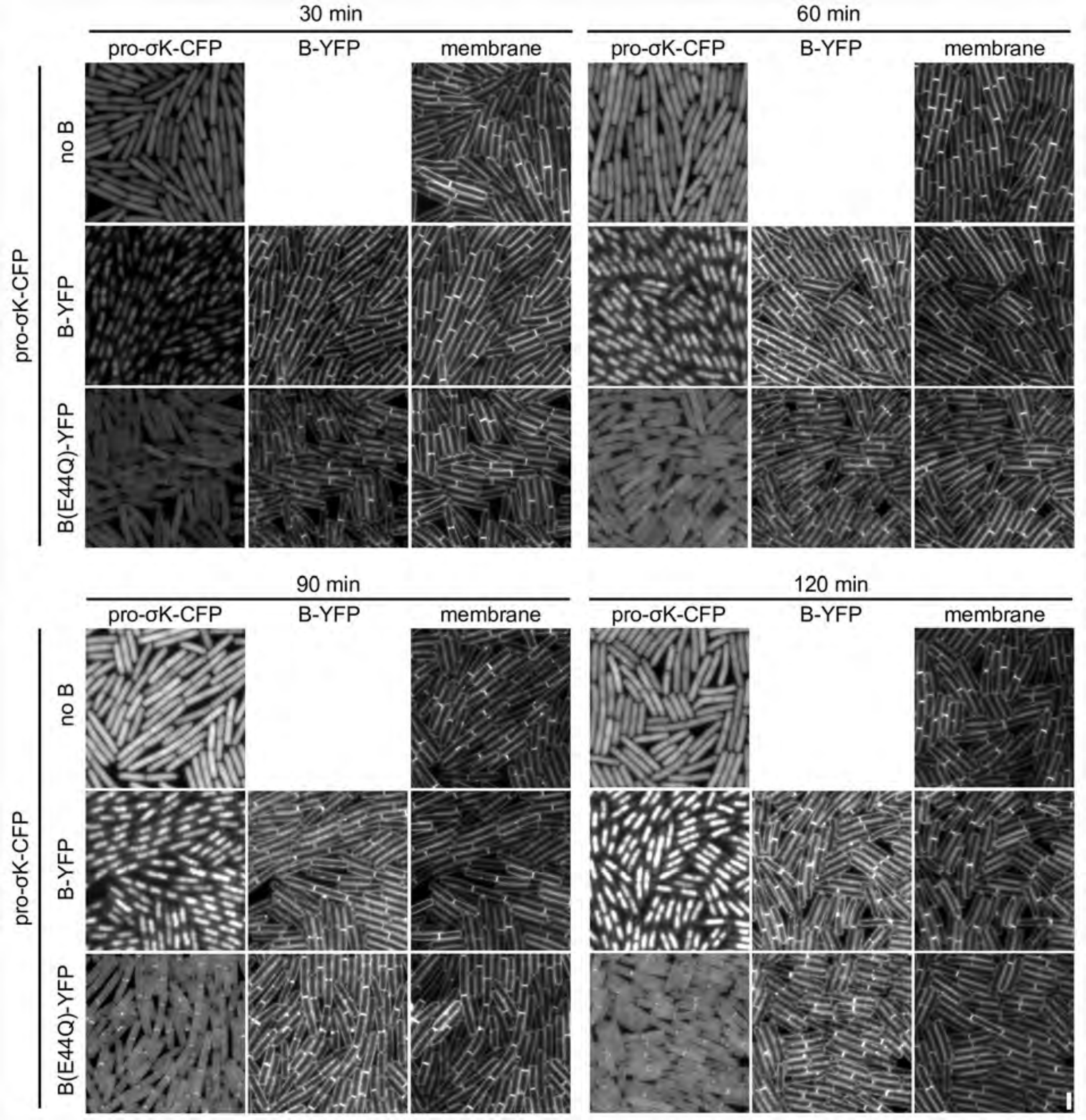
strain	sporulation (%)
WT	100
B-YFP	105.9 ± 8.8
pro-sigK-CFP (spoIIIC-CFP)	102.5 ± 4.0
B-YFP, pro-sigK-CFP	105.3 ± 8.6



pro-σK-CFP
B(E44Q)-YFP
membrane
CFP/membrane
T3.5
IVB+
ΔIVB
T4
IVB+
ΔIVB
T4.5
IVB+
ΔIVB

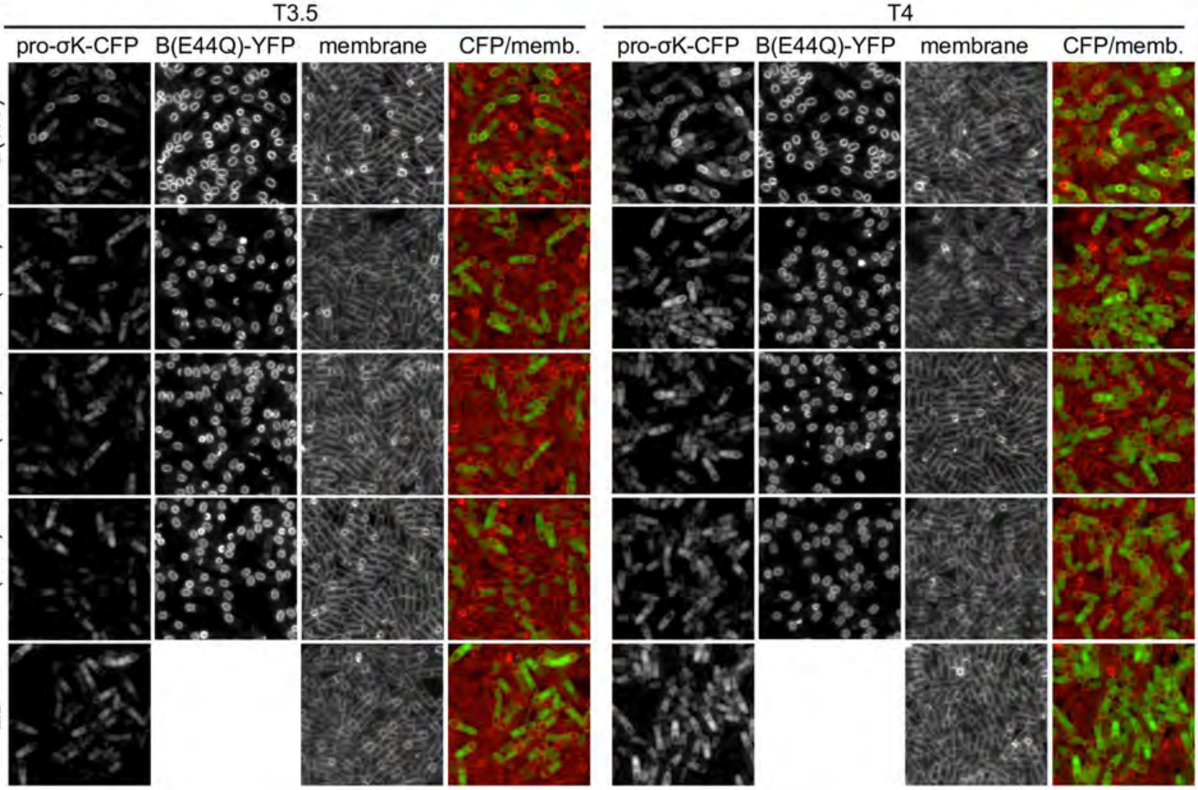

A
pro-sigK-CFP localization patterns
strong localization
weak localization
"beard"
diffuse
B
pro-sigK-CFP, B(E44Q)-YFP
IVB+
ΔIVB
IVB(S378A)
% of each class
n = 222
n = 206
n = 212
strong
weak
"beard"
diffuse
C
pro-sigK-CFP, B(E44Q)-YFP
pro-sigK-CFP
-
ΔA
ΔbofA
ΔA ΔbofA
ΔB
% of each class
n = 280
n = 257
n = 268
n = 218
n = 207
D
pro-sigK-CFP
B(E44Q)-YFP
B(E44Q)Δ10-YFP
B(E44Q)Δ66-YFP
B(E44Q)Δ85-YFP
ΔB
% of each class
n = 384
n = 396
n = 264
n = 289
n = 214

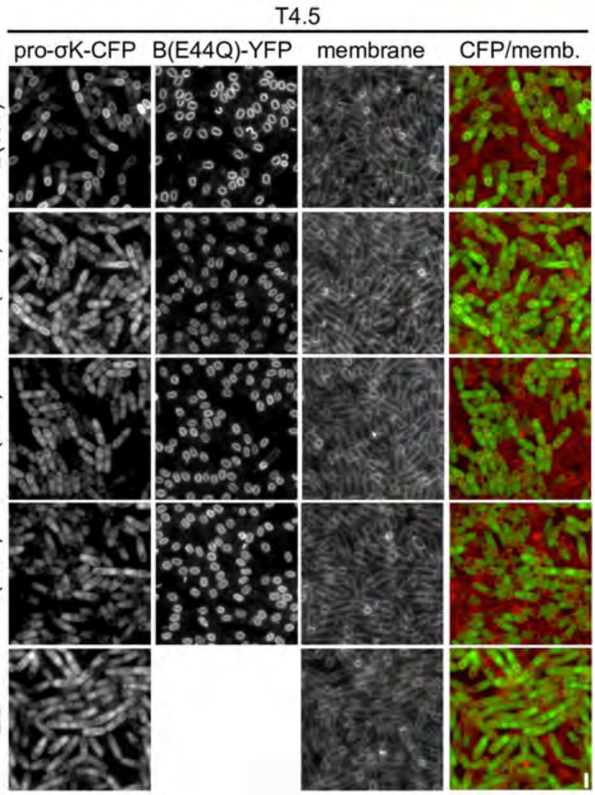

T3.5
pro-σK-CFP
B(E44Q)-YFP
membrane
CFP/memb.
WT
ΔA
ΔbofA
ΔA ΔbofA
ΔB
T4
pro-σK-CFP
B(E44Q)-YFP
membrane
CFP/memb.
T4.5
pro-σK-CFP
B(E44Q)-YFP
membrane
CFP/memb.
WT
ΔA
ΔbofA
ΔA ΔbofA
ΔB



30 min
60 min
pro-σK-CFP
B-YFP
membrane
pro-σK-CFP
B-YFP
membrane
pro-σK-CFP
no B
B-YFP
B(E44Q)-YFP
90 min
120 min
pro-σK-CFP
B-YFP
membrane
pro-σK-CFP
B-YFP
membrane
pro-σK-CFP
no B
B-YFP
B(E44Q)-YFP

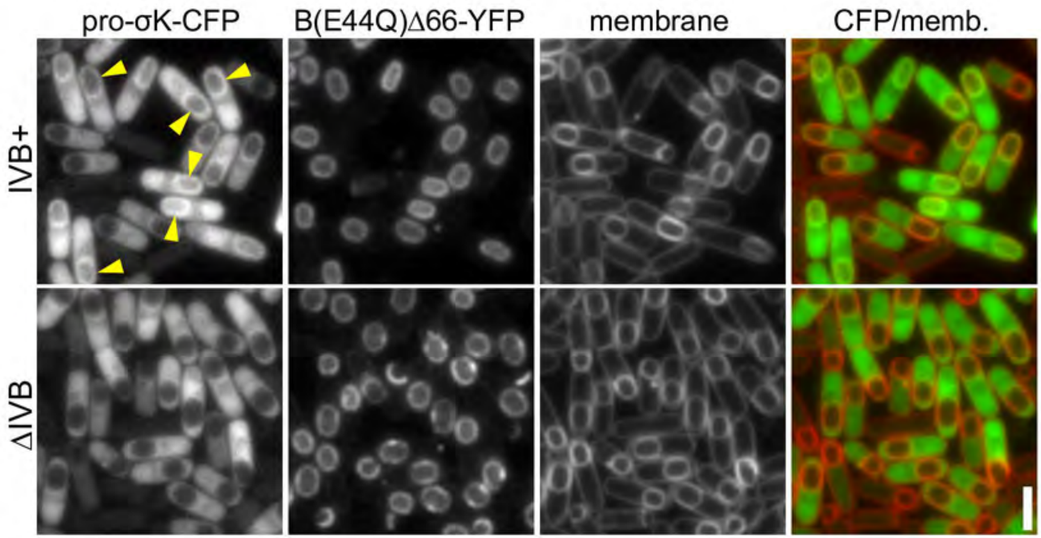
A

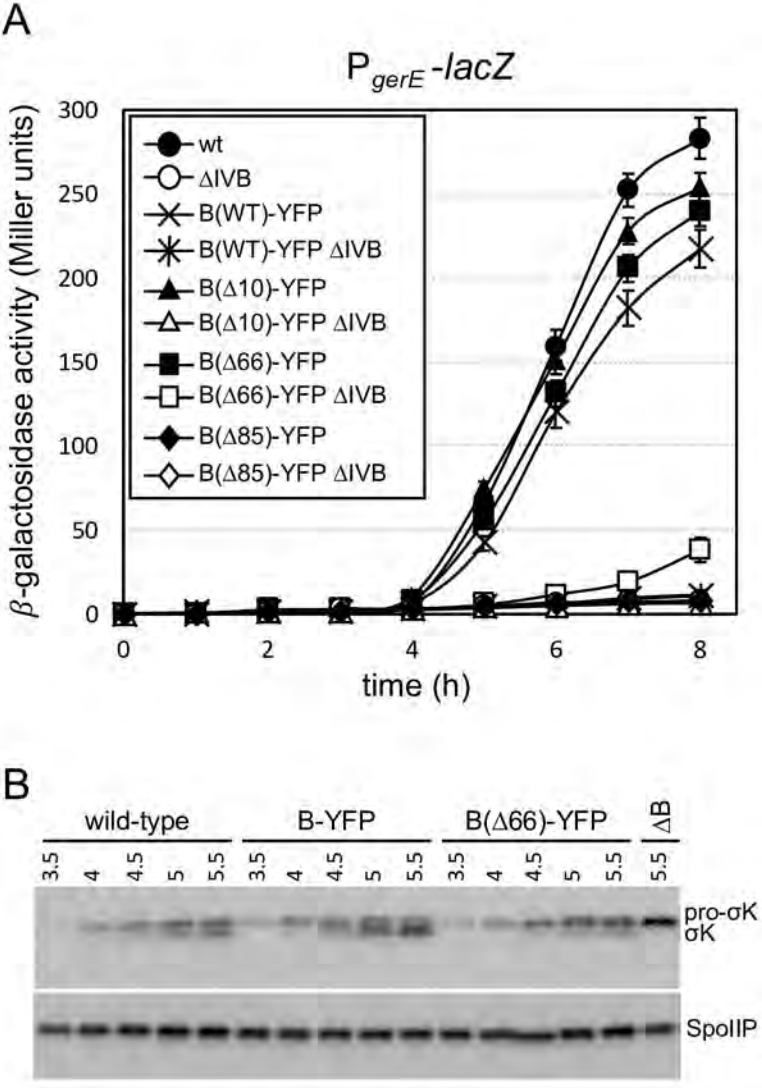


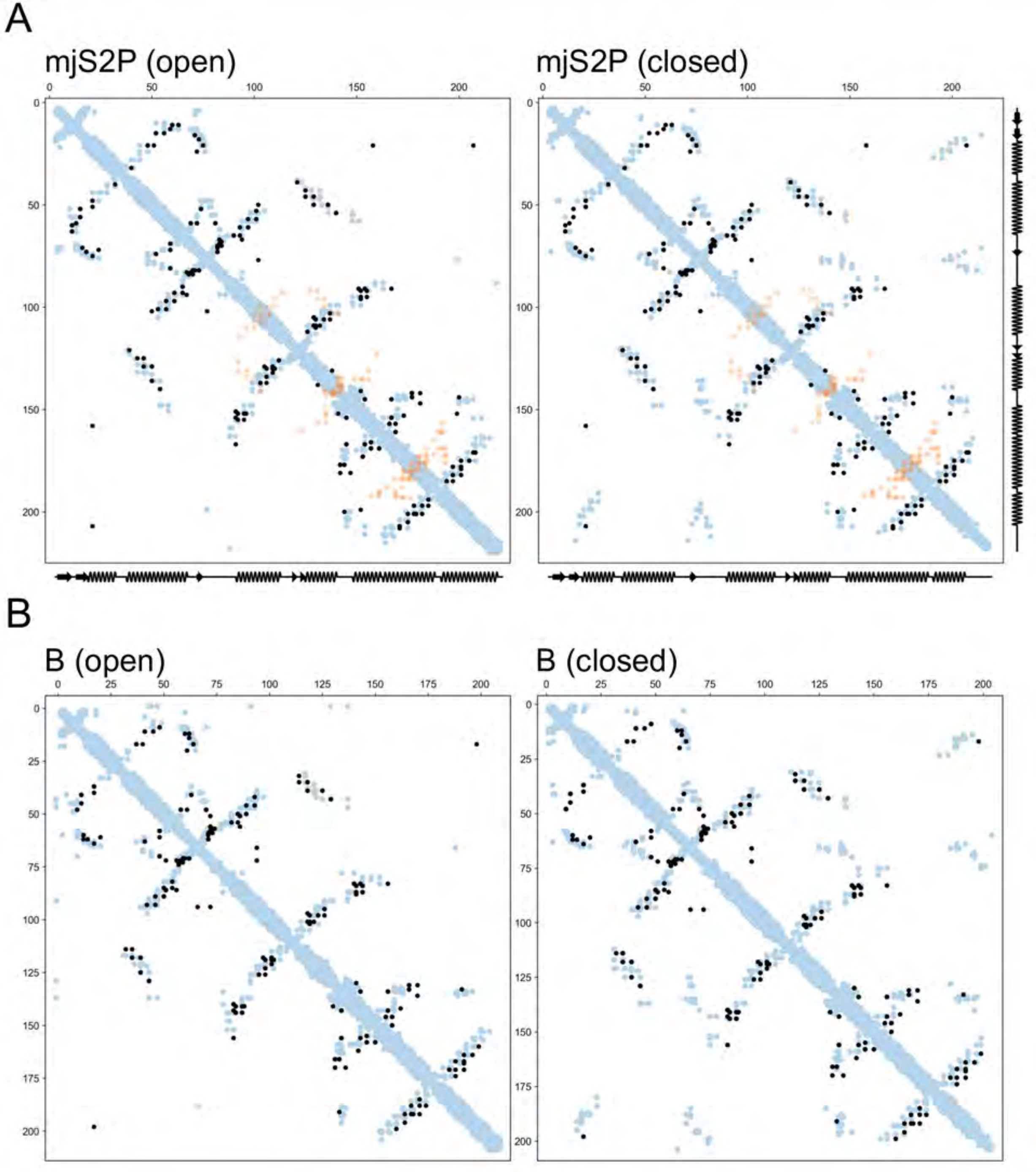


B

strain	forespore-associated fluorescence (au)
B-YFP	1553.2 ± 203.0
B(Δ10)-YFP	1457.2 ± 198.6
B(Δ66)-YFP	1461.8 ± 207.8
B(Δ85)-YFP	1594.9 ± 170.7
ΔIVB, B-YFP	1534.5 ± 164.6
ΔIVB, B(F66A)-YFP	1593.4 ± 213.3

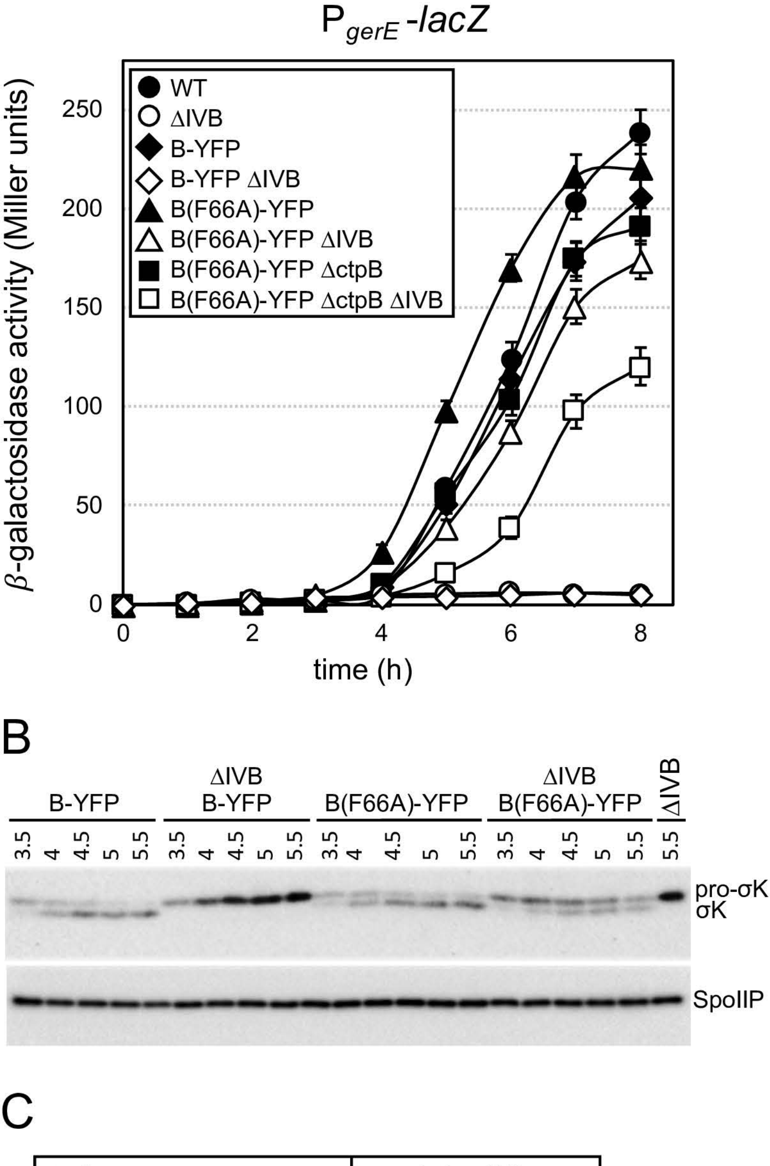

pro-σK-CFP
B(E44Q)Δ66-YFP
membrane
CFP/memb.
IVB+
ΔIVB


A
P gerE -lacZ
β-galactosidase activity (Miller units)
time (h)
wt
ΔIVB
B(WT)-YFP
B(WT)-YFP ΔIVB
B(Δ10)-YFP
B(Δ10)-YFP ΔIVB
B(Δ66)-YFP
B(Δ66)-YFP ΔIVB
B(Δ85)-YFP
B(Δ85)-YFP ΔIVB
0
50
100
150
200
250
300
0
2
4
6
8
B
wild-type
B-YFP
B(Δ66)-YFP
ΔB
3.5
4
4.5
5
5.5
pro-σK
σK
SpoIIP



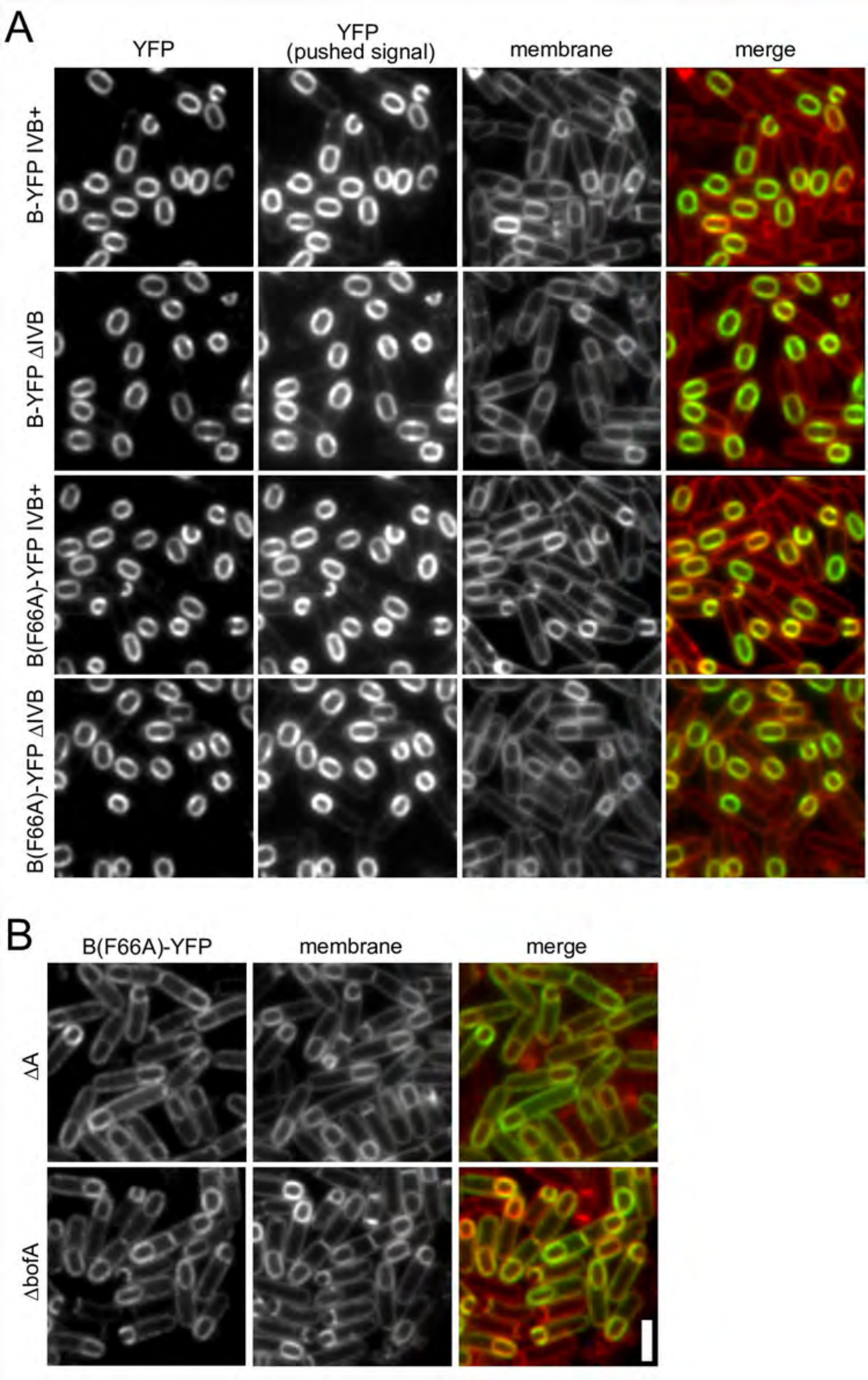
A
mjS2P (open)
mjS2P (closed)
B
B (open)
B (closed)

A



C

strain	sporulation (%)
WT	100
ΔbofA	31.9 ± 4.7
B-YFP	94.7 ± 3.5
B(F66A)-YFP	56.6 ± 2.9

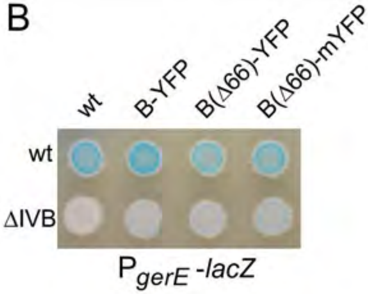


A
YFP
YFP (pushed signal)
membrane
merge
B-YFP IVB+
B-YFP ΔIVB
B(F66A)-YFP IVB+
B(F66A)-YFP ΔIVB
B
B(F66A)-YFP
membrane
merge
ΔA
ΔbofA

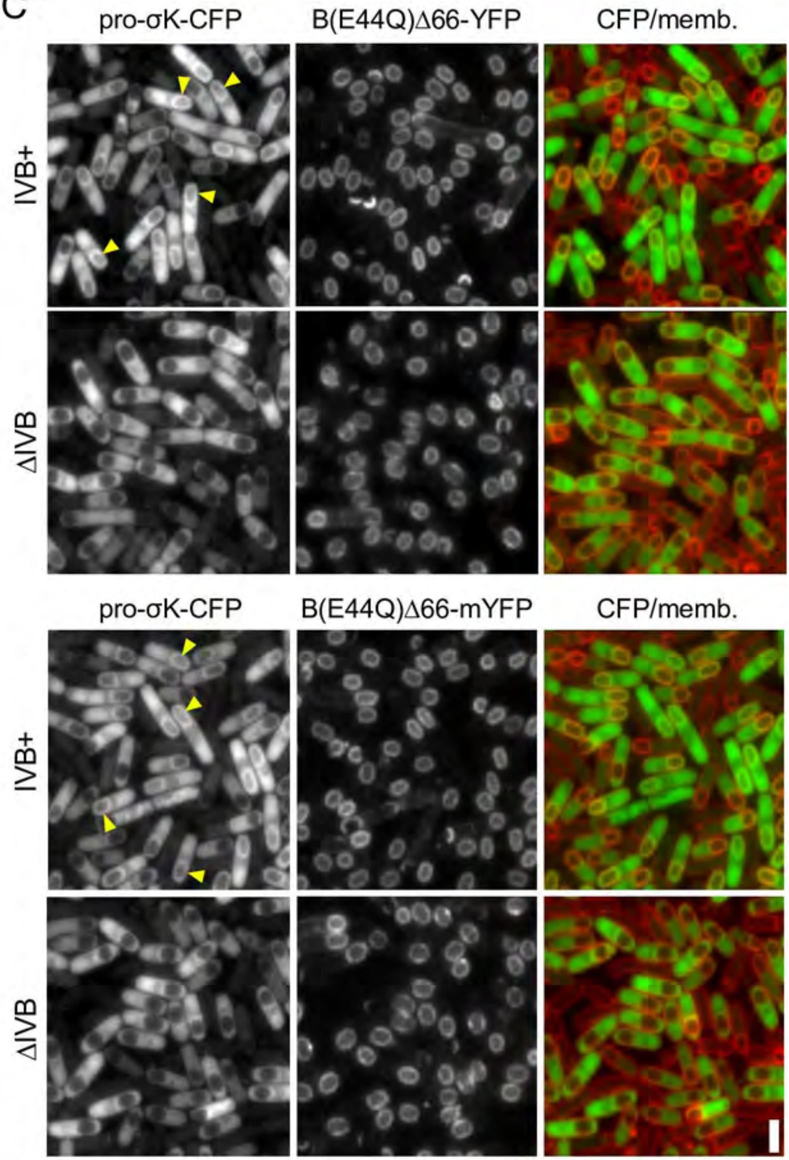
A

strain	sporulation (%)
wt	100
ΔB	0.0004 ± 0.0002
B(wt)-YFP	96.3 ± 2.1
B(Δ66)-YFP	85.4 ± 2.4
B(Δ66)-mYFP	84.8 ± 3.1

B



C



S1 Table. *Bacillus subtilis* strains used in this study

Strain	Genotype	Source
PY79	Prototrophic wild-type	Youngman *et al.,* 1983
BNC279	Δ*bofA::tet*	Campo & Rudner, 2006
BNC689	Δ*spoIVB::phleo*	Campo & Rudner, 2006
BKM880	*spoIVFB-yfp (spec)*	This work
BKM846	*spoIVF::cat, amyE::spoIVFAB(E44Q)-gfp (spec) (tet)*	This work
BKM868	*spoIVF::cat, amyE::spoIVFAB(E44Q)-gfp (spec) (tet), spoIVB::erm*	This work
BKM1020	*spoIVF::cat, amyE::spoIVFAB(E44Q)-gfp (spec) (tet), spoIVC::erm*	This work
BKM1351	*ycgO::PspoIVF-spoIVFB (erm)*	This work
BKM1411	*spoIIIC-cfp (cat)*	This work
BKM1425	*spoIVF::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q)-yfp (erm), spoIIIC-cfp(cat)*	This work
BKM1501	*spoIVF::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q)-yfp (erm), spoIIIC-cfp(cat), amyE::spoIVFA (spec)*	This work
BKM1507	*spoIVF::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q)-yfp (erm), spoIIIC-cfp(cat), amyE::spoIVFA (spec) spoIVB::phleo*	This work
BKM1557	*spoIVFAB::cat::neo, spoIIIC-cfp (cat)*	This work
BKM1595	*spoIVFAB::cat::neo, ycgO::PspoIVF-B(E44Q)-yfp (erm), spoIIIC-cfp (cat), amyE::spoIVFA (spec), bofA::tet*	This work
BCR1604	*spoIIIC-cfp (cat), spoIVFB-yfp (spec)*	This work
BCR1606	*spoIIIC-cfp (cat), spoIVFB-yfp (spec), spoIVB::phleo*	This work
BCR1621	*spoIVF::cat::neo, ycgO::PspoIVF-B(E44Q)-yfp (erm), spoIIIC-cfp (cat), bofA::tet*	This work
BCR1635	*amyE::Pspank-pro-sigK-cfp (spec)*	This work
BCR1639	*ycgO::Pspank-spoIVFB(E44Q)-yfp (erm), amyE::Pspank-pro-sigK-cfp (spec)*	This work
BCR1641	*ycgO::Pspank-spoIVFB-yfp (erm), amyE::Pspank-pro-sigK-cfp (spec)*	This work
BCR1646	*spoIIIC-cfp (cat), spoIVFB::spec*	This work
BCR1658	*spoIVFAB::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q)*Δ*10-yfp (erm), spoIIIC-cfp (cat), amyE::spoIVFA (spec)*	This work
BCR1659	*spoIVFAB::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q)*Δ*66-yfp (erm), spoIIIC-cfp (cat), amyE::spoIVFA (spec)*	This work
BCR1660	*spoIVFAB::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q)*Δ*85-yfp (erm), spoIIIC-cfp (cat), amyE::spoIVFA (spec)*	This work
BDR63	*spoIVF*Δ*AB::cat*	Lu & Kroos, 1994
BDR64	Δ*spoIVFB::spec*	Resnekov *et al.,* 1996
BDR622	Δ*ctpB::tet*	Pan *et al.,* 2003
BDR1454	*spoIVB::erm, amyE::spoIVB(S378A) (cat)*	Campo & Rudner 2006
BDR3565	Δ*spoIVFB::spec ycgO::cat*	This work
BDR3787	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB-yfp (erm)*	
BDR3568	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB*Δ*10-yfp (erm)*	This work
BDR3569	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB*Δ*66-yfp (erm)*	This work
BDR3570	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB*Δ*85-yfp (erm)*	This work
BDR169	*sp*β*::gerE-lacZ (erm, cat)*	Losick collection
BDR3681	*spoIVFB-yfp (spec) sp*β*::gerE-lacZ(erm, cat)*	This work
BDR3682	Δ*spoIVFB::spec ycgO::PspoIVFB-spoIVFB*Δ*10-yf (erm) sp*β*::gerE-lacZ (erm, cat)*	This work
BDR3683	Δ*spoIVFB::spec ycgO::PspoIVFB-spoIVFB*Δ*66-yfp(erm) sp*β*::gerE-lacZ (erm, cat)*	This work
BDR3684	Δ*spoIVFB::spec ycgO::PspoIVFB-spoIVFB*Δ*85-yfp(erm) sp*β*::gerE-lacZ (erm, cat)*	This work
BDR3685	Δ*spoIVB::kan*	This work
BDR3687	*spoIVFB-yfp (spec)* Δ*spoIVB::kan sp*β*::gerE-lacZ (erm, cat)*	This work
BDR3689	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB*Δ*10-yfp (erm) sp*β*::gerE-lacZ (erm, cat)* Δ*spoIVB::kan*	This work
BDR3691	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB*Δ*66-yfp (erm) sp*β*::gerE-lacZ (erm, cat)* Δ*spoIVB::kan*	This work
BDR3693	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB*Δ*85-yfp (erm) sp*β*::gerE-lacZ (erm, cat)* Δ*spoIVB::kan*	This work
BDR3698	*sp*β*::gerE-lacZ (erm, cat)* Δ*spoIVB::kan*	This work
BDR3729	*ycgO::Pspank-spoIVF-yfp (erm), amyE::Pspank-pro-sigK-cfp (spec), yvbJ::Physpank-spoIVFA (cat), yhdG::Physpank-bofA (kan)*	This work
BDR3763	*lacA::spoIVB(S378A) (tet)*	This work
BDR3765	*spoIVFAB::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q)-yfp (erm), spoIIIC-cfp (cat), amyE::spoIVFA (spec), spoIVB::phleo, lacA::spoIVB(S378A) (tet)*	This work
BDR3791	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB*Δ*66-myfp (erm)*	This work
BDR3799	*spoIVFAB::cat::neo ycgO::PspoIVF-spoIVFB(E44Q)*Δ*66-yfp (erm) spoIIIC-cfp (cat), amyE::spoIVFA (spec), spoIVB::phleo*	This work
BDR3802	*spoIVFAB::cat::neo ycgO::PspoIVF-spoIVFB(E44Q)*Δ*66-myfp (erm), spoIIIC-cfp (cat), amyE::spoIVFA (spec)*	This work
BDR3804	*spoIVFAB::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q)*Δ*66-myfp (erm), spoIIIC-cfp (cat), amyE::spoIVFA (spec), spoIVB::phleo*	This work
BDR3810	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB*Δ*66-myfp (erm) sp*β*::gerE-lacZ (erm, cat)*	This work
BDR3820	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB*Δ*66-myfp (erm) sp*β*::gerE-lacZ (erm, cat)* Δ*spoIVB::kan*	This work
BDR3829	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB(F66A)-yfp (erm)*	This work
BDR3832	Δ*spoIVFB::spec ycgO::PspoIVF-spoIVFB(F66A)-yfp (erm)* Δ*spoIVB::kan*	This work

BDR3836	*ΔspoIVFB::spec ycgO::PspoIVF-spoIVFB(F66A)-yfp (erm) spβ::gerE-lacZ (erm, cat)*	This work
BDR3839	*ΔspoIVFB::spec ycgO::PspoIVF-spoIVFB(F66A)-yfp (erm) spβ::gerE-lacZ (erm, cat) ΔspoIVB::kan*	This work
BDR3847	*ΔspoIVFB::spec ycgO::PspoIVF-spoIVFB(F66A)-yfp (erm) spβ::gerE-lacZ (erm, cat) ΔctpB::tet*	This work
BDR3849	*ΔspoIVFB::spec ycgO::PspoIVF-spoIVFB(F66A)-yfp (erm) spβ::gerE-lacZ (erm, cat) ΔctpB::tet ΔspoIVB::kan*	This work
BDR3856	*ΔctpB::tet spβ::gerE-lacZ (erm, cat)*	This work
BDR3857	*ΔctpB::tet ΔspoIVB::kan spβ::gerE-lacZ (erm, cat)*	This work
BDR3858	*spoIVFB-yfp ΔctpB::tet (spec) spβ::gerE-lacZ (erm, cat)*	This work
BDR3859	*spoIVFB-yfp ΔctpB::tet ΔspoIVB::kan (spec) spβ::gerE-lacZ (erm, cat)*	This work
BDR3864	*ΔspoIVFB::spec ycgO::PspoIVF-spoIVFB-yfp (erm) ΔspoIVB::kan*	This work
BDR3866	*spoIVFAB::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q, F66A)-yfp (erm), spoIIIC-cfp (cat), amyE::spoIVFA (spec)*	This work
BDR3867	*spoIVFAB::cat::neo, ycgO::PspoIVF-spoIVFB(E44Q, F66A)-yfp (erm), spoIIIC-cfp (cat), amyE::spoIVFA (spec) spoIVB::phleo*	This work
BDR4044	*spoIIIC-cfp (cat), spoIVFB-yfp (spec), spoIVB::phleo, lacA::spoIVB(S378A) (tet)*	This work
BDR4047	*ΔspoIVFB::spec ycgO::PspoIVF-spoIVFB(Δ66) (erm)*	This work
BDR4051	*ΔspoIVFB::spec ycgO::PspoIVF-spoIVFB (erm)*	This work

S2 Table. Plasmids used in this study

Plasmids	Description	Source
pKM190	*spoIVFB-yfp (spec)* (single crossover integration)	This work
pKM261	*ycgO::PspoIVF-spoIVFB(E44Q)-yfp (erm)*	This work
pKM266	*spoIIIC-cfp (cat)* (single crossover integration)	This work
pKM283	ycgO::PspoIVF-*spoIVFB-yfp (erm)*	This work
pCR275	*ycgO::Pspank-spoIVFB(E44Q)-yfp (erm)*	This work
pCR276	*ycgO::Pspank-spoIVFB-yfp (erm)*	This work
pCR278	*amyE::Pspank-pro-sigK-cfp (spec)*	This work
pCR286	*ycgO::PspoIVF-spoIVFB(E44Q)Δ10-yfp (erm)*	This work
pCR287	*ycgO::PspoIVF-spoIVFB (E44Q)Δ66-yfp (erm)*	This work
pCR288	*ycgO::PspoIVF-spoIVFB (E44Q)Δ85-yfp (erm)*	This work
pFR20	*ycgO::PspoIVF-spoIVFBΔ10-yfp (erm)*	This work
pFR21	*ycgO::PspoIVF-spoIVFBΔ66-yfp (erm)*	This work
pFR22	*ycgO::PspoIVF-spoIVFBΔ85-yfp (erm)*	This work
pFR28	*yvbJ::Phyperspank-spoIVFA (cat)*	This work
pFR29	*yhdG::Phyperspank-bofA (kan)*	This work
pFR30	*lacA::spoIVB(S378A) (tet)*	This work
pFR31	*ycgO::PspoIVF-spoIVFB(E44Q)Δ66-myfp (erm)*	This work
pFR32	*ycgO::PspoIVF-spoIVFBΔ66-myfp (erm)*	This work
pFR36	*ycgO::PspoIVF-spoIVFBΔ66 (erm)*	This work
pCB061	*ycgO::PspoIVF-spoIVFB(F66A)-yfp (erm)*	This work

S3 Table. Oligonucleotide primers used in this study

Primers	Sequence*
oDR078	gccGGATCCttatttgtatagttcatccatgcc
oDR079	gcgCTCGAGggttccggaatgag
oDR106	ggcAAGCTTacataaggaggaactactatgaataaatggctcgaccttatc
oDR481	gcggCAATTGcgtcgctctgcttctgcctgc
oDR482	cggCTCGAGgtagggcagaagcagttcctc
oDR594	gccGAATTCgggaatgaaataagcctgattgacg
oDR595	cggCTCGAGtttccccttcgccttcttccg
oCR599	gcgAAGCTTacataaggaggaactactatggtgacaggtgttttcgcagc
oCR600	cgcGCTAGCtttccccttcgccttcttccgctt
oCR601	cgcGCTAGCatggtttcaaaaggcgaagaactgt
oCR603	gcgAAGCTTacataaggaggaactactatgaataaatggctcgaccttat
oCR604	cgcGCTAGCttatttgtatagttcatccatgccatg
oCR605	cgcGCATGCttatttccccttcgccttcttccgct
oCR606	cgcGCATGCttacttataaagttcgtccatgccaag
oCR619	cggCTCGAGattcgtccgcttatcggcaaag
oCR620	cggCTCGAGctccctgtttttccgtaatacc
oCR621	cggCTCGAGgtgcctttgccgatattcctc
oFR48	gaagggtaccagcggtatgt
oFR49	ctgagcgagggagcagaaatcgggcattcactact
oFR50	gttgaccagtgctccctggcaagctgactgccgga
oFR51	gaatggaccaagagtgcagg
oFR58	gagcggataacaattaagcttacataaggaggaactactatgagtcacagagcagatga
oFR59	ttagcttgcatgcgagctagcttattcaaatgaaatcacc
oFR62	cggACTAGTacataaggaggaactactatggagcctatttttattattggg
oFR66	gccGCATGCttaaatgataaattgcttaatgacgactaa
oFR72	gccCTCGAGttgccgatgataagctgtc
oFR73	gccGCTAGCActggtaatggtagcgaccg
oFR77	gccCTCGAGggttccggagtttcaaaaggcgaagaactg
oFR78	gccGGATCCttacttataaagttcgtccatgcc
oFR83	ggcGGATCCTTActcaagctccctgttttttccgta
oFR84	ggcAAGCTTacataaggaggaactactatgaataaatggctcgaccttatc
oCB038	gaatcaagcgtgtttttttgctgccggctggcggaacggtcgaagtggaag
oCB039	cttccacttcgaccgttccgccagccggcagcaaaaaaacacgcttgattc

* Capital letters indicate restriction endonuclease sites and underlined letters indicate mutated bases