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Supplemental Data

Recruitment of SMC by ParB-*parS* Organizes the Origin Region and Promotes Efficient Chromosome Segregation

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmid construction

pKM011 [*lacA::PspoIIQ-cfp (erm)*] was generated by inserting *PspoIIQ-cfp* from pKM008 (Doan et al., 2005) into pDR183 between *Eco*RI and *Bam*HI. pDR183 [*lacA::erm*] is an ectopic integration vector for double crossover insertions into the nonessential *lacA* locus (K.A.M. and D.Z.R., unpublished).

pKM173 [*ywjI::PspoIIQ-cfp (cat)*] was generated by inserting *PspoIIQ-cfp* from pKM008 into pKM171 between *Eco*RI and *Bam*HI. pKM171 [*ywjI::cat*] is an ectopic integration vector for double crossover insertions into the nonessential *ywjI* locus (K.A.M. and D.Z.R. unpublished).

pKM186 [355˚(*parS**) in pMAD (erm)] was generated by inserting a BamHI-SalI PCR product containing the *yycG* gene (oligonucleotide primers oDR473 and oDR474 and wild-type genomic DNA as template) into pER19 (Ricca et al., 1992). Synonymous changes in the *parS* site were introduced by site-directed mutagenesis using oDR475. Seven of the 16 bases in the *parS* site were changed. The *yycG** mutant was then inserted into pMAD (Arnaud et al., 2004) between BamHI and SalI.

pKM230 [354˚(*parS*Δ) in pMAD (erm)] was generated in a three-way ligation with an EcoRI-XhoI PCR product (oligonucleotide primers oDR509 and oDR516 and wild-type genomic DNA as template), an XhoI-BamHI PCR product (oligonucleotide primers oDR510 and oDR517 and

wild-type genomic DNA as template), and pMAD cut with EcoRI and BamHI. The resulting plasmid replaces the 354˚ *parS* site with an XhoI site.

pKM231 [356˚(*parS*Δ) in pMAD (erm)] was generated in a three-way ligation with an EcoRI-XhoI PCR product (oligonucleotide primers oDR520 and oDR521 and wild-type genomic DNA as template), an XhoI-BamHI PCR product (oligonucleotide primers oDR522 and oDR523 and wild-type genomic DNA as template), and pMAD cut with EcoRI and BamHI. The resulting plasmid replaces the 356˚ *parS* site with an XhoI site.

pKM232 [4˚(*parS*Δ) in pMAD (erm)] was generated in a three-way ligation with an EcoRI-XhoI PCR product (oligonucleotide primers oDR526 and oDR527 and wild-type genomic DNA as template), an XhoI-BamHI PCR product (oligonucleotide primers oR528 and oDR529 and wild-type genomic DNA as template), and pMAD cut with EcoRI and BamHI. The resulting plasmid replaces the 4˚ *parS* site with an XhoI site.

pKM234 [Δ*(soj-spo0J)::cat)*] was generated by inserting an EcoRI-XbaI PCR product containing the 3' end of the *spo0J* gene (oligonucleotide primers oNS071 and oNS072 and wild-type genomic DNA as template) into pKM233 between EcoRI and XbaI. pKM233 was generated by inserting an EagI-SalI PCR product containing the 5' end of the *soj* gene (oligonucleotide primers oNS069 and oNS070 and wild-type genomic DNA as template) into pKM074 between EagI and SalI. pKM074 is a plasmid containing multiple restriction sites flanking the *cat* gene (K.A.M. and D.Z.R. unpublished).

pKM252 [15˚(*parS*Δ) in pminiMAD (erm)] was generated in a three-way ligation with an EcoRI-XhoI PCR product (oligonucleotide primers oDR549 and oDR550 and wild-type genomic DNA as template), an XhoI-BamHI PCR product (oligonucleotide primers oDR551 and oDR552 and wild-type genomic DNA as template), and pminiMAD (a gift from Dan Kearns, Indiana University) cut with EcoRI and BamHI. The resulting plasmid replaces the 15˚ *parS* site with an XhoI site.

pKM253 [334˚(*parS*Δ) in pminiMAD (erm)] was generated in a three-way ligation with an EcoRI-EagI PCR product (oligonucleotide primers oDR555 and oDR556 and wild-type genomic DNA as template), an EagI-BamHI PCR product (oligonucleotide primers oDR557 and oDR558

and wild-type genomic DNA as template), and pminiMAD cut with EcoRI and BamHI. The resulting plasmid replaces the 334˚ *parS* site with an EagI site.

pKM255 [40˚(*parS*Δ) in pminiMAD (erm)] was generated in a three-way ligation with the EcoRI and EagI PCR product (oligonucleotide primers oDR561 and oDR562 and wild-type genomic DNA as template), an EagI-BamHI PCR product (oligonucleotide primers oDR563 and oDR564 and wild-type genomic DNA as template), and pminiMAD cut with EcoRI and BamHI. The resulting plasmid replaces the 40˚ *parS* site with an EagI site.

pKM256 [*pelB::Psoj-gfp-spo0J(parS*) (cat)*] was generated in a three-way ligation with an XhoI-HindIII fragment containing *gfp* and an optimized RBS (Vellanoweth and Rabinowitz, 1992) from pDR095 (Rudner and Losick, 2002), an XhoI-BamHI PCR product containing *spo0J(parS*)* (oligonucleotide primers oDR472 and oNS025 and genomic DNA from a derivative of DCL468 as template) into pKM170 between HindIII and BamHI. pKM170 [*pelB::Psoj (cat)*] was generated by inserting an EcoRI-HindIII PCR product containing the *soj* promoter (oligonucleotide primers oDR470 and oDR471 and wild-type genomic DNA as template) into pKM020. pKM020 [*pelB::cat*] is an ectopic integration vector for double crossover insertions into the nonessential *pelB* locus (K.A.M. and D.Z.R., unpublished).

pKM287 [*ytoI::Psmc-gfp-smc (erm)*] was generated in a three-way ligation with an XhoI-NheI PCR product containing *gfp* (oligonucleotide primers oDR630 and oDR631 and pKL147 (Lemon and Grossman, 1998) as template), an NheI-BamHI PCR product containing *smc* (oligonucleotide primers oDR632 and oDR627 and wild-type genomic DNA as template) and pKM284 cut with XhoI and BamHI. pKM284 [*ytoI::Psmc (erm)*] was generated by inserting an EcoRI-XhoI PCR product containing the *smc* promoter (oligonucleotide primers oDR628 and oDR629 and wild-type genomic DNA as template) into pBB285. pBB285 [*ytoI::erm*] is an ectopic integration vector for double crossover insertions into the nonessential *ytoI* locus (B. Burton and D.Z.R., unpublished).

pKM299 [*lacA::Psoj-cfp(d)-spo0J(parS*) (erm)*] was generated by inserting *Psoj-cfp(d)-spo0J(parS*)* from pNS137 into pDR183 between EcoRI and BamHI. pNS137 [*pelB::Psoj-cfp(d)-spo0J(parS*) (cat)*] is described below.

pKM309 [*smc*-(*his*)$_6$ in pET24b (+) (*kan*)] was generated in a two-way ligation with an NdeI-XhoI PCR product containing *smc* (oligonucleotide primers oDR682 and oDR683 and wild-type genomic DNA as template) and pET24b (+) (Novagen).

pKM315 [*spo0J*-(*his*)$_6$ in pET24b (+) (*kan*)] was generated in a two-way ligation with an NheI-XhoI PCR product containing *spo0J* (oligonucleotide primers oDR689 and oDR690 and wild-type genomic DNA as template) and pET24b (+).

pKM323 [*lacI*Δ11-(*his*)$_6$ in pRsetA (*amp*)] was generated in a two-way ligation with an NdeI-HindIII PCR product containing *lacI*Δ11-(*his*)$_6$ (oligonucleotide primers oDR703 and oDR704 and pET24b (+) as template) and pRsetA (Invitrogen).

pNS005 [*lacA::PspoIIQ-cfp + parS (erm)*] was generated by inserting *parS* linkers (oTD040 and oTD041) into pKM011 at the BamHI site.

pNS007 [*amyE::PspoIIQ-cfp (cat)*] was generated by inserting *PspoIIQ-cfp* from pKM008 (Doan et al., 2005) into pDG364 (Karmazyn-Campelli et al., 1992) between EcoRI and BamHI.

pNS009 [*amyE::PspoIIQ-cfp + parS (cat)*] was generated by inserting *parS* linkers (oTD040 and oTD041) into pNS007 at the BamHI site.

pNS010 [*lacA::PspoIIQ-cfp + parS* (erm*] was generated by inserting *parS** linkers (oTD048 and oTD049) into pKM011 at the BamHI site.

pNS012 [*amyE::PspoIIQ-cfp + parS* (cat)*] was generated by inserting *parS** linkers (oTD048 and oTD049) into pNS007 at the BamHI site.

pNS013 [*ycgO::PspoIIQ-cfp (kan)*] was generated by inserting *PspoIIQ-cfp* from pKM008 into pKM087 between *Eco*RI and *Bam*HI. pKM087 [*ycgO::kan*] is an ectopic integration vector for double crossover insertions into the nonessential *ycgO* locus (K.A.M. and D.Z.R., unpublished).

pNS021 [*pelB::soj*Δ *spo0J+ (kan)*] was generated by inserting the *soj spo0J* operon containing an in-frame deletion in *soj* from pIK212 (Ireton et al., 1994) into pKM069 between EcoRI and

BamHI. pKM069 [*pelB::kan*] is an ectopic integration vector for double crossover insertions into the nonessential *pelB* locus (K.A.M. and D.Z.R., unpublished).

pNS038 [*yvbJ::PspoIIQ-cfp (spec)*] was generated by inserting *PspoIIQ-cfp* from pKM008 into pNS028 between EcoRI and BamHI. pNS028 [*yvbJ::spec*] is an ectopic integration vector for double crossover insertions into the nonessential *yvbJ* locus (N.L.S. and D.Z.R., unpublished).

pNS051 [*yycR::PspoIIQ-yfp (phleo)*] was generated by inserting *PspoIIQ-yfp* from pKM003 into pNS042 between *Eco*RI and *Bam*HI. pKM003 [*amyE::PspoIIQ-yfp (spec)*] was generated by inserting a PCR product containing *yfp* (oligonucleotides oDR078 and oDR079 with pKL183 (Lemon and Grossman, 2000) as template) into pKM001 cut with HindIII and BamHI. pKM001 [*amyE::PspoIIQ (spec)*] was generated by inserting a PCR product containing *PspoIIQ (*oligonucleotides oDR234 and oDR235 with wild-type genomic DNA) into pLD30 (Garsin et al., 1998) between EcoRI and HindIII. pNS042 [*yycR::phleo*] is an ectopic integration vector for double-crossover insertions into the nonessential *yycR* locus (N.L.S. and D.Z.R., unpublished).

pNS056 [*pelB::PspoIIQ-yfp (kan)*] was generated by inserting *PspoIIQ-cfp* from pKM008 into pKM069.

pNS059 [*yycR::PspoIIQ-yfp (spec)*] was generated by inserting *PspoIIQ-yfp* from pKM003 into pNS044 between *Eco*RI and *Bam*HI. pNS044 [*yycR::spec*] is an ectopic integration vector for double-crossover insertions into the nonessential *yycR* locus (N.L.S. and D.Z.R., unpublished).

pNS066 [*pelB::(soj, spo0J(parS*)-yfp) (cat)*] was generated in a three-way ligation with an XhoI-EcoRI PCR product containing *(soj spo0J(parS*)* (oligonucleotide primers oNS024 and oDR418 with genomic DNA from a derivative of DCL468 as template), an XhoI-BamHI PCR product containing *yfp* (oligonucleotide primers oTD078 and oTD079 and pKL183 as template) and pKM020 cut with BamHI and EcoRI.

pNS069 [*amyE::PspoIIQ-cfp (kan)*] was generated by inserting *PspoIIQ-cfp* from pKM008 into pER82 between EcoRI and BamHI. pER82 [*amyE::kan*] is an *amyE* integration vector derived from pDG364 and created by Ezio Ricca.

pNS087 [*ydaD::PspoIIQ-cfp (erm)*] was generated by inserting a BamHI-XhoI PCR product containing *PspoIIQ-cfp* (oligonucleotide primers oNS032 and oTD020 and pKM008 as template) into pNS080 between XhoI and BamHI. pNS080 [*ydaD::erm*] is an ectopic integration vector for double crossover insertions into the nonessential *ydaD* locus (N.L.S. and D.Z.R., unpublished).

pNS109 [*yycR::PspoIIQ-yfp + parS (spec)*] was generated by inserting *parS* linkers (oTD040 and oTD041) into pNS059 at the BamHI site.

pNS110 [*yycR::PspoIIQ-yfp + parS* (spec)*] was generated by inserting *parS** linkers (oTD048 and oTD049) into pNS059 at the BamHI site.

pNS131 [*yycR::parS (spec)*] was generated by inserting *parS* linkers (oTD040 and oTD041) into pNS044 at the BamHI site.

pNS132 [*yycR::parS* (spec)*] was generated by inserting *parS** linkers (oTD048 and oTD049) into pNS044 at the BamHI site.

pNS137 [*pelB::Psoj-cfp(d)-spo0J(parS*) (cat)*] was generated in a three-way ligation with an XhoI-BamHI fragment containing *spo0J(parS*)* from pKM256, a HindIII-XhoI PCR product containing *cfp(d)* (oligonucleotide primers oTD004 and oTD005 and pNS103 as template), and pKM170 cut with HindIII and BamHI. pNS103 [*cfp(d)*] was generated by site-directed mutagenesis using pDR200 (Doan et al., 2005) and oNS063. This generated CFP(A206K), which allows weak dimerization of CFP.

Fluorescence microscopy

Fluorescence microscopy was performed as previously described (Doan et al., 2005). Fluorescent signals were visualized with a phase contrast objective UplanFLN 100X and captured with a monochrome CoolSnapHQ digital camera (Photometrics) using Metamorph software version 6.1 (Universal Imaging). Exposure times were typically 500-1000 ms for GFP/YFP/CFP protein fusions. Exposure times for promoter fusions to CFP or YFP were typically 100-200 ms. Membranes were stained with either TMA-DPH or FM4-64 (Molecular Probes), at a final concentration of 0.02 mM and 3 μg/ml respectively, and imaged with exposure times of 200-300 ms. DNA was stained with DAPI (Molecular Probes), at a final concentration of 2μg/ml and

imaged with an exposure time of 200 ms. Fluorescence images were analyzed, adjusted and cropped using Metamorph v 6.1 software (Molecular Devices).

Construction of the Δ8 *parS* strain

The unmarked *spo0J(parS*)* mutation (Lin and Grossman, 1998) in an *amyE::cat* strain was introduced into *spoIIIE36, Δ(soj, spo0J)::spec* by congression selecting for Cm(R) and screening for loss of Spec(R). Cm(R) and Spec(S) transformants were sequenced to confirm the presence of the *parS* mutation.

The next seven *parS* deletions were introduced sequentially by allelic replacement using single cross-over integration plasmids derived from pMAD (Arnaud et al., 2004). Transformants were grown in the absence of selection to allow "loop-out" of the plasmid, and screened for loss of MLS(R). For initial diagnosis, MLS(S) strains were analyzed by PCR and direct sequencing. A similar approach was used to delete all eight *parS* sites in a wild-type background. Subsequent confirmation of the unmarked deletions was achieved by PCR using oligonucleotide primers (Table S4) that overlapped the mutation. For each site, a second primer was used that generated a product of unique size. All 8 products could be identified in a single PCR reaction.

Immunoblot analysis

During logarithmic growth or sporulation the OD_{600} was measured (for equivalent loading) and samples (1.0 ml) were collected by centrifugation. Whole cell extracts were prepared by resuspension of cell pellets in 50 µl lysis buffer [20 mM Tris pH 7.0, 10 mM EDTA, 1 mg/ml lysozyme, 10 µg/ml DNase I, 100 µg/ml RNase A, with protease inhibitors: 1 mM PMSF, 1 µg/ml leupeptin, 1 µg/ml pepstatin] and incubation at 37˚C for 10 min followed by addition of 50 µl sodium dodecyl sulfate (SDS) sample buffer [0.25 M Tris pH 6.8, 4% SDS, 20% glycerol, 10 mM EDTA] containing 10% 2-Mercaptoethanol. Samples were heated for 5 min at 80˚C prior to loading. Proteins were separated by SDS-PAGE on 15% polyacrylamide gels, electroblotted onto Immobilon-P membrane (Millipore) and blocked in 5% nonfat milk in phosphate-buffered saline (PBS)-0.5% Tween-20. The blocked membrane was probed with anti-Spo0J (Lin et al., 1997), anti-Soj (Quisel et al., 1999), anti-SMC (Lindow et al., 2002), anti-σ^F (Pan et al., 2001), anti-σ^A (Fujita, 2000), or affinity-purified anti-GFP (Rudner and Losick, 2002) antibodies diluted into 3%

BSA in PBS-0.05% Tween-20. The primary antibodies were detected using horseradish peroxidase-conjugated goat, anti-rabbit immunoglobulin G (BioRad) with the Supersignal Substrate as described by the manufacturer (Perkin Elmer).

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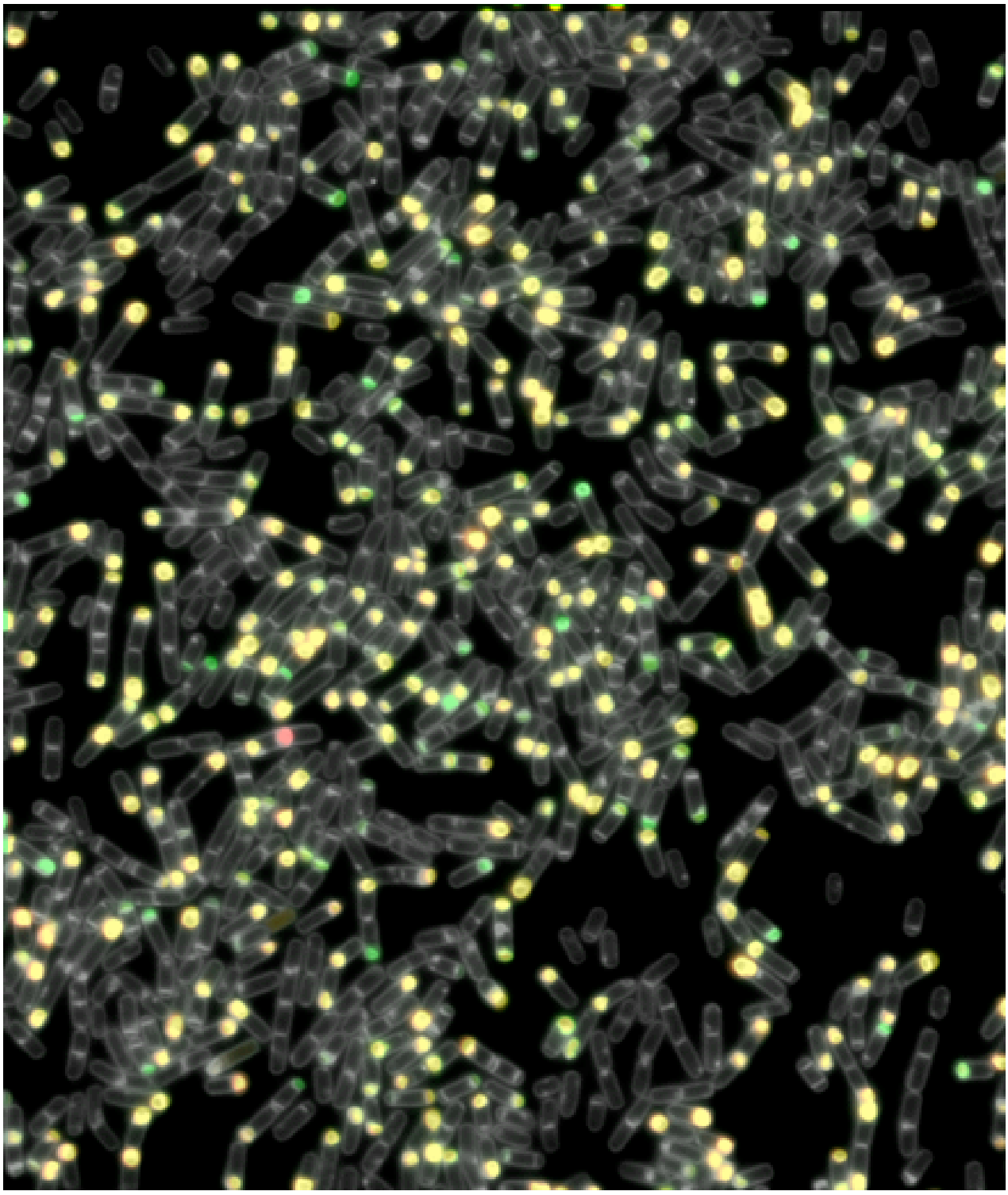
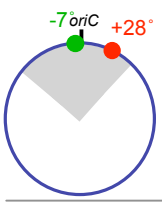


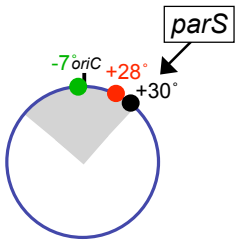
Figure S1 Fluorescent micrograph of a typical field of sporulating cells used to analyze chromosome organization. The *spoIIIE36* mutant contained the YFP reporter (false-colored red) at -7˚ and the CFP reporter (false-colored green) at +28˚.

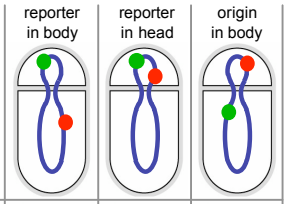


	reporter in body	reporter in head	origin in body	forespores counted
+28°	9%	90%	0%	1191
	13%	86%	1%	702
	11%	88%	1%	1087
	13%	85%	1%	915
	11%	88%	1%	806
	15%	84%	1%	972

Figure S2 Reproducibility of the chromosome organization assay. Six independent experiments analyzing the +28° CFP reporter (red circle) and the -7° YFP reporter (green circle). The number of sporulating cells counted in each experiment is indicated in the right column.

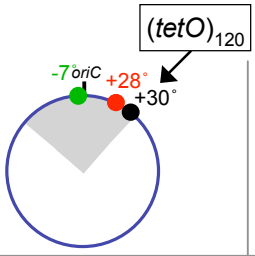
A

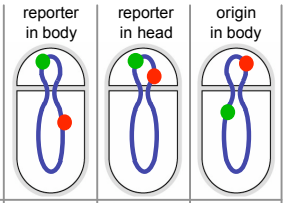




		reporter in body	reporter in head	origin in body
+28˚	WT	11%	89%	0%
	+30˚::*parS*	33%	67%	1%
	+30˚::*parS**	10%	90%	1%

B





		reporter in body	reporter in head	origin in body
+28˚	WT	12%	88%	1%
	tetR	11%	88%	1%
	+30˚::$(tetO)_{120}$	12%	88%	1%
	tetR, +30˚::$(tetO)_{120}$	10%	89%	0%

Figure S3 (**A**) An ectopic *parS* site disrupts the organization of a reporter 30 kb away. A consensus *parS* site or a mutated site (*parS**) was inserted at +30˚ (black circle). The CFP reporter inserted at +28˚ (red circle) was analyzed relative to the -7˚ YFP reporter (green circle). (**B**) TetR-GFP bound to an array of *tetO* sites does not disrupt chromosome organization. Analysis of the CFP reporter at +28˚ relative to the -7˚ YFP reporter in a strain containing $(tetO)_{120}$ inserted at +30˚ and *tetR-gfp* under the control of the P_{spoIIE} promoter such that TetR-GFP was synthesized at the onset of sporulation.

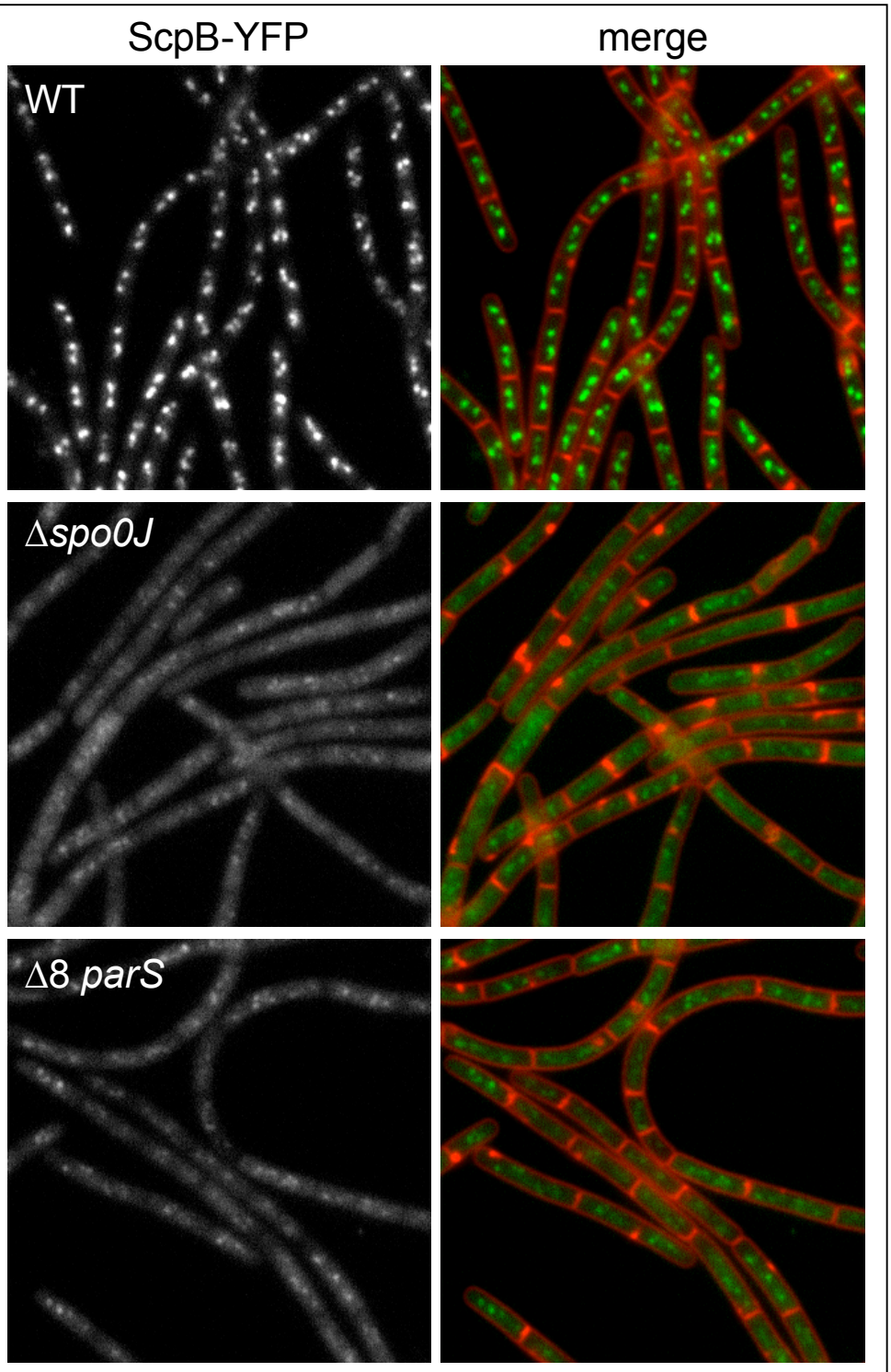


Figure S4 Proper localization of the SMC complex requires Spo0J and the *parS* sites. (**A**) Localization of ScpB-YFP in large fields of cells in wild-type and strains lacking Spo0J (Δ*spo0J*) and the 8 origin-proximal *parS* sites (Δ*parS*). The signal intensities in the three images were normalized for direct comparison The membranes (false-colored red) were stained with TMA-DPH.

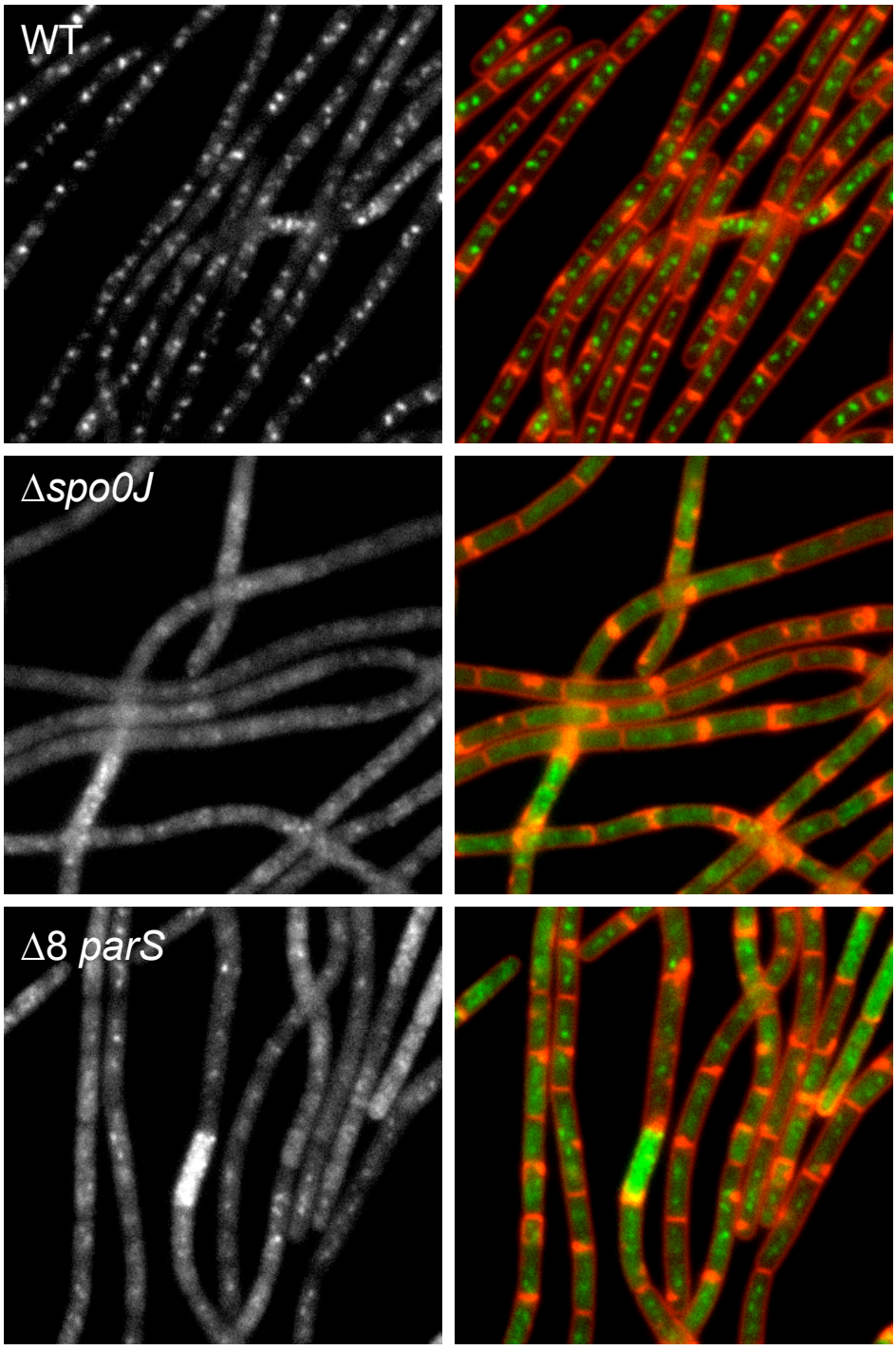
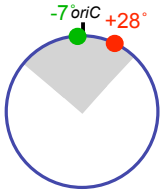


Figure S4 Proper localization of the SMC complex requires Spo0J and the *parS* sites. (**B**) Localization of GFP-SMC in wild-type and strains lacking Spo0J and the eight origin-proximal *parS* sites. The signal intensities in the three images were normalized for direct comparison. The membranes (false-colored red) were stained with TMA-DPH.



+28˚	reporter in body	reporter in head	origin in body	both loci in the body
WT	11%	88%	1%	0%
Δ(*soj spo0J*)	35%	30%	15%	20%
Δ(*soj spo0J*), *spo0J93*	37%	31%	14%	18%
Δ(*soj spo0J*), *spo0J+*	8%	66%	20%	6%

Figure S5 Chromosome organization is impaired in a Spo0J93 mutant. Chromosome organization was analyzed in a Δ(*soj spo0J*) mutant containing a wild-type copy of *spo0J* or the *spo0J93* allele inserted at an ectopic locus. The CFP reporter inserted at +28˚ was analyzed relative to the -7˚ YFP reporter. A wild-type strain and the Δ(*soj spo0J*) mutant were included as controls. The strain that contained the wild-type copy of *spo0J* is the same as the Δ*soj* mutant and therefore has a defect in origin re-positioning.

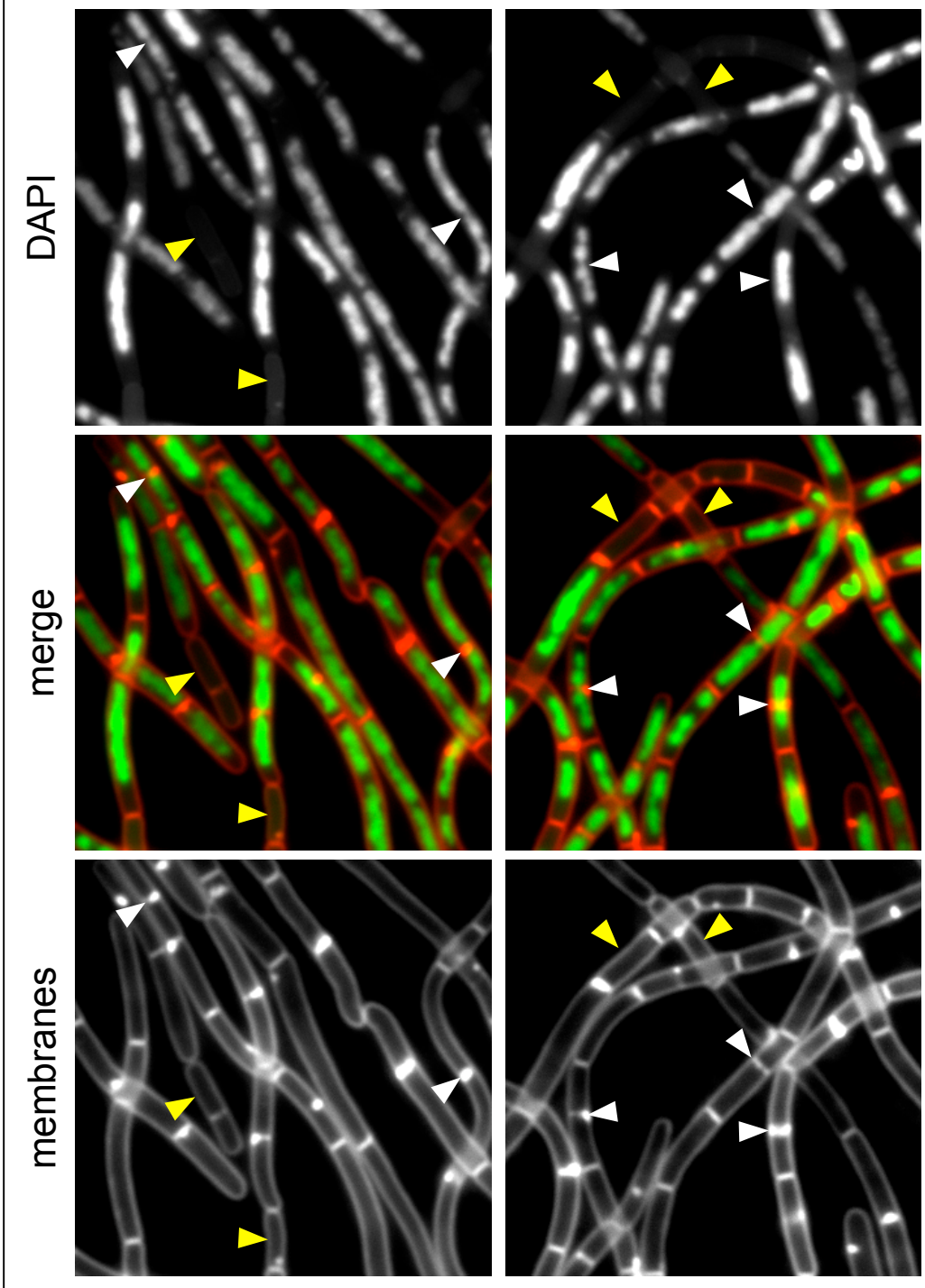
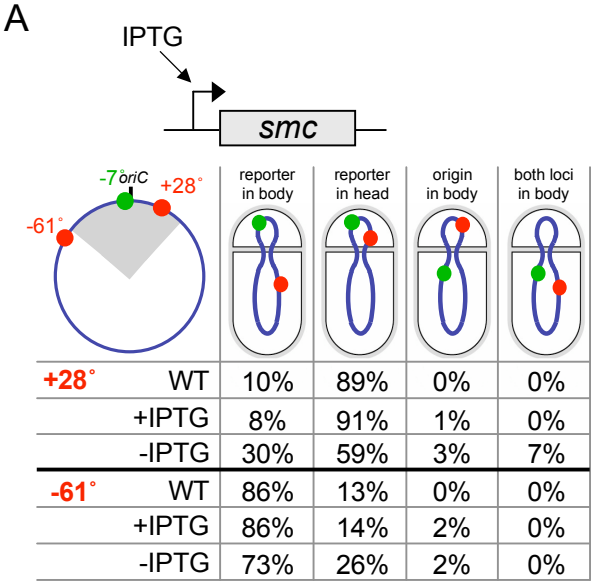


Figure S6 Recruitment of SMC to an ectopic site impairs chromosome organization and DNA segregation. Nucleoid morphology in two fields of cells from a strain (BKM1661) lacking the eight origin-proximal *parS* sites and containing the *parS* array near the terminus. Anucleate cells (yellow caret) and cells with bisected chromosomes (white caret) are highlighted.



		reporter in body	reporter in head	origin in body	both loci in body
+28˚	WT	10%	89%	0%	0%
	+IPTG	8%	91%	1%	0%
	-IPTG	30%	59%	3%	7%
-61˚	WT	86%	13%	0%	0%
	+IPTG	86%	14%	2%	0%
	-IPTG	73%	26%	2%	0%

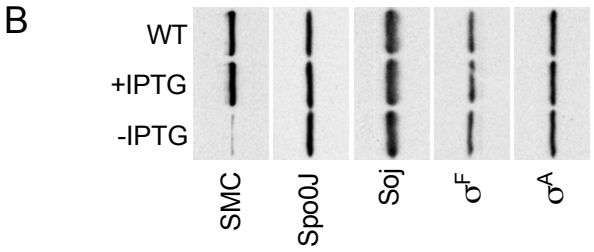


Figure S7 Depletion of SMC impairs chromosome organization. (**A**) The *smc* gene was fused to an IPTG-inducible promoter (P_{spac}). The inducible strains were grown in the presence or absence of 500μM IPTG for 3.5 generations before initiating sporulation. The CFP reporters at -61˚ and +28˚ were analyzed relative to the -7˚ YFP reporter. (**B**) The levels of SMC protein were analyzed by immunoblot at an early stage of sporulation. For comparison, the levels of Spo0J and Soj were analyzed. All strains efficiently entered sporulation as judged by the levels of the sporulation transcription factor σ^F. σ^A was used to control for loading. DAPI staining indicates that the chromosome mass was not yet decondensed after 3.5 hours of depletion and appeared normal at the time of sporulation induction (data not shown). The degree of disorganization in the SMC depletion strain was less severe than the Spo0J mutant and we suspect that this is due to the residual SMC protein still present in the cells. Further depletion of SMC resulted in greater defects in chromosome organization, however, under these conditions, entry into sporulation was much less efficient and both cell and chromosome morphology were perturbed (data not shown).

Table S1

Strains Used in this study

Strain	Genotype	Reference	Figure
BNS891	*yycR::P_{spoIIQ}-yfp (phleo), lacA::P_{spoIIQ}-cfp (erm)*	This work	1A
BNS270	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), lacA::P_{spoIIQ}-cfp (erm)*	This work	1A, 1B, 1D, 2
BNS652	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), ywjI::P_{spoIIQ}-cfp (erm)*	This work	1B, 1D
BNS268	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), amyE::P_{spoIIQ}-cfp (cat)*	This work	1B, 1D, 2
BNS272	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), ycgO::P_{spoIIQ}-cfp (kan)*	This work	1B
BNS490	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), ydaD::P_{spoIIQ}-cfp (erm)*	This work	1B
BNS274	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), yvbJ::P_{spoIIQ}-cfp (spec)*	This work	1B
BNS276	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), pelB::P_{spoIIQ}-cfp (kan)*	This work	1B
BNS620	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), amyE::P_{spoIIQ}-cfp (cat), Δ(soj-spo0J)::spec, pelB::sojΔ spo0J^{+}(kan)*	This work	1D
BNS306	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), amyE::P_{spoIIQ}-cfp (cat), Δ(soj-spo0J)::spec*	This work	1D
BNS1329	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), ywjI::P_{spoIIQ}-cfp (erm), Δ(soj-spo0J)::spec, pelB::sojΔ spo0J^{+} (kan)*	This work	1D
BNS1159	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), ywjI::P_{spoIIQ}-cfp (erm), Δ(soj-spo0J)::spec*	This work	1D
BNS622	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), lacA::P_{spoIIQ}-cfp (erm), Δ(soj-spo0J)::spec, pelB::sojΔ spo0J^{+} (kan)*	This work	1D
BNS314	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), lacA::P_{spoIIQ}-cfp (erm), Δ(soj-spo0J)::spec*	This work	1D
BNS286	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), amyE::P_{spoIIQ}-cfp + parS (cat)*	This work	2
BNS288	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), amyE::P_{spoIIQ}-cfp + parS* (cat)*	This work	2
BNS294	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), lacA::P_{spoIIQ}-cfp + parS (erm)*	This work	2
BNS332	*spoIIIE36, yycR::P_{spoIIQ}-yfp (phleo), lacA::P_{spoIIQ}-cfp + parS* (erm)*	This work	2
BNS436	*spoIIIE36, yycR::P_{spoIIQ}-yfp (spec), amyE::P_{spoIIQ}-cfp (cat)*	This work	3B, 3C
BNS1661	*spoIIIE36, yycR::P_{spoIIQ}-yfp (spec), amyE::P_{spoIIQ}-cfp (cat), Δ3 parS^*	This work	3B
BNS1735	*spoIIIE36, yycR::P_{spoIIQ}-yfp (spec), ywjI::P_{spoIIQ}-cfp (erm)*	This work	3B, 3C
BNS1809	*spoIIIE36, yycR::P_{spoIIQ}-yfp (spec), ywjI::P_{spoIIQ}-cfp (erm), Δ3 parS^*	This work	3B
BNS1433	*spoIIIE36, yycR::P_{spoIIQ}-yfp (spec), lacA::P_{spoIIQ}-cfp (erm)*	This work	3B, 3C
BNS1808	*spoIIIE36, yycR::P_{spoIIQ}-yfp (spec), lacA::P_{spoIIQ}-cfp (erm), Δ3 parS^*	This work	3B
BNS1480	*spoIIIE36, yycR::P_{spoIIQ}-yfp (spec), amyE::P_{spoIIQ}-cfp (cat), Δ8 parS†*	This work	3C
BNS1567	*spoIIIE36, yycR::P_{spoIIQ}-yfp + parS (spec), amyE::P_{spoIIQ}-cfp (cat), Δ8 parS†*	This work	3C
BNS1571	*spoIIIE36, yycR::P_{spoIIQ}-yfp + parS* (spec), amyE::P_{spoIIQ}-cfp (cat), Δ8 parS†*	This work	3C
BNS1749	*spoIIIE36, yycR::P_{spoIIQ}-yfp (spec), ywjI::P_{spoIIQ}-cfp(erm), amyE::cat, Δ8 parS†*	This work	3C
BNS1755	*spoIIIE36, yycR::P_{spoIIQ}-yfp + parS (spec), ywjI::P_{spoIIQ}-cfp(erm), amyE::cat, Δ8 parS†*	This work	3C
BNS1756	*spoIIIE36, yycR::P_{spoIIQ}-yfp + parS* (spec), ywjI::P_{spoIIQ}-cfp(erm), amyE::cat, Δ8 parS†*	This work	3C
BNS1747	*spoIIIE36, yycR::P_{spoIIQ}-yfp (spec), lacA::P_{spoIIQ}-cfp(erm), amyE::cat, Δ8 parS†*	This work	3C
BNS1754	*spoIIIE36, yycR::P_{spoIIQ}-yfp + parS (spec), lacA::P_{spoIIQ}-cfp(erm), amyE::cat, Δ8 parS†*	This work	3C
BNS1748	*spoIIIE36, yycR::P_{spoIIQ}-yfp + parS* (spec), lacA::P_{spoIIQ}-cfp(erm), amyE::cat, Δ8 parS†*	This work	3C
BNS1437	*pelB::Psoj-gfp-spo0J(parS*) (cat)*	This work	3D, 3E
BNS1672	*pelB::Psoj-gfp-spo0J(parS*) (cat), Δ8 parS†*	This work	3D, 3E
BNS1695	*pelB::Psoj-gfp-spo0J(parS*) (cat), Δ8 parS†, yycR::parS (spec)*	This work	3D, 3E
BNS1773	*pelB::Psoj-gfp-spo0J(parS*) (cat), Δ8 parS†, yycR::parS* (spec)*	This work	3D, 3E
BKM1634	*scpB-yfp (spec)*	This work	4A, 4B
BKM1683	*scpB-yfp (spec), Δ(soj-spo0J)::cat, thrC::soj (erm)*	This work	4A, 4B
BKM1662	*scpB-yfp (spec), Δ(soj-spo0J)::cat, thrC::soj (erm), pelB::sojΔ spo0J+ (kan)*	This work	4A, 4B
BKM1657	*scpB-yfp (spec), Δ8 parS†*	This work	4A, 4B
BKM1688	*scpB-yfp (spec), Δ8 parS†, yycR::parS (cat)*	This work	4A, 4B
BKM1675	*scpB-yfp (spec), pelB::P_{soj}-cfp(d)-spo0J(parS*)(cat)*	This work	4C
BKM1725	*pelB::P_{soj}-cfp(d)-spo0J(parS*)(cat), dnaX-yfp (spec)*	This work	4D
BKM1729	*scpB-yfp (spec), Δ8 parS†, lacA::P_{soj}-cfp(d)-spo0J(parS*) (erm)*	This work	4E
BKM1730	*scpB-yfp (spec), Δ8 parS†, lacA::P_{soj}-cfp(d)-spo0J(parS*) (erm), cgeD(181°)Ω(parS)$_{16}$ (cat)*	This work	4E
BKM1646	*ytoI::Psmc-gfp-smc(erm), trpC2, pheA1*	This work	4F
BKM1647	*ytoI::Psmc-gfp-smc(erm), spo0J93, trpC2, pheA1*	This work	4F
PY79	wild-type	Youngman et al,1983	6
BNS1657	*Δ8 parS†*	This work	6
BKM1661	*Δ8 parS†, yqkF(210°)Ω(parS)$_{16}$ (cat)*	This work	6
BDR1050	*spoIIIE36*	Wu Errington, 1994	
AG1505	*Δ(soj, spo0J)::spec, trpC2, pheA1*	Ireton et al., 1994	
DCL468	*spo0J (parS*), trpC2, pheA1*	Lin Grossman, 1998	
KI913	*Δ(soj-spo0J)::spec, thrC::soj (erm), trpC2, pheA1,*	Ireton et al., 1994	
JH693	*spo0J93, trpC2, pheA1*	Hoch Mathews,1973	

= Δ8 *parS* contains deletions or synonymous mutations in the eight origin proximal *parS* sites.
^ Δ3 *parS* contains deletions in the three dispersed *parS* sites (-26°, +15°, +40°).

Table S2
Plasmids used in this study

plasmid	description	reference
pDG364	*amyE::cat*	Karmazyn-Campelli et al, 1992
pDL141	*cgeD(181˚)ΩparS$_{16}$(cat)*	Lee et al, 2003
pJCL075	*ypuH-yfp (scpB-yfp) (spec)*	Lindow et al, 2002
pKM011	*lacA::P$_{spoIIQ}$-cfp (erm)* (-61˚)	This work
pKM173	*ywjI::P$_{spoIIQ}$-cfp (cat)* (-35˚)	This work
pKM186	-5˚(parS*) in pMAD (erm)	This work
pKM230	-6˚(parSΔ) in pMAD (erm)	This work
pKM231	-4˚(parSΔ) in pMAD (erm)	This work
pKM232	+4˚(parSΔ) in pMAD (erm)	This work
pKM234	*Δ(soj spo0J)::cat* (contains *parS* site at -1˚)	This work
pKM252	+15˚(parSΔ) in pminiMAD(erm)	This work
pKM253	-26˚(parSΔ) in pminiMAD(erm)	This work
pKM255	+40˚(parSΔ) in pminiMAD(erm)	This work
pKM256	*pelB::P$_{soj}$-gfp-spo0J (parS*) (cat)*	This work
pKM287	*ytoI::P$_{smc}$-gfp-smc (erm)*	This work
pKM299	*lacA::P$_{soj}$-cfp(d)-spo0J(parS*) (erm)*	This work
pKM309	*smc-(his)$_6$ (kan)*	This work
pKM315	*spo0J-(his)$_6$ (kan)*	This work
pKM323	*lacIΔ11-his$_6$ (amp)*	This work
pNS005	*lacA::P$_{spoIIQ}$-cfp, parS (erm)*	This work
pNS007	*amyE::P$_{spoIIQ}$-cfp (cat)* (+28˚)	This work
pNS009	*amyE::P$_{spoIIQ}$-cfp, parS (cat)*	This work
pNS010	*lacA::P$_{spoIIQ}$-cfp, parS* (erm)*	This work
pNS012	*amyE::P$_{spoIIQ}$-cfp, parS* (cat)*	This work
pNS013	*ycgO::P$_{spoIIQ}$-cfp (kan)* (+30˚)	This work
pNS021	*pelB::sojΔ spo0J+ (kan)*	This work
pNS038	*yvbJ::P$_{spoIIQ}$-cfp (spec)* (-63˚)	This work
pNS051	*yycR::P$_{spoIIQ}$-yfp (phleo)* (-7˚)	Burton et al, 2007
pNS056	*pelB::P$_{spoIIQ}$-cfp (kan)* (+174˚)	Burton et al, 2007
pNS059	*yycR::P$_{spoIIQ}$-yfp (spec)*	This work
pNS066	*pelB::soj spo0J(parS*)-yfp (cat)*	This work
pNS069	*amyE::P$_{spoIIQ}$-cfp (kan)*	This work
pNS087	*ydaD::P$_{spoIIQ}$-cfp (erm)* (+40˚)	This work
pNS109	*yycR::P$_{spoIIQ}$-yfp, parS (spec)*	This work
pNS110	*yycR::P$_{spoIIQ}$-yfp, parS* (spec)*	This work
pNS131	*yycR::parS (spec)*	This work
pNS132	*yycR::parS* (spec)*	This work
pNS137	*pelB::P$_{soj}$-cfp(d)-spo0J(parS*) (cat)*	This work
pPSL3A	*yqkF(210˚)ΩparS$_{16}$(cat)*	Lee et al, 2003

Table S3 Oligonucleotides used in this study

Primer	Sequence	Use
oDR234	gccGAATTCcatgcttcgtcaatgtatatgctg	pNS059
oDR235	cggAAGCTTagcaacattctgaacacttttctg	pNS059
oDR418	ggcgCTCGAGtgattctcgttcagacaaaagctc	pNS066
oDR470	gccGAATTCaatccggctttaatgatcagat	pKM256
oDR471	cgccAAGCTTtcacatgaacatgtactatct	pKM256
oDR472	ggcgCTCGAGatggctaaaggccttggaaaaggg	pKM256
oDR473	cgcGGATCCtggatcgtcacacgccgtggtg	pKM186
oDR474	cgccGTCGAcacacgaacagtagactctgttga	pKM186
oDR475	cggcgctggaactgttgaa**cgtgagccgggagacc**gccttagaaatgccgattacgagt	pKM183
oDR509	gccGAATTCgctgtcgaccagcccggcctg	pKM230
oDR510	gccGGATCCagcggcgaggctgggagcgg	pKM230
oDR516	cggCTCGAGgcaaatggcagttagctgcaaatac	pKM230
oDR517	cggCTCGAGtccatcttgtatttttcttcatattcg	pKM230
oDR520	cgcGAATTCgacagcaatcacagccgcagc	pKM231
oDR521	gccCTCGAGttagcgtcattttacttgcgatttgg	pKM231
oDR522	gccCTCGAGtgaaaaaggaaaagacattctttgaaaagaatgccc	pKM231
oDR523	cgcGGATCCaccaggtgccgcccaaggtag	pKM231
oDR526	cgcGAATTcagccagaatcacgcaaaaacgaaatg	pKM232
oDR527	gccCTCGAGcctttgtttatcattatttaattcttgtg	pKM232
oDR528	gccCTCGAGattcgtcgaacacctttgtgtttcg	pKM232
oDR529	cgcGGATCCgcctccttctcctcggagag	pKM232
oDR549	gccGAATTcggccctgaacacggcgtc	pKM252
oDR550	gcgCTCGAGtacgatgcttatgttctaataaatg	pKM252
oDR551	gcgCTCGAGtattcttattgaacgaaagacccttac	pKM252
oDR552	gccGGATCCgagatggcccattgtgccc	pKM252
oDR555	cgcGGATCCggcattatgcctgttaccggg	pKM253
oDR556	gcgtCGGCCGtaaaaacccctccagacggagag	pKM253
oDR557	gcgtCGGCCGtattccgacaaatagggacatgc	pKM253
oDR558	ggcGTCGAcagccgctccttaacctgttc	pKM253
oDR561	cgcGGATCcaggatatgtagatgaagatggc	pKM255
oDR562	gcgtCGGCCGctgagaggaaattaactcaaaaagg	pKM255
oDR563	gcgtCGGCCGtttttctgataaagcaatgcgaagg	pKM255
oDR564	ggcGTCGACgttcacttcttctttcgtgatgcc	pKM255
oDR627	cgcGGATCCgtttcttgtttttcaaggccatcc	pKM287
oDR630	cggCTCGaggaggatcgctatgagtaaaggagaagaacttttc	pKM287
oDR631	gccGCTAGCtttgtatagttcatccatgccatg	pKM287
oDR632	gccGCTAGCggttccggaatgttcctcaaacgtttagacg	pKM287
oDR682	gcaggCATATGttcctcaaacgtttagacgttatag	pKM309
oDR683	cggCTCGAGctgaacgaattcttttgtttcttccag	pKM309
oDR689	gccGCTAGCaaaggccttggaaaaggg	pKM315
oDR690	cggCTCGAGtgattctcgttcagacaaaagc	pKM315
oDR686	gcgaatcatttaagactgctgacac	gel shift
oDR688	ccgttgcaaaggctcactgg	gel shift
oDR703	caggCATATGaaaccagtaacgttatacgatg	pKM323
oDR704	gccAAGCTTagtgatggtggtgatggtgcagctgcattaatgaatcggc	pKM323
oDR705	ctctagttgctagagcatgtgtctc	gel shift
oDR706	ccaggaactctagcaagcaagcaat	gel shift
oNS024	gccGAATTCgtttccacgttctgtactgtg	pNS066
oNS025	gccGGATCCcagagtggaggcaagaacgcc	pKM256
oNS032	cggCTCGAGcatgcttcgtcaatgtatatgc	pNS087
oNS063	ccattacttaagcacgcaatctgcgctgtcaaaagaccctaacga	pKM299
oNS069	cgcGGCCGgtttccacgttctgtactgtg	pKM234
oNS070	cgcGTCGAcgcccaatgacggcggac	pKM234
oNS071	gcgGAATTCgactgctgacactgccag	pKM234
oNS072	gccTCTAGAgatcggacagagtggaggc	pKM234

oTD004	gcgCTCGAGcttataaagttcgtccatgcc	pKM299
oTD005	gttgctAAGCTtacataaggagg	pKM299
oTD020	gccGGATCCttacttataaagttcgtccatgccaag	pNS087
oTD040	GATCaatcagaa**tgttacacgtgaaaca**aagaaaaaC	consensus *parS*
oTD041	GATCGtttttctt**tgtttcacgtgtaaca**ttctgatt	consensus *parS*
oTD048	GATCaatcagaa**cgtgcccagggagacc**aagaaaaaC	*parS* mutant
oTD049	GATCGtttttctt**ggtctcctgggcacg**ttctgatt	*parS* mutant
oTD078	gtgGGTACCgtcagcagcatatttgatgcc	pNS059, pNS066
oTD079	gtgCTCGAGtgatactctcatttgcgttggaatcag	pNS059, pNS066

restriction endonuclease sites are capitalized
parS and *parS** sites are in bold face.
mutations are underlined

Table S4

Oligonucleotide primers used for PCR confirmation of the *parS* deletions.

Primer	**Sequence**	***parS* site**
oDR585	gcagctaactgccatttgcCTCG	-6˚
oDR515	gtcggtgaccgggtgaaggtg	-6˚
oDR476	ttctaaggcGgtCtcCcgGCTC	-5˚
oDR586	ggcaagaaccattacccacccgc	-5˚
oDR587	tcgcaagtaaaatgacgctaaCTCG	-4˚
oDR588	gccaatgttctgcgttcatccatg	-4˚
oDR589	tttcttgGTcTCcCtgGGcAC	-1˚ (*spo0J*)
oDR590	tgcgggtgaacggcgttttcg	-1˚ (*spo0J*)
oDR591	gcattacagctttcggccagctg	+4˚
oDR592	cacaaaaggtgttcgacgaatCTCG	+4˚
oDR598	gtgtttgtcatccgccaaatcatgc	-26˚
oDR599	ccgtctggaggggtttttaCGGC	-26˚
oDR600	cgattgctgaatgtgtatccgtttg	+15˚
oDR601	tcatttattagaacataagcatcgtaCTCG	+15˚
oDR602	cgcgactgcatttacacggatgc	+40˚
oDR603	tttgagttaatttcctctcagCGGC	+40˚