Inventory of Supplemental information

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Supplemental Experimental Procedures: This provides a detailed description of the methods applied in this study.

Supplemental References: This includes the references cited in the Supplemental Information.

Figure S1.

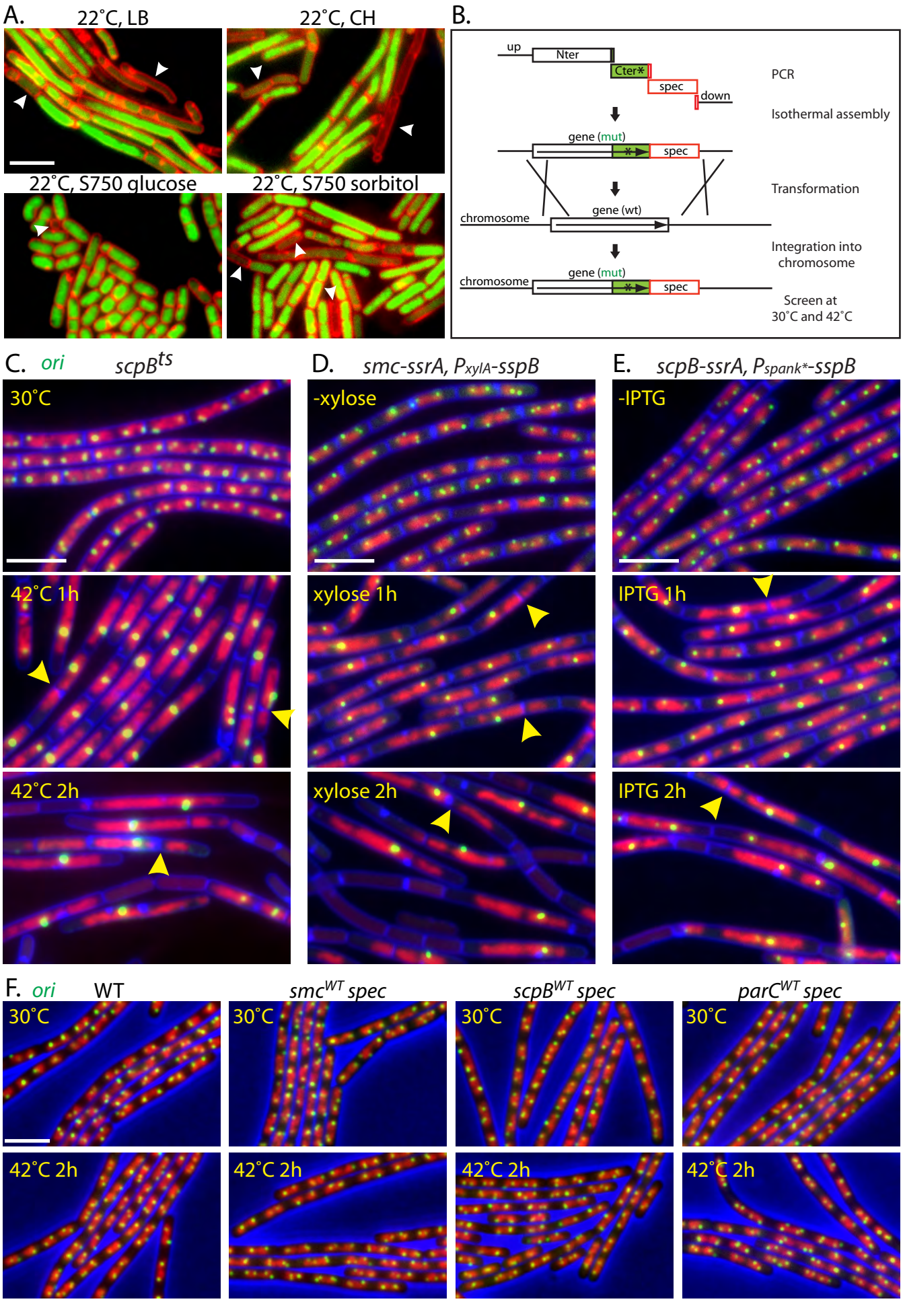
A.
22˚C, LB
22˚C, CH
22˚C, S750 glucose
22˚C, S750 sorbitol
B.
up
Nter
Cter*
spec
down
PCR
Isothermal assembly
gene (mut)
spec
Transformation
chromosome
gene (wt)
Integration into chromosome
chromosome
gene (mut)
spec
Screen at 30˚C and 42˚C
C. ori
scpB^ts
30˚C
42˚C 1h
42˚C 2h
D. smc-ssrA, P_xylA-sspB
-xylose
xylose 1h
xylose 2h
E. scpB-ssrA, P_spank*-sspB
-IPTG
IPTG 1h
IPTG 2h
F. ori
WT
smc^WT spec
scpB^WT spec
parC^WT spec
30˚C
42˚C 2h

Figure S1. SMC complexes are required for origin segregation, Related to Figure 1.

(A) Anucleate-cell formation and heterogeneous nucleoid morphologies in the SMC null mutant grown under permissive conditions. Representative images of Δ*smc* (strain BWX2208) grown at 22˚C in LB, casein hydrolysates (CH), minimal medium (S750) supplemented with glucose or sorbitol. The membranes (red) were stained with TMA-DPH, and nucleoids (green) were labeled using HbsU-GFP. Cells that lack DNA (white carets) often have a faint cytoplasmic Hbsu-GFP signal. This likely results from nucleoid bisection and DNA degradation. In the most permissive growth condition (S750 sorbitol at 22˚C) we observed 11.5% anucleate cells (n=1563).

(B) Schematic flow chart depicting the generation of temperature-sensitive mutants. The gene of interest (or part of it) was amplified by error-prone PCR (green) and assembled by isothermal assembly (see Supplemental Experimental Procedures) with an antibiotic resistance gene (spec) and upstream (up) and downstream (down) fragments. The product was transformed directly into *B. subtilis* replacing the wild-type gene selecting for Spectinomycin resistance. Transformants were arrayed and then screened for colony formation at permissive (30˚C) and restrictive (42˚C) temperatures.

(C) Inactivation of ScpB impairs origin resolution and chromosome segregation. Representative images of nucleoids (labeled with HbsU-GFP, false-colored red), origin foci (green), and membranes (blue) in an $scpB^{ts}$ mutant (BWX2092) grown in CH medium at 30˚C and after shift to 42˚C for 1 and 2 hours. Membranes were stained with TMA-DPH. Yellow carets highlight septum formation on top of the nucleoid.

(D) Degradation of SMC-SsrA blocks origin resolution. Representative images of DAPI-stained nucleoids (false-colored red), origin foci (green), and membranes (blue) in cells (BWX1497) harboring an *smc-ssrA* allele before and after the addition of 0.5% xylose to induce expression of the *E. coli* SspB adaptor protein.

(E) Degradation of ScpB-SsrA blocks origin resolution. Representative images of cells (BWX1120) harboring an *scpB-ssrA* allele before and after the addition of 0.5 mM IPTG to induce expression of the *E. coli* SspB adaptor protein.

(F) Origin resolution and chromosome segregation are not altered at 42˚C in cells harboring a spectinomycin resistance gene linked to *smc*, *scpB*, or *parC*. Representative micrographs of nucleoids (HbsU-GFP, false-colored red), origin foci (green), and phase contrast (false-colored blue) in wild-type (BWX2006) and in strains harboring a spectinomycin resistance gene linked to *smc* (BWX2492), *scpB* (BWX2491), or *parC* (BWX2493). Cells were imaged at 30˚C and after shift to 42˚C for 2 hours. Scale bars are 4 μm.

Figure S2.

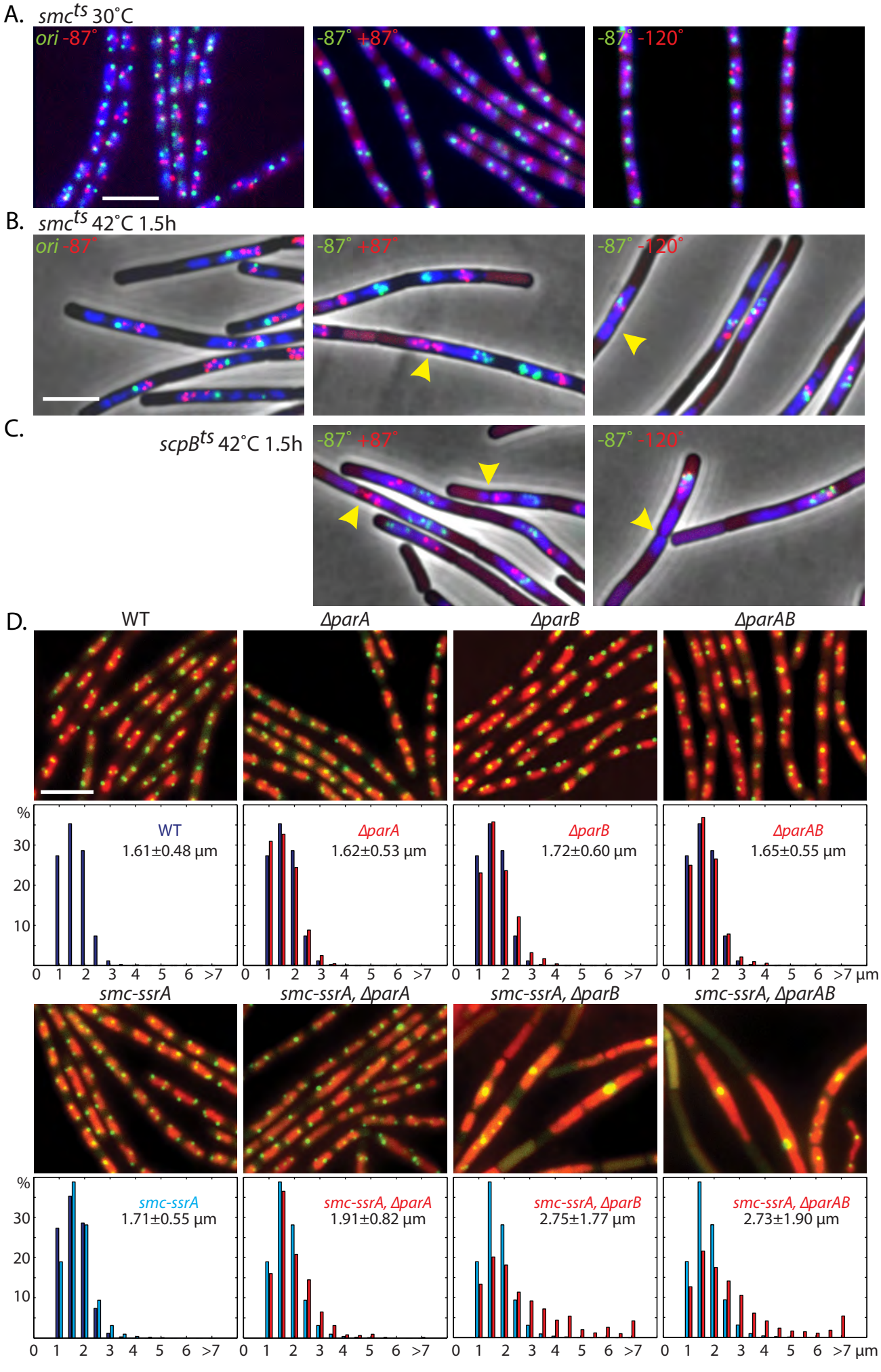
A. smc^ts 30°C
ori -87°
-87° +87°
-87° -120°
B. smc^ts 42°C 1.5h
ori -87°
-87° +87°
-87° -120°
C.
scpB^ts 42°C 1.5h
-87° +87°
-87° -120°
D.
WT
ΔparA
ΔparB
ΔparAB
%
WT
1.61±0.48 µm
ΔparA
1.62±0.53 µm
ΔparB
1.72±0.60 µm
ΔparAB
1.65±0.55 µm
0 1 2 3 4 5 6 >7 µm
smc-ssrA
smc-ssrA, ΔparA
smc-ssrA, ΔparB
smc-ssrA, ΔparAB
smc-ssrA
1.71±0.55 µm
smc-ssrA, ΔparA
1.91±0.82 µm
smc-ssrA, ΔparB
2.75±1.77 µm
smc-ssrA, ΔparAB
2.73±1.90 µm

Figure S2. ParB-mediated recruitment of SMC promotes chromosome segregation, Related to Figure 2.

(A) Representative images of DAPI-stained nucleoids (blue) and indicated chromosomal loci (green and red) in the *smc*^{ts} mutant (BWX2378, BWX2116, BWX2110) grown in CH medium under permissive conditions (30˚C.)

(B) Bulk chromosome segregation is blocked upon SMC inactivation. Images from Figure 2A with phase contrast (gray) to highlight the cell bodies and the fragmented nucleoids within them. Representative images of DAPI-stained nucleoids (blue), phase contrast image (gray) and indicated chromosomal loci (green and red) in the *smc*^{ts} mutant (BWX2378, BWX2116, BWX2110) grown in CH medium for 1.5 h after shifting to 42˚C. Yellow carets highlight septa on top of nucleoids.

(C) Bulk chromosome segregation is blocked upon ScpB inactivation. Images of DAPI-stained nucleoids (blue), phase contrast image (gray) and indicated chromosomal loci (green and red) in *scpB*^{ts} strains (BWX2114 and BWX2108) grown at 42˚C for 1.5 hours. Replicated left and right chromosome arms cluster separately.

(D) ParB-mediated recruitment of SMC promotes efficient origin segregation. Upper panel: Origin segregation is mildly impaired in cells lacking *parB*. Representative micrographs of DAPI-stained nucleoids (false-colored red) and origin loci (green) in wild-type (BWX811) and cells lacking *parA* (BWX2549), *parB* (BWX945) or *parAB* (BWX943). Lower panel: Origin segregation is severely impaired in cells lacking *parB* when SMC levels are reduced. Representative micrographs of strains harboring an *smc-ssrA* fusion that results in a 2.5-fold reduction in SMC levels in wild-type (BWX1497); an in-frame deletion of *parA* (BWX2551); a *parB* mutant (BWX1569); and a *parAB* deletion (BWX1571). Bar graphs of nucleoid lengths are shown below the micrographs. Average nucleoid length is indicated within the graphs. Scale bars are 4 μm.

Figure S3.

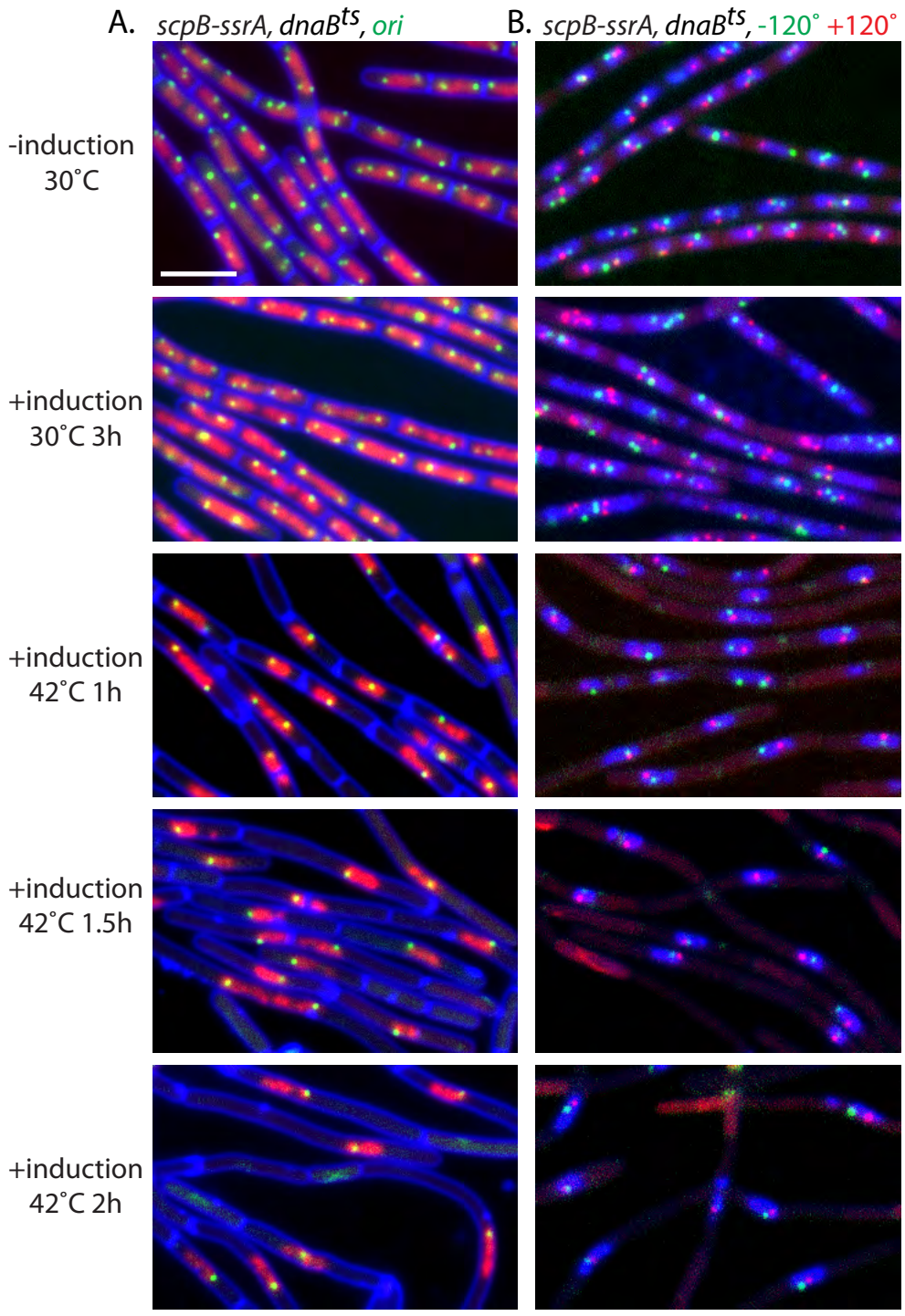
A. scpB-ssrA, dnaB^ts, ori
B. scpB-ssrA, dnaB^ts, -120° +120°
-induction 30°C
+induction 30°C 3h
+induction 42°C 1h
+induction 42°C 1.5h
+induction 42°C 2h

Figure S3. Chromosome resolution and segregation occur in the absence of ScpB when new rounds of replication are blocked, Related to Figure 4.

(A) Representative micrographs of DAPI-stained nucleoids (false-colored red), origin foci (green), and membranes (blue) in a strain (BWX1345) harboring the *scpB-ssrA* degradable allele and a temperature-sensitive replication initiation mutant (*dnaB*^{ts}). After induction of ScpB-SsrA degradation for 3 h at 30˚C most cells have unsegregated nucleoids with poorly resolved origin foci. Inhibition of replication leads to resolution and segregation of the chromosomes. The defect in origin segregation upon ScpB-SsrA degradation is less severe at 30˚C (this experiment) than at 37˚C (the experiment shown in Figure S1E).

(B) Representative micrographs of DAPI-stained nucleoids (blue) and indicated chromosomal loci (red and green) in a strain (BWX1172) harboring *scpB-ssrA* and *dnaB*^{ts}. After induction of ScpB-SsrA degradation for 3 h at 30˚C most cells have large unsegregated nucleoids with clusters of replicated chromosomal loci. After inhibition of replication the chromosomes segregate and each nucleoid contains a single -120˚ and +120˚ focus. Scale bar is 4 μm.

Table S1. Strains used in this study

strain	genotype	reference	figure
BWX2208	*yycR(-7˚)::tetO120 (erm), ycgO::P_{ftsW} tetR-mcherry (phleo), sacA::hbsu-mgfpmut3 (cat), Δsmc::neo*	This study	1A, S1A
BWX811	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo)*	This study	1C, S2D
BWX2090	*yycR(-7˚)::tetO120 (erm), ycgO::P_{ftsW} tetR-mcherry (phleo), sacA::hbsu-mgfpmut3 (cat), smcts02 (spec)*	This study	1D-G
BWX2092	*yycR(-7˚)::tetO120 (erm), ycgO::P_{ftsW} tetR-mcherry (phleo), sacA::hbsu-mgfpmut3 (cat), scpBts02 (spec)*	This study	1H, S1C
BWX1497	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), lacA::P_{xylA} (Ec) sspB (loxP no a.b.), smc-ssrA (loxP-kan-loxP)*	This study	1I, 2B, 4C, S1D, S2D
BWX1771	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), lacA::P_{xylA} (Ec) sspB (loxP no a.b.), smc-ssrA (loxP-kan-loxP), dnaX-yfp (spec)*	This study	1J
BWX2378	*yycR(-7˚)::tetO48 (cat), yuxG(-87˚)::lacO48 (erm), ycgO::P_{ftsW} tetR-cfp (kan) terminators P_{ftsW} lacI-mypet, smcts02 (spec)*	This study	2A
BWX2116	*yuxG(-87˚)::lacO48 (phleo), yhdG(+87˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (kan) terminators P_{ftsW} lacI-mypet, smcts02 (spec)*	This study	2A
BWX2110	*yuxG(-87˚)::lacO48 (phleo), yrvN(-120˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (kan) terminators P_{ftsW} lacI-mypet, smcts02 (spec)*	This study	2A
BWX2551	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), lacA::P_{xylA} (Ec) sspB (loxP no a.b.), smc-ssrA (loxP-kan-loxP), Δsoj132 (loxP-spec-loxP)*	This study	2B, 4C, S2D
BWX1569	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), lacA::P_{xylA} (Ec) sspB (loxP no a.b.), smc-ssrA (loxP-kan-loxP), Δspo0J::spec*	This study	2B, S2D
BWX2112	*yuxG(-87˚)::lacO48 (phleo), yhdG(+87˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (kan) terminators P_{ftsW} lacI-mypet, parCts01 (spec)*	This study	3A
BWX2106	*yuxG(-87˚)::lacO48 (phleo), yrvN(-120˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (kan) terminators P_{ftsW} lacI-mypet, parCts01 (spec)*	This study	3A
BWX2082	*yycR(-7˚)::tetO120 (erm), ycgO::P_{ftsW} tetR-mcherry (phleo), sacA::hbsu-mgfpmut3 (cat), parCts01 (spec)*	This study	3B
BWX2574	*yycR(-7˚)::tetO120 (erm), ycgO::P_{ftsW} tetR-mcherry (phleo), sacA::hbsu-mgfpmut3 (cat), Δsoj132 (loxP-kan-loxP), parCts01 (spec)*	This study	3C
BWX1527	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), lacA::P_{xylA} (Ec) sspB (loxP no a.b.), smc-ssrA (loxP-kan-loxP), dnaB134 (ts)-zhb83::Tn917 (erm)*	This study	4A
BWX2558	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), lacA::P_{xylA} (Ec) sspB (loxP no a.b.), smc-ssrA (loxP-kan-loxP), Δsoj132 (loxP-spec-loxP), dnaB134 (ts) - zhb83::Tn917 (erm)*	This study	4B
BWX1120	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), scpB-ssrA (kan) , amyE::P_{spank}(T-7A) (Ec) sspB (spec)*	This study	S1E
BWX2006	*yycR(-7˚)::tetO120 (erm), ycgO::P_{ftsW} tetR-mcherry (phleo), sacA::hbsu-mgfpmut3 (cat)*	This study	S1F
BWX2492	*yycR(-7˚)::tetO120 (erm), ycgO::P_{ftsW} tetR-mcherry (phleo), sacA::hbsu-mgfpmut3 (cat), smc^{WT} (loxP-spec-loxP)*	This study	S1F
BWX2491	*yycR(-7˚)::tetO120 (erm), ycgO::P_{ftsW} tetR-mcherry (phleo), sacA::hbsu-mgfpmut3 (cat), $scpAB^{WT}$ (loxP-spec-loxP)*	This study	S1F
BWX2493	*yycR(-7˚)::tetO120 (erm), ycgO::P_{ftsW} tetR-mcherry (phleo), sacA::hbsu-mgfpmut3 (cat), $parEC^{WT}$ (loxP-spec-loxP)*	This study	S1F
BWX2114	*yuxG(-87˚)::lacO48 (phleo) yhdG(+87˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (kan) terminators P_{ftsW} lacI-mypet, scpBts02 (spec)*	This study	S2C

BWX2108	*yuxG(-87˚)::lacO48 (phleo), yrvN(-120˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (kan) terminators P_{ftsW} lacI-mypet, scpBts02 (spec)*	This study	S2C
BWX2549	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), Δsoj132 (loxP-spec-loxP)*	This study	S2D
BWX945	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), Δspo0J::spec*	This study	S2D
BWX943	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), Δ(soj spo0J)::spec*	This study	S2D
BWX1571	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), lacA::P_{xylA} (Ec) sspB (loxP no a.b.), smc-ssrA (loxP-kan-loxP), Δ(soj spo0J) (spec)*	This study	S2D
BWX1345	*yycR(-7˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (phleo), scpB-ssrA (kan) , amyE::P_{spank}(T-7A) (Ec) sspB (spec), dnaB134 (ts) - zhb83::Tn917 (erm)*	This study	S3A
BWX1172	*yrvN(-120˚)::lacO48 (phleo), ykoW(+120˚)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (spec) terminators P_{ftsW} lacI-mypet, lacA::P_{xylA} (Ec) sspB (loxP no a.b.), scpB-ssrA (kan), dnaB134 (ts) - zhb83::Tn917 (erm)*	This study	S3B
PY79	*wild-type*	[S1]	
AG1468	*Δspo0J::spec, trpC2, pheA1*	[S2]	
AG1505	*Δ(soj spo0J)::spec, trpC2, pheA1*	[S2]	
BKM1725	*pelB::Psoj-cfp(d)-spo0J(parS*)(cat), dnaX-yfp (spec)*	[S3]	
KPL69	*dnaB134 (ts) - zhb83::Tn917 (erm), trpC2, pheA1*	[S4]	
RB35	*Δsmc::neo, trpC2, pheA1*	[S5]	
SV132	*Δsoj132 (no a.b.), trpC2, pheA1*	[S6]	

Table S2. Plasmids used in this study

Plasmid	description	reference
pWX116S	*ykoW(+120˚)::tetO48 (cat)*	This study
pWX118S	*yrvN(-120˚)::tetO48 (cat)*	This study
pWX151	*yrvN(-120˚)::lacO48 (phleo)*	This study
pWX154	*yhdG(+87˚)::tetO48 (cat)*	This study
pWX157	*yuxG(-87˚)::lacO48 (erm)*	This study
pWX159	*yuxG(-87˚)::lacO48 (phleo)*	This study
pWX178	*yycR(-7˚)::tetO48 (cat)*	This study
pWX361	*ycgO::P_{ftsW} tetR-cfp (spec) terminators P_{ftsW} lacI-mypet*	This study
pWX419	*ycgO::P_{ftsW} tetR-cfp (phleo)*	This study
pWX425	*ycgO::P_{ftsW} tetR-cfp (kan) terminators P_{ftsW} lacI-mypet*	This study
pWX477	*scpB$_{Cter}$-ssrA (kan)*	This study
pWX480	*lacA::P_{xylA} (Ec) sspB loxP-erm-loxP*	This study
pWX510	*ycgO::P_{ftsW} tetR-mcherry (phleo)*	This study
pWX570	*yycR(-7˚)::tetO120 (erm)*	This study

Table S3. Oligonucleotides used in this study

oligos	sequence	use
odr198	gccCTCGAGttttccggcaactgcgtctttaagcgc	BWX2006
odr214	gccGAATTCaaatcaccttaaatccttgacgagc	BWX2006
oWX345	tttGGATCCatGAATTCaacgttcttgccattgctgc	pWX361
oWX346	cgcGTCGACatgactctctagcttgaggcatc	pWX361
oWX347	gcgGAATTCataaccgctatttttctctcatctc	pWX361
oWX348	gcgCTGCAGttactacttataaagttcgtccatgcc	pWX361
oWX421	gcgGAATTCagtcttgtcgcgaaggcgctattg	pWX477
oWX422	cgcGCTAGCttttatatcttcgaaggtttggttaaag	pWX477
oWX426	cgcGGTACCcagggagcactggtcaac	pWX480
oWX427	cgcAGATCTttctgctccctcgctcag	pWX480
oWX438	gaccagggagcactggtcaac	antibiotic cassettes
oWX439	tccttctgctccctcgctcag	antibiotic cassettes
oWX507	cgtgcttgaattttcaattatttccc	BWX2549, BWX2574
oWX508	acccgttgcaaaggctcactgggcgc	BWX2549, BWX2574
oWX509	cgttcttctagcgataaacgtgacgc	*parC* mutant library
oWX525	aggttaaagcagcaggatgtgaaaggc	BWX1497
oWX526	gaatagttctcatcattcgctgcctgaacgaattcttttgtttcttcc	BWX1497
oWX527	ggaagaaacaaaagaattcgttcaggcagcgaatgatgagaactattc	BWX1497
oWX528	ctcatcttttaacctctttcctcgttagaccagggagcactggtcaactac	BWX1497
oWX529	gtagttgaccagtgctccctggtctaacgaggaaagaggttaaaagatgag	BWX1497
oWX530	cggacagccagtgccaaacgcgcccg	BWX1497
oWX570	gtcacgtttatcgctagaagaacg	*parC* mutant library
oWX671	aaaCTCGAGggatctggcggatcaggcatgagtaaaggagaagaacttttcactgg	BWX2006
oWX672	cgcGGATCCAAGCTTttactatttgtatagttcatccatgccatg	BWX2006
oWX762	ctgaaaattgagctgattgatgaacgc	*parC* mutant library
oWX789	gatgaaagtcggcgcccagcctcc	*parC* mutant library
oWX792	ctgagcgagggagcagaaggatcccgctcatatagtatgagcgggttttttatta	*parC* mutant library
oWX793	cggtagttgaccagtgctccctggtccgtttgtttttatcagctttcatgg	*parC* mutant library
oWX821	taaaatccccctatgactcaggggg	*smc* mutant library
oWX823	cagtaacgaggaaagaggttaaaagggatccttctgctccctcgctcag	*smc* mutant library
oWX840	cttgcgccaagaaggacgaggcatcgg	*scpAB* mutant library
oWX841	catctcaccattttcaagtatcactc	*scpAB* mutant library
oWX842	gagtgatacttgaaaatggtgagatg	*scpAB* mutant library
oWX843	ctgagcgagggagcagaaggatccaagcgcgattcattaaacttcta	*scpAB* mutant library
oWX844	gttgaccagtgctccctggtcgcgctttttcatcatcataagatataagg	*scpAB* mutant library
oWX845	ccgattccggaaataagcgttgcc	*scpAB* mutant library
oWX847	cttttcagatacggcagagagctcttc	*smc* mutant library
oWX848	gaagagctctctgccgtatctgaaaag	*smc* mutant library
oWX849	ctgagcgagggagcagaaggatccctttaacctctttcctcgttac	*smc* mutant library
oWX850	gttgaccagtgctccctggtctaacgaggaaagaggttaaaagatgagc	*smc* mutant library
oWX851	cgtcagcctcaagcagcgcaagacgg	*smc* mutant library
oWX894	cgcggcacagacttgatgaaacgtcc	BWX2549, BWX2574
oWX895	ctgagcgagggagcagaaggatccttaaaaatataaaaagctctcctgcttttc	BWX2549, BWX2574
oWX896	gttgaccagtgctccctggtccaaaaggtaatcacttactttttagtgaatat	BWX2549, BWX2574
oWX897	gatttttcccacgatgtcacctactttc	BWX2549, BWX2574

Restriction endonuclease sites are capitalized.

Supplemental Experimental Procedures

Generation of mutant libraries.

The *smc* mutant library was obtained by direct transformation of an isothermal assembly [S7] product that contained four PCR fragments (Figure S1B): 1) a 2.4 kb fragment containing the 5' half of the *smc* gene (amplified from wild-type genomic DNA using Phusion polymerase and oligonucleotides oWX821 and oWX847). 2) the 3' half of the *smc* gene (1.2 kb) generated by error-prone PCR (GeneMorph II Random Mutagenesis Kit, Stratagene) and oligonucleotides oWX848 and oWX849 under conditions in which each amplicon had on average 1 mutation per kb. 3) a spectinomycin resistance cassette (amplified from pWX466 using oligonucleotides oWX823 and oWX438) and 4) a 2.2 kb fragment downstream of the *smc* gene (amplified from wild-type genomic DNA using Phusion polymerase and oligonucleotides oWX850 and oWX851). The transformation was plated on LB agar plates supplemented with spectinomycin (100 μg/ml) at 30˚C. More than 40,000 colonies were pooled, aliquoted, and frozen in LB medium containing 17% glycerol.

The *scpAB* mutant library was generated by the same method using oligonucleotides oWX840 and oWX841 for fragment 1; oWX842 and oWX843 for fragment 2; oWX438 and oWX439 for fragment 3; and oWX844 and oWX845 for fragment 4.

The *parC* mutant library was generated by the same method using primers oWX762 and oWX570 for fragment 1; oWX509 and oWX792 for fragment 2; oWX438 and oWX439 for fragment 3: and oWX793 and oWX789 for fragment 4.

Temperature-sensitive mutant screens.

Frozen aliquots of the mutant libraries were thawed, diluted and plated onto rectangular plates (Omnitray, Thermo Scientific) containing LB agar supplemented with spectinomycin at a density of ~200 colonies per plate. The plates were incubated 16-18 h at 30˚C. A colony-picking robot (BioMatrix, S&P Robotics) picked and arrayed independent transformants onto rectangular LB agar plates. These were incubated

overnight at 30˚C. The re-arrayed colonies were replica-plated onto two LB agar plates. One was placed at 30˚C and the other at 42˚C. Mutants that grew well at 30˚C and did not grow at 42˚C were streaked for single colonies and re-tested. Genomic DNAs from mutants that bred true were used to transform wild-type to confirm linkage and the mutagenized region was then sequenced. ~2300 transformants were screened for each gene. 8 temperature sensitive alleles of *smc,* 13 alleles of *scpAB,* and 21 alleles of *parC* were isolated. Strains harboring wild-type copies of *smc*, *scpB*, or *parC* and a linked spectinomycin resistance gene displayed normal chromosome organization and segregation when shift to 42˚C (Figure S1F). Mutants with growth rates and nucleoid morphologies most similar to wild-type at 30˚C were characterized further. One mutant for each gene (*smcts02*, *scpBts02, parCts01*) was selected for analysis. Two amino acid changes were present in *smcts02* (L903P and E1125D), 3 mutations were found in *scpBts02* coding sequence (L133S, K161I, F193Y).

Immunoblot analysis.

Vegetatively growing cells were harvested at OD_{600} between 0.4 and 0.5. Whole cell lysates were prepared as described [S8]. Samples were heated for 5 min at 80˚C prior to loading. Equivalent loading was based on OD_{600} at the time of harvest. Proteins were separated by SDS-PAGE on 10% (for SMC and SigA) or 12.5% (for ScpB) polyacrylamide gels, electroblotted onto Immobilon-P membranes (Millipore) and blocked in 5% nonfat milk in phosphate-buffered saline (PBS) containing 0.5% Tween-20. The blocked membranes were probed with anti-SMC (1:5,000) [S9], anti-ScpB (1:20,000) or anti-SigA (1:10,000) [S10], diluted into 3% BSA in PBS-0.05% Tween-20. Primary antibodies were detected using horseradish peroxidase-conjugated goat anti-rabbit IgG (BioRad) and the Super Signal chemiluminscence reagent as described by the manufacturer (Pierce).

Image analysis.

Image analysis for Figure S2D was performed using the MATLAB-based program, MicrobeTracker [S11]. The nucleoid was stained with the DNA fluorescent dye (DAPI). The background fluorescence intensity was determined by averaging the fluorescence

intensity in cell-free regions of the image and subtracted from the image in MetaMorph. After background subtraction, the images were then inverted in MicrobeTracker and the outline of the nucleoid was determined using built-in algorithms in MicrobeTracker. MicrobeTracker generated a co-ordinate system for each nucleoid, called a mesh, in which each point was described by two co-ordinates: the distance to a cell pole that was randomly selected and the distance to the mid-line along the cell length. The mesh was used to calculate nucleoid parameters such as the length, width, and area.

Plasmid construction.

pWX116s [*ykoW (+120˚)::tetO48 (cat)*] was generated by inserting *tetO48* (liberated with *Nhe*I and *Hind*III from pLAU29, I. Lau and D. J. Sherratt, unpublished) into pWX102 between *Nhe*I and *Hind*III. pWX102 [*ykoW::cat*] is an ectopic integration vector for double crossover insertions into the *ykoW* locus (X.W. and D.Z.R., unpublished).

pWX118s [*yrvN (-120˚)::tetO48 (cat)*] was generated by inserting *tetO48* (liberated with *Nhe*I and *Hind*III from pLAU29, I. Lau and D. J. Sherratt, unpublished) into pWX109 between *Nhe*I and *Hind*III. pWX109 [*yrvN::cat*] is an ectopic integration vector for double crossover insertions into the *yrvN* locus (X.W. and D.Z.R., unpublished).

pWX151 [*yrvN (-120˚)::lacO48 (phleo)*] was generated by inserting *lacO48* (liberated with *Nhe*I and *Hind*III from pLAU23, I. Lau and D. J. Sherratt, unpublished) into pWX112 between *Nhe*I and *Hind*III. pWX112 [*yrvN::phleo*] is an ectopic integration vector for double crossover insertions into the *yrvN* locus (X.W. and D.Z.R., unpublished).

pWX154 [*yhdG(+87˚)::tetO48 (cat)*] was generated by inserting *tetO48* (liberated with *Nhe*I and *Hind*III from pLAU29) into pBB275 between *Nhe*I and *Hind*III. pBB275 [*yhdG::cat*] is an ectopic integration vector for double crossover insertions into the *yhdG* locus (B. Burton and D.Z.R., unpublished).

pWX157 [*yuxG (-87˚)::lacO48 (erm)*] was generated by inserting *lacO48* (liberated with *Nhe*I and *Hind*III from pLAU23) into pWX146 between *Nhe*I and *Hind*III. pWX146 [*yuxG::erm*] is an ectopic integration vector for double crossover insertions into the *yuxG* locus (X.W. and D.Z.R., unpublished).

pWX159 [*yuxG (-87˚)::lacO48 (phleo)*] was generated by inserting *lacO48* (liberated with *Nhe*I and *Hind*III from pLAU23) into pWX147 between *Nhe*I and *Hind*III. pWX147 [*yuxG::phleo*] is an ectopic integration vector for double crossover insertions into the *yuxG* locus (X.W. and D.Z.R., unpublished).

pWX178 [*yycR (-7˚)::tetO48 (cat)*] was generated by inserting *tetO48* (liberated with *Nhe*I and *Hind*III from pLAU29) into pNS037 between *Nhe*I and *Hind*III. pNS037 [*yycR::cat*] is an ectopic integration vector for double crossover insertions into the *yycR* locus (N. Sullivan and D.Z.R., unpublished).

pWX361 [*ycgO::*P_{ftsW}*-tetR-cfp (spec)* terminators P_{ftsW}*-lacI-mypet*] was generated in a 3-way ligation to insert transcription terminators (amplified from pDR111 using oWX345 and oWX346 and digested with *Sal*I and *Eco*RI) and P_{ftsW}*-tetR-cfp* (amplified from pWX193 using oWX347 and oWX348 and digested with *Eco*RI and *Pst*I) into pWX309 between *Sal*I and *Pst*I. pDR111 contains *amyE::*$P_{hyperspank}$ *(spec)* with transcription terminators (D.Z.R. unpublished). pWX309 contains *ycgO::*P_{ftsW}*-lacI-mypet (spec)* (X.W and D.Z.R., unpublished).

pWX419 [*ycgO::*P_{ftsW}*-tetR-cfp (phleo)*] was generated by cloning the phleomycin resistance gene (liberated from pNC015 using *Bam*HI and *Sal*I] into pWX193 [*ycgO::*P_{ftsW}*-tetR-cfp (spec)*] between *Bam*HI and *Sal*I to replace the spectinomycin resistance gene. pNC015 contains *sacA::phleo* (N. Campo and D.Z.R., unpublished).

pWX425 [*ycgO::*P_{ftsW}*-tetR-cfp (kan) terminators* P_{ftsW}*-lacI-mypet*] was constructed by cloning kanamycin resistance gene (liberated from pBB283 using *Bam*HI and *Sal*I] into

pWX361 [*ycgO::*P_{ftsW}*-tetR-cfp (spec)* terminators P_{ftsW}*-lacI-mypet*] between *Bam*HI and *Sal*I to replace the spectinomycin resistance gene. pBB283 [*yhdG::kan*] is an ectopic integration vector with a kanamycin resistance gene for double crossover insertions into the *yhdG* locus (B. Burton and D.Z.R., unpublished).

pWX477 [$scpB_{Cter}$*-ssrA (kan)*] was generated by cloning the C-terminus of *scpB* gene (without the stop codon, amplified using primers oWX421 and oWX422 and digested with *Eco*RI and *Nhe*I) into pWX475 between *Eco*RI and *Nhe*I. pWX475 [*ssrA (kan)*] (X.W and D.Z.R., unpublished) is a pUC19 derivative containing a kanamycin resistance gene and an *E. coli ssrA* tag plus a 4 amino acid linker AANDENYSENYALGG [S12].

pWX480 [*lacA::*P_{xylA} *(Ec) sspB loxP-erm-loxP*] was generated by cloning a *loxP-erm-loxP* cassette (amplified from pWX467 using primers oWX426 and oWX427 and digested with *Kpn*I and *Bgl*II) into pKG1267 [*lacA::PxylA (Ec) sspB (tet)*] [S12] between *Kpn*I and *Bgl*II to replace the tetracycline resistance gene.

pWX510 [*ycgO::*P_{ftsW}*-tetR-mcherry (phleo)*] was generated by cloning phleomycin resistance gene (liberated from pNC015 using *Bam*HI and *Sal*I] into pWX192 between *Bam*HI and *Sal*I to replace the spectinomycin resistance gene. pNC015 contains *sacA::phleo* (N. Campo and D.Z.R., unpublished). pWX192 contains *ycgO::*P_{ftsW}*-tetR-mcherry (spec)* (X.W. and D.Z.R., unpublished).

pWX570 [*yycR (-7˚)::tetO120 (erm)*] was generated by inserting *tetO120* (liberated with *Nhe*I and *Hind*III from pLAU39, I. Lau and D. J. Sherratt, unpublished) into pNS043 between *Nhe*I and *Hind*III. pNS043 [*yycR::erm*] is an ectopic integration vector for double crossover insertions into the *yycR* locus (N. Sullivan and D.Z.R., unpublished).

Strain construction.

The in-frame deletion of *parA* called *Δsoj132* linked to *loxP-spec-loxP* in BWX2549 and BWX2551 was generated as follows. BWX2538 *(Δsoj132 loxP-spec-loxP* in the JH642

background) was obtained by direct transformation of an isothermal assembly product [S7] into SV132 [S6] to link the unmarked in-frame deletion *Δsoj132* allele to a spectinomycin resistance gene inserted between *noc* and *yyaB* (0.7 kb upstream of *Δsoj132*). The isothermal assembly reaction contained three PCR fragments: 1) *noc* and its upstream region (amplified from wild-type genomic DNA using primers oWX894 and oWX895); 2) *loxP-spec-loxP* cassette (amplified from pWX466 using universal primers oWX438 and oWX439) and 3) a region downstream of *noc* and upstream of *Δsoj132* containing the *yyaB* gene (amplified from wild-type genomic DNA using primers oWX896 and oWX897). pWX466 contains a *loxP-spec-loxP* cassette (X.W. and D.Z.R., unpublished). *Δsoj132 loxP-spec-loxP* was then backcrossed to PY79 twice. The resulting construct was sequenced across the *soj-spo0J* region using primers oWX507 and oWX508.

Δsoj132 (loxP-kan-loxP) in BWX2574 was constructed using the same method described above except that a *loxP-kan-loxP* cassette was amplified from pWX470 (X.W. and D.Z.R., unpublished) using primers oWX438 and oWX439.

sacA::hbsU-mgfp mut3 (cat) was constructed by direct transformation of a 3-way ligation into *B. subtilis*, in which the *hbsU* gene with its native promoter (amplified using primers odr198 and odr214 and digested with *Eco*RI and *Xho*I) was fused to *mgfp mut3* (amplified from pDHL580 [S13]) using primers oWX671 and oWX672 and digested with *Xho*I and *Bam*HI) and inserted into pKM064 between *Eco*RI and *Bam*HI. pKM064 [*sacA::cat*] is an ectopic integration vector for double crossover insertions into the *sacA* locus (K. Marquis and D.Z.R., unpublished).

smc-ssrA (kan) from BWX1497 was obtained by direct transformation of an isothermal assembly product [S7] into *B. subtilis*. The isothermal assembly reaction contained three PCR fragments: 1) the C-terminus of *smc* (without the stop codon, amplified from wild-type genomic DNA using primers oWX525 and oWX526); 2) *ssrA loxP-kan-loxP* (amplified from pWX499 using primers oWX527 and oWX528) and 3) downstream of

smc (amplified from wild-type genomic DNA using primers oWX529 and oWX530). pWX499 (X.W. and D.Z.R., unpublished) contains an *E. coli ssrA* tag plus 4 amino acid linker AANDENYSENYALGG [S12] and a *loxP-kan-loxP* cassette.

lacA::P_{xylA} *(Ec) sspB* without an antibiotic marker *(no a.b.)* in BWX1497 was obtained by transforming pWX480 [*lacA::*P_{xylA} *(Ec) sspB loxP-erm-loxP*] into *B. subtilis* and subsequently looping out *loxP-erm-loxP* cassette using a *cre*-expressing plasmid pDR244, which contains a spectinomycin resistance gene and a temperature-sensitive replication origin (D.Z.R, unpublished).

Supplemental References

S1. Youngman, P.J., Perkins, J.B., and Losick, R. (1983). Genetic transposition and insertional mutagenesis in Bacillus subtilis with Streptococcus faecalis transposon Tn917. Proc Natl Acad Sci U S A *80*, 2305-2309.

S2. Ireton, K., Gunther, N.W., and Grossman, A.D. (1994). spo0J is required for normal chromosome segregation as well as the initiation of sporulation in Bacillus subtilis. J Bacteriol *176*, 5320-5329.

S3. Sullivan, N.L., Marquis, K.A., and Rudner, D.Z. (2009). Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation. Cell *137*, 697-707.

S4. Rokop, M.E., Auchtung, J.M., and Grossman, A.D. (2004). Control of DNA replication initiation by recruitment of an essential initiation protein to the membrane of Bacillus subtilis. Mol Microbiol *52*, 1757-1767.

S5. Britton, R.A., Lin, D.C., and Grossman, A.D. (1998). Characterization of a prokaryotic SMC protein involved in chromosome partitioning. Genes Dev *12*, 1254-1259.

S6. Lee, P.S., and Grossman, A.D. (2006). The chromosome partitioning proteins Soj (ParA) and Spo0J (ParB) contribute to accurate chromosome partitioning, separation of replicated sister origins, and regulation of replication initiation in Bacillus subtilis. Mol Microbiol *60*, 853-869.

S7. Gibson, D.G., Young, L., Chuang, R.Y., Venter, J.C., Hutchison, C.A., 3rd, and Smith, H.O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods *6*, 343-345.

S8. Doan, T., and Rudner, D.Z. (2007). Perturbations to engulfment trigger a degradative response that prevents cell-cell signalling during sporulation in Bacillus subtilis. Mol Microbiol *64*, 500-511.

S9. Lin, D.C., Levin, P.A., and Grossman, A.D. (1997). Bipolar localization of a chromosome partition protein in Bacillus subtilis. Proc Natl Acad Sci U S A *94*, 4721-4726.

S10. Fujita, M. (2000). Temporal and selective association of multiple sigma factors with RNA polymerase during sporulation in Bacillus subtilis. Genes Cells *5*, 79-88.

S11. Sliusarenko, O., Heinritz, J., Emonet, T., and Jacobs-Wagner, C. (2011). High-throughput, subpixel precision analysis of bacterial morphogenesis and intracellular spatio-temporal dynamics. Mol Microbiol *80*, 612-627.

S12. Griffith, K.L., and Grossman, A.D. (2008). Inducible protein degradation in Bacillus subtilis using heterologous peptide tags and adaptor proteins to target substrates to the protease ClpXP. Mol Microbiol *70*, 1012-1025.

S13. Landgraf, D., Okumus, B., Chien, P., Baker, T.A., and Paulsson, J. (2012). Segregation of molecules at cell division reveals native protein localization. Nat Methods *9*, 480-482.