

1 The ParB homologs, Spo0J and Noc, together prevent premature midcell Z ring
2 assembly when the early stages of replication are blocked in *Bacillus subtilis*.

SUPPLEMENTARY MATERIALS

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Running title: ParB homologs block premature midcell Z ring assembly

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1 **Table S1: *Bacillus subtilis* strain list used in this study**

Strain	Genotype	Source
SU5	168 <i>trpC2</i>	E. Nester
SU492	168 <i>trpC2 amyE::(spc P_{xyt}-ftsZ-yfp)</i>	Lab stock
SU504	168 <i>trpC2 amyE::Pspachy-ftsZ (cat)</i>	Rodrigues & Harry, 2012
SU533	168 <i>trpC2 Δnoc::cat</i>	Lab stock
SU629	168 <i>trpC2 Δnoc::tet amyE::(spc P_{xyt}-noc-yfp)</i>	Lab stock
SU656	168 <i>trpC2 Δnoc::tet</i>	Lab stock
SU661	168 <i>trpC2+ dna-1</i>	Rodrigues & Harry, 2012
SU682	168 <i>trpC2 amyE::(spc Pxyl-ftsZ-yfp) ΔminCD::cat</i>	Lab stock
SU746	168 <i>trpC2+ dna-1 amyE::(spc P_{xyt}-ftsZ-yfp)</i>	This work
SU747	<i>trpC2 Δsoj::neo</i>	Scholefield <i>et al.</i> , 2011
SU748	<i>trpC2 Δspo0J::neo, amyE::spo0J (cat)</i>	Gruber & Errington, 2009
SU765	168 <i>trpC2 Δsoj-spo0J::tet</i>	This work
SU766	168 <i>trpC2+ dna-1 Δsoj-spo0J::tet</i>	This work
SU767	168 <i>trpC2 amyE::(spc P_{xyt}-ftsZ-yfp) Δsoj-spo0J::tet</i>	This work
SU768	168 <i>trpC2+ dna-1 amyE::(spc P_{xyt}-ftsZ-yfp) Δsoj-spo0J::tet</i>	This work
SU769	168 <i>trpC2 Δspo0J::kan</i>	This work
SU770	168 <i>trpC2+ dna-1 Δspo0J::kan</i>	This work
SU771	168 <i>trpC2 Δsoj::kan</i>	This work
SU772	168 <i>trpC2+ dna-1 Δsoj::kan</i>	This work
SU802	168 <i>trpC2+ dna-1 Δnoc::cat</i>	This work
SU803	168 <i>trpC2 Δnoc::cat Δsoj-spo0J::tet</i>	This work
SU804	168 <i>trpC2+ dna-1 Δnoc::cat Δsoj-spo0J::tet</i>	This work
SU823	168 <i>trpC2 Δsoj-spo0J::tet hutM(345°)::lacO(cat), thrC (283°)::lacI-cfp(erm) amyE::(spc P_{xyt}-ftsZ-yfp)</i>	This work
SU824	168 <i>trpC2+ dna-1 Δsoj-spo0J::tet hutM(345°)::lacO(cat), thrC (283°)::lacI-cfp(erm) amyE::(spc P_{xyt}-ftsZ-yfp)</i>	This work
SU827	168 <i>trpC2 Δnoc::cat Δsoj::kan</i>	This work
SU828	168 <i>trpC2+ dna-1 Δnoc::cat Δsoj::kan</i>	This work
SU829	168 <i>trpC2 Δnoc::cat Δspo0J::kan</i>	This work
SU830	168 <i>trpC2+ dna-1 Δnoc::cat Δspo0J::kan</i>	This work
SU831	168 <i>trpC2 Δnoc::cat amyE::(spc P_{xyt}-noc-yfp)</i>	This work
SU832	168 <i>trpC2+ dna-1 Δnoc::cat amyE::(spc P_{xyt}-noc-yfp)</i>	This work
SU833	168 <i>trpC2 Δnoc::cat Δspo0J::kan amyE::(spc P_{xyt}-noc-yfp)</i>	This work
SU834	168 <i>trpC2+ dna-1 Δnoc::cat Δspo0J::kan amyE::(spc P_{xyt}-noc-yfp)</i>	This work
SU835	168 <i>trpC2 Δnoc::cat Δsoj-spo0J::tet amyE::(spc P_{xyt}-ftsZ-yfp)</i>	This work
SU836	168 <i>trpC2+ dna-1 Δnoc::cat Δsoj-spo0J::tet amyE::(spc P_{xyt}-ftsZ-yfp)</i>	This work
SU849	<i>smc-ssrA loxP (kan), lacA::P_{xyt}A Ec sspB loxP (erm)</i>	Wang <i>et al.</i> 2014a
SU850	168 <i>trpC2 smc-ssrA loxP (kan), lacA::PxylA Ec sspB loxP (erm)</i>	This work
SU851	168 <i>trpC2+ dna-1 smc-ssrA loxP (kan), lacA::PxylA Ec sspB loxP (erm)</i>	This work
SU874	168 <i>trpC2 Δnoc::cat, smc-ssrA loxP (kan), lacA::PxylA Ec sspB loxP (erm)</i>	This work
SU875	168 <i>trpC2+ dna-1 Δnoc::cat, smc-ssrA loxP (kan), lacA::PxylA Ec sspB loxP (erm)</i>	This work
SU876	168 <i>trpC2 Δsoj-spo0J::tet, smc-ssrA loxP (kan), lacA::PxylA Ec sspB loxP (erm)</i>	This work
SU877	168 <i>trpC2+ dna-1 Δsoj-spo0J::tet, smc-ssrA loxP (kan), lacA::PxylA Ec sspB loxP (erm)</i>	This work
SU878	168 <i>trpC2 Δnoc::cat, Δsoj-spo0J::tet, smc-ssrA loxP (kan), lacA::PxylA Ec sspB loxP (erm)</i>	This work
SU879	168 <i>trpC2+ dna-1 Δnoc::cat, Δsoj-spo0J::tet, smc-ssrA loxP (kan), lacA::PxylA Ec sspB loxP (erm)</i>	This work
SU887	168 <i>trpC2 amyE::Pspachy-ftsZ (cat) Δspo0J::kan</i>	This work
SU888	168 <i>trpC2 amyE::Pspachy-ftsZ (cat) Δnoc::tet</i>	This work
SU889	168 <i>trpC2 amyE::Pspachy-ftsZ (cat) Δspo0J::kan Δnoc::tet</i>	This work
SU890	168 <i>trpC2 amyE::(spc Pxyl-ftsZ-yfp) Δspo0J::kan</i>	This work
SU891	168 <i>trpC2 amyE::(spc Pxyl-ftsZ-yfp) Δnoc::cat</i>	This work
SU892	168 <i>trpC2 amyE::(spc Pxyl-ftsZ-yfp) Δspo0J::kan Δnoc::cat</i>	This work
SU893	168 <i>trpC2 amyE::(spc Pxyl-ftsZ-yfp) Δspo0J::kan ΔminCD::cat</i>	This work
SU894	168 <i>yuxG(-87°)::lacO48 (phleo), yhdG(+87°)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (spec) terminators P_{ftsW} lacI-mypet</i>	Wang <i>et al.</i> 2014a
SU895	168 <i>trpC2+ dna-1 yuxG(-87°)::lacO48 (phleo), yhdG(+87°)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (spec) terminators P_{ftsW} lacI-mypet</i>	This work
SU897	168 <i>trpC2+ dna-1 soj-spo0J::tet, yuxG(-87°)::lacO48 (phleo), yhdG(+87°)::tetO48 (cat), ycgO::P_{ftsW} tetR-cfp (spec) terminators P_{ftsW} lacI-mypet</i>	This work
SU899	168 <i>trpC2+ dna-1 Δspo0J::kan, ycgO::spo0J (erm)</i>	This work
SU901	168 <i>trpC2+ dna-1 Δnoc::cat Δspo0J::kan, ycgO::spo0J (erm)</i>	This work
SU903	168 <i>trpC2+ dna-1 Δsoj-spo0J::tet, ycgO::spo0J (erm)</i>	This work
SU905	168 <i>trpC2+ dna-1 Δnoc::cat Δsoj-spo0J::tet, ycgO::spo0J (erm)</i>	This work

1 **Table S2: Plasmids and oligonucleotides used in this study**

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Plasmid	Description	Source
pKM084	<i>ycgO::cat</i>	D. Rudner
plH003	<i>ycgO::Psoj-optRBS-spo0J (erm)</i>	This work
Oligonucleotides	Sequence ^a	Source
olH004	cgc G AATTCAAACCATTCTCACCATCCTG	This work
olH005	cgc G CCTAGCCTTCACATGAACATGTACTATC	This work
olH008	cgc G CTAGCACATAAGGAGGAACTACTATGGCtaaAGGCCTTGGAAAAGGGAT	This work
olH0010	gcg GG ATCCTTATGATTCTCGTTAGACA	This work

3 ^a Bold letters indicate the recognition sequence for restriction enzymes

4 **Plasmid construction**

5 **plH003** [*ycgO::Psoj-optRBS-spo0J (erm)*] was generated by a two-way ligation of a
 6 EcoRI-NheI PCR product containing the promoter region for the *soj-spo0J* operon
 7 (amplified with oligonucleotide primers olH004 and olH005), and a NheI-BamHI PCR
 8 product containing the *spo0J* gene (amplified with oligonucleotide primers olH008 and
 9 olH0010) into pKM084 cut with EcoRI-BamHI. Both PCR products were amplified from
 10 SU5 genomic DNA as the template. pKM084 is an ectopic integration vector for
 11 recombination into the *ycgO* locus (David Rudner).

12
 13
 14 **SUPPLEMENTARY FIGURE LEGENDS**

15 **Figure S1: Z ring positioning when initiation of DNA replication is blocked in**
 16 **vegetatively grown fixed cells.** (A-D) Vegetative cells were grown in PAB to the mid-
 17 exponential phase at the permissive temperature (34°C), then shifted to the non-
 18 permissive temperature (48°C) for a further 60 min: (A) wild-type; SU5, (B) *dna-1*;
 19 SU661, (C) Δ *soj-spo0J*; SU765 and (D) *dna-1* Δ *soj-spo0J*; SU766. Percentages
 20 shown are the frequencies of Z rings occurring at midcell in the range of 0.45 – 0.5 on
 21 the x-axis.

22 **Figure S2: Z ring positioning when initiation of DNA replication is blocked in**
 23 **vegetatively grown fixed cells of individual *soj* or *spo0J* mutants.** (A-F) Vegetative cells were grown in PAB to the mid-exponential phase at the permissive
 24 temperature (34°C), then shifted to the non-permissive temperature (48°C) for a
 25 further 60 min: (i) wild-type; SU5, (ii) *dna-1*; SU661, (iii) Δ *soj*; SU769, (iv) *dna-1* Δ *soj*;
 26 SU770, (v) Δ *spo0J*; SU771 and, (vi) *dna-1* Δ *spo0J*; SU772. Percentages shown are
 27 the frequencies of Z rings occurring at midcell in the range of 0.45 – 0.5 on the x-axis.

1 **Figure S3: Z ring positioning in outgrown spore cells at the permissive**
2 **temperature.** Z ring positioning was examined in outgrown spores at the permissive
3 temperature (34°C) for 120 min in strains: (A) wild-type; SU492, (B) *dna-1*; SU746, (C)
4 Δ *soj-spo0J*; SU767 and (D) *dna-1* Δ *soj-spo0J*; SU768. Percentages shown are the
5 frequencies of Z rings occurring at midcell in the range of 0.45 – 0.5 on the x-axis.

6 **Figure S4: Whole field of view of Z rings overlayed with DAPI and phase contrast**
7 **when initiation of DNA replication is blocked in live outgrown spores.**
8 Microscopic images of an overlay of phase contrast, DAPI (red) and FtsZ-YFP (green)
9 in (A) *dna-1*; SU746, and (B) *dna-1* Δ *soj-spo0J*; SU768. Right side images show closer
10 details of selected cells with different nucleoid morphologies and varying Z ring
11 positions. Scale bar represents 2 μ m.

12 **Figure S5: Flow cytometry profiles of Δ *soj-spo0J* strains when initiation of DNA**
13 **replication is blocked via *dna-1* mutant or addition of HPUrA.** Flow cytometry
14 profiles in controls where initiation of DNA replication is progressing normally (wild-
15 type; SU492 and Δ *soj-spo0J*; SU767) or blocked (controls: *dna-1*; SU746 and wild-
16 type +HPUrA; and test strains: *dna-1* Δ *soj-spo0J*; SU768 and Δ *soj-spo0J* +HPUrA. n
17 = 10,000.

18 **Figure S6: Noc localisation in the absence of *spo0J* when initiation of DNA**
19 **replication is blocked.** Strains (A) wild type; SU831, (B) *dna-1*; SU832, (C) Δ *spo0J*
20 Δ *noc*; SU833, and (D) *dna-1* Δ *spo0J* Δ *noc*; SU834, possessing Noc tagged with a
21 yellow fluorescent protein (*noc-yfp*; falsely coloured cyan) were grown vegetatively
22 supplemented with 0.3% xylose. DNA stained with DAPI (falsely coloured red). Scale
23 bar = 2 μ m.

24 **Figure S7: Z ring positioning when initiation of DNA replication is blocked**
25 **during spore outgrowth.** Z ring positioning was examined in two conditions: in the
26 temperature-sensitive *dna-1* background (A) and with the addition of the DNA
27 polymerase III inhibitor HPUrA (B), in spore outgrown spore cells. A: (i) wild-type;
28 SU492, (ii) *dna-1*; SU746, (iii) Δ *noc*; SU831, (iv) *dna-1* Δ *noc*; SU832, (v) Δ *soj-spo0J*
29 Δ *noc*; SU835, and (vi) *dna-1* Δ *soj-spo0J* Δ *noc*; SU836; B: (i) wild-type +HPUrA and
30 (ii) Δ *soj-spo0J* Δ *noc* +HPUrA.

31 **Figure S8: Z ring positioning in the individual mutants, *soj* or *spo0J*, in the *dna-***
32 **1 Δ *noc* mutant at the non-permissive temperature.** Scatter plots showing Z ring

1 positioning and average cell length of vegetatively grown, fixed cells of (A) wild-type;
2 SU5, (B) *dna-1*; SU661, (C) Δ *soj* Δ *noc*; SU829, (D) *dna-1* Δ *soj* Δ *noc*; SU830, (E)
3 Δ *spo0J* Δ *noc*; SU827, and (F) *dna-1* Δ *spo0J* Δ *noc*; SU828.

4 **Fig S9. Complementation of spo0J in the temperature sensitive strains.** (A) Z ring
5 positioning, and (B) nucleoid morphologies in strains (i) *dna-1 spo0J ycgo::spo0J*
6 (SU899), (ii) *dna-1 spo0J noc ycgo::spo0J* (SU901), (iii) *dna-1 soj-spo0J ycgo::spo0J*
7 (SU903), and (iv) *dna-1 soj-spo0J noc ycgo::spo0J* (SU905), grown vegetatively at the
8 non-permissive temperature and examined via IFM and ethanol fixation, respectively.
9 n = 200.

10 **Figure S10: Z ring positioning when initiation of DNA replication is blocked**

11 during spore outgrowth in the *dna-1* temperature-sensitive SMC-depleted

12 mutant. Scatter plots showing Z ring positioning and average cell length of outgrown

13 spores grown in the presence of xylose (1% v/v) to induce SMC degradation in

14 replicating cells (left column) and non-replicating cells (*dna-1* mutant; right column). Z

15 ring positioning was examined in strains (A) wild-type; SU850, (B) *dna-1*; SU851, (C)

16 Δ *noc*; SU874, (D) *dna-1* Δ *noc*; SU875, (E) Δ *soj-spo0J*; SU876, (F) *dna-1* Δ *soj-spo0J*;

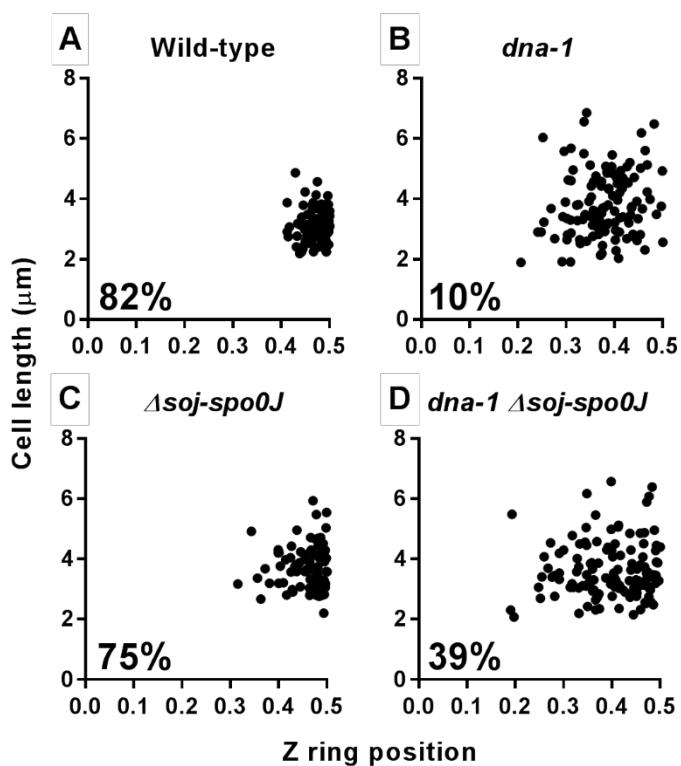
17 SU877, (G) Δ *noc* Δ *soj-spo0J*; SU878, and (H) *dna-1* Δ *noc* Δ *soj-spo0J*; SU879.

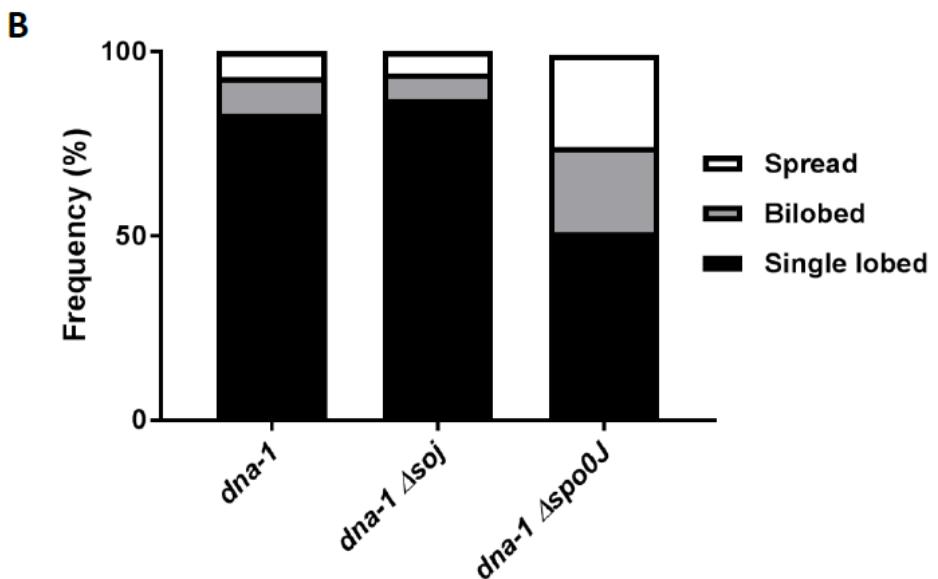
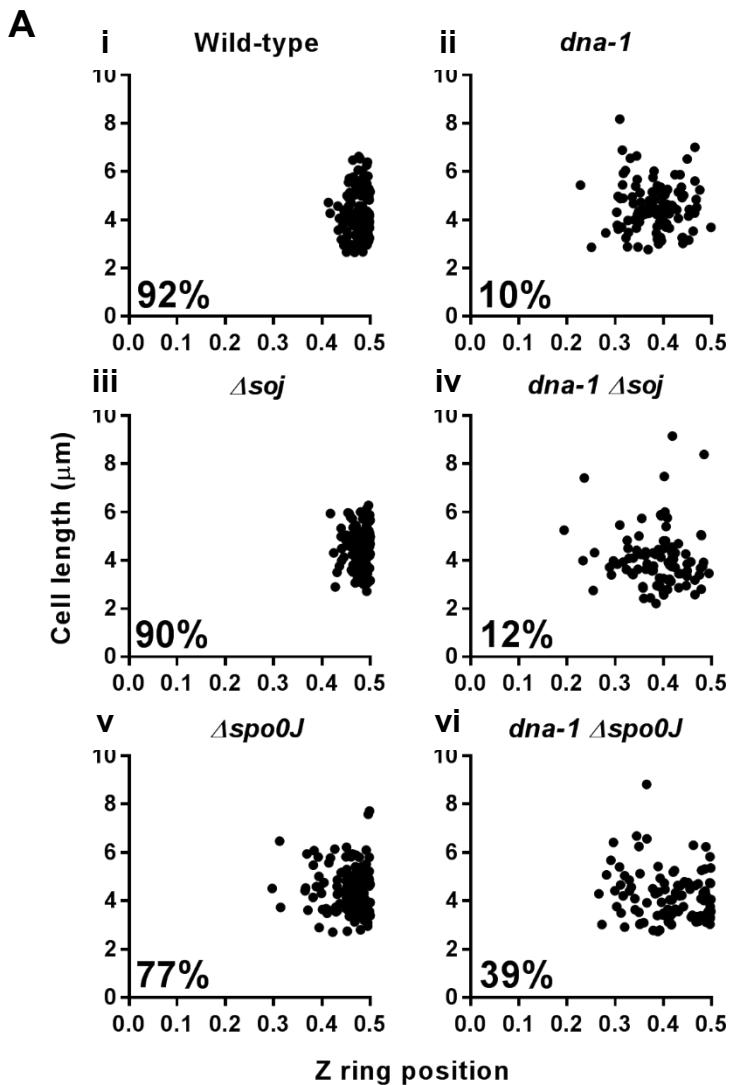
18 **Figure S11: Cell division is not inhibited in a *minCD* *spo0J* double mutant.** Phase

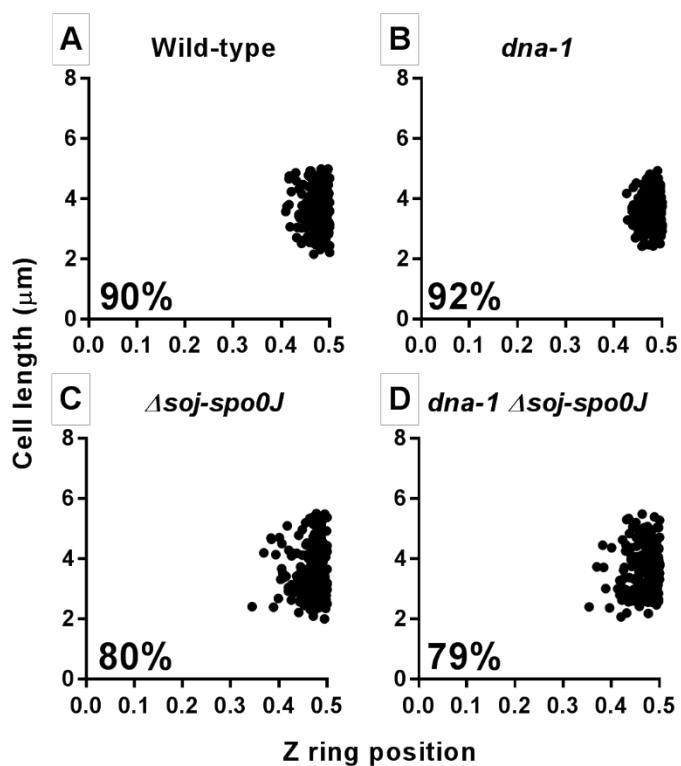
19 contrast images of mid-exponentially growing cells of strains (A) wild-type; SU492, (B)

20 Δ *spo0J*; SU890, (C) Δ *minCD*; SU682 and (D) Δ *spo0J* Δ *minCD*; SU893 grown at 30°C

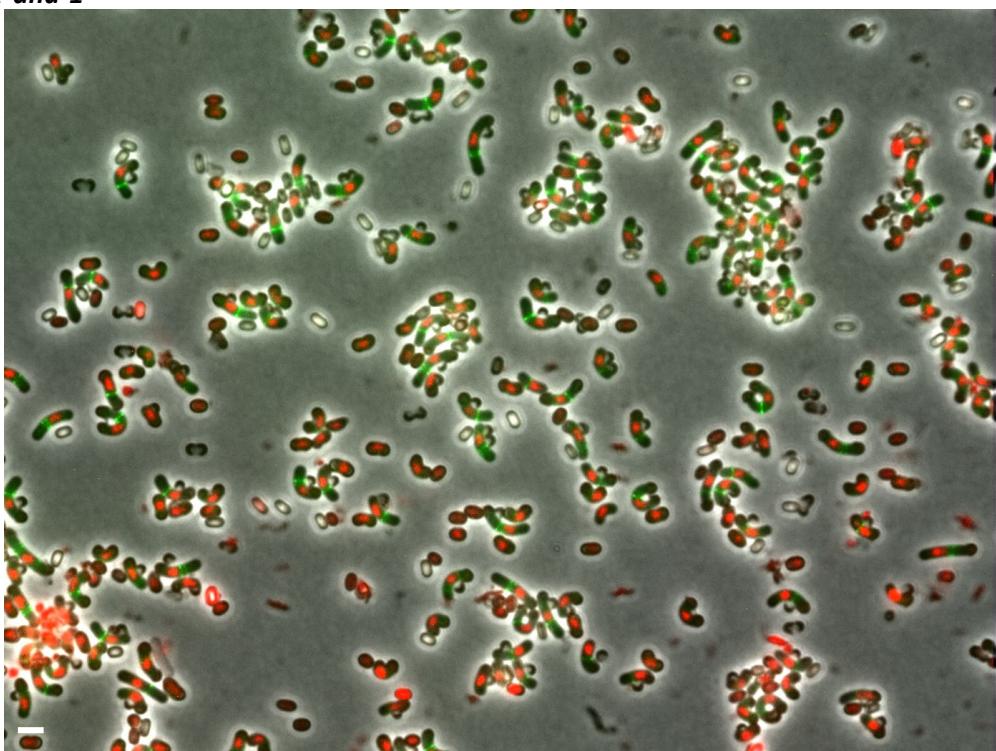
21 and 37°C. Scale bar represents 2 μ m.



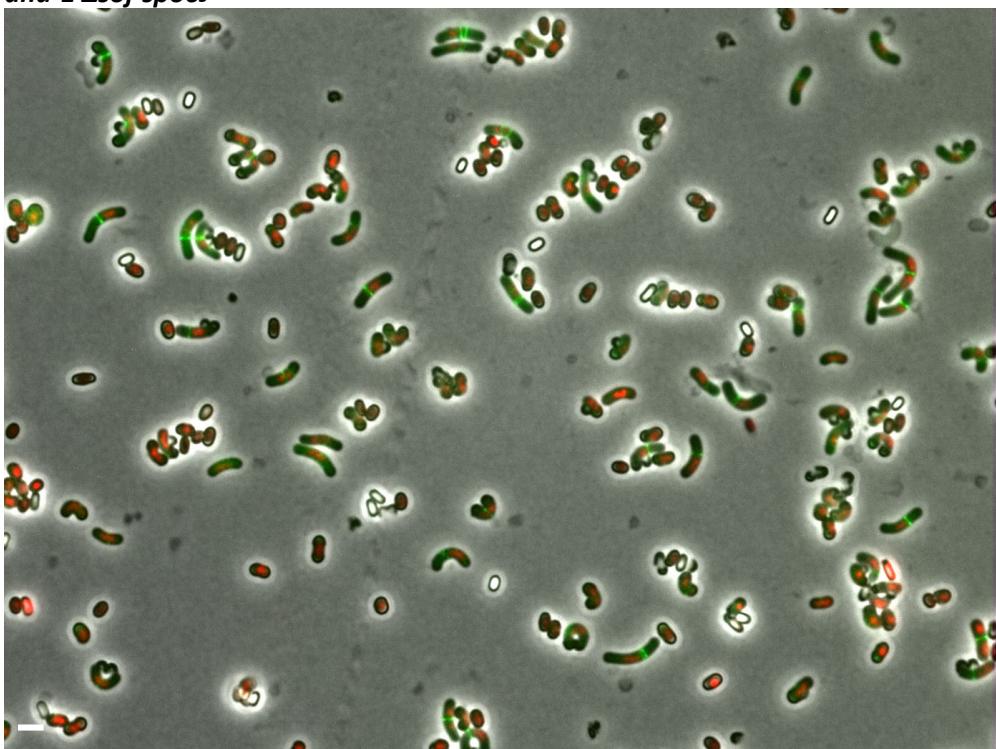


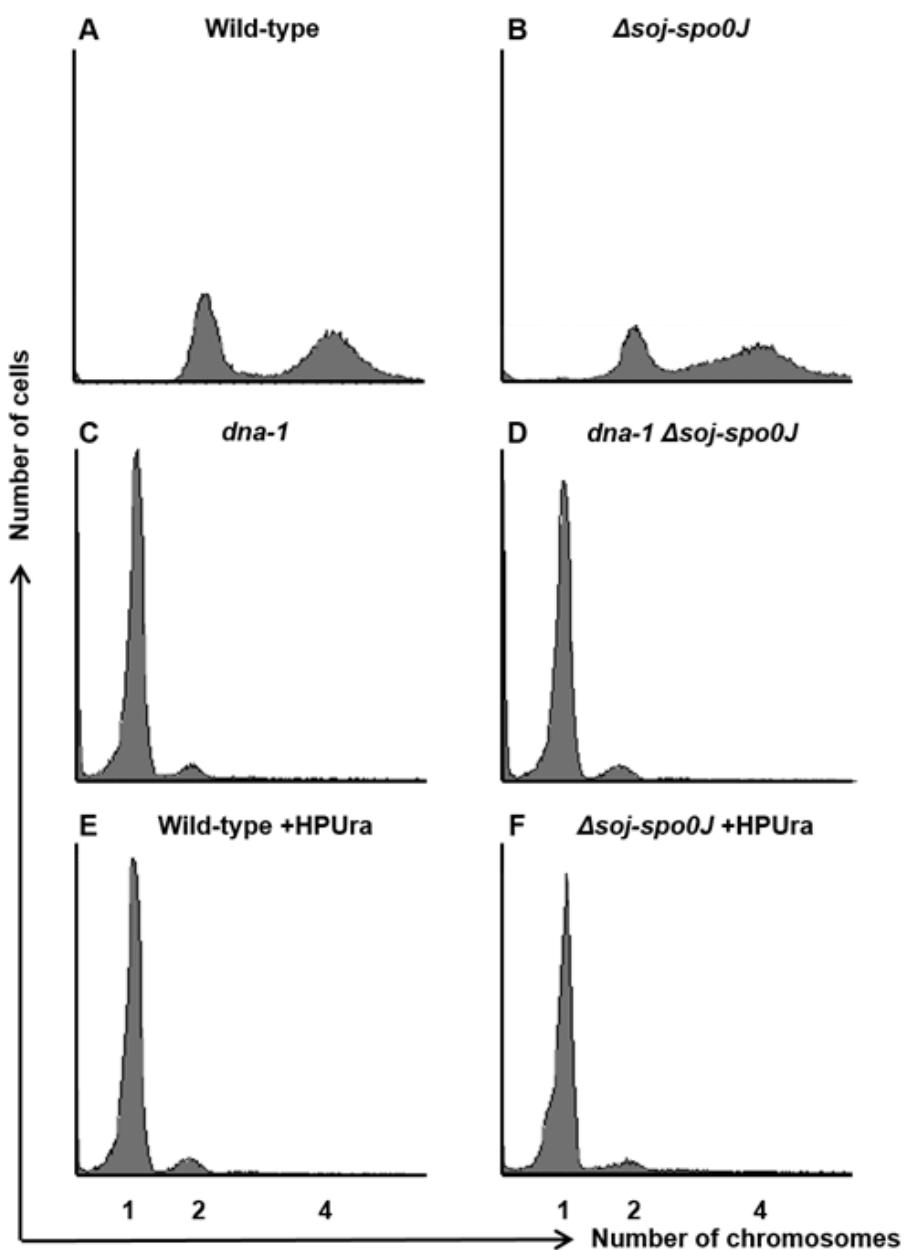


A *dna-1*

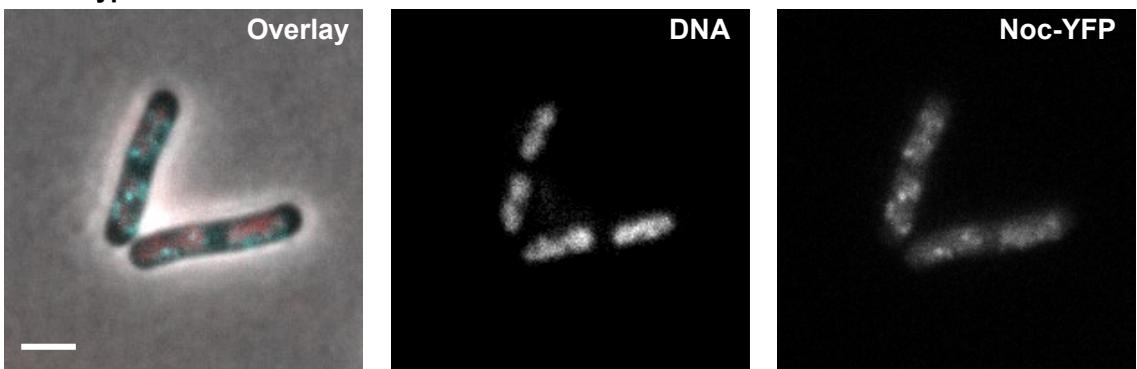


B *dna-1 Δsoj-spo0J*

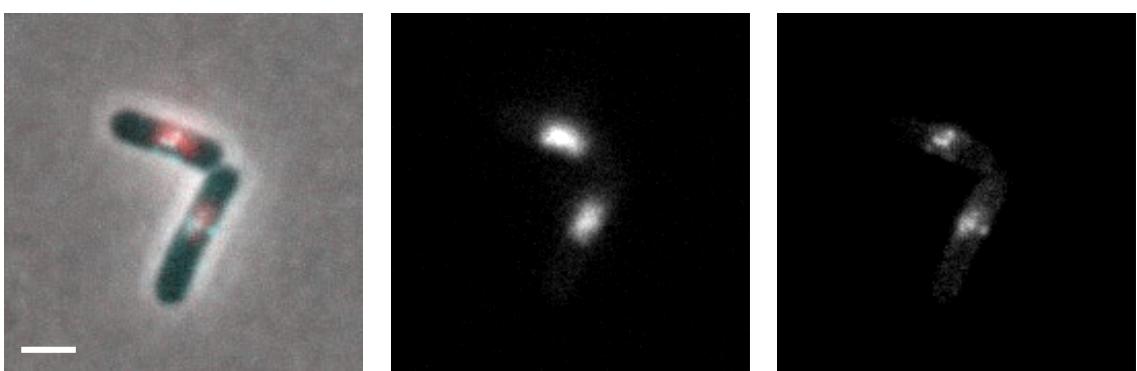




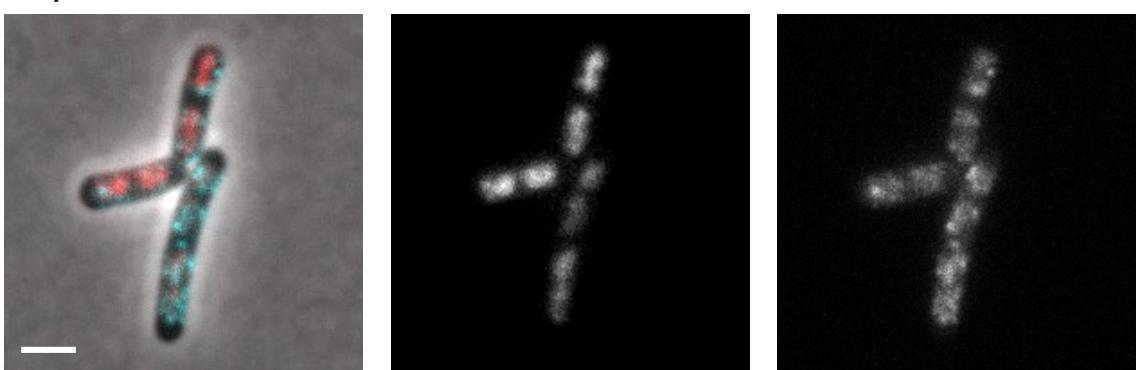
A Wild-type



B *dna-1*



C $\Delta spo0J$



D *dna-1* $\Delta spo0J$

