A network of *cis* and *trans* interactions is required for ParB spreading

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**Supplementary Information**

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SUPPLEMENTARY MATERIALS AND METHODS

Immunoblot analysis

Vegetatively growing cells were harvested at OD$_{600}$ between 0.3 and 0.4, and 1 ml of cells were collected and resuspended in lysis buffer (20 mM Tris at pH 7.5, 1 mM EDTA, 10 mM MgCl$_2$, 1 mg/ml lysozyme, 1 mM PMSF, 10 µg/ml DNase I and 100 µg/ml RNase A) to a final OD$_{600}$ of 20 for equivalent loading. The cells were incubated at 37°C for 10 min followed by addition of equal volume of sodium dodecyl sulfate (SDS) sample buffer (0.25 M Tris at pH 6.8, 4% SDS, 20% glycerol, 10 mM EDTA and 1% bromophenol blue) containing 10% 2-mercaptoethanol. Samples were heated for 5 min at 80°C prior to loading. Proteins were separated by SDS-PAGE on 12% precast polyacrylamide gels (BioRad), electroblotted onto polyvinylidine fluoride membranes, and blocked in 5% nonfat milk in 1x phosphate-buffered saline (PBS) for 15 min. The blocked membranes were probed with anti-GFP (1:10,000) (Rudner et al. 1999), anti-Spo0J (1:5,000) (Lin et al. 1997), or anti-SigA (1:10,000) (Fujita 2000) antibodies diluted into 3% BSA in PBS. Primary antibodies were detected using 1:20,000 horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (H + L) secondary antibody (Jackson ImmunoResearch) in 5% nonfat milk in PBS. Blots were exposed to chemiluminescent HRP substrate (HyGLO Quick Spray, Denville Scientific) and imaged on an Amersham Imager 600 (GE Healthcare Life Sciences). Blots with weak signal were re-imaged using SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific).

Size exclusion chromatography with multi-angle light scattering

Wild type BsSpo0J proteins at 120 µg ml$^{-1}$ and BSA standard at 2 mg ml$^{-1}$ were run at a flow rate of 0.5 ml min$^{-1}$ in storage buffer (20 mM Tris at pH 8.0, 350 mM NaCl, 10% glycerol, 10 mM imidazole and 5 mM BME) on an AdvanceBio 300Å size exclusion chromatography column (Agilent Technologies) attached to a Wyatt Dawn Heleos II Multi-Angle Light Scattering detector (Wyatt Technology) and a Wyatt Optilab T-rex Refractive Index Detector (Wyatt Technology). Chromatograms were analyzed using the ASTRA 7 software (Wyatt Technology) to determine the molecular weights.

Plasmid construction

Variants of pKM304 (Graham et al. 2014) encoding mutants of His6-SUMO-BsSpo0J were generated by site-directed mutagenesis (either QuickChange or Round-the-Horn method) with primers listed in Supplementary Table S4. Sequences of the resulting constructs were confirmed with universal T7-sequencing primers.

Variants of pWX563 (Graham et al. 2014) encoding mutants of mGFPmut3-BsSpo0J were generated by site-directed mutagenesis (either QuickChange or Round-the-Horn method) with primers listed in Supplementary Table S4. Sequences of the resulting constructs were confirmed with either primer oTG237 or primer oTG004R.

pLS063 was generated from pLS050 by site-directed mutagenesis (QuickChange method) with primers oTG169 and oTG170.

pLS064 was generated from pLS050 by site-directed mutagenesis (QuickChange method) with primers oLS077F and oLS077R.

pLS066 was generated from pTG240 by site-directed mutagenesis (QuickChange method) with primers oTG169 and oTG170.

pLS067 was generated from pTG240 by site-directed mutagenesis (QuickChange method) with primers oLS077F and oLS077R.
### Supplementary Table S1: Mutations introduced in this study

| Residue in HpSpo0J | Residue in BsSpo0J | Identity | Mutation(s) in BsSpo0J | References |
|--------------------|-------------------|----------|------------------------|------------|
| R49                | R39               | Interaction hub | R39A                  | This work |
| Q62                | E52               | Interacting partner | E52R            | This work |
| H67                | H57               | Interacting partner | H57E            | This work |
| L70                | L60               | Interaction hub | L60E              | This work |
| Q71                | Q61               | Interaction hub | Q61A, Q61R     | This work |
| P72                | P62               | Interaction hub | P62A              | This work |
| Y82                | Y72               | ParB Box II | Y72A                | This work |
| L84                | I74               | Interaction hub; ParB Box II | I74A         | This work |
| I85                | V75               | Interaction hub; ParB Box II | V75A, V75E | This work |
| G87                | G77               | Interacting partner; ParB Box II | G77S          | Breier and Grossman 2007; Graham et al. 2014 |
| E88                | E78               | Interaction hub; ParB Box II | E78R          | This work |
| R89                | R79               | Interaction hub; ParB Box II | R79A          | Graham et al. 2014 |
| R90                | R80               | Interaction hub; ParB Box II | R80A          | Autret et al. 2001; Graham et al. 2014 |
| L91                | F81               | Interaction hub; ParB Box II | F81A          | This work |
| R92                | R82               | Interaction hub; ParB Box II | R82A          | Graham et al. 2014 |
| M114               | M104              | Interacting partner | M104A         | This work |
| R115               | R105              | Interaction hub | R105A, R105E     | This work |
| N122               | N112              | Interacting partner | N112S         | Gruber and Errington 2009 |
| E150               | Q140              | Interaction hub | Q140A, Q140R     | This work |

### Supplementary Table S2: Strains used in this study

| Strain | Genotype | Source | Figure |
|--------|----------|--------|--------|
| PY79   | wild type | Youngman et al. 1983 | S2     |
| BDR2292| Δspo0J::spec | Wang et al. 2015 |        |
| BWX523 | sacA::hbsu-mcherry kan | X.W. and D.Z.R., unpublished |        |
| BDR2798| pelB::Psoj-mgfpmut3-spo0J WT (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | Graham et al. 2014 | 2, S3 – S5, S20 |
| BDR2796| pelB::Psoj-mgfpmut3-spo0J G77S (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | Graham et al. 2014 | 2, S4 |
| BDR2797| pelB::Psoj-mgfpmut3-spo0J R79A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | Graham et al. 2014 | 2, S3 |
| BDR2799| pelB::Psoj-mgfpmut3-spo0J R80A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | Graham et al. 2014 | S3 |
| BDR2866| pelB::Psoj-mgfpmut3-spo0J R82A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | Graham et al. 2014 | S3 |
| BLS005 | pelB::Psoj-mgfpmut3-spo0J H57E (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work | S3 |
| BLS007 | pelB::Psoj-mgfpmut3-spo0J L60E (ΔparS) tet, | This work | 2, S3 |
| Strain   | Genotype                                                                 | Source         | Figure |
|----------|---------------------------------------------------------------------------|----------------|--------|
| BLS008   | pelB::Psoj-mgfp3mut3-spo0J R39A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | 2, S3  |
| BLS014   | pelB::Psoj-mgfp3mut3-spo0J Q140A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | 2, S3  |
| BLS015   | pelB::Psoj-mgfp3mut3-spo0J Q61A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S3     |
| BLS016   | pelB::Psoj-mgfp3mut3-spo0J R105A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S3     |
| BLS017   | pelB::Psoj-mgfp3mut3-spo0J E52R (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S5     |
| BLS018   | pelB::Psoj-mgfp3mut3-spo0J M104A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | 2, S3  |
| BLS021   | pelB::Psoj-mgfp3mut3-spo0J E78R (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | 2, S4  |
| BLS022   | pelB::Psoj-mgfp3mut3-spo0J P62A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S4     |
| BLS024   | pelB::Psoj-mgfp3mut3-spo0J N112S (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S5     |
| BLS032   | pelB::Psoj-mgfp3mut3-spo0J Q61R (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S5     |
| BLS033   | pelB::Psoj-mgfp3mut3-spo0J I74A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | 2, S4  |
| BLS034   | pelB::Psoj-mgfp3mut3-spo0J V75A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S3     |
| BLS035   | pelB::Psoj-mgfp3mut3-spo0J F81A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S3     |
| BLS036   | pelB::Psoj-mgfp3mut3-spo0J R105E (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | 2, S3  |
| BLS038   | pelB::Psoj-mgfp3mut3-spo0J Y72A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | 2, S4  |
| BLS045   | pelB::Psoj-mgfp3mut3-spo0J Q140R (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S3     |
| BLS046   | pelB::Psoj-mgfp3mut3-spo0J V75E (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | 2, S4  |
| BLS050   | pelB::Psoj-mgfp3mut3-spo0J Q61R + R82A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S20    |
| BLS051   | pelB::Psoj-mgfp3mut3-spo0J Q61R + R105E (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S20    |
| BLS052   | pelB::Psoj-mgfp3mut3-spo0J G77S + R79A (ΔparS) tet, Δspo0J::spec, sacA::hbsu-mcherry kan | This work      | S20    |
| BDR2711  | pelB::Psoj-mgfp3mut3-spo0J WT (ΔparS) tet, Δspo0J::spec                         | Graham et al. 2014 | S2     |
| BDR2709  | pelB::Psoj-mgfp3mut3-spo0J G77S (ΔparS) tet, Δspo0J::spec                      | Graham et al. 2014 | S2     |
| BDR2710  | pelB::Psoj-mgfp3mut3-spo0J R79A (ΔparS) tet, Δspo0J::spec                     | Graham et al. 2014 | S2     |
| BDR2713  | pelB::Psoj-mgfp3mut3-spo0J R80A (ΔparS) tet, Δspo0J::spec                     | Graham et al. 2014 | S2     |
| BDR2849  | pelB::Psoj-mgfp3mut3-spo0J R82A (ΔparS) tet, Δspo0J::spec                     | Graham et al. 2014 | S2     |
| BLS001   | pelB::Psoj-mgfp3mut3-spo0J H57E (ΔparS) tet, Δspo0J::spec                     | This work       | S2     |
| BLS003   | pelB::Psoj-mgfp3mut3-spo0J L60E (ΔparS) tet, Δspo0J::spec                     | This work       | S2     |
| Strain | Genotype | Source | Figure |
|--------|----------|--------|--------|
| BLS004 | pelB::psoj-mgfpmut3-spo0J R39A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS009 | pelB::psoj-mgfpmut3-spo0J Q140A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS010 | pelB::psoj-mgfpmut3-spo0J Q61A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS011 | pelB::psoj-mgfpmut3-spo0J R105A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS012 | pelB::psoj-mgfpmut3-spo0J E52R (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS013 | pelB::psoj-mgfpmut3-spo0J M104A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS019 | pelB::psoj-mgfpmut3-spo0J E78R (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS020 | pelB::psoj-mgfpmut3-spo0J P62A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS027 | pelB::psoj-mgfpmut3-spo0J Q61R (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS028 | pelB::psoj-mgfpmut3-spo0J I74A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS029 | pelB::psoj-mgfpmut3-spo0J V75A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS030 | pelB::psoj-mgfpmut3-spo0J F81A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS031 | pelB::psoj-mgfpmut3-spo0J R105E (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS037 | pelB::psoj-mgfpmut3-spo0J Y72A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS043 | pelB::psoj-mgfpmut3-spo0J Q140R (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS044 | pelB::psoj-mgfpmut3-spo0J V75E (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS047 | pelB::psoj-mgfpmut3-spo0J Q61R + R82A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS048 | pelB::psoj-mgfpmut3-spo0J Q61R + R105E (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS049 | pelB::psoj-mgfpmut3-spo0J G77S + R79A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS037 | pelB::psoj-mgfpmut3-spo0J Q61R + R105E (ΔparS) tet, Δspo0J::spec | This work | S2 |
| BLS049 | pelB::psoj-mgfpmut3-spo0J G77S + R79A (ΔparS) tet, Δspo0J::spec | This work | S2 |
| TG309  | pelB::psoj-mgfpmut3-spo0J N112S (ΔparS) tet, Δspo0J::spec | This work | S2 |

Supplementary Table S3: Plasmids used in this study

| Plasmid  | Description    | Source                      |
|----------|----------------|-----------------------------|
| pKM304   | His6-SUMO-Spo0J WT | Graham et al. 2014          |
| pLS021   | His6-SUMO-Spo0J L60E  | This work                   |
| pLS022   | His6-SUMO-Spo0J Q61A  | This work                   |
| pLS023   | His6-SUMO-Spo0J R39A  | This work                   |
| pLS025   | His6-SUMO-Spo0J R105A | This work                   |
| pLS032   | His6-SUMO-Spo0J M104A | This work                   |
| pLS034   | His6-SUMO-Spo0J E52R  | This work                   |
| pLS035   | His6-SUMO-Spo0J P62A  | This work                   |
| Plasmid   | Description                        | Source               |
|-----------|------------------------------------|----------------------|
| pLS036    | His6-SUMO-Spo0J Q140A               | This work            |
| pLS037    | His6-SUMO-Spo0J E78R               | This work            |
| pLS038    | His6-SUMO-Spo0J H57E               | This work            |
| pLS055    | His6-SUMO-Spo0J R105E              | This work            |
| pLS057    | His6-SUMO-Spo0J F81A               | This work            |
| pLS061    | His6-SUMO-Spo0J Q140R              | This work            |
| pLS062    | His6-SUMO-Spo0J V75E               | This work            |
| pLS066    | His6-SUMO-Spo0J Q61R + R82A        | This work            |
| pLS067    | His6-SUMO-Spo0J Q61R + R105E       | This work            |
| pLS068    | His6-SUMO-Spo0J G77S + R79A        | This work            |
| pTG037    | His6-SUMO-Spo0J R80A               | Graham et al. 2014   |
| pTG052    | His6-SUMO-Spo0J G77S               | Graham et al. 2014   |
| pTG105    | His6-SUMO-Spo0J N112S              | This work            |
| pTG114    | His6-SUMO-Spo0J Y72A               | This work            |
| pTG115    | His6-SUMO-Spo0J I74A               | This work            |
| pTG116    | His6-SUMO-Spo0J V75A               | This work            |
| pTG118    | His6-SUMO-Spo0J R79A               | Graham et al. 2014   |
| pTG119    | His6-SUMO-Spo0J R82A               | Graham et al. 2014   |
| pTG240    | His6-SUMO-Spo0J Q61R               | This work            |
| pWX563    | pelB::Psoj-mgfmutil3-spo0J WT ΔparS tet | Graham et al. 2014   |
| pLS039    | pelB::Psoj-mgfmutil3-spo0J H57E ΔparS tet | This work   |
| pLS041    | pelB::Psoj-mgfmutil3-spo0J L60E ΔparS tet | This work   |
| pLS042    | pelB::Psoj-mgfmutil3-spo0J R39A ΔparS tet | This work   |
| pLS043    | pelB::Psoj-mgfmutil3-spo0J Q140A ΔparS tet | This work   |
| pLS044    | pelB::Psoj-mgfmutil3-spo0J Q61A ΔparS tet | This work   |
| pLS045    | pelB::Psoj-mgfmutil3-spo0J R105A ΔparS tet | This work   |
| pLS046    | pelB::Psoj-mgfmutil3-spo0J E52R ΔparS tet | This work   |
| pLS047    | pelB::Psoj-mgfmutil3-spo0J M104A ΔparS tet | This work   |
| pLS048    | pelB::Psoj-mgfmutil3-spo0J E78R ΔparS tet | This work   |
| pLS049    | pelB::Psoj-mgfmutil3-spo0J P62A ΔparS tet | This work   |
| pLS050    | pelB::Psoj-mgfmutil3-spo0J Q61R ΔparS tet | This work   |
| pLS051    | pelB::Psoj-mgfmutil3-spo0J I74A ΔparS tet | This work   |
| pLS052    | pelB::Psoj-mgfmutil3-spo0J V75A ΔparS tet | This work   |
| pLS053    | pelB::Psoj-mgfmutil3-spo0J F81A ΔparS tet | This work   |
| pLS054    | pelB::Psoj-mgfmutil3-spo0J R105E ΔparS tet | This work   |
| pLS056    | pelB::Psoj-mgfmutil3-spo0J Y72A ΔparS tet | This work   |
| pLS059    | pelB::Psoj-mgfmutil3-spo0J Q140R ΔparS tet | This work   |
| pLS060    | pelB::Psoj-mgfmutil3-spo0J V75E ΔparS tet | This work   |
| pLS063    | pelB::Psoj-mgfmutil3-spo0J Q61R + R82A ΔparS tet | This work   |
| pLS064    | pelB::Psoj-mgfmutil3-spo0J Q61R + R105E ΔparS tet | This work   |
| pLS065    | pelB::Psoj-mgfmutil3-spo0J G77S + R79A ΔparS tet | This work   |
| pTG138    | pelB::Psoj-mgfmutil3-spo0J G77S ΔparS tet | Graham et al. 2014 |
| pTG140    | pelB::Psoj-mgfmutil3-spo0J R79A ΔparS tet | Graham et al. 2014 |
| pTG141    | pelB::Psoj-mgfmutil3-spo0J R80A ΔparS tet | Graham et al. 2014 |
| pTG142    | pelB::Psoj-mgfmutil3-spo0J R82A ΔparS tet | Graham et al. 2014 |
| pTG184    | pelB::Psoj-mgfmutil3-spo0J N112S ΔparS tet | This work   |

**Supplementary Table S4: Oligonucleotides used in this study**

| Oligo     | Sequence            | Use                 |
|-----------|---------------------|---------------------|
| oLS032F   | gacgccgctctatgcagaa | pLS021; pLS041      |
| oLS031R   | aatgccatgctgacgca  | pLS021; pLS041      |
| oLS033F   | gcgccgctctatgcagaaatctttaaaag | pLS022; pLS044      |
| Oligo     | Sequence                        | Use                  |
|----------|---------------------------------|----------------------|
| oLS034R  | aagaatgccatgctgacgc             | pLS022; pLS044       |
| oLS039F  | gcggaaatgctttatatagaaaccttcagcg | pLS025               |
| oLS040R  | cattaacgcctctgataattaacccg      | pLS025               |
| oLS058F  | gagggaattccttacggctca          | pLS038; pLS039       |
| oLS029R  | ctgcagcagatcttttagtctc         | pLS038; pLS039       |
| oLS052F  | gcggaaatgctttatatagaaaccttcagcg | pLS032; pLS047       |
| oLS052R  | taacgcctctgataataacccg         | pLS032; pLS047       |
| oLS054F  | cggctctgtgcagcatggctca        | pLS034               |
| oLS053R  | ttttagtcagcataagcctc          | pLS034               |
| oLS055F  | gcgttcacgcaaaatctttta         | pLS035; pLS049       |
| oLS055R  | ctaaagcagatgctctgacca         | pLS035; pLS049       |
| oLS056F  | gcagggctgttcgagc              | pLS037; pLS048       |
| oLS056R  | acccgcaacattatctagctctttt     | pLS037; pLS048       |
| oLS057F  | gcggcttgccaaacgtctgga          | pLS036               |
| oLS057R  | ctctgtgtgagatcttaagg         | pLS036               |
| oLS063F  | aatctttatgacacggacaaacactttgatgac | pLS023; pLS042   |
| oLS063R  | gtcataaagctttgctgataaggatt    | pLS023; pLS042       |
| oLS065F  | gatctcacaacagggcggcttaacgcttt  | pLS043               |
| oLS065R  | aagacgtttgcaagccctctttgtgatc  | pLS043               |
| oLS067F  | tcagggcagtaagccgaaatgttttat    | pLS047               |
| oLS067R  | taataaagcagctttgagaaatgtcttt   | pLS047               |
| oLS068F  | ttagctgtaactaaacggctgtgctgcacat | pLS046             |
| oLS068R  | atgctgacacagacgctttgatcgcca   | pLS046               |
| oLS077F  | tcagggcggctttgcattta         | pLS054; pLS055; pLS064; pLS067 |
| oLS077R  | taatataaagctttgacacgctctgga  | pLS054; pLS055; pLS064; pLS067 |
| oLS080F  | gatctcacaacagggcggcttaacgcttt  | pLS059; pLS061       |
| oLS080R  | aagacgtttgcaagccctctttgtgatc  | pLS059; pLS061       |
| oLS081F  | taataaagcagctttgagaaatgtcttt   | pLS060; pLS062       |
| oLS081R  | ttagctgtaactaaacggctgtgctgcacat | pLS060; pLS062   |
| oLS082F  | gatattgtgcagatgacgcttttgcagcg  | pLS065; pLS068       |
| oLS082R  | cgcctgaaatcgcgttactgcaaatatctc  | pLS065; pLS068       |
| oTG027F  | ggctggctggaaagctgttcttgacgcgaag  | pTG037; pTG141      |
| oTG027R  | cttccgctgtaaagccttgctcggcccaaac  | pTG037; pTG141      |
| oTG049F  | latgatatgttgctggaagcgcttttcc  | pTG052; pTG138       |
| oTG049R  | gaaacngcgttcagctcagcaaaatctttac  | pTG052; pTG138       |
| oTG143  | attgttattgagaaagctttgcaacgcttt  | pTG150; pTG184       |
| oTG144  | attgttattgagaaagctttgcaacgcttt  | pTG150; pTG184       |
| oTG159  | gaaatcatttaaaaggcgctgatatttgtcgggt  | pTG114; pLS056      |
| oTG160  | accgacaacaatcgcgcttttaaaatgttttc  | pTG114; pLS056      |
| oTG161  | ctttaaaagctgtatcttgtgcttggtgacag  | pTG115; pLS051      |
| oTG162  | cggctcgcgccagacgcatatactagcttttaaag  | pTG115; pLS051      |
| oTG163  | taatataaagcagctttgagaaatgtcttt   | pTG116; pLS052      |
| oTG164  | aacggcgttcacgcaaccaaatatcagccttttaa  | pTG116; pLS052      |
| oTG167  | attgttattgagaaagctttgcaacgcttt  | pTG118; pTG140      |
| oTG168  | tggccgctgtaaagacgcgttcacgcagcaacaat  | pTG118; pTG140      |
| oTG169  | ggtgaaagcggcttttgcagcgcagcttaaaacgccttcagc  | pTG119; pTG142; pLS063; pLS066 |
| oTG170  | tcgccagcttttgcagccagaaagctttgcagc  | pTG119; pTG142; pLS063; pLS066 |
| oTG211  | gcgggaatgctttttgtggctcgagcgcaagcgtctgcag   | pLS053; pLS057      |
| oTG223  | cagcctttcgtgacagcgcggctttgcagc  | pLS053; pLS057      |
| oTG379  | cagcagctttcgtgacagcgcggctttgcagc  | pTG240; pLS050      |
| oTG380  | tctgagataaagcggcgcgaagatcatgctgtgctgcag   | pTG240; pLS050      |
| oTG004R | gcactcaggtgattttcctgctcagacaaagcctc  | sequencing         |
Oligo | Sequence | Use |
--- | --- | --- |
oTG237 | atgctaaagccttgga | sequencing |
oTG489 | TGAGGGATATCGAATTCCTGCAGGgctattcctcgagggaggtc | single-molecule PIFE (first round of PCR amplification) |
oTG491 | GAGCGCAATTATTTTGATGGCCGgatctgccgcatgatc | single-molecule PIFE (first round of PCR amplification) |
oTG437 | /5Biosg/TGAGGGATATCGAATTCCTGCAGG | single-molecule PIFE (second round of PCR amplification) |
oTG491 | /5DigN/GACGCGAATTATTTTGATGGCGG | single-molecule PIFE (second round of PCR amplification) |
oLS044F | agaaagtctccacgtgaacaaga | EMSA (24-bp parS) |
oLS044R | tttttcgcttcacgtgaacattct | EMSA (24-bp parS) |
oLS046F | agaacgtccacggagacaaaaaga | EMSA (24-bp scrambled parS) |
oLS046R | tcttgcagggctctgattcact | EMSA (24-bp scrambled parS) |
oTG041F | cagttgaatcatcaagaga | EMSA (39-bp parS) |
oTG041R | tttttcgttcacgtgaacattctgattcaactg | EMSA (39-bp parS) |
oTG043F | cagttgaatctgacaaatgactaacaatgagcaaaaa | EMSA (39-bp scrambled parS) |
oTG043R | tttttgcagggctctgattcact | EMSA (39-bp scrambled parS) |

- All oligonucleotides were obtained from Integrated DNA Technologies (IDT) and the sequences are given in the 5' – 3' direction.
- Capitalized text in oTG489 and oTG491 indicate adapter sequences that are complementary to oTG437 and oTG488, respectively.
- Red text in oLS044F, oLS044R, oTG041F and oTG041R indicates parS sequence.

Modifications:

/5Biosg/ - 5' biotin
/5DigN/ - 5' digoxigenin

**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1.** Sequence alignment between ParB homologs. Alignment was generated using Clustal Omega with ParB sequences from *Bacillus subtilis* (BsSpo0J), *Helicobacter pylori* (HpSpo0J), *Thermus thermophilus* (ThSpo0J), *Caulobacter crescentus* (CcParB), *Pseudomonas aeruginosa* (PaParB), *Streptococcus pneumoniae* (SpParB), *Vibrio cholerae* chromosome I (VcParB), RP4 plasmid (RP4KorB) and *Enterobacteria* phage P1 (P1ParB). ParB Box I (S53 – R66 in BsSpo0J) and Box II (Y72 – A83 in BsSpo0J) are marked. Colours indicate levels of conservation. Arrows on the bottom of the alignment indicate selected residues in the mutagenesis: arrows in magenta and black indicate hubs and interacting residues, respectively, in the 2D network map as shown in Figure 1F. Asterisks indicate residues previously identified to be essential for ParB spreading in *B. subtilis*: G77 (Breier and Grossman 2007), R79 (Graham et al. 2014), R80 (Autret et al. 2001), and R82 (Graham et al. 2014).

**Figure S2.** Immunoblot analysis of fluorescent protein fusions to BsSpo0J variants. All fusion proteins are intact and expressed at similar levels. Strain PY79 expresses endogenous wild type BsSpo0J without a fluorescent tag. mGFPmut3-BsSpo0J was detected using anti-GFP antibodies and the predicted size of free GFP is indicated (arrowhead). σ^A levels are shown as a control for loading.

**Figure S3.** Localization of mGFPmut3-tagged BsSpo0J Group I mutants (see Table 2). Some panels are duplicated here from Figure 2 to facilitate comparison. Nucleoid (false-coloured red) was labelled with HBsu-mCherry. Scale bar = 5 µm.
Figure S4. Localization of mGFPmut3-tagged BsSpo0J Group II mutants (see Table 2). Some panels are duplicated here from Figure 2 to facilitate comparison. Nucleoid (false-coloured red) was labelled with HBsu-mCherry. Scale bar = 5 μm.

Figure S5. Localization of mGFPmut3-tagged BsSpo0J Group III mutants (see Table 2). Images for the wild type are duplicated here from Figure 2 to facilitate comparison. Nucleoid (false-coloured red) was labelled with HBsu-mCherry. Scale bar = 5 μm.

Figure S6. Interaction between H67 and E88 in the HpSpo0J-parS complex. (A) Cartoon representation of the C-terminally truncated HpSpo0J-parS crystal structure (Chen et al. 2015) (PDB code: 4UMK). Figure is not drawn to scale. (B) Interaction (magenta dashed line) between H67 on chain A (blue) and E88 on chain B (orange) in trans. Figure was prepared in PyMOL.

Figure S7. Protein purification of BsSpo0J. (A) Coomassie-stained SDS-PAGE gel showing wild type BsSpo0J and mutants purified via His6-SUMO expression system (see Methods). (B) Light scattering signals represented in Rayleigh ratio for wild type BsSpo0J (black solid line) and a BSA standard (black dashed line). Signals were monitored using SEC-MALS and normalized to the maximum in each curve. A single peak was observed for wild type BsSpo0J (red solid line) with a calculated molecular weight (MW) of 63.5 ± 0.4 kDa, corresponding to a dimeric protein. Three major peaks with calculated MWs of 62.2 ± 0.3 kDa, 123.6 ± 0.2 kDa, and 201.2 ± 0.6 kDa were observed for BSA (red dashed lines), corresponding to monomeric, dimeric, and higher oligomeric proteins, respectively.

Figure S8. Quantification of the kinetics of DNA compaction by wild type BsSpo0J at 100 nM. (A) Trajectories of fold increase in integrated fluorescence intensity (blue) and DNA length (green) of a single Cy3-labelled DNA compacted by wild type BsSpo0J. Time zero was defined as the starting point of protein association. Lag time (tlag) is the time between protein binding and the initiation of DNA compaction. tlag = 5.1 s as shown here. (B) Histogram of lag times (tlag) fitted with a Gaussian distribution (red) for wild type BsSpo0J. (C) Trajectory of DNA length (green) of a single Cy3-labelled DNA compacted by wild type BsSpo0J. Rate of DNA compaction (kc) was estimated by linear fitting of the trajectory (red dashed line) between maximum and minimum DNA lengths (purple dashed lines). The slope shown here is 0.44 ± 0.02 μm s⁻¹ (fit ± error estimate). (D) Histogram of rates of DNA compaction (kc) fitted with a Gaussian distribution (red) for wild type BsSpo0J.

Figure S9. Single-molecule DNA compaction by BsSpo0J Group I mutants (see Table 2). (A) Fold increase in integrated fluorescence intensity and DNA length trajectories for the wild type BsSpo0J (black; reproduced in each panel) and mutants (red) at a protein concentration of 100 nM. Each trajectory was averaged over 20 – 30 DNAs. Some panels are re-plotted here from Figure 3C to facilitate comparison. Note that in comparison to Figure 3C, trajectories shown here were plotted over a longer time scale to capture the complete DNA compaction by mutants. The fold change in integrated intensity showed a decreasing trend after reaching equilibrium due to photobleaching rather than protein dissociation (see part B). (B) Overlay of a trajectory showing association of BsSpo0J R80A to Cy3-labelled DNAs (red; reproduced from part A) and a photobleaching curve of Cy3-labelled DNAs in the absence of protein (blue). Each trajectory was averaged over 20 – 30 DNAs. Integrated fluorescence intensities of Cy3-labelled DNAs were normalized by the maximum values. Plots were aligned at the time point marked by the purple dashed line when the integrated intensity started to decrease.

Figure S10. Group I mutants of BsSpo0J (see Table 2) are defective in DNA compaction even at a higher protein concentration. Fold increase in integrated fluorescence intensity and DNA length trajectories for the wild type BsSpo0J (black; reproduced in each panel) and mutants (red) at a protein concentration of 300 nM. Some compaction activity is observed for the V75A and F81A mutants, consistent with their ability to form wild type-like foci in vivo (Supplementary Figure S3). Each trajectory was averaged over 20 – 30 DNAs.

Figure S11. DNA compaction by BsSpo0J mutants in low salt. Fold increase in integrated fluorescence intensity and DNA length trajectories for R80A (blue), R105E (green), or R82A (red) at a protein
concentration of 100 nM in binding buffer containing 50 mM NaCl. Each trajectory was averaged over 20 – 30 DNAs.

**Figure S12.** Single-molecule DNA compaction by BsSpo0J Group II and Group III mutants (see Table 2). (A) Fold increase in integrated fluorescence intensity and DNA length trajectories for the wild type BsSpo0J (black; reproduced in each panel) and mutants (red) at a protein concentration of 100 nM. Each trajectory was averaged over 20 – 30 DNAs. Some panels are re-plotted here from Figure 3C to facilitate comparison. (B) Trajectory of the mutant N112S at a protein concentration of 300 nM.

**Figure S13.** In vitro characterization of the specific binding of BsSpo0J to the 24-bp parS DNA duplexes without competitor DNA. Protein concentrations were 0.2, 0.4, 0.8 µM in (A) – (E), and 0.2, 0.4, 0.8, and 1.0 µM in (F) – (J). Asterisk and arrow indicate position of the wells and free DNA respectively in each gel. Some panels are duplicated here from Figure 4 to facilitate comparison.

**Figure S14.** In vitro characterization of the specific binding of BsSpo0J to the 39-bp parS DNA duplexes supplemented with cold 39-bp scrambled parS competitor DNA. Protein concentrations were 0.2, 0.4, 0.8 µM in (A) – (E), and 0.2, 0.4, 0.8, and 1.0 µM in (F) – (J). Asterisk and arrow indicate position of the wells and free DNA respectively in each gel. Some panels are duplicated here from Figure 4 to facilitate comparison.

**Figure S15.** In vitro characterization of the non-specific binding of BsSpo0J to the 39-bp DNA duplexes containing a scrambled parS site. Protein concentrations were 0.2, 0.4, 0.8 µM in (A) – (E), and 0.2, 0.4, 0.8, and 1.0 µM in (F) – (J). Asterisk and arrow indicate position of the wells and free DNA respectively in each gel.

**Figure S16.** Crystal structure of C-terminally truncated TtSpo0J (Leonard et al. 2004) (PDB code: 1VZ0). C-terminally truncated TtSpo0J monomers (chain A in magenta and chain B in green) form an antiparallel dimer through interactions between the alpha helix “H2” of one monomer and the globular domain of the other. Dimerization is further stabilized by hydrophobic interactions between the extended N-terminal chain of one monomer and multiple β-sheets of the other at each end of the dimer. The helix-turn-helix DNA-binding domain is highlighted in each monomer for orientation (orange in chain A and yellow in chain B, respectively). Residues affected by Group I and Group III mutations (see Table 2), which are highlighted in red, are not involved in the N-terminal dimerization interface. Residues affected by Group II mutations including P62A (see Table 2), which are highlighted in blue, are involved in stabilizing the N-terminal dimerization. A 180° reverse view of the dimer is shown for comparison. Figure was prepared in PyMOL.

**Figure S17.** Residues in the highly conserved ParB Box II region coordinate multiple interactions between ParB dimers both in cis and in trans. (A) Cartoon representation of the C-terminally truncated HpSpo0J-parS crystal structure (Chen et al. 2015) (PDB code: 4UMK). Figure is not drawn to scale. (B) Residues R89 and I85 in ParB Box II (shown in yellow) of chain D (green) interact in cis with E150 in the helix-turn-helix (HTH) domain (shown in pink) of chain B (orange). Interactions between other residues in ParB Box II (shown in yellow) of chain D (green) and residues on chain B (orange) outside the HTH domain are also displayed. Location of residue P72 on chain D (green) is indicated. (C) Multiple interactions in trans between chain A (blue) and chain B (orange) coordinated by residues in ParB Box II on each chain (ParB Box II on chain A is shown in cyan and ParB Box II on chain B is shown in yellow). Residues Y82 – E88 (Y72 – E78 in BsSpo0J) in ParB Box II are located on the β-sheet and loop region. Residues R89 – R92 (R79 – R82 in BsSpo0J) in ParB Box II are located on the α-helix. Residue P72 (P62 in BsSpo0J) on chain B (orange) also interacts with L74 on chain A (blue) in trans. Yellow dashed lines indicate hydrogen bonds, and magenta dashed lines indicate hydrophobic interactions. Figures were prepared in PyMOL.

**Figure S18.** Thermal stability of BsSpo0J mutants in Group I (A) subgroup A, (B) subgroup B, and (C) subgroup C (see Table 2). Thermal denaturation curves for wild type BsSpo0J (black dashed line; reproduced in each panel) and mutants (red) were measured with differential scanning fluorimetry at a protein concentration of 100 µg ml⁻¹ (see Methods). Fluorescence intensities were normalized to the
maximum in each curve. Only one replicate of each protein is shown. Some data are re-plotted here from Figure 5A to facilitate comparison.

**Figure S19.** Thermal stability of BsSpo0J (A) Group II mutants and (B) Group III mutants (see Table 2). Thermal denaturation curves for wild type BsSpo0J (black dashed line; reproduced in each panel) and mutants (red) were measured with differential scanning fluorimetry at a protein concentration of 100 µg ml⁻¹ (see Methods). Fluorescence intensities were normalized to the maximum in each curve. Only one replicate of each protein is shown. Some data are re-plotted here from Figure 5A to facilitate comparison.

**Figure S20.** In vivo and in vitro characterization of BsSpo0J double mutants. (A) Localization of mGFPmut3-tagged BsSpo0J double mutants. Nucleoid (false-colored red) was labelled with HBsu-mCherry. Scale bar = 5 µm. (B) Fold increase in integrated fluorescence intensity and DNA length trajectories for the wild type BsSpo0J (black; reproduced in each panel) and double mutants (red) at a protein concentration of 100 nM. Each trajectory was averaged over 20 – 30 DNAs. (C) Fold increase in integrated fluorescence intensity and DNA length trajectories for the wild type BsSpo0J (black; reproduced in each panel) and mutants (red) at a protein concentration of 300 nM. Each trajectory was averaged over 20 – 30 DNAs. (D) EMSA of wild type BsSpo0J and double mutants binding to the 24-bp parS DNA duplexes without competitor DNA. Protein concentrations were 0.2, 0.4, 0.8, and 1.0 µM. (E) EMSA of wild type BsSpo0J and double mutants binding to the 39-bp parS DNA duplexes supplemented with cold 39-bp scrambled parS competitor DNA. Protein concentrations were 0.2, 0.4, 0.8, and 1.0 µM. (F) EMSA of wild type BsSpo0J and double mutants binding to the 39-bp DNA duplexes containing a scrambled parS site. Protein concentrations were 0.2, 0.4, 0.8, and 1.0 µM. Asterisk and arrow in (D) – (F) indicate position of the wells and free DNA respectively in each gel. (G) Thermal denaturation curves for wild type BsSpo0J (black dashed line; reproduced in each panel) and double mutants (red) at a protein concentration of 100 µg ml⁻¹ measured with differential scanning fluorimetry (see Methods). Fluorescence intensities were normalized to the maximum in each curve. Only one replicate of each protein is shown.

**Figure S21.** Summary of mutagenesis results annotated on the 2D network map generated from the crystal structure (see Methods) indicating cis (blue) and trans (green) interactions within the HpSpo0J-parS tetrameric complex (Chen et al. 2015). Interactions between residues within the same HpSpo0J monomer are shown in grey. Highly conserved residues that act as hubs for multiple interactions are circled in magenta. Colours indicate different levels of severity in ParB spreading based on the loss of mGFPmut3-BsSpo0J foci after the highlighted residue was mutated (see Supplementary Table S1 and Table 2 for specific mutations). Non-highlighted residues were not included in the mutagenesis studies. Residue number corresponds to that in HpSpo0J.
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SUPPLEMENTARY FIGURES

Supplementary Figure S1

ParB Box I

ParB Box II

Weak

Strong

13
Supplementary Figure S2

A

mGFP-Spo0J

σA

PY79  WT  H57E  L60E  R39A  Q61R  N112S  E78R  P52A  I74A  R105E

B

mGFP-Spo0J

σA

PY79  WT  R79A  R80A  R82A  G77S

C

mGFP-Spo0J

σA

PY79  WT  M104A  Q61A  Q140A  Q140R  R105A  V75A  F81A  Y72A  V75E

D

mGFP-Spo0J

σA

PY79  E62R  Q61R + R82A  Q61R + R105E  G77S + R79A
Supplementary Figure S3

- **No foci**
  - WT
  - mGFP-Spo0J
  - merge

- **Fuzzy foci**
  - H57E
  - R82A
  - R105E
  - Q140R
  - mGFP-Spo0J
  - merge

- **WT like**
  - Q61A
  - V75A
  - F81A
  - M104A
  - R105A
  - Q140A
  - mGFP-Spo0J
  - merge
Supplementary Figure S5

![Image of a cellular structure with different genotypes: WT, Q61R, N112S, E52R. The images show mGFP-Spo0J and a merge view, with annotations for No foci and WT like.]
Supplementary Figure S6
Supplementary Figure S7

A

B

Relative Rayleigh Ratio

0.10

0.08

0.06

0.04

0.02

0.00

12 13 14 15 16

Time (min)

Molar mass (g mol⁻¹)

10⁴

10⁵

10⁶

SpoOJ WT

BSA

19
Supplementary Figure S8

A. Fold increase in integrated intensity over DNA length (μm) vs. time (s).

B. Histogram of DNA length vs. $t_{lag}$ (s).

C. DNA length (μm) vs. time (s) showing $L_{max}$, $k_c$, and $L_{min}$.

D. Histogram of DNA number vs. $k_c$ (μm s$^{-1}$).
Supplementary Figure S10

No compaction

Fold increase in integrated intensity

Normalized DNA length

Time (s)

-10 0 10 20 30 40 50

No compaction

Slower compaction

Fold increase in integrated intensity

Normalized DNA length

Time (s)

-10 0 10 20 30 40 50

-10 0 10 20 30 40 50

-10 0 10 20 30 40 50

-10 0 30 60 90 120

-10 0 30 60 90 120
Supplementary Figure S12

A

Faster compaction

Fold increase in integrated intensity

Normalized DNA length

Time (s)

B

Faster compaction

Fold increase in integrated intensity

Normalized DNA length

Time (s)
Supplementary Figure S13
Supplementary Figure S14
Supplementary Figure S15
Supplementary Figure S16
Supplementary Figure S17

A

C-terminal (truncated)
DNA-binding domain
N-terminal

B

HTH of chain B

C

Pro 72
Leu 74

Arg 88
Glu 150
Ile 85
Supplementary Figure S18

A

Relative Fluorescence vs Temperature (°C)

- R39A
- H57E
- L60E
- R79A
- R80A
- WT

B

Relative Fluorescence vs Temperature (°C)

- R82A
- R105E
- WT

C

Relative Fluorescence vs Temperature (°C)

- Q61A
- V75A
- F81A
- M104A
- R105A
- WT

Relative Fluorescence vs Temperature (°C)

- Q140A
- Q140R
- WT
Supplementary Figure S20

A

No foci

WT
G77S + R79A
Q61R + R82A
Q61R + R105E

merge

B

Faster compaction

No compaction

Fold increase in integrated intensity

Normalized DNA length

-10 0 10 20 30 40 50 60 70 80 90 100

Time (s)

C

Slower compaction

Fold increase in integrated intensity

Normalized DNA length

0 30 60 90 120

Time (s)

D

E

F

G

Relative Fluorescence

G77S + R79A
Q61R + R82A
Q61R + R105E

WT
Supplementary Figure S2