Supplemental Information

Nucleoid occlusion prevents cell division during replication fork arrest in *Bacillus subtilis*

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. TetR bound to *tetO* arrays causes cell division inhibition and cell death. Images before and after induction of a replication roadblock at +7˚ (81.7 kb from the origin of replication). Time (in min) after removal of the inducer (aTC) is indicated. Images show membranes stained with FM4-64 (red), DAPI-stained DNA (blue), and TetR-GFP (green) bound to $(tetO)_{120}$. A phase contrast image shows cell lysis after 140 min. Similar results were obtained using TetR-YFP or TetR-CFP and with the *tetO* array at +130˚ (data not shown). White bar is 1 μm.

Figure S2. Replication of the left chromosome arm continues during a block on the right arm. Images of cells during a replication roadblock at +7˚ (81.7 kb from the origin of replication). Images show membranes stained with FM4-64 (red), DAPI-stained DNA (blue), and LacI-CFP bound to a small array of *lacO* operators at –7˚ (green). LacI-CFP binding to the *lacO* array does not impair replication (data not shown). The bottom set of images shows membranes (red) and TetR-YFP foci (green) to visualize the replication roadblock at +7˚. Time (in min) after removal of aTC is indicated. White bar is 1 μm.

Figure S3. Genomic microarray analysis before and after replication roadblocks at +7˚ and +130˚. Gene dosage ($\log_2$) relative to a reference DNA is shown on the y-axis. All the probed genes in the *B. subtilis* chromosome arranged from -188˚ to +172˚ (*ter-oriC-ter*) are shown (grey dots). The smooth line was generated by plotting the average gene dosage of the 25 genes before and 25 genes after each gene probed. Black arrows indicate the genomic positions surrounding the site of insertion of the *tetO* array. The red arrow indicates an increase in DNA content for genes located adjacent to the *ter* site on the blocked arm. Time (in min) after induction of the roadblock is indicated on the left. The upper panels are cells prior to the induction of the roadblock. The lower panels are after induction of the roadblock. Schematic representations of the two conditions are shown to the left of the graphs.

Figure S4. Efficient origin segregation after release of the replication roadblock. Images show a strain harboring LacI-CFP and a small *lacO* array inserted adjacent to the origin at –

7°. This strain also contains TetR-YFP and a *tetO* array at +7° that is capable of blocking replication. The replication roadblock was induced as described previously for 90 min. aTC was added to the culture to release the replication roadblock and cells were visualized by fluorescence microscopy. Images show FM4-64 stained membranes (red), DAPI-stained DNA, and LacI-CFP (false-colored green) bound to a small lacO array at –7°. Time (in min) after releasing the roadblock is indicated. Segregated nucleoids (green carets) and cell division events (yellow carets) are highlighted. White bar is 1 μm.

Figure S5. Comparison of RecA-GFP foci and filaments during treatment with HPUra and after induction of the replication roadblock. Time (in min) after addition of HPUra or removal of aTC is indicated. Images show RecA-GFP (green), FM4-64 stained membranes (red) and DAPI-stained DNA (blue).

Figure S6. Cell division inhibition during fork arrest does not require the SOS response. Representative fields of cells containing wild-type LexA or an uninducible LexA mutant ($LexA^{ind-}$) (Fabret et al., 2002) following treatment with HPUra (upper panels) or after induction of a replication roadblock (lower panels). Membranes were visualized with FM4-64. Time (in min) after drug treatment or induction of the roadblock is indicated. Average cell lengths and standard deviations of 500 cells for each strain are shown. White bar is 1 μm.

Figure S7. Transcriptional repression of *ftsL* does not contribute to cell division inhibition during the replication roadblock. Cells harboring an IPTG-inducible promoter fusion to *ftsL* (P_{spac}-*ftsL*) and cells containing wild-type *ftsL* were subjected to the replication roadblock as described previously with the only difference that the medium used for pre-culture, washing, and growth was supplemented with 1mM IPTG to maintain expression of *ftsL*. At time points after induction of the roadblock, cells were visualized by fluorescence microscopy using the membrane dye FM4-64 and cell length was quantified. Histograms show length distributions at indicated time (in min). >1000 cells were measured for each strain and each time point.

Figure S8. Cells lacking Spo0J have a greater size distribution than wild-type during fork arrest. The replication roadblock was induced as described previously in isogenic strains with (black) or without (grey) Spo0J. Cells were visualized by fluorescence microscopy using the membrane dye FM4-64 and cell length was quantified. Histograms show length distributions at 90 minutes after induction of the roadblock. Cells smaller than 5μm were binned together to highlight the increased number of small cells in the Spo0J mutant strain. >1500 cells were measured for each strain.

Figure S9. The SMC condensin complex is partially mislocalized in the absence of Spo0J during fork arrest. Images show the localization of ScpB-YFP (a subunit of the SMC condensin complex) during the replication roadblock in the presence or absence of Spo0J. The replication roadblock was induced as described previously and cells were visualized by fluorescence microscopy. Images show representative fields obtained 90 min after removal of aTC with ScpB-YFP (false-colored green), FM4-64-stained membranes (red), DAPI-stained DNA (blue). Septa that bisect the DNA mass in the absence of Spo0J are highlighed (yellow carets). White bar is 1 μm.

Figure S1
Bernard et al.

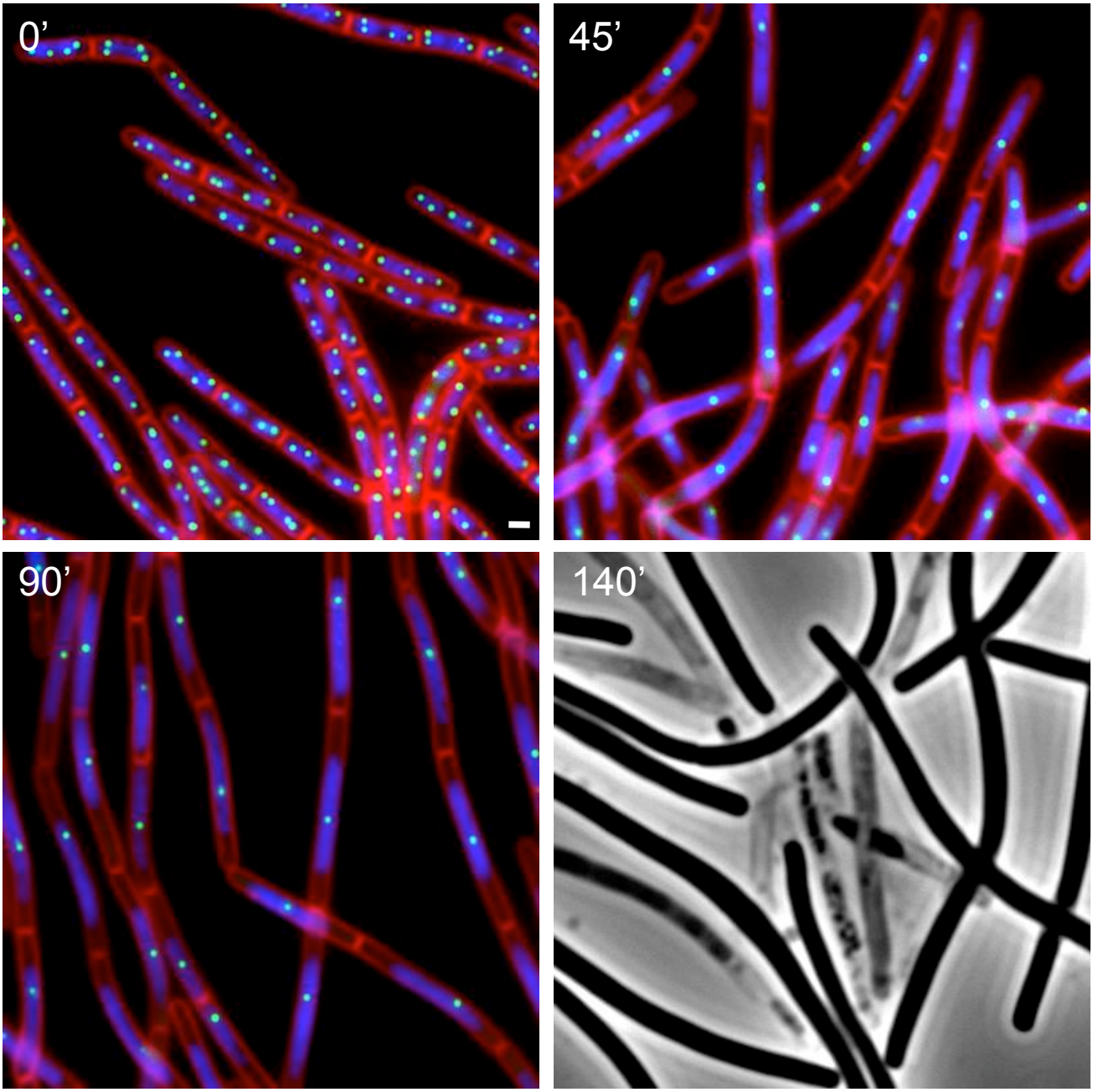


Figure S2
Bernard et al.

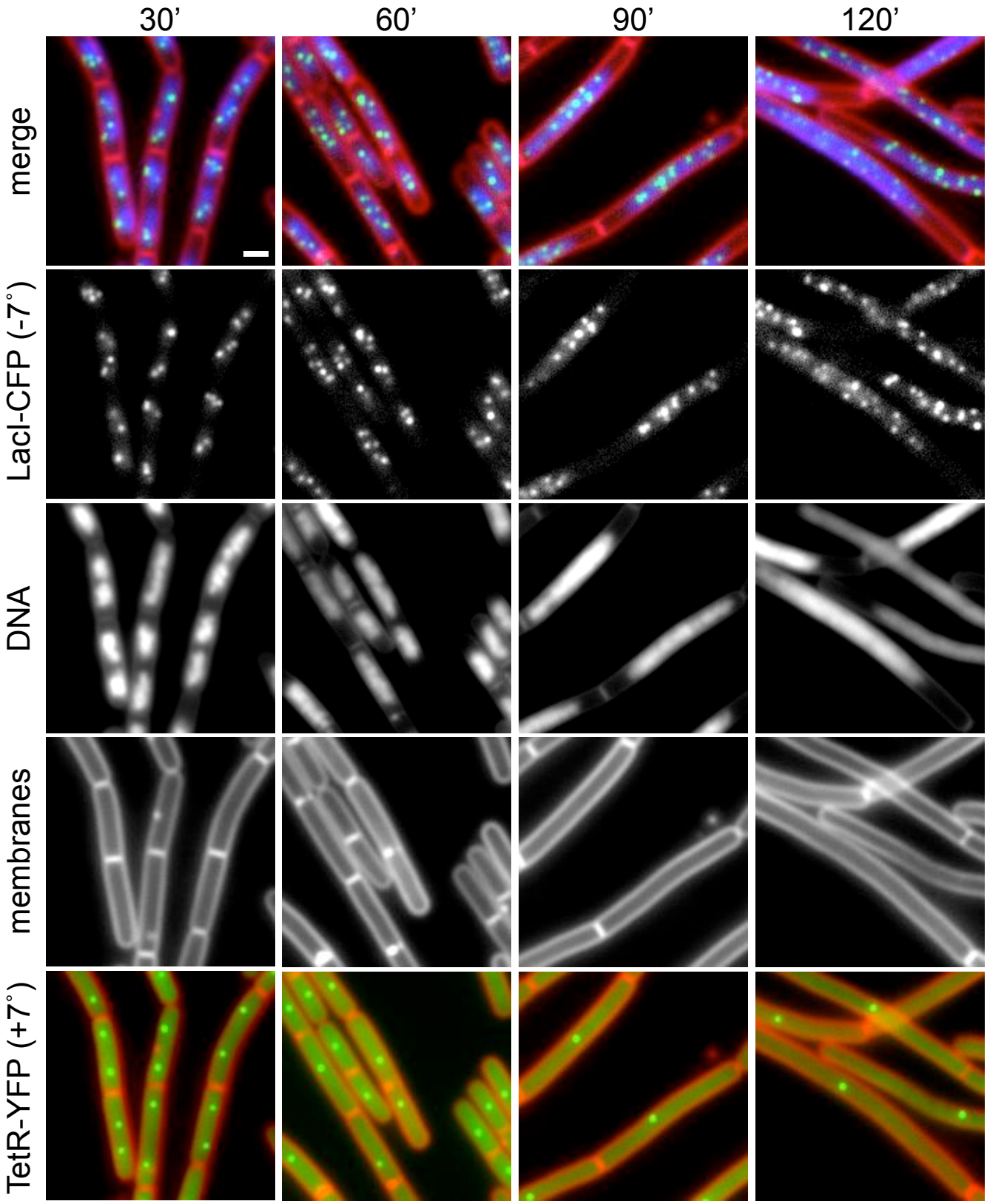
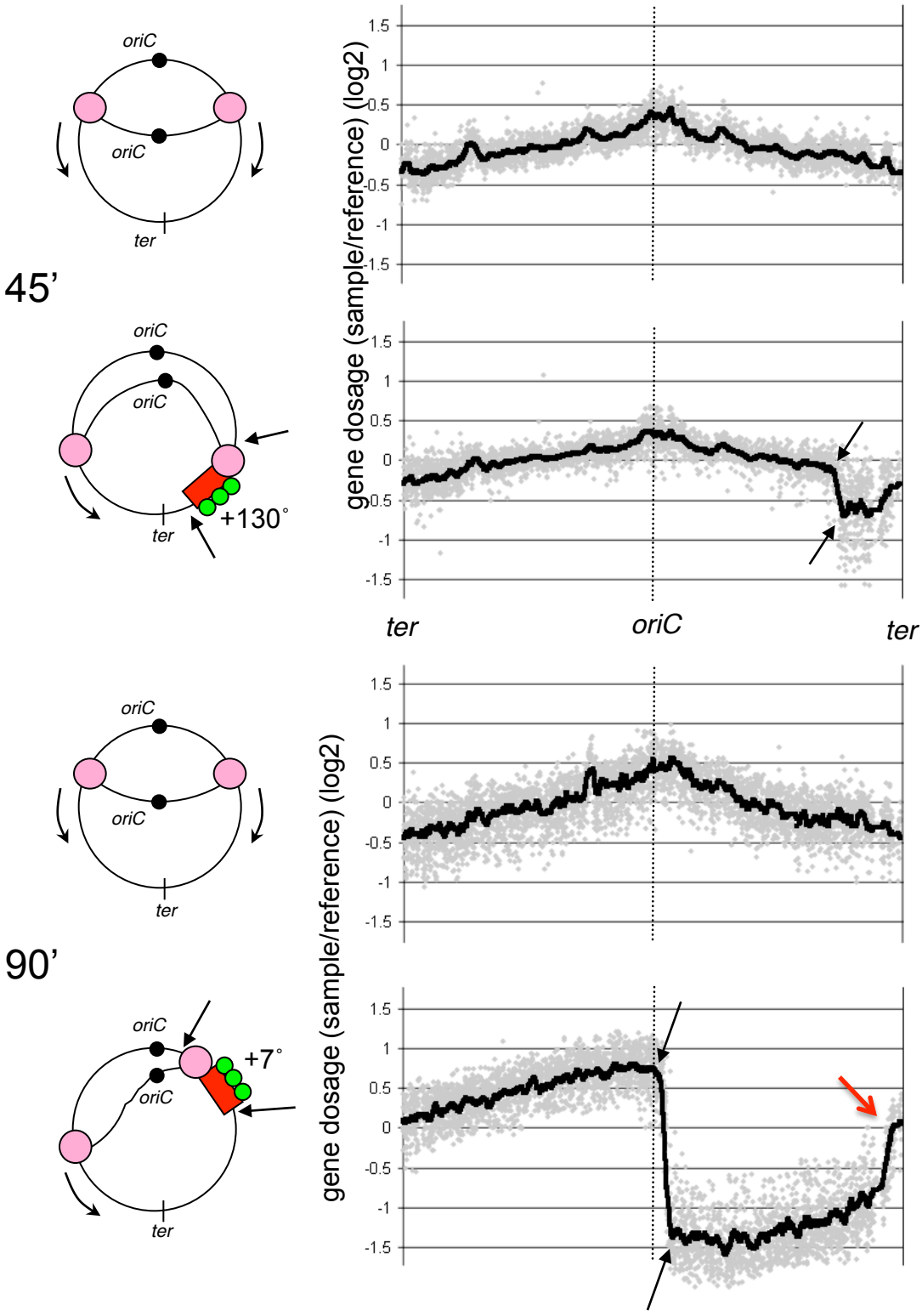


Figure S3
Bernard et al.



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Bernard et al.

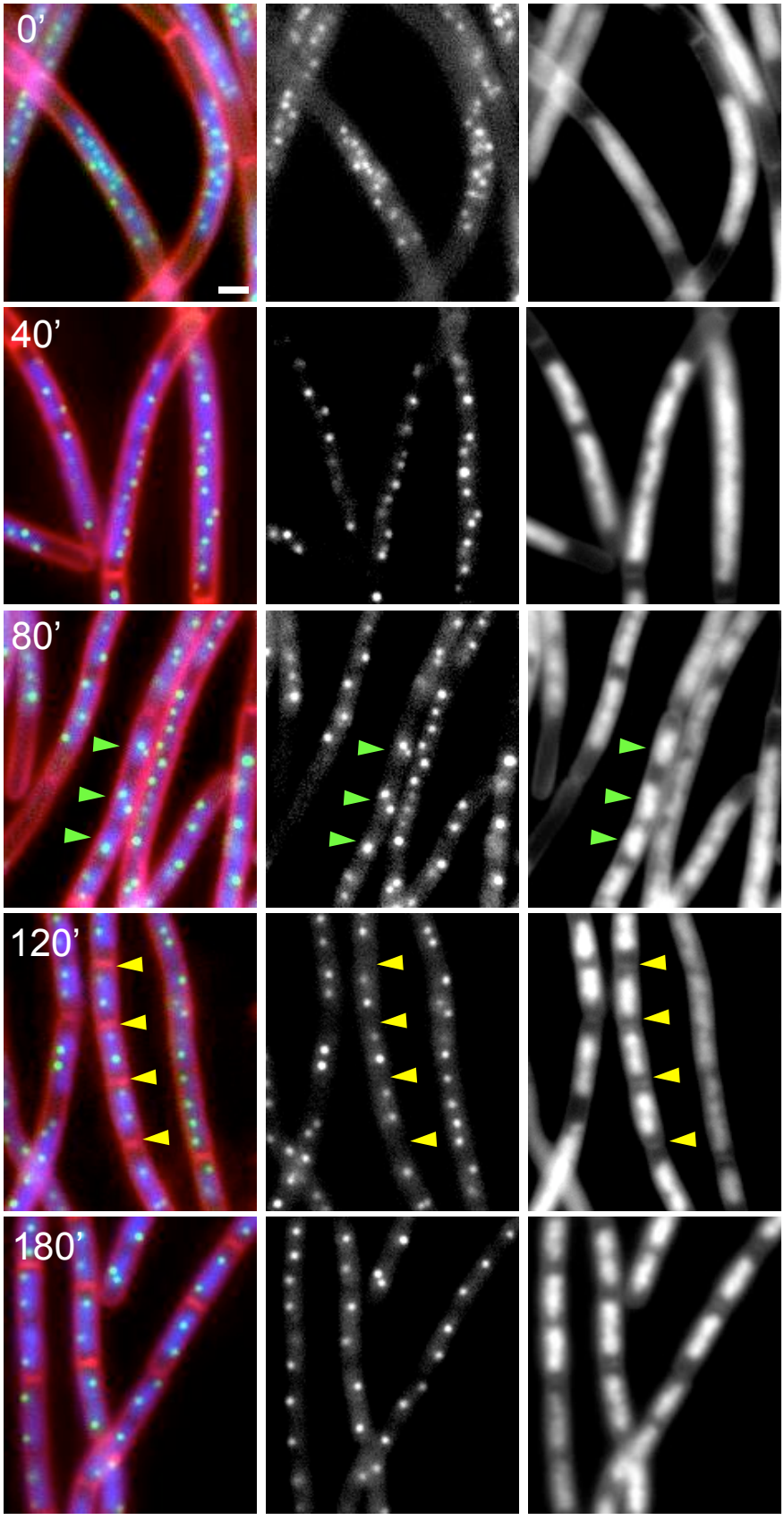

Figure S5
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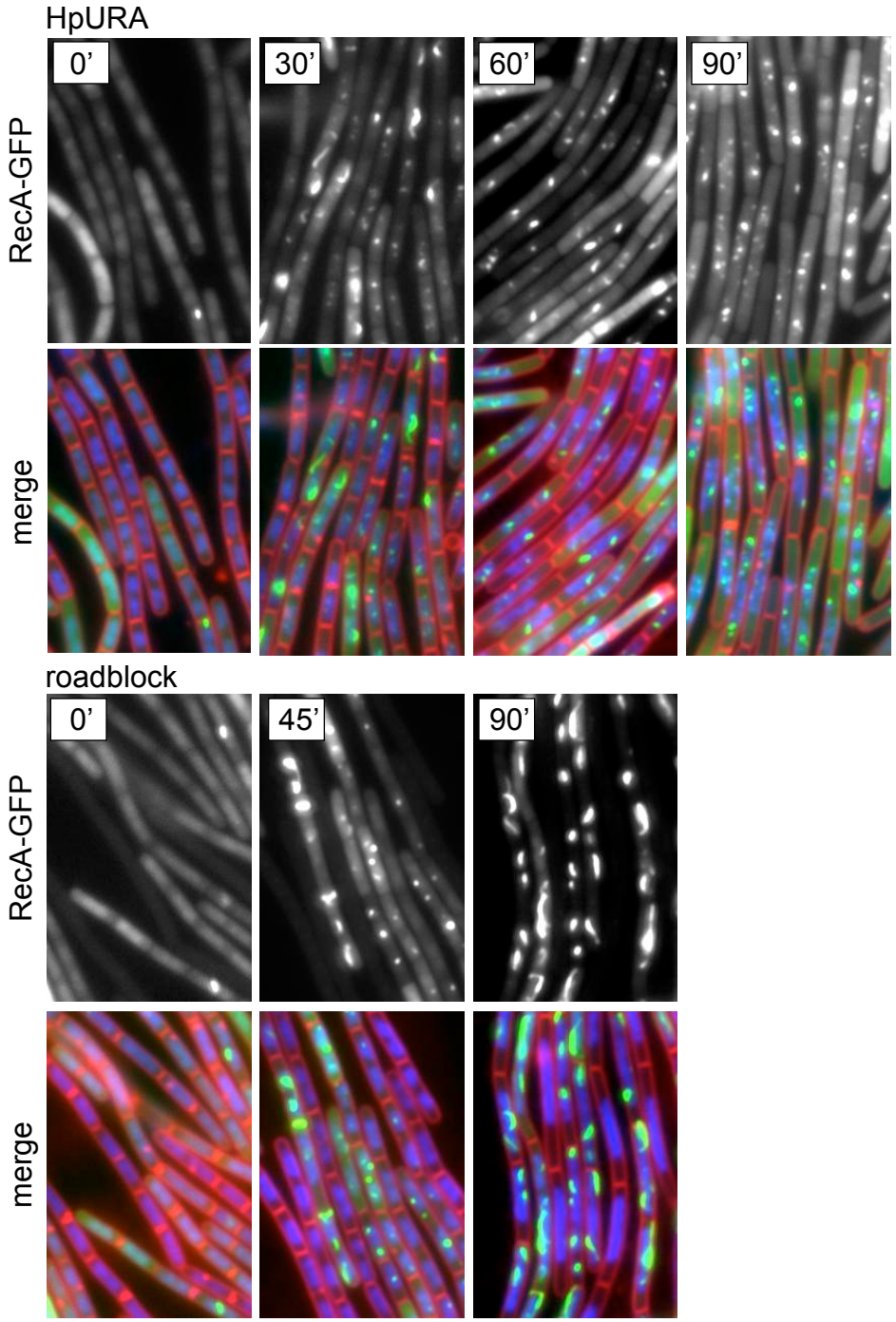

Figure S6
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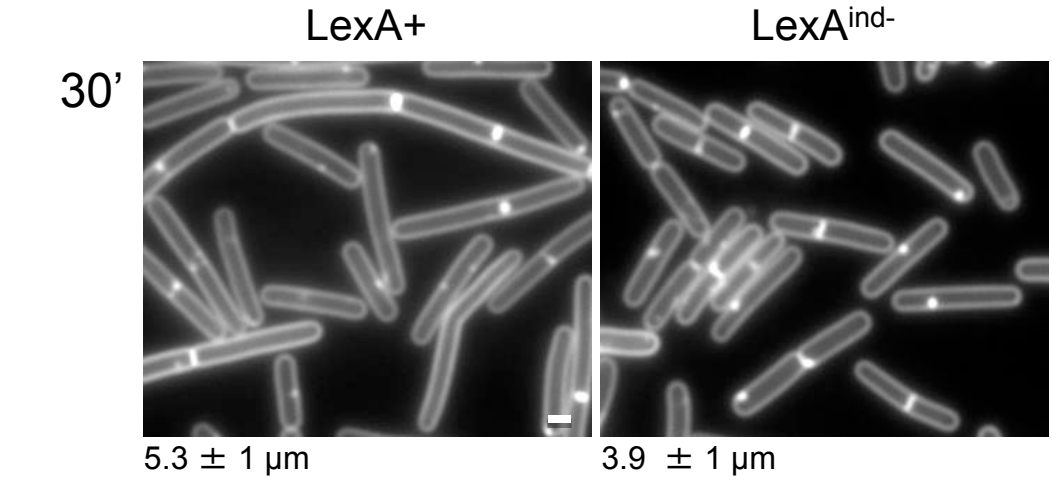
HPUra



replication roadblock

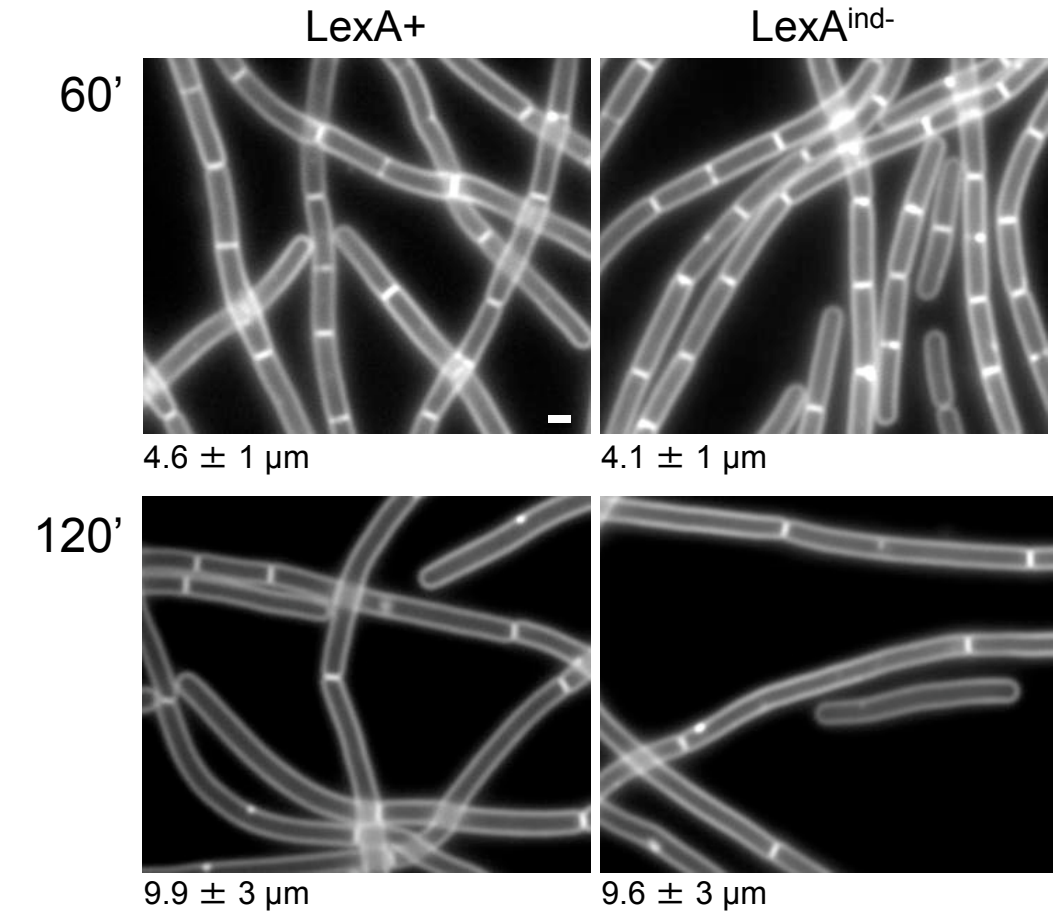


Figure S7
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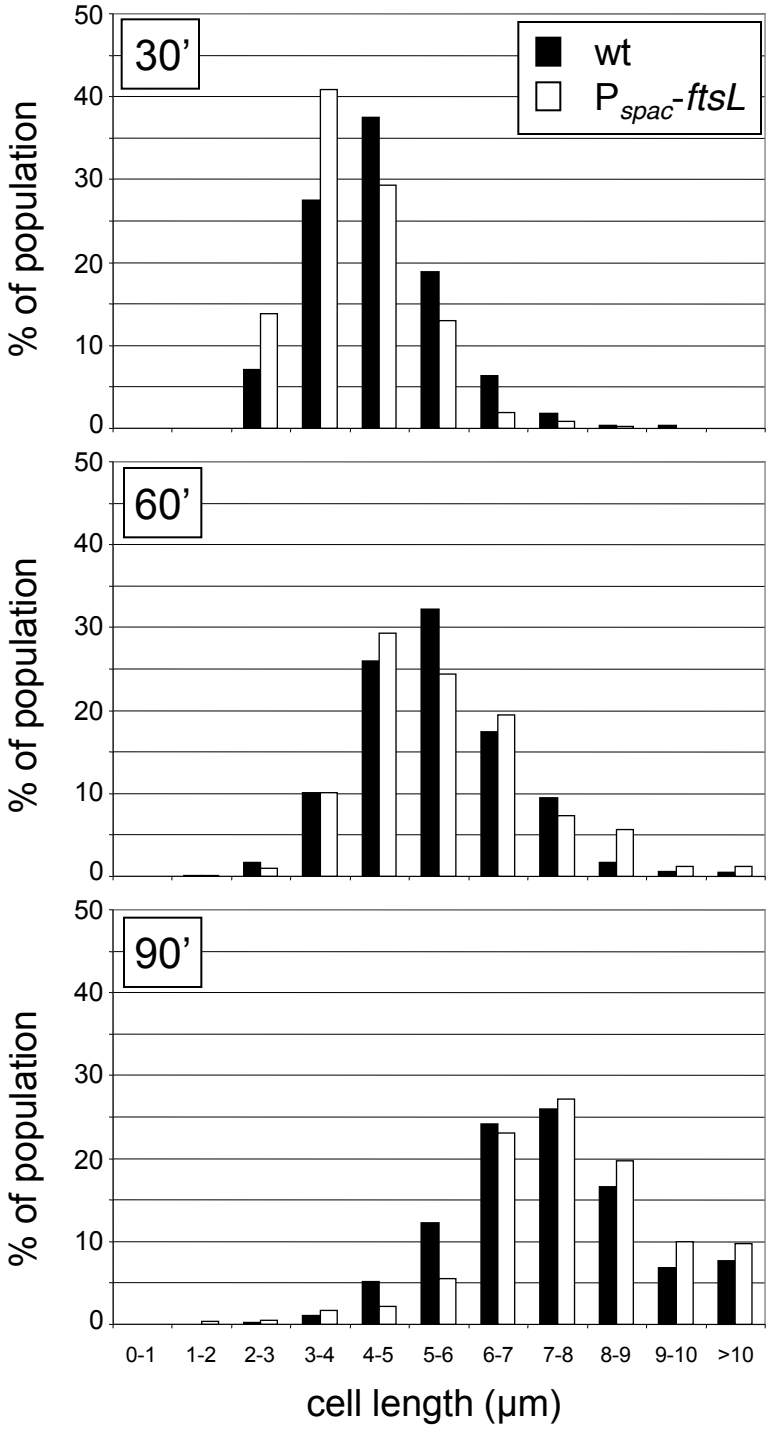

Figure S8
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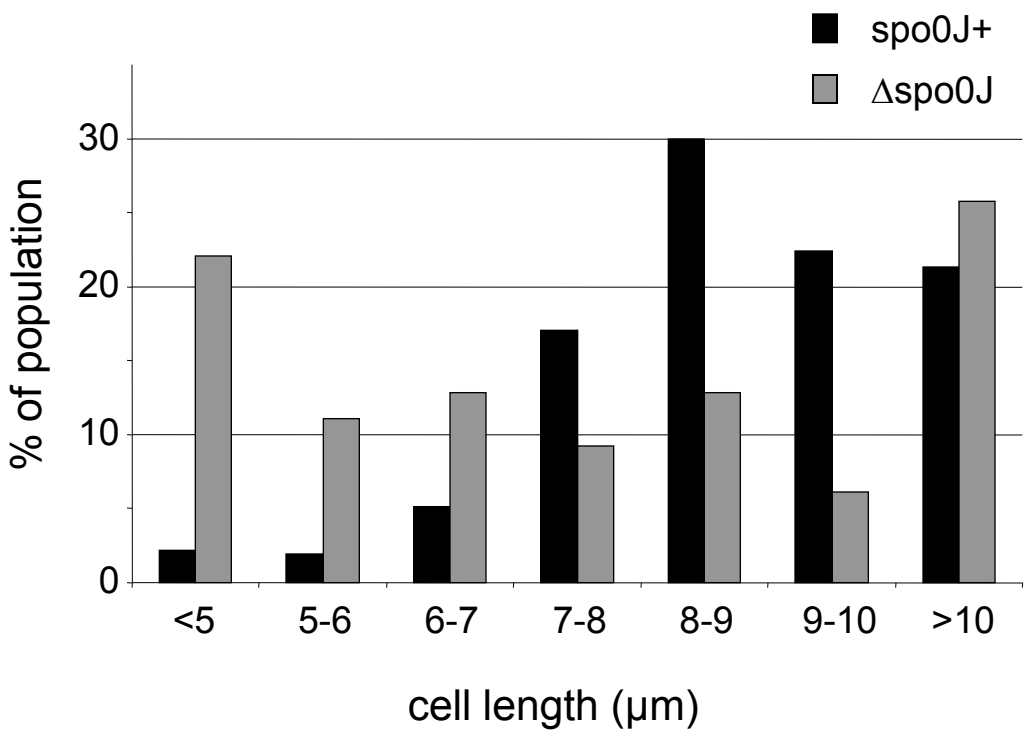

Figure S9
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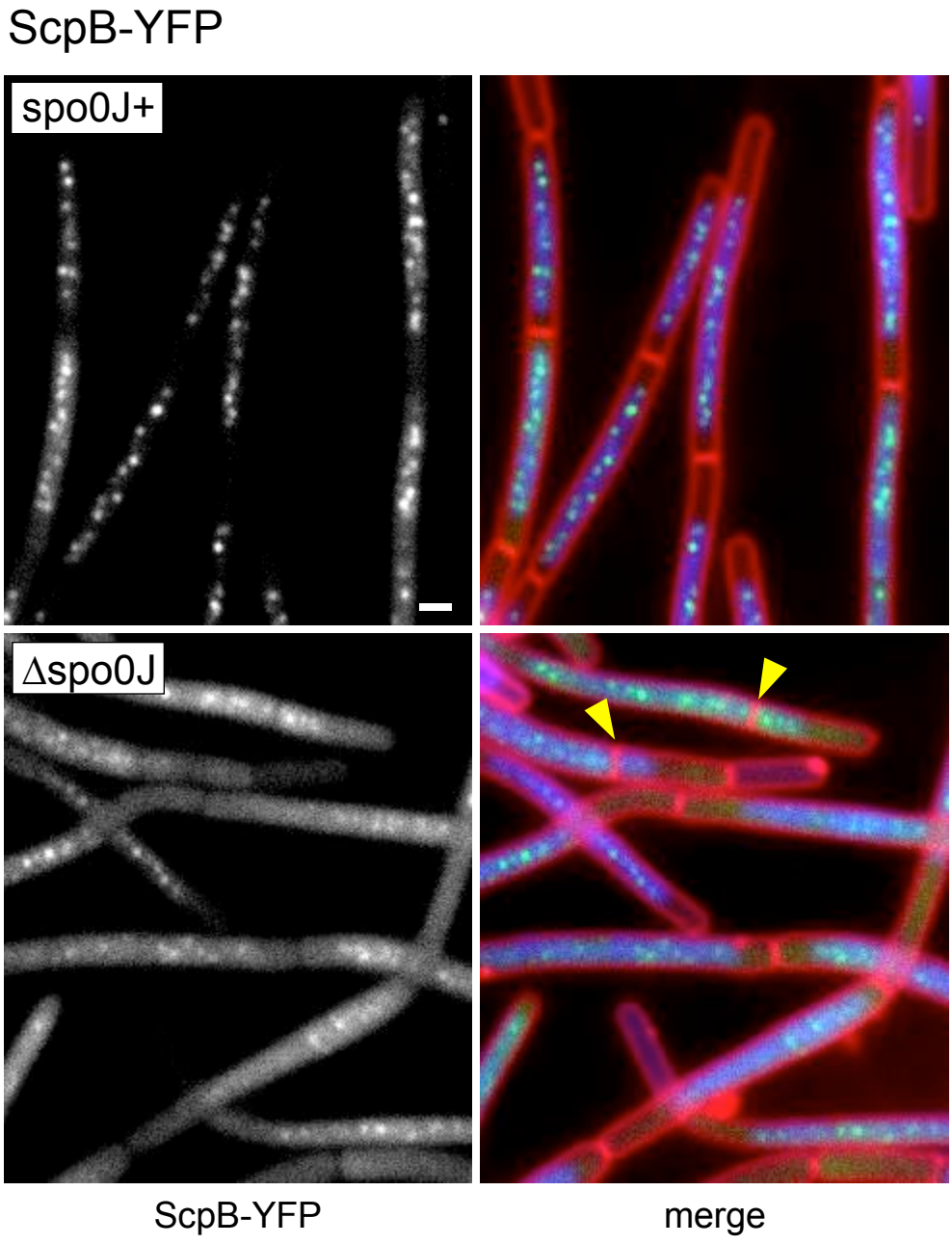

Table S1
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Induced genes

genes	Fold change ± SD	Function or putative function
ypvA	2.95 ± 2.5	Unknown, putative DNA helicase
cotD	2.79 ± 0.8	Spore coat
proH	2.57 ± 2.3	Metabolism
yqxJ	2.49 ± 1.4	Unknown
yqfW	2.39 ± 1.0	Unknown
scpA	2.34 ± 0.9	Condensin complex
yxaI	2.32 ± 1.1	Unknown
ywbH	2.19 ± 1.2	Unknown
ytbJ	2.17 ± 1.0	Unknown
yphF	2.16 ± 0.5	Unknown
lonA	2.16 ± 1.1	Protease
ansR	2.15 ± 0.7	Metabolism
fur	2.14 ± 1.0	Iron uptake
yxbB	2.07 ± 0.9	Unknown
ruvA	2.06 ± 0.8	SOS-induced, recombintational repair
swrA	1.95 ± 0.3	Motility
yrvM	1.95 ± 0.7	Unknown
yaaK	1.93 ± 0.6	Unknown
yqeG	1.93 ± 0.7	Unknown
amyC	1.92 ± 0.8	Metabolism
ypsC	1.92 ± 0.4	Unknown
yqkE	1.90 ± 0.2	Unknown
yphE	1.89 ± 0.4	Unknown
rocR	1.81 ± 0.5	Metabolism
yqfS	1.79 ± 0.6	putative endonuclease
dnaD	1.79 ± 0.6	DNA replication, helicase loader
yvcL	1.78 ± 0.3	Unknown
ytmQ	1.76 ± 0.6	Unknown
yqfR	1.75 ± 0.5	putative RNA helicase
uvrB	1.72 ± 0.3	SOS-induced, Excision repair
yqfF	1.70 ± 0.5	Unknown
pbpA	1.70 ± 0.5	Cell wall, PBP2a
sspA	1.69 ± 0.6	Small spore protein SspA
degU	1.68 ± 0.4	Global regulator
yyaQ	1.67 ± 0.2	Unknown
ypsA	1.65 ± 0.3	Unknown
yvcK	1.65 ± 0.1	Unknown
yqjE	1.64 ± 0.6	Unknown
ypbG	1.62 ± 0.2	Unknown
Obg	1.62 ± 0.5	General stress response, essential GTP binding protein
dgkA	1.61 ± 0.3	Cell wall, dyacylglycerol kinase
ywfO	1.60 ± 0.1	Unknown

Repressed genes

genes	Fold change ± SD	Function or putative function
ykgA	- 4.63 ± 0.8	Unknown, general stress response
hag	- 3.32 ± 0.6	Flagellin, motility
ykgB	- 3.04 ± 0.9	Unknown, operon with *ykgA*
ylaL	- 2.88 ± 0.2	Unknown
prkC	- 2.81 ± 0.5	Kinase, cell growth
ykzL	- 2.58 ± 0.6	Unknown
yloV	- 2.44 ± 0.3	Unknown
yloN	- 2.23 ± 0.8	Unknown
yknX	- 2.23 ± 0.3	Unknown, general stress response
ykqC	- 2.17 ± 0.6	Rnase J1, essential
yisN	- 1.80 ± 0.2	Unknown
ylaF	- 1.74 ± 0.4	Unknown
yonV	- 1.72 ± 0.1	Unknown
ykrP	- 1.67 ± 0.1	Unknown
yloU	- 1.61 ± 0.1	Unknown

Table S2
Strains Used in this study

Strain	Genotype	Reference
BDR11	wild-type (PY79)	Youngman et al,1983
BRB1	*amyE::$P_{spac(C)}$-tetR-gfp (spec), 130°Ω $(tetO)_{120}$ (cat)*	This work
BRB12	*(yneA-yneB-ynzC)::erm*	This work
BRB35	*(yneA-yneB-ynzC)::phleo, amyE::$P_{spac(c)}$-tetR-gfp(spec),130°Ω $(tetO)_{120}$ (cat)*	This work
BRB63	*amyE::$P_{spac(C)}$-tetR-gfp (spec), 7°Ω $(tetO)_{120}$ (cat)*	This work
BRB73	*noc::tet*	Wu Errington, 2004
BRB89	*noc::tet, (yneA-yneB-ynzC)::erm*	This work
BRB114	*noc::tet, (yneA-yneB-ynzC)::erm, amyE::$P_{spac(C)}$-tetR-gfp (spec), 130°Ω $(tetO)_{120}$ (cat)*	This work
BRB117	*noc::tet, amyE::$P_{spac(C)}$-tetR-gfp (spec), 130°Ω $(tetO)_{120}$ (cat)*	This work
BRB150	*amyE::$P_{spac(C)}$-tetR-gfp (spec), 7°Ω $(tetO)_{120}$ (Phleo)*	This work
BRB175	*yndNΩ pRB033a (kan), sacA::P_{yneA}-cfp (erm)*	This work
BRB190	*yndNΩ pRB033a (kan), sacA::P_{yneA}-cfp (erm), amyE::$P_{spac(C)}$-tetR-yfp (spec), 7°Ω $(tetO)_{120}$ (cat)*	This work
BRB206	*noc::tet, amyE::$P_{spac(C)}$-tetR-gfp (spec), 7°Ω $(tetO)_{120}$ (cat)*	This work
BRB225	*Δ(soj-spo0J)::cat, thrC::soj^{+} (erm), amyE::$P_{spac(C)}$-tetR-gfp (spec), 7°Ω $(tetO)_{120}$ (phleo)*	This work
BRB291	*(zapA-yshB)::tet, yhdG::P_{xylA}-zapA-yfp (erm), amyE::$P_{spac(C)}$-tetR-cfp (spec), 7°Ω $(tetO)_{120}$ (cat)*	This work
BRB359	*P_{spac}-smc (cat) Ω, yhdG::P_{pen}-tetR-gfp (spec), 7°Ω $(tetO)_{120}$ (phleo)*	This work
BRB636	*recA-gfp-mut2(A206K) (spec), yhdG::$P_{spac(C)}$-tetR-cfp (erm), 7°Ω $(tetO)_{120}$ (phleo)*	This work
BNS1733	*dnaB134 (ts) zhb-83::Tn917 (erm)*	Wagner et al, 2009
BDR2429	*recA-gfp-mut2(A206K) (spec)*	Simmons et al, 2009

Table S3
Plasmids used in this study

plasmid	description	reference
pRB012	*Δ(yneA-yneB-ynzC)::phleo*	This work
pRB013	*Δ(yneA-yneB-ynzC)::erm*	This work
pRB015	*sacA::P_{yneA}-cfp (erm)*	This work
pRB020	*+7° Ω $(tetO)_{120}$ (cat)*	This work
pRB032	*+7° Ω $(tetO)_{120}$ (phleo)*	This work
pRB033	*yndN Ω kan*	This work
pRB046	*yhdG::P_{xylA}-zapA-yfp (erm)*	This work
pKM218	*+130° Ω $(tetO)_{120}$ (cat)*	Marquis et al, 2008

Table S4 Oligonucleotides used in this study

Primer	Sequence
oRB001	cgcGAATTCccattgttcgctgtgttttgc
oRB002	cgcGGATCCtcatgatcataacctccaacag
oRB003	cgccGTCGACgaggaatacggcaatatcgtattc
oRB004	cctgCGGCcgactacgttaaatgaatcgcg
oRB005	gcttcatcatcttctgtcatcg
oRB006	tccattaaatcaccatcaccgc
oRB010	gccgCAATTGttaaaactttatcaacagaagcgg
oRB011	cggAAGCTTattatttgctgccataagcaac
oRB014	gccGAATTCcaaacgggccaaatggc
oRB015	ggcAAGCTTaacaggaatgtttgttcgc
oRB018	cgcGGATCCgtactcgagatcatggaacattacctgagc
oRB019	cgcGAATTCatgattggcggcactactg
oRB029	caggtattcttaggccgtgatttc
oRB032	gcacagatgcgtaaggagaaaatac
oRB035	gccGAATTCggcatcgttgaacaagtcggg
oRB036	cgcGGATCCgaaccagacatcccgagaaatgac
oRB039	cgcAAGCTTacataaggaggaactactatgtctgacggcaaaaaacaaaaac
oRB040	cggCTCGAGatccttttctttaagctgacgctc
oDR078	gccGGATCCttatttgtatagttcatccatgcc
oDR079	gcgCTCGAGggttccggaatgag
oDR699	gcgtcGAATTCccggtggaaacgaggtcatc
oDR700	cgcAAGCTTgaatatttgattgatcgtaaccag

restriction endonuclease sites are capitalized