SUPPLEMENTAL MATERIAL

Plasmid construction

pKM288 [*dnaX-yfp (phleo)]* was generated by subcloning an EcoRI-PstI fragment (*dnaX-yfp*) from pKL183 (Lemon and Grossman, 1998) into pDT2. pDT2 is a pUC19 derivative containing a phleomycin resistance cassette between EcoRI and NdeI (T. Doan and D.Z.R., unpublished).

pKM322 [*amyE::Psweet-dnaA-gfp (spec)*] was generated in a three-way ligation with a NheI-PstI PCR product containing *dnaA* (oligonucleotide primers odr694 and odr701 with PY79 genomic DNA as template) and a PstI-BamHI PCR product containing *gfp* (oligonucleotide primers oDR702 and oDR78 with pKL147 (Lemon and Grossman, 1998) as template DNA) and pDR160 cut with BamHI and EcoRI. pDR160 [*amyE::Psweet-xylR (spec)*] is an ectopic integration vector containing the xylose-inducible Psweet promoter (Bhavsar *et al.*, 2001) (D.R.Z., unpublished).

pJW002 [*amyE::Phyperspank-opt.rbs-sirA (spec)*] was generated in a two-way ligation with a HindIII-NheI PCR product containing *sirA* (oligonucleotide primers oJW001 and oJW002 with PY79 genomic DNA as template) and pDR111 [*amyE*::*Phypespank (spec)*] cut with HindIII and NheI (D.R.Z., unpublished).

pJW005 [*yhdG::Phyperspank-opt.rbs-sirA (phleo)*] was generated in a two-way ligation with a PCR product containing *sirA* by cloning *yneE* (oligonucleotide primers oJW001 and oJW002 with PY79 genomic DNA as template) and pJW004 cut with HindIII and NheI. pJW004 [*yhdG::Phyperspank (phleo)*] was generated in a two way ligation by cloning the 1770 bp BamHI –EcoRI fragment containing *Phyperspank* and *lacI* from pDR111 into pBB280 cut with EcoRI and BamHI. pBB280 [*yhdG::phleo*] is an ectopic integration vector for double crossover insertions into the nonessential *yhdG* locus (B. Burton and D.Z.R., unpublished).

pJW006 [*amyE::Phyperspank-gfp-sirA (spec)*] was generated in a two-way ligation with a HindIII-NheI PCR product containing *gfp-sirA* (oligonucleotide primers oLM022 and oJW002 and pLM021 as template DNA) and pDR111 cut with HindIII and NheI.

pJW020 [*ycgO::PxylA-gfp-sirA (erm)*] was generated by subcloning the HindIII-BamHI fragment containing *gfp-yneE* from pJW006 into the pRB036. pRB036 [*ycgO::PxylA-xylR (erm)*] was generated by cloning the EcoRI-BamHI *PxylA-xylR* fragment from pDR150 into pKM084 cut with EcoRI and BamHI. pDR150 [amyE::PxylA-xylR (spec)] is an ectopic integration containing the xylose-inducible promoter PxylA (D.Z.R., unpublished). pKM084 [*ycgO::erm*] is an ectopic integration vector for double crossover insertions into the nonessential *ycgO* locus (K.M. and D.Z.R., unpublished).

pJW022 [*BK-dnaA (kan)*] was generated by cloning a NdeI-BamHI PCR product containing *dnaA* (oligonucleotide primers oJW021 and oDR692 with PY79 genomic DNA as template) into the pGBKT7 (Clontech) cut with NdeI and BamHI.

pJW023 [*ADT7-sirA (amp)*] was generated by cloning an EcoRI-BamHI PCR product containing *sirA* (oligonucleotide primers oJW009 and oJW002 with PY79 genomic DNA as template) into pGADT7 (Clontech) cut with EcoRI BamHI.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. SirA expression is lethal to vegetatively growing cells. Cells (strain BJW38) harboring an IPTG-inducible promoter ($P_{hyperspank}$) fused to *sirA* were analyzed before and after the addition of IPTG. Time (in min) after induction is indicated. Membranes were stained with the fluorescent dye FM4-64 (red) and DNA was stained with DAPI (false-colored green). Examples of anucleate cells (white carets) and guillotined nucleoids (yellow carets) are indicated.

Figure S2. Replisome and origin foci are unaffected by the addition of IPTG. To control for the loss of DnaX-YFP foci (and reduction in number of TetR-GFP origin foci) upon the induction of SirA, cells (BKM1585 and BKM865) lacking $P_{hyperspank}$-*sirA* were treated with IPTG for 60 min. The replisome and origin foci were indistinguishable before and after addition of IPTG. Time (in min) before and after induction is indicated. DnaX-YFP and TetR-GFP foci were

false-colored green. Membranes were stained with fluorescent dye FM4-64 (red) and DNA was stained with DAPI (blue).

Figure S3. SirA inhibits DNA replication. (*A*) Cells were analyzed for ongoing replication using an SSB-GFP fusion. The Images show cells (strain BKM1797) before and after SirA induction. Time (in minutes) after induction is indicated. Prior to SirA induction all cells are actively replicating as evidenced by SSB-GFP (green) foci. Membranes were stained with fluorescent dye FM4-64 (red) and DNA was stained with DAPI (blue). Immunoblot analysis shows that SSB-GFP remains intact after SirA induction. SSB-GFP was analyzed using anti-GFP antibodies and the caret identifies the predicted size of free GFP. EzrA and σ^A were used to control for loading. (*B*) Cells were analyzed for origin content using TetR-GFP bound to an array of *tet* operators adjacent to *oriC*. Images show cells (strain BJW141) before and after SirA induction. Prior to SirA induction, most cells have 2 or 4 origin foci per DNA mass. After SirA induction, cells have a single origin focus per nucleoid.

Figure S4. GFP-SirA is less potent inhibitor of growth than SirA. Cells harboring an IPTG-inducible promoter ($P_{hyperspank}$) fused to *sirA* (P_{hy}-*sirA*; strain BJW38), *gfp-sirA* (P_{hy}-*gfp-sirA*; strain BJW101) or *gfp* (P_{hy}-*gfp*; strain BDR1003) were streaked on LB agar plates with and without IPTG.

Figure S5. The localization of GFP-SirA produced at low and high levels. At low levels, GFP-SirA does not efficiently inhibit replication and localizes in nucleoid-associated foci. At high levels, GFP-SirA inhibits replication and is present as a diffuse cytosolic haze. *(A)* Cells harboring an IPTG-inducible promoter ($P_{hyperspank}$) (strain BJW101) or a xylose-inducible promoter (P_{xylA}) (strain BJW196) fused to *gfp-sirA* were visualized after 60 min of induction. Membranes were stained with the fluorescent dye FM4-64 (red) and DNA was stained with DAPI (blue). *(B)* Comparison of protein levels in the two strains shown in A. Upper panel: Immunoblot analysis of GFP-SirA over an induction time course. Time (in min) is indicated. GFP-SirA was analyzed using anti-GFP antibodies. Lower Panel: Quantitative comparison of GFP-SirA levels. Immunoblot analysis of serial dilutions of whole-cell extracts from strain BJW101 ($P_{hyperspank}$-*gfp-sirA*) and strain BJW196 (P_{xylA}-*gfp-sirA*). The lysates were from cells

induced for 60 min. The lysate from strain BJW196 was serially diluted into a whole-cell extract from wild-type cells (lacking GFP) such that each lane contained the same amount of total protein. Expression from the P_{xylA} promoter resulted in ~10-fold lower steady-state levels of the GFP-SirA than expression from $P_{hyperspank}$.

Figure S6. GFP-SirA co-localizes with *oriC*. Cells (strain BJW252) harboring a *tetO* array adjacent to *oriC* and producing low levels of GFP-SirA and TetR-mCherry were visualized by fluorescence microscopy. Membranes were stained with TMA-DPH (blue). GFP-SirA (green), TetR-mCherry (red) bound near *oriC*, and the merged image are shown. Carets highlight prominent foci present in all channels.

Figure S7. KinA induction in nutrient replete conditions results in over-replication and production of twins. (*A*) Cells sporulated in rich medium by induction of the sensor kinase KinA were analyzed for origin content using TetR-GFP bound to a *tetO* array adjacent to *oriC*. The images show the SirA mutant (strain BJW279) after 2 hrs of KinA induction. Yellow carets highlight cells with more than two origin foci (green) in the forespore. White carets highlight cells with more than two origin foci in the mother cell. The pink caret highlights a representative twin, possessing two developing forespores with four origin markers and four nucleoids. Membranes were stained with FM4-64 (red) and DNA was stained with DAPI (blue). (*B*) An example of a mother cell and twin forespores after 4 hr of KinA induction. White carets indicate the location of phase-grey endospores. At this late stage, DAPI is unable to access the forespore chromosomes.

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Figure S1
Wagner et al.

SirA induction

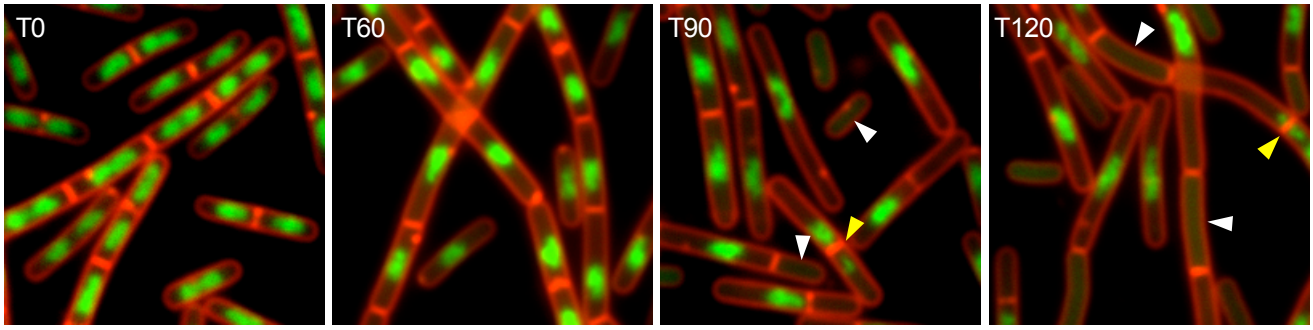
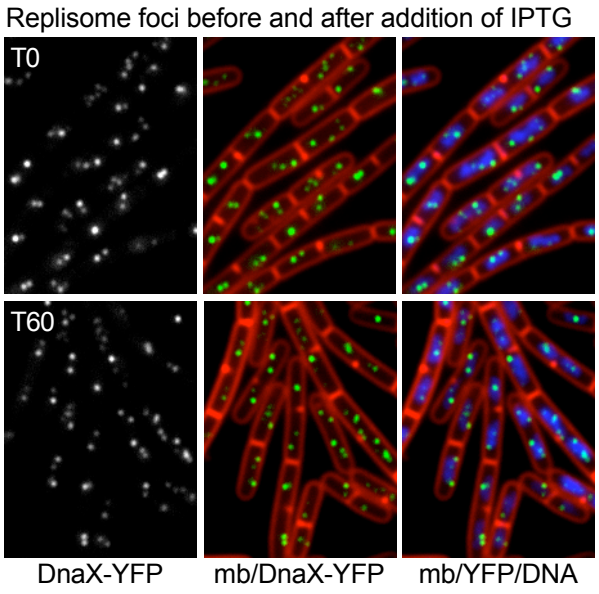


Figure S2
Wagner et al.



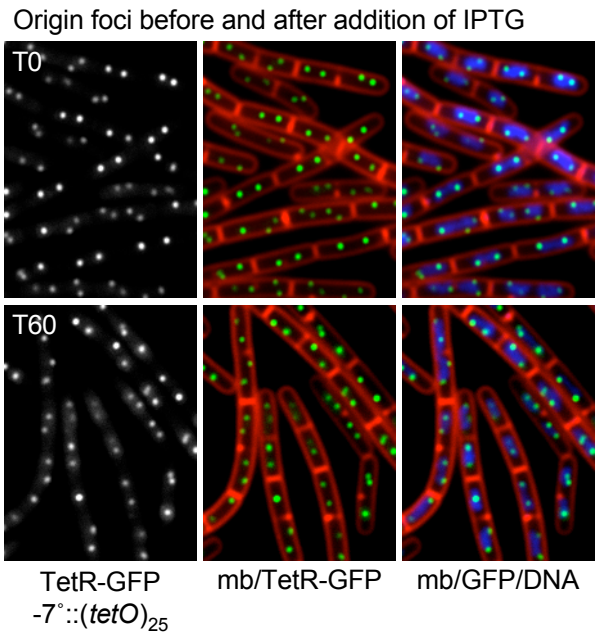
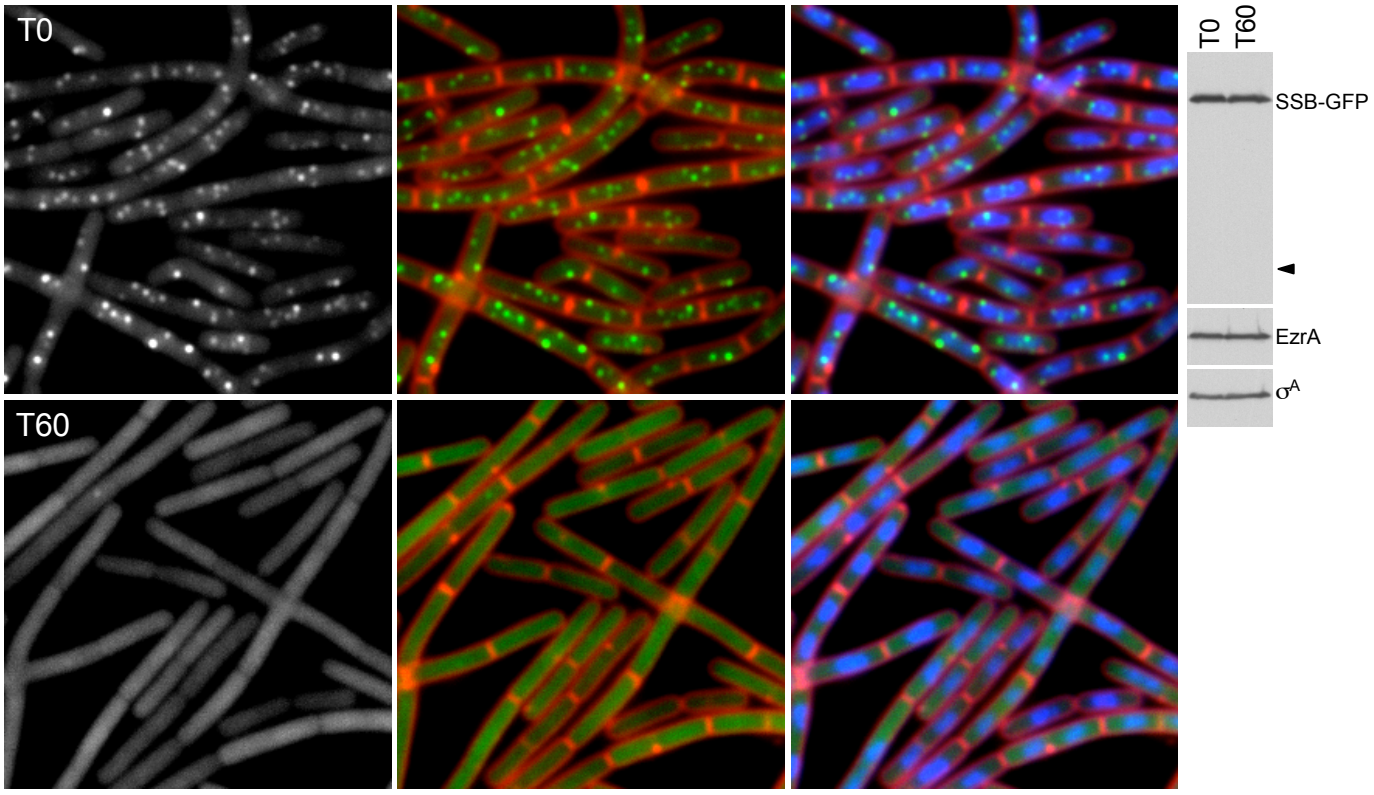


Figure S3
Wagner et al.

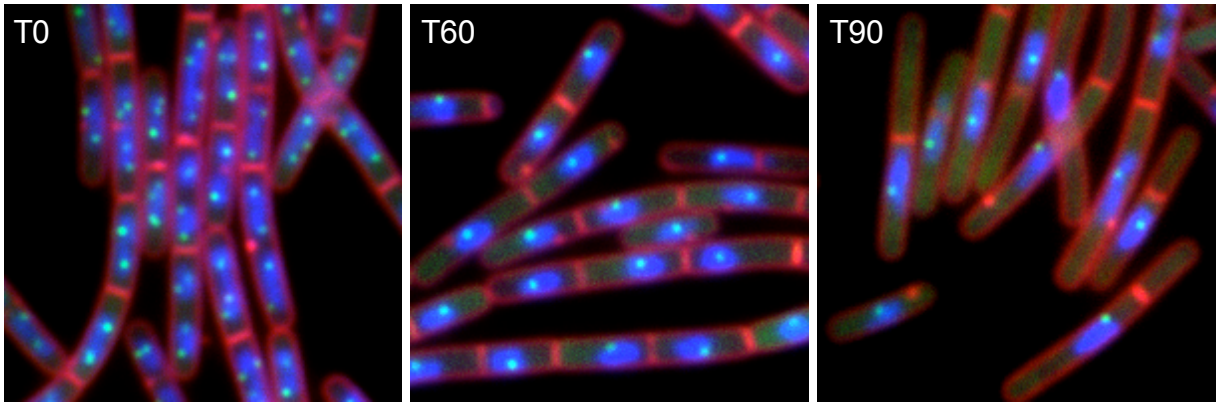
A

SirA induction



B

SirA induction



-7°::(*tetO*)$_{25}$, TetR-GFP

Figure S4
Wagner et al.

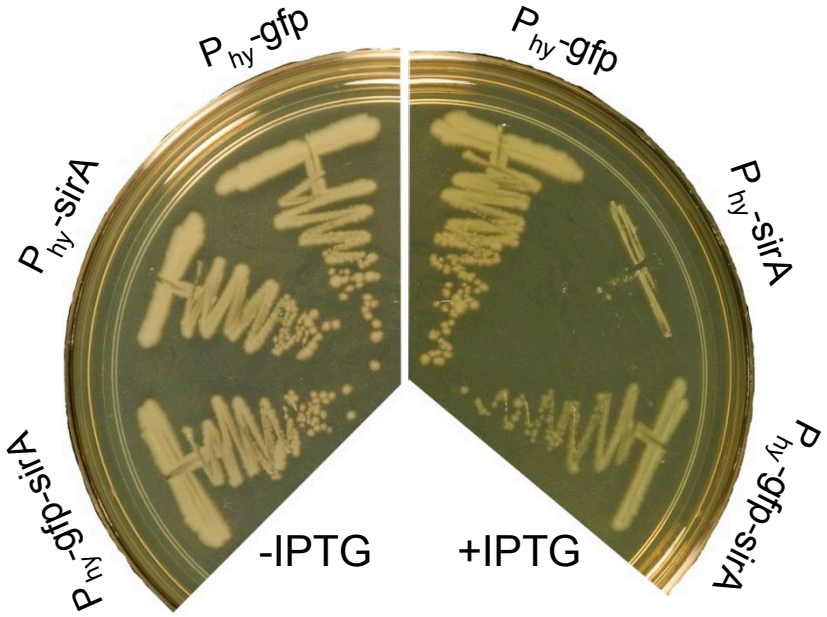
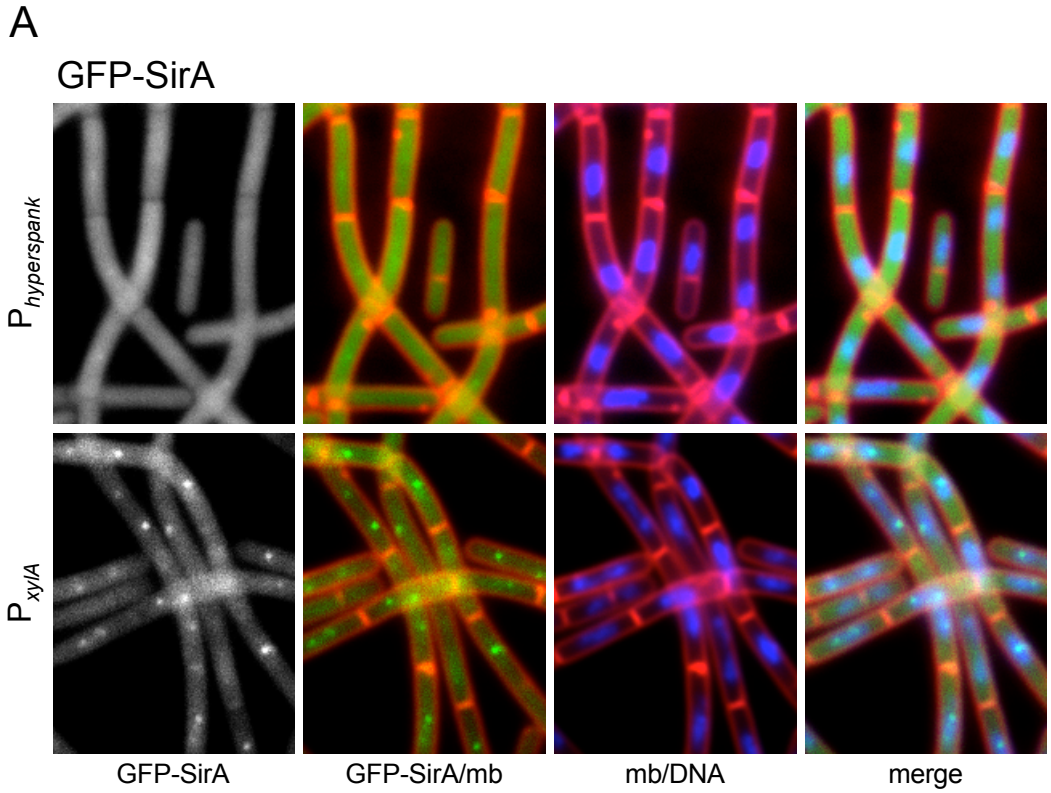


Figure S5
Wagner et al.



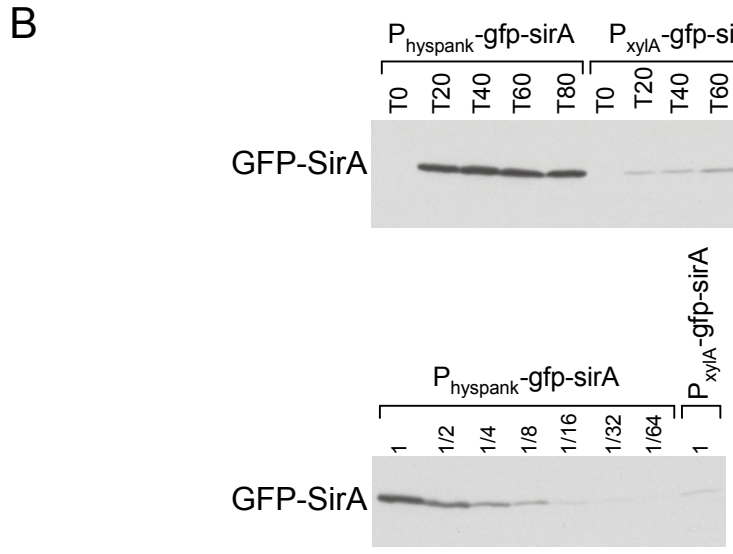


Figure S6
Wagner et al.

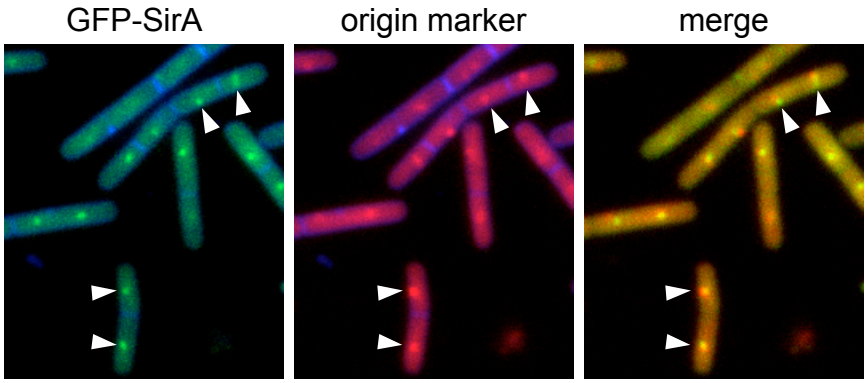
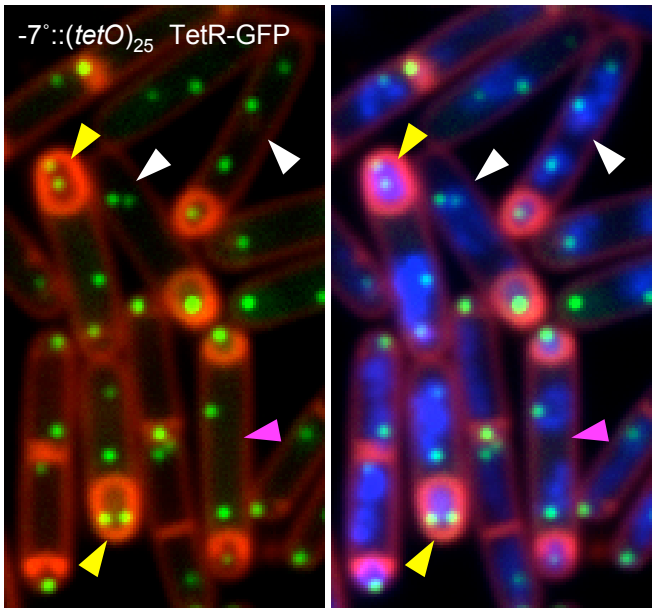


Figure S7
Wagner et al.

A

KinA induction (hour 2)



B

KinA induction (hour 4)

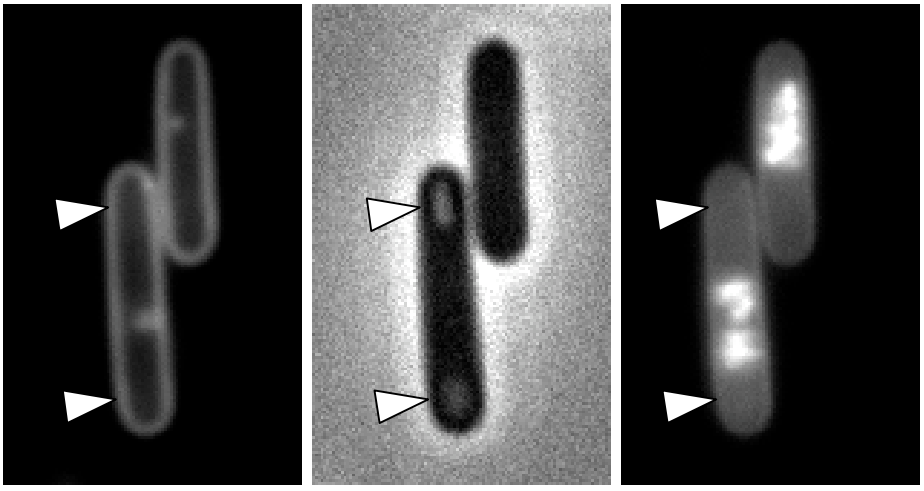


Table S1
Strains Used in this study

Strain	Genotype	Reference	Figure
PY79	wild-type	Youngman et al. 1983	
BDR1003	*amyE::Phyperspank-gfp(spec)*	This work	2A, S3
LAS223	*dnaAΩPspac-dnaA (tet)*	Simmons et al. 2007	2B, 3C
BJW38	*amyE::Phyperspank-sirA (spec)*	This work	2A, 2B, 3C, S1
BJW82	*dnaAΩPspac-dnaA (tet), dnaXΩ-yfp (phleo)*	This work	
BJW84	*amyE::Phyperspank-sirA (spec),dnaXΩ-yfp (phleo)*	This work	3A
BJW91	*dnaXΩ-yfp (phleo), sirA::tet*	This work	7A
BJW101	*amyE::Phyperspank-gfp-sirA (spec)*	This work	S3, S4A, S4B, S4C
BJW118	*amyE::Pspac(c)-tetR-gfp (spec), yycR::(tetO)25 (erm), sirA::tet*	This work	
BJW136	*amyE::Pspac(c)-tetR-gfp (spec), yycR::(tetO)25 (erm), dnaAΩPspac-dnaA (tet)*	This work	
BJW141	*amyE::Pspac(c)-tetR-gfp (spec), yycR::(tetO)25 (erm), yhdG::Phyperspank-sirA (phleo)*	This work	3B, S2B
BJW145	*amyE::Pspac(c)-tetR-gfp (spec), ywjI::(tetO)25 (erm)*	This work	
BJW156	*amyE::Pspac(c)-tetR-gfp (spec), ywjI::(tetO)25 (erm), sirA::tet*	This work	
BJW173*	*pheA1, 359°::oriN repN (kan), ΔoriC-S, yhdG::Phypespank-sirA (phleo)*	This work	4
BJW174*	*trpC2, pheA1, 359°::oriC dnaAN (kan),Δ(oriC-L)::spec, yhdG::Phyperspank-sirA (phleo)*	This work	4
BJW192	*yhdG::Phyperspank-sirA (phleo), amyE::Pspac(c)-tetR-gfp (spec), ywjI::(tetO)25 (erm)*	This work	
BJW196	*ycgO::PxylA-gfp-sirA (erm)*	This work	6A, S4A, S4B, S4C
BJW197	*dnaAΩPspac-dnaA (tet), ycgO::PxylA-gfp-sirA (erm)*	This work	5B, 5C, 6B
BJW218	*amyE::Psweet-dnaA-gfp (spec), yhdG::Phyperspank-sirA (phleo)*	This work	5A
BJW230	*kinAΩPhyperspank-kinA (cat), dnaXΩ-yfp (phleo)*	This work	7C
BJW232	*kinAΩPhyperspank-kinA (cat), dnaXΩ-yfp (phleo), sirA::tet*	This work	7C
BJW252	*amyE::PxylA-tetR-mcherry (spec), yycR::(tetO)120 (cat), ycgO::PxylA-gfp-sirA (erm)*	This work	S5
BJW271	*amyE::PxylA-ssb-gfp (cat), spo0AΩPhyperspank-spo0A(sad67) (spec)*	This work	7B
BJW275	*amyE::PxylA-ssb-gfp (cat), spo0AΩPhyperspank-spo0A(sad67) (spec), sirA::tet*	This work	7B
BJW279	*amyE::Pspac(c)-tetR-gfp(spec), yycR::(tetO)25 (erm), kinAΩPhyperspank-kinA (cat)*	This work	7D
BJW280	*amyE::Pspac(c)-tetR-gfp(spec), yycR::(tetO)25 (erm), kinAΩPhyperspank-kinA (cat), sirA::tet*	This work	7D, S6
BKM830	*amyE::Pspac(c)-tetR-gfp (spec)*	Marquis et al. 2008	
BKM838	*yycR::(tetO)120 (cat)*	Marquis et al. 2008	
BKM865	*amyE::Pspac(c)-tetR-gfp (spec), yycR::(tetO)25 (cat)*	This work	
BKM918	*yycR::(tetO)25(erm)*	This work	
BKM1585	*dnaXΩ-yfp (phleo)*	This work	7A
BKM1797	*yhdG::Phyperspank-sirA (phleo), amyE::PxylA-ssb-gfp (cat)*	This work	S2A
BNS1733	*dnaB134 (ts-) zhb-83::Tn917 (erm)*	This work	3C
HM422	*amyE::PxylA-tetR-mcherry (spec), trpC2*	Murray & Errington 2008	
MF1913	*kinAΩPhyperspank-kinA(cat)*	Fujita & Losick 2005	
MF2146	*spo0AΩPhyperspank-spo0A(sad67) (spec)*	Fujita & Losick 2005	
MMB208*	*pheA1, 359°::oriN repN (kan),ΔoriC-S*	Berkmen & Grossman 2007	
MMB612*	*trpC2, pheA1, 359°::oriC dnaAN (kan), Δ(oriC-L)::spec*	Berkmen & Grossman 2007	
RL2285	*amyE::PxylA-ssb-gfp (cat)*	a gift from M Yang & R Losick	
AH109	*MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4D, gal80D, LYS2::GAL1UAS-GAL1TATA-HIS3, GAL2UAS-GAL2TATA-ADE2, URA3::MEL1UAS-MEL1TATA-lacZ, MEL1*	Clontech	

* Derived from the wild-type strain JH642 (*trpC2*, *pheA1*)

Table S2
Plasmids used in this study

plasmid	description	reference
pKM288	*dnaX-yfp (phleo)*	This work
pKM322	*amyE::Psweet-dnaA-gfp (spec)*	This work
pJW002	*amyE::Phyperspank-opt.rbs-sirA (spec)*	This work
pJW005	*yhdG::Phyperspank-opt.rbs-sirA (phleo)*	This work
pJW006	*amyE::Phyperspank-gfp-sirA (spec)*	This work
pJW020	*ycgO::PxylA-gfp-sirA (erm)*	This work
pJW022	*BK-dnaA (kan)*	This work
pJW023	*ADT7-sirA (amp)*	This work

Table S3
Oligonucleotides used in this study

Primer	Sequence (5' to 3')
oDR078	gccggatccttatttgtatagttcatccatgcc
oDR098	cggctgcagcaacgttcttgccattgctgc
oDR458	gccaagcttagtgatagagaagacgaaccgtcc
oDR459	cggctcgagcattacagtttacgaaccgaacagg
oDR692	cgcggatcctatttaagctgttctttaatttc
oDR701	cggctgcagtttaagctgttctttaatttcttttac
oDR702	cggctgcagggttccggaatgagtaaaggag
oJW001	cgcaagcttacataaggaggaactactatggaacgtcactactatacg
oJW002	gccgctagcggatccggttttagacaaaatttctttc
oJW009	gccgaattcatggaacgtcactactatacg
oJW014	cgcaagcttacataaggaggaactactatgaaagtaagcaccaaagacaaa
oJW015	tttgctagcggatcccggtgctagttggtgagcgccac
oJW021	cggcatatggaaaatatattagacctgtgg
oJW022	aatacgactcactatagggc
oJW023	agatggtgcacgatgcac
oLM022	gccgaattccttctcagttccgaacg