Supplemental Information

Centromere interactions promote the maintenance of the multipartite genome in *Agrobacterium tumefaciens*

Zhongqing Ren, Qin Liao, Ian S. Barton, Emma Wiesler, Clay Fuqua, Xindan Wang

Inventory of Supplemental information

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- Table S1C: Oligonucleotides used in this study.
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Supplemental Materials and Methods: This provides a detailed description of the methods applied in this study.

Ren Figure S1

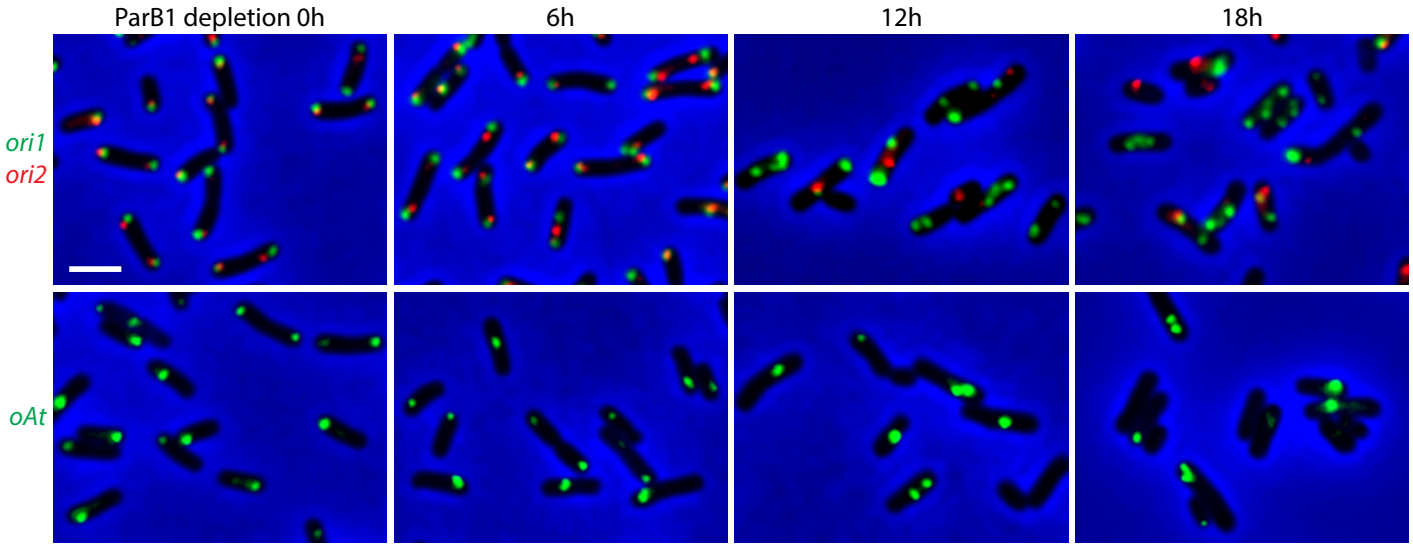
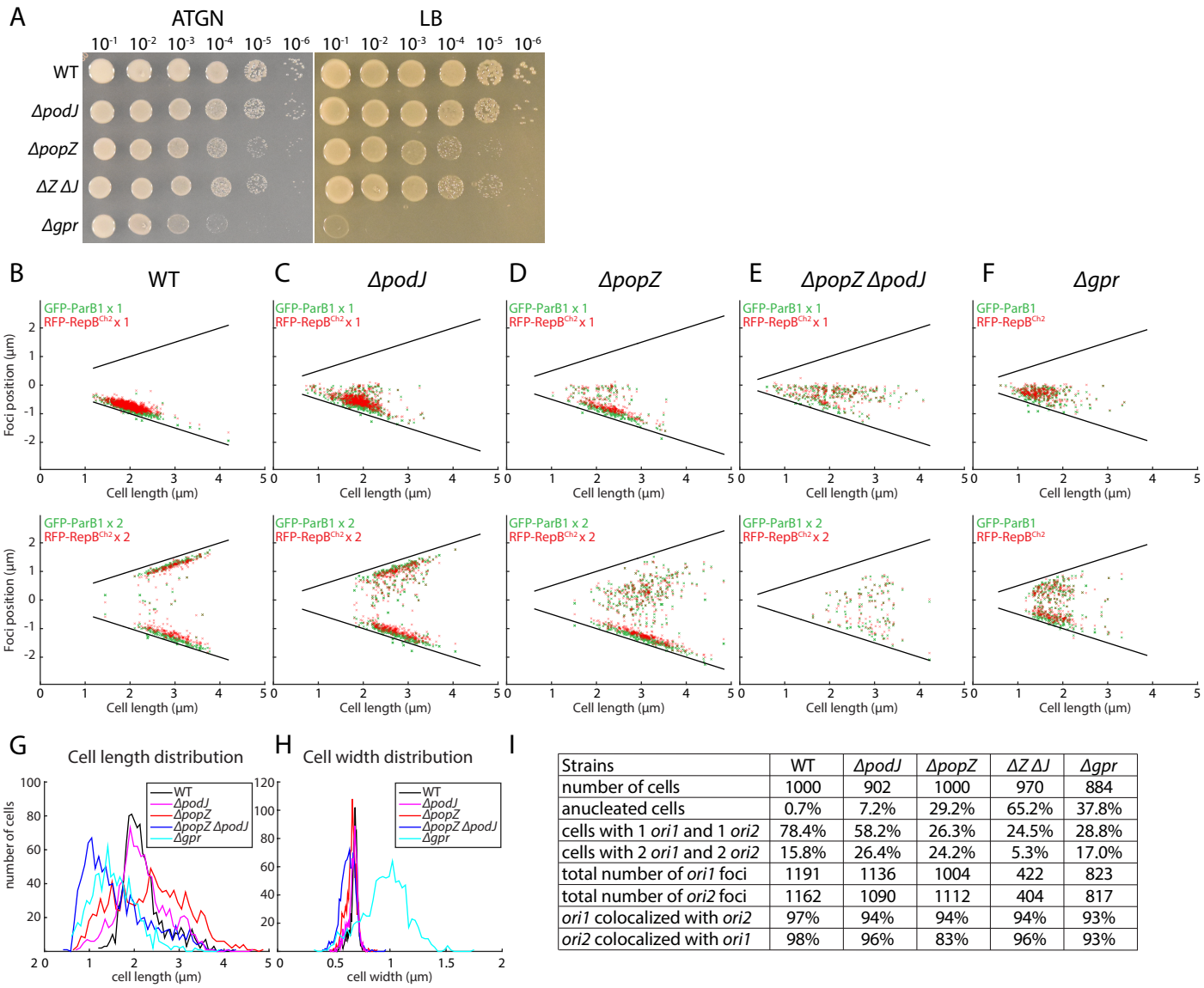


Figure S1. ParB1 is important for genome maintenance.

Visualization of origin localization when ParB1 was depleted for the indicated durations in AtWX496 (top) and AtWX498 (bottom). The origins are labeled using *mcherry-parB*^{P1}-*parS*^{P1} at 50 kb away from *ori1* (green, top panel), or *ygfp-parB*^{pMT1}-*parS*^{pMT1} at 57 kb away from *ori2* (red, top panel) or 11 kb away from *oAt* (green, bottom panel). Pseudo-colors were assigned as indicated. Protein levels can be found in **Figure 1B**. Scale bar represents 2 μm. ParB1 depletion was performed by washing away the inducers (1 μM AHL and 2 mM theophylline).

Ren Figure S2



I

Strains	WT	*ΔpodJ*	*ΔpopZ*	*ΔZ ΔJ*	*Δgpr*
number of cells	1000	902	1000	970	884
anucleated cells	0.7%	7.2%	29.2%	65.2%	37.8%
cells with 1 *ori1* and 1 *ori2*	78.4%	58.2%	26.3%	24.5%	28.8%
cells with 2 *ori1* and 2 *ori2*	15.8%	26.4%	24.2%	5.3%	17.0%
total number of *ori1* foci	1191	1136	1004	422	823
total number of *ori2* foci	1162	1090	1112	404	817
ori1 colocalized with *ori2*	97%	94%	94%	94%	93%
ori2 colocalized with *ori1*	98%	96%	83%	96%	93%

Figure S2. The polar organizers are required for polar localization of the origins.
(A) 10-fold serial dilutions of the indicated strains spotted on ATGN plate (left) or LB plate (right).
(B-F) Plots of the localization of *ori1* (green) and *ori2* (red) in **(C)** WT (AtWX263), **(C)** Δ*podJ* (AtWX307), **(D)** Δ*popZ* (AtWX303), **(E)** Δ*popZ* Δ*podJ* (AtWX305), **(F)** Δ*gpr* (AtW309) for cells containing a single *ori1* and *ori2* focus (top), or two *ori1* and *ori2* foci (bottom). The percentage of cells in these subpopulations can be found in **(H).**
(G-H) Distribution of cells length **(G)** and cell width **(H)** in the indicated strains.
(I) Image analysis. Colocalization was defined as a pair of green and red foci that are with an inter-focal distance of less than 6 pixels. Images were analyzed using Oufti software, see Materials and Methods.

Ren Figure S3

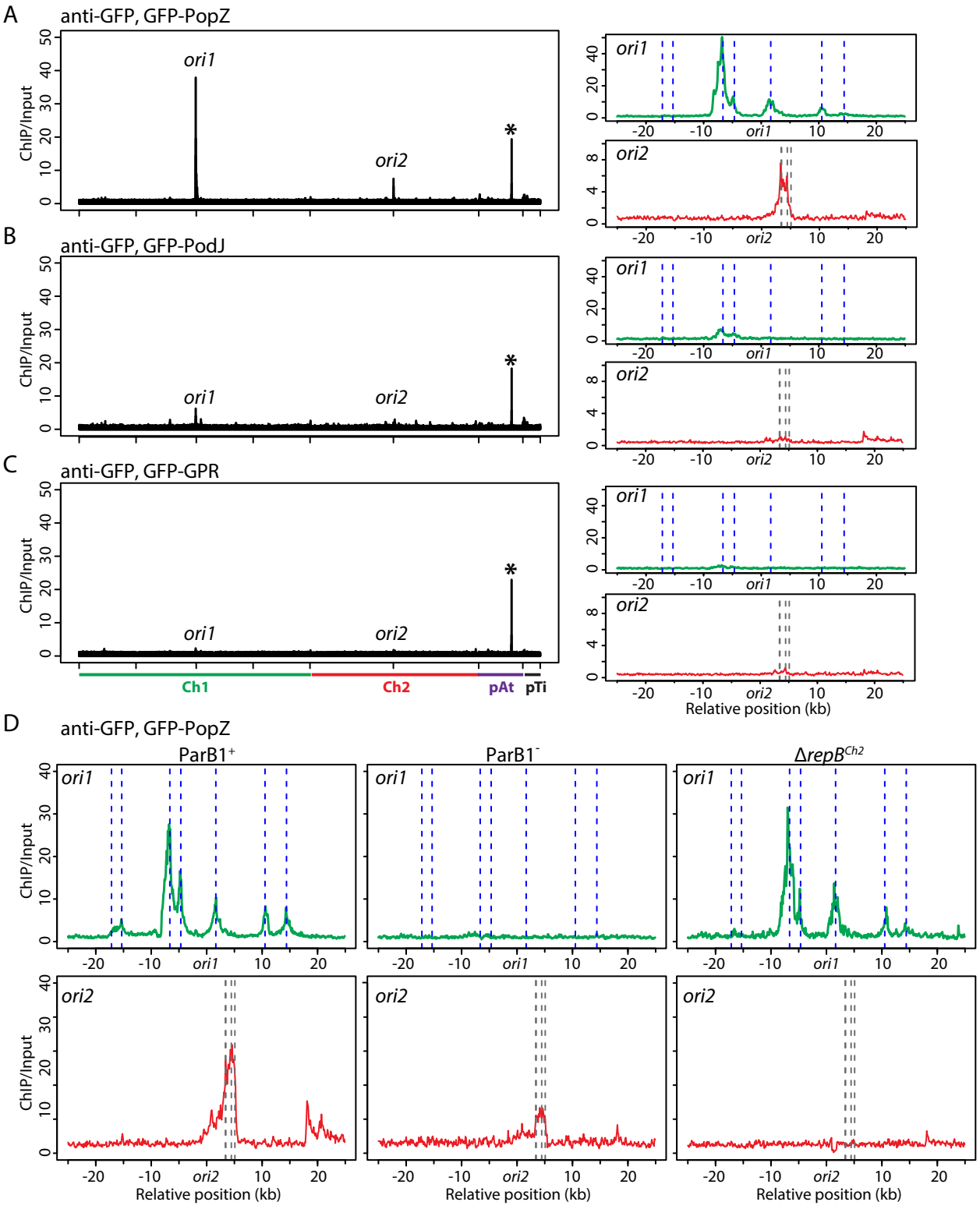


Figure S3. PopZ is enriched at *ori1* and *ori2*, which requires ParB1 and RepB^{Ch2} respectively.

(A-C) ChIP enrichment (ChIP/input) of (**A)** GFP-PopZ (AtWX234), (**B)** GFP-PodJ (AtWX263) and **(C)** GFP-GPR (AtWX236). Whole-genome profiles are shown on the left in 1-kb bins and high-resolution plots of *ori1* and *ori2* regions are shown on the right in 100-bp bins. Black asterisks indicate an enrichment peak present in all of our anti-GFP ChIP-seq experiments regardless of the fusion protein. Blue and gray dotted lines indicate the *parS1* and *parS2* sites, respectively.

(D) High-resolution ChIP enrichment (ChIP/input) of GFP-PopZ at *ori1* (top) and *ori2* (bottom) in ParB1^{+} (AtWX289 with inducers 1 μM AHL and 2 mM theophylline), ParB1^{-} (AtWX289 without inducers), and Δ*repB^{Ch2}* (AtWX291). The enrichment of GFP-PopZ at the *ori1* and *ori2* regions depends on the presence of ParB1 and RepB^{Ch2}, respectively.

Ren Figure S4

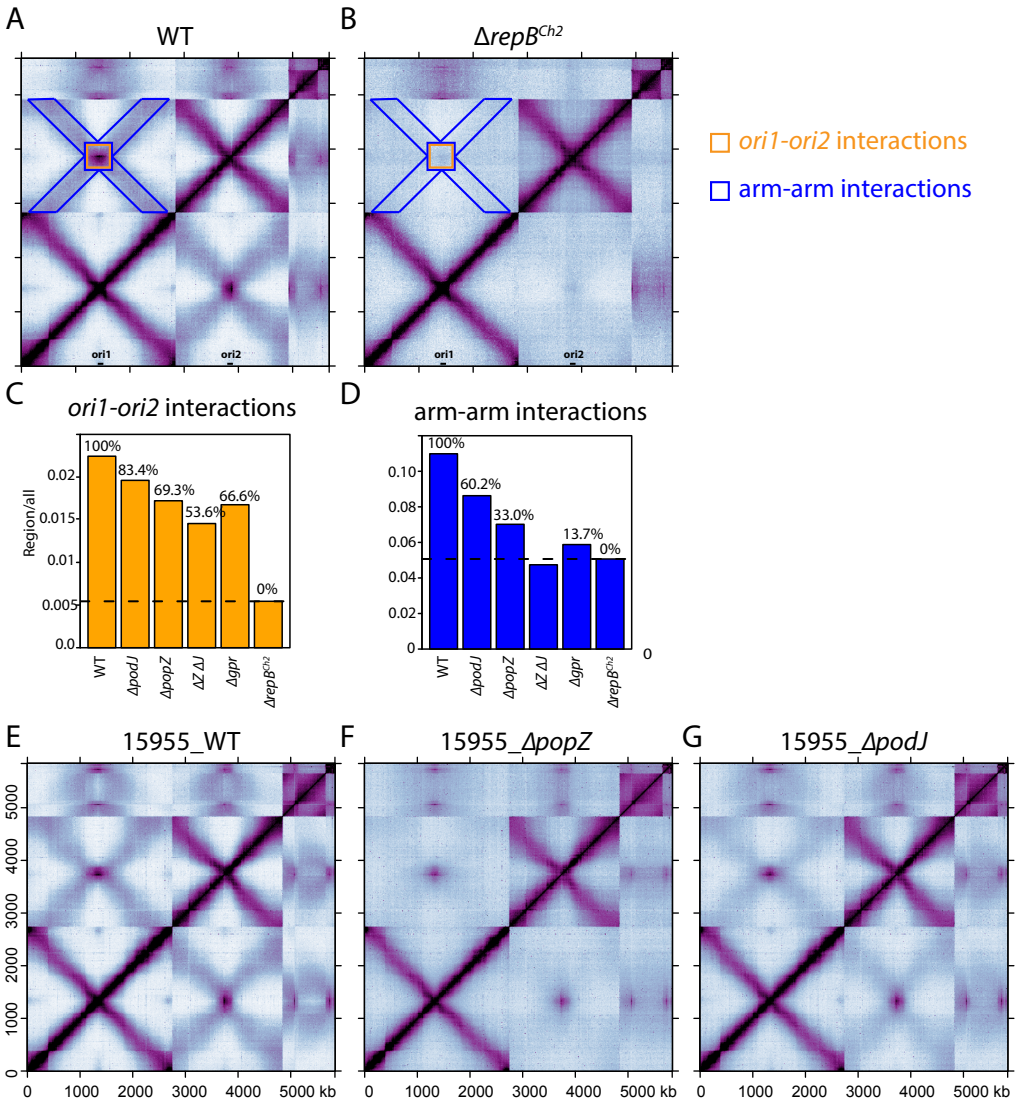


Figure S4. Quantification of inter-replicon interactions.

(A-D) Quantifications of *ori1-ori2* interactions (orange region) and Ch1-Ch2 alignment (blue regions) in different strains. Regions used for quantification are shown in **(A-B)**. Details can be found in Materials and Methods. Interactions in Δ*repB*^{Ch2} is set as the background (0%, black dotted lines). After subtracting background, the percentage of interactions relative to the WT (100%) is shown.

(E-G) *A. tumefaciens* 15955 strain showed similar phenotype. Normalized Hi-C contact maps for **(E)** 15955 WT (11) **(F)** 15955 Δ*popZ* (IB173), **(G)** 15955 Δ*podJ* (IB172) grown in ATGN.

Ren Figure S5

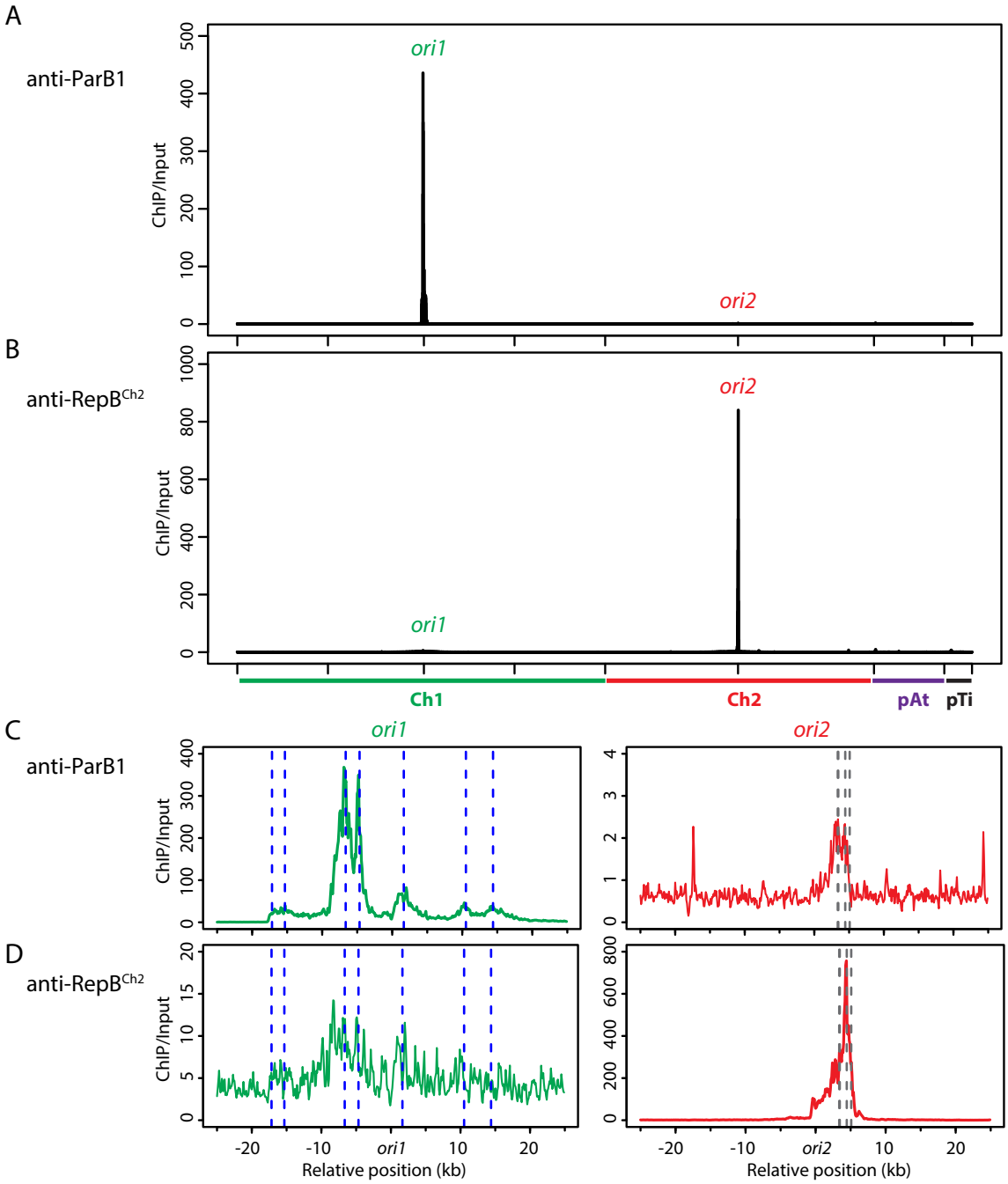


Figure S5. ChIP-seq enrichment of ParB1 and RepB^{Ch2} at cognate sites and reciprocal sites.

(A) ParB1 enrichment in wild-type cells (11). Sequencing reads from ChIP and input samples were normalized to the total number of reads and plotted in1-kb bins. x-axis shows genome positions.

(B) RepB^{Ch2} enrichment in wild-type cells (11).

(C) High-resolution plots of ParB1 enrichment from **(A)** at 50-kb regions in encompassing *ori1* (left panel) and *ori2* (right panel). *parS1* and *parS2* sites are indicated by blue and gray dotted lines, respectively. Data are plotted in 100-bp bins.

(D) High-resolution plots of RepB^{Ch2} enrichment from **(B)** at 50-kb regions in encompassing *ori1* (left panel) and *ori2* (right panel).

Ren Figure S6

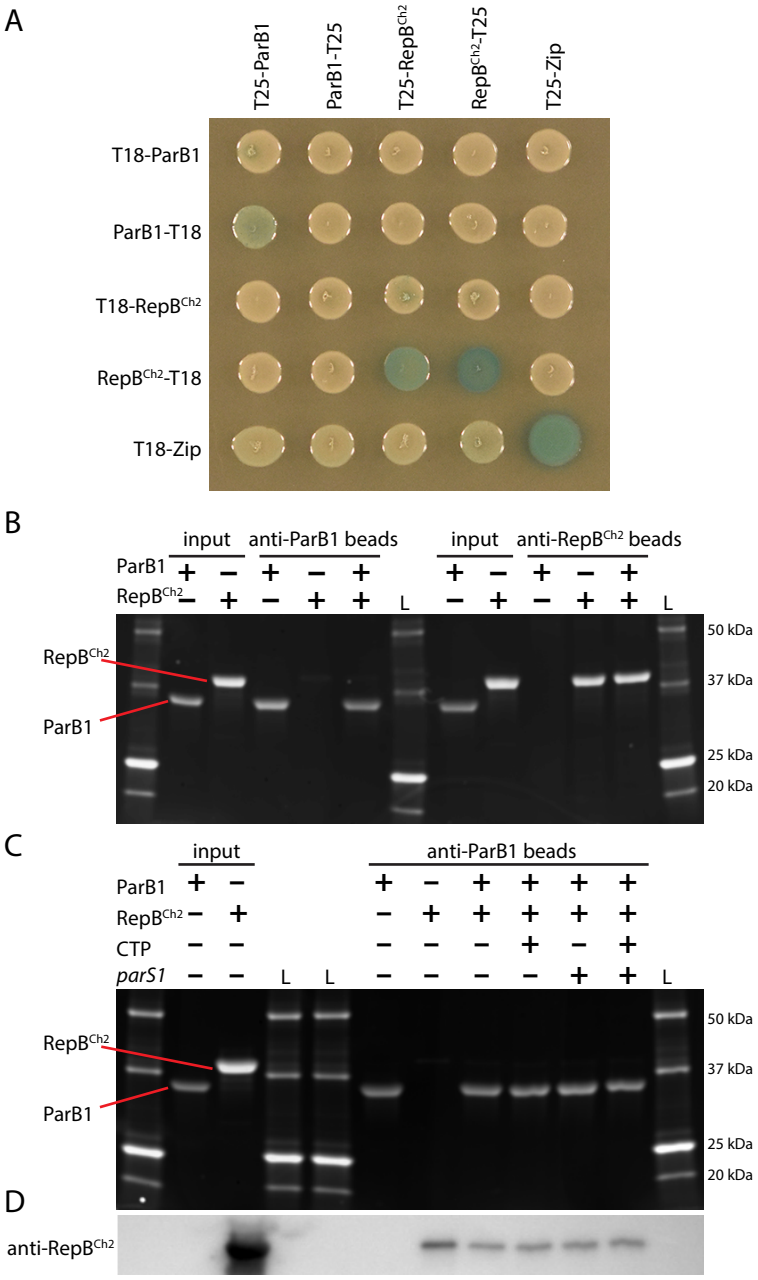


Figure S6. ParB1-RepB^{Ch2} interactions could not be detected in BACTH or *in vitro* pulldown assays.

(A) BACTH interactions between ParB1 and RepB^{Ch2}. *E. coli* strain BTH101 (56) expressing protein fusions to different domains (T25 and T18) of an adenylate cyclase. T25 and T18 fused to the same leucine zipper domain ("zip") from yeast GCN4 serve as both positive and negative controls (38). This experiment detected interactions between ParB1 and ParB1, and between RepB^{Ch2} and RepB^{Ch2}, but not between ParB1 and RepB^{Ch2}.

(B) An SDS-PAGE gel of an *in vitro* pulldown experiment. Affinity-purified ParB1 polyclonal antibodies were crosslinked to magnetic ProteinA Sepharose beads, which were then incubated with 50 µg of ParB1 protein and 50 µg of RepB^{Ch2} proteins in 1 ml 1xPBS solution, singly or doubly. Similarly, beads crosslinked with purified RepB^{Ch2} antibodies were incubated with proteins. Proteins were eluted in sample loading buffer at 65°C and separated by stain-free precast 4-20% polyacrylamide gradient gels (Bio-Rad 4561096). L is for protein ladder (BioRad 1610363). The gels were imaged using ProteinSimple Fluorchem R gel documentation system.

(C) An SDS-PAGE gel of an *in vitro* pulldown experiment using ParB1 beads similar to that in **(B).** The beads were incubated with ParB1, RepB^{Ch2}, 1 mM CTP, 3 µM *parS1* and *parS2* DNA fragments. ParB1-RepB^{Ch2} interactions were not detected.

(D) The SDS-PAGE gel in **(C)** was immunoblotted using RepB^{Ch2} antibodies. A low amount of RepB^{Ch2} protein could be detected when incubated with ParB1 beads alone, but this level did not increase in the presence of the ParB1 protein.

Table S1A. Bacterial strains used in this study.

Strain	Genotype	Reference	Figure
***A. tumefaciens* used in main figures**			
AtWX063	C58, wild type	(1)	1B, 3A, S2A, S4ACD, S5ABCD
AtWX089	C58, Δ*repB*^{Ch2}(Atu3923/ATU_RS18280)::*amp*	(2)	1B, 3B, S2A, S4BCD
AtWX192	C58, Δ*traI*, *tetRA*::*gen PtraI-riboswitch-parB1*(Atu2828/ATU_RS13770) *traR*	(2)	1B
AtWX356	C58, *mcherry-parB*^{P1}*-parS*^{P1} inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1*, *ygfp-parB*^{pMT1}*-par*^{pMT1} inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from *ori2*	This study	1CF
AtWX359	C58, *ygfp-parB*^{pMT1}*-parS*^{pMT1} inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from *oAt*	(2)	1CF
AtWX402	C58, Δ*repB*^{Ch2}(Atu3923/ATU_RS18280)::*amp*, *mcherry-parB*^{P1}*-parS*^{P1} inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1*, *ygfp-parB*^{pMT1}*-par*^{pMT1} inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from *ori2*	This study	1DF
AtWX500	C58, Δ*repB*^{Ch2}(Atu3923/ATU_RS18280)::*amp*, *ygfp-parB*^{pMT1}*-parS*^{pMT1} inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from *oAt*	This study	1DF
AtWX496	C58, Δ*traI*, *tetRA*::*gen PtraI-riboswitch-parB1*(Atu2828/ATU_RS13770) *traR*, *mcherry-parB*^{P1}*-parS*^{P1} inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1, ygfp-parB*^{pMT1}*-par*^{pMT1} inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from *ori2*	This study	1EF, S1
AtWX498	C58, Δ*traI*, *tetRA*::*gen PtraI-riboswitch-parB1*(Atu2828/ATU_RS13770) *traR*, *ygfp-parB*^{pMT1}*-parS*^{pMT1} inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from *oAt*	This study	1EF, S1
AtWX277	C58, *ygfp-parB*^{pMT1}*-parS*^{pMT1} inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1*	(2)	1F
AtWX295	C58, *ygfp-parB*^{pMT1}*-parS*^{pMT1} inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from *ori2*	(2)	1F

AtWX351	C58, *ygfpP-parB^{pMT1}-parS^{pMT1}* inserted between Atu6047/ATU_RS23235 and Atu6048/ATU_RS23240, 4 kb from *oTi*	(2)	1F
AtWX263	C58, carrying pWX970, pSRKKm *Plac rfp-repB^{Ch2}* (Atu3923/ATU_RS18280) *terminator Plac egfp-parB1* (Atu2828/ATU_RS13770) *terminators*	(2)	2A, S2B
AtWX307	C58, Δ*podJ* (Atu0499/ATU_RS02460), containing pWX970	This study	2B, S2C
AtWX303	C58, Δ*popZ* (Atu1720/ATU_RS08420), containing pWX970	This study	2C, S2D
AtWX305	C58, Δ*popZ* (Atu1720/ATU_RS08420) Δ*podJ* (Atu0499/ATU_RS02460), containing pWX970	This study	2D, S2E
AtWX309	C58, Δ*gpr* (Atu1348/ATU_RS06650), containing pWX970	This study	2E, S2F
AtWX283	C58, Δ*podJ* (Atu0499/ATU_RS02460)	This study	3C, S2A, S4CD
AtWX110	C58, Δ*popZ* (Atu1720/ATU_RS08420)	This study	3D, S2A, S4CD
AtWX121	C58, Δ*popZ* (Atu1720/ATU_RS08420) Δ*podJ* (Atu0499/ATU_RS02460)	This study	3E, S2A, S4CD
AtWX286	C58, Δ*gpr* (Atu1348/ATU_RS06650)	This study	3F, S2A, S4CD
***A. tumefaciens* used for strain building and in supplemental figures**			
AtWX234	C58, containing pWX822, pSRKKm *msfgfp-popZ* (Atu1720/ATU_RS08420)	This study	S3A
AtWX265	C58, containing pMAT3, pSRKKm *msfgfp-podJ* (Atu0499/ATU_RS02460)	This study	S3B
AtWX236	C58, containing pJZ253, pSRKGm *gfp-gpr* (Atu1348/ATU_RS06650)	This study	S3C
AtWX289	C58, Δ*traI*, *tetRA*::*gen PtraI-riboswitch-parB1*(Atu2828/ATU_RS13770) *traR*, pSRKKm *msfgfp-popZ* (Atu1720/ATU_RS08420)	This study	S3D
AtWX291	C58, Δ*repB^{Ch2}*, pSRKKm *msfgfp-popZ* (Atu1720/ATU_RS08420)	This study	S3D
AtWX486	C58, Δ*traI*, *tetRA*::*gen PtraI-riboswitch-parB1*(Atu2828/ATU_RS13770) *traR*, *ygfp-parB^{P1}-parS^{P1}* inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1*	This study	
AtWX050	15955, wild type	(3)	S4E
IB172	15955, Δ*podJ* (ISGA_411)	This study	S4F
IB173	15955, Δ*popZ* (ISGA_1749)	This study	S4G
***B. subtilis* strains used in main figures**			
BWX5333	*pelB::Psoj mcherry-parB1$_{At}$ tet, parS2$_{At}$* cluster at -91° *kan, ΔparB$_{Bs}$ spec*	This study	4BCD
BWX5359	*ycgO::Phyperspank-optRBS-mgfpmut3-repB^{Ch2}$_{At}$ cat*, *parS2$_{At}$* cluster at -91° *kan, ΔparB$_{Bs}$ spec*	This study	4BCD
BWX5341	*pelB::Psoj mCherry-parB1$_{At}$ tet*, *ycgO::Phyperspank-optRBS-mgfpmut3-repB^{Ch2}$_{At}$ cat*, *ΔparB$_{Bs}$ spec*	This study	4C

BWX5353	*pelB::Psoj mCherry-parB1$_{At}$ tet*, *ycgO::Phyperspank-optRBS-mgfpmut3-repB^{Ch2}$_{At}$ cat*, *parS2$_{At}$* cluster at -91° *kan, parSΔ9, ΔparB$_{Bs}$ (ΔparS) spec*	This study	4D
***B. subtilis* strains used for strain building and in supplemental figures**			
AG1468	*Δspo0J::spec, trpC2, pheA1*	(4)	
BWX2423	*ΔparB (ΔparS) spec*	(5)	
BWX3212	*parS*Δ9 no a.b.	(6)	
BWX3379	*parS*Δ9 no a.b., *parS* at -91˚ *ytuF kan*	(7)	
BWX5258	*pelB::Psoj mcherry-parB1$_{At}$ tet*	This study	
BWX5260	*ycgO::Psoj mgfpmut3-repB^{Ch2}$_{At}$ cat*	This study	
BWX5265	*parS2$_{At}$* cluster at -91° *kan*	This study	
BWX5309	*parSΔ9 no a.b., ycgO::Phyperspank-optRBS-mgfpmut3-repB^{Ch2}$_{At}$ cat*	This study	
BWX5329	*ycgO::Phyperspank mgfpmut3-repB^{Ch2}$_{At}$ cat*, *parSΔ9*	This study	
BWX5349	*pelB::Psoj mCherry-parB1$_{At}$ tet*, *ycgO::Phyperspank-optRBS-mgfpmut3-repB^{Ch2}$_{At}$ cat*, *parS2$_{At}$* cluster at -91° *kan, ΔparB$_{Bs}$ spec*	This study	

Table S1B. Plasmids used in this study.

Plasmid	Description	Reference
pFHC2973	The plasmid carries *cfp-parB^{P1}* and *ygfp-parB^{pMT1}*	(8)
pGM9	pNPTS138 Δ*podJ* (Atu0499/ATU_RS02460) (*kan*)	Fuqua Lab, unpublished
pIB315	pNPTS138 15955 Δ*popZ* (ISGA_1749) (*kan*)	This study
pIB316	pNPTS138 15955 Δ*podJ* (ISGA_411) (*kan*)	This study
pJW005	*yhdG::Phyperspank-opt.rbs-sirA (phleo)*	(9)
pJZ253	pSRKGm *Plac gfp-gpr* (Atu1348/ATU_RS06650) (*kan*)	(10)
pJZ298	pBSKII+ plasmid with *sacB* carb carrying 2kb sequencing homologous to *gpr* (Atu1348/ATU_RS06650)	(10)
pKNT18	BACTH plasmid contains MCS *t18* (*amp*)	(11)
pKNT25	BACTH plasmid contains MCS *t25* (*kan*)	(11)
pKT18	BACTH plasmid contains *t18* MCS (*amp*)	(11)
pKT25	BACTH plasmid contains *t25* MCS (*kan*)	(11)
pKT25zip	BACTH Plasmid was used to express *t25-zip* (*kan*)	(11)
pUT18Czip	BACTH Plasmid was used to express *t18-zip* (*amp*)	(11)
pMAT3	pSRKKm *Plac msfgfp-podJ* (Atu0499/ATU_RS02460) (*kan*)	Fuqua Lab, unpublished
pNPTS138	*oriT sacB kan*	(12)
pSRKKm	Broad host-range, *Plac* (*kan*)	(13)
pSRKKm msfGFP	pSRKKm *Plac msfgfp* (*kan*)	(14)
mini-Tn7	pUC18-mini-Tn7T *gen Plac ha*	(14)
pWX294	pACYC origin with MCS (*amp*)	This study
pWX563	*pelB::Psoj-mgfpmut3-spo0J (parS*)* (*tet*)	(5)
pWX564	*pelB::Psoj-mcherry-spo0J (parS*) (tet)*	(15)
pWX588	*ycgO::Pspank* (optRBS) gfp-spo0J (parS*) cat*	This study
pWX822	pSRKKm *Plac msfgfp-popZ* (Atu1720/ATU_RS08420) (*kan*)	(13)
pWX839	pNPTS138 Δ*popZ* (Atu1720/ATU_RS08420) (*kan*)	This study
pWX845	BACTH Plasmid was used to express *t25-parB1* (kan)	This study
pWX846	BACTH Plasmid was used to express *t25-repB^{Ch2}* (*kan*)	This study
pWX847	BACTH Plasmid was used to express *parB1-t25* (*kan*)	This study
pWX848	BACTH Plasmid was used to express *repB^{Ch2}-t25* (*kan*)	This study
pWX849	BACTH Plasmid was used to express *t18-parB1* (*amp*)	This study
pWX850	BACTH Plasmid was used to express *t18-repB^{Ch2}* (*amp*)	This study
pWX851	BACTH Plasmid was used to express *parB1-t18* (*amp*)	This study
pWX852	BACTH Plasmid was used to express *repB^{Ch2}-t18* (*amp*)	This study
pWX854	pNPTS138 *repB^{Ch2}* (Atu3923/ATU_RS18280)::*ampR* (*kan*)	This study
pWX915	pACYC *terminator Ppen* (*amp*)	This study
pWX916	pACYC *terminator Ppen cfp-parB^{P1}-parS^{P1}* (*amp*)	This study
pWX930	pNPTS138 *Ppen cfp-parB^{P1}-parS^{P1}* kan at Atu3054/ATU_RS14060	This study
pWX936	pNPTS138 PT7strong *cfp-parB^{P1}-parS^{P1}* at Atu3054/ATU_RS14060	This study

pWX962	pNPTS138 PT7strong *cfp-parB^{P1}-parS^{P1}* at Atu0048/ATU_RS00235	This study
pWX967	pNPTS138 PT7strong *yGFP-parB^{pMT1}-parS^{pMT1}* at Atu3973/ATU_RS18530	This study
pWX970	pSRKKm *Plac rfp-repB^{Ch2}* (Atu3923/ATU_RS18280) *terminator* (Atu2828/ATU_RS13770) *parB1-egfp Plac terminators*	(2)
pWX995	pNPTS138 terminators PT7strong *mcherry-parB^{P1}-parS^{P1}* at Atu0048/ATU_RS00235	This study
pWX1005	pNPTS138 *yGFP-parB^{pMT1}-parS^{pMT1}* Atu5337/ATU_RS25505	(2)

Table S1C. Oligonucleotides used in this study.

Oligo	Sequence	Use
oML83	CCTCATCCTCTTCATCCTC	sequencing
oML85	AATAGCGTCCTTGCTCTCGT	sequencing
IBE140	GGATCCAGTAGCCTCGATCATGTCGGGG	IB172
IBE141	CATCCGTTGCGAAACGGTTACATCTTCTCGCTCGCTTCGC	IB172
IBE142	GCGAAGCGAGCGAGAAGATGTAACCGTTTCGCAACGGATG	IB172
IBE143	GCTAGCAGCCAGCGTTCCGCGCCGGAAA	IB172
IBE144	TTCAGCGGGAAAAGCCGCTC	IB172
IBE145	CGTAGCGCCCACGAGACCGC	IB172
IBE146	GGATCCACTGGTCGTCGTTGTCGGATA	IB173
IBE147	ATGCGGACGAACAGAGCCTACATATCAATCCCCGGTTTCC	IB173
IBE148	GGAAACCGGGGATTGATATGTAGGCTCTGTTCGTCCGCAT	IB173
IBE149	GCTAGCACTGCTGTTCCATCAGCTTGC	IB173
IBE150	CAGACCTTGTCCACGAAGGC	IB173
IBE151	TCGATGAAGTGCCGGCGGCA	IB173
oWX439	TCCTTCTGCTCCCTCGCTCAG	BWX5265
oWX776	ATGGGCTGGGAAGCCAGCAGCGAG	sequencing
oWX998	AAACCCGGGACATAAGGAGGAACTACTATGAGTAAAGG	pWX588
oWX999	TTTGCTAGCCAGAGTGGAGGCAAGAACGCCTTAACCC	pWX588
oWX1279	CTAATCCGACAGCTAACCTCGTAGGCG	BWX5265
oWX1282	CGATAAAGTCGGACCAGGGATGCTCGG	BWX5265
oWX1283	TCCTATTTTCAGGCAGTGACGCCG	sequencing
oWX1782	TGAGTTAGCTCACTCATTAGGC	sequencing
oWX1783	ACCAGGCGGAACATCAATGTGG	sequencing
oWX1789	CATCTGTCCAACTTCCGCGAC	sequencing
oWX1790	CCTCTTCGCTATTACGCCAGC	sequencing
oWX1835	GCCAGGGTTTTCCCAGTCACGA	sequencing
oWX1854	CGCCAGGGTTTTCCCAGTCACGAC	sequencing
oWX1855	TCACACAGGAAACAGCTATGAC	sequencing
oWX2044	CAATTTCACACAGGAAACAGCATATGAGTAAAGGTGAAGAACTGTTCACC	pWX822
oWX2046	GGATCCTCCAGATCCTTTGTATAGTTCATCCATGCCGTG	pWX822
oWX2051	TATACAAAGGATCTGGAGGATCCGCTCAGCCAAGTGTCGCGCGTGAAC	pWX822
oWX2052	CTCGAGGTCGACGGTATCGATAAGCTTTTAGCGGCGCGAGCCGCGCGCCACACG	pWX822
oWX2060	GGAAAGCGGGCAGTGAGCGC	sequencing
oWX2061	GAAGGTTATGTACAGGAGCGCACC	sequencing
oWX2076	TGGCGCCAAGCTTCTCTGCAGGATATGACACAGAGTGCCGATTTAAG	sequencing
oWX2077	GCTAGCGAATTCGTGGATCCAGATCTAACCCGCCATGCCCACCTCC	sequencing

oWX2160	CTGGCGCCAAGCTTCTCTGCAGGATTGCCAAGGCAACTGTCTATCG	pWX839, sequencing
oWX2161	GGCAGCGATTGTAGCCTGCGGAATATCAATCCCCGGTTTCTACTC	pWX839
oWX2162	GAGTAGAAACCGGGGATTGATATTCCGCAGGCTACAATCGCTGCC	pWX839, sequencing
oWX2163	AGCTAGCGAATTCGTGGATCCAGATAGCTGGTTTTCCATCAGCTTGC	pWX839, sequencing
oWX2190	CGCTCTAGAGTAACACACAGGAAACAGCTATGAGTGATGATCTTTCG AAGCG	pWX847, pWX851
oWX2191	ATGCCCGGGCCGAACCGCTACCTTTCTGCTCCAGCAGCC	pWX847, pWX851
oWX2193	ATGCCCGGGTTATTTCTGCTCCAGCAGCC	pWX845, pWX849
oWX2194	CGCTCTAGAGTAACACACAGGAAACAGCTATGAGCAGAAAACAGATA TTCGC	pWX848, pWX852
oWX2195	ATGCCCGGGCCGAACCGCTACCCTGCTTGGACCGGTATTCG	pWX848, pWX852
oWX2197	ATGCCCGGGTTACTGCTTGGACCGGTATTCG	pWX846, pWX850
oWX2202	CGCTCTAGAGGGCAGCGGTAGTGATGATCTTTCGAAGCG	pWX845, pWX849
oWX2203	CGCTCTAGAGGGCAGCGGTAGCAGAAAACAGATATTCGC	pWX846, pWX850
oWX2291	GGCTGATTGGCATGACAATATTTGACGTGCG	sequencing
oWX2292	GTTCTGCGATCGGCAGATAGACAGTCACGG	sequencing
oWX2377	GGCTTCCTTTGTTATCAAGCGCAG	sequencing
oWX2385	GCTGAATTCCCCGCGAAAGCGGGGTTTTTTTTTCCGGTGGAAACGAG GTCATCATTTC	pWX915
oWX2386	TTTAAGCTTGAATATTTGATTGATCGTAACCAGATGAAGC	pWX915
oWX2387	TCAATCAAATATTCAAGCTTAAAGGAGGTGGAAACATGAGTAAAGGAG AAGAACTTTTC	pWX916
oWX2388	TCTTAAATGACTCGCGAGAACTCGAGTTAATAGTGAAATTTGAATGGC GAAAG	pWX916
oWX2389	CTTTCGCCATTCAAATTTCACTATTAACTCGAGTTCTCGCGAGTCATTT AAGACCG	pWX916
oWX2390	GCCGATACTGCAGATGTCGACATGGATCCGTGAAATCGTGGCGATTT CACCTTG	pWX916
oWX2395	TCTTCGCTATTACGCCAGATCC	sequencing
oWX2396	CCGTCAATTGTCTGATTCGTTACC	sequencing
oWX2397	GATGACGGTAACTACAAAACCC	sequencing
oWX2407	CTCTAGATAGCGCATGCTGAATTC	pWX930, pWX962
oWX2408	GGTTATGCTAGTTATTGCTCAGCC	pWX930, pWX962
oWX2420	GGCGCCAAGCTTCTCTGCAGGATATCCAGTTACGTGCTGGCGGCAG GATC	pWX930

oWX2421	GAATTCAGCATGCGCTATCTAGAGCTATTTTGGGATAGCTCGAACCGTG	pWX930
oWX2422	GGCTGAGCAATAACTAGCATAACCCGGCAGGCATATGAAACCGGATTG	pWX930
oWX2423	CTAGCGAATTCGTGGATCCAGATATCTGCCATGTGGAACGATGGTGAGGG	pWX930
oWX2424	TTCTGCCGCTCCGATCAAAACAGG	sequencing
oWX2425	CCGGACTCCACATCCGCAGATTTC	sequencing
oWX2426	GGCCTTCTGCTTAGCTAGAGCGGC	sequencing
oWX2431	TAATACGACTCACTATAGGGAGACCACAACGCTTCATCTGGTTACGATCAATC	pWX936
oWX2432	GGTCTCCCTATAGTGAGTCGTATTAATTTCGAAATGATGACCTCGTTTCCACC	pWX936
oWX2497	AAGTCAAGTTTGAAGGTGATACCC	sequencing
oWX2502	CGCCAAGCTTCTCTGCAGGATATCGAACGTCGATATTGGCCTCGAATG	pWX962, sequencing
oWX2503	AATTCAGCATGCGCTATCTAGAGTCAGCCCGCCTGCTTTGCTTTCAG	pWX962
oWX2504	GGCTGAGCAATAACTAGCATAACCAGCGGCCGCCTGGTATTTCCAG	pWX962
oWX2505	TAGCGAATTCGTGGATCCAGATATCTGCGGGTTATGCGCTGCCGGCC	pWX962, sequencing
oWX2506	ACAATATTGGCCTGATGGAGGACC	sequencing
oWX2507	AAAAGGGACAGGACACGCTGTTCC	sequencing
oWX2508	CGCCAAGCTTCTCTGCAGGATATCAACGCGCGAAAAACTGTTGACG	sequencing
oWX2511	AGCGAATTCGTGGATCCAGATATCGACACTGGAGGATATGGGAACATTC	sequencing
oWX2530	CGGAGAAGGTCATGTCGAGCG	sequencing
oWX2530	CGGAGAAGGTCATGTCGAGCG	sequencing
oWX2531	AAGGCGTTCAGCGTGAAACTCAGG	sequencing
oWX2560	CATGAGCTCGAGGCCTGATCCGCCAGATCCTTACTTAACTGCGTCTTTCAGTGCC	sequencing
oWX2563	CCGAATCGTATAGAGATTCTTCG	BWX5258
oWX2564	GGACTCGAGATGAGTGATGATCTTTCGAAGCG	BWX5258
oWX2566	GGACTCGAGATGAGCAGAAAACAGATATTCGC	BWX5260
oWX2567	ATGGGATCCTCACTGCTTGGACCGGTATTCGG	BWX5260
oWX2568	TTTAAAGGATTTGAGCGTAGCG	sequencing
oWX2569	ATGTGATTCTTCCACAATGCCTCGAGTCACCCTGTAAACACTTCGCCATC	BWX5265
oWX2570	GCGAAGTGTTTACAGGGTGACTCGAGGCATTGTGGAAGAATCACATTTGC	BWX5265
oWX2571	CGAACGGTACTGAGCGAGGGAGCAGAAGGAGTAATCGGTATCTTGCAAGTATCC	BWX5265
oWX2584	TTCAAGCTTAAAGGAGGTGGAAACATGGTCAGCAAGGGAGAGGAAG	pWX995
oWX2585	CTGCTCGACCATGAGCTCGAATTCTTTGTATAATTCGTCCATTCCACC	pWX995
oWX2589	CTTCCTCTCCCTTGCTGACCATGTTTCCACCTCCTTTAAGCTTG	pWX995

oWX2590	GGTGGAATGGACGAATTATACAAAGAATTCGAGCTCATGGTCGAGCAG	pWX995
oWX2597	CGCCAAGCTTCTCTGCAGGATATCCCAGGGATGGCATTAAGGTCC	sequencing
oWX2600	AGCGAATTCGTGGATCCAGATATCCTTCGCCAACGTGCTGGATGCC	sequencing
oWX2649	AAGCTTACATAAGGAGGAACTACTATGAGTAAAGGAGAAGAACTTTTCAC	BWX5309
oWX2650	CAGCTATGACAAACAAATGAAACAGC	BWX5309, BWX5329
oWX2651	GGATGCCGATACGGCTGAAGCG	BWX5309, BWX2651
oWX2655	ATAGTAGTTCCTCCTTATGTAAGCTTAATTGTTATCCGCTCAC	BWX5309
oWX2668	TGCCTCAAGCTAGAGAGTCGATGTTCAGACGCTCAGCTTCAG	BWX5309
oWX2669	GAAGCTGAGCGTCTGAACATCGACTCTCTAGCTTGAGGCATC	BWX5309
oWX2674	CCGAATTAGCTTGCATGCGATCACTGCTTGGACCGGTATTCGG	BWX5329
oWX2675	AATACCGGTCCAAGCAGTGATCGCATGCAAGCTAATTCGGTGG	BWX5329

Table S1D. Next generation sequencing samples used in this study.

Sample name	Figure	Reference	Identifier
401_Wang_HiC_AtWX063_ATGN	3A, S4ACD	(2)	GSM5542437
408_Wang_HIC_AtWX089_ATGN	3B, S4BCD	(2)	GSM5542444
443_Wang_HiC_AtWX283_ATGN	3C, S4CD	This study	GSM5870438
444_Wang_HiC_AtWX110_ATGN	3D, S4CD	This study	GSM5870439
445_Wang_HiC_AtWX121_ATGN	3E, S4CD	This study	GSM5870440
446_Wang_HiC_AtWX286_ATGN	3F, S4CD	This study	GSM5870441
447_Wang_ChIP_anti_mCherry_BWX5333_CH	4CD	This study	GSM5870442
448_Wang_input_BWX5333_CH	4CD	This study	GSM5870443
449_Wang_ChIP_anti_GFP_BWX5359_CH_20uMIPTG1h	4CD	This study	GSM5870444
450_Wang_input_BWX5359_CH_20uMIPTG1h	4CD	This study	GSM5870445
451_Wang_ChIP_anti_GFP_BWX5341_CH_20uMIPTG1h	4C	This study	GSM5870446
452_Wang_input_BWX5341_CH_20uMIPTG1h	4C	This study	GSM5870447
453_Wang_ChIP_anti_mCherry_BWX5353_CH_20uMIPTG1h	4D	This study	GSM5870448
454_Wang_input_BWX5353_CH_20uMIPTG1h	4D	This study	GSM5870449
458_Wang_ChIP_anti_GFP_AtWX234_ATGN_halfmMIPTG4h	S3A	This study	GSM5870453
459_Wang_input_AtWX234_ATGN_halfmMIPTG4h	S3A	This study	GSM5870454
460_Wang_ChIP_anti_GFP_AtWX236_ATGN_halfmMIPTG4h	S3C	This study	GSM5870455
461_Wang_input_AtWX236_ATGN_halfmMIPTG4h	S3C	This study	GSM5870456
462_Wang_ChIP_anti_GFP_AtWX265_ATGN_halfmMIPTG4h	S3B	This study	GSM5870457
463_Wang_input_AtWX265_ATGN_halfmMIPTG4h	S3B	This study	GSM5870458
464_Wang_ChIP_anti_GFP_AtWX289_LB_2mMTheo_1uMAHL_halfmMIPTG4h	S3D	This study	GSM5870459
465_Wang_input_AtWX289_LB_2mMTheo_1uMAHL_halfmMIPTG4h	S3D	This study	GSM5870460
466_Wang_ChIP_anti_GFP_AtWX289_LB_halfmMIPTG4h	S3D	This study	GSM5870461
467_Wang_input_AtWX289_LB_halfmMIPTG4h_4h	S3D	This study	GSM5870462
468_Wang_ChIP_anti_GFP_AtWX291_ATGN_halfmMIPTG4h	S3D	This study	GSM5870463
469_Wang_input_AtWX291_ATGN_halfmMIPTG4h	S3D	This study	GSM5870464
415_Wang_HiC_AtWX050_LB	S4E	(2)	GSM5542451
470_Wang_HiC_IB173_ATGN	S4F	This study	GSM5870465
471_Wang_HiC_IB172_ATGN	S4G	This study	GSM5870466
403_Wang_input_AtWX063_ATGN_rep2	S5A-D	(2)	GSM5542439
404_Wang_ChIP_anti_AtParB_AtWX063_ATGN	S5AC	(2)	GSM5542440
405_Wang_ChIP_anti_AtRepBCh2_AtWX063_ATGN	S5BD	(2)	GSM5542441

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SUPPLEMENTAL MATERIALS AND METHODS

Plasmid construction

pWX588 [*ycgO::Pspank* (optRBS) gfp-spo0J (parS*)* (*cat*)] was constructed by a ligation reaction containing two DNA fragments: 1) pAM12 (1) was digested by XmaI and NheI to give *ycgO::Pspank* cat*; 2) *(optRBS) gfp-spo0J (parS*)* was amplified using oWX998 and oWX999 from pKM256 (2).

pWX822 [pSRKKm *msfgfp-popZ* (Atu1720/ATU_RS08420) (kan)] was constructed by an isothermal assembly reaction containing three DNA fragments: 1) pSRKKm digested by NdeI and HindIII; 2) *msfgfp* amplified using oWX2044 and oWX2046 from pSRKKm *msfgfp*; 3) *At popZ* amplified using oWX2051 and oWX2052 from C58 genomic DNA. The construct was sequenced using oWX1835, oWX2060 and oWX2061.

pWX839 [pNPTS138 Δ*popZ* (Atu1720/ATU_RS08420) (*kan*)] was constructed by an isothermal assembly reaction containing three gel-purified fragments: 1) pNPTS138 digested by EcoRV; 2) *At popZ* upstream region amplified using oWX2160 and oWX2161 from C58 genomic DNA; 3) *At popZ* downstream region amplified using oWX2162 and oWX2163 from C58 genomic DNA. The construct was sequenced using oWX1854 and oWX1855.

pWX845 [pKT25 *t25-parB1* (Atu2828/ATU_RS13770) (*kan*)] was constructed by ligating two DNA fragments: 1) pKT25 digested by XbaI and XmaI; 2) *At parB1* amplified using oWX2202 and oWX2193 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1789 and oWX1790.

pWX846 [pKT25 *t25-repB*^{Ch2} (Atu3923/ATU_RS18280) (*kan*)] was constructed by ligating two DNA fragments: 1) pKT25 digested by XbaI and XmaI; 2) *At repB*^{Ch2} amplified using oWX2203 and oWX2197 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1789 and oWX1790.

pWX847 [pKNT25 *parB1-t25* (Atu2828/ATU_RS13770) (*kan*)] was constructed by ligating two DNA fragments: 1) pKNT25 digested by XbaI and XmaI; 2) *At parB1* amplified using oWX2190 and oWX2191 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1782 and oWX1783.

pWX848 [pKNT25 *repB^{Ch2}-t25* (Atu3923/ATU_RS18280) (*kan*)] was constructed by ligating two DNA fragments: 1) pKT25 digested by XbaI and XmaI; 2) *At repB^{Ch2}* amplified using oWX2194 and oWX2195 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1782 and oWX1783.

pWX849 [pKT18 *t18-parB1* (Atu2828/ATU_RS13770) (*amp*)] was constructed by ligating two DNA fragments: 1) pKT18 digested by XbaI and XmaI; 2) *At parB1* amplified using oWX2202 and oWX2193 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1789 and oWX1790.

pWX850 [pKT18 *t18-repB^{Ch2}* (Atu3923/ATU_RS18280) (*amp*)] was constructed by ligating two DNA fragments: 1) pKT18 digested by XbaI and XmaI; 2) *At repB^{Ch2}* amplified using oWX2203 and oWX2197 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1789 and oWX1790.

pWX851 [pKNT18 *parB1-t18* (Atu2828/ATU_RS13770) (*amp*)] was constructed by ligating two DNA fragments: 1) pKNT18 digested by XbaI and XmaI; 2) *At parB1* amplified using oWX2190 and oWX2191 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1782 and oWX1783.

pWX852 [pKNT18 *repB^{Ch2}-t18* (Atu3923/ATU_RS18280) (*amp*)] was constructed by ligating two DNA fragments: 1) pKNT18 digested by XbaI and XmaI; 2) *At repB^{Ch2}* amplified using oWX2194 and oWX2195 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1782 and oWX1783.

pWX915 [pACYC *terminator Ppen*] was constructed by ligating two DNA fragments: 1) pWX294 digested by EcoRI and HindIII; 2) *Ppen* amplified using oWX2385 and oWX2386 from gWX46. pWX294 is an empty cloning vector with pACYC origin. Ppen is a constitutive promoter the penicillinase gene from *B. lycheniformis*. The construct was sequenced using oWX2395.

pWX916 [pACYC *terminator Ppen cfp-parB^{P1}-parS^{P1}*] was constructed by an isothermal assembly reaction containing three gel-purified fragments: 1) pWX915 digested by HindIII and BamHI; 2) *rbs-cfp-parB^{P1}* amplified using oWX2387 and oWX2388 from pFHC2973 (3); 3) *parS^{P1}* amplified using oWX2389 and oWX2390 from gDNA of TND1379 (4). The construct was sequenced using oWX2395, oWX2396, oWX2397 and 2377.

pWX930 [pNPTS138 *Ppen cfp-parB^{P1}-parS^{P1} kan* at Atu3054/ATU_RS14060] was constructed by an isothermal assembly reaction containing four gel-purified fragments: 1) pNPTS138 digested by EcoRV; 2) a part of Atu3054/ATU_RS14060 amplified using oWX2420 and oWX2421 from C58 gDNA; 3) *cfp-parB^{P1}-parS^{P1}* amplified using oWX2407 and oWX2408 from pWX916 4) a part of Atu3055/ATU_RS14065 amplified using oWX2422 and oWX2423 from C58 gDNA. The construct was sequenced using oWX2424, oWX2426, oWX2377 and oWX2425.

pWX936 [pNPTS138 *PT7strong cfp-parB^{P1}-parS^{P1}* at Atu3054/ATU_RS14060] was constructed by an isothermal assembly reaction containing one gel-purified fragments: pWX930 backbone amplified using oWX2431 and oWX2432. The construct was sequenced using oWX2424, oWX2426, oWX2377 and oWX2425.

pWX962 [pNPTS138 *PT7strong cfp-parB^{P1}-parS^{P1}* at Atu0048/ATU_RS00235] was constructed by an isothermal assembly reaction containing four gel-purified fragments: 1) pNPTS138 digested by EcoRV; 2) *PT7strong cfp-parB^{P1}-parS^{P1}* amplified using oWX2407 and oWX2408 from pWX936; 3) a part of Atu0047/ATU_RS00230 amplified using oWX2502 and oWX2503 from C58 gDNA; 4) a part of Atu0048/ATU_RS00235

amplified using oWX2504 and oWX2505 from C58 gDNA. The construct was sequenced using oWX2506, oWX2377, oWX2426, oWX2507.

pWX995 [pNPTS138 terminators *PT7strong mcherry-parB^{P1}-parS^{P1}* at Atu0048/ATU_RS00235] was constructed by an isothermal assembly reaction containing two gel-purified fragments: 1) pWX962 backbone amplified using oWX2589 and oWX2590 on pWX962; 2) *mcherry* amplified using oWX2584 and oWX2585 from gDNA of BWX2208 (5). The construct was sequenced using oWX2506, oWX2377, oWX2426 and oWX2507.

pIB315 [pNPTS138 15955 Δ*popZ* (ISGA_1749) (*kan*)] was constructed in two steps. First, *At* 15955 *popZ* upstream amplified using IPB140 and IBP141 and *At* 15955 *popZ* downstream amplified using IPB142 and IBP143 from 15955 gDNA were stitched together by PCR and then ligated into pGEM T-easy (Promega), confirmed by sequencing (6). Next the stitched fragment digested using BamH1 and Nhe1 and pNPTS138 digested with the same enzymes were ligated together.

pIB316 [pNPTS138 15955 Δ*podJ* (ISGA_411) (*kan*)] was constructed in two steps. First, *At* 15955 *podJ* upstream amplified using IPB146 and IBP147 and *At* 15955 *podJ* downstream amplified using IPB148 and IBP149 from 15955 gDNA were stitched together by PCR and then ligated into pGEM T-easy (Promega), confirmed by sequencing (6). Next the stitched fragment digested using BamH1 and Nhe1 and pNPTS138 digested with the same enzymes were ligated together.

A. tumefaciens Strain construction

In general, in-frame deletions of C58 *A. tumefaciens* strains were constructed using a previously described allelic replacement method (6). Briefly, regions flanking the gene to be deleted were PCR amplified using Phusion (NEB M0530) or Q5 polymerase (NEB M0491) and cloned into pNPTS138 (7), a ColE1 suicide plasmid that confers kanamycin resistance and sucrose sensitivity, by isothermal assembly reactions. See Plasmid construction for details. pNPTS138 deletion constructs were then introduced into *A.*

tumefaciens C58 via mating with *E. coli* S17-1/λpir (8) carrying the appropriate construct. Screening for plasmid integration and target gene deletion was performed as previously described (6, 9). Colony PCR was used to amplify the region to confirm the deletion mutants. Specifically,

C58*, mcherry-parB^{P1}-parS^{P1}* inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1*, *ygfp-parB^{pMT1}-parS^{pMT1}* inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from *ori2* (AtWX356) was generated in two steps. First, pWX967 was used to insert *ygfp-parB^{MT1}-parS^{MT1}* between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from *ori2*, generating AtWX295. This strain was confirmed using oWX2508 and oWX2511. Next, pWX995 was used to insert *mcherry-parB^{P1}-parS^{P1}* between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1*, generating AtWX356. This strain was confirmed using oWX2502 and oWX2505.

C58*, ∆repB^{Ch2}, mcherry-parB^{P1}-parS^{P1}* inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1*, *ygfp-parB^{pMT1}-parS^{pMT1}* inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from *ori2* (AtWX402) was generating using pWX854 on AtWX356 (see above), and conformed using oWX2076 and oWX2077.

C58, ∆*traI*, *tetRA*::*gen Ptral-riboswitch-parB1*(Atu2828/ATU_RS13770) *traR*, *mcherry-parB^{P1}-parS^{P1}* inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1*, *ygfp-parB^{pMT1}-parS^{pMT1}* inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from *ori2* (AtWX496) was generated in two steps. First, pWX967 was used to insert *ygfp-parB^{MT1}-parS^{MT1}* between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from *ori2* on AtWX192 (10), generating AtWX486. This strain was confirmed using oWX2508 and oWX2511. Next, pWX995 was used to insert *mcherry-parB^{P1}-parS^{P1}* between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from *ori1* on AtWX486, generating AtWX496. This strain was confirmed using oWX2502 and oWX2505.

AtWX192 contains Δ*traI*, *tetRA*::*gen PtraI-riboswitch-parB1*(Atu2828/ATU_RS13770) *traR* (10).

C58, Δ*traI*, *tetRA*::*gen PtraI-riboswitch-parB1*(Atu2828/ATU_RS13770) *traR*, *ygfp-parB^{pMT1}-parS^{pMT1}* inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from *oAt* (AtWX498) was generated using pWX1005 on AtWX192 (10), and confirmed using oWX2597 and oWX2600.

C58, Δ*repB^{Ch2}*, *ygfp-parB^{pMT1}-parS^{pMT1}* inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from *oAt* (AtWX500) was generated using pWX1005 on AtWX089 (10), and confirmed using oWX2597 and oWX2600. AtWX089 contains Δ*repB^{Ch2}* (10).

C58, Δ*podJ* (Atu0499/ATU_RS02460) (AtWX283) was generated using *pGM9*, and confirmed using oWX2291 and oWX2292.

C58, Δ*popZ* (Atu1720/ATU_RS08420) (AtWX110) was generated using pWX839, and confirmed using oWX2160 and oWX2163.

C58, Δ*popZ* (Atu1720/ATU_RS08420) Δ*podJ* (Atu0499/ATU_RS02460) (AtWX121) was generated using pGM9 on AtWX110, and confirmed using oWX2291 and oWX2292.

C58, Δ*gpr* (Atu1348/ATU_RS06650) (AtWX286) was generated using pJZ298 (11), and confirmed using oWX2530 and oWX2531.

15955, Δ*podJ* (ISGA_411) (IB172) was generated using pIB316, and confirmed using IBP144 and IBP145.

15955, Δ*popZ* (ISGA_1749) (IB173) was generated using pIB315, and confirmed using IBP150 and IBP151.

Replicative plasmids were introduced to *A. tumefaciens* by electroporation as previously described (6). pWX822, pJZ253, pMAT3 were electroporated into C58 WT, generating AtWX234, AtWX236, AtWX265. pWX970 was electroporated into AtWX110, AtWX121, AtWX283 and AtWX286 to generate AtWX303, AtWX305, AtWX307 and AtWX309. pWX822 was electroporated into AtWX089 (10) and AtWX192 (10), generating AtWX291 and AtWX289, respectively.

***B. subtilis* Strain construction**

***pelB::Psoj mCherry-parB1$_{At}$ tet* (BWX5258)** A ligation reaction containing the following two DNA fragments was directly transformed to PY79: 1) pWX564 [*pelB::Psoj-mcherry-spo0J (parS*) (tet)*] (12) cut with BamHI and XhoI to remove *spo0J (parS*)*; 2) *parB1$_{At}$* (amplified from C58 genomic DNA using oWX2563 and oWX2564, and then cut with BamHI and XhoI). The transformants were amplified using oWX776 and oML85 and sequenced using oWX776 and oML85.

***ycgO::Psoj mgfpmut3-RepB$^{Ch2}_{At}$ cat* (BWX5260)** A ligation reaction containing the following three DNA fragments was directly transformed to PY79: 1) an empty cloning vector pKM077 [*ycgO::cat*] cut with EcoRI and BamHI; 2) *repB$^{Ch2}_{At}$* (amplified from C58 genomic DNA using oWX2566 and oWX2567, and then cut with BamHI and XhoI); 3) *Psoj mgfpmut3* liborated from pWX563 using EcoRI and XhoI. The transformants were amplified and sequenced using oWX2497 and oWX2568. pWX563 (13) contains *pelB::Psoj-mgfpmut3-spo0J (parS*) tet*.

***parS2$_{At}$* cluster at -91° *kan* (BWX5265)** An isothermal assembly reaction containing the following three PCR products was directly transformed to PY79: 1) the region containing *ytuF* upstream region (amplified from PY79 genomic DNA using oWX1279 and oWX2569); 2) the *parS2$_{At}$* region (amplified from C58 genomic DNA using oWX2570 and oWX2571); 3) the region containing *kan*, *ytuF* and *ytuF* downstream (amplified from BWX3379 genomic DNA (14) using primers oWX439 and oWX1282).

The transformants were amplified using oWX1283 and oML83 and sequenced using oWX1283 and oML83.

***parS∆9 no a.b., ycgO::Phyperspank-optRBS-mgfpmut3- repB$^{Ch2}_{At}$ cat* (BWX5309)** An isothermal assembly reaction containing the following three PCR products was directly transformed to BWX3212 (15): 1) the region containing *ycgO* downstream (amplified from PY79 genomic DNA using oWX2668 and oWX2650); 2) the *Phyperspank* promotor amplified from pJW005 (16) using oWX2655 and oWX2669); 3) the region containing *mgfpmut3-repB*$^{Ch2}_{At}$, *cat*, *ycgO* downstream (amplified from genomic DNA of BWX5260 using primers oWX2649 and oWX2651). The transformants were amplified using oWX2568 and oWX2560 and sequenced using oWX2568 and oWX2497.

***ycgO::Phyperspank mgfpmut3-repB*$^{Ch2}_{\mathrm{At}}$ *cat, parS∆9* (BWX5329)** An isothermal assembly reaction containing the following three PCR products was directly transformed to BWX3212 (15): 1) the region containing *ycgO* downstream and *Phyperspank mgfpmut3-repB*$^{Ch2}_{\mathrm{At}}$ (amplified from genomic DNA of BWX5309 (see above) using oWX2674 and oWX2650); 2) the *lacI-cat* and *ycgO* upstream (amplified from pWX588 using oWX2675 and oWX2651). pWX588 contains *ycgO::Pspank* (optRBS) gfp-spo0J (parS*) cat*. The transformants were amplified using oWX2568 and oWX2560 and sequenced using oWX2568 and oWX2497.

After individual *B. subtlis* constructs were built as above, their genomic DNA was extracted and used in successive transformations to build BWX5333, BWX5341, BWX5349, BWX5353, BWX5359.

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