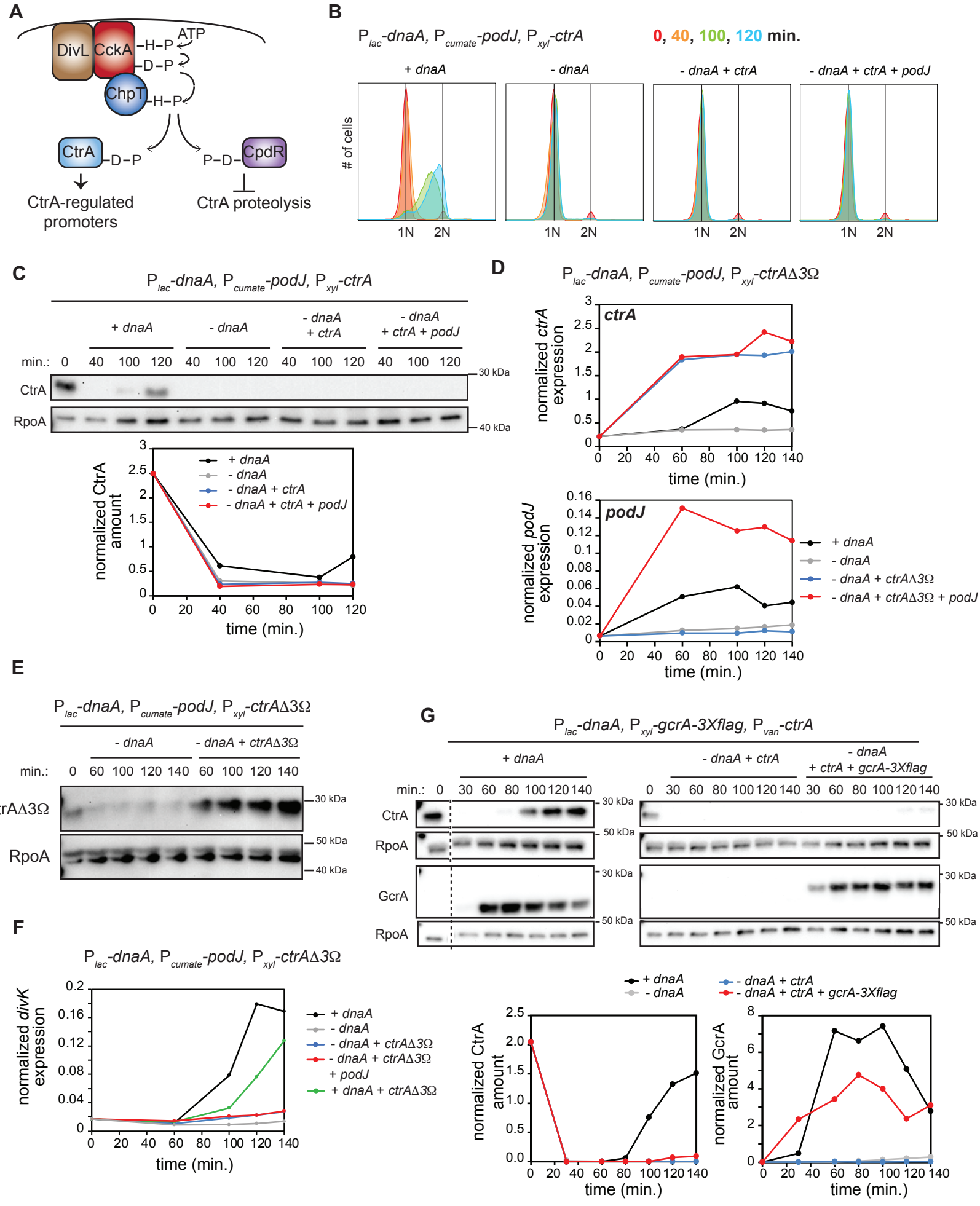
A
DivL
CckA
ChpT
ATP
-H-P
-D-P
CtrA
CpdR
CtrA-regulated promoters
CtrA proteolysis
B
P lac -dnaA, P cumate -podJ, P xyl -ctrA
0, 40, 100, 120 min.
+ dnaA
- dnaA
- dnaA + ctrA
- dnaA + ctrA + podJ
# of cells
1N
2N
C
P lac -dnaA, P cumate -podJ, P xyl -ctrA
min.: 0 40 100 120 40 100 120 40 100 120 40 100 120
CtrA
RpoA
30 kDa
40 kDa
normalized CtrA amount
time (min.)
D
P lac -dnaA, P cumate -podJ, P xyl -ctrAΔ3Ω
ctrA
podJ
normalized ctrA expression
normalized podJ expression
time (min.)
+ dnaA
- dnaA
- dnaA + ctrAΔ3Ω
- dnaA + ctrAΔ3Ω + podJ
E
P lac -dnaA, P cumate -podJ, P xyl -ctrAΔ3Ω
- dnaA
- dnaA + ctrAΔ3Ω
min.: 0 60 100 120 140 60 100 120 140
CtrAΔ3Ω
RpoA
30 kDa
50 kDa
40 kDa
F
P lac -dnaA, P cumate -podJ, P xyl -ctrAΔ3Ω
normalized divK expression
time (min.)
+ dnaA
- dnaA
- dnaA + ctrAΔ3Ω
- dnaA + ctrAΔ3Ω + podJ
+ dnaA + ctrAΔ3Ω
G
P lac -dnaA, P xyl -gcrA-3Xflag, P van -ctrA
+ dnaA
- dnaA + ctrA
- dnaA + ctrA + gcrA-3Xflag
min.: 0 30 60 80 100 120 140
CtrA
RpoA
GcrA
30 kDa
50 kDa
normalized CtrA amount
normalized GcrA amount
time (min.)
- dnaA + ctrA + gcrA-3Xflag

Figure S1, related to Fig. 1: Polarization of CckA and DivL is not sufficient to activate CtrA in predivisional cells in the absence of DNA replication.

(A) Schematic of the CckA-ChpT phosphorelay activity at the new pole in predivisional cells.

(B) Flow cytometry profiles after SYTOX staining showing DNA content of synchronized cells expressing *dnaA* (+IPTG) or depleted of *dnaA* (-IPTG) with ectopic expression of *ctrA* (+0.075% xyl) and *podJ* (+cumate) when indicated.

(C) Biological replicate of Fig. 1B.

(D) mRNA levels of the *ctrA* and *podJ* genes measured by qRT-PCR and normalized to *rpoA* mRNA levels in cells expressing *dnaA* (+IPTG) or depleted for *dnaA* (-IPTG) with ectopic expression of the stable *ctrA* point mutant *ctrAΔ3Ω* (+0.075% xyl) and *podJ* (+cumate) when indicated.

(E) CtrAΔ3Ω protein levels in cells depleted for *dnaA* (-IPTG) with ectopic expression of the stable *ctrA* point mutant *ctrAΔ3Ω* (+0.075% xyl) when indicated.

(F) Data represent one of the biological replicates from Fig. 1C with addition of the *+dnaA +ctrAΔ3Ω* condition.

(G) CtrA and GcrA protein levels at the times indicated post-synchronization in cells expressing *dnaA* (+IPTG) or depleted of *dnaA* (-IPTG) with ectopic expression of wild-type *ctrA* (+van) and *gcrA-3xflag* (+xyl) when indicated. Graphs show CtrA and GcrA protein band intensity normalized to RpoA.

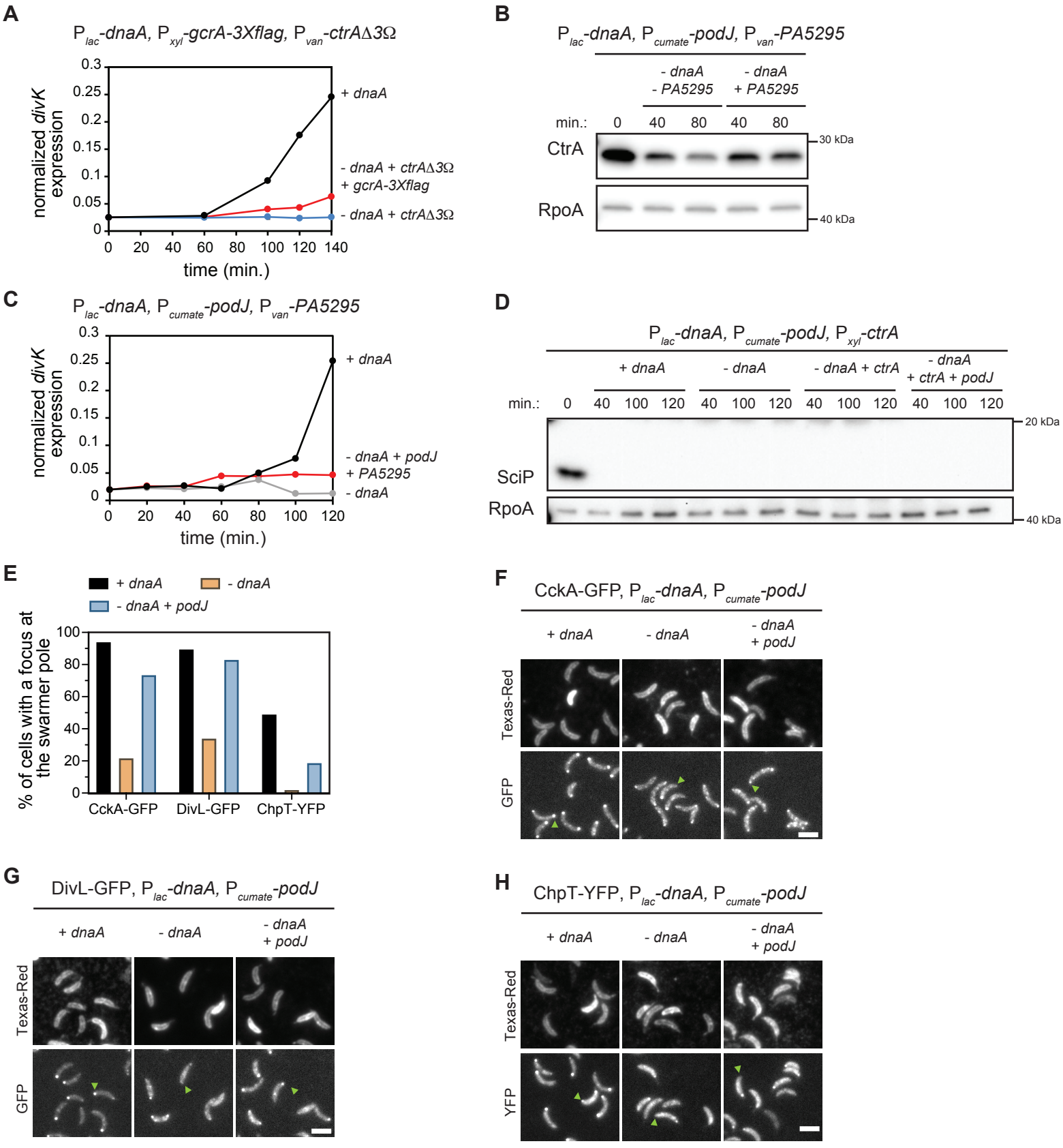
A
P lac -dnaA, P xyl -gcrA-3Xflag, P van -ctrAΔ3Ω
normalized divK expression
time (min.)
+ dnaA
- dnaA + ctrAΔ3Ω + gcrA-3Xflag
- dnaA + ctrAΔ3Ω
B
P lac -dnaA, P cumate -podJ, P van -PA5295
- dnaA - PA5295
- dnaA + PA5295
min.: 0 40 80 40 80
CtrA
RpoA
30 kDa
40 kDa
C
P lac -dnaA, P cumate -podJ, P van -PA5295
normalized divK expression
time (min.)
+ dnaA
- dnaA + podJ + PA5295
- dnaA
D
P lac -dnaA, P cumate -podJ, P xyl -ctrA
+ dnaA
- dnaA
- dnaA + ctrA
- dnaA + ctrA + podJ
min.: 0 40 100 120 40 100 120 40 100 120 40 100 120
SciP
RpoA
20 kDa
40 kDa
E
+ dnaA
- dnaA
- dnaA + podJ
% of cells with a focus at the swarmer pole
CckA-GFP
DivL-GFP
ChpT-YFP
F
CckA-GFP, P lac -dnaA, P cumate -podJ
+ dnaA
- dnaA
- dnaA + podJ
Texas-Red
GFP
G
DivL-GFP, P lac -dnaA, P cumate -podJ
+ dnaA
- dnaA
- dnaA + podJ
Texas-Red
GFP
H
ChpT-YFP, P lac -dnaA, P cumate -podJ
+ dnaA
- dnaA
- dnaA + podJ
Texas-Red
YFP

Figure S2, related to Fig. 1: Polarization of CckA and DivL is not sufficient to activate CtrA in predivisional cells in the absence of DNA replication.

(A) mRNA levels of the CtrA-activated gene *divK* measured by qRT-PCR and normalized to *rpoA* mRNA levels at the times indicated post-synchronization in cells expressing *dnaA* (+IPTG) or depleted of *dnaA* (-IPTG) with ectopic expression of the proteolytically stable mutant *ctrAΔ3Ω* (+van) and with (+xyl) or without ectopic expression of *gcrA-3xflag*.

(B) CtrA protein levels in cells expressing (+ 50 μM van) or not (-van) the PA5295 phosphodiesterase in cells depleted for *dnaA* (-IPTG).

(C) mRNA levels of the CtrA-activated gene *divK* measured by qRT-PCR and normalized to *rpoA* mRNA levels at the times indicated post-synchronization in cells expressing *dnaA* (+IPTG) or depleted of *dnaA* (-IPTG) and the *P. aeruginosa* phosphodiesterase PA5295 (+50 μM van) when indicated. This strain also expressed an ectopic copy of *podJ (*+cumate), when indicated.

(D) SciP protein levels at the times indicated post-synchronization in cells expressing *dnaA* (+IPTG) or depleted of *dnaA* (-IPTG) with ectopic expression of *ctrA* (+xyl) and *podJ* (+cumate) when indicated.

(E-G) Same as Fig. 1D-F but with additional Texas-Red-X succinimidyl ester (TRSE) staining to label the stalk by non-specifically labeling surface-exposed proteins (Hughes *et al.*, 2013). Scale bars = 2 μm.

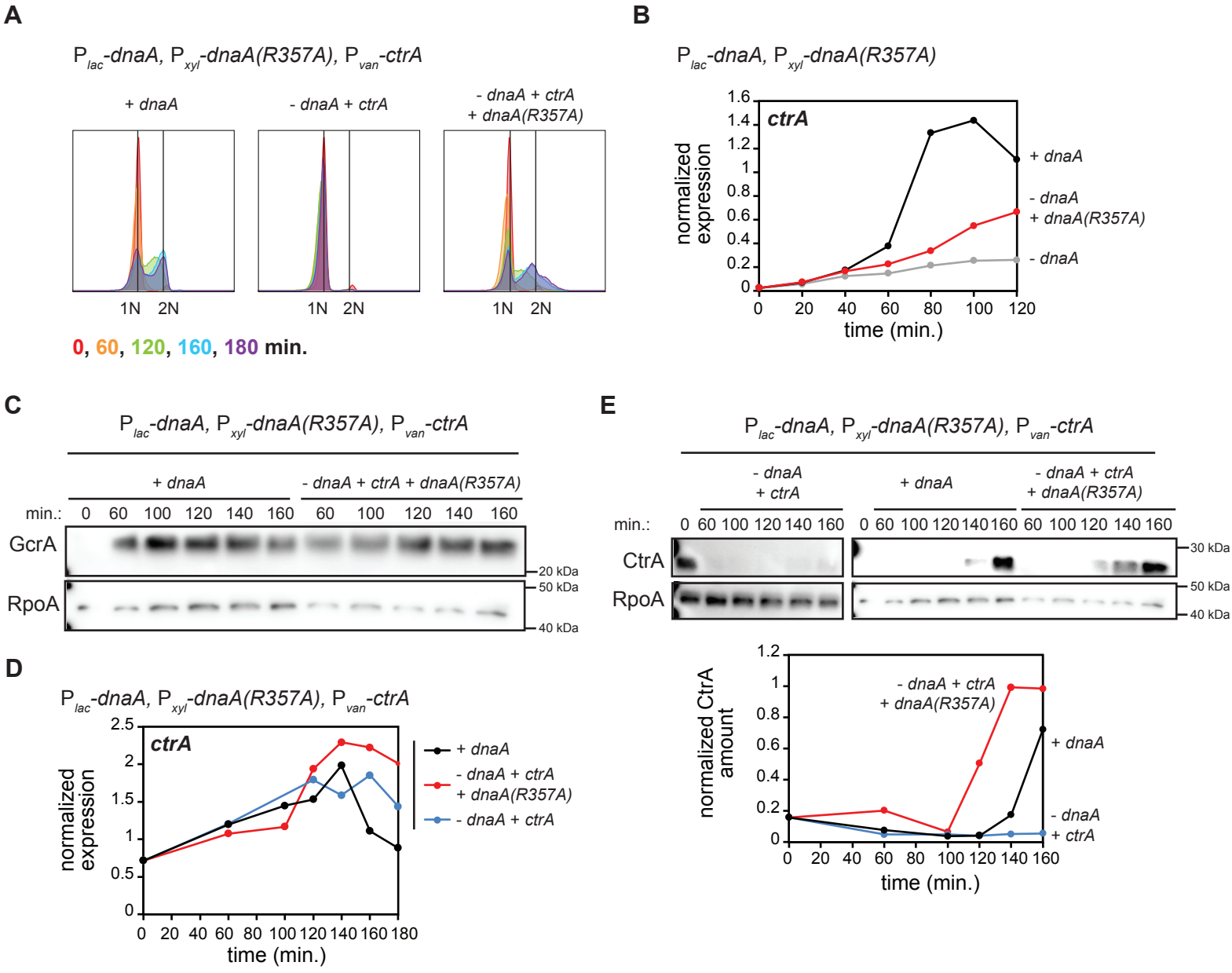
A
P lac -dnaA, P xyl -dnaA(R357A), P van -ctrA
+ dnaA
- dnaA + ctrA
- dnaA + ctrA + dnaA(R357A)
1N 2N
0, 60, 120, 160, 180 min.
B
P lac -dnaA, P xyl -dnaA(R357A)
ctrA
normalized expression
time (min.)
+ dnaA
- dnaA + dnaA(R357A)
- dnaA
C
P lac -dnaA, P xyl -dnaA(R357A), P van -ctrA
+ dnaA
- dnaA + ctrA + dnaA(R357A)
min.: 0 60 100 120 140 160 60 100 120 140 160
GcrA
RpoA
20 kDa
50 kDa
40 kDa
D
P lac -dnaA, P xyl -dnaA(R357A), P van -ctrA
ctrA
normalized expression
time (min.)
+ dnaA
- dnaA + ctrA + dnaA(R357A)
- dnaA + ctrA
E
P lac -dnaA, P xyl -dnaA(R357A), P van -ctrA
- dnaA + ctrA
+ dnaA
- dnaA + ctrA + dnaA(R357A)
min.: 0 60 100 120 140 160 0 60 100 120 140 160 60 100 120 140 160
CtrA
RpoA
30 kDa
50 kDa
40 kDa
normalized CtrA amount
time (min.)
- dnaA + ctrA + dnaA(R357A)
+ dnaA
- dnaA + ctrA

Figure S3, related to Fig. 2: Replication of the full chromosome is not required for CtrA activation.

(A) Flow cytometry profiles after SYTOX staining showing DNA content of synchronized cells expressing *dnaA* (+IPTG) or depleted of *dnaA* (-IPTG) with ectopic expression of *ctrA* (+van) and *dnaA(R357A)* (+xyl) when indicated.

(B) *ctrA* mRNA levels measured by qRT-PCR and normalized to *rpoA* mRNA levels in cells expressing dnaA (+IPTG) or depleted of *dnaA* (-IPTG) with ectopic expression of *dnaA(R357A)* (+xyl) when indicated.

(C) GcrA protein levels in cells expressing *dnaA* (+IPTG) or depleted of *dnaA* (-IPTG) with ectopic expression of wild-type *ctrA* (+van) and *dnaA(R357A)* (+xyl). RpoA is shown as a loading control.

(D) mRNA levels of *ctrA* measured by qRT-PCR and normalized to *rpoA* mRNA levels in cells expressing dnaA (+IPTG) or depleted of *dnaA* (-IPTG) with ectopic expression of *ctrA* (+van) and *dnaA(R357A)* (+xyl) when indicated.

(E) Biological replicate of Fig. 2B.

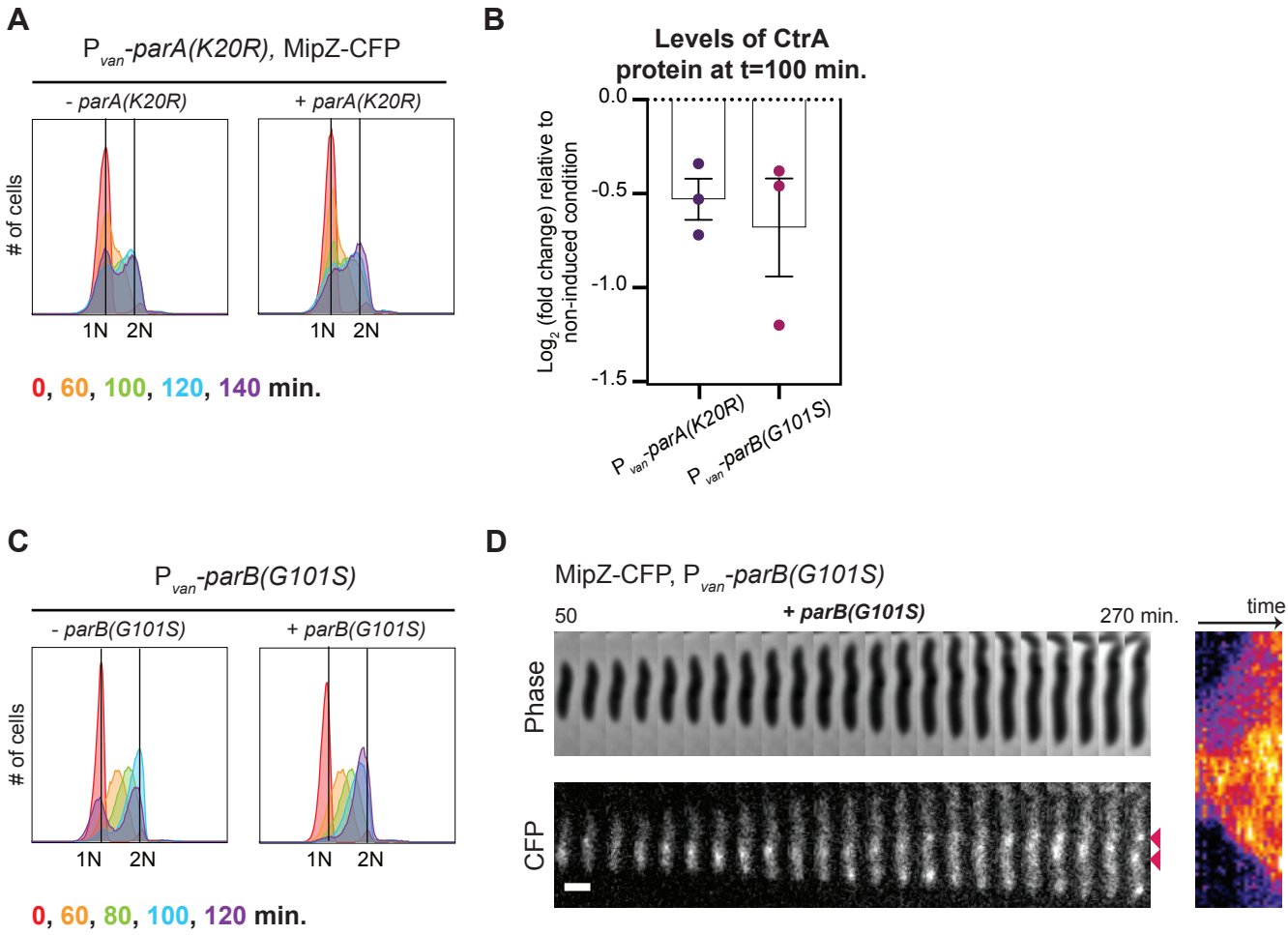
A
P van -parA(K20R), MipZ-CFP
- parA(K20R)
+ parA(K20R)
# of cells
1N 2N
1N 2N
0, 60, 100, 120, 140 min.
B
Levels of CtrA protein at t=100 min.
Log2 (fold change) relative to non-induced condition
0.0
-0.5
-1.0
-1.5
P van -parA(K20R)
P van -parB(G101S)
C
P van -parB(G101S)
- parB(G101S)
+ parB(G101S)
# of cells
1N 2N
1N 2N
0, 60, 80, 100, 120 min.
D
MipZ-CFP, P van -parB(G101S)
50
+ parB(G101S)
270 min.
time
Phase
CFP

Figure S4, related to Fig. 3: CtrA activation in predivisional cells is reduced when chromosome segregation is perturbed.

(A) Flow cytometry profiles after SYTOX staining showing DNA content of synchronized cells with (+van) or without (-van) ectopic expression of *parA(K20R)*. Expression of *parA(K20R)* was induced 60 min. before synchronization and after synchronization with 500 μM vanillate.

(B) Relative fold-change in CtrA protein levels normalized to RpoA levels 100 min. post-synchronization, when inducing *parA(K20R)* or *parB(G101S)* 60 min. pre-synchronization compared to the uninduced condition. Bars indicate mean ± SEM from three biological replicates shown as individual datapoints.

(C) Flow cytometry profiles after SYTOX staining showing DNA content of synchronized cells with (+van) or without (-van) ectopic expression of *parB(G101S)*. Expression of *parB(G101S)* was induced 60 min. before synchronization and after synchronization with 500 μM vanillate.

(C) Time lapse of MipZ-CFP dynamics within a single cell expressing the *parB(G101S)* mutant. *parB(G101S)* was induced 30 min. before synchronization and during imaging post-synchronization in the agarose pad with addition of 500 μM vanillate. Red arrows show MipZ-CFP internal clusters next to the newly replicated chromosomal origins. Scale bar = 1 μm.

Figure S5

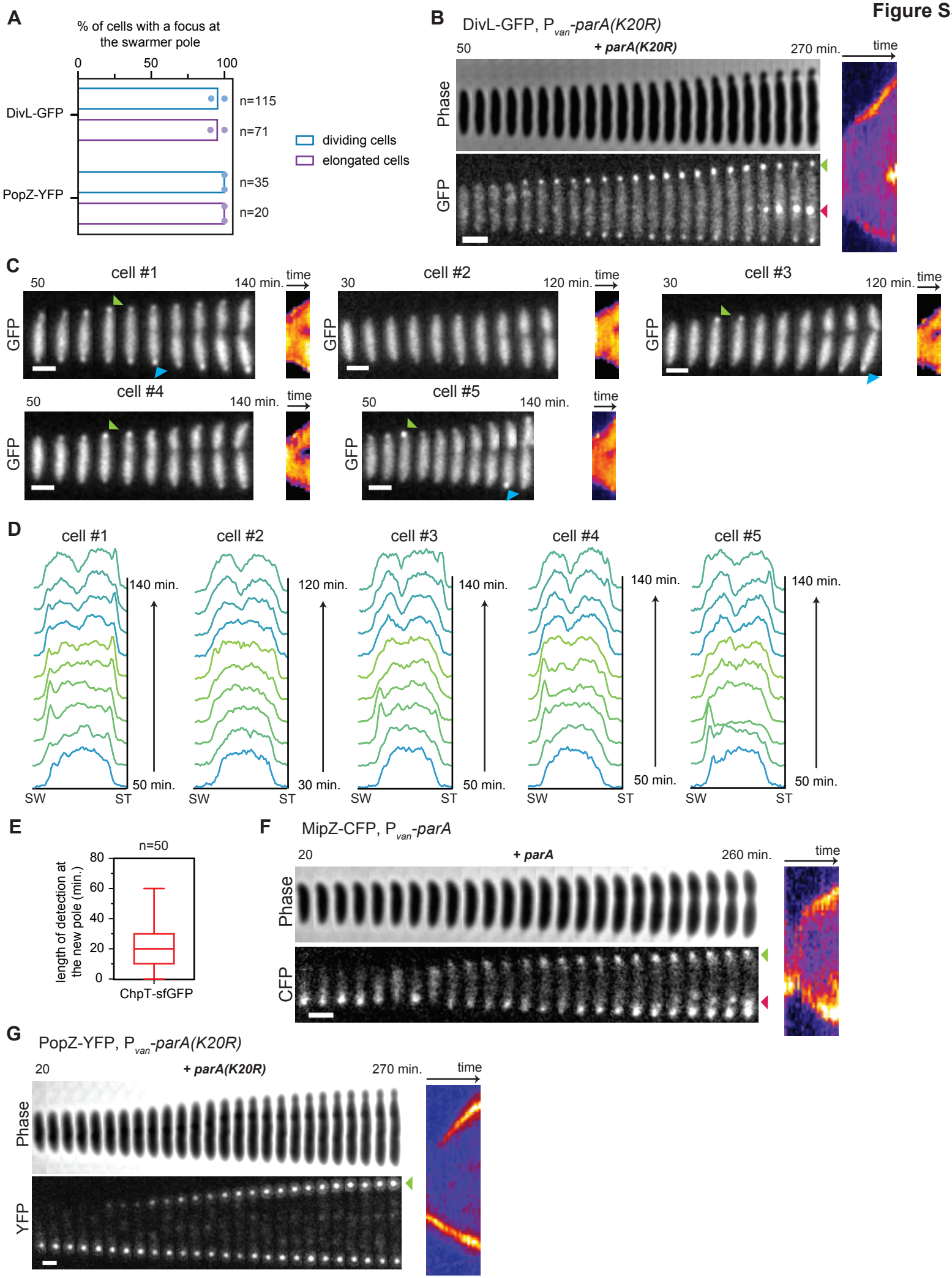
A
% of cells with a focus at the swarmer pole
0
50
100
DivL-GFP
PopZ-YFP
n=115
n=71
n=35
n=20
dividing cells
elongated cells
B
DivL-GFP, P van -parA(K20R)
50
+ parA(K20R)
270 min.
time
Phase
GFP
C
cell #1
cell #2
cell #3
cell #4
cell #5
50
140 min.
30
120 min.
time
GFP
D
cell #1
cell #2
cell #3
cell #4
cell #5
140 min.
120 min.
50 min.
30 min.
SW
ST
E
n=50
length of detection at the new pole (min.)
0
20
40
60
80
ChpT-sfGFP
F
MipZ-CFP, P van -parA
20
+ parA
260 min.
time
Phase
CFP
G
PopZ-YFP, P van -parA(K20R)
20
+ parA(K20R)
270 min.
time
Phase
YFP

Figure S5, related to Fig. 4: ChpT access to the new swarmer pole is affected when chromosome segregation is disrupted.

(A) Percentage of cells with detectable foci at the swarmer pole at any time during time-lapse imaging of DivL-GFP and PopZ-YFP without *parA(K20R)* induction (dividing cells; -van) and with *parA(K20R)* induction (elongated cells only; +van). *parA(K20R)* was induced 30 min. pre- and post-synchronization with 500 μM vanillate. Bars indicate mean from two biological replicates shown as individual datapoints. Total # of cells examined from two biological replicates indicated in each case.

(B) Example from time lapse imaging of cells quantified in (A) for DivL-GFP. Green arrow shows DivL-GFP localization at the new swarmer pole. Red arrow shows DivL-GFP accumulation in a single internal cluster. Scale bar = 1 μm.

(C) Time-lapses of ChpT-sfGFP in wild-type cells. Additional examples of individual cells from the one shown in Fig. 4A. See also Movie S1. Green arrow shows ChpT-sfGFP localization at the new swarmer pole and blue arrow shows ChpT-sfGFP localization at the old stalked pole. Scale bar = 1 μm.

(D) Average fluorescence intensity profiles from the new pole to the old pole of the five individual cells shown in (C).

(E) Boxplot representing the length of detection of ChpT-sfGFP in wild-type cells at the new pole, based on the number of consecutive frame with a detectable ChpT-sfGFP polar cluster. The lower and upper boundaries of the boxes correspond to the 25% and 75% percentiles, respectively. The median is shown as a thick black line and the whiskers represent the minimum and maximum values.

(F) Timelapse of MipZ-CFP dynamics within a single cell expressing ectopic wild-type *parA*, induced 30 min. before synchronization and during imaging post-synchronization in the agarose pad with addition of 500 μM vanillate. Green arrow shows MipZ-CFP localization at the new swarmer pole . Red arrow shows MipZ-CFP internal cluster. Scale bar = 1 μm.

(G) As in (B) but for PopZ-YFP dynamics. The green arrow shows PopZ-YFP localization at the new swarmer pole. Scale bar = 1 μm.

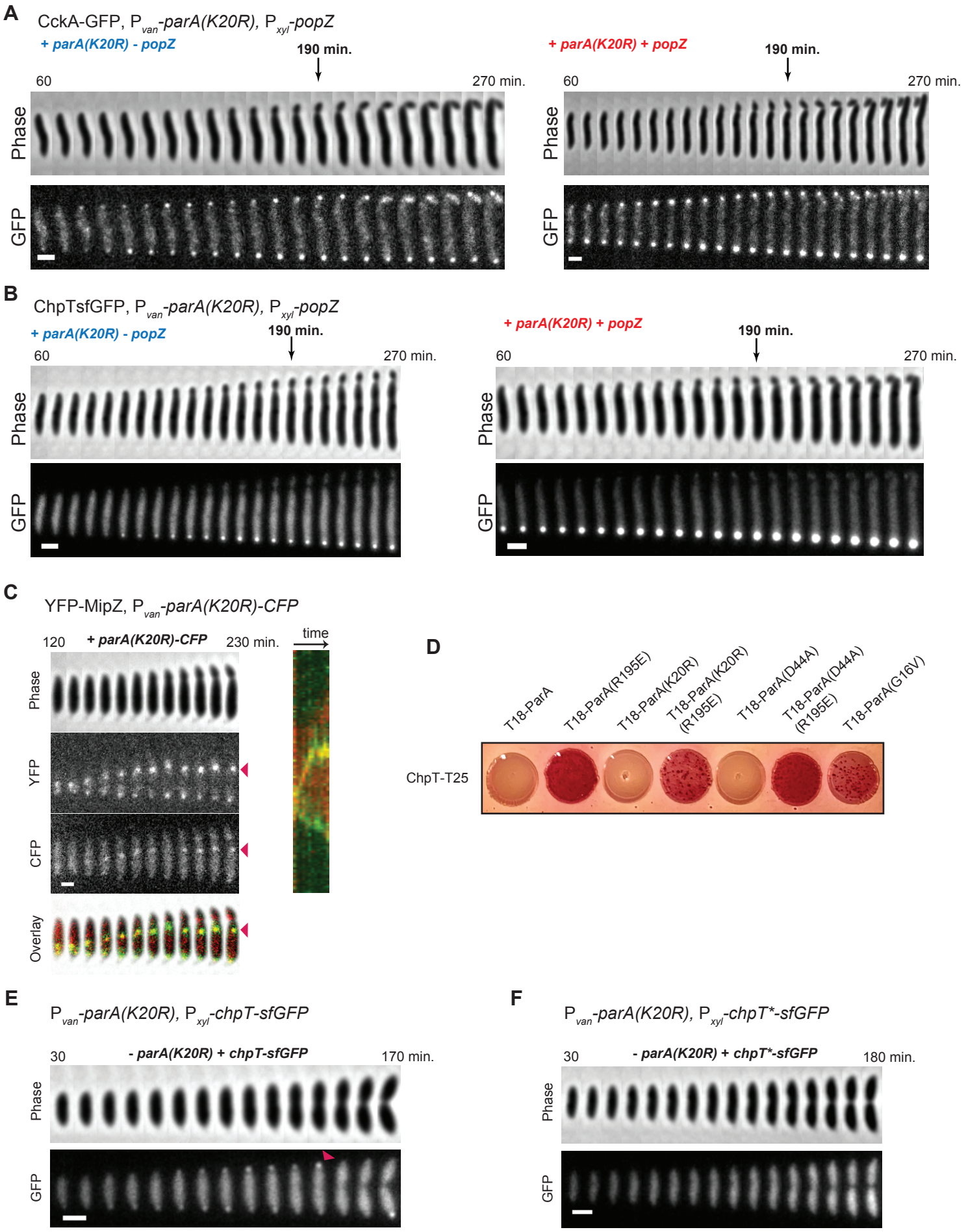
A
CckA-GFP, P van -parA(K20R), P xyl -popZ
+ parA(K20R) - popZ
190 min.
60
270 min.
Phase
GFP
+ parA(K20R) + popZ
190 min.
60
270 min.
Phase
GFP
B
ChpTsfGFP, P van -parA(K20R), P xyl -popZ
+ parA(K20R) - popZ
190 min.
60
270 min.
Phase
GFP
+ parA(K20R) + popZ
190 min.
60
270 min.
Phase
GFP
C
YFP-MipZ, P van -parA(K20R)-CFP
120
+ parA(K20R)-CFP
230 min.
time
Phase
YFP
CFP
Overlay
D
T18-ParA
T18-ParA(R195E)
T18-ParA(K20R)
T18-ParA(K20R) (R195E)
T18-ParA(D44A)
T18-ParA(D44A) (R195E)
T18-ParA(G16V)
ChpT-T25
E
P van -parA(K20R), P xyl -chpT-sfGFP
30
- parA(K20R) + chpT-sfGFP
170 min.
Phase
GFP
F
P van -parA(K20R), P xyl -chpT*-sfGFP
30
- parA(K20R) + chpT*-sfGFP
180 min.
Phase
GFP

Figure S6, related to Fig. 5: ParA not bound to DNA recruits ChpT to the swarmer pole

(A-B) Full time lapses of CckA-GFP (A) and ChpT-sfGFP (B) dynamics in cells expressing *parA(K20R)* (+van) with (+xyl) or without (-xyl) induction of *popZ* expression, corresponding to Fig. 5A and B respectively. Scale bar = 1 μm.

(C) YFP-MipZ internal clusters colocalize with ectopically produced ParA(K20R)-CFP. Time lapse imaging of YFP-MipZ and ParA(K20R)-CFP dynamics within a single cell expressing *parA(K20R)* (+van). *parA(K20R)* was induced 30 min. before synchronization and during imaging post-synchronization in the agarose pad with addition of 500 μM vanillate. In the overlay, YFP-MipZ is shown in green and ParA(K20R)-CFP in red. Red arrows show the YFP-MipZ and ParA(K20R)-CFP internal cluster. Scale bar = 1 μm.

(D) Bacterial two-hybrid assay testing the interaction of ChpT fused to the T25 *Bordetella* adenylate cyclase domain with ParA and ParA point mutants fused to the *Bordetella* T18 adenylate cyclase domain. When spotted on MacConkey agar plates supplemented with IPTG and maltose, interactions between the two fusion proteins result in a red color shift of the bacterial colony.

(E-F) Time lapse of the dynamics of ectopic ChpT-sfGFP and the ChpT(R167E,R169E,R171E)-sfGFP (ChpT*-sfGFP) mutant (+xyl) in cells that are not expressing *parA(K20R)*. Compare to Fig. 5E and F, respectively. Note that the ChpT*-sfGFP point mutant fails to accumulate at the new pole prior to cell division, compared to ectopically produced wild-type ChpT-sfGFP (red arrow). Scale bar = 1 μm.

Supplementary Table S1, related to STAR Methods, strain construction: Bacterial strains used in this study

***Caulobacter crescentus* strains**		
ML1071	ChpT-YFP	This study
ML3415	eYFP-mipZ::mipZ	This study
ML3359	*hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{cum}-*podJ* P_{xyl}-*ctrA*	This study
ML3360	*hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{cum}-*podJ* P_{xyl}-*ctrA*$\Delta 3\Omega$	This study
ML3361	CckA-GFP *hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{cum}-*podJ*	This study
ML3362	DivL-GFP *hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{cum}-*podJ*	This study
ML3363	ChpT-YFP *hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{cum}-*podJ*	This study
ML3364	*hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{xyl}-*gcrA-3xflag* P_{van}-*ctrA*	This study
ML3365	*hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{xyl}-*gcrA-3xflag* P_{van}-*ctrA*$\Delta 3\Omega$	This study
ML3366	*cckA(Y514D)* P_{van}-*hdaA*	This study
ML3367	*hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{cum}-*podJ* P_{van}-*PA5295*	This study
ML3368	DivL-GFP *hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{xyl}-*dnaA(R357A)*	This study
ML3369	DivL-GFP *hfa*::P_{lacI}-*lacI* P_{dnaA}::P_{lac} P_{xyl}-*dnaA(R357A)* P_{van}-*ctrA*	This study
ML3370	MipZ-CFP P_{van}-*parA(K20R)*	This study
ML3371	CckA-GFP P_{van}-*parA(K20R)*	This study
ML3372	DivL-GFP P_{van}-*parA(K20R)*	This study
ML3373	ChpT-sfGFP	This study
ML3374	ChpT-sfGFP P_{van}-*parA(K20R)*	This study
ML3375	ChpT-sfGFP P_{van}-*parA*	This study
ML3376	MipZ-CFP P_{van}-*parA*	This study
ML3377	YFP-MipZ P_{van}-*parA(K20R)-cfp*	This study
ML3378	ChpT-YFP P_{van}-*parA(K20R)-cfp*	This study
ML3379	PopZ-YFP P_{van}-*parA(K20R)*	This study
ML3380	ChpT-sfGFP P_{van}-*parA(K20R)* P_{xyl}-*popZ*	This study
ML3381	CckA-GFP P_{van}-*parA(K20R)* P_{xyl}-*popZ*	This study
ML3382	*xyl::*P_{xyl}-*chpT-sfgfp* P_{van}-*parA(K20R)*	This study
ML3383	*xyl::*P_{xyl}-*chpT(R167E)(R169E)(R171E)-sfgfp* P_{van}-*parA(K20R)*	This study
ML3384	MipZ-CFP *van::*P_{van}-1Xflag-*parB(G101S)*	This study
***E. coli* strains**		
ML3385	BTH101 *p*KNT25-ChpT	This study
ML3386	BTH101 *p*KNT25-$ChpT_{DHP}$	This study

ML3387	BTH101 *p*KT25-CckA$_{HK}$	This study
ML3388	BTH101 *p*KNT25-ChpT(R167E)(R169E)(R171E)-T25	This study
ML3389	BTH101 *p*UT18C-ParB	This study
ML3390	BTH101 *p*UT18C-ParA	This study
ML3391	BTH101 *p*UT18C-ParA(R195E)	This study
ML3392	BTH101 *p*UT18C-ParA(K20R)	This study
ML3393	BTH101 *p*UT18C-ParA(K20R)(R195E)	This study
ML3394	BTH101 *p*UT18C-ParA(D44A)	This study
ML3395	BTH101 *p*UT18C-ParA(D44A)(R195E)	This study
ML3396	BTH101 *p*UT18C-ParA(G16V)	This study
ML3397	BTH101 *p*UT18-ChpT	This study
ML3398	BTH101 *p*UT18C-CckA$_{RD}$	This study
ML3399	BTH101 *p*UT18C-MipZ	This study
ML3400	BTH101 *p*UT18C-MipZ(R194A)	This study

Supplementary Table S2, related to STAR Methods, plasmid construction and quantitative PCR: Primers used in this study

Primers	
AAGCTTTCGCGAGACGTCCAATTGCATATGACCGAGACCGTCACCGAGACCAC CGCCCCC	pYFPC2_chpT_up_F
GGGGGCGGTGGTCTCGGTGACGGTCTCGGTCATATGCAATTGGACGTCTCGCG AAAGCTT	pYFPC2_chpT_up_R
GACCCCCGTGAAGAGCTCCTCGCCCTTCGACATGGTGGCGCCCGAGCCCCGC GCCGGGACCCAGGCGGCGATCGAGGCGCG	chpT_dwn_sfGFP_up_R
ACGCATGGCATGGATGAACTCTACAAGTAAGCTAGCTGCAGCCCGGGGGATCC ACTAGTT	sfGFP_dwn_pYFPC2_F
cagacgctcgagttttggggagacgaccatATGACCGAGACCGTCACCGAGACCACCGCC	pBX_chpT_up_F
AACTAGTGGATCCCCCGGGCTGCAGCTAGCTTACTTGTAGAGTTCATCCATGCC ATGCGT	sfGFP_pXGFPC_down_R2
ACGCATGGCATGGATGAACTCTACAAGTAAGCTAGCTGCAGCCCGGGGGATCC ACTAGTT	sfGFP_pXGFPC_down_F2
GGCGGTGGTCTCGGTGACGGTCTCGGTCATatggtcgtctccccaaaactcgagcgtctg	pBX_chpT_up_R
CTGTTTGTAACTAGTAGAGGAAGCTTCCGCATGACGGCGGCTTCGCCATGGAG C	podJ_4pQF_1..24
GAGCTCGGTACCTCGAGATCTTCTAGAGTCttattaTTAGCGCGCGTAGACCGACA GGCG	podJ_4pQF_last24
CATGCGGAAGCTTCCTCTACTAGT	pQF_CPEC_up
GACTCTAGAAGATCTCGAGGTACCG	pQF_CPEC_noFLAG_down
GGAATTCCATATGCGCGTACTGTTGATCGA	ctrA_NdeI_F
CTA**GCTAGC**TCATCAGGCGGCGTTAACCTGCT	ctrA-extrastop-NheI-R
tgtatcGCTAGCtcatcaatcaatcaccggatccc	ctrAΔ3W -NheI-R
GACGCTGTCGGTGAAACGGTC	dnaA 1068..1048
gcCGAGCTGGAAGGCGCGCTGAACACC	dnaA 1069..1095 R357A
CGGgaattcTTATTATTAGCCCCGCAGCTTGCGCG	DnaA_rev_3xTAA_EcoRI
AGATCATATGACCATGAAGGGCGGGGTTGC	NdeI_DnaA_1..23
GGAGACGACCATATGGTGTCCGCTAAT	pXYFP_ParA_NdeI_up_F
CGAGCTCTTATTAGGCGGCCTTGGCCTGGCG	ParA_TAATAA_SacI_R
GCCGAACCACGATGCGAGGAAACGCATATGGTGTCCGCTAATCCTCTCCGCGT TCTGGCT	pRVMCS-6_parA_up_F
GCACCGGTACGTAGATCTTAAGAGCTCTTATTAGGCGGCCTTGGCCTGGCGAT CGCGTTC	pRVMCS-6_parA_down_R
GAACGCGATCGCCAGGCCAAGGCCGCCTAATAAGAGCTCTTAAGATCTACGTA CCGGTGC	pRVMCS-6_parA_down_F
GGTGCCCTTAAACGCCTGGTTCTACGCCTG	pRVMCS6_upcat_R
AGCCAGAACGCGGAGAGGATTAGCGGACACCATATGCGTTTCCTCGCATCGTG GTTCGGC	pRVMCS6_parA_up_R
CCCCTTTCGCCAGCAGATCCGTCGGTCCCT	pRMCS_mid_F
accatgcatattaattaaggcgcctgcaggGGCGGCCTTGGCCTGGCGATCGCGTTCGCG	parA_CFP_R
CGCGAACGCGATCGCCAGGCCAAGGCCGCCcctgcaggcgccttaattaatatgcatggt	parA_CFP_F
CTCTAGAACTAGTGGATCCCCCGGGCTGCAGCTAGCttacttgtacagctcgtccatgcc	CFP_pRVMCS6_dwn_R
ggcatggacgagctgtacaagtaaGCTAGCTGCAGCCCGGGGGATCCACTAGTTCTAGAG	CFP_pRVMCS6_dwn_F
cagacgctcgagttttggggagacgaccatATGTCCGATCAGTCTCAAGAACCTACAATG	pBX_popZ_up_F
actagtggatcccccgggctgcaggaattcTTAGGCGCCGCGTCCCCGAGAGATACGCTG	pB_popZ_down_R
CAGCGTATCTCTCGGGGACGCGGCGCCTAAgaattcctgcagcccgggggatccactagt	pB_popZ_down_F

CATTGTAGGTTCTTGAGACTGATCGGACATatggtcgtctccccaaaactcgagcgtctg	pBX_popZ_up_R
acaatttcacacaggaaacagctATGACCGAGACCGTCACCGAGACCACCG	pKNT25_chpT_up_F
CGGTGGTCTCGGTGACGGTCTCGGTCATagctgtttcctgtgtgaaattgt	pKNT25_chpT_up_R
CTCGATCGCCGCCTGGGTCCCGGCGTCGAATTCAATGACCATGCAGCAAT	pKNT25_chpT_down_F
ATTGCTGCATGGTCATTGAATTCGACGCCGGGACCCAGGCGGCGATCGAG	pKNT25_chpT_down_R
CCGAGTGGCCTTCGGCGCCTCGGCCTCGAATTCAATGACCATGCAGCAAT	pKNT25_chpTDHP_down_F
ATTGCTGCATGGTCATTGAATTCGAGGCCGAGGCGCCGAAGGCCACTCGG	pKNT25_chpTDHP_down_R
GGGATCCTCTAGAGTCGACCCTGCAGCCCG	pKT25_up_R
TAActaagaattcggccgtcgttttacaac	pKT25_down_F
CGGGCTGCAGGGTCGACTCTAGAGGATCCCCGCGGCTCAGCGCTTTCCGGCG GCGACGCC	pKT25_cckA_HK_up_F
gttgtaaaacgacggccgaattcttagTTAGGCGCCCGACAGGTCGCGAGCGGCGCGCGG	pKT25_cckA_HK_down_R
CTAAAGGGCGAGCCGCTGGCCGAGGGCCT	R167E169E171E_short2_F
TTGGCGTCGGCGATGATCGAGAAGCGCCCG	R167E169E171E_short2_R
CGGGCGCTTCTCGATCATCGCCGACGCCAAGGGCCCGGAGGCGGAGCTGGAG CCGGAGGTGCTGGCGGGCCTAAAGGGCGAGCCGCTGGCCGAGGGCCT	R167E169E171E_ultramer
AGGCCCTCGGCCAGCGGCTCGCCCTTTAGGCCCGCCAGCACCTCCGGCTCCA GCTCCGCCTCCGGGCCCTTGGCGTCGGCGATGATCGAGAAGCGCCCG	R167E169E171E_ultramer_ R
CTGCAGGTCGACTCTAGAGGATCCCGAGTCCGTCGTGGTGGGAGAGCCCG	pUT18C_parB_up_F
CGGGCTCTCCCACCACGACGGACTCGGGATCCTCTAGAGTCGACCTGCAG	pUT18C_parB_up_R
CAACCGACTGACGCGCGGGATCTAACTAAGTAAtatggtgcactctcagt	pUT18C_parB_down_F
actgagagtgcaccataTTACTTAGTTAGATCCCGCGCGTCAGTCGGTTG	pUT18C_parB_down_R
CTGCAGGTCGACTCTAGAGGATCCCTCCGCTAATCCTCTCCGCGTTCTGG	pUT18C_parA_up_F
CCAGAACGCGGAGAGGATTAGCGGAGGGATCCTCTAGAGTCGACCTGCAG	pUT18C_parA_up_R
ACGCGATCGCCAGGCCAAGGCCGCCTAACTAAGTAAtatggtgcactctc	pUT18C_parA_down_F
gagagtgcaccataTTACTTAGTTAGGCGGCCTTGGCCTGGCGATCGCGT	pUT18C_parA_down_R
TCGTACATGGTCAGCACAACCCCTTGGATC	ParAR195E_R
CAGCTTGTCGGAACAGGTCGCCAAGGACGT	ParAR195E_F
GATCCAAGGGGTTGTGCTGACCATGTACGACCGCGAGAACAGCTTGTCGGAAC AGGTCGCCAAGGACGT	ultramer_parAR195E_F
ACGTCCTTGGCGACCTGTTCCGACAAGCTGTTCTCGCGGTCGTACATGGTCAG CACAACCCCTTGGATC	ultramer_parAR195E_R
AGGGCAACTGTTCCACGGGGCTTGGCATTG	ParAD44A_F
TCGATCAGCAGCACGCGCTCGCCGCAGGCG	ParAD44A_R
CGCCTGCGGCGAGCGCGTGCTGCTGATCGACGCCGCCCCGCAGGGCAACTGT TCCACGGGGCTTGGCATTG	ParAD44A_ultramer_F
CAATGCCAAGCCCCGTGGAACAGTTGCCCTGCGGGGCGGCGTCGATCAGCAG CACGCGCTCGCCGCAGGCG	ParAD44A_ultramer_R
TGGGGAAGACCACGACCGCGATCAATCTGG	ParAG16V_F
GATTGGCGATAGCCAGAACGCGGAGAGGAT	ParAG16V_R
ATCCTCTCCGCGTTCTGGCTATCGCCAATCAAAAGGTCGGGGTGGGGAAGACC ACGACCGCGATCAATCTGG	ParAG16V_ultramer_F
CCAGATTGATCGCGGTCGTGGTCTTCCCCACCCCGACCTTTTGATTGGCGATAG CCAGAACGCGGAGAGGAT	ParAG16V_ultramer_R
tATGACCATGATTACGCCAAGCTTGACCGAGACCGTCACCGAGACCACCG	pUT18_chpT_up_F
CGGTGGTCTCGGTGACGGTCTCGGTCAAGCTTGGCGTAATCATGGTCATa	pUT18_chpT_up_R
CTCGATCGCCGCCTGGGTCCCGGCGTCGAATTCAGCCGCCAGCGAGGCCA	pUT18_chpT_down_F
TGGCCTCGCTGGCGGCTGAATTCGACGCCGGGACCCAGGCGGCGATCGAG	pUT18_chpT_down_R

CTGCAGGTCGACTCTAGAGGATCCCCGCATCCTGTTCGTCGAGGACGAGG	pUT18C_cckA_RD_up_F
CCTCGTCCTCGACGAACAGGATGCGGGGATCCTCTAGAGTCGACCTGCAG	pUT18C_cckA_RD_up_R
CAAGCAGCAGCTGCAGGCGGCGTAACTAAGTAAtatggtgcactctcagt	pUT18C_cckA_RD_down_F
actgagagtgcaccataTTACTTAGTTACGCCGCCTGCAGCTGCTGCTTG	pUT18C_cckA_RD_down_R
CTGCAGGTCGACTCTAGAGGATCCCGCCGAAACGCGCGTTATCGTCGTCG	pUT18C_mipZ_up_F
CGACGACGATAACGCGCGTTTCGGCGGGATCCTCTAGAGTCGACCTGCAG	pUT18C_mipZ_up_R
CGAGACGATGCTGGCGGCGCAGTAACTAAGTAAtatggtgcactctcagt	pUT18C_mipZ_down_F
actgagagtgcaccataTTACTTAGTTACTGCGCCGCCAGCATCGTCTCG	pUT18C_mipZ_down_R
AAGCGTCTGGAGGACCGCCTCAACGCTTTG	mipZR194A_F
TGGTGGCCAGGCGGTTGCGCAGCACCACCC	mipZR194A_R
GGGTGGTGCTGCGCAACCGCCTGGCCACCACCGAGGCGGCCAACCGCAAGCG TCTGGAGGACCGCCTCAACGCTTTG	ultramer_mipZR194A_F
CAAAGCGTTGAGGCGGTCCTCCAGACGCTTGCGGTTGGCCGCCTCGGTGGTG GCCAGGCGGTTGCGCAGCACCACCC	ultramer_mipZR194A_R
GAGCAATGCGACCGTTGATC	Cori_2_F
CGGTTGCTTAACCACTTGCC	Cori_2_R
GGGCATGTCGTTGTAGTGGA	CCNA_03518_F
AACAAGAAGGCGAATCCGGT	CCNA_03518_R
GACGACGGAATGCTCGAGAT	CCNA_00623_F
GTGTCATCCACCTGCGAGAA	CCNA_00623_R
CGGCCATAGATGTCGAAGCT	CCNA_02846_F
TCGCAATCTGAACGACACCA	CCNA_02846_R
ATGGGCTTTGAGACCTCTGC	CCNA_02189_F
CGAAGCGCCTCATAGATCGT	CCNA_02189_R
ACTATCGCAGCGTCTTCTCG	CCNA_02567_F
GTCCATCATTTCGGCTTCGC	CCNA_02567_R
TTCGTCTGGTTCTGGCTGTC	CCNA_01869_F
CGTCAGCGTCTACGACTACC	CCNA_01869_R
ACATCGTCTACATCGGCGAC	rpoA_qPCR_1
GGCGAGCACTTCCTTGATCT	rpoA_qPCR_2
GTACGACCCTGACCAAGGAA	ctrA_qPCR_1
GCAGATGAAGACGTCGATGA	ctrA_qPCR_5
GTTACGAGACCCTGCAGACC	divK_qPCR_1
TCGTCTTCCTTCAGCCACTT	divK_qPCR_2
GGATCTGGATCTGGAAGTCG	pdeA_qPCR_3
AAGCCTTGGCCATAGTCACA	pdeA_qPCR_5
ATCACCGGCATCGATCACTC	podJ_qPCR_1
CTGCTCGGTCTTCAGTTCGT	podJ_qPCR_2

Supplementary Table S3, related to STAR Methods, plasmid construction: Plasmids used in this study

Plasmids		
ML3401	*p*YFPC-2-*chpT-sfgfp*	This study
ML3402	*p*RVMCS-5-*ctrAΔ3Ω*	This study
ML3403	*p*QF-*podJ*	This study
ML3405	*p*RVMCS-5-*ctrA*	This study
ML3406	*p*BXMCS-2-*dnaA(R357A)*	This study
ML3407	*p*RVMCS-6-*parA*	This study
ML3408	*p*RVMCS-6-*parA(K20R)*	This study
ML3409	*p*RVMCS-6-*parA(K20R)-cfp*	This study
ML3410	*p*BXMCS-2-*popZ*	This study
ML3411	*p*BXMCS-4-*popZ*	This study
ML3412	*p*XGFPC-2-*chpT-sfgfp*	This study
ML3413	*p*XGFPC-2-*chpT(R167E)(R169E)(R171E)-sfgfp*	This study