

Supplementary Information

Chromosome organization by a conserved condensin-ParB system in the actinobacterium *Corynebacterium glutamicum*

Kati Böhm¹, Giacomo Giacomelli^{1,2}, Andreas Schmidt³, Axel Imhof³, Romain
Koszul^{4,5}, Martial Marbouty^{4*}, and Marc Bramkamp^{1,2*}

¹ Ludwig-Maximilians-Universität München, Fakultät Biologie, Großhaderner Straße
2-4, 82152 Planegg-Martinsried, Germany

² Christian-Albrechts-Universität zu Kiel, Institut für allgemeine Mikrobiologie, Am
Botanischen Garten 1-9, 24118 Kiel, Germany

³ Ludwig-Maximilians-Universität München, Zentrallabor für Proteinanalytik,
Biomedizinisches Centrum München, 82152 Planegg-Martinsried, Germany

⁴ Institut Pasteur, Unité Régulation Spatiale des Génomes, Paris, France/ CNRS,
UMR 3525, Paris, France.

⁵ Institut Pasteur, Center of Bioinformatics, Biostatistics and Integrative Biology
(C3BI), Paris, France

* Corresponding authors:

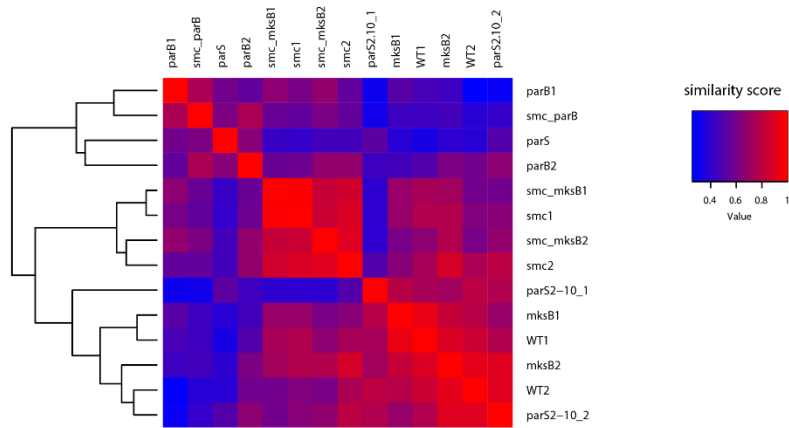
Marc Bramkamp: Email: bramkamp@ifam.uni-kiel.de; Phone: +49-(0)431-8804341;
Fax: +49(0)431-880-2198

Martial Marbouty: Email: martial.marbouty@pasteur.fr; Phone: +33-144389365

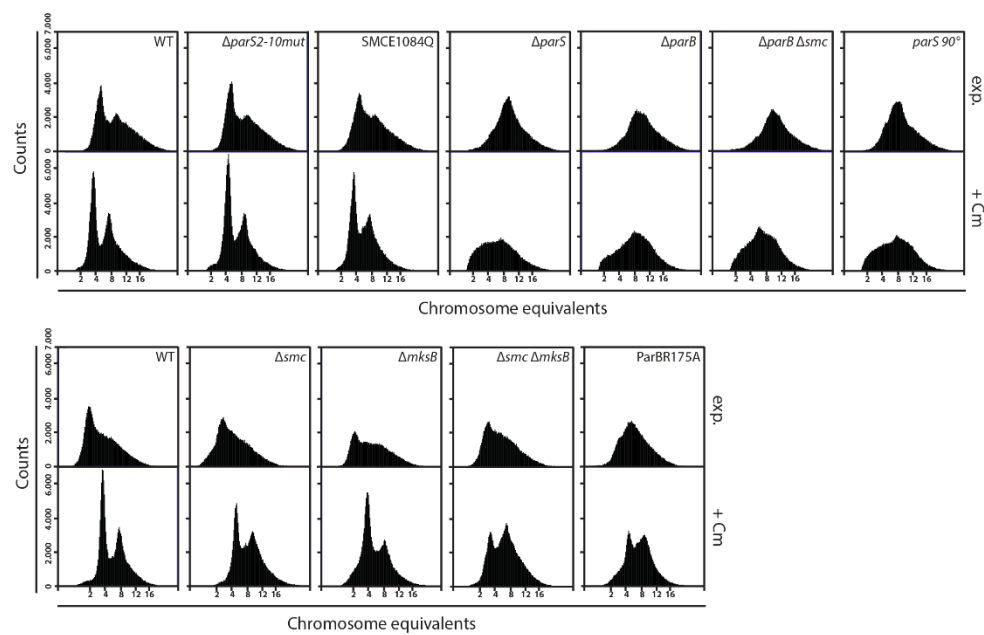
Supplementary Figure 1: Chromosome organization and domains analysis in *Corynebacterium glutamicum*.

A) From top to bottom: i) Whole genome anti-mCherry ChIP-seq data of strain CKB006 harboring ParB-mCherry. ii) Normalized genomic contact map derived from asynchronously grown cells (fast growth $\mu \geq 0.6 \text{ h}^{-1}$, exponential phase). X- and Y-axes indicate chromosomal coordinates binned in 5 Kb; *oriC*-centered as in Fig. 1. Color scales, indicated above the contact map, reflect contact frequency between two genomic loci from white to red (rare to frequent contacts). iii) Transcription signal using 5Kb bins ¹. iv) long genes (black bars) more than 3 Kb. v) domain signals at 200 Kb resolution (DI analysis) with up- and downstream regions marked in green and red are displayed. vi) domain signals at 100 Kb resolution. **B)** Correlation between transcription and short-range chromosome contacts captured by 3C. Genomic coordinates are indicated on X-axis and contacts between neighboring bins are plotted (blue curve) along the transcription pattern (orange curve) ².

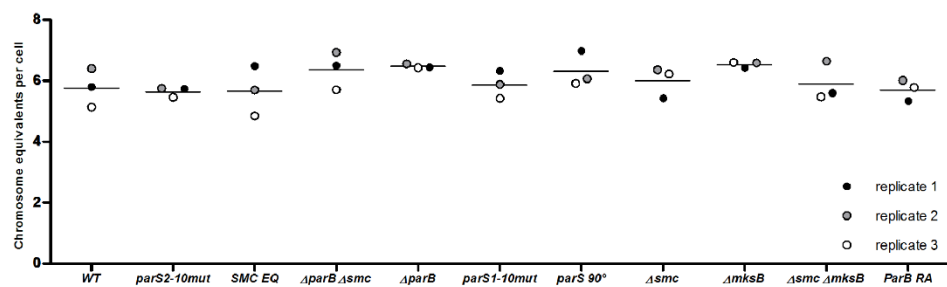
A



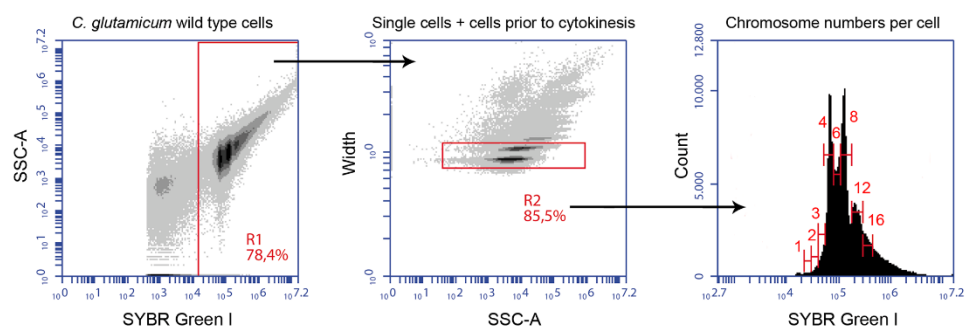
B



C



D



42

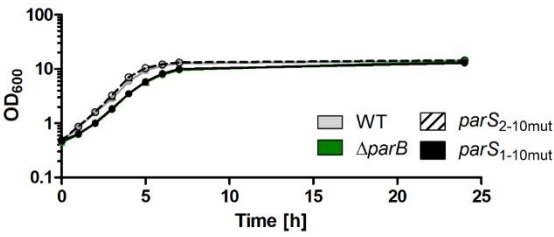
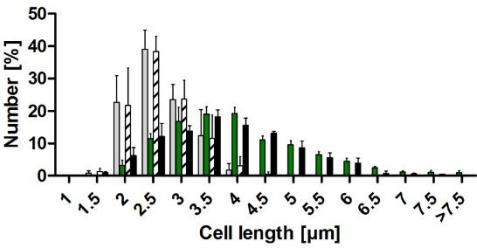
43 **Supplementary Figure 2. Replicates of chromosomal contact maps in *C.***
44 ***glutamicum* mutants.**

45 **A)** Similarity matrix between the different constructed libraries. The distance between
46 the different libraries was calculated using HiCRep³. Color scale indicates the similarity
47 score between libraries. **B)** Determination of DNA amount per cell of *C. glutamicum*
48 strains used for Hi-C. Distributions of DNA content in wild-type and mutant strains as
49 indicated (top: CBK023, CBK050, CBK024, CDC003, CBK002, CBK037; below:
50 CDC026, CBK001, CBK004, CBK047) Flow cytometry was performed with SYBR
51 Green-stained samples of exponential BHI-grown cultures (exp.) and after > 4 h of
52 incubation with chloramphenicol (+Cm). Chromosome numbers are shown in
53 histograms vs the green channel FI1-A. **C)** Averaged chromosome numbers per cell
54 determined by flow cytometry after replication runout for above-named strains (mean,
55 n = 3). DNA amount per cell does not significantly differ between wild-type and mutant
56 strains (two sample t test, 5 % significance level). **D)** Gating strategy for *C. glutamicum*
57 cells via flow cytometry. Gating is exemplified using wild-type *C. glutamicum* cells at
58 replication run-out conditions. Left: 200000 cells were monitored in R1 in order to
59 distinguish DNA-containing cells (SYBR Green I⁺) from dust particles and non-stained
60 debris using SYBR Green I versus side scatter (SSC-A) gating. Middle panel: Cell
61 aggregates derived from fixation processes and incomplete separation after cell
62 division were excluded from R1 in SSC-A versus width plots in gate R2. Note that two
63 populations were taken into account in order to include single cells as well as long cells
64 prior to cytokinesis. Right: Histogram showing distribution of SYBR Green I⁺ cells
65 (labels represent increasing number of chromosome equivalents) within gate R2.
66 Source data are provided as a Source Data file.

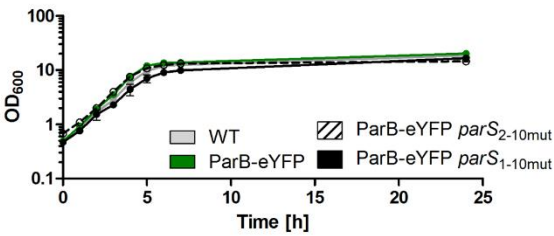
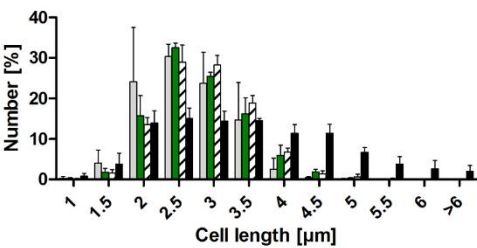
A

<i>parS</i> _{WT}	TGTTTCACGTGAAACA	PmlI
<i>parS</i> _{mut}		
1	GGTGTCCCGGAGACT	XmaI
2-4, 6-8, 10	CGAATCCCGGATACT	
5, 9	CGAATGTCGACATACT	Sall

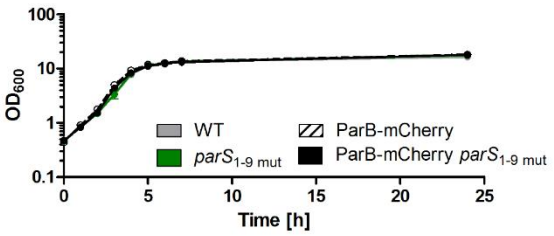
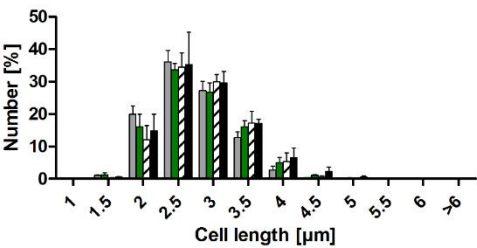
B



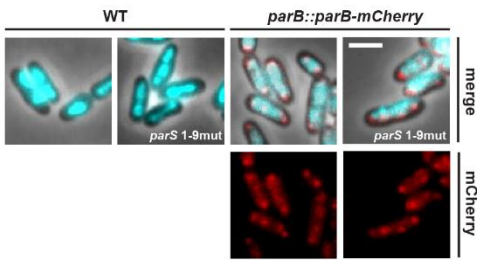
C



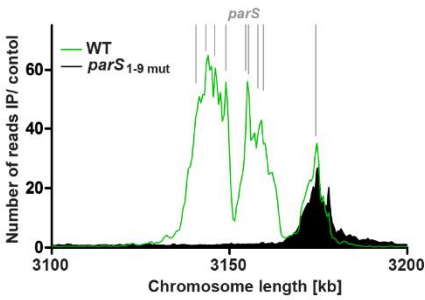
D



E



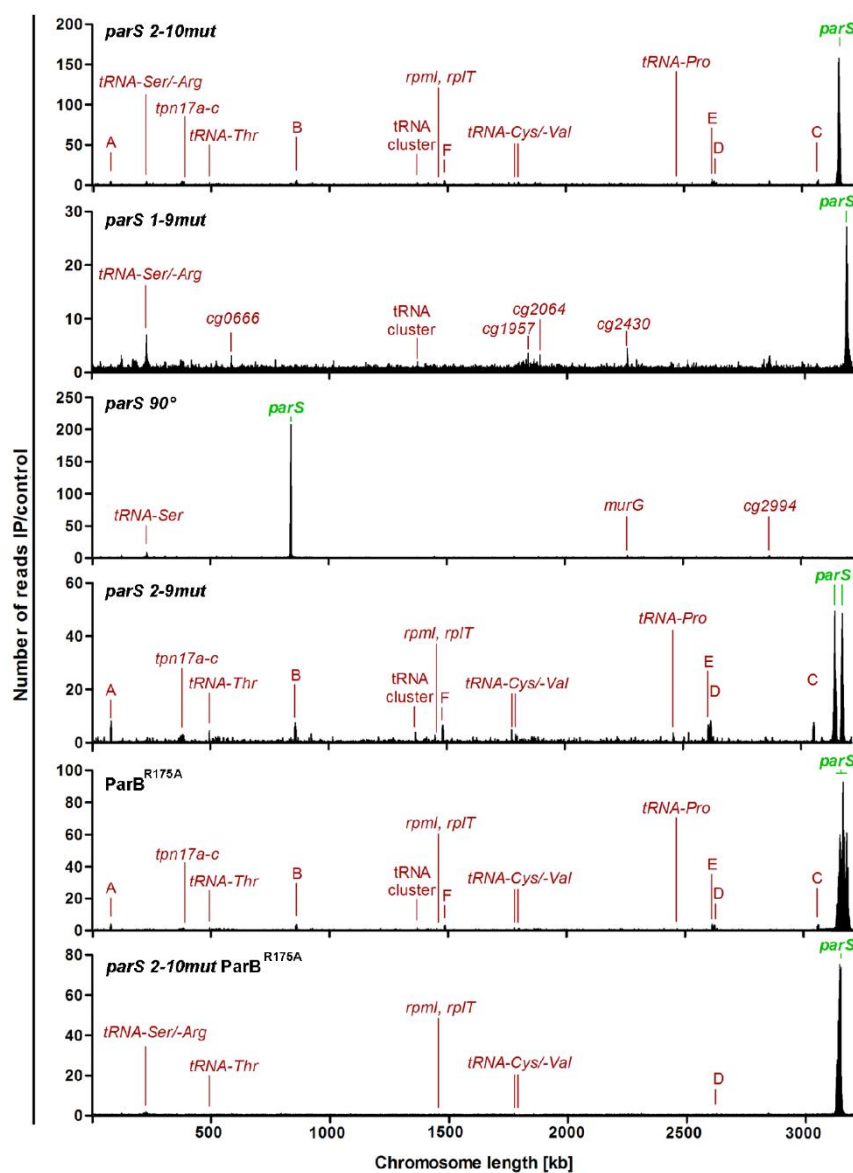
F



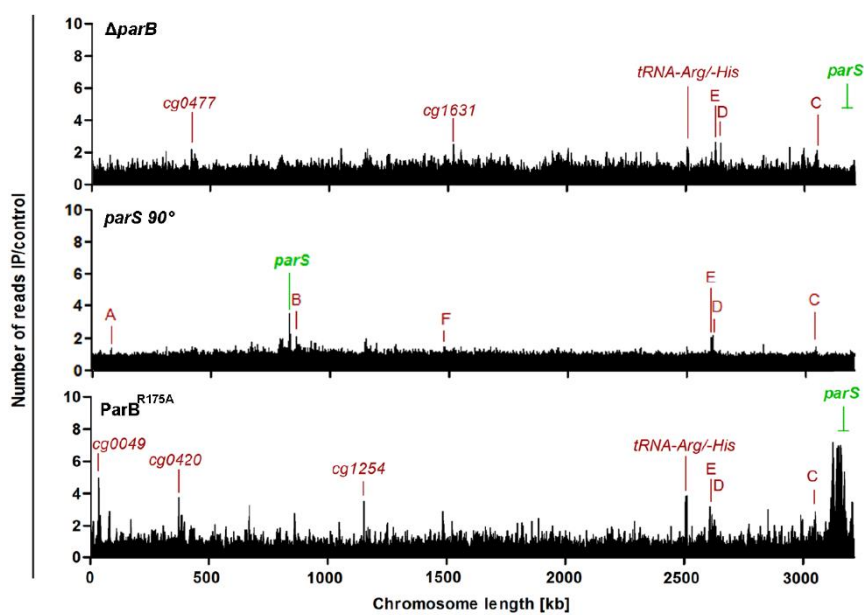
Supplementary Figure 3. Mutation and shifting of *parS* sites affects growth and morphology.

A) Point mutations render native *parS* sequences nonfunctional. Base pair exchanges (red letters) generate new restriction sites (underscored) for mutation screening as indicated. Notably, point mutations of the intragenic *parS* site are silent. **B), C)** Mutation of *parS* sites mimics growth and morphology of $\Delta parB$ cells. Comparison of cell length distributions (mean $\pm$ s.d., n of each biological replicate listed below) and growth curves (mean $\pm$ s.d., n = 3) of *parS* mutants and *parB* deletion strain grown in BHI. Growth rates derive from triplicates: $\mu_{WT}=0.65 \text{ h}^{-1}$ ($n_{b1} = 446$, $n_{b2} = 387$, $n_{b3} = 355$, $n_{c1} = 633$, $n_{c2} = 381$, $n_{c3} = 325$), $\mu_{\Delta parB}=0.57 \text{ h}^{-1}$ (CDC003) ($n_1 = 376$, $n_2 = 628$, $n_3 = 518$), $\mu_{parS2-10mut}=0.69 \text{ h}^{-1}$ (CBK023) ($n_1 = 503$, $n_2 = 502$, $n_3 = 952$), $\mu_{parS1-10mut}=0.57 \text{ h}^{-1}$ (CBK024) ($n_1 = 383$, $n_2 = 261$, $n_3 = 506$), $\mu_{ParB-eYFP}=0.65 \text{ h}^{-1}$ (CBK007) ($n_1 = 421$, $n_2 = 403$, $n_3 = 487$), $\mu_{ParB-eYFP \text{ } parS2-10mut}=0.59 \text{ h}^{-1}$ (CBK025) ($n_1 = 710$, $n_2 = 408$, $n_3 = 302$), $\mu_{ParB-eYFP \text{ } parS1-10mut}=0.55 \text{ h}^{-1}$ (CBK026) ($n_1 = 402$, $n_2 = 209$, $n_3 = 257$). **D)** Growth curves (mean $\pm$ s.d., n = 3) and cell length measurements (mean $\pm$ s.d., n listed below) for *C. glutamicum* wild-type (WT, $\mu=0.68$) ($n_1 = 1545$, $n_2 = 1247$, $n_3 = 1170$), *parS1-9mut* (CBK90, $\mu=0.69$) ($n_1 = 2300$, $n_2 = 975$, $n_3 = 959$), *parB::parB-mCherry* (CBK006, $\mu=0.68$) ($n_1 = 1427$, $n_2 = 1772$, $n_3 = 884$), *parB::parB-mCherry parS1-9mut* (CBK091, $\mu=0.69$) ($n_1 = 670$, $n_2 = 576$, $n_3 = 734$). **E)** Fluorescence microscopy of above-named strains. ParB-mCherry fluorescence (red) is shown in separate channels and as overlays with bright field and Hoechst fluorescence (DNA stain, cyan). Scale bar; 2 μ m. **F)** ParB-mCherry ChIP-seq in strain CBK0 harboring a single *parS10* site (black) compared to wild-type (green). Enrichment signals are shown in a 0.1 Kb range surrounding the *parS* cluster; chromosomal *parS* positions are indicated by gray lines. Source data are provided as a Source Data file.

A

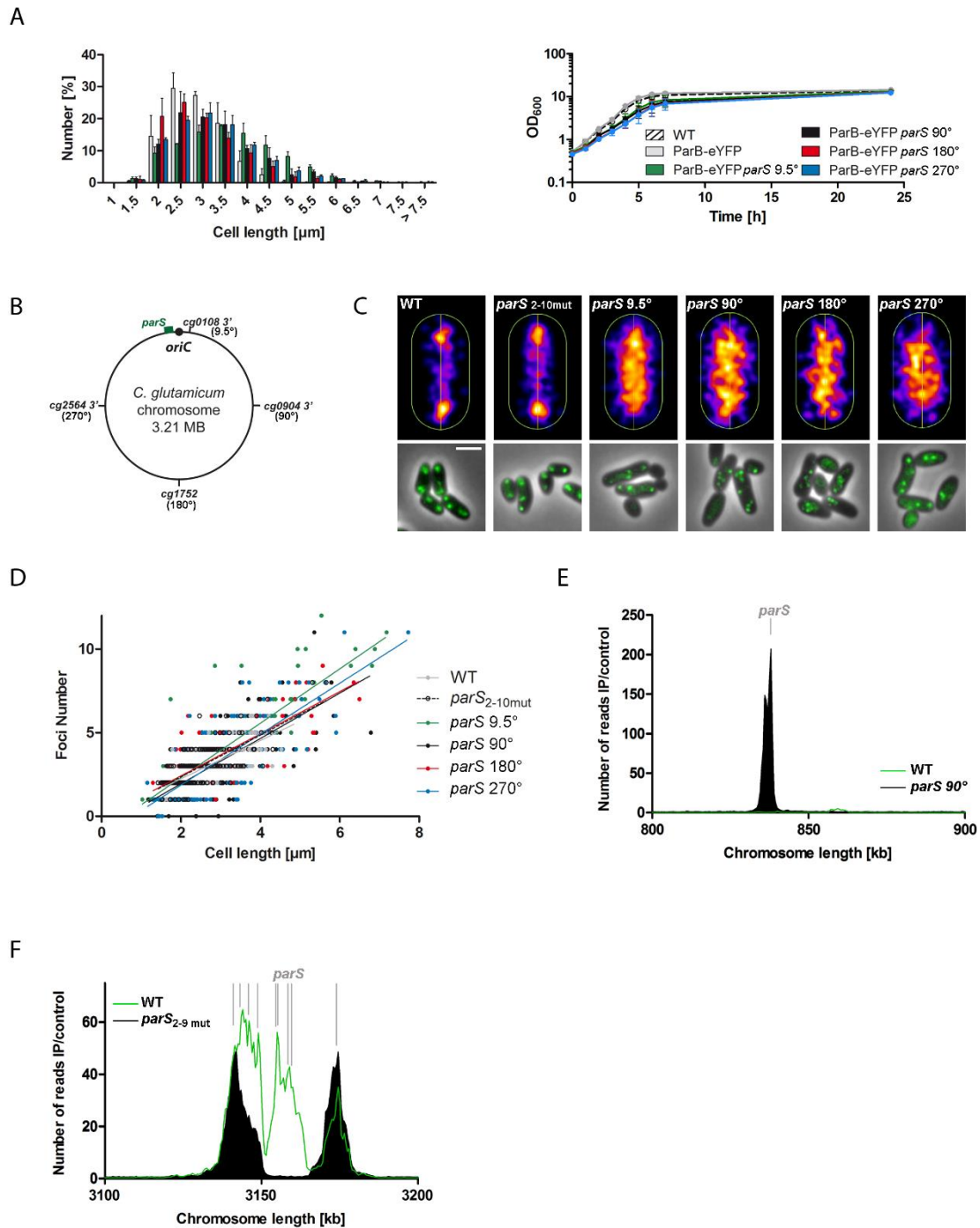


B



Supplementary Figure 4. Whole genome ParB- and SMC-ChIP-seq data.

A) ParB -ChIP-seq analyses in different mutant backgrounds; enrichments at *parS* sites (green) and highly transcribed genes (red) are depicted with a bin size of 0.5 Kb. Fold enrichments of immunoprecipitation relative to extract samples (IP/control) are shown for the entire genome lengths, respectively; x-axes are centered at *terC*. Strains analyzed from top down are: *parB::parB-mCherry parS_{2-10mut}* (CBK029), *parB::parB-mCherry parS_{1-9mut}* (CBK091), *parB::parB-mCherry parS* at 90° chromosomal position (3' *cg0904*, CBK042), *parB::parB-mCherry parS_{2-9mut}* (CBK030), *parB::parB^{R175A}-mCherry* (CBK47) and *parB::parB^{R175A}-mCherry parS_{2-10mut}* (CBK048). **B)** DNA-enrichment patterns were determined for SMC via ChIP-seq as described above. Genotypes analyzed are *smc::smc-mCherry* in combination with either Δ *parB* (CBK014), *parS* at 90° chromosomal position (3' of *cg0904*, CBK045) or *parB::parB^{R175A}* (CBK049).



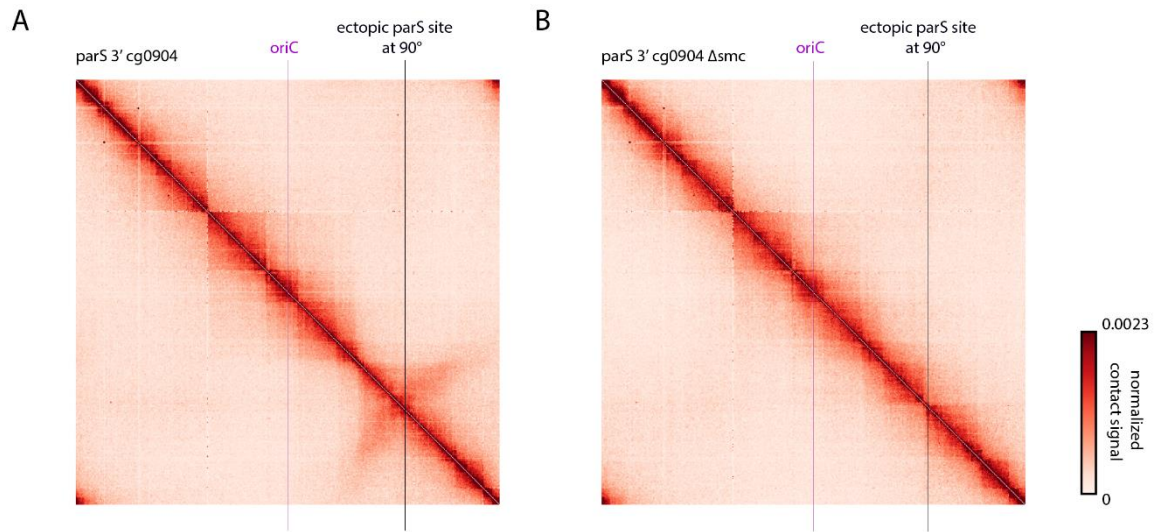
110

111 **Supplementary Figure 5. Confined chromosomal positioning of *parS* impacts**
 112 **on its function.**

113 **A)** Cell lengths (mean $\pm$ s.d., n for each replicate listed below) and growth curves
 114 (mean $\pm$ s.d., n=3) determined for mutant cells ParB-eYFP in combination with a
 115 single misplaced *parS* site at different chromosomal location in degree, growth rates:

116 $\mu_{WT}=0.57 \text{ h}^{-1}$ ($n_1 = 410$, $n_2 = 276$, $n_3 = 286$), $\mu_{\text{ParB-eYFP}}=0.61 \text{ h}^{-1}$ (CBK007) ($n_1 = 1126$, n_2
 117 $= 1126$, $n_3 = 378$), $\mu_{\text{ParB-eYFP } parS 9.5^\circ}=0.46 \text{ h}^{-1}$ (CBK040) ($n_1 = 549$, $n_2 = 468$, $n_3 = 271$), μ
 118 $\text{ParB-eYFP } parS 90^\circ}=0.46 \text{ h}^{-1}$ (CBK041) ($n_1 = 527$, $n_2 = 527$, $n_3 = 252$), $\mu_{\text{ParB-eYFP } parS 180^\circ}=0.40$
 119 h^{-1} (CBK044) ($n_1 = 415$, $n_2 = 498$, $n_3 = 363$), $\mu_{\text{ParB-eYFP } parS 270^\circ}=0.41 \text{ h}^{-1}$ (CBK043) ($n_1 =$
 120 414 , $n_2 = 409$, $n_3 = 311$). **B)** Scheme of chromosomal *parS* insertions, native *parS*
 121 cluster shown in green, *parS* shifted to intergenic regions 3' of *cg0108*, 3' of *cg0904*,
 122 3' of *cg2563* or *cg1702::parS* (CBK040, CBK041, CBK043, CBK044). **C)** ParB
 123 recruitment to *parS* sites at any chromosomal position remains intact. Top: average
 124 ParB-*parS* cluster localizations of *parB::parB-eYFP* cells with native *parS* cluster (WT,
 125 CBK007) or one single *parS* site (*parS*_{2-10mut}, CBK025) and *parS* sequences located
 126 at respective chromosomal loci illustrated via MicrobeJ ⁴ Below: ParB-eYFP clusters
 127 (green) of representative mutant cells harboring *parS* sites at above-mentioned
 128 chromosomal positions. Scale bar, 2 μm . **D)** ParB foci number in relation to cell length
 129 in *parS*-shifted mutant strains ($n>150$). Linear regression lines are shown, $r(\text{WT})=0.71$,
 130 $r(\text{parS}_{2-10\text{mut}})=0.74$, $r(\text{parS } 9.5^\circ)=0.86$, $r(\text{parS } 90^\circ)=0.68$, $r(\text{parS } 180^\circ)=0.75$, $r(\text{parS}$
 131 $270^\circ)=0.79$. **E)** α -mCherry-ChIP-seq of *C. glutamicum parB::parB-mCherry parS* at 90°
 132 chromosomal position (black, CBK042) compared to wild-type enrichment signal
 133 (green, CBK006) within genomic region 0.8 -0.9 Mb including mutant *parS* location
 134 (gray), bin size 0.5 Kb. **F)** ChIP-seq of cells harboring *parS1* and 10 (*parS*_{2-9mut},
 135 CBK30), performed and compared to the wild-type-signal as in E). Source data are
 136 provided as a Source Data file.

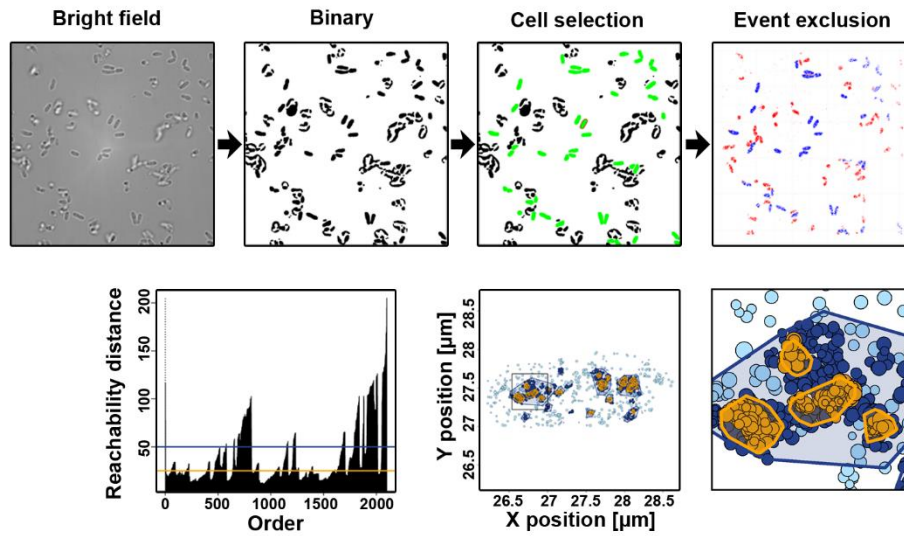
137



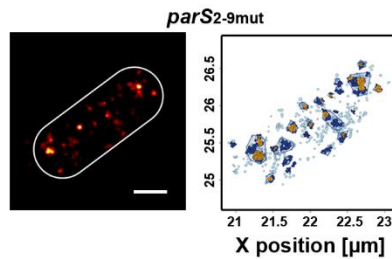
Supplementary Figure 6. Whole chromosome organization of *C. glutamicum* mutants.

Normalized contact maps of mutant strains. **A)** Chromosomal contacts of *parS* at 90° chromosomal position in a wild-type (CBK037). **B)** Chromosomal contacts of *parS* at 90° chromosomal position in a Δ smc background (CBK046). Contact maps are *ori* centered (purple lines).

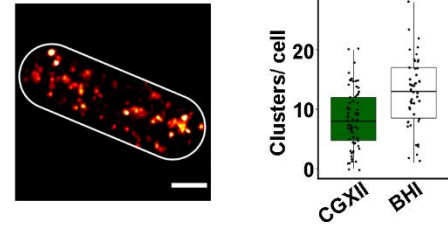
A



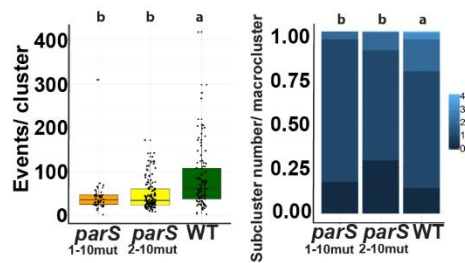
B



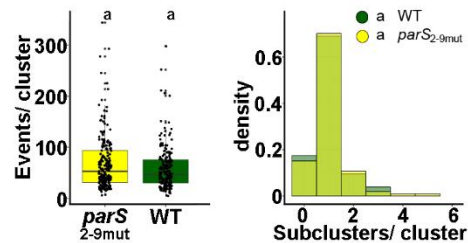
C



D



E

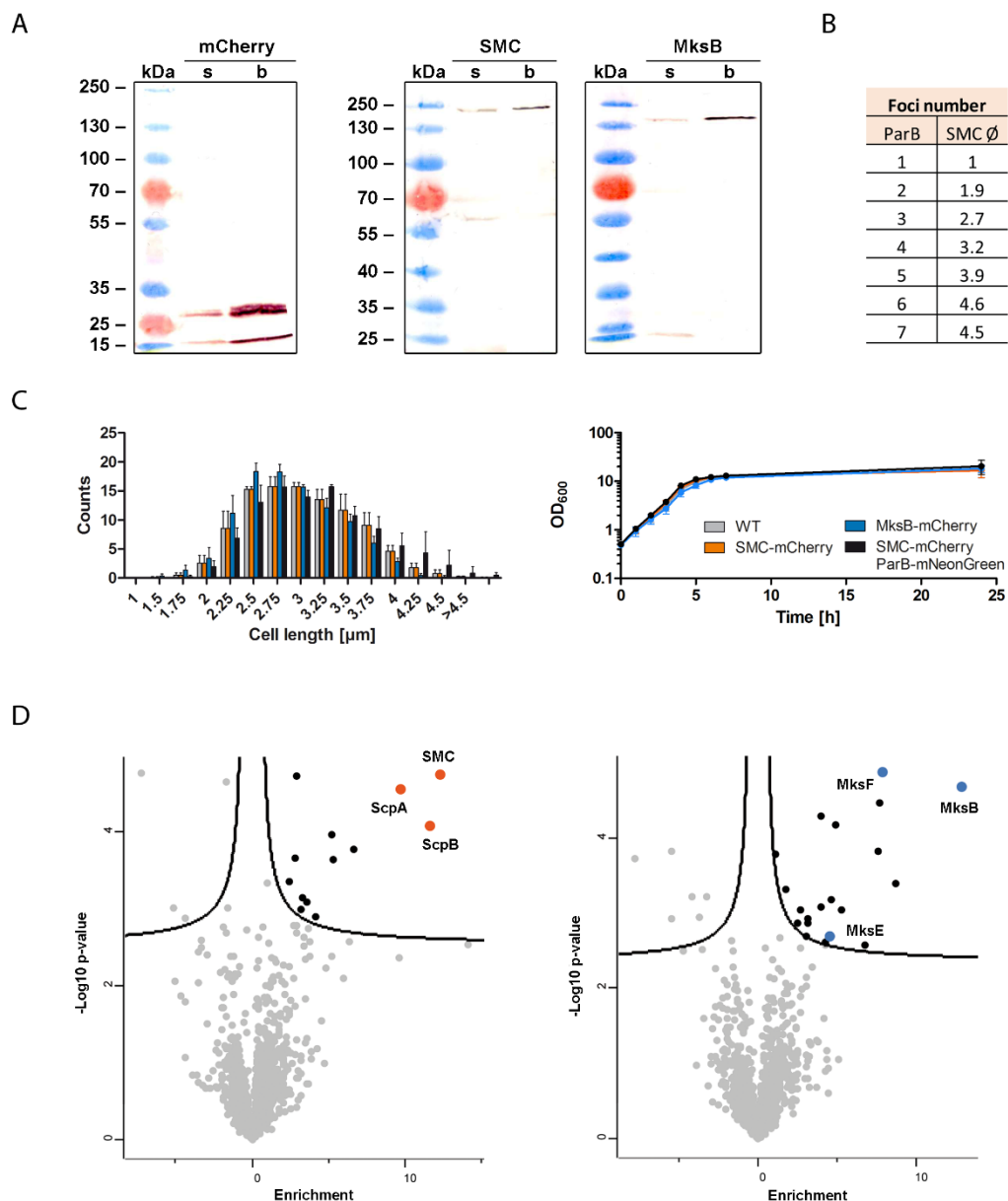


146

147 **Supplementary Figure 7. PALM analyses on cellular ParB localization.**

148 **A)** Workflow for protein cluster analysis. **B)** Representative *parS2-9mut* mutant cell
 149 (CBK031) analyzed via single molecule localization microscopy. Left: Gaussian
 150 rendering of ParB-PAMCherry signals, right: color-coded representation of ParB-
 151 PAMCherry events within corresponding cells as described in Fig. 2G. **C)** Left:
 152 Gaussian rendered PALM microscopy image (0.71 PSF, 1 px = 10 nM) exemplifying
 153 ParB-PAMCherry localization in fast-grown *C. glutamicum* cell, strain CBK009. Scale

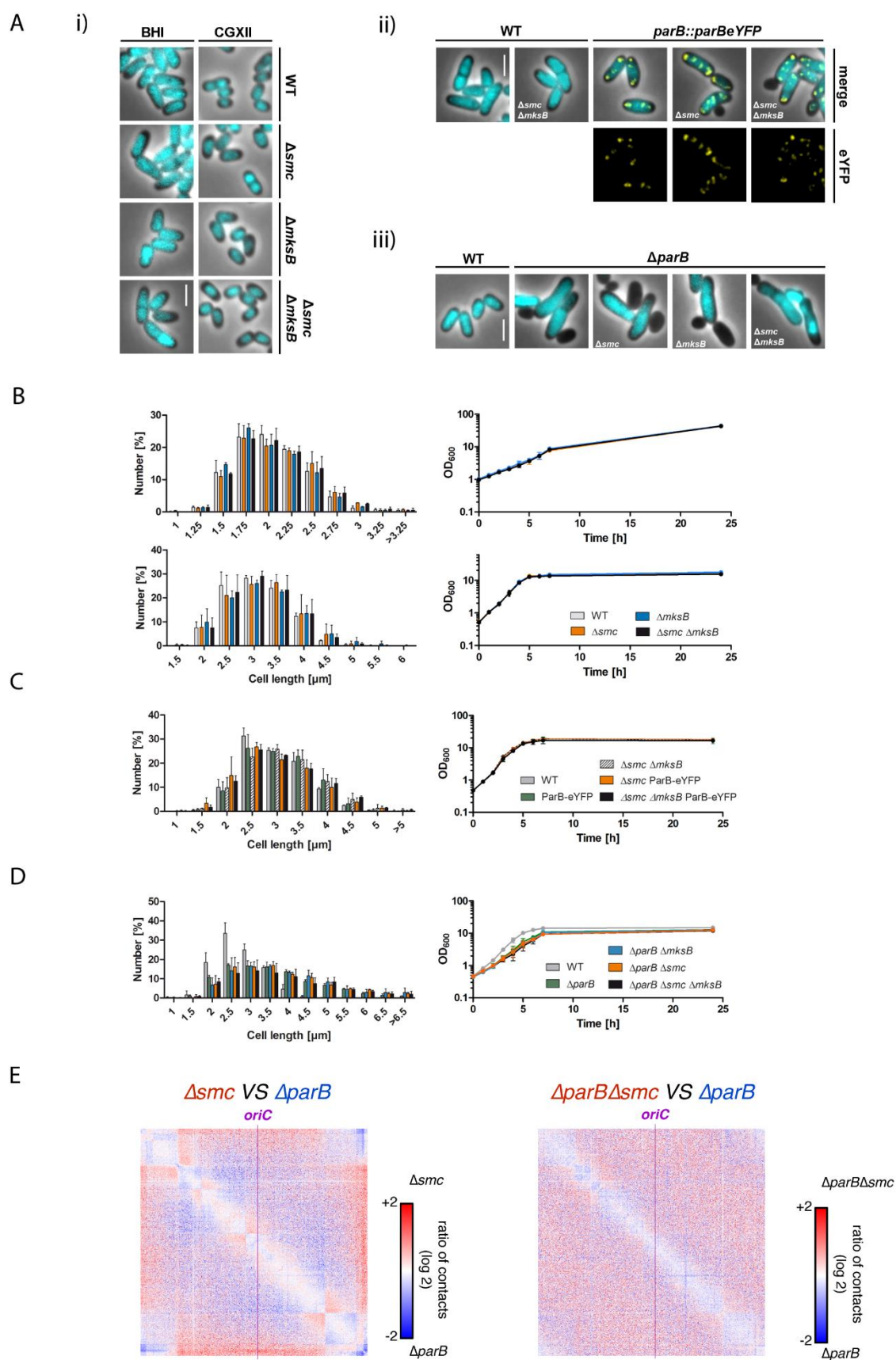
bars, 0.5 μ m. Right: more ParB-PAmCherry macroclusters are present in fast-growing (BHI medium) compared to slow-growing cells (CGXII medium), (significance by two-tailed Kruskal-Wallis Rank Sum Test: chi-squared = 14.576, df = 1, p = 0.0001346, n_{BHI} = 47, n_{CGXII} = 68). Medians are indicated as solid lines and whiskers mark 1.5 IQR (Inter Quartile Ranges). **D)** Properties of the two biggest ParB-PAmCherry clusters per cell compared between strains harboring all (wild-type, WT), a single *parS* 1 site (1 *parS*) or no *parS* site (no *parS*, CBK087) (clusters_{wild-type}: n = 114, clusters_{*parS*2-10mut}: n = 145, clusters_{*parS*1-10mut}: n = 46). Left: two-tailed Kruskal-Wallis Rank Sum test yields significant differences in number of events per macro-cluster of wild-type cells and cells harboring one *parS* (chi-squared = 27.582, df = 1, p-value = 1.506e-07) or no *parS* site (chi-squared = 20.167, df = 1, p-value = 7.096e-06), as indicated by letters above data sets. Medians are indicated as solid lines and whiskers mark 1.5 IQR. Right: Quantity of ParB sub-clusters per macro-cluster depends on number of *parS* sites. Data sets differ significantly between wild-type and cells harboring a single *parS*1 (two-tailed Kruskal-Wallis Rank Sum test: chi-squared = 12.284, df = 1, p-value = 0.0004569) or no *parS* site (two-tailed Kruskal-Wallis Rank Sum test: chi-squared = 4.6317, df = 1, p-value = 0.03139). **E)** Comparison of the two biggest ParB-PAmCherry clusters in wild-type and *parS*2-9mut (n_{wildtype} = 178, $n_{\text{parS2-9mut}}$ = 205). Two-tailed Kruskal-Wallis Rank Sum test yielded no significant differences of macrocluster sizes (medians are indicated as solid lines and whiskers mark 1.5 IQR) (left, chi-squared = 1.7848, df = 1, p = 0.1816) or subcluster numbers (right, chi-squared = 0.38145, df = 1, p = 0.5368) amongst both strain backgrounds. Source data are provided as a Source Data file.



Supplementary Figure 8. Validation of full-length fusion proteins SMC-/ MksB-mCherry and co-immunoprecipitation of subunits.

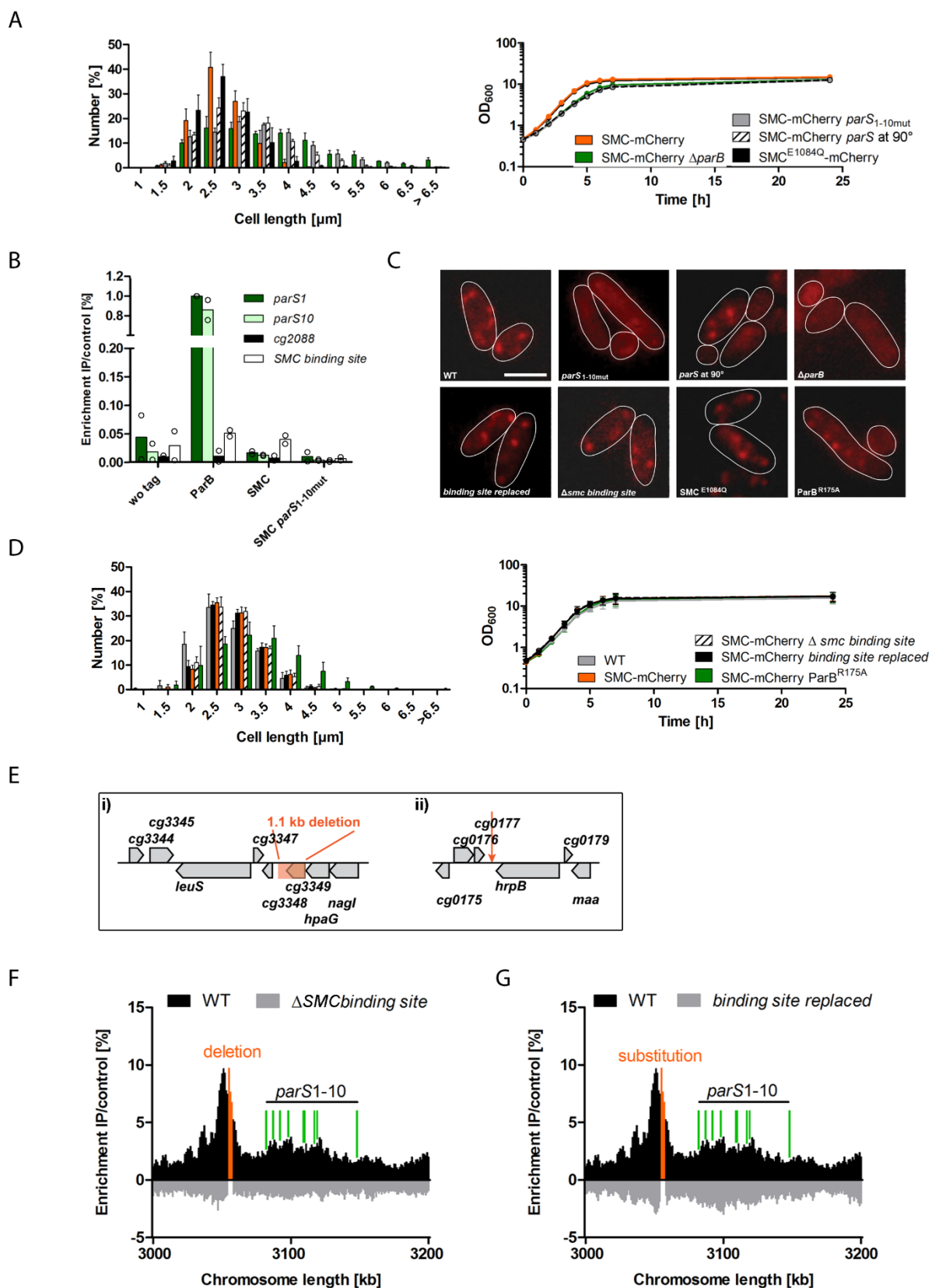
A) Full length fluorescent fusion proteins and their enrichment during immunoprecipitation were validated via western blotting. Whole cell lysates of *C. glutamicum* strains CBK052 (pEKEx2-mCherry), CBK012 (*smc::smc-mCherry*) and CBK015 (*mksB::mksB-mCherry*) strains were used for pulldown experiments. Western blots show proteins SMC-mCherry (155 kDa), MksB-mCherry (151 kDa) and mCherry (26.7 kDa) trapped on 10 μ l magnetic RFP-Trap® beads (b) and in the 10 μ l

supernatant (s) detected via polyclonal α -mCherry antibody. **B)** Average SMC foci numbers increase in dependence of ParB- clusters per cell in strain *smc::smc-mCherry parB::parB-mNeonGreen* (CBK013), $n > 200$. **C)** Growth curves (mean $\pm$ s.d., $n = 3$) and cell length distributions (mean $\pm$ s.d., $n > 1000$) of *C. glutamicum* strains grown in BHI harboring allelic replacements of condensin subunits or ParB by fluorescent versions as indicated. $\mu_{WT} = 0.64 \text{ h}^{-1}$, $\mu_{smc-mCherry} = 0.67 \text{ h}^{-1}$ (CBK012), $\mu_{mksB-mCherry} = 0.60 \text{ h}^{-1}$ (CBK015) $\mu_{smc-mCherry parB-mNeonGreen} = 0.68 \text{ h}^{-1}$ (CBK013). Source data are provided as a Source Data file. **D)** Condensin subunit interactions identified by pull-down experiments and mass spectrometry. Cell lysates of *C. glutamicum smc::smc-mCherry*, *mksB::mksB-mCherry* and a negative control strain (CBK052) were used for co-immunoprecipitations via anti-mCherry agarose beads. Volcano plots show the difference in means (enrichment) plotted against the $-\log_{10}$ adjusted p value for each protein identified by mass spectrometry in three independent samples; condensin subunits are highlighted respectively. The cutoff curves indicate significant hits (two-tailed t-test, $p < 0.05$, FC > 0.1).



Supplementary Figure 9. Characterization of growth and cell length phenotypes of condensin deletion strains.

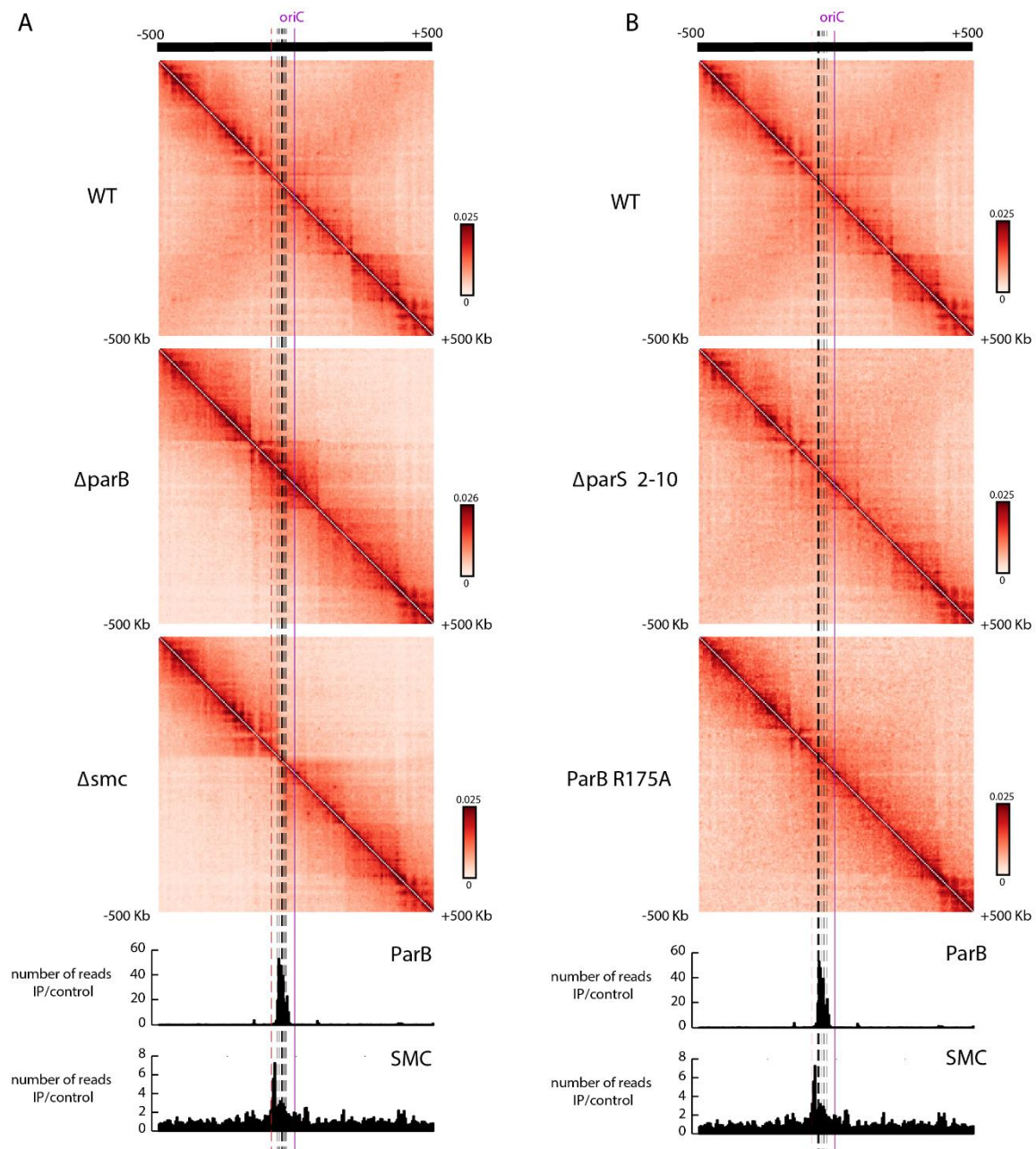
A) Phenotypes of exponentially grown *C. glutamicum* mutant cells exemplified in overlays of ParB-eYFP fluorescence (yellow) with Hoechst-stained DNA (cyan) and the phase contrast image, respectively. The eYFP fluorescence channel is additionally depicted in separate images. Scale bar, 2 μm . i) Wild-type and mutant strains CDC026, CBK001, CBK004 grown in CGXII and BHI medium. ii) Wild-type and strains CBK004, CBK007, CBK010, CBK011 and iii) Wild-type, CDC003, CBK002, CBK003, CBK005 grown in BHI medium. **B)-D)** Growth curves (mean $\pm$ s.d., $n = 3$) and cell length distributions (mean $\pm$ s.d., $n > 1000$) of *C. glutamicum* mutant strains displayed above. **B)** Growth experiments performed in CGXII medium (top): $\mu_{\text{WT}}=0.26 \text{ h}^{-1}$, $\mu_{\Delta\text{smc}}=0.22 \text{ h}^{-1}$ (CDC026), $\mu_{\Delta\text{mksB}}=0.27 \text{ h}^{-1}$ (CBK001), $\mu_{\Delta\text{smc} \Delta\text{mksB}}=0.25 \text{ h}^{-1}$ (CBK004); in BHI medium (below): $\mu_{\text{WT}}=0.71 \text{ h}^{-1}$, $\mu_{\Delta\text{smc}}=0.70 \text{ h}^{-1}$, $\mu_{\Delta\text{mksB}}=0.72 \text{ h}^{-1}$, $\mu_{\Delta\text{smc} \Delta\text{mksB}}=0.70 \text{ h}^{-1}$. **C)** Analysis in BHI medium: $\mu_{\text{WT}}=0.70 \text{ h}^{-1}$, $\mu_{\text{ParB-eYFP}}=0.69 \text{ h}^{-1}$ (CBK007), $\mu_{\Delta\text{smc} \Delta\text{mksB}}=0.72 \text{ h}^{-1}$ (CBK004), $\mu_{\Delta\text{smc} \text{ ParBeYFP}}=0.70 \text{ h}^{-1}$ (CBK010), $\mu_{\Delta\text{smc} \Delta\text{mksB} \text{ ParBeYFP}}=0.71 \text{ h}^{-1}$ (CBK011). **D)** Analysis in BHI medium: $\mu_{\text{WT}}=0.67 \text{ h}^{-1}$, $\mu_{\Delta\text{parB}}=0.51 \text{ h}^{-1}$ (CDC003), $\mu_{\Delta\text{parB} \Delta\text{smc}}=0.46 \text{ h}^{-1}$ (CBK002), $\mu_{\Delta\text{parB} \Delta\text{mksB}}=0.49 \text{ h}^{-1}$ (CBK003), $\mu_{\Delta\text{parB} \Delta\text{smc} \Delta\text{mksB}}=0.43 \text{ h}^{-1}$ (CBK005). Source data of B-D are provided as a Source Data file. **E)** Differential maps between $\Delta\text{smc}-\Delta\text{parB}$ (left) and $\Delta\text{parB}\Delta\text{smc}-\Delta\text{parB}$ (right) contact maps indicating the log of the ratio as in Fig. 2.



Supplementary Figure 10. Characterization of *C. glutamicum smc::smc-mCherry*

A) Growth (mean $\pm$ s.d., $n = 3$) and cell length analysis (mean $\pm$ s.d., n listed below) in BHI medium: $\mu_{\text{SMC-mCherry}} = 0.71 \text{ h}^{-1}$ (CBK012) ($n_1 = 493, n_2 = 380, n_3 = 434$), $\mu_{\text{SMC-mCherry } \Delta \text{parB}} = 0.60 \text{ h}^{-1}$ (CBK014) ($n_1 = 445, n_2 = 404, n_3 = 401$), $\mu_{\text{SMC-mCherry } \text{parS1-10mut}} = 0.56 \text{ h}^{-1}$ (CBK032) ($n_1 = 616, n_2 = 252, n_3 = 246$), $\mu_{\text{SMC-mCherry } \text{parS } 3' \text{ cg904}} = 0.55 \text{ h}^{-1}$ (CBK045) ($n_1 = 626, n_2 = 415, n_3 = 464$), $\mu_{\text{SMC-mCherryE1084Q}} = 0.72 \text{ h}^{-1}$ (CBK050) ($n_1 = 367, n_2 = 531, n_3 = 339$). **B)** ChIP-qPCR of the wild-type and cells harboring ParB- or SMC-mCherry tagged proteins in wild-type (SMC) or a *parS*-mutation (*parS*_{1-10mut}) background. **C)** Localization of SMC-mCherry foci in strain backgrounds described in A) and D). Images show mCherry fluorescence in representative cells; cell outlines are indicated (white lines). Scale bar, 2 μm . **D)** Analysis of cell lengths distributions (mean $\pm$ s.d., n listed below) (left) and growth (mean $\pm$ s.d., $n = 3$) in BHI medium (right) of *smc::smc-mCherry* mutant strain derivatives, growth rates: $\mu_{\text{WT}} = 0.63 \text{ h}^{-1}$ ($n_1 = 199, n_2 = 508, n_3 = 284$), $\mu_{\text{SMC-mCherry}} = 0.69 \text{ h}^{-1}$ (CBK012) ($n_1 = 306, n_2 = 362, n_3 = 550$), $\mu_{\text{SMC-mCherry } \Delta \text{SMC binding site}} = 0.68 \text{ h}^{-1}$ (CBK034) ($n_1 = 745, n_2 = 482, n_3 = 232$), $\mu_{\text{SMC-mCherry SMC binding site replaced}} = 0.68 \text{ h}^{-1}$ (CBK035) ($n_1 = 612, n_2 = 452, n_3 = 268$), $\mu_{\text{SMC-mCherry ParBR175A}} = 0.69 \text{ h}^{-1}$ (CBK049) ($n_1 = 379, n_2 = 339, n_3 = 379$). Source data of A, B and D are provided in the Source Data file. **E)** Scheme showing mutation sites within the *C. glutamicum* CBK034 genome with a partially deleted SMC-enrichment region upstream of *parS* reinserted into intergenic region 3' of *cg0177*. i) ChIP-seq enrichment site of SMC-mCherry protein covers genes *cg3345* to *hpaG*. Deletion is indicated in orange (31.3 Mb). ii) Arrow points to intergenic insertion site of the 1.1 Kb fragment (0.15 Mb). **F)** Partial deletion of SMC-enrichment site collapses entire SMC-clustering nearby. *In vivo* ChIP-seq was performed using exponentially grown *C. glutamicum* SMC-mCherry expressing wild-type (upper y-axis, black) or CBK034 cells (lower y-axis, gray). The

251 deletion site and *parS* sequences are indicated in orange and by green lines,
252 respectively. **G)** SMC-ChIP-seq analysis in CBK035 cells with a partial substitution of
253 the SMC-binding site by a non-coding *B. subtilis* genome sequence of identical size
254 abolishes SMC enrichment at this site.



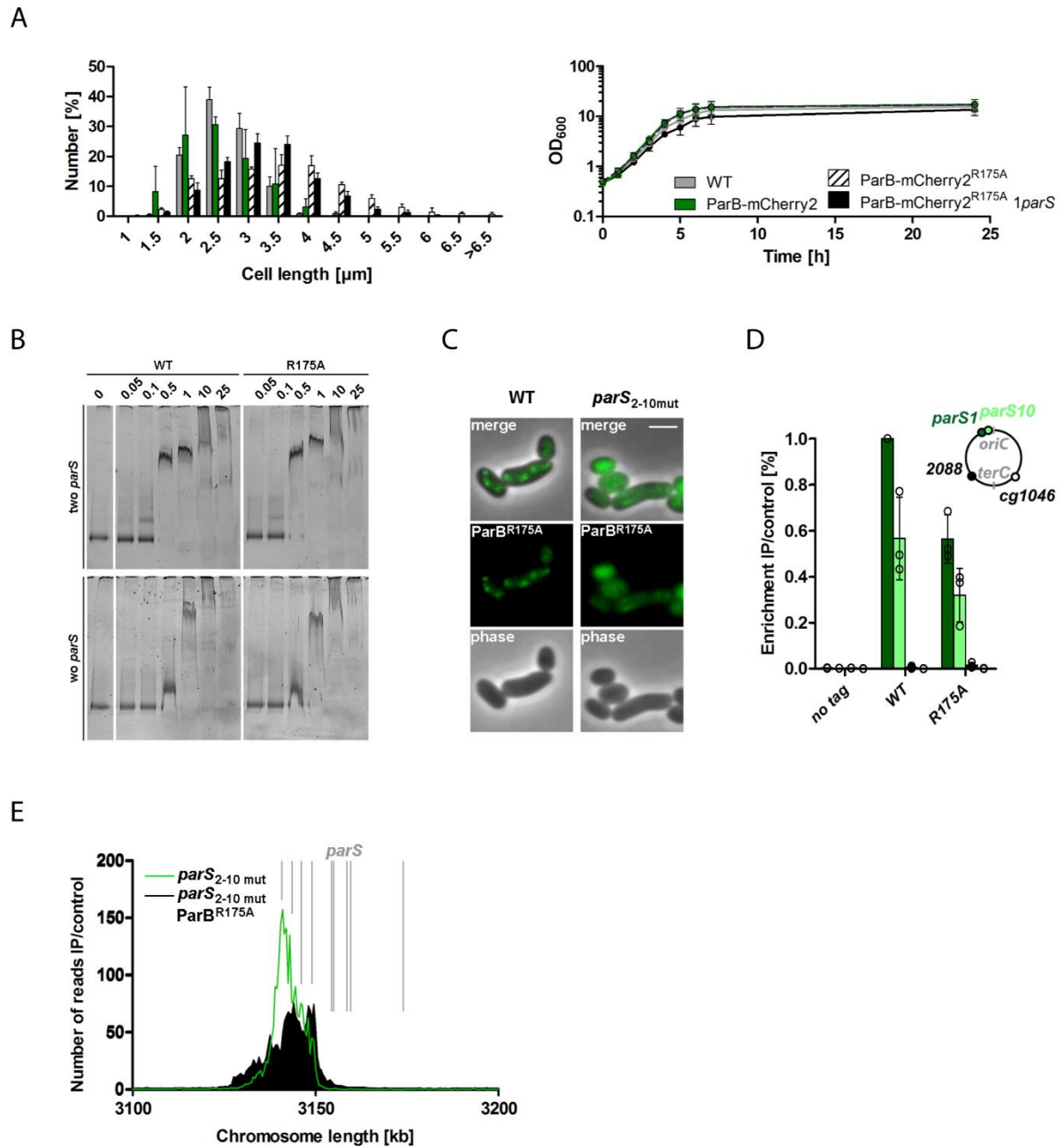
255

256 **Supplementary Figure 11. Disorganization of *oriC*-regions in Par and SMC**
 257 **mutants.**

258 **A)** Magnification of normalized genomic contact matrices of wild-type, $\Delta parB$ and
 259 Δsmc , (CDC003, CDC026) encompassing 500 Kb regions surrounding *oriC* (purple
 260 line). *parS* sites are indicated as dashed lines. Color codes as in Fig. 1 were applied.
 261 ParB and SMC enrichment zones are shown below the contact maps (ChIP signal

262 relative to the input in 5 Kb bins). **B)** Magnification of normalized genomic contact
263 matrices of wild-type, *parS*_{2-10mut} and *parB::parB*^{R175A} (CBK023, CBK047) as in A.

264

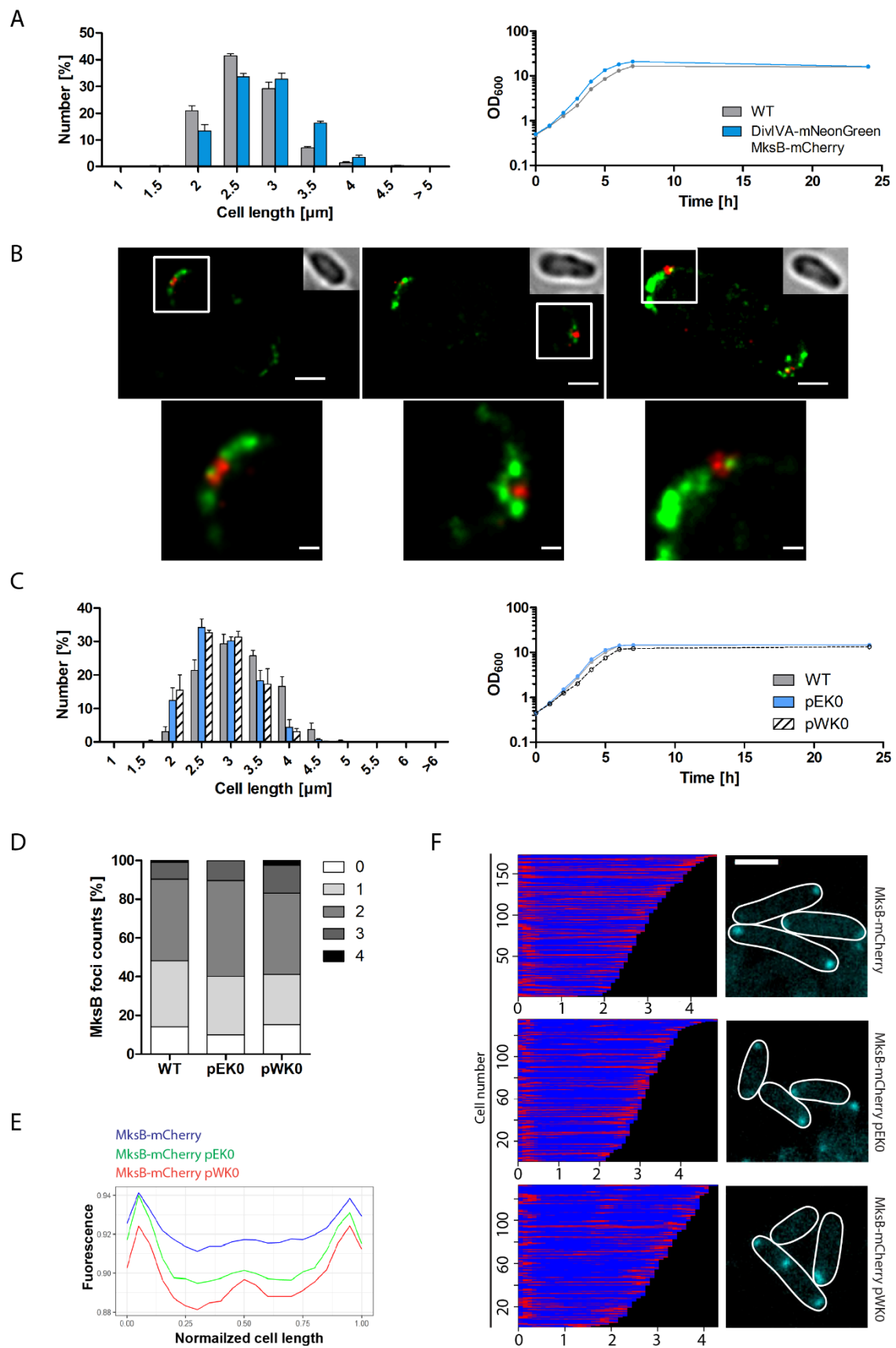


Supplementary Figure 12. ParBR175A compromises DNA binding *in vivo*.

A) Growth curves (mean $\pm$ s.d., $n = 3$) and cell length distributions (mean $\pm$ s.d., n listed below) of strain *parB::parB-mCherry* derivatives harboring ParB^{R175A} mutant protein grown in BHI medium are shown; growth rates: $\mu_{WT}=0.63 \text{ h}^{-1}$ ($n_1 = 199$, $n_2 = 508$, $n_3 = 284$), $\mu_{ParB-mCherry}=0.69 \text{ h}^{-1}$ (CBK006) ($n_1 = 225$, $n_2 = 538$, $n_3 = 555$), $\mu_{ParBR175A-mCherry}=0.68 \text{ h}^{-1}$ (CBK047) ($n_1 = 339$, $n_2 = 564$, $n_3 = 297$), $\mu_{ParBR175A-mCherry parS2-10mut}=0.57 \text{ h}^{-1}$ (CBK048) ($n_1 = 403$, $n_2 = 243$, $n_3 = 272$). **B)** Recombinant ParB^{WT} and ParB^{R175A}

273 proteins bind *parS* sites and nonspecific DNA. Electrophoretic mobility shift assay of
274 ParB proteins pre-incubated with 100 ng DNA sized 1084 bp with or without two *parS*
275 sites. **C)** ParB^{R175A}-mCherry localization in wild-type and *parS*_{2-10mut} mutant cells
276 (CBK47, CBK048). Shown are phase contrast, mCherry fluorescence and an overlay
277 of both channels. Scale bar, 2 μm. **D)** ParB-mCherry ChIP-qPCR at specified
278 chromosomal markers performed with cells harboring ParB wild-type or R175A mutant.
279 Standard deviations derive from biological triplicates; data normalization to wild-type
280 *parS*1 signals. **E)** ChIP-seq enrichment at region of native *parS* cluster (gray); ParB^{WT}-
281 (green, CBK027) or ParB^{R175A}-mCherry protein (black, CBK047) in strain background
282 *parS*_{2-10mut}. Source data are provided as a Source Data file.

283

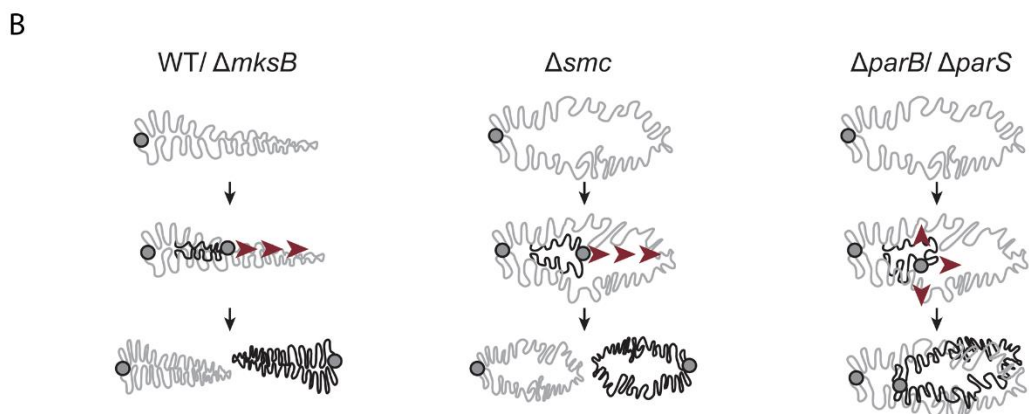
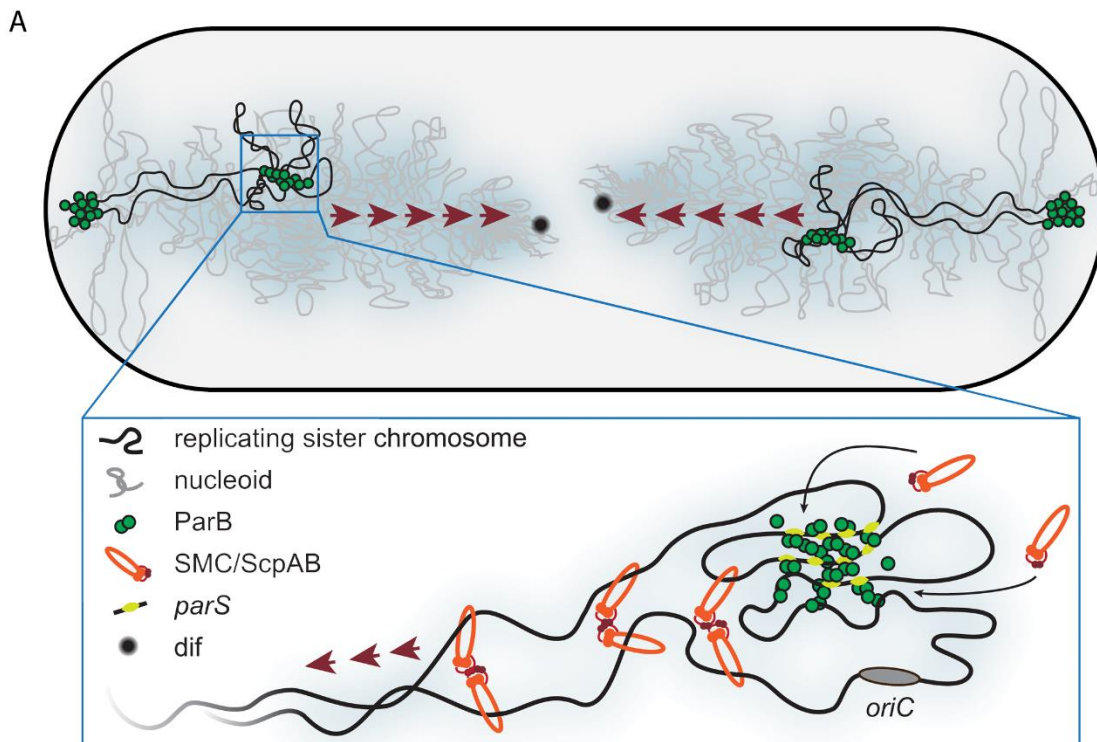


284

285

Supplementary Fig. 13 Polar MksB-mCherry localization in presence and absence of plasmids.

A) Cell length distribution (mean $\pm$ s.d., n listed below) (left) and growth curves (mean $\pm$ s.d., n = 3) (right) of wild-type and *divIVA::divIVA-mNeonGreen mksB::mksB-mCherry* cells (CBK092). Analyses were performed in biological triplicates using BHI medium; growth rates: $\mu_{WT}=0.68 \text{ h}^{-1}$ ($n_1 = 588$, $n_2 = 1646$, $n_3 = 820$), $\mu_{DivIVA-mNeonGreen \text{ MksB-mCherry}}=0.69 \text{ h}^{-1}$ ($n_1 = 1513$, $n_2 = 727$, $n_3 = 1096$). **B)** Super resolution imaging of MksB (red) and DivIVA localization (green). Top: Gaussian rendering and bright field of three exemplary *C. glutamicum divIVA::divIVA-mNeonGreen mksB::mksB-PAmCherry* cells (scale bar: 0.5 μm). Below: Magnification of polar regions (scale bar, 0.1 μm). See “Material and Methods: PALM microscopy” for detailed description of imaging conditions and sample preparation. **C)** Cell length (mean $\pm$ s.d., n listed below) and growth (mean $\pm$ s.d., n = 3) in BHI medium of strains *mksB::mksB-mCherry* without plasmid (CBK015) or containing either pEK0 or pWK0 (CBK089, CBK088) were analyzed in biological triplicates; growth rates: $\mu_{WT}=0.68$ ($n_1 = 1292$, $n_2 = 1312$, $n_3 = 1289$), $\mu_{pEK0}=0.69$ ($n_1 = 760$, $n_2 = 848$, $n_3 = 583$), $\mu_{pWK0}=0.68$ ($n_1 = 824$, $n_2 = 585$, $n_3 = 1498$). **D)** MksB-mCherry foci numbers per cell were determined in strains named above, $n > 100$. **E)** MksB-mCherry fluorescence profiles along longitudinal cell axes were extracted from above-named strains, normalized to cell length ($n>100$) and binned (bin = 0.05 μm). Fluorescence (a.u.) is plotted normalized to maximal intensity values. **F)** Right: Cellular MksB fluorescence profiles summarized in E) are depicted in demographs sorted by cell length with a color scheme ranging from blue (low intensity values) to red (high intensity values). Left: Microscopy images showing MksB-mCherry fluorescence (cyan) of exemplary cells with contours indicated by white lines; scale bar, 2 μm . Source data are provided as a Source Data file.



Supplementary Figure 14. Model of ParB- and SMC-mediated chromosome organization in *C. glutamicum*.

A) Top: Illustration of chromosome segregation in a diploid *C. glutamicum* cell. Newly replicated ParB-*oriC*s complexes translocate from cell poles towards *terC*s at septal positions via a ParABS system, where ParB nucleocomplexes use ParA-bound sister chromosomal loci as transient tethers for translocation across the nucleoid. Below: Condensins SMC/ScpAB are loaded ParB-dependently at *parS* sites and relocate to

321 distant chromosomal regions (red arrows) causing inter-linkage of chromosomal arms;
322 here illustration is based on a two-translocator model. **B)** Cartoon showing mutant
323 phenotypes of overall chromosome folding and segregation compared to wild-type
324 (WT). Replication *oriC* (grey mark), chromosome (grey line) and replicating sister DNA
325 (black line) are indicated; red arrows indicate direction of DNA segregation.

326

Supplementary Table 1. Percentage of DNA-free cells of relevant *C. glutamicum* RES167 strains used in this study.

Color code visualizes severity of nucleoid missegregation: virtually none (0*), up to 10 % (<10) and more than 20 % (>20) DNA-free cells, (n>1000).

Strain	Genotype	Anucleate cells [%]	Missegregation group
RES 167	wild-type	0	0*
CDC003	$\Delta parB$	26.14	>20
CDC026	Δsmc	0.1	0*
CBK001	$\Delta mksB$	0	0*
CBK002	$\Delta parB \Delta smc$	27.08	>20
CBK003	$\Delta parB \Delta mksB$	24.95	>20
CBK004	$\Delta smc \Delta mksB$	0	0*
CBK005	$\Delta parB \Delta smc \Delta mksB$	25.62	>20
CBK006	<i>parB::parB-mCherry2</i>	0	0*
CBK007	<i>parB::parB-eYFP</i>	0.1	0*
CBK010	$\Delta smc parB::parB-eYFP$	4.25	<10
CBK011	$\Delta smc \Delta mksB parB::parB-eYFP$	3.98	<10
CBK012	<i>smc::smc-mCherry</i>	0	0*
CBK014	<i>smc::smc-mCherry</i> $\Delta parB$	24.18	>20
CBK015	<i>mksB::mksB-mCherry</i>	0	0*
CBK023	<i>parS2-10mut</i>	0.19	0*
CBK024	<i>parS1-10mut</i>	29.27	>20
CBK025	<i>parS2-10mut parB::parB-eYFP</i>	6.71	<10
CBK026	<i>parS1-10mut parB::parB-eYFP</i>	28.5	>20
CBK032	<i>parS1-10mut smc::smc-mCherry</i>	30.38	>20
CBK034	<i>smc::smc-mCherry</i> Δ SMC loading site	0	0*
CBK035	<i>smc::smc-mCherry</i> SMC loading site replaced	0	0*
CBK040	<i>parS1-10mut parS 3' cg0108 (9.5°)</i> <i>parB::parB-eYFP</i>	25.33	>20
CBK041	<i>parS1-10mut parS 3' cg0904 (90°)</i> <i>parB::parB-eYFP</i>	23.48	>20
CBK043	<i>parS1-10mut parS 3' cg2563 (270°)</i> <i>parB::parB-eYFP</i>	24.28	>20
CBK044	<i>parS1-10mut int::parS (180°)</i> <i>parB::parB-eYFP</i>	24.98	>20
CBK045	<i>parS1-10mut parS 3' cg0904 (90°)</i> <i>smc::smc-mCherry</i>	25.07	>20
CBK047	<i>parB::parB^{R175A}-mCherry2</i>	17.83	>20
CBK048	<i>parB::parB^{R175A}-mCherry2 parS2-10mut</i>	31.74	>20
CBK049	<i>smc::smc-mCherry parB::parB^{R175A}</i>	19.09	>20

CBK051	<i>smc::smc^{E1084Q}-mCherry</i>	0	0*
CBK090	<i>parS_{1-9mut}</i>	0.18	0*
CBK091	<i>parS_{1-9mut} parB::parB-mCherry2</i>	1.74	<10
CBK092	<i>divIVA::divIVA-mNeonGreen, mksB::mksB-mCherry</i>	0	0*

332

333

Supplementary Table 2. Average DNA amount per cell after replication runout determined by flow cytometry in triplicates, including standard deviation.

Stain	Genotype	Chromosome numbers per cell
RES167	Wild-type	5.99 ± 0.50
CDC002	$\Delta parB$	6.47 ± 0.07
CDC026	Δsmc	6.00 ± 0.51
CBK001	$\Delta mksB$	6.53 ± 0.10
CBK002	$\Delta parB \Delta smc$	6.38 ± 0.62
CBK004	$\Delta smc \Delta mksB$	5.90 ± 0.64
CBK025	<i>parS</i> _{2-10mut}	5.65 ± 0.16
CBK026	<i>parS</i> _{1-10mut}	5.87 ± 0.45
CBK043	<i>parS</i> _{1-10mut} <i>parS</i> 3' <i>cg0904</i> (90°) <i>parB::parB-eYFP</i>	6.32 ± 0.57
CBK051	<i>parB::parB</i> ^{R175A} -mCherry2	5.71 ± 0.35
CBK054	<i>smc::smc</i> ^{E1084Q}	5.67 ± 0.82

338 **Supplementary References**

- 339 1 Pfeifer-Sancar, K., Mentz, A., Rückert, C. & Kalinowski, J. Comprehensive analysis of the
340 *Corynebacterium glutamicum* transcriptome using an improved RNAseq technique. *BMC*
341 *Genomics* **14**, 888, doi:10.1186/1471-2164-14-888 (2013).
- 342 2 Lioy, V. S. *et al.* Multiscale structuring of the *E. coli* chromosome by nucleoid-associated and
343 condensin proteins. *Cell* **172**, 771-783.e718, doi:10.1016/j.cell.2017.12.027 (2018).
- 344 3 Yang, T. *et al.* HiCRep: assessing the reproducibility of Hi-C data using a stratum-adjusted
345 correlation coefficient. *Genome Res* **27**, 1939-1949, doi:10.1101/gr.220640.117 (2017).
- 346 4 Ducret, A., Quardokus, E. M. & Brun, Y. V. MicrobeJ, a tool for high throughput bacterial cell
347 detection and quantitative analysis. *Nat Microbiol* **1**, 16077, doi:10.1038/nmicrobiol.2016.77
348 (2016).

349