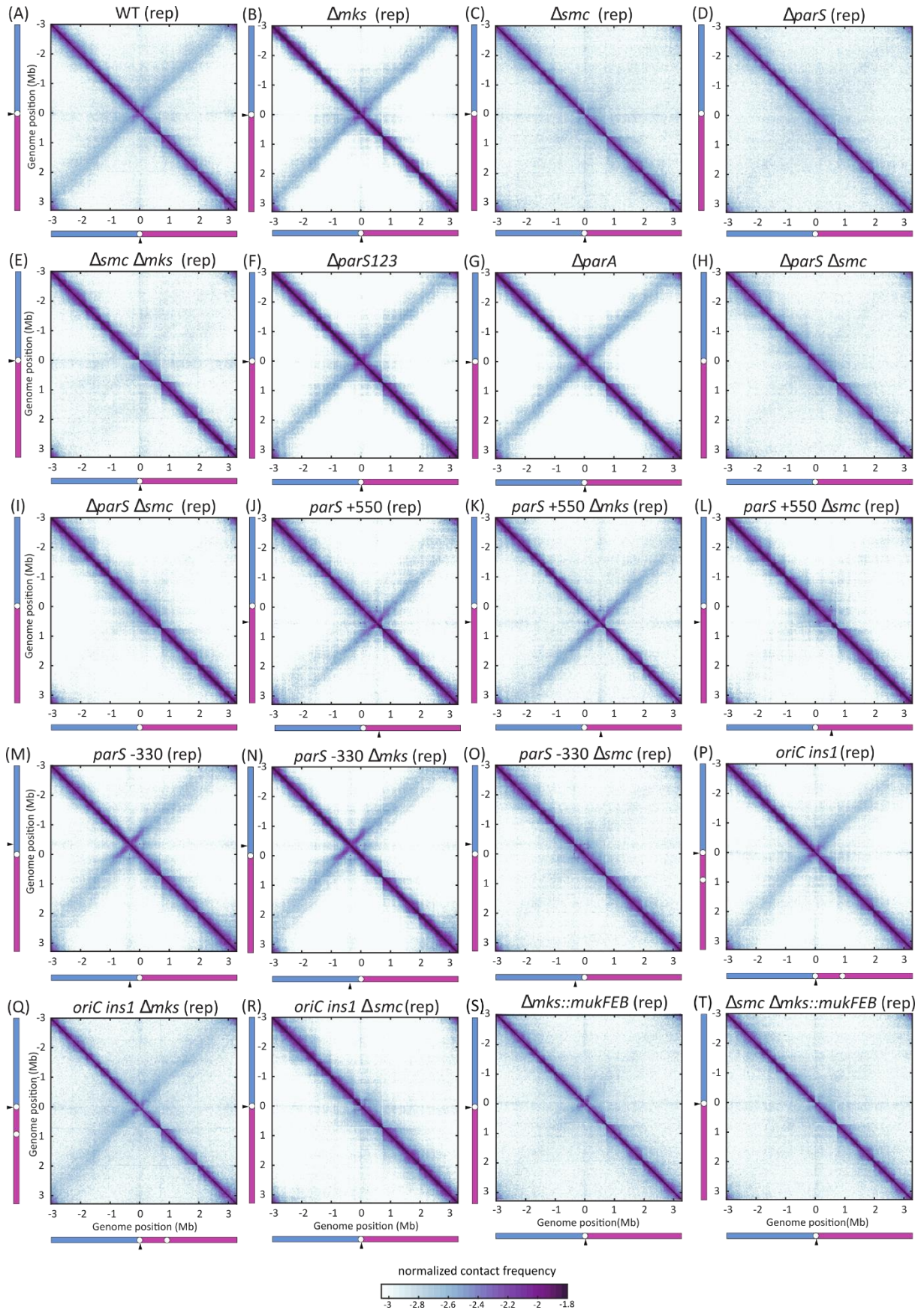
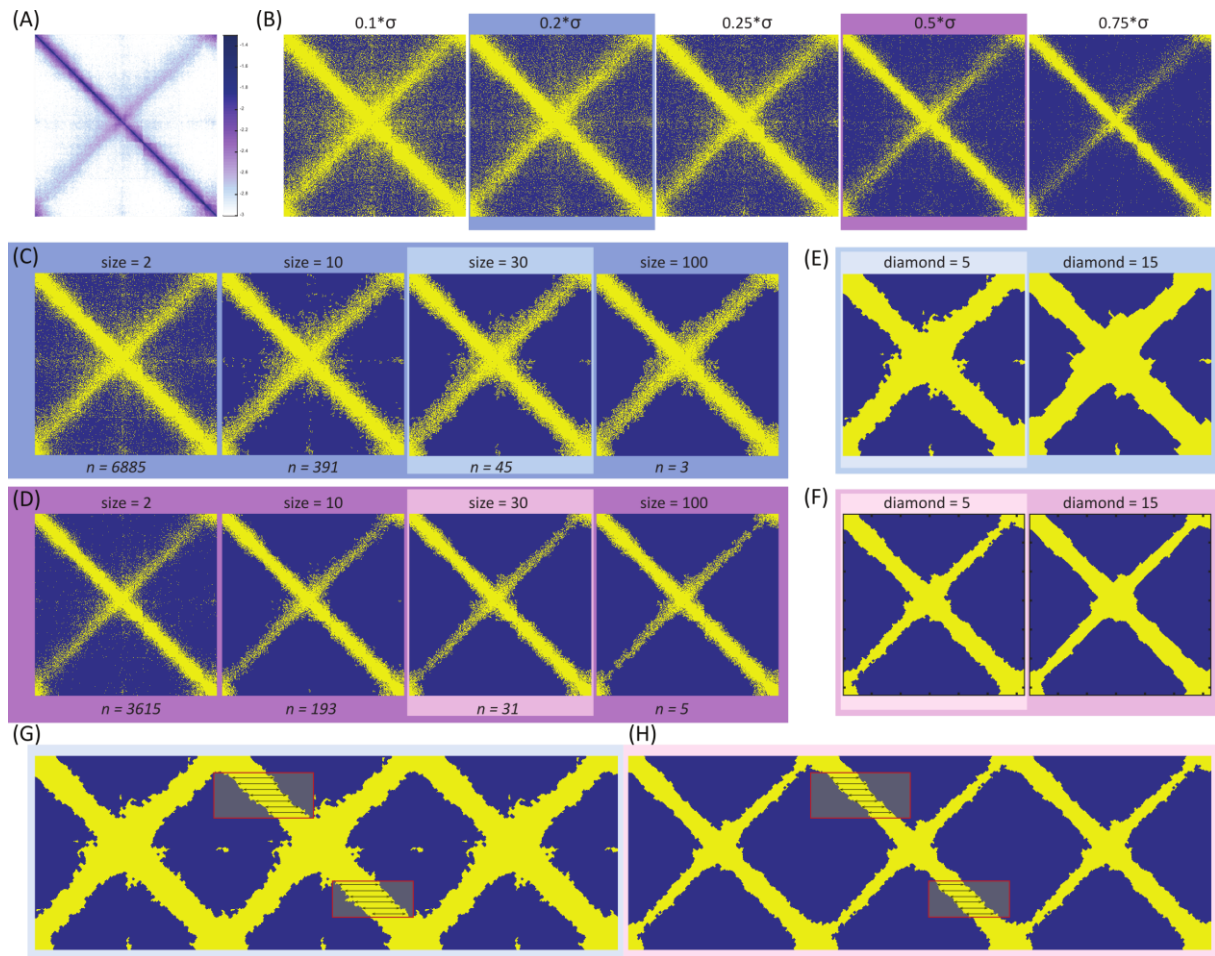


Supplementary Figure 1. Differential impact of ParABS, MksBEF and Smc-ScpAB on chromosome segregation and positioning. (A) Percentage of anucleate cells (white bars) in cultures of the different mutants grown in minimal medium supplemented with glucose and casamino acids at 30°C (left panel), in LB at 30°C (middle panel) and in LB at 37°C (right panel). Histograms and error bars represent the mean and standard deviation for at least three independent experiments. **(B)** (Left) Position of the two copies of a chromosomal locus located near the *parS* sites (82-R) in two-foci cells, in different genetic backgrounds (indicated above each graph). The relative position of the two copies of the fluorescent tag inside the cell are represented according to cell length. Cells are arbitrarily oriented, with the 0

pole being the one closest to a fluorescent tag. (Right) Distance between the two copies of a chromosomal locus located near the *parS* sites (82-R) in two-foci cells (whose position are represented in Figure 1D), in different genetic backgrounds (indicated above each graph). The relative distance is represented according to cell length. **(C)** Schematic representation of the position of the chromosomal loci studied. The two chromosomal loci whose relative position inside the cells are represented in **(D)** are highlighted in green and red. **(D)** Top panels: relative position inside the cells of two chromosomal loci located at similar distance from *oriC* (highlighted in green and red in (C)), in the wild type strain (left panels) and in the Δsmc mutant (right panels). Boxplot representations are used, indicating the median (horizontal bar), the 25th and the 75th percentile (open box) and the rest of the population except for the outliers (whiskers). Outliers are defined as $1.5 \times IQR$ or more above the 75th percentile or $1.5 \times IQR$ or more below the first 25th percentile quartile. Cells are arbitrarily oriented, the 0 pole being the one closest to the 1,812-L locus. Experiments have been performed at least twice independently; one representative example is shown here. Bottom panels: interfocal distances (in micrometers) between the two chromosomal loci, in cells containing one copy of each locus (top panel) or two copies of each locus (bottom panel). The percentage of cells in which the distance is of a certain value is plotted, for the wild type strain (white) and the Δsmc mutant (yellow). Histograms and error bars represent the mean and standard deviation for two independent experiments.



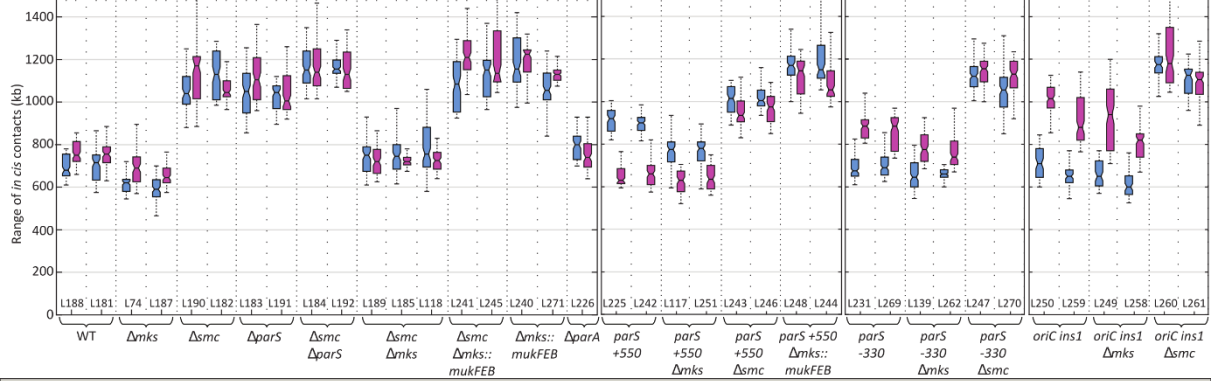
Supplementary Figure 2: Normalized contact maps obtained for different strains grown in minimal medium supplemented with citrate at 30°C. Abscissa and ordinate axis represent genomic coordinates. The colorscale reflects the frequency of contacts between two regions of the genome, from white (rare contacts) to dark purple (frequent contacts). Maps obtained for the wild type strain **(A)**, the Δmks mutant **(B)**, the Δsmc mutant **(C)**, the $\Delta parS$ mutant **(D)**, the $\Delta smc \Delta mks$ mutant **(E)**, the $\Delta parS123$ mutant **(F)**, the $\Delta parA$ mutant **(G)**, the $\Delta parS \Delta smc$ mutant **(H and I)**, the $parS^{+550}$ **(J)**, $parS^{+550} \Delta mks$ **(K)**, $parS^{+550} \Delta smc$ **(L)**, $parS^{-330}$ **(M)**, $parS^{-330} \Delta mks$ **(N)**, $parS^{-330} \Delta smc$ **(O)**, $oriC ins1$ **(P)**, $oriC ins1 \Delta mks$ **(Q)**, $oriC ins1 \Delta smc$ **(R)** $\Delta mks::mukFEB$ **(S)** and $\Delta smc \Delta mks::mukFEB$ **(T)** strains.



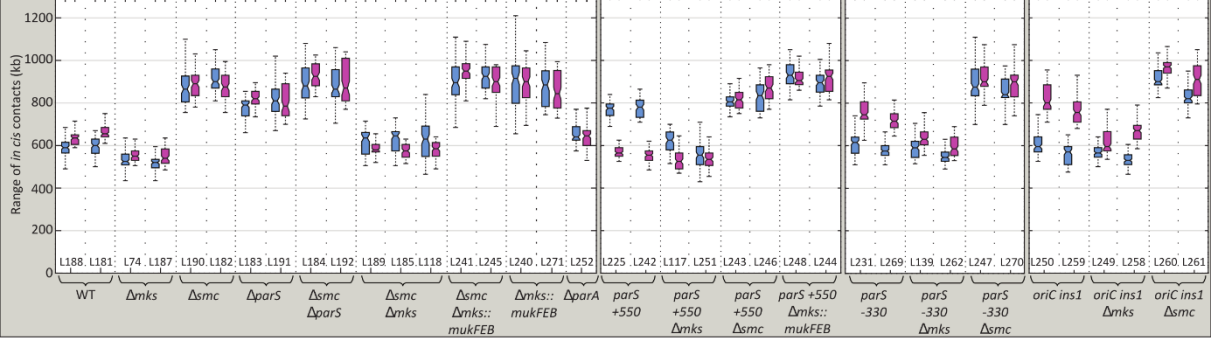
Supplementary Figure 3: Illustration of the three step process used to determine the width of the primary diagonal, i.e. the range of *cis* contacts of chromosomal loci. (A) Contact map to be analyzed. **(B)** Binary contact maps obtained after setting the threshold for significant interactions at 0.1, 0.2, 0.25, 0.5 and 0.75 times the standard deviation (defined as $\sigma = 1.4826 \cdot \text{mad}$ (median absolute deviation)) above the median. Contact frequencies above or below the threshold were assigned a value of 1 or 0, respectively, generating a binary contact map in which significant interactions between chromosomal loci are represented in yellow whereas non-significant interactions are represented in blue. Abscissa and ordinate axis represent genomic coordinates. **(C)** and **(D)** Binary contact maps obtained by varying the size of the connected element considered as significant (smaller elements are discarded as non-significant background noise). (C) starting point is the $0.2 \cdot \sigma$ map whereas (D) starting point is the $0.75 \cdot \sigma$ one, as indicated by the colored area surrounding the maps. Yellow dots indicate a significant interaction whereas blue dots indicate a non-significant interaction. Abscissa and ordinate

axis represent genomic coordinates. **(E)** and **(F)** Binary contact maps obtained using different sizes of the diamond shape used to fill out the empty points comprised in the connected elements. As indicated by the colored area surrounding the maps, (E) comes from (C) size = 30 and (F) comes from (D) size = 30. **(G)** and **(H)** Schematic representations of the measurement of the range of *cis* contacts for chromosomal loci belonging to chromosome arms. Region in which significant interactions were measured for each chromosomal locus (black arrows) are represented as red rectangles.

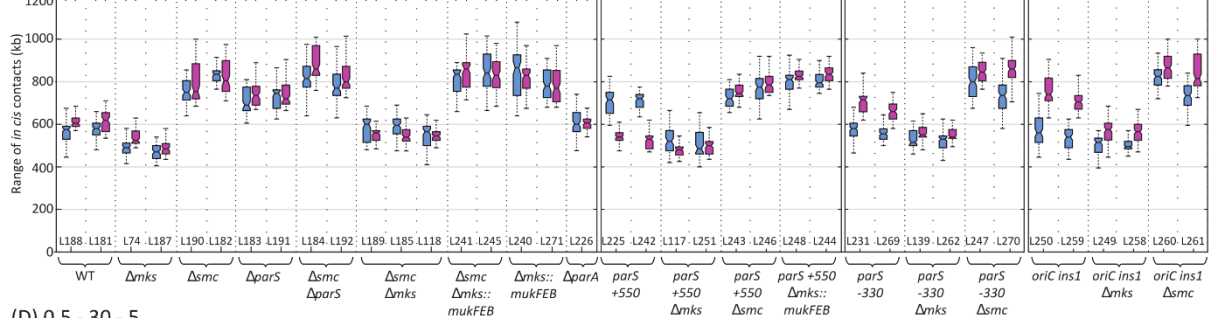
(A) 0.1 - 30 - 5



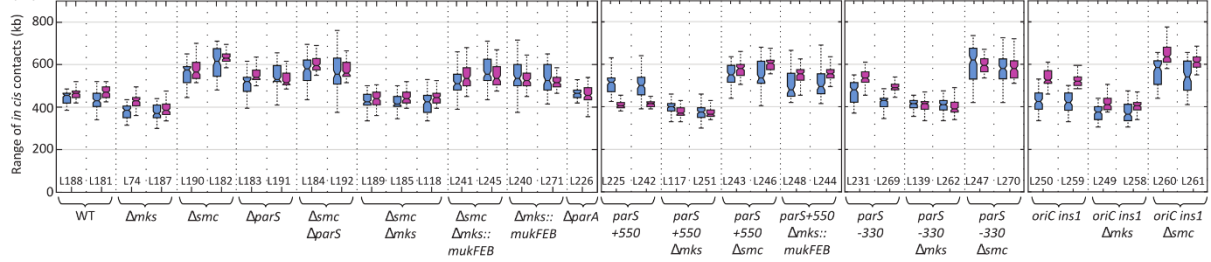
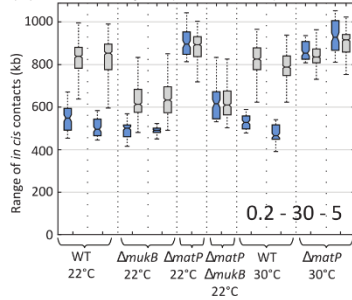
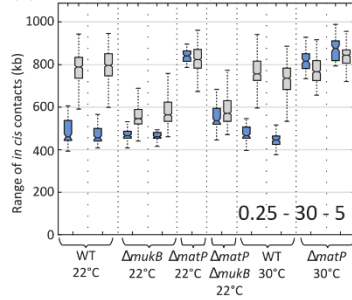
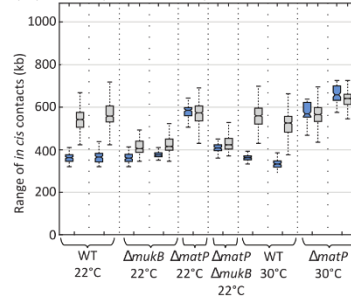
(B) 0.2 - 30 - 5



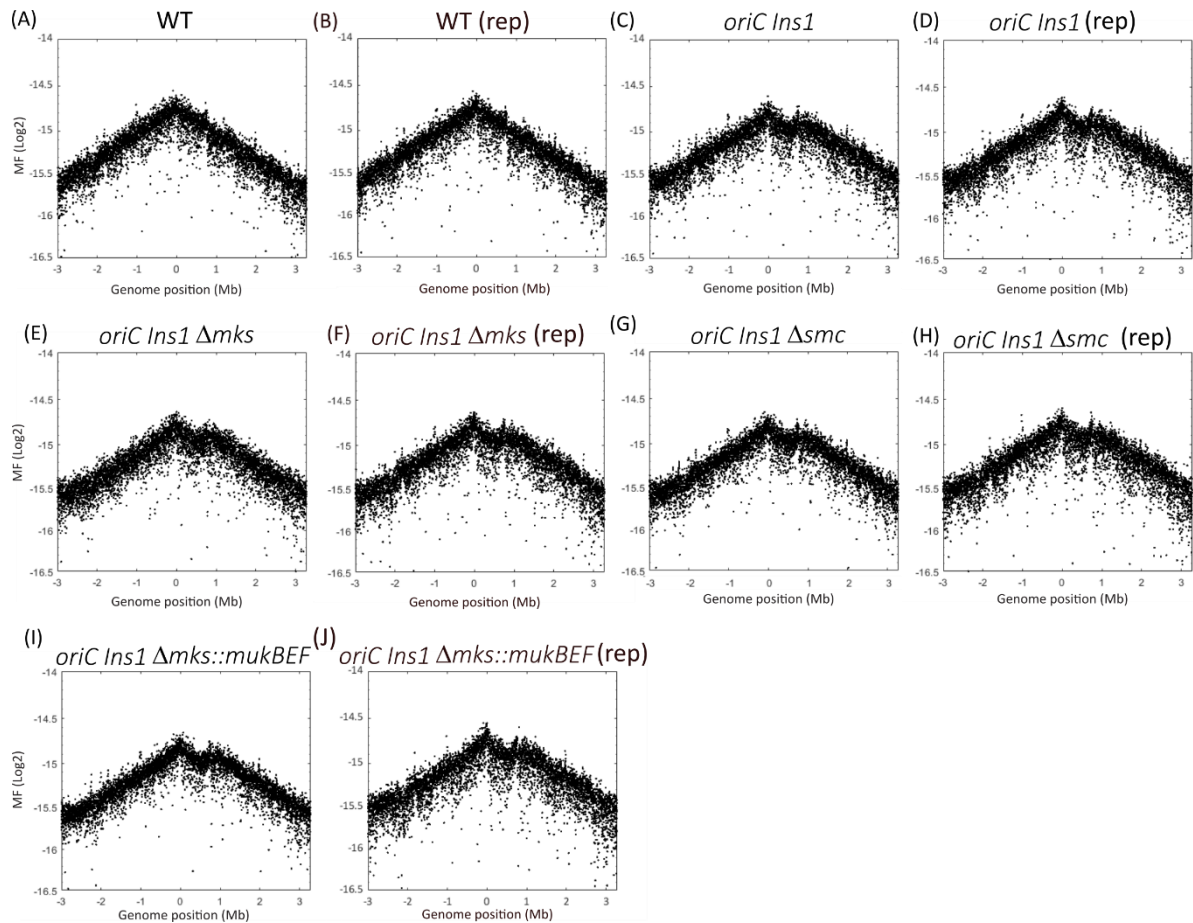
(C) 0.25 - 30 - 5



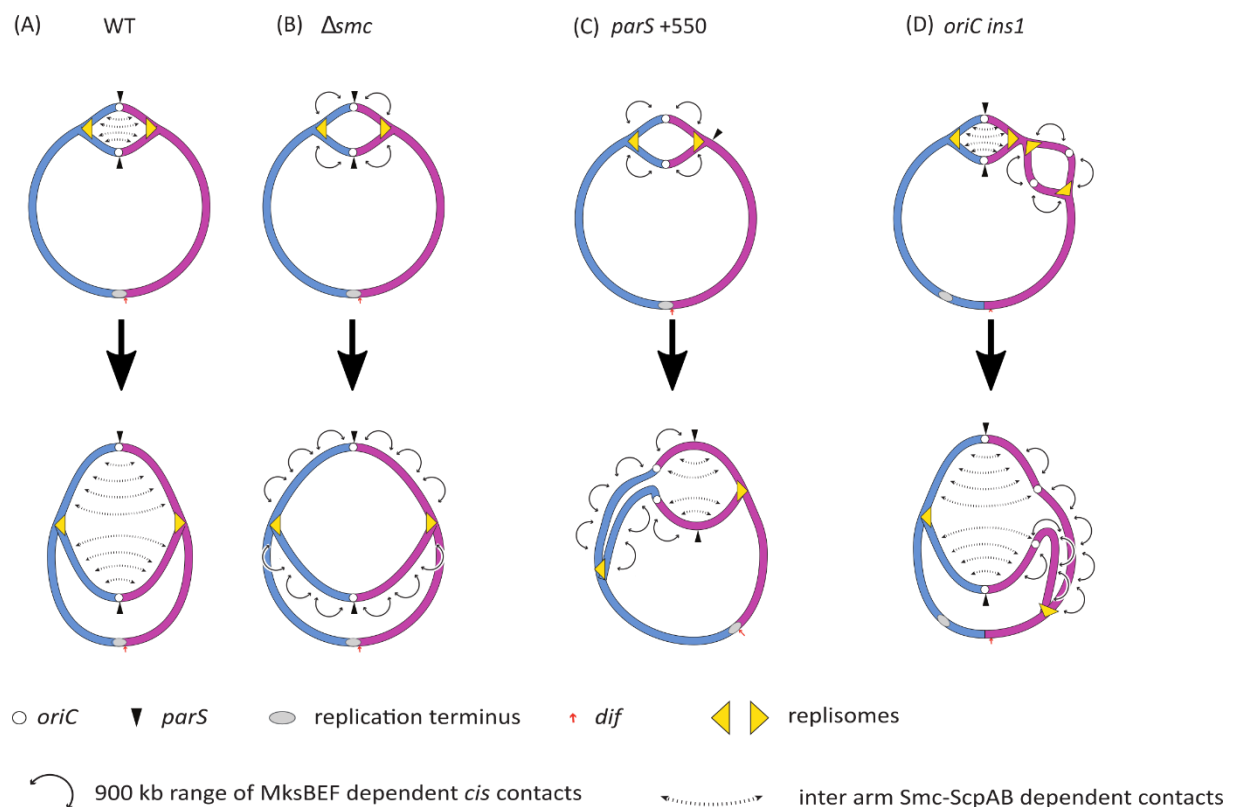
(D) 0.5 - 30 - 5

(E) *E. coli* analysis (0.2 - 30 - 5)(F) *E. coli* (0.25 - 30 - 5)(G) *E. coli* (0.5 - 30 - 5)

Supplementary Figure 4: (A-D) Quantification of the range of *cis* contacts for all contact maps of this study and for *E. coli* contact maps of the wild type strain, the $\Delta matP$ and the $\Delta mukB$ mutants. Using different thresholds for a significant interaction: $0.1 \times \sigma$ **(A)**, $0.2 \times \sigma$ **(B)**, $0.25 \times \sigma$ **(C)** and $0.5 \times \sigma$ **(D)**. Boxplot representations are used, indicating the median (horizontal bar), the 25th and the 75th percentile (open box) and the rest of the population except for the outliers (whiskers). Outliers are defined as $1.5 \times IQR$ or more above the 75th percentile or $1.5 \times IQR$ or more below the first 25th percentile quartile. The $0.2 \times \sigma$ threshold is highlighted in grey as it is the threshold chosen for the main figures. **(E-G)** Quantification of the range of *cis* contacts for *E. coli* contact maps of the wild type strain, the $\Delta matP$ and the $\Delta mukB$ mutants, previously published in Lioy et al., 2018. Strains were grown in Minimal medium supplemented with glucose and casamino acids, at the temperature indicated below the graphs with the genetic background. Different thresholds were used, $0.2 \times \sigma$ **(E)**, $0.25 \times \sigma$ **(F)** and $0.5 \times \sigma$ **(G)**.



Supplementary Figure 5: Marker frequency analyses for different strains grown in minimal medium supplemented with citrate at 30°C. Marker frequencies for WT (A) and (B), *oriC ins1* (C) and (D), *oriC ins1 Δmks* (E), *oriC ins1 Δsmc* (R) *Δmks::mukFEB* (S) and *Δsmc Δmks::mukFEB* (T) strain marker frequencies (black dots after trimming and normalisation on the total number of reads, see STAR methods) are represented in Log2 as a function of the genome position.



Supplementary Figure 6: Recapitulative schematization of the different chromosomal contacts observed in *P. aeruginosa* according to different chromosome configurations during the replication cycle. Overall contacts in the WT strain (A), in the Δsmc mutant (B), in the strain with the displaced *parS* site ($parS^{+550}$) (C) and in the strain with the additional ectopic *oriC* ($oriC\ ins1$) (D).