Supplementary Figures and Tables

Chromosome segregation dynamics during the cell cycle of *Staphylococcus aureus*

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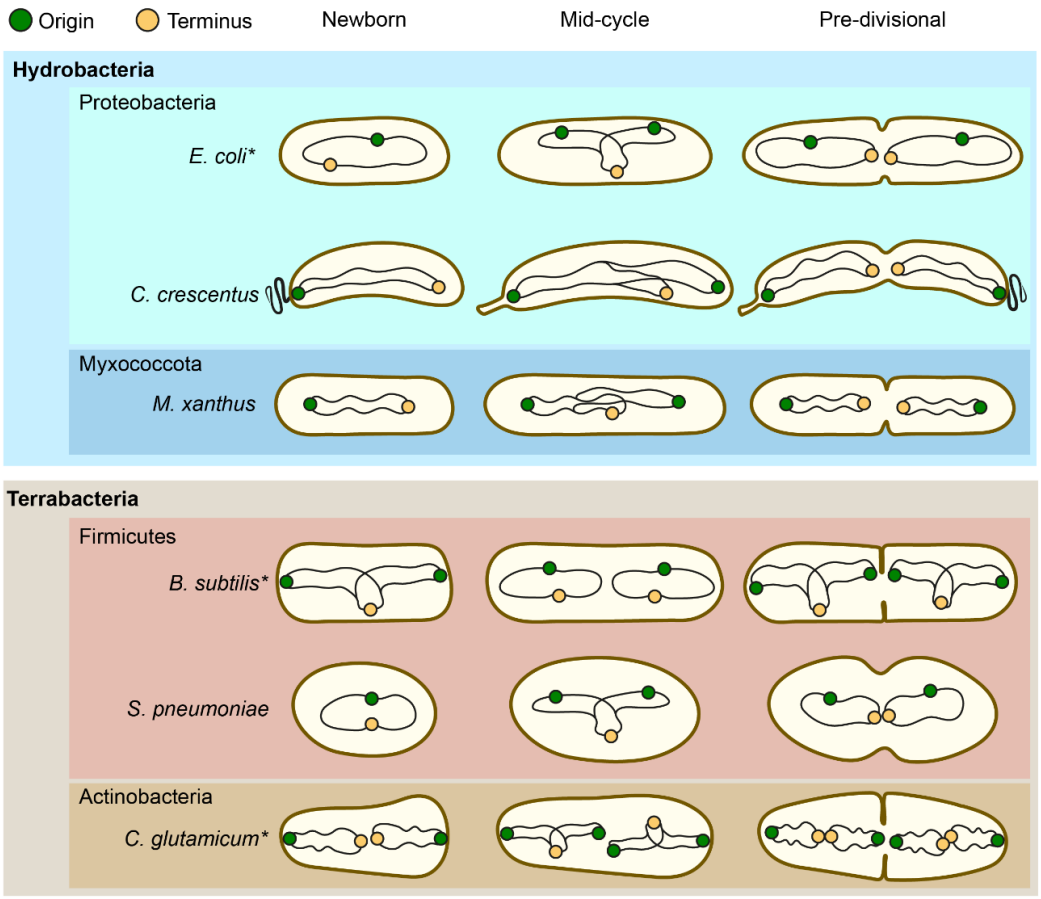
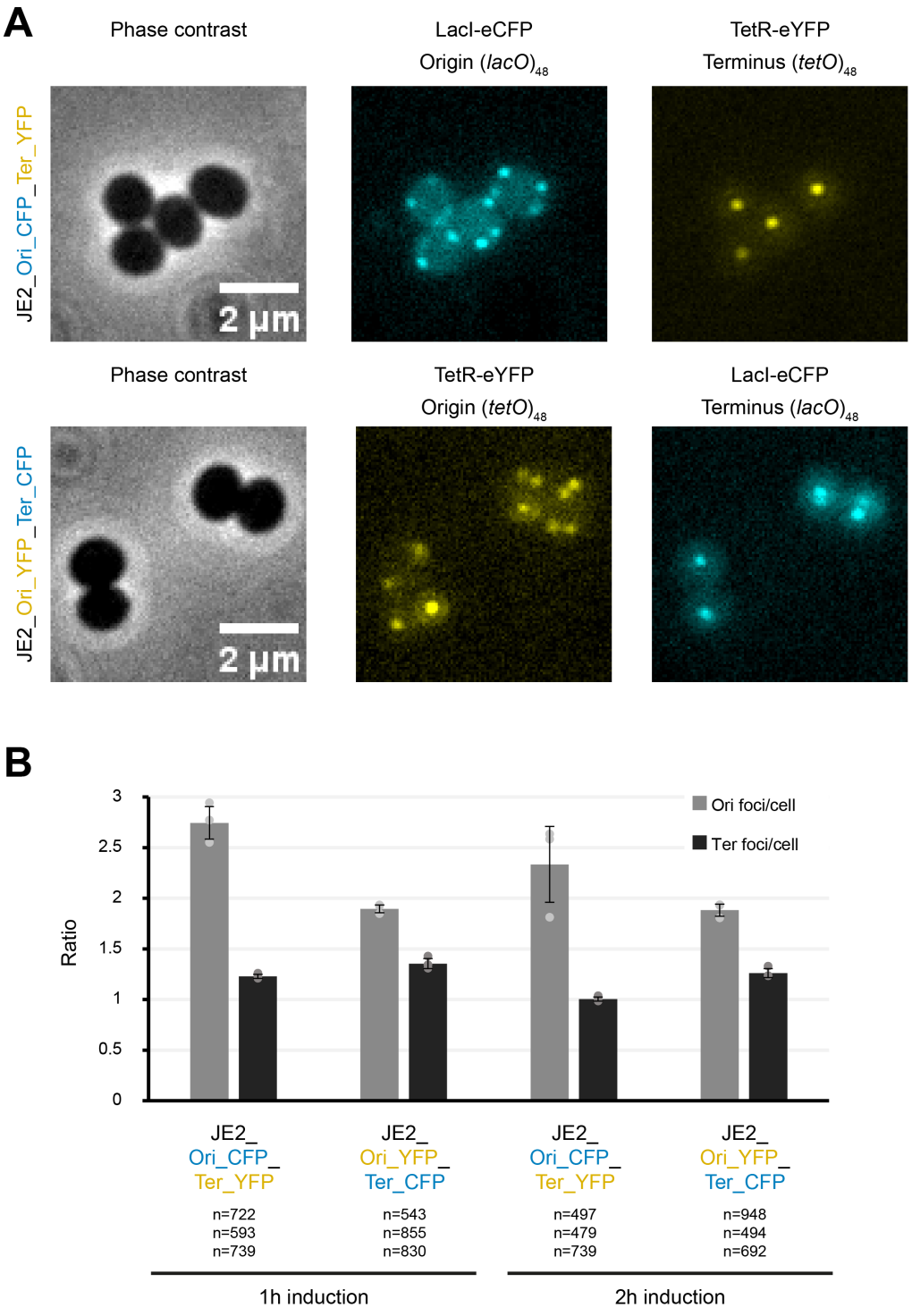
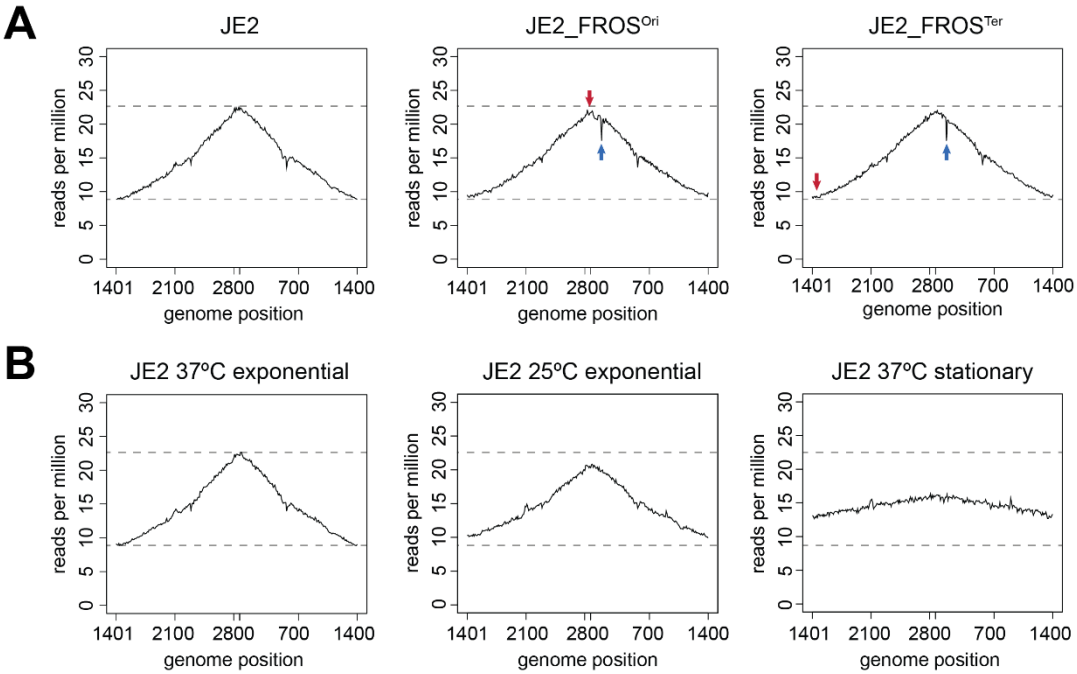


Supplementary Figure 1. Chromosome organization and dynamics in various bacteria. Chromosomes are depicted as black lines, with the origin and terminus as green and yellow dots, respectively. Species are arranged according to their taxonomy, as indicated by the colored boxes labelled with the corresponding phyla. Cells are represented, from left to right, as newborn, in an intermediate stage and pre-divisional. For species marked with an asterisk, the diagram represents chromosome dynamics under slow growing conditions (in the absence of multifork replication). References are provided in the main text.

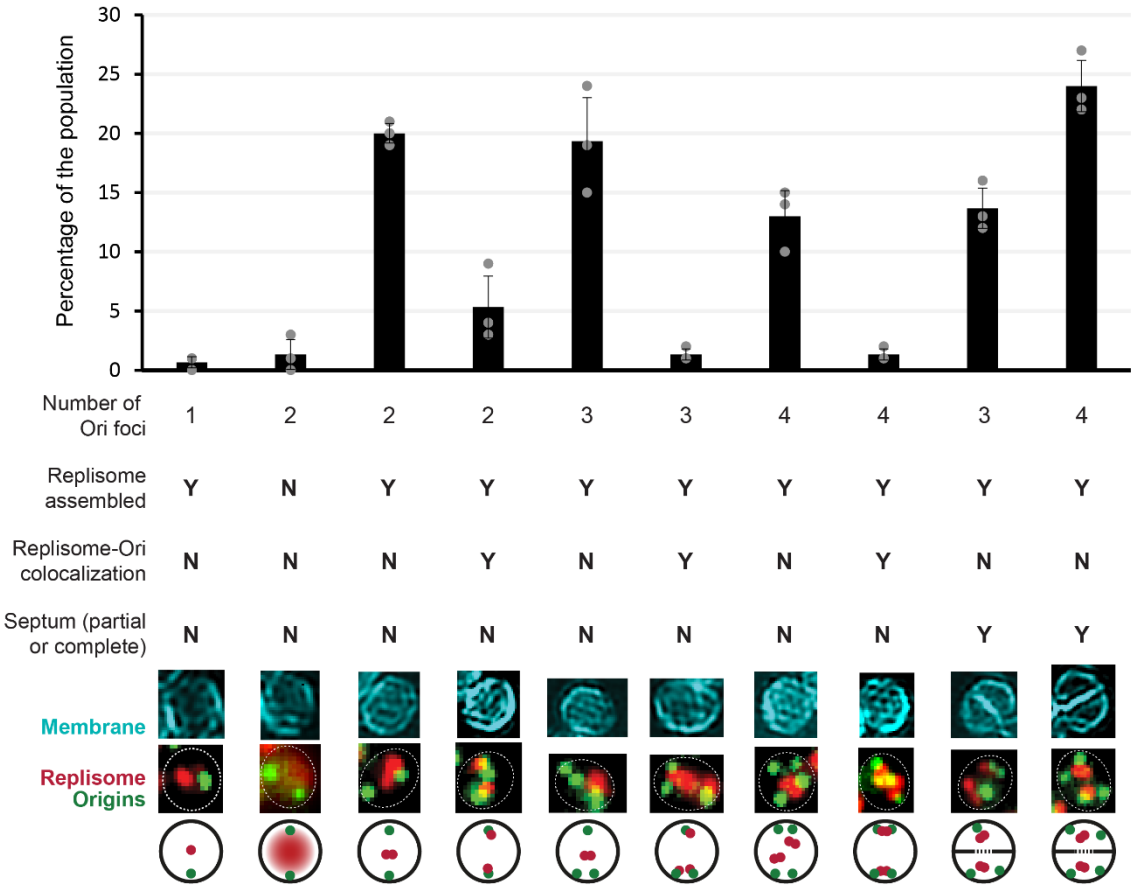


Supplementary Figure 2. Dual labeling of origins and termini using the FROS system. A Phase contrast (left) and fluorescence (middle and right) microscopy images of cells expressing TetR-eYFP and LacI-eCFP in strain JE2_Ori_CFP_Ter_YFP (top) with a $(lacO)_{48}$ array integrated near the

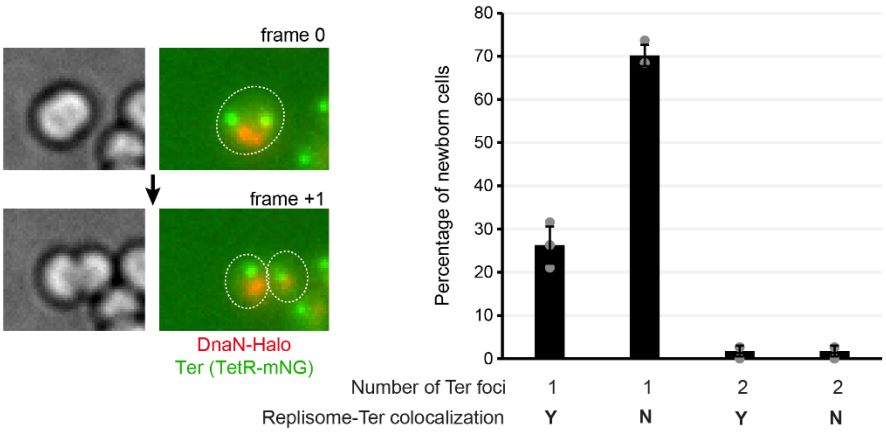
origin and a $(tetO)_{48}$ array near the terminus, and in strain JE2_Ori_YFP_Ter_CFP (bottom) with a $(lacO)_{48}$ array integrated near the terminus and $(tetO)_{48}$ array near the origin. The expression of the fluorescent protein was induced by adding 1µM of $CdCl_2$ to the culture. **B** Bar chart showing the number of Ori/cell and Ter/cell for the strains shown in **A**, after 1 or 2 hours of induction with 1µM of $CdCl_2$. Bars indicate the mean of three biological replicates, error bars the standard deviation and are centered on the mean; individual data points are represented as grey circles. Source data available in Source data file.



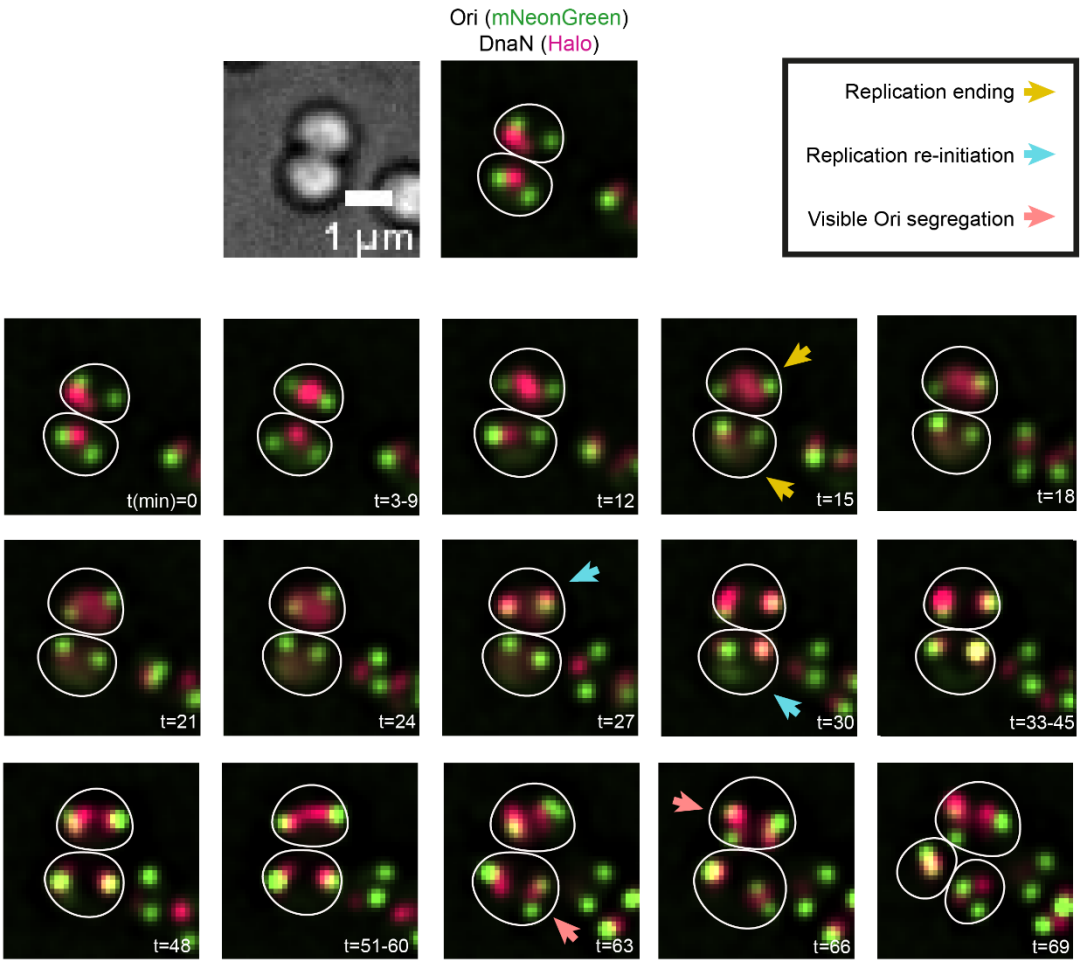
Supplementary Figure 3. Maker frequency analysis of *S. aureus* JE2 in different growth conditions and of the FROS strains. Marker frequency analysis based on whole genome sequencing of strains **(A)** JE2, JE2_FROS^{Ori} and JE2_FROS^{Ter} grown to exponential phase at 37°C and **(B)** JE2 grown to exponential phase at 37°C or 25°C, or to stationary phase at 37°C. The x-axis represents the genomic position, with the origin at the center. The y-axis shows the number of reads at each genomic location. A higher y value indicates that the corresponding genomic position has a higher copy number. Sequencing reads were normalized to the total number of reads for each sample (reads per million) before plotting. Data are plotted in 10-kb bins. Dashed grey lines indicate the highest and lowest number of reads for the JE2 control strain grown at 37°C. Red arrows mark the location of the *tetO* arrays in the JE2_FROS^{Ori} and JE2_FROS^{Ter} strains. Blue arrows indicate the location of the *spa* gene, which has been replaced with *tetR-mNG* in both FROS strains.



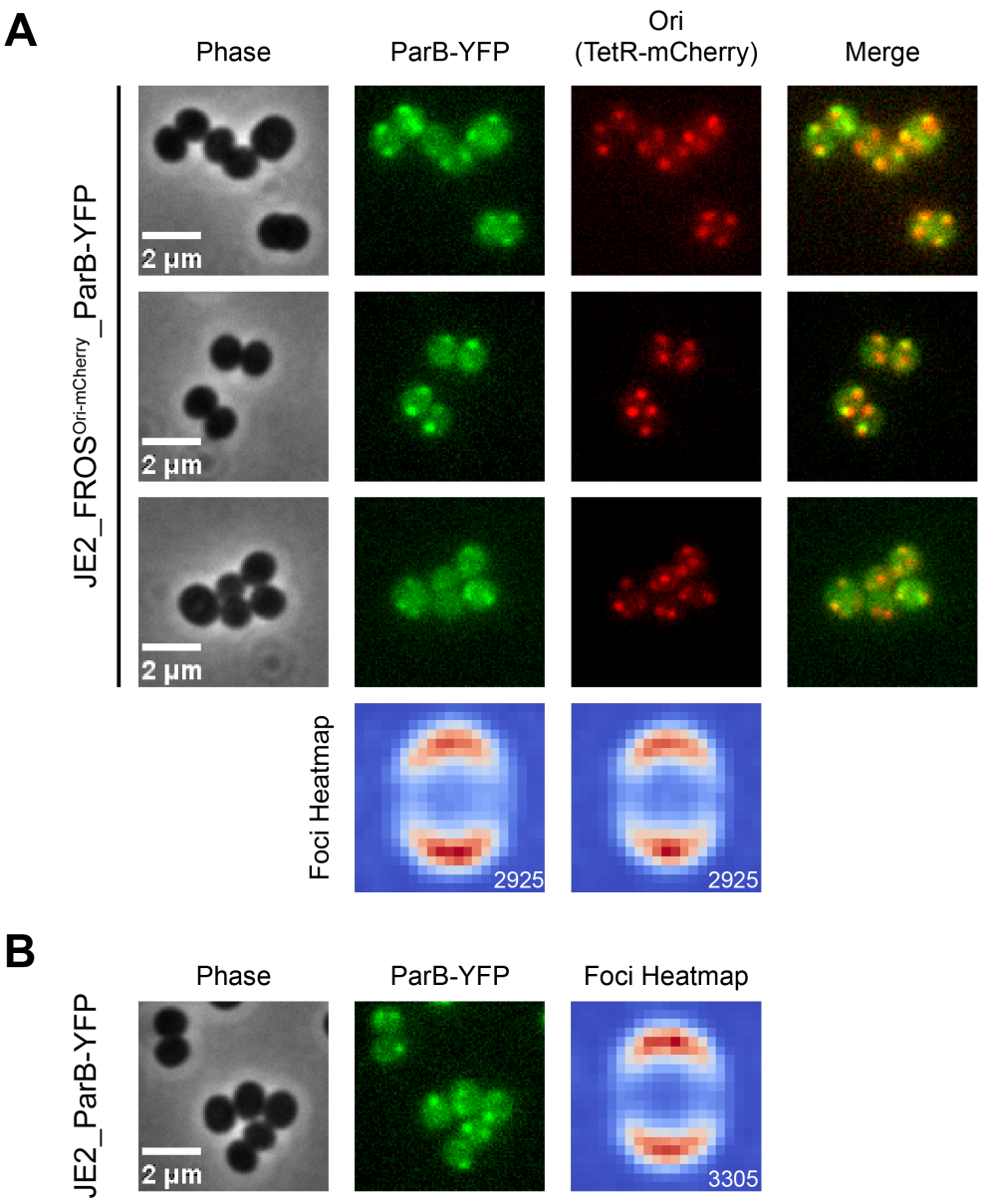
Supplementary Figure 4. Origin number and co-localization with replisome during the *S. aureus* cell cycle. Bar chart showing the distribution of JE2_FROS^{Ori}_DnaN-Halo cells manually classified according to the number of origin foci, replisome assembly, colocalization of DnaN-Halo with origins and presence of a visible septum (partial or complete). Data shown is from three biological replicates, error bars indicate standard deviation and are centered on the mean; individual data points are represented as grey circles, n=100 each replicate. Below the chart are images of representative cells with origins labelled by TetR-mNG (green), DnaN-Halo labelled with JF549 (red) and membrane labelled with CellBrite Fix 640 dye (cyan). The bottom row shows schematic representations of these cells to illustrate the classification. Source data available in Source data file.



Supplementary Figure 5. Classification of newborn cells by Ter number and Ter/replisome colocalization. Left, brightfield and fluorescence microscopy images of a representative cell from JE2_FROS^{Ter}_DnaN-Halo strain that underwent division between frame 0 and frame +1, showing two newborn cells with a single Ter each in the latter frame (3 min interval between frames). JF549-labelled DnaN-Halo signal in red and TetR-mNG (Ter) signal in green. Right, bar chart showing the relative frequency of each class of newborn cells, categorized by Ter number and Ter/replisome colocalization. Data from three biological replicates (n= 38 each), error bars indicate standard deviation and are centered on the mean; individual data points are represented as grey circles. Source data available in Source data file.

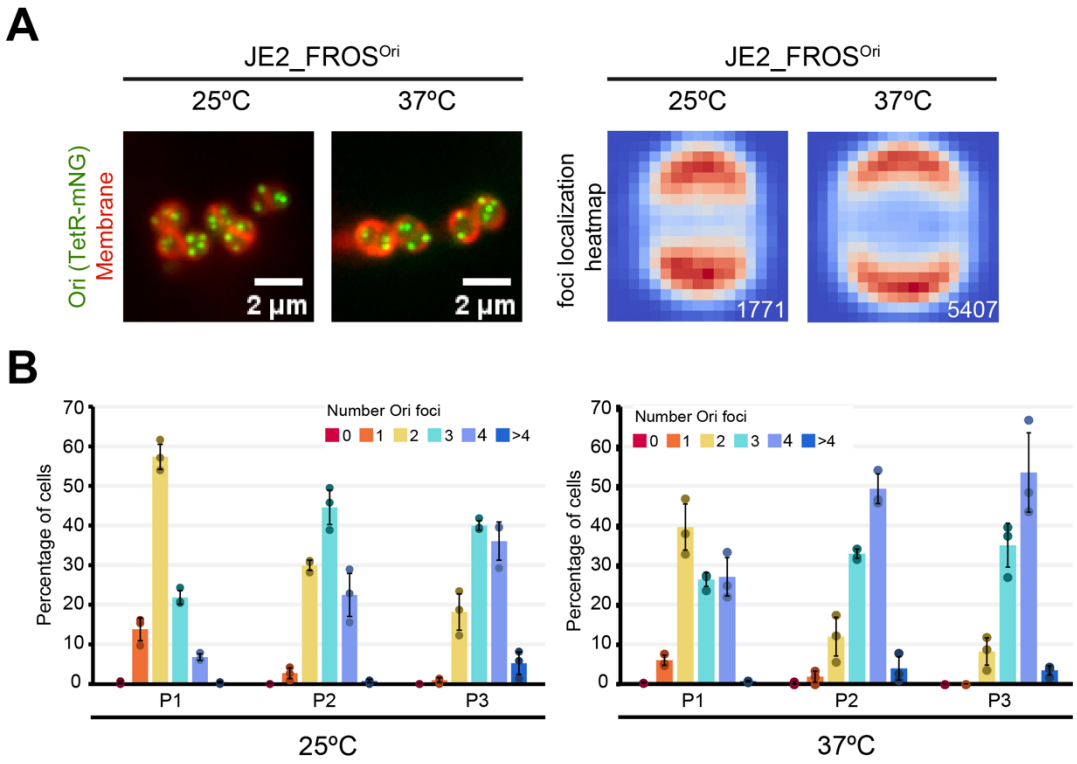


Supplementary Figure 6. Representative time-lapse of the *S. aureus* chromosome cycle. Images from time-lapse microscopy of *S. aureus* cells of the strain JE2_FROS^{Ori}_DnaN-Halo with origins labelled by TetR-mNeonGreen (green) and DnaN-Halo fusion labelled with JF549 (red). Frames were captured at 3 min intervals. For periods without relevant changes, only the initial frame is shown and the range is indicated in the lower right corner. Cell outlines are shown in white. Arrows mark key events: replication termination (yellow), re-initiation (blue) and visible origin segregation (red).

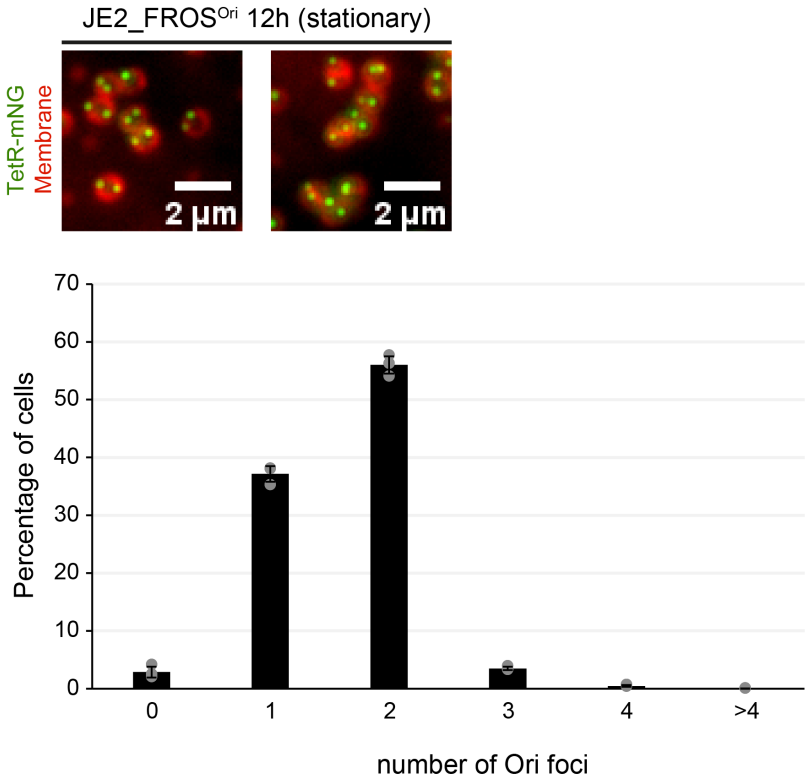


Supplementary Figure 7. ParB localizes in the polar region of cell periphery and colocalizes with FROS-labelled Ori. A Fluorescence microscopy images of the JE2_FROS$^{Ori\text{-}mCherry}$_ParB-YFP strain grown in M9 minimal medium showing localization of Ori labelled with the FROS system (red) and ParB-YFP (green). Heatmaps of the average localization of detected fluorescence spots of each fluorescent protein are shown below the corresponding images. The color scale in each dataset ranges from red (maximum spot density) to dark blue (no spots detected), n number indicated in the lower right corner of each heatmap. **B** Fluorescence microscopy images of the JE2_ParB-YFP strain grown in M9 minimal medium showing localization of ParB-YFP (green).

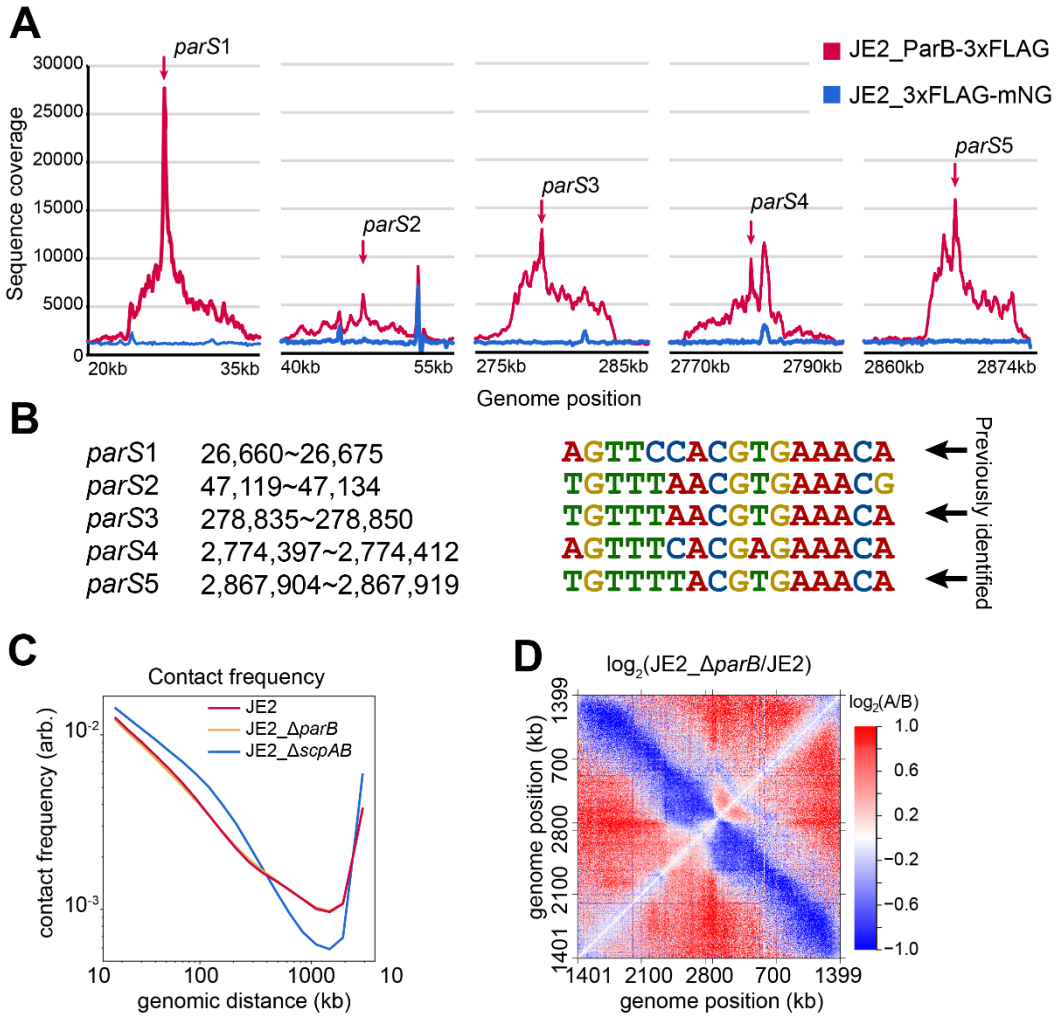
Heatmap of the average localization of detected fluorescence spots of ParB-YFP is shown on the right. The color scale ranges from red (maximum spot density) to dark blue (no spots detected), n number indicated in the lower right corner of each heatmap.



Supplementary Figure 8. Localization and number of Ori foci for *S. aureus* cells grown at 25°C versus 37°C. A Left side, fluorescence microscopy images of the JE2_FROS^{Ori} strain grown at 25°C or 37°C in TSB, showing localization of Ori labelled with the FROS system (green) and membrane labelled with FM 4-64 dye (red). Heatmaps of the average localization of detected fluorescence spots of the JE2_FROS^{Ori} strain grown at 25°C or 37°C are shown on the right side. The color scale in each dataset ranges from red (maximum spot density) to dark blue (no spots detected), n number shown in the lower right corner of each heatmap. **B** Bar chart showing the relative distribution of number of origin foci in cells of the JE2_FROS^{Ori} strain grown at 25°C or 37°C (the latter is the same data as shown in Fig. 2D). Data from three biological replicates, error bars indicate standard deviation and are centered on the mean; individual data points are represented as colored circles. For growth at 25°C, n=402, 563, 545; for growth at 37°C, n=2309, 2001, 1097. Source data available in Source data file.

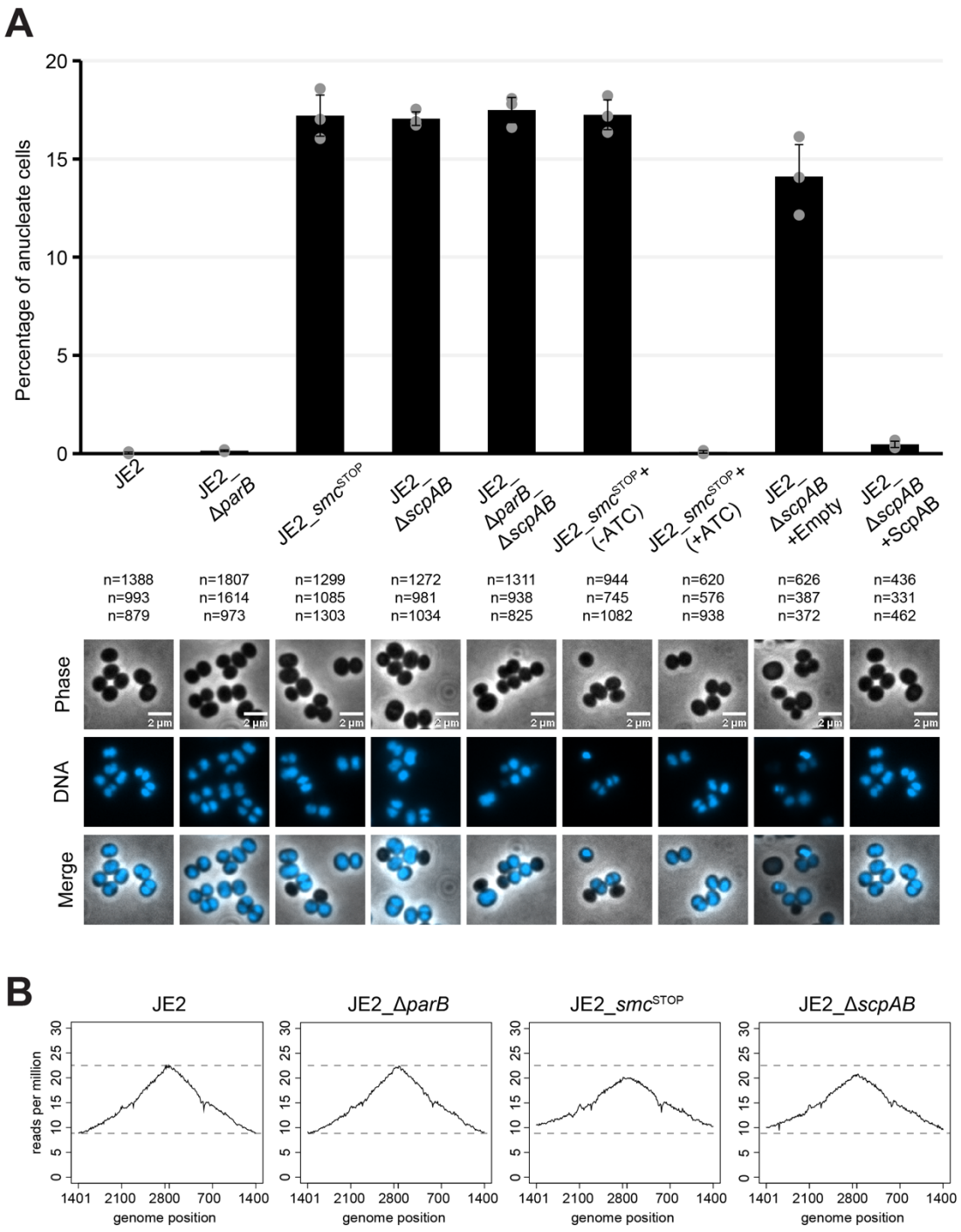


Supplementary Figure 9. Localization and number of Ori foci for *S. aureus* cells in stationary phase. Top, fluorescence microscopy images of the JE2_FROS^{Ori} strain grown to stationary phase (12 hours of incubation in TSB media at 37°C with agitation) showing localization of Ori labelled with the FROS system (green) and membrane labelled with FM 4-64 dye (red). Bottom, bar chart representing the relative distribution of number of origin foci in cells of the JE2_FROS^{Ori} strain grown to stationary phase. Data from three biological replicates, error bars indicate standard deviation and are centered on the mean; individual data points are represented as grey circles, n=2734, 2618, 2840. Source data available in Source data file.



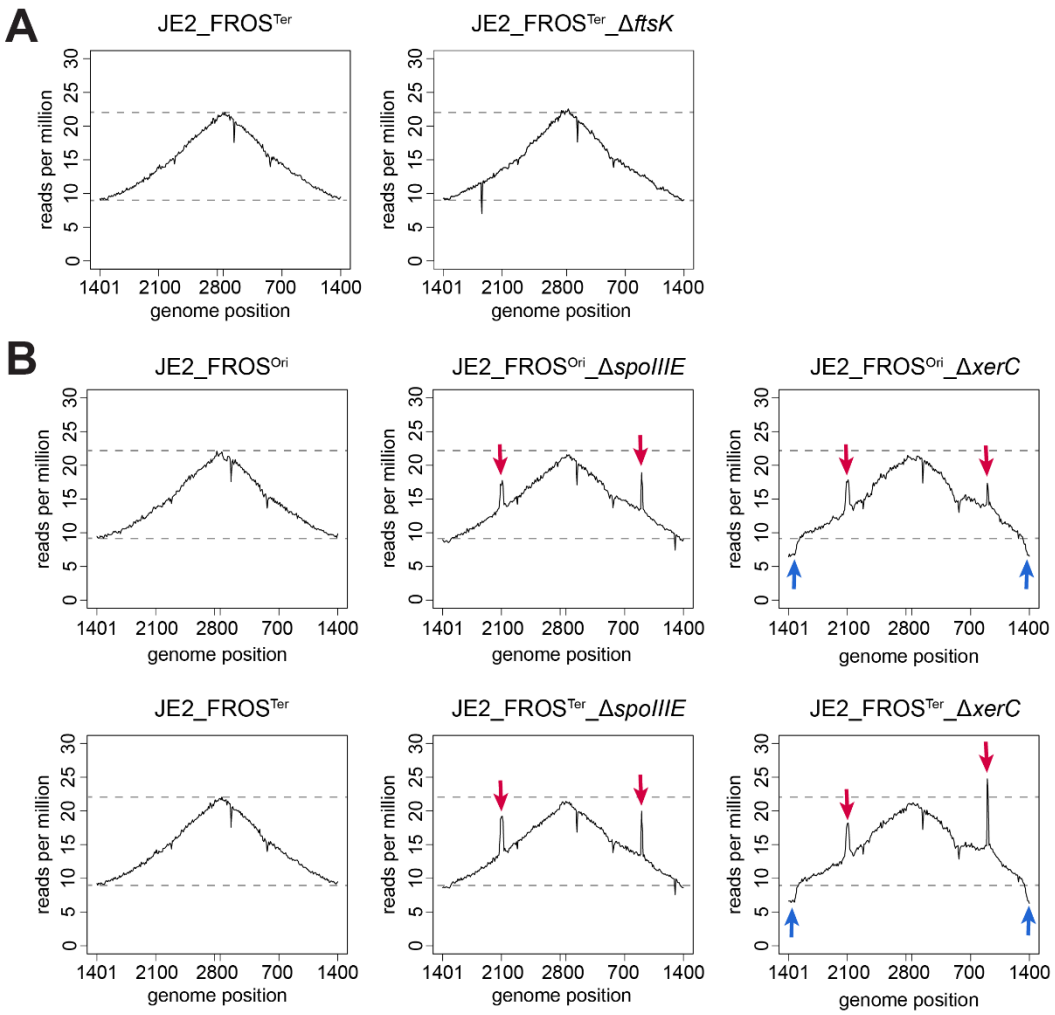
Supplementary Figure 10. Identification of ParB binding sites and role of ParB and SMC-ScpAB complex in chromosome organization. A Genomic regions enriched by anti-FLAG chromatin immunoprecipitation in JE2_ParB-3xFLAG (red) compared to JE2_3xFLAG-mNG control (blue). The x-axis indicates genomic position and the y-axis represents the number of reads for each position. Red arrows mark the position of *parS* sequences. Data from a single experiment. **B** List of the five identified *parS* sites in *S. aureus*, including genomic position and sequence. The sites *parS*1, *parS*3 and *parS*5 were previously predicted (*1*) **C** Contact probability curves for JE2 (red), JE2_Δ*parB* (yellow) and JE2_Δ*scpAB* (blue) strains. The curves represent the averaged probability of a contact (y-axis) between two loci separated by a given genomic distance indicated in the x-axis. Sequences separated by shorter distances (left side) have a higher probability of being in contact, and as the distance grows (moving towards the right side), the probability of contact decreases. Since the chromosome is circular, the curve rises again at its

rightmost end. Sequencing reads from biological replicates were combined before mapping and analysis (2 replicates for JE2, 3 replicates for JE2_*ΔparB*, and 3 replicates for JE2_*ΔscpAB*). Note that the yellow line (JE2_*ΔparB*) is almost perfectly overlapped by the red line (JE2). **D** Hi-C matrices were compared by calculating Log_2(JE2_*ΔparB*/JE2) and plotting as a heatmap. Blue pixels indicate regions with decreased interactions in *ΔparB*. Red pixels indicate regions with increased interactions in *ΔparB*. The color scale is shown on the right.



Supplementary Figure 11. Quantification of anucleate cells in mutants lacking ParB or SMC-ScpAB. A Top, bar graph showing the average fraction of anucleate cells in the indicated strains. Data from three biological replicates, n number indicated under each strain's name. Strain JE2_*smc*^{STOP}+ is a conditional mutant with an ectopic copy of *3xflag-smc* under control of an anhydrotetracycline (ATC)-inducible promoter to allow for complementation of the *smc*

mutation. Cells were grown in the presence or absence of 50 ng/ml ATC before imaging. Strains JE2_Δ*scpAB*+ScpAB and Δ*scpAB*+Empty lack the native *scpAB* genes and contain either a plasmid that expresses *scpAB* from a xylose-inducible promoter (no inducer was added, leaky expression was sufficient for complementation), or the empty plasmid (negative control), respectively, for *scpAB* complementation experiments. Error bars indicate standard deviation and are centered on the mean; individual data points are represented as grey circles. Source data available in Source data file. Bottom, representative images of each strain labelled with Hoechst DNA dye showing, from top to bottom, a phase contrast image, the fluorescent signal from the DNA dye and a merge of the two channels. Strain JE2_*smc*^{STOP}+ is shown in the presence or absence of the inducer ATC. **B** Marker frequency analysis of the indicated strains. The x-axis represents the genomic position, with the origin at the center. The y-axis shows the number of reads at each genomic location. A higher y value indicates that the corresponding genomic position has a higher copy number. Sequencing reads were normalized to the total number of reads for each sample (reads per million) before plotting. The data are plotted in 10-kb bins. Dashed grey lines indicate the highest and lowest number of reads for the JE2 control strain.



Supplementary Figure 12. Genome-wide marker frequency analysis of *S. aureus* mutants lacking FtsK, SpoIIIE or XerC. The x-axis represents the genomic position, with the origin at the center. The y-axis shows the number of reads at each genomic location. A higher y value indicates that the corresponding genomic position has a higher copy number. Sequencing reads were normalized to the total number of reads for each sample (reads per million) before plotting. The data are plotted in 10-kb bins. Dashed grey lines indicate the highest and lowest number of reads for the JE2 control strain used for comparison with each mutant. Red arrows mark peaks that are absent in the reference strain. Blue arrows indicate drops that are absent in the reference strain.

Supplementary Table 1: Strains used in the study

Strain name	Relevant information	Construction/Source
***E. coli* DC10B**	*dam+ dcm+ ΔhsdRMS endA1 recA1*	(*2*)
***S. aureus* JE2**	Community acquired MRSA	(*3*)
***S. aureus* RN4220**	Restriction deficient derivative of *S. aureus* NCTC8325-4	(*4*)
JE2_ori-*tetO*48	*Ori-(tetO)$_{48}$*	Plasmid pBCBSS289 was transduced into strain JE2, followed by an integration/excision process to introduce *(tetO)$_{48}$* near the origin region (between genes SAUSA300_2631 and SAUSA300_2632, approx. 14Kb away from Ori).
JE2_ter-*tetO*48	*Ter-(tetO)$_{48}$*	Plasmid pBCBSS285 was transduced into strain JE2, followed by an integration/excision process to introduce *(tetO)$_{48}$* near the Ter region (between genes SAUSA300_1326 and SAUSA300_1327, approx. 21Kb away from the 180° position).
JE2_left-*tetO*48	*Left-(tetO)$_{48}$*	Plasmid pBCBSS315 was transduced into strain JE2, followed by an integration/excision process to introduce *(tetO)$_{48}$* in the left arm of the chromosome (between genes SAUSA300_1984 and SAUSA300_1985).
JE2_right-*tetO*48	*Right-(tetO)$_{48}$*	Plasmid pBCBSS317 was transduced into strain JE2, followed by an integration/excision process to introduce *(tetO)$_{48}$* in the right arm of the chromosome (between genes SAUSA300_0660 and SAUSA300_0661).
JE2_ori-*lacO*48	*Ori-(lacO)$_{48}$*	Plasmid pBCBSS290 was transduced into strain JE2, followed by an integration/excision process to *(lacO)$_{48}$* near the origin region (between genes SAUSA300_2631 and SAUSA300_2632).
JE2_ori-*tetO*48_ter-*lacO*48	*Ori-(tetO)$_{48}$ Ter-(lacO)$_{48}$*	Plasmid pBCBSS283 was transduced into strain JE2_ori-*tetO*48, followed by an integration/excision process to introduce *(lacO)$_{48}$* near the Ter region (between genes SAUSA300_1326 and SAUSA300_1327).
JE2_ori-*lacO*48_ter-*tetO*48	*Ori-(lacO)$_{48}$ Ter-(tetO)$_{48}$*	Plasmid pBCBSS285 was transduced into strain JE2_ori-*lacO*48, followed by an integration/excision process to introduce *(tetO)$_{48}$* near the Ter region (between genes SAUSA300_1326 and SAUSA300_1327).
JE2_Ori_YFP_Ter_CFP	Δ*spa::tetR-eYFP lacI-eCFP Ori-(tetO)$_{48}$ Ter-(lacO)$_{48}$*	Plasmid pBCBSS292 was transduced into strain JE2_ori-*tetO*48_ter-*lacO*48, followed by an integration/excision process to introduce in the *spa* locus a *laci-ecfp* and *tetR-eyfp* fusions under P*cad* control.
JE2_Ori_CFP_Ter_YFP	Δ*spa::tetR-eYFP lacI-eCFP Ori-(lacO)$_{48}$ Ter-(tetO)$_{48}$*	Plasmid pBCBSS292 was transduced into strain JE2_ori-*lacO*48_ter-*tetO*48, followed by an integration/excision process to introduce in the *spa* locus a *laci-ecfp* and *tetR-eyfp* fusions under P*cad* control.
JE2_FROS^{Ori}	Δ*spa*::*tetR-mneongreen Ori-(tetO)$_{48}$*	Plasmid pBCBAIM018 was transduced into strain JE2_ori-*tetO*48, followed by an integration/excision process to replace *spa* by a *tetR-mNG* fusion under P*cad* control.

JE2_FROS^{Ter}	Δ*spa::tetR-mneongreen Ter-(tetO)$_{48}$*	Plasmid pBCBAIM018 was transduced into strain JE2_ter-*tetO*48, followed by an integration/excision process to replace *spa* by a *tetR-mNG* fusion under P*cad* control.
JE2_FROS^{Left}	Δ*spa::tetR-mneongreen Left-(tetO)$_{48}$*	Plasmid pBCBAIM018 was transduced into strain JE2_left-*tetO*48, followed by an integration/excision process to replace *spa* by a *tetR-mNG* fusion under P*cad* control.
JE2_FROS^{Right}	Δ*spa::tetR-mneongreen Right-(tetO)$_{48}$*	Plasmid pBCBAIM018 was transduced into strain JE2_right-*tetO*48, followed by an integration/excision process to replace *spa* by a *tetR-mNG* fusion under P*cad* control.
JE2_FROS^{Ori}_DnaN-Halo	Δ*spa*::*tetR-mneongreen Ori-(tetO)$_{48}$ dnaN*::*dnaN-halo*	Plasmid pBCBAIM001 was transduced into strain JE2_FROS^{Ori}, followed by an integration/excision process to introduce a *dnaN-halo* fusion into the native locus.
JE2_FROS^{Ter}_DnaN-Halo	Δ*spa*::*tetR-mneongreen Ter-(tetO)$_{48}$ dnaN*::*dnaN-halo*	Plasmid pBCBAIM001 was transduced into strain JE2_FROS^{Ter}, followed by an integration/excision process to introduce a *dnaN-halo* fusion into the native locus.
JE2_FROS^{Ori}_Δ*parB*	Δ*spa::tetR-mneongreen Ori-(tetO)$_{48}$ parB::ΔparB*	Plasmid pBCBMS073 was transduced into strain JE2_FROS^{Ori}, followed by an integration/excision process to delete *parB.*
JE2_FROS^{Ori}_*smc*^{STOP}	Δ*spa*::*tetR-mneongreen Ori-(tetO)$_{48}$ smc^{STOP}*	Plasmid pBCBDB013 was transduced into strain JE2_FROS^{Ori}, followed by an integration/excision process to introduce an array of STOP codons near the start of the *smc* gene. Positive clones were identified by colony PCR followed by BglII digestion of the PCR product.
JE2_FROS^{Ori}_Δ*scpAB*	Δ*spa::tetR-mneongreen Ori-(tetO)$_{48}$ ΔscpAB*	Plasmid pBCBMS086 was transduced into strain JE2_FROS^{Ori}, followed by an integration/excision process to delete *scpA* and *scpB.*
JE2_FROS^{Ori}_Δ*ftsK*	Δ*spa*::*tetR-mneongreen Ori-(tetO)$_{48}$ ΔftsK*	Plasmid pBCBHV012 was transduced into strain JE2_FROS^{Ori}, followed by an integration/excision process to delete *ftsK.*
JE2_FROS^{Ori}_Δ*noc*	Δ*spa*::*tetR-mneongreen Ori-(tetO)$_{48}$ Δnoc*	Plasmid pBCBHV001 was transduced into strain JE2_FROS^{Ori}, followed by an integration/excision process to delete *noc.*
JE2_FROS^{Ori}_Δ*spoIIIE*	Δ*spa*::*tetR-mneongreen Ori-(tetO)$_{48}$ ΔspoIIIE*	Plasmid pBCBHV009 was transduced into strain JE2_FROS^{Ori}, followed by an integration/excision process to delete *spoIIIE.*
JE2_FROS^{ori}_Δ*xerC*	Δ*spa*::*tetR-mneongreen Ori-(tetO)$_{48}$ ΔxerC*	Plasmid pBCBHV046 was transduced into strain JE2_FROS^{Ori}, followed by an integration/excision process to delete *xerC.*
JE2_FROS^{Ter}_Δ*parB*	Δ*spa*::*tetR-mneongreen* Ter-*(tetO)$_{48}$ ΔparB*	Plasmid pBCBMS073 was transduced into strain JE2_FROS^{Ter}, followed by an integration/excision process to delete *parB.*
JE2_FROS^{Ter}_*smc*^{STOP}	Δ*spa*::*tetR-mneongreen* Ter-*(tetO)$_{48}$ smc^{STOP}*	Plasmid pBCBDB013 was transduced into strain JE2_FROS^{Ter}, followed by an integration/excision process to introduce an array of STOP codons near the start of the *smc* gene. Positive clones were identified by colony PCR followed by BglII digestion of the PCR product.
JE2_FROS^{Ter}_Δ*scpAB*	Δ*spa*::*tetR-mneongreen* Ter-*(tetO)$_{48}$ ΔscpAB*	Plasmid pBCBMS086 was transduced into strain JE2_FROS^{Ter}, followed by an integration/excision process to delete *scpA* and *scpB.*

JE2_FROS^{Ter}_Δ*ftsK*	Δ*spa*::*tetR-mneongreen* Ter-*(tetO)$_{48}$ ΔftsK*	Plasmid pBCBHV012 was transduced into strain JE2_FROS^{Ter}, followed by an integration/excision process to delete *ftsK.*
JE2_FROS^{Ter}_Δ*noc*	Δ*spa*::*tetR-mneongreen* Ter-*(tetO)$_{48}$ Δnoc*	Plasmid pBCBHV001 was transduced into strain JE2_FROS^{Ter}, followed by an integration/excision process to delete *noc.*
JE2_FROS^{Ter}_Δ*spoIIIE*	Δ*spa*::*tetR-mneongreen* Ter-*(tetO)$_{48}$ ΔspoIIIE*	Plasmid pBCBHV009 was transduced into strain JE2_FROS^{Ter}, followed by an integration/excision process to delete *spoIIIE.*
JE2_FROS^{Ter}_Δ*xerC*	Δ*spa*::*tetR-mneongreen* Ter-*(tetO)$_{48}$ ΔxerC*	Plasmid pBCBHV046 was transduced into strain JE2_FROS^{Ter}, followed by an integration/excision process to delete *xerC.*
JE2_Δ*parB*	*ΔparB*	Plasmid pBCBMS073 was transduced into strain JE2, followed by an integration/excision process to delete *parB.*
JE2_*smc*^{STOP}	*smc^{STOP}*	Plasmid pBCBDB013 was transduced into strain JE2, followed by an integration/excision process to introduce an array of STOP codons near the start of the *smc* gene. Positive clones were identified by colony PCR followed by BglII digestion of the PCR product.
JE2_Δ*scpAB*	*ΔscpAB*	Plasmid pBCBMS086 was transduced into strain JE2, followed by an integration/excision process to delete *scpA* and *scpB.*
JE2_Δ*scpAB*_Δ*parB*	*ΔscpAB ΔparB*	Plasmid pBCBMS086 was transduced into strain JE2_Δ*parB*, followed by an integration/excision process to delete *scpA* and *scpB.*
JE2_Δ*parS*(-1°)	Δ*parS*(-1°)	Plasmid pBCBSS322 was transduced into strain JE2, followed by an integration/excision process to delete the *parS* site at -1° (*parS*5). Positive clones were identified by colony PCR followed by BglII digestion and sequencing of the PCR product.
JE2_Δ2*parS*	Δ*parS*(-1°) **parS*(+3°)	Plasmid pBCBSS319 was transduced into strain JE2_Δ*parS*(-1°), followed by an integration/excision process to mutate the *parS* site at +3° (*parS*1). Positive clones were identified by colony PCR followed by PmlI digestion and sequencing of the PCR product.
JE2_Δ3*parS*	Δ*parS*(-1°) **parS*(+3°) Δ*parS*(-12°)	Plasmid pBCBSS321 was transduced into strain JE2_Δ2*parS*, followed by an integration/excision process to delete the *parS* site at -12° (*parS*4). Positive clones were identified by colony PCR followed by BglII digestion and sequencing of the PCR product.
JE2_Δ4*parS*	Δ*parS*(-1°) **parS*(+3°) Δ*parS*(-12°) Δ*parS*(+35°)	Plasmid pBCBSS320 was transduced into strain JE2_Δ3*parS*, followed by an integration/excision process to delete the *parS* site at +35° (*parS*3). Positive clones were identified by colony PCR followed by BglII digestion and sequencing of the PCR product.
JE2_Δ5*parS*	Δ*parS*(-1°) **parS*(+3°) Δ*parS*(-12°) Δ*parS*(+35°) **parS*(+6°)	Plasmid pBCBSS379 was transduced into strain JE2_Δ4*parS*, followed by an integration/excision process to mutate the *parS* site at +6° (*parS*2). Positive clones were identified by colony PCR followed by AclI digestion and sequencing of the PCR product.
JE2_ParB-3xFLAG	*parB::*pMUTIN4-*parB-3xflag*	Plasmid pBCBSS269 was electroporated into strain RN4220 and subsequently transduced into strain JE2 to

		express a *parB-3xflag* fusion from the native *parB* locus. Clones that acquired the plasmid were obtained after selection with erythromycin.
JE2_3xFLAG-mNG	Δ*spa::*P_{spac}*-3xflag-mneongreen*	Plasmid pBCBSS135 was transduced into strain JE2, followed by an integration/excision process replace *spa* by a *3xflag-mNG* fusion. Positive clones were identified by colony PCR.
JE2_ParB-YFP	*parB::*pMUTIN-*parB-yfp*	Plasmid pBCBHV004 was electroporated into strain RN4220 and subsequently transduced into strain JE2. Clones that integrated the plasmid into the chromosome to express a *parB-yfp* fusion from the native *parB* locus were obtained after selection with erythromycin.
JE2_FROS$^{Ori\text{-}mCherry}$	Δ*spa*::*tetR-mCherry Ori-*$(tetO)_{48}$	Plasmid pBCB13-TetRmCh was transduced into strain JE2_ori-*tetO*48, followed by an integration/excision process to replace *spa* by a *tetR-mCherry* fusion under P*spac* control.
JE2_FROS$^{Ori\text{-}mCherry}$_ParB-YFP	Δ*spa*::*tetR-mCherry Ori-*$(tetO)_{48}$ *parB::*pMUTIN-*parB-yfp*	Plasmid pBCBHV004 was transduced from RN4220_ParB-YFP into strain JE2_FROS$^{Ori\text{-}mCherry}$ to express a *parB-yfp* fusion from the native *parB* locus. Clones that acquired the plasmid were obtained after selection with erythromycin.
JE2_Δ*scpAB*+Empty	*ΔscpAB* pEPSA5	Plasmid pEPSA5 was transduced into strain JE2_ Δ*scpAB* and clones that acquired the plasmid were obtained after selection with chloramphenicol.
JE2_Δ*scpAB*+ScpAB	*ΔscpAB* pEPSA5-*scpAB*	Plasmid pBCBSS327 was transduced into strain JE2_Δ*scpAB* and clones that acquired the plasmid were obtained after selection with chloramphenicol.
JE2_3xFLAG-Smc	*smc::3xflag-smc*	Plasmid pBCBSS281 was transduced into strain JE2, followed by an integration/excision process to introduce a *3xflag-smc* fusion into the native locus.
JE2_spa-3xFLAG-Smc	Δ*spa::*$P_{xyl\text{-}tetO}$*-3xflag-smc*	Plasmid pBCBDB014 was transduced into strain JE2, followed by an integration/excision process to replace *spa* by a *3xflag-smc* fusion.
JE2_*smc*^{STOP}+	Δ*spa::*$P_{xyl\text{-}tetO}$*-3xflag-smc smc*^{STOP}	Plasmid pBCBDB013 was transduced into strain JE2_spa-3xFLAG-Smc, followed by an integration/excision process to introduce an array of STOP codons near the start of the *smc* gene. Positive clones were identified by colony PCR followed by BglII digestion of the PCR product.

Plasmids were initially introduced by electroporation into RN4220 and then transduced into the required strain using phage 80α as described in materials and methods. The integration/excision process used for allelic replacement is described in the methods section. Except where stated otherwise, positive clones were identified by colony PCR.

Supplementary Table 2: Plasmids used in the study.

Plasmid name	Resistance in *E. coli*	Resistance in *S. aureus*	Construction/Source
pMAD	Ampicillin	Erythromycin	*E. coli-S. aureus* shuttle vector with a thermosensitive origin of replication for Gram-positive bacteria (*5*).
pCN51	Ampicillin	Erythromycin	*E. coli-S. aureus* shuttle replicative vector containing the staphylococcal cadmium-inducible promoter *cad* (*6*).
pBCB13	Ampicillin	Erythromycin	pMAD-spa derivative with P*spac-lacI* region (*7*).
pEPSA5	Chloramphenicol	Chloramphenicol	*E. coli-S. aureus* shuttle replicative vector containing the xylose-inducible promoter T5X (*8*).
pBCB43	Ampicillin	Erythromycin	pBCB13 derivative containing the ATC-inducible promoter *xyl-tetO* (*9*).
pBCB13-TetRmCh	Ampicillin	Erythromycin	pBCB13 derivative containing *tetR-mCherry* under P*spac* control (*7*).
pBCBSS135	Ampicillin	Erythromycin	pBCB13 containing 3XFLAG-mNeonGreen under P*spac* control (*10*).
pMUTIN4	Ampicillin	Erythromycin	Integration vector containing *lacZ* (*11*).
pBCBHV004	Ampicillin	Erythromycin	pMUTINYFP containing *spo0J–yfp* (*12*).
pMAD-Δ*spa*	Ampicillin	Erythromycin	pMAD containing *spa* upstream region-NotI restriction site-*spa* downstream region (*10*).
pLAU53	Ampicillin	-	Plasmid containing a module with the *lacI-eCFP* and *tetR-eYFP* fusions (*13*).
pLAU29	Ampicillin	-	Plasmid containing a $(tetO)_{48}$ array (*13*).
pLAU23	Ampicillin	-	Plasmid containing a $(lacO)_{48}$ array (*13*).
pBCBHV012	Ampicillin	Erythromycin	pMAD derivative to delete *ftsK* (*14*).
pBCBHV001	Ampicillin	Erythromycin	pMAD derivative to delete *noc* (*12*)
pBCBHV009	Ampicillin	Erythromycin	pMAD derivative to delete *spoIIIE* (*14*).
pBCBHV046	Ampicillin	Erythromycin	pMAD derivative to delete *xerC* (*14*).
psav-mSc-I	Chloramphenicol	-	N. Meiresonne and T. den Blaauwen. The plasmid contains the sequence of the mScarletI fluorescent protein and was only used as template to amplify said sequence in the construction of plasmid pBCBSS311.

pBCBAIM001	Ampicillin	Erythromycin	pMAD derivative to introduce a *dnaN-halo* fusion in the native locus. Three fragments were amplified: A (oligos OBCBAIM003/ OBCBAIM004), halo (oligos OBCBAIM005/ OBCBAIM006) and B (oligos OBCBAIM007/ OBCBAIM008). Fragments were combined by extension PCR into a single fragment, which was cloned, using Gibson Assembly, into pMAD, previously digested with EcoRI and NcoI.
pBCBSS270	Ampicillin	Erythromycin	pMUTIN4 derivative, intermediate plasmid to make C-terminal FLAG fusions. A DNA fragment generated by hybridization between the two oligos 3xFLAG_oligo1 and 3xFLAG_oligo2 was ligated into EagI/BamHI-digested pMUTIN4.
pBCBSS269	Ampicillin	Erythromycin	pMUTIN4 derivative to introduce a *parB-flag* fusion in the native locus. A DNA fragment containing *parB* was removed from pBCBHV004 by HindIII/EagI digestion and ligated into equally digested pBCBSS270.
pBCB33	Ampicillin	Erythromycin	pMAD containing up- and downstream regions of the *spa* gene and Cadmium inducible promoter P_{cad}. Primers P1_pBCB33 and P2_pBCB33 were used to amplify the P_{cad} promoter from pCN51, which was cloned into pBCB13 via EcoRI & NheI.
pBCBSS311	Ampicillin	Erythromycin	pMAD derivative, to replace spa by a *laci-mScI* and *tetR-mNG* fusions under a cadmium inducible promoter. Four DNA fragments were amplified: *lacI* from pLAU53 (oligos GA_st7lacIcfp-st7tetRyfp_1/ GA_lacI_rev), mScarletI from psav-mSc-I (oligos GA_mScarletI_fwd/ GA_mScarletI_rev), *tetR* from pLAU53 (oligos GA_tetR_fwd/ GA_tetR_rev), and mNeonGreen from pBCBSS135 (oligos GA_mNG_fwd/ GA_mNG_rev). The fragments were cloned into XhoI/SmaI-digested pBCB33 using Gibson Assembly.
pBCBAIM018	Ampicillin	Erythromycin	pMAD derivative, to replace *spa* by a *tetR-mNG* fusion under a cadmium inducible promoter. pBCBSS311 was amplified by PCR with oligos OBCBAIM082/ OBCBAIM083 to remove the *lacI-scI* module. Amplification product was treated with DpnI and directly used for transformation.
pBCBMS073	Ampicillin	Erythromycin	pMAD derivative to delete *parB*. Two fragments were amplified: A (oligos mNG-parB P1 fw/ d-parB P2 rv) and B (oligos d-parB P3 fw/ parB-fusion P4 rv). Fragments were combined by extension PCR into a single fragment, which was cloned, using Gibson Assembly, into pMAD previously digested with EcoRI and NcoI.
pBCBMS086	Ampicillin	Erythromycin	pMAD derivative to delete *scpA* and *scpB*. Two fragments were amplified: A (oligos d-scpAscpB P1 fw/ d-scpAscpB P2 rv) and B (oligos d-scpAscpB P3 fw/ d-scpAscpB P4 fw). Fragments were combined by extension PCR into a single fragment, which was cloned, using Gibson Assembly, into pMAD previously digested with EcoRI and NcoI.
pBCBDB013	Ampicillin	Erythromycin	pMAD derivative to introduce an array of STOP codons near the start of the *smc* gene. Two fragments were amplified: A (oligos GA_Smc-up_1/ smc+24_stop-BglII_rev) and B (oligos smc+24_stop-BglII_fwd/ GA_Smc-N_6). Fragments were combined by extension PCR into a single fragment, and cloned into pMAD via EcoRI and BamHI.

pBCBDB014	Ampicillin	Erythromycin	pBCB43 derivative containing *3xflag-smc* under P*xyl-tetO* control. *3xflag-smc* was amplified from JE2_3xFLAG-Smc genomic DNA using the oligos smcRBS_SmaI_fwd2/ smc_NheI_rev2, and cloned into pBCB43 via SmaI and NheI.
pBCBSS279	Ampicillin	Erythromycin	pMAD derivative, intermediate plasmid to introduce operator arrays near the Ter region. Two DNA fragments were amplified: SAUSA300_1326 (oligos 1326_Bam_fwd/ 1326_MCS_rev) and SAUSA300_1327 (oligos 1327_MCS_fwd/ 1327_PvuII_rev). The fragments were digested with BamHI/SalI and SalI/PvuII, respectively, and ligated into BamHI/SmaI-digested pMAD.
pBCBSS281	Ampicillin	Erythromycin	pMAD derivative, to replace native *smc* with *3xflag-smc*. Two fragments were amplified using the oligos GA_Smc-up_1/GA_flag-Smc-up_2 and GA_flag-Smc_3/GA_Smc-N_6. Fragments were combined by extension PCR into a single fragment, and cloned into pMAD via EcoRI and BamHI.
pBCBSS283	Ampicillin	Erythromycin	pMAD derivative to introduce a *lacO* array near the Ter region. A DNA fragment containing the $(lacO)_{48}$ array was removed from pLAU23 by NheI/SalI digestion and ligated into equally digested pBCBSS279.
pBCBSS285	Ampicillin	Erythromycin	pMAD derivative to introduce a *tetO* array near the Ter region. A DNA fragment containing the $(tetO)_{48}$ array was removed from pLAU29 by NheI/SalI digestion and ligated into equally digested pBCBSS279.
pBCBSS288	Ampicillin	Erythromycin	pMAD derivative, intermediate plasmid to introduce operator arrays near the Ori region. Two DNA fragments were amplified: SAUSA300_2631 (oligos 2631_Bam_fwd/ 2631_MCS_rev) and SAUSA300_2632 (oligos 2632_MCS_fwd/ 2632_PvuII_rev). The fragments were digested with BamHI/SalI and SalI/PvuII, respectively, and ligated into BamHI/SmaI-digested pMAD.
pBCBSS289	Ampicillin	Erythromycin	pMAD derivative to introduce a *tetO* array near the Ori region. A DNA fragment containing the $(tetO)_{48}$ array was removed from pLAU29 by NheI/SalI digestion and ligated into equally digested pBCBSS288.
pBCBSS290	Ampicillin	Erythromycin	pMAD derivative to introduce a *lacO* array near the Ori region. A DNA fragment containing the $(lacO)_{48}$ array was removed from pLAU23 by NheI/SalI digestion and ligated into equally digested pBCBSS288.
pBCBSS292	Ampicillin	Erythromycin	pMAD derivative to replace *spa* by a *laci-ecfp* and *tetR-eyfp* fusions under a cadmium inducible promoter. Two DNA fragments were amplified from pLAU53: lacIeCFP (oligos GA_st7lacIcfp-st7tetRyfp_1/ GA_st7lacIcfp-st7tetRyfp_2) and tetReYFP (oligos GA_st7lacIcfp-st7tetRyfp_3/ GA_st7lacIcfp-st7tetRyfp_4). The fragments were cloned into XhoI/SmaI-digested pBCB33 using Gibson Assembly.
pBCBSS313	Ampicillin	Erythromycin	pMAD derivative, intermediate plasmid to introduce operator arrays in the left arm of the chromosome. Two DNA fragments were amplified: SAUSA300_1984 (oligos 1984_Bam_fwd/ 1984_MCS_rev) and SAUSA300_1985 (oligos 1985_MCS_fwd/ 1985_PvuII_rev). The fragments were digested with BamHI/SalI and SalI/PvuII, respectively, and ligated into BamHI/SmaI-digested pMAD.

pBCBSS314	Ampicillin	Erythromycin	pMAD derivative, intermediate plasmid to introduce operator arrays in the right arm of the chromosome. Two DNA fragments were amplified: SAUSA300_0660 (oligos 0660_Bam_fwd/ 0660_MCS_rev) and SAUSA300_0661 (oligos 0661_MCS_fwd/ 0661_PvuII_rev). The fragments were digested with BamHI/SalI and SalI/PvuII, respectively, and ligated into BamHI/SmaI-digested pMAD.
pBCBSS315	Ampicillin	Erythromycin	pMAD derivative to introduce a *tetO* array in the left arm of the chromosome. A DNA fragment containing the $(tetO)_{48}$ array was removed from pLAU29 by NheI/SalI digestion and ligated into SpeI/SalI-digested pBCBSS313.
pBCBSS317	Ampicillin	Erythromycin	pMAD derivative to introduce a *tetO* array in the right arm of the chromosome. A DNA fragment containing the $(tetO)_{48}$ array was removed from pLAU29 by NheI/SalI digestion and ligated into SpeI/SalI-digested pBCBSS314.
pBCBSS319	Ampicillin	Erythromycin	pMAD derivative to mutate the *parS* site at +3° (*parS*1). Two DNA fragments were amplified: A (oligos parS1-1_up_Bam_fwd/ parS1-1_up_EFHVK_rev) and B (oligos parS1-1_down_EFHVK_fwd/ parS1-1_down_Eco_rev). The fragments were combined by extension PCR into a single fragment, digested with BamHI/EcoRI and ligated into equally digested pMAD.
pBCBSS320	Ampicillin	Erythromycin	pMAD derivative to delete the *parS* site at +35° (*parS*3). Two DNA fragments were amplified: A (oligos parS1-2_up_Bam_fwd/ parS1-2_up_BglII_rev) and B (oligos parS1-2_down_BglII_fwd/ parS1-2_down_Sma_rev). The fragments were digested with BamHI/BglII and BglII/SmaI, respectively, and ligated into BamHI/SmaI-digested pMAD.
pBCBSS321	Ampicillin	Erythromycin	pMAD derivative to delete the *parS* site at -12° (*parS*4). Two DNA fragments were amplified: A (oligos parS1-4_up_Bam_fwd/ parS1-4_up_BglII_rev) and B (oligos parS1-4_down_BglII_fwd/ parS1-4_down_Eco_rev). The fragments were digested with BamHI/BglII and BglII/EcoRI, respectively, and ligated into BamHI/EcoRI-digested pMAD.
pBCBSS322	Ampicillin	Erythromycin	pMAD derivative to delete the *parS* site at -1° (*parS*5). Two DNA fragments were amplified: A (oligos parS1-3_up_Bam_fwd/ parS1-3_up_BglII_rev) and B (oligos parS1-3_down_BglII_fwd/ parS1-3_down_Eco_rev). The fragments were digested with BamHI/BglII and BglII/EcoRI, respectively, and ligated into BamHI/EcoRI-digested pMAD.
pBCBSS327	Chloramphenicol	Chloramphenicol	pEPSA5 derivative containing *scpAB* under PT5X control. *scpAB* was amplified using the oligos scpA_-27_Eco_fwd and scpB_Kpn_rev, and cloned into pEPSA5 via EcoRI and KpnI.
pBCBSS379	Ampicillin	Erythromycin	pMAD derivative to mutate the *parS* site at +6° (*parS*2). Two DNA fragments were amplified: A (oligos parS+6_up_Sma_rev/ parS+6_up_RFTLN_fwd) and B (oligos parS+6_down_RFTLN_rev/ parS+6_down_Bam_fwd). The fragments were combined by extension PCR into a single fragment, digested with SmaI/EcoRI and ligated into equally digested pMAD.

Except if stated otherwise, JE2 genomic DNA was used as a template for PCR reactions

Supplementary Table 3: oligonucleotides used in the study.

Name	Sequence (5'->3')
OBCBAIM003	CTAGACAGATCTATCGATGCATGCCATGGCAGCGACTGACTCACACCGC
OBCBAIM004	CCTCCACTTCCACCGTAAGTTCTGATTGGTAAAATTAATTGCGTTACCG
OBCBAIM005	ATCAGAACTTACGGTGGAAGTGGAGGTGGAAGT
OBCBAIM006	ATATTTATTTTTATTAACCACTGATTTCTAAAGTAGATAACC
OBCBAIM007	ATCAGTGGTTAATAAAAATAAATATAAATAAAGGATGACGTGATTAATT
OBCBAIM008	CAGCCTCGCGTCGGGCGATATCGGATCCGAAAGAACCAGCATCTTCAGG
OBCBAIM082	CGGGAAAAAATAAGGAGGAAAAAAAATGGTGTCTAGA
OBCBAIM083	CCATTTTTTTTCCTCCTTATTTTTTCCCGGG
parB-fusion P1 fw	CGATGCATGCCATGGTACCCAAAATTGCTGGTCTAAAATACG
parB-fusion P4 rv	CTTCTAGAATTCGAGCTCCCTGTACCACCGTTATTTATCTTAAC
mNG-parB P1 fw	CGATGCATGCCATGGTACCCATAAAAAGGACGAAAGCTTATG
d-parB P2 rv	CTGTTGTTATCAAATAAAAAGTGAT
d-parB P3 fw	TATCAAATAAAAAGTGATTTACACAATTTTATATAATAACTCTTTGTG
parB-fusion P4 rv	GTTAAGATAAATAACGGTGGTACAGGGAGCTCGAATTCTAGAAG
d-scpAscpB P1 fw	ATGCATGCCATGGTACCCTGGCGATTACGACCTTCTGTAATTG
d-scpAscpB P2 rv	CATTATTTTCTCCTTTTTGATTGACAATATCTACCTCGTATTG
d-scpAscpB P3 fw	AATATCTACCTCGTATTGCGTC
d-scpAscpB P4 fw	GAAAACATAGACGTAATTAATCGGGAGCTCGAATTCTAGA
GA_Smc-up_1	GTATCGATAAGCTTGATATCGAATTCGTCTTGAAAAATAATAAATCTTACATC
smc+24_stop-BglII_rev	CCAATGGCAGATCTTTATCACTATTAATCTATTGATTTTAAATAAACC
smc+24_stop-BglII_fwd	TAATAGTGATAAAGATCTGCCATTGGATTTAAGTCTTTTGC
GA_Smc-N_6	GCGGCCGCTCTAGAACTAGTGGATCCTTTGTTTACTTTTCAGAGACTTATAAG
1326_Bam_fwd	ATATGGATCCTCGTCGTCCCACCCCAACTTGCATTGTCTGT
1326_MCS_rev	ATATGTCGACACCCGGGAGCTAGCACTCGAGCGATTGACATCACATCAGTCGGTGCTCCT
1327_MCS_fwd	ATATCTCGAGTGCTAGCTCCCGGGTGTCGACGTGCTCCTCTATTTATCAAAGAAACAAATTA
1327_PvuII_rev	ATATCAGCTGAGAGATATTAATAATGCGCATACATTACAGCA
2631_Bam_fwd	ATATGGATCCATGAATATTGCGAAGTTAGAGAATTATTTAC
2631_MCS_rev	ATATGTCGACACCCGGGAGCTAGCACTCGAGAAGCATAAAAAAGGGGCGCTACCTACAATAAG
2632_MCS_fwd	ATATCTCGAGTGCTAGCTCCCGGGTGTCGACCATAAACACAACAAAAAGGATATGACACAAACTTC
2632_PvuII_rev	ATATCAGCTGAACATGTTGCACTGATAATATCGTCATAGTC
GA_st7lacIcfp-st7tetRyfp_1	CAATGTCTGAACCTGCACCCGGGAAAAAATAAGGAGGAAAAAAAATGGTGGTGAATGTGAAACC
GA_st7lacIcfp-st7tetRyfp_2	TTATCTAGACTTGTACAGCTCG
GA_st7lacIcfp-st7tetRyfp_3	AGCTGTACAAGTCTAGATAATGAATAGCTAAGGTAATAAAAAATAAGGAGGAAAAAAAATGGTGTCTAGATTAGATAAAAGTAAAGTG
GA_st7lacIcfp-st7tetRyfp_4	ATTAATGCAGCGCTAGCTACTCGAGGGTACCCGGCCGTCTCATCCGCCAAAACAGCC

1984_Bam_fwd	ATATGGATCCCATCATTGCTAACTGTTATT
1984_MCS_rev	ATATGTCGACACCCGGGAACTAGTACTCGAGCGTGTGTGATTCGTTTTTTT
1985_MCS_fwd	ATATCTCGAGTACTAGTTCCCGGGTGTCGACGATTATCGTCGCTGTGATTCG
1985_PvuII_rev	ATATCAGCTGCCAGATCCAGATAAACCAAAGCCA
0660_Bam_fwd	ATATGGATCCTCCAGGATACGCTTCAACAC
0660_MCS_rev	ATATGTCGACACCCGGGAACTAGTACTCGAGCTAAATTACAGTTACCGAA
0661_MCS_fwd	ATATCTCGAGTACTAGTTCCCGGGTGTCGACTGATTTTATCATTAACAGTAC
0661_PvuII_rev	ATATCAGCTGCGTTTAATATGATATGATTGACCT
parS1-1_up_Bam_fwd	ATATGGATCCGCAACAGACCGCCGTGGAC
parS1-1_up_EFHVK_rev	GTGGATTTTGCTTTACATGAAATTCGACACGTTTATCGCCTCT
parS1-1_down_EFHVK_fwd	AATTTCATGTAAAGCAAAATCCACTTTATAATCGAATG
parS1-1_down_Eco_rev	ATATGAATTCTTGAAATTGAAATGATTTGGTAC
parS1-2_up_Bam_fwd	ATATGGATCCGTTATTAGCTAAAGATGGTTATAC
parS1-2_up_BglII_rev	ATATAGATCTAATAGTGACAATTAGATTTATATAAAATG
parS1-2_down_BglII_fwd	ATATAGATCTCTTTTTTAACTCCAAAAAGTATTCCTATTCCACTC
parS1-2_down_Sma_rev	ATATCCCGGGGGATTATGGGTCATGGCAGCA
parS1-3_up_Bam_fwd	ATATGGATCCGGATGATGAAGAGACGGCTGTTG
parS1-3_up_BglII_rev	ATATAGATCTAAAAAAAAGACAAAGCTGTTATGATCTTAGC
parS1-3_down_BglII_fwd	ATATAGATCTTAAAAATTTATATTTATATGTTGATCAGG
parS1-3_down_Eco_rev	ATATGAATTCGACAATTGCTCCAGTACTAAG
parS1-4_up_Bam_fwd	ATATGGATCCATGCTAACATGGCATATGGTCAT
parS1-4_up_BglII_rev	ATATAGATCTTTTTTGTGTATCAGCAAATTGCGCC
parS1-4_down_BglII_fwd	ATATAGATCTTAGATTTAGCTTATAGTTTTATCATC
parS1-4_down_Eco_rev	ATATGAATTCGATGTGTTGAAACTGAGTTCAATT
parS+6_up_Sma_rev	ATATCCCGGGCGATAGCCATGTCCTTTGGCATA
parS+6_up_RFTLN_fwd	AGATTTACACTTAATATATTGTACCCCTATATTGAAAC
parS+6_down_RFTLN_rev	GGGTACAATATATTAAGTGTAAATCTTTTTTGAATAATATTTTGAATGATGTG
parS+6_down_Bam_fwd	ATATGGATCCACCCTATCAAGTTCGTAACATTATC
GA_lacI_rev	ACCAGAACCTTGACCAGATCCTGGTCCTTGTCCTGATCCTTCCAAGCCCAGCTGCATTAATGAATC

GA_mScarletI_fwd	TTGGAAGGATCAGGACAAGGACCAGGATCTGGTCAAGGTTCTGGTGTGAGCAAGGGCGAGGCAGTG
GA_mScarletI_rev	TTTTTTTCCTCCTTATTTTTTATTACCTTAGCTATTCATTACTTGTACAGCTCGTCCATGCC
GA_tetR_fwd	TGAATAGCTAAGGTAATAAAAAATAAGGAGGAAAAAAAATGGTGTCTAGATTAGATAAAAGTAAAG
GA_tetR_rev	AGAGCCACCTCCGCCAGAACCGCCTCCACCGATGTCAGACCCACTTTCAC
GA_mNG_fwd	GGTGGAGGCGGTTCTGGCGGAGGTGGCTCTATAATTAAAGTATCAAAAGGTGAAGAAG
GA_mNG_rev	ATTAATGCAGCGCTAGCTACTCGAGTTATTTGTATAACTCATCCATGCC
P1_pBCB33	GCCGAATTCGCATGCGCACTTATTCAAGTG
P2_pBCB33	GCCGCTAGCTACTCGAGTACCCGGGTGCAGGTTCAGACATTGAC
3xFLAG_oligo1	GGCCGTCCTGCGGCGCCTCCGATTACAAAGATGATGATGACAAAGATTATAAAGATGACGACGATAAAGACTACAAGGATGATGACGATAAATAAG
3xFLAG_oligo2	GATCCTTATTTATCGTCATCATCCTTGTAGTCTTTATCGTCGTCATCTTTATAATCTTTGTCATCATCATCTTTGTAATCGGAGGCGCCGCAGGAC
GA_flag-Smc-up_2	CCTCCACCTTTATCGTCATCATCCTTGTAGTCTTTATCGTCGTCATCTTTATAATCTTTGTCATCATCATCTTTGTAATCCATCCTAAACTCCTTATCAC
GA_flag-Smc_3	GATGATGACGATAAAGGTGGAGGCGGTTCTGGCGGAGGTGGCTCTGTTTATTTAAAATCAATAGATGCCATTGG
scpA_-27_Eco_fwd	ATATGAATTCCATATGACGCAATACGAGGTAGATATT
scpB_Kpn_rev	ATATGGTACCTTAGTCATTATTTTCTCCTTTTTGATTGAC
smcRBS_SmaI_fwd2	AGCTCTCTATCATTGATAGAGTCCCGGGCTAAAATATCGTATACATGTGTTCTTTAAAATTG
smc_NheI_rev2	ACATACAGGGGGTATTAATGCAGCGCTAGCTTATTGCTCCTCCTTCAACAC

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