# Transcription rate and transcript length drive formation of chromosomal interaction domain boundaries

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## APPENDIX MATERIALS AND METHODS

### Strains, media, and growth conditions

*E. coli* and *C. crescentus* were grown in LB and PYE, respectively. When appropriate, media were supplemented with antibiotics at the following concentrations (liquid/solid media for *C. crescentus*; liquid/solid media for *E. coli* [μg/ml]): chloramphenicol (1/2; 20/30), kanamycin (5/25; 30/50), oxytetracycline (1/2; 12/12), spectinomycin (25/100; 50/50 and gentamycin (0.5/5; 15/20).

Synchronizations were performed on mid-exponential phase cells using Percoll (GE Healthcare) and density gradient centrifugation. Briefly, 250 ml cultures of the wild-type CB15N or its derivatives were grown in PYE at 30°C to an $OD_{600}$ of ~0.4 and pelleted via centrifugation. Cells were resuspended in 6 ml of 1x M2 salts (6.1 mM $Na_2HPO_4$, 3.9 mM $KH_2PO_4$, 9.3 mM $NH_4Cl$, 0.5 mM $MgSO_4$, 10 μM $FeSO_4$, 0.5 mM $CaCl_2$) via pipetting and 6 ml of ice-cold Percoll (Sigma) was added to the resuspension. The resulting mixture was transferred to a 15 ml Falcon tube that was subsequently centrifuged for 20 min (10000 *g*) at 4 °C. Swarmer cells formed a discrete band near the bottom of the tube. This band was removed via pipetting and the cells within this band were washed three times with 1 ml of ice-cold 1x M2 salts.

After synchronization, swarmer cells were released into PYE+1% formadehyde for fixation for chromosome conformation capture and Hi-C analysis. For antibiotic treatment, swarmer cells were incubated with 50 μg/ml chloramphenicol (final concentration) for 30 minutes before fixing with formadehyde. For starvation experiments, swarmer cells were released into an 1xM2 salts solution for 90 min before fixation for Hi-C.

For formadehyde fixation of elongated cells, strains ML2000 were grown to $OD_{600}$=0.4 in the presence of IPTG before the cells were collected by centrifugation and washed of residual IPTG twice with fresh PYE. Cells were then resuspended in fresh PYE (IPTG omitted) to deplete DnaA for 90 min before synchronization, thereby inducing cell elongation. Cells were formadehyde-fixed at 0 hr post synchronization (90 min after IPTG withdrawal), 1 hr post synchronization (150 min after IPTG withdrawal), 2 hr post synchronization (210 min after IPTG withdrawal) and 3 hr post synchronization (270 min after IPTG withdrawal).

The same general fixation procedure was used for CtrA(D51E)Δ3Ω overexpression strains (ML1675). Cells were grown to $OD_{600}$=0.4 in the presence of chloramphenicol and glucose before the cells were collected by centrifugation and washed of residual glucose twice with fresh

PYE and synchronized. Cells were then resuspended in PYE plus xylose (0.3% final concentration) to induce the production of CtrA(D51E)Δ3Ω. Cells were collected and formadehyde-fixed at 60, 120 and 180 min after the addition of xylose.

**Chromosome conformation capture with deep sequencing (Hi-C)**

Hi-C experiments were performed exactly as described previously (Le *et al*, 2013). Restriction enzymes (BglII or NcoI) used for Hi-C are listed for each sample in Appendix Table S3. Cells at $OD_{600}$ of 0.2 were fixed with 1% formadehyde for 30 minutes before quenching by 0.125 M glycine. Fixed cells were washed twice in 1xM2 buffer before being resuspended in 1xTE buffer (10 mM Tris-HCl pH8 and 1 mM EDTA) and subjected to the Hi-C procedure (Le *et al*, 2013).

**Generation of Hi-C contacts maps and directional preference plots**

Each end of paired-end sequencing reads were mapped independently to the genome of *Caulobacter crescentus* NA1000 using Bowtie 2.1.0 and an algorithm used that iteratively increases truncation length to maximize the yield of valid Hi-C interactions (Imakaev *et al*, 2012). The *Caulobacter* NA1000 genome was then divided into restriction fragments (700 BglII fragments and 2025 NcoI fragments). Each read of a read pair was sorted into its corresponding restriction fragment. Read pairs were classified as valid Hi-C products, non-ligation, or self-ligation products (Imakaev *et al*, 2012). Only valid Hi-C products that are uniquely aligned to the *Caulobacter* genome were employed to generate Hi-C contact maps (Appendix Table S3). To create interaction matrices, the *Caulobacter* genome was first divided into 405 10-kb bins. We then assigned valid Hi-C products to the 10-kb bins and normalised using an iterative correction procedure as described previously (Imakaev *et al*, 2012, Le *et al*, 2013). Subsequent analysis and visualization of the contact maps was done using Python and R scripts.

Independent Hi-C experiments were reproducible, as judged by a comparison of biological replicates ($r$=0.98, $p<10^{-15}$) (Appendix Fig S1B-C and Fig EV3A). To calculate Pearson correlation coefficients between Hi-C experiments, two-dimensional matrices representing Hi-C contact maps were decomposed to one-dimensional vectors row-by-row. R was then used to compute the Pearson correlation coefficient between vectors.

To quantify the degree of directional preference for a given 10-kb bin, we extracted the vector of interactions between that bin and bins at regular 10-kb intervals, either to the left or right, up to 100 kb. We then compared $\log_2$(Hi-C scores) of the two vectors by a paired t-test to assess whether the strength of interactions were significantly stronger in one direction compared to the

other (Le *et al*, 2013). The more significant the difference, the higher the absolute t-value. Negative t-values indicated a bin interacts more with bins to the left than to the right and vice versa. A p-value of 0.05 (dashed horizontal line) was used as a threshold to assess statistical significance. Directional preferences for each bin along the chromosome were then represented as a bar plot with positive and negative t-values shown as red and green bars, respectively. At CID boundaries the directional preference of bins changed most abruptly from consecutive negative values to consecutive positive values with an absolute change > 4. Bins near the middle of a CID also changed their preferred direction but did so more gradually than those at the boundaries and from positive to negative (Le *et al*, 2013).

**RT-PCR**

*C. crescentus* strains were harvested after reaching $OD_{600}$ ~0.2-0.4 and RNA extracted using RNeasy Mini Kit (Qiagen). 1 µg of total RNA was reverse transcribed into cDNA using Superscript-II (Invitrogen). RNA template was degraded with RNAseH (NEB), and nucleotides removed using the QIAquick PCR purification kit (Qiagen). PCR was then used to test for the presence or absence of DNA products. The presence or absence of PCR products was used to determine the extent of *rsaA* transcription in *Caulobacter* strains where transcription terminators were inserted at various positions on *rsaA* coding sequence. Product number 1 is 112 bp long and starts 154 bp from *rsaA* transcription start site. Product number 2 is 118 bp long and starts 769 bp from *rsaA* transcription start site. Product number 3 is 99 bp long and starts 1691 bp from *rsaA* transcription start site. Product number 4 is 115 bp long and starts 2257 bp from *rsaA* transcription start site. Product number 5 is a control and is within the *ruvA* gene (Le *et al*, 2013). Primers for these PCR products are listed in Appendix Table S2. A sample without added reverse transcriptase served as a control for genomic DNA contamination.

**RNA-Seq**

Exponentially-growing *Caulobacter* cells were collected by centrifugation, released into 1xM2 salts for 90 minutes before being pelleted and snap-frozen in liquid nitrogen in preparation for RNA extraction. 400 µl of 65$^{o}$C-preheated Trizol (Thermoscientific) was added to the cell pellet and mixed at 200 rpm for 10 minutes on a thermomixer (Eppendorf). The Trizol mixture was then frozen at -80$^{o}$C for 30 minutes before being centrifuged at 14000 rpm for 5 minutes at 4$^{o}$C. Trizol supernatant was then aspirated and added directly to 400 µl of 100% ethanol. The Trizol/ethanol mixture was then applied to an RNA-extraction spin column (Zymo Research). Subsequently, the column was spun at 10000 rpm for 30 s and the flow-through discarded. The

spin column was then washed with 400 µl of RNA Prewash solution twice and finally with 700 µl of RNA Wash buffer. Residual RNA Wash buffer was removed by an additional centrifugation at 13000 rpm for 1 minute. RNA was eluted out with 90 µl of DEPC-treated water. Contaminated genomic DNA was removed from this RNA prep by DNaseI treatment. Briefly, 10 µl of 10x Turbo DNaseI buffer and 2 µl of DNaseI enzyme (Ambion) was added to the 90 µl RNA prep. The reaction was incubated for 20 minutes at 37 $^{o}$C. This DNaseI digestion step was repeated one additional time to ensure all genomic DNA was digested and removed. RNA was then purified and concentrated using acidic phenol-chloroform extraction. The integrity of the RNA was checked via agarose gel and by Bioanalyzer before being submitted for Illumina library prep and deep-sequencing (BioMicroCenter, MIT).

For analysis of RNA-seq data, Hiseq 2500 Illumina short reads (40 bp) were mapped back to the *Caulobacter* NA1000 reference genome (NCBI Reference Sequence: NC-011916.1) using Bowtie 1 using the following command:

bowtie -m 1 -n 1 --best --strata -p 4 --chunkmbs 512 NA1000-2014-bowtie --sam *.fastq > output.sam

The sequencing coverage was computed using BEDTools (Quinlan & Hall, 2010). The general feature format (gff) file for *Caulobacter* NA1000 was downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/Caulobacter-crescentus-NA1000-uid59307/). The normalized value of reads per kb per million mapped reads (RPKPM) was calculated for each gene by a custom R script to enable comparison of gene expression within and between RNA-seq datasets (Dataset EV2).

To identify genes that are potentially responsible for the differences in CID boundaries in exponentially-growing vs. starved cells (Dataset EV1-2), we considered highly-expressed genes with RPKPM values greater than 1000 and gene length > 1 kb. Highly-expressed genes < 1 kb in length were also considered if they are in an operon and the total length of the operon was > 1 kb or if there were multiple highly-expressed genes in a 10-kb bin near a given CID boundary and the total length of these genes was > 1 kb.

To construct a gene expression * length plot (Fig. 1B-C), we computed the product of gene expression (rpkpm) and the gene length (kb) for every genes on the *Caulobacter* chromosomes. We then summed up the rpkpm*length values of all genes that reside in each 10-kb bin along the chromosome (405 bins in total) to give the total rpkpm*length value for each bin.

**Strain and plasmid construction**

All strains and plasmids used are listed in Appendix Table S1. All primers used in strain and plasmid construction are listed in Appendix Table S2.

*pVMCS-4-478-dnaA:*

A 478 bp N-terminal fragment of *Caulobacter dnaA* was amplified by PCR using primer CA7 and CA12, gel extracted and digested with NdeI and XmaI before being ligated to the NdeI-XmaI-cut pVMCS4 (Thanbichler *et al*, 2007). Electro-competent *Caulobacter* cells were electroporated with pVMCS-4-478-*dnaA* and selected on gentamycin + vanillate plates to allow for a single integration that replaces the native *dnaA* promoter with a vanillate-inducible promoter.

*pJS14-tetracyclineR-empty:*

PCR was used to amplify a tetracycline resistance cassette *(tetAR*) from pMCS5 (Thanbichler *et al*, 2007) using primers tetARmodJS14F and tetARmodJS14R. The plasmid backbone was amplified by PCR using primers JS14chlRinternaltailF and JS14chlRinternaltailR with pJS14 serving as a template. The two PCR fragments were DpnI-treated to remove template plasmid, gel-purified and assembled together using a Gibson 2x master mix (NEB). Briefly, 2.5 µl of each DNA fragment at equimolar concentration was added to 5 µl Gibson master mix (NEB), and the mixture was incubated at 50°C for 60 minutes. 5 µl was used to transform chemically-competent *E. coli* DH5α cells. Gibson assembly was driven by a 23 bp sequence shared between the two PCR fragments. This homology was incorporated during primer design.

*pJS14-tetracyclineR-$P_{xyl}$-ctrAD51EΔ3Ω:*

The same procedure and primers were used as above, except that pJS14-$P_{xyl}$-*ctrAD51EΔ3*Ω (Domian *et al*, 1997) was used as template for PCR to generate the plasmid backbone.

*pMT553-$P_{hup1}$-hup1-gfp:*

An HU-encoding gene (*CC2331*) together with its promoter was amplified by PCR using primers L553-P-HU-GFP-1 and L553-P-HU-GFP-2. A gene encoding GFP was amplified from pXGFPC-2 (Thanbichler *et al*, 2007) by PCR using primers L553-P-HU-GFP-3 and L553-P-HU-GFP-4. The two PCR fragments were gel purified and assembled together and to an NdeI-NheI-cut pMT553 (Thanbichler *et al*, 2007) by Gibson assembly. Briefly, 1.6 µl of each fragment at

equimolar concentration was added to 5 µl Gibson master mix and the mixture was incubated at 50°C for 60 minutes before 5 µl was used to transform competent *E. coli* DH5α cells. Gibson assembly was possible due to a 23 bp region shared among the two PCR fragments and the NdeI-NheI-cut pMT553 backbone (Thanbichler *et al*, 2007). This homology was incorporated during primer design. The resulting plasmid was sequence verified by Sanger sequencing (Genewiz). *Caulobacter* cells were electroporated with pMT553-$P_{hup1}$-*hup1-gfp* to allow for a single integration of $P_{hup1}$-*hup1-gfp* at the van locus.

*pMT619::parS$^{pMT1}$:*

For insertion of *parS$^{pMT1}$* at + 400kb in the *Caulobacter* genome, primers label400-NdeI-F and label400-SacI-R were used to amplified a ~500 bp fragment by PCR, using *Caulobacter* genomic DNA as template. This fragment was 5' phosphorylated by T4 PNK (NEB) before being blunt-end ligated to a SmaI-cut pUC19 (Fermentas). The resulting construct was sequence verified. The NdeI-SacI-ended fragment was then liberated from the pUC19-based plasmid by NdeI and SacI double digestion before being cloned into the same sites on pMT619::*parS$^{pMT1}$* (Badrinarayanan *et al*, 2015). The constructions of pMT619::*parS$^{pMT1}$* for insertion at +800kb, +1400kb, and +1800kb were carried out essentially as above, except the pairs of primers used were: label800-NdeI-F and label800-SacI-R; label1400-NdeI-F and label1400-SacI-R; and label1800-NdeI-F and label1800-SacI-R, respectively. Electro-competent *Caulobacter* cells were electroporated with these plasmids to allow for a single integration at the site of interest. The correct integration was verified by PCR using a primer specific to the *parS$^{pMT1}$* site and another primer upstream of the ~500 bp homologous region used to drive integration.

*pMT632::parS$^{P1}$:*

To insert *parS$^{P1}$* sites at +600 kb and +1600 kb in the *Caulobacter* genome, the same procedure as above was used, except that the pairs of primers used were: label600-NdeI-F and label600-SacI-R; and label1600-NdeI-F and label1600-SacI-R, respectively. Also, the NdeI-SacI-ended fragment was then liberated from the pUC19-based plasmid by NdeI and SacI double digestion before being cloned into the same sites on pMT632::*parS$^{P1}$* (Badrinarayanan *et al*, 2015) instead of pMT619::*parS$^{pMT1}$*. *Caulobacter* cells were electroporated with these plasmids to allow for a single integration at the site of interest. The correct integration was verified by PCR using a primer within the *parS$^{P1}$* site and another upstream of the ~500 bp homologous region used to drive insertion.

*pUC::$P_{rsaA}$-full-length-rsaA and its derivatives:*

The coding sequence of *rsaA* and 264 bp upstream encompassing its core promoter were amplified by PCR using primers rsaA-NdeI-F and rsaA-NheI-R and cloned into the SmaI site of pUC19 (Fermentas). The resulting construct was verified by Sanger sequencing (Genewiz). This construct served as the starting construct for insertion of transcription terminators at various positions in *rsaA*.

For the construction of a cassette containing 2 x TAA stop codon and 2 x *rrnBT1* transcription terminators, primers rrnBT1-100F and rrnBT1-100R were used to amplify the *rrnBT1* sequence from pVMCS-2 (Thanbichler *et al*, 2007). The resulting PCR fragment was 5'-phosphorylated by T4 PNK (NEB) and cloned into SmaI-cut pUC19 to give pUC19-rrnBT1 plasmid. Two pairs of primers (Gib-rrnB-part1F and Gib-rrnB-part1R; Gib-rrnB-part2F and Gib-rrnB-part2R) were used in a PCR to generate two *rrnBT1*-containg fragments (fragment 1 and fragment 2), using pUC19-rrnBT1 as a template. Fragment 1 and 2 share 23 bp identity that allows for a Gibson assembly to result in a cassette containing two transcription terminators (fragment 3). Primer pair pUCTleftrrnB-d-TAA and pUCTrightrrnB was used in a PCR to attach a double stop codons TAA to fragment 3, resulting in fragment 4. In summary, fragment 4 has 2 consecutive stop codons TAA, following by 2 identical *rrnBT1* transcription terminators that are separated by a 20-bp linker. The sequence of this 271 bp 2xTAA2xter is listed below:

TAATAAGGTCGACTCTGGAGGATGATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGC
CTTTCGTTTTATCTGTTGTTTGTCGCATTATACGCAAGGCGACAAGGTGCTGATGCCGCTG
GCGACTGTGGAAGAGCTCCTACGATAGCAAATAAAACGAAAGGCTCAGTCGAAAGACTGG
GCCTTTCGTTTTATCTGTTGTTTGTCGCATTATACGCAAGGCGACAAGGTGCTGATGCCGC
TGGCGAATCTCACTGGCCGTCGTTTTAC

To insert the 2xTAA2xter cassette at various positions within *rsaA*, Gibson assembly was used. For insertion 60 bp from the *rsaA* transcription start site, a pair of primers (rsaA-ter-0F and rsaA-2stopter-0R) was used to generate the plasmid backbone by PCR using pUC::$P_{rsaA}$-full-length-*rsaA* as a template. The PCR reaction was DpnI-treated to remove the plasmid template and the DNA fragment (~5.5 kb) was then gel-purified and assembled with the 2xTAA2xter cassette in a 2x Gibson master mix (NEB). Competent *E. coli* DH5α was transformed with 5 µl of the master mix and spread on LB + carbenicillin plates for selection. The resulting plasmid was verified by Sanger sequencing. For insertion 560 bp from the *rsaA* transcription start site the same procedure was used as before, except primers rsaA-ter-500F and rsaA-2stopter-500R were used to generate the plasmid backbone. For insertion 1060 bp from the *rsaA* transcription start site the same procedure was used as before, except primers rsaA-ter-1kF and rsaA-

2stopter-1kR were used to generate the plasmid backbone. For insertion 2060 bp from the *rsaA* transcription start site the same procedure was used as before, except primers rsaA-ter-2kF and rsaA-2stopter-2kR were used to generate the plasmid backbone.

For the construction of plasmid pUC::$P_{rsaA}$-*rsaA*-ATGtoTAA, a PCR-based mutagenesis procedure was used. A primer pair: rsaA-ATGmutTAAF and rsaA-ATGmutTAAR was used to PCR amplify the backbone of pUC::$P_{rsaA}$-full-length-*rsaA*. The resulting fragment was DpnI-treated, 5'-phosphorylated, and ligated together. Competent *E. coli* DH5α was transformed with 5 µl of the master mix and spread on LB + carbenicillin plates for selection. The resulting plasmid (pUC::$P_{rsaA}$-*rsaA*-ATGtoTAA) was verified by Sanger sequencing. For the construction of plasmid pUC::$P_{rsaA}$-*rsaA*-antitermination-ATGtoTAA, a native *Caulobacter* anti-termination sequence (Arnvig *et al*, 2008) was first amplified from *Caulobacter* genomic DNA using primers amplify-CC-AT-F and amplify-CC-AT-R. PCR was used to generate the plasmid backbone from pUC::$P_{rsaA}$-full-length-rsa*A* template using primers rsaA-insertAT-aroundcir-F and rsaA-insertAT-aroundcir-R. The two PCR fragments were gel purified and assembled together in a 2x Gibson master mix (NEB). The resulting plasmid was verified by Sanger sequencing.

*pMT675::$P_{rsaA}$* -full-length-*rsaA and its derivatives:*

For the construction of pMT675::$P_{rsaA}$-full-length-*rsa*A: a 3-fragment ligation was used to ligate together NdeI-NheI-cut pMT675, NheI-ended 2xTAA2xter cassette and NdeI-NheI-cut $P_{rsaA}$-full-length-*rsaA* fragment which had been liberated from pUC::$P_{rsaA}$-full-length-*rsaA* by NdeI and NheI double digestion.

To generate a series of plasmids: pMT675::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-60bp (with respect to transcription start site of *rsaA*); pMT675::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-560bp; pMT675::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-1060bp; pMT675::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-2060bp; pMT675::$P_{rsaA}$-*rsaA*-antiterminator-ATGtoTAA; pMT675::$P_{rsaA}$-*rsaA*-ATGtoTAA, NdeI/NheI fragments were dropped out of their corresponding pUC19-based plasmids by NdeI and NheI double digestion, then ligated to the NdeI-NheI-cut pMT675 (Thanbichler *et al*, 2007). The pMT675-based plasmid allows for a single integration that inserts these plasmids at the *van* locus.

For the construction of plasmid: pMT675::promoterless-*rsaA*, around-the-horn PCR was used to remove the promoter region of *rsaA* from plasmid pMT675::$P_{rsaA}$-full-length-*rsaA*. Briefly, a pair of primers (675cutrsAP-R and 675cutrsAP-F) was used to PCR amplify the plasmid pMT675::$P_{rsaA}$-full-length-*rsaA*. This PCR amplified *rsaA* and the whole plasmid pMT675 backbone without a 150 bp region that encompasses the core -10 and -35 elements and the

Shine Dalgarno site of *rsaA*. The PCR reaction was subsequently DpnI-treated to remove the plasmid template. The DNA fragment was then gel-purified, 5'-phosphorylated and self-ligated. The re-circularized plasmid was used to transform competent *E. coli* DH5α. The resulting plasmid was verified by Sanger sequencing.

For the construction of plasmid pMT533::$P_{rsaA}$-FLAG-full-length-*rsaA*, around-the-horn PCR was used to insert a sequence encoding the FLAG tag at the 5' end of *rsaA*. Briefly, a pair of primers, FLAG-rsaAF and FLAG-rsaAR, was used to PCR amplify the plasmid pUC::$P_{rsaA}$-full-length-*rsaA*. Parts of the FLAG tag were incorporated into the 5' end of the primers. The whole FLAG tag was reconstituted when the amplified plasmid was recircularized by T4 ligase. The resulting plasmid was verified by Sanger sequencing. FLAG-*rsaA* was then liberated from the pUC-based plasmid by double digestion with NdeI and NheI before ligating into NdeI-NheI-cut pMT533 (Thanbichler *et al*, 2007) to give pMT533::$P_{rsaA}$-FLAG-full-length-*rsaA*.

For the construction of plasmid: pMT553::$P_{rsaA}$-FLAG-*rsaA*-2xTAA2xter-at-560bp; pMT553::$P_{rsaA}$-FLAG-*rsaA*-2xTAA2xter-at-1060bp; pMT553::$P_{rsaA}$-FLAG-*rsaA*-2xTAA2xter-at-2060bp; the same procedure as described above for pMT533::$P_{rsaA}$-FLAG-full-length-*rsaA* was used, except that the DNA templates were: pUC::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-560bp; pUC::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-1060bp; pUC::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-2060bp, respectively.

*pMT687-Pc-γδtnpR$^{CCDAS4}$ and derivatives:*

Gibson assembly was used to assemble 3 individual DNA fragments together (fragments 1 to 3). Fragment 1 contains the γδ *tnpR* gene, fragment 2 contains the cumate-inducible promoter (Kaczmarczyk *et al*, 2013) and fragment 3 contains the kanamycin-resistant plasmid backbone which is derived from pMT687 (Thanbichler *et al*, 2007). To generate fragment 1: PCR was used to amplify *tnpR* from the genomic DNA of DH5α F' *E. coli* using primers deltagammaR-NdeIF and deltagammaR-NheIR. The resulting PCR product (fragment 1A) was gel-purified and used as a template in a subsequent PCR amplification using primers deltagammaR-NdeIF and deltagammaR-CCDAS4P1 to generate fragment 1B that contains half of the *Caulobacter crescentus* ClpXP-degron tag (DAS4). To generate the *tnpR* fragment with a complete DAS4 tag (fragment 1C), PCR was again used with primers deltagammaR-NdeIF and deltagammaR-CCDAS4P2 and fragment 1B as template. To generate fragment 2, primers cumR-p-1 and cumR-p-2 were used for PCR amplification with pQF5 (Kaczmarczyk *et al*, 2013) as template. To generate fragment 3, primers pMT687-cir-R and pMT687-cir-F were used for PCR amplification with pMT687 as template. The resulting PCR product was DpnI-treated to remove

the circular pMT687 template and subsequently gel-purified. Fragments 1C, 2 and 3 were assembled together using an NEB Gibson master mix.

For the construction of plasmid pMT687-Pc-*γδtnpR*$^{\Delta DBD}$, PCR-based point mutagenesis was used to convert codon 160 in *tnpR* to a TAA stop codon, thereby truncating the *tnpR* coding sequence just before the DNA-binding domain.

*pMT675::res-tetAR-res:*

Plasmid pMT675::*res-res* was first constructed with a NdeI-NheI cloning site in between two consecutive resolution (*res*) site. An NheI-ended *tetAR* fragment was then ligated to NheI-cut pMT675::*res-res*. The first resolution (*res*) site (112bp) (fragment 1) was amplified by PCR using primers GD-res-left-F and GD-res-left-R and genomic DNA of DH5α F' *E. coli* as template. The second resolution site (fragment 2) was amplified by PCR using primers GD-res-right-F and GD-res-right-R and genomic DNA of DH5α F' *E. coli* as template. The plasmid backbone (fragment 3) was amplified by PCR using primers around675R and around675F and plasmid pMT675 as template. The PCR mix was DpnI-treated to remove the circular pMT675 template and fragment 3 was subsequently gel-purified. Fragment 1, 2 and 3 were assembled together using Gibson assembly driven by a ~23 bp identity shared among the 3 fragments. The resulting plasmid pMT675::*res-res* was sequenced verified before being digested by NheI. To generate NheI-ended tetAR fragment, PCR was used to amplify *tetAR* from pKO3 (George Church, Harvard), using primers TetAR-NheIF and TetAR-NheIR. The resulting PCR product was NheI digested, gel purified and subsequently ligated to NheI-cut pMT675::*res-res* to result in pMT675::*res-tetAR-res*.

*pMT675::res-P$_{rsaA}$-full-length-rsaA-ter-tetAR-res and derivatives:*

A 3-fragment ligation was used to ligate together NdeI-NheI-cut pMT675::*res-res*, NheI-cut *tetAR* and NdeI-NheI-cut P$_{rsaA}$-full-length-*rsaA* fragment which had been liberated from pUC::P$_{rsaA}$-full-length-*rsaA* by NdeI and NheI double digestion. The resulting plasmid with a correct orientation of the *tetAR* cassette was screened for using PCR. For all subsequent derivatives of pMT675::*res*-P$_{rsaA}$-full-length-*rsaA-ter-tetAR-res* plasmid, the same 3-way ligation was used except for the NdeI-NheI-ended *rsaA* derivates being liberated from their corresponding pUC-based vector.

*pMT675::res-lacO$_{(10)}$-tetAR-res:*

As above, a 3-way ligation was used. A fragment containing 10 consecutive *lacO* operators was PCR amplified using primers lac10-array-NdeIF and lac10-array-NheIR and pLAU43 as template (Lau *et al*, 2003).

*Strains for labeling individual loci:*

For strain ML2527 (CB15N *parS*$^{pMT1}$::400kb *parS*$^{P1}$::600kb *xyl*::P$_{xyl}$-*mcherry-parB*$^{P1}$-y*fp-parB*$^{pMT1}$ pJS14-tetracyclineR-P$_{xyl}$-empty): First, *parS*$^{pMT1}$ at 400kb (marked with spectinomycin$^{R}$) was transduced into CB15N, then *parS*$^{P1}$ at 600kb (marked with chloramphenicol$^{R}$) was transduced into the resulting strain, and then *xyl*::P$_{xyl}$-*mcherry-parB*$^{P1}$-*yfp-parB*$^{pMT1}$ (marked with kanamycin$^{R}$) was transduced in. Finally, the plasmid pJS14-tetracyclineR-P$_{xyl}$-empty was introduced by electroporation.

For strain ML2529 (CB15N *parS*$^{pMT1}$::400kb *parS*$^{P1}$::600kb *xyl*::P$_{xyl}$-*mcherry-parB*$^{P1}$-y*fp-parB*$^{pMT1}$ pJS14-tetracyclineR-P$_{xyl}$-*ctrAD51EΔ3*Ω), the same procedure as above was used except that plasmid pJS14-tetracyclineR-P$_{xyl}$-*ctrAD51EΔ3*Ω was introduced instead.

For strain ML2528 (CB15N *parS*$^{pMT1}$::800kb *parS*$^{P1}$::600kb *xyl*::P$_{xyl}$-*mcherry-parB*$^{P1}$-*yfp-parB*$^{pMT1}$ pJS14-tetracyclineR-P$_{xyl}$-empty): First, *parS*$^{pMT1}$ at 800kb (marked with spectinomycin$^{R}$) was transduced into CB15N, then *parS*$^{P1}$ at 600kb (marked with chloramphenicol$^{R}$) was transduced into the resulting strain, and then *xyl*::P$_{xyl}$-*mcherry-parB*$^{P1}$-*yfp-parB*$^{pMT1}$ (marked with kanamycin$^{R}$) was transduced in. Finally, the plasmid pJS14-tetracyclineR-P$_{xyl}$-empty was introduced by electroporation

For strain ML2530 (CB15N *parS*$^{pMT1}$::800kb *parS*$^{P1}$::600kb *xyl*::P$_{xyl}$-*mcherry-parB*$^{P1}$-*yfp-parB*$^{pMT1}$ pJS14-tetracyclineR-P$_{xyl}$-*ctrAD51EΔ3*Ω): The same procedure was used as above, except that the plasmid pJS14-tetracyclineR-P$_{xyl}$-*ctrAD51EΔ3*Ω was used instead.

For strain ML2531 (CB15N *parS*$^{pMT1}$::400kb *parS*$^{P1}$::600kb *dnaA*::P$_{van}$-*dnaA xyl*::P$_{xyl}$-*mcherry-parB*$^{P1}$-*yfp-parB*$^{pMT1}$*parB*::*parB-cfp*): First, *parS*$^{pMT1}$ at 400kb (marked with spectinomycin$^{R}$) was transduced into CB15N, then *parS*$^{P1}$ at 600kb (marked with chloramphenicol$^{R}$) was transduced into the resulting strain, and then *xyl*::P$_{xyl}$-*mcherry-parB*$^{P1}$-*yfp-parB*$^{pMT1}$ (marked with kanamycin$^{R}$) was transduced in. Finally, *dnaA*::P$_{van}$-*dnaA* (gentamycin$^{R}$) with the genetically-linked *parB::parB-cfp* was transduced into produce the final strain.

For ML2532 (CB15N *parS*$^{pMT1}$::800kb *parS*$^{P1}$::600kb *dnaA*::P$_{van}$-*dnaA xyl*::P$_{xyl}$-*mcherry-parB*$^{P1}$-*yfp-parB*$^{pMT1}$*parB*::*parB-cfp*): First, *parS*$^{pMT1}$ at 800kb (marked with spectinomycin$^{R}$) was transduced into CB15N, then *parS*$^{P1}$ at 600kb (marked with chloramphenicol$^{R}$) was transduced

into the resulting strain, and then *xyl*::$P_{xyl}$-*mcherry-parB*$^{P1}$-*yfp-parB*$^{pMT1}$ (marked with kanamycin$^R$) was transduced in. Finally, *dnaA*::$P_{van}$-*dnaA* (gentamycin$^R$) with the genetically-linked *parB*::*parB-cfp* was transduced in to produced the final strain.

For the construction of strains ML2533, ML2534, ML2535, ML2536, ML2537 and ML2538, essentially the same transduction procedure was used as described above for ML2527-ML2532, except that *parS*$^{PMT1/P1}$ were labeled at 1400 kb, 1600 kb and 1800 kb.

*Strains expressing rsaA variants:*

For the construction of strain ML2539, plasmid *pMT675::*$P_{rsaA}$-full-length-*rsaA* was electroporated into ML2121 (*ΔrsaA*). The correct single-crossover integration at the *van* locus was verified by PCR.

For the construction of strains ML2540; ML2541; ML2542; ML2543; ML2544; ML2545; ML2546; ML2547; ML2548; ML2549 and ML2550: The plasmids pMT675::promoterless-*rsaA*; pMT675::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-560bp; pMT675::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-2060bp; pMT675::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-60bp; pMT675::$P_{rsaA}$-*rsaA*-2xTAA2xter-at-1060bp; pMT675::$P_{rsaA}$-*rsaA*-ATGtoTAA; pMT675::$P_{rsaA}$-*rsaA-antitermination*-ATGtoTAA; pMT553::FLAG-$P_{rsaA}$-full-length-*rsaA;* pMT553::FLAG-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-560bp; pMT553::FLAG-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-1060bp; pMT553::FLAG-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-2060bp were individually electroporated into ML2121 (*ΔrsaA*). The correct single-crossover integration at the *van* locus was verified in each case by PCR.

*Strains for recombination assays:*

For strain ML2551 (CB15N *van*::pMT675::*res-tetAR-res* and pMT687-Pc-*γδtnpR*$^{CCDAS4}$): First, plasmid pMT675::*res-tetAR-res* was electroporated into CB15N. The correct single-crossover integration at the *van* locus was verified by PCR. Subsequently, the replicating plasmid pMT687-Pc-*γδtnpR*$^{CCDAS4}$ was introduced by conjugation.

For the construction of strain ML2552 (CB15N *van*::pMT675::*res-tetAR-res* and pMT687-Pc-*γδtnpR*$^{ΔDBD}$): First, plasmid pMT675::*res-tetAR-res* was electroporated into CB15N. The correct single-crossover integration at the *van* locus was verified by PCR. Subsequently, the replicating plasmid pMT687-Pc-*γδtnpR*$^{ΔDBD}$ was introduced by conjugation.

For the construction of strain ML2554 (CB15N Δ*rsaA van*::pMT675-*res*-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-60bp-tetAR-*res* and pMT687-Pc-*tnpR*$^{CCDAS4}$): First, plasmid pMT675::*res*-$P_{rsaA}$-*rsaA*-

2xTAA2xter-at-60bp-tetAR-*res* was electroporated into ML2121 (*ΔrsaA*). The correct single-crossover integration at the *van* locus was verified by PCR. Subsequently, the replicating plasmid pMT687-Pc-*γδtnpR*$^{CCDAS4}$ was introduced by conjugation. For the construction of strains ML2555; ML2556; ML2557; ML2558; ML2559 and ML2561: the same procedure was carried out as for ML2554, except that plasmid pMT675::*res*-P$_{rsaA}$-*rsaA*-2xTAA2xter-at-560bp-*tetAR-res;* pMT675::*res*-P$_{rsaA}$-*rsaA*-2xTAA2xter-at-1060bp-*tetAR-res;* pMT675::res-P$_{rsaA}$-*rsaA*-2xTAA2xter-at 2060bp-*tetAR-res*; pMT675::*res*-P$_{rsaA}$-*rsaA*-antitermination-ATGtoTAA-*tetAR-res;* pMT675::*res*-P$_{rsaA}$-*rsaA*-ATGtoTAA-*tetAR-res* or pMT675::*res*-promoterless-*rsaA-tetAR-res* was used, respectively.

For the construction of strain ML2560 (CB15N *ΔrsaA van*::pMT675-*res-lacO$_{(10)}$-tetAR-res* and *xyl*::P$_{xyl}$-*tetR-cfp-lacI-yfp* and pMT687-Pc-*tnpR*$^{CCDAS4}$): Plasmid pMT675::*res-lacO$_{(10)}$-tetAR-res* was electroporated into ML2121 (*ΔrsaA*). The correct single-crossover integration at the *van* locus was verified by PCR. Subsequently, *xyl*::P$_{xyl}$-*tetR-cfp-lacI-yfp* (marked with spectinomycin$^{R}$) (Viollier *et al*, 2004) was transduced into the resulting strain. Finally, the replicating plasmid pMT687-Pc-*γδtnpR*$^{CCDAS4}$ was introduced by conjugation.

**Determination of subcellular positions of chromosomal loci by orthogonal ParB/*parS* systems**

*C. crescentus* strains with *parS*$^{pMT1}$ or *parS*$^{P1}$ inserted at various locations on the chromosome was created as described above. These strains also harboured a P$_{xyl}$-m*cherry-parB*$^{P1}$*-yfp-parB*$^{pMT1}$ cassette at the *xyl* locus. Strains were grown to $OD_{600}$=0.4 in the presence of appropriate antibiotics, vanillate, and glucose before the cells were collected by centrifugation and washed of residual vanillate, antibiotics and glucose twice with fresh PYE. Cells were then resuspended in PYE plus xylose (0.3% final concentration) (vanillate omitted) to deplete DnaA for 90 min before synchronization, thereby inducing cell elongation. Cells were imaged at 0 hr, 1 hr, 2 hr and 3 hr post synchronization. Phase contrast (150 ms exposure) and fluorescence images (2000 ms exposure) were collected. MicrobeTracker (http://microtracker.org) was used to detect cell outlines and SpotFinderZ to detect fluorescent foci positions. Only cells with a single CFP-ParB, Mcherry-ParB$^{P1}$ and YFP-ParB$^{pMT1}$ focus were used for construction of boxplots. Cells were then sorted into bins (<2.5 μm, 2.5-3.5 μm, 3.5-4.5 μm and 4.5-5.5 μm) according to their length. The number of cells used for construction of boxplots in Fig 6A and C (cell length μm / loci measured kb / number of cells) were: (<2.5/400-600/206), (<2.5/600-800/254), (2.5-3.5/400-600/1438), (2.5-3.5/600-800/998), (3.5-4.5/400-600/803), (3.5-4.5/600-800/651), (4.5-5.5/400-600/422), (4.5-5.5/600-800/458), (<2.5/1400-1600/90), (<2.5/1600-

1800/152), (2.5-3.5/1400-1600/131), (2.5-3.5/1600-1800/714), (3.5-4.5/1400-1600/401), (3.5-4.5/1600-1800/346), (4.5-5.5/1400-1600/308), (4.5-5.5/1600-1800/520).

The same general procedure was used for CtrA(D51E)Δ3Ω overexpression strains. Briefly, cells were grown to $OD_{600}$=0.4 in the presence of appropriate antibiotics and glucose before the cells were collected by centrifugation and washed of residual glucose twice with fresh PYE and synchronized. Cells were then resuspended in PYE plus xylose (0.3% final concentration) to induce the production of YFP-$ParB^{pMT1}$, mCherry-$ParB^{P1}$ and CtrA(D51E)Δ3Ω. Cells were imaged at 60, 120 and 180 min after the addition of xylose. For the 0 min time point, strains harboring pJS14-$P_{xyl}$-empty plasmid were used instead as 60 min induction by xylose is required for a sufficient production and fluorophore maturation of YFP-$ParB^{pMT1}$, mCherry-$ParB^{P1}$. Only cells with a single Mcherry-$ParB^{P1}$ and YFP-$ParB^{pMT1}$ focus were used for construction of boxplots. Cells were then sorted into bins (<2.5 μm, 2.5-3.5 μm, 3.5-4.5 μm and 4.5-5.5 μm) according to their length. The number of cells used for construction of boxplots in Fig 6B and 6D (cell length μm / loci measured kb / number of cells) were: (<2.5/400-600/968), (<2.5/600-800/804), (2.5-3.5/400-600/1268), (2.5-3.5/600-800/1337), (3.5-4.5/400-600/579), (3.5-4.5/600-800/529), (4.5-5.5/400-600/189), (4.5-5.5/600-800/199), (<2.5/1400-1600/511), (<2.5/1600-1800/306), (2.5-3.5/1400-1600/1058), (2.5-3.5/1600-1800/993), (3.5-4.5/1400-1600/732), (3.5-4.5/1600-1800/861), (4.5-5.5/1400-1600/375), (4.5-5.5/1600-1800/443).

**S-layer extraction and Western blotting**

A low pH extraction method was used to determine whether the full-length S-layer protein RsaA was secreted and assembled on the surface of various strains of *C. crescentus* (Walker *et al*, 1992). Briefly, cells were collected by centrifugation and washed twice gently in 750 μl of 10 mM Hepes pH 7.2. Subsequently, the supernatant was removed and the pellet resuspended in 100 μl of 100 mM Hepes pH 2.0 for 5 minutes. The solution was then neutralized by addition of 1.6 μl of 6N NaOH. Cell debris was removed by centrifugation and the supernatant containing S-layer proteins transferred to a fresh Eppendorf tubes for SDS-PAGE analysis.

SDS sample buffer was added to the solution of S-layer extracted proteins to 1x and heated to 42°C for 1 min before loading onto a SDS polyacrylamide gel. For total protein analysis or Western blot analysis, *Caulobacter* cells were pelleted and resuspended directly in 1x SDS sample buffer, then heated to 95°C for 5 min before loading. Equal amounts of total protein were run on 10% Tris-HCl gels (Bio-Rad Laboratories, Inc.) at 150 V for separation. Resolved proteins were transferred to polyvinylidene fluoride membranes and probed with 1:5,000 dilution

of primary anti-FLAG antibodies (Sigma-Aldrich) and subsequently by a secondary horseradish peroxidase–conjugated antibody (1:5,000). Blots were imaged using a FluorChem M scanner (ProteinSimple).

**γδ TnpR recombination assay**

*Caulobacter* strains used for the recombination assay were first grown to early exponential phase in PYE supplemented with tetracycline and kanamycin. Just before inducing TnpR, cells were collected by centrifugation, washed of tetracycline and resuspended in fresh PYE + kanamycin to an $OD_{600}$ of ~0.2. Cumate was then added to a final concentration of 100 μM and cultures were incubated with shaking at 30°C for 2 hours. Subsequently, cultures were 10-fold serially diluted and 5 μl of each dilution was spotted on both tetracycline + kanamycin and kanamycin PYE plates. To measure colony forming units (CFU), *Caulobacter* cultures were serially (20-fold) diluted with 50 μl of appropriately diluted culture spread on tetracycline + kanamycin or kanamycin PYE plates. Plates were incubated at 30°C for two days before counting CFUs.

For a recombination assay where rifampicin or novobiocin was added, TnpR was induced by adding cumate for 60 minutes before rifampicin (1 μg/ml final concentration) or novobiocin (25 μg/ml final concentration) was added for another 60 minutes. Afterwards, *Caulobacter* cells were washed of antibiotics by centrifugation and resuspended in fresh PYE six times before spotting or spreading for CFU determination.

For a recombination assay of *res-(lacO)$_{10}$-tetAR-res* strains, *Caulobacter* was grown to mid-exponential phase in PYE supplemented with kanamycin, tetracycline and 0.1% glucose to repress the production of LacI-YFP protein. Cells were then washed in plain PYE three times. Cells were then divided into 2 cultures, one with LacI-YFP produced by addition of 0.3% xylose while the other one had glucose added to 0.1% final concentration. Cultures were left incubated with shaking at 30 °C for an additional 1 hour to allow for LacI-YFP production. Subsequently, cells were washed of tetracycline by centrifugation and resuspension in fresh PYE + kanamycin + xylose/glucose before cumate was added to 100 μM. After another 2 hours at 30°C, cultures were spreaded on tetracycline + kanamycin or kanamycin PYE plates to determine CFUs.

## APPENDIX REFERENCES


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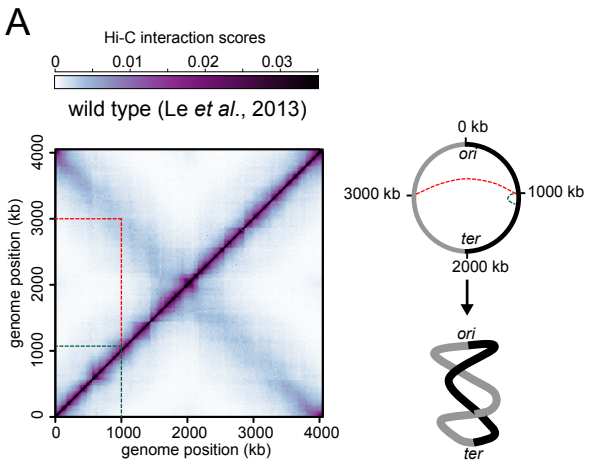
## APPENDIX FIGURE LEGENDS

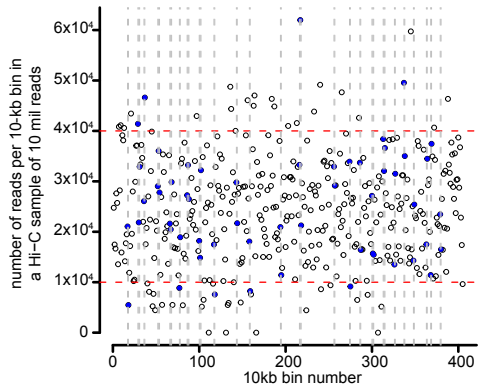
**Figure S1. Domain boundaries are not an artifact of non-uniformities in the cross-linking efficiencies of genomic DNA.** (A) *C. crescent*us Hi-C contact map (Le *et al*, 2013) showing intra- and inter-chromosomal arm interactions (left panel). Model of *in vivo* configuration of *C. crescentus* chromosome consistent with the Hi-C data (right panel). (B-C) Comparison of two independent repeats of the BglII Hi-C maps for starved swarmer cells (panel B is the same as that shown in Fig 1C). Directional preference plots are shown below each Hi-C map. (D) Scatter plot showing numbers of Hi-C reads in each 10-kb bin along the *Caulobacter* genome (Hi-C dataset for wild-type swarmer cells). Blue dots represent numbers of reads from bins residing at CID boundaries. Red dashed lines show thresholds for calling read-depleted and read-enriched 10-kb bins.

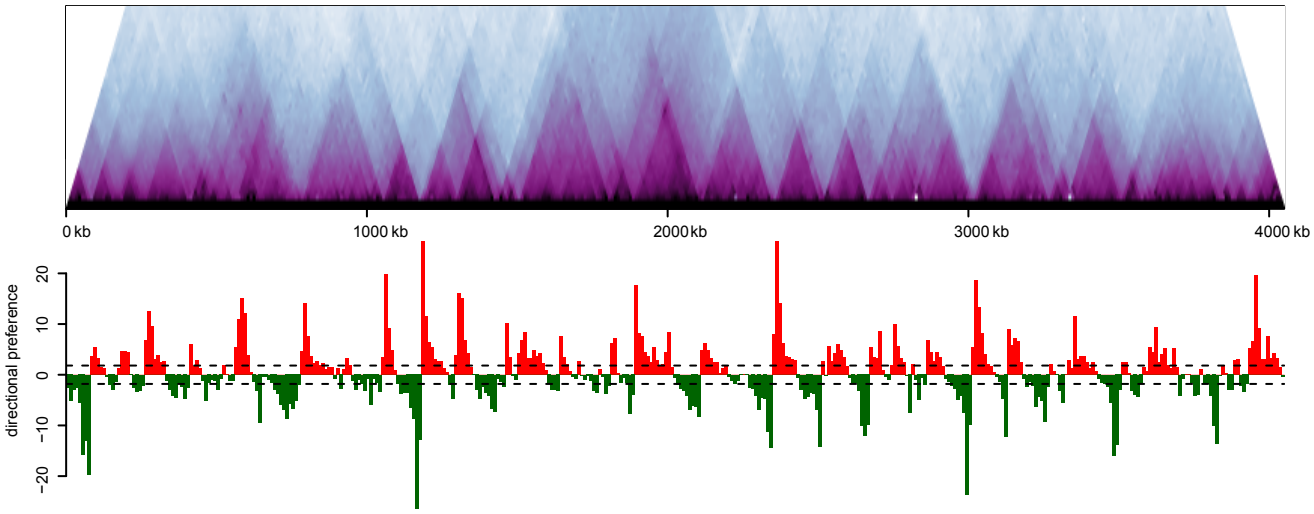
**Figure S2. Validation of the γδ TnpR recombination assay for measuring supercoil diffusion.** Tetracycline-resistance, which requires excision of a $tet^R$ cassette by TnpR, was used as a proxy for the extent of supercoil diffusion between two *res* sites (see Fig 5). (A) A strain producing full-length γδ TnpR recombinase was compared to a strain producing a TnpR derivative lacking a DNA-binding domain ($tnpR^{\Delta DBD}$). TnpR constructs were expressed (or not) using a cumate-inducible promoter. Each spot is a 10-fold dilution. Presence or absence of tetracycline in plates is indicated. (B) Strains tested harbored the indicated construct between one of the *res* sites and the $tet^R$ cassette and were treated with novobiocin to inhibit negative supercoiling or with (C) rifampicin to inhibit transcription. Each strain expresses full-length TnpR and was plated on tetracycline.

**Figure S3. Intra- and inter-domain DNA expand differentially in elongated *Caulobacter* cells.** (A) Cells harbouring *dnaA*::$P_{van}$-*dnaA* were grown without vanillate for 90 minutes, synchronized, and then released into medium without vanillate. Cells were imaged 0, 1, 2 and 3 hrs post-synchronization. The nucleoid was labeled with HU1-GFP produced from the native *hup1* promoter. The average cell length is indicated for each time point. Scale bars represent 2 μm. (B-E) Representative fields of view for cells imaged when calculating the inter-focus distances reported in Fig 6.

FIG. S1


A
Hi-C interaction scores
0
0.01
0.02
0.03
wild type (Le et al., 2013)
genome position (kb)
0
1000
2000
3000
4000
0 kb
ori
1000 kb
ter
2000 kb
3000 kb
ori
ter


D
number of reads per 10-kb bin in a Hi−C sample of 10 mil reads
6x10⁴
5x10⁴
4x10⁴
3x10⁴
2x10⁴
1x10⁴
0
0
100
200
300
400
10kb bin number


B
BglII Hi-C of Caulobacter swarmer cells, starved in M2 salts for 90 min - repeat 1
0 kb
1000 kb
2000 kb
3000 kb
4000 kb
directional preference
20
10
0
−10
−20

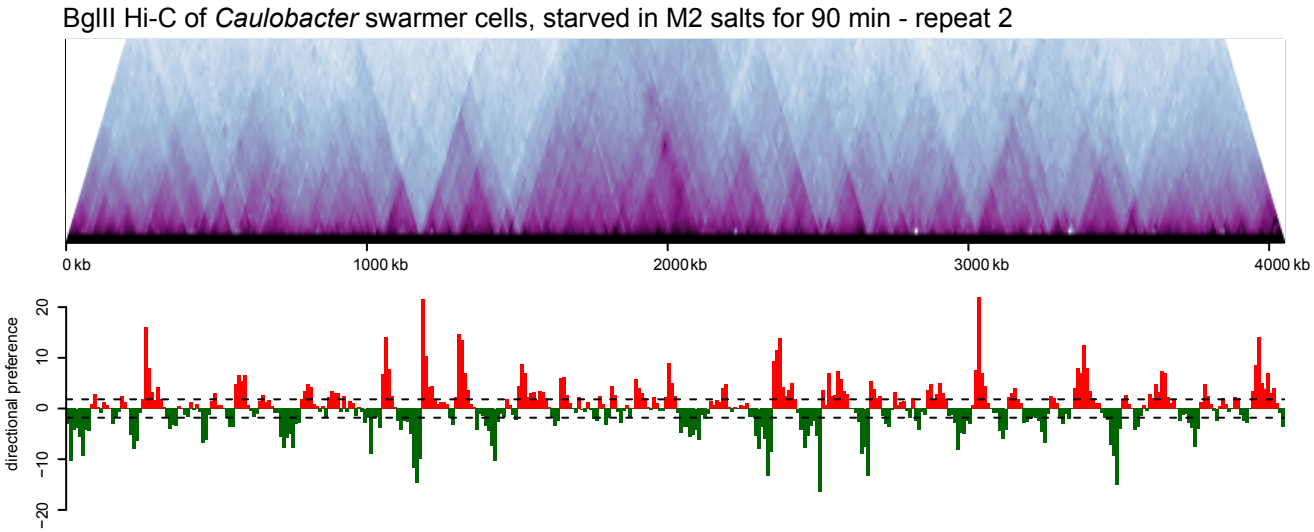
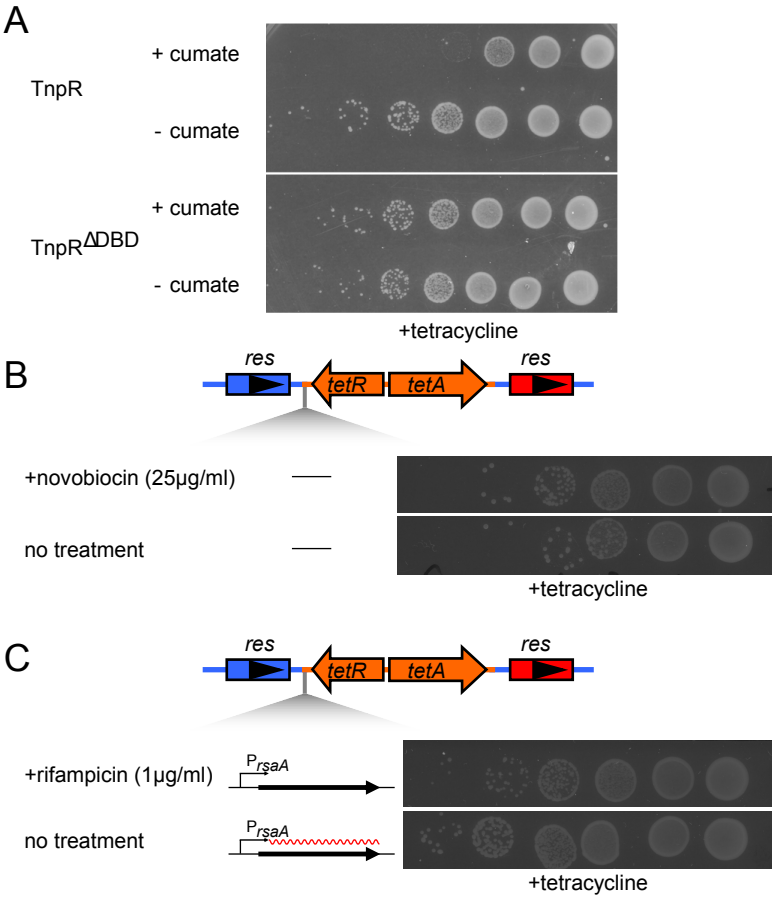
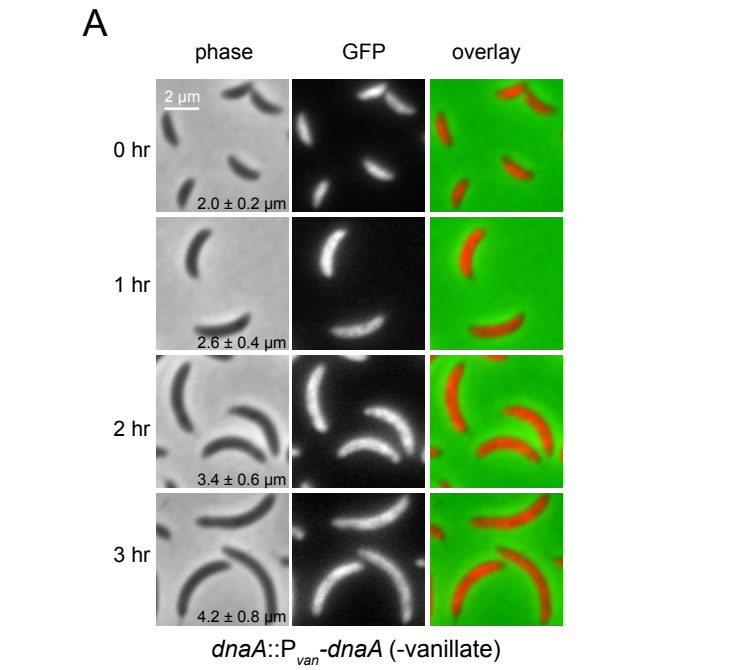
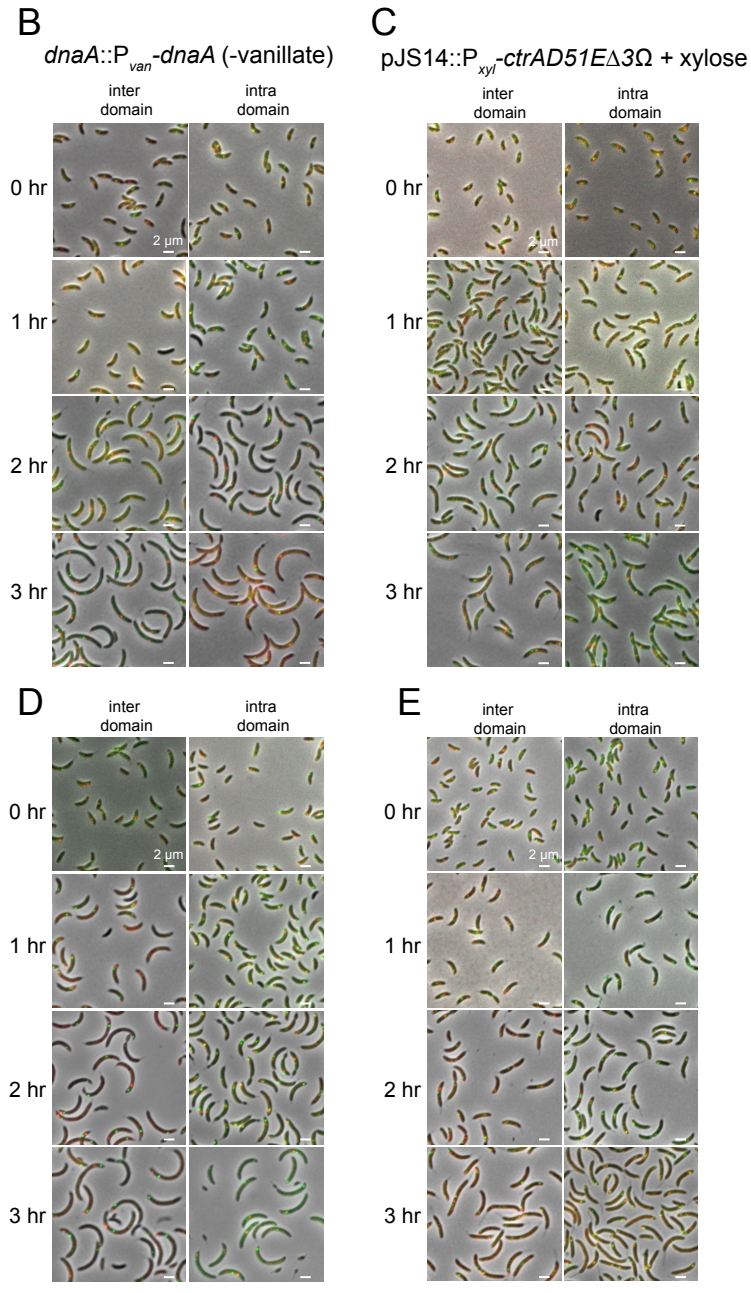

C
BglII Hi-C of Caulobacter swarmer cells, starved in M2 salts for 90 min - repeat 2
0 kb
1000 kb
2000 kb
3000 kb
4000 kb
directional preference
20
10
0
−10
−20

FIG. S2


A
+ cumate
TnpR
- cumate
+ cumate
TnpR^ΔDBD
- cumate
+tetracycline
B
res
tetR
tetA
res
+novobiocin (25µg/ml)
no treatment
+tetracycline
C
res
tetR
tetA
res
+rifampicin (1µg/ml)
P rsaA
no treatment
P rsaA
+tetracycline


A
phase
GFP
overlay
2 μm
0 hr
2.0 ± 0.2 μm
1 hr
2.6 ± 0.4 μm
2 hr
3.4 ± 0.6 μm
3 hr
4.2 ± 0.8 μm
dnaA::Pvan-dnaA (-vanillate)


B
dnaA::Pvan-dnaA (-vanillate)
C
pJS14::Pxyl-ctrAD51EΔ3Ω + xylose
inter domain
intra domain
0 hr
1 hr
2 hr
3 hr
2 μm
D
E

## APPENDIX TABLE S1. PLASMIDS AND STRAINS

| ML number | Strains/Plasmids | Description | Source |
|---|---|---|---|
| | | | |
| | **Plasmids** | | |
| | pUC19 | General cloning vector (carbenicilin$^R$) | Fermentas |
| | pENTR-D-TOPO | ENTRY vector for gateway cloning (kanamycin$^R$) | Invitrogen |
| | pVMCS-4 | integrative vector (gentamycin$^R$) | Thanbichler *et al*, 2007 |
| | pMT675 | pVYFPC-6, integrative vector to the *vanA* locus (chloramphenicol$^R$) | Thanbichler *et al*, 2007 |
| | pMT619 | pMCS-1, integrative vector (spectinomycin$^R$) | Thanbichler *et al*, 2007 |
| | pMT632 | pMCS-6, integrative vector (chloramphenicol$^R$) | Thanbichler *et al*, 2007 |
| | pMT553 | pVYFPC-2, integrative vector to the *vanA* locus (kanamycin$^R$) | Thanbichler *et al*, 2007 |
| | pMT687 | pRXMCS-2, low copy replicative vector (kanamycin$^R$) | Thanbichler *et al*, 2007 |
| 2482 | pVMCS-4-478-*dnaA* | pCT628 or plasmid habours 478bp N-terminal fragment of *dnaA* gene, for replacement of native $P_{dnaA}$ with $P_{van}$-*dnaA* (gentamycin$^R$) | C. Tsokos, lab collection |
| 2483 | pJS14-$P_{xyl}$-*ctrAD51EΔ3Ω* | expressing a non-proteolysable phosphorylation-mimicking *ctrAD51EΔ3Ω* from xylose-inducible promoter on a high-copy number plasmid (chloramphenicol$^R$) | Domian *et al*, 1997 |
| 2484 | pJS14-tetracyclineR-$P_{xyl}$-*ctrAD51EΔ3Ω* | expressing a non-proteolysable phosphorylation-mimicking *ctrAD51EΔ3Ω* from xylose-inducible promoter on a high-copy number plasmid (tetracycline$^R$ instead of chloramphenicol$^R$) | This study |
| 2485 | pJS14-tetracyclineR-empty | high-copy number plasmid, tetracycline-resistant derivatives of pJS14 | This study |
| 2432 | pMT697::$P_{xyl}$-*mcherry-parB*$^{P1}$-$P_{xyl}$-*yfp-parB*$^{pMT1}$ | integrative vector to the *xylX* locus, for expression of mCherry-ParB$^{P1}$ YFP-ParB$^{pMT1}$ under xylose-inducible promoter (kanamycin$^R$) | Badrinarayanan *et al*, 2015 |
| 2486 | pMT553-$P_{hup1}$-*hup1-gfp* | integrative vector, for expression of HU-GFP under *hup1* native promoter | This study |
| 2487 | pMT619::*parS*$^{pMT1}$ at +400kb | label +400kb with *parS*$^{pMT1}$ site | This study |
| 2488 | pMT632::*ParS*$^{P1}$ at +600kb | label +600kb with *ParS*$^{P1}$ site | This study |

| | | | |
|---|---|---|---|
| 2489 | pMT619::*parS*$^{pMT1}$ at +800kb | label +800kb with *parS*$^{pMT1}$ site | This study |
| 2490 | pMT619::*parS*$^{pMT1}$ at +1400kb | label +1400kb with *parS*$^{pMT1}$ site | This study |
| 2491 | pMT632::*ParS*$^{P1}$ at +1600kb | label +1600kb with *ParS*$^{P1}$ site | This study |
| 2492 | pMT619::*parS*$^{pMT1}$ at +1800kb | label +1800kb with *parS*$^{pMT1}$ site | This study |
| 2493 | pUC::P$_{rsaA}$ full-length-*rsaA* | wild-type *rsaA* together with its promoter | This study |
| 2494 | pUC::P$_{rsaA}$-*rsaA*-2xTAA2xter-at-60bp | 2xTAA 2x terminators inserted 60 bp from *rsaA* transcription start site | This study |
| 2495 | pUC::P$_{rsaA}$ *rsaA*-2xTAA2xter-at-560bp | 2xTAA 2x terminators inserted 560 bp from *rsaA* transcription start site | This study |
| 2496 | pUC::P$_{rsaA}$-*rsaA*-2xTAA2xter-at-1060bp | 2xTAA 2x terminators inserted 1060 bp from *rsaA* transcription start site | This study |
| 2497 | pUC::P$_{rsaA}$-*rsaA*-2xTAA2xter-at-2060bp | 2xTAA 2x terminators inserted 2060 bp from *rsaA* transcription start site | This study |
| 2498 | pUC::P$_{rsaA}$-*rsaA*- anti-termination ATGtoTAA | anti-termination sequence preceding the *rsaA* coding sequence, start codon ATG mutated to TAA | This study |
| 2499 | pUC::P$_{rsaA}$-*rsaA*-ATGtoTAA | start codon ATG of *rsaA* mutated to TAA | This study |
| 2500 | pMT675::P$_{rsaA}$-full-length-*rsaA* | wild-type *rsaA* together with its promoter, for integration at the *van* locus | This study |
| 2501 | pMT675::P$_{rsaA}$-*rsaA*-2xTAA2xter-at-60bp | 2xTAA 2x terminators inserted 60 bp from *rsaA* transcription start site, for integration at the *van* locus | This study |
| 2502 | pMT675::P$_{rsaA}$-*rsaA*-2xTAA2xter-at-560bp | 2xTAA 2x terminators inserted 560 bp from *rsaA* transcription start site, for integration at the *van* locus | This study |
| 2503 | pMT675::P$_{rsaA}$-*rsaA*-2xTAA2xter-at-1060bp | 2xTAA 2x terminators inserted 1060 bp from *rsaA* transcription start site, for integration at the *van* locus | This study |
| 2504 | pMT675::P$_{rsaA}$-*rsaA*-2xTAA2xter-at-2060bp | 2xTAA 2x terminators inserted 2060 bp from *rsaA* transcription start site, for integration at the *van* locus | This study |
| 2505 | pMT675::P$_{rsaA}$-*rsaA*-antiterminator-ATGtoTAA | anti-termination sequence preceding the *rsaA* coding sequence, start codon ATG mutated to TAA, for integration at the *van* locus | This study |
| 2506 | pMT675::P$_{rsaA}$-*rsaA*-ATGtoTAA | start codon ATG of *rsaA* mutated to TAA, for integration at the *van* locus | This study |
| 2507 | pMT675::promoterless-*rsaA*-full length | promoter of *rsaA* was removed, for integration at the *van* locus | |
| 2508 | pMT553::P$_{rsaA}$-FLAG-full-length-*rsaA* | N-terminal FLAG-tagged *rsaA* together with its promoter, for integration at the *van* locus | This study |

| | | | |
|---|---|---|---|
| 2509 | pMT553::$P_{rsaA}$-FLAG-*rsaA*-2xTAA2xter-at-560bp | N-terminal FLAG-tagged *rsaA*, 2xTAA 2x terminator inserted 560 bp from *rsaA* transcription start site, for integration at the *van* locus | This study |
| 2510 | pMT553::$P_{rsaA}$-FLAG-*rsaA*-2xTAA2xter-at-1060bp | N-terminal FLAG-tagged *rsaA*, 2xTAA 2x terminator inserted 1060 bp from *rsaA* transcription start site, for integration at the *van* locus | This study |
| 2511 | pMT553::$P_{rsaA}$-FLAG-*rsaA*-2xTAA2xter-at-2060bp | N-terminal FLAG-tagged *rsaA*, 2xTAA 2x terminator inserted 2060 bp from *rsaA* transcription start site, for integration at the *van* locus | This study |
| 2512 | pMT687-Pc-γδ-$tnpR^{CCDAS4}$ | expressing γδ *tnpR*-degron | This study |
| 2513 | pMT687-Pc-γδ-$tnpR^{\Delta DBD}$ | expressing γδ $tnpR^{\Delta DBD}$, DNA-binding domain of *tnpR* was removed | This study |
| | | | |
| 2517 | pMT675::*res-tetAR-res* | for integration of a *res-tetAR-res* cassette at the *van* locus | This study |
| 2518 | pMT675::*res*-$P_{rsaA}$-full-length-*rsaA-ter-tetAR-res* | full length *rsaA* driven by $P_{rsaA}$ in between 2 *res* sites, for integration at the *van* locus | This study |
| 2519 | pMT675::*res*-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-60bp-*tetAR-res* | $P_{rsaA}$-*rsaA*-2xTAA2xter at 60 bp in between 2 *res* sites, for integration at the *van* locus | This study |
| 2520 | pMT675::*res*-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-560bp-*tetAR-res* | $P_{rsaA}$-*rsaA*-2xTAA2xter at 560 bp in between 2 *res* sites, for integration at the *van* locus | This study |
| 2521 | pMT675::*res*-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-1060bp-*tetAR-res* | $P_{rsaA}$-*rsaA*-2xTAA2xter at 1060 bp in between 2 *res* sites, for integration at the *van* locus | This study |
| 2522 | pMT675::*res*-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-2060bp-*tetAR-res* | $P_{rsaA}$-*rsaA*-2xTAA2xter at 2060 bp in between 2 *res* sites, for integration at the *van* locus | This study |
| 2523 | pMT675::*res*-$P_{rsaA}$-*rsaA* anti-termination ATGtoTAA-*tetAR-res* | $P_{rsaA}$-*rsaA* anti-termination start codon ATG mutated to TAA in between 2 *res* sites, for integration at the *van* locus | This study |
| 2524 | pMT675::*res*-$P_{rsaA}$-*rsaA* ATGtoTAA-*tetAR-res* | $P_{rsaA}$-*rsaA* ATGtoTAA in between 2 *res* sites, for integration at the *van* locus | This study |
| 2525 | pMT675::*res-lacO*$_{(10)}$-*tetAR-res* | *lacO*$_{(10)}$ in between 2 *res* sites, for integration at the *van* locus | This study |
| 2526 | pMT675::*res*-promoterless-*rsaA-tetAR-res* | promoterless *rsaA* in between 2 *res* sites, for integration at the *van* locus | This study |

| Strains | Description | Source |
|---|---|---|
| CB15N | Wild-type synchronizable *Caulobacter crescentus* | lab collection |
| 2000 | CB15N *hfa::lacI dnaA::P$_{lac}$-dnaA* | Aakre *et al*, 2013 |
| 1675 | CB15N pJS14::p$_{Xyl}$-*ctrAD51EΔ3Ω* | Domian *et al*, 1997 |
| 2527 | CB15N *parS$^{pMT1}$*::400kb *parS$^{P1}$*::600kb *xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* pJS14-tetracyclineR-P$_{xyl}$-empty | This study |
| 2528 | CB15N *parS$^{pMT1}$*::800kb *parS$^{P1}$*::600kb *xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* pJS14-tetracyclineR-P$_{xyl}$-empty | This study |
| 2529 | CB15N *parS$^{pMT1}$*::400kb *parS$^{P1}$*::600kb *xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* pJS14-tetracyclineR-P$_{xyl}$-*ctrAD51EΔ3Ω* | This study |
| 2530 | CB15N *parS$^{pMT1}$*::800kb *parS$^{P1}$*::600kb *xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* pJS14-tetracyclineR-P$_{xyl}$-*ctrAD51EΔ3Ω* | This study |
| 2531 | CB15N *parS$^{pMT1}$*::400kb *parS$^{P1}$*::600kb *dnaA*::P*van-dnaA xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$ parB::parB-cfp* | This study |
| 2532 | CB15N *parS$^{pMT1}$*::800kb *parS$^{P1}$*::600kb *dnaA*::P$_{van}$-*dnaA xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$ parB::parB-cfp* | This study |
| 2533 | CB15N *parS$^{pMT1}$*::1400kb *parS$^{P1}$*::1600kb *xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* pJS14-tetracyclineR-P$_{xyl}$-empty | This study |
| 2534 | CB15N *parS$^{pMT1}$*::1800kb *parS$^{P1}$*::1600kb *xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* pJS14-tetracyclineR-P$_{xyl}$-empty | This study |
| 2535 | CB15N *parS$^{pMT1}$*::1400kb *parS$^{P1}$*::1600kb *xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* pJS14-tetracyclineR-P$_{xyl}$-*ctrAD51EΔ3Ω* | This study |
| 2536 | CB15N *parS$^{pMT1}$*::1800kb *parS$^{P1}$*::1600kb *xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* pJS14-tetracyclineR-P$_{xyl}$-*ctrAD51EΔ3Ω* | This study |
| 2537 | CB15N *parS$^{pMT1}$*::1400kb *parS$^{P1}$*::1600kb *dnaA*::P$_{van}$-*dnaA xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* | This study |
| 2538 | CB15N *parS$^{pMT1}$*::1800kb *parS$^{P1}$*::1600kb *dnaA*::P$_{van}$-*dnaA xyl*::P$_{xyl}$-*mcherry-parB$^{P1}$-yfp-parB$^{pMT1}$* | This study |
| 2121 | CB15N Δ*rsaA::markerless* | Le *et al*, 2013 |
| 2539 | CB15N Δ*rsaA van*::pMT675-full-length-*rsaA* | This study |
| 2540 | CB15N Δ*rsaA van*::pMT675-promoterless-*rsaA* | This study |
| 2541 | CB15N Δ*rsaA van*::pMT675-*rsaA* 2xTAA2xter-at-560bp | This study |
| 2542 | CB15N Δ*rsaA van*::pMT675-*rsaA*-2xTAA2xter-at-2060bp | This study |
| 2543 | CB15N Δ*rsaA van*::pMT675-*rsaA*-2xTAA2xter-at-60bp | This study |

| | | |
|---|---|---|
| 2544 | CB15N Δ*rsaA van*::pMT675-*rsaA*-2xTAA2xter-at-1060bp | This study |
| 2545 | CB15N Δ*rsaA van*::pMT675-*rsaA* start-ATGtoTAA | This study |
| 2546 | CB15N Δ*rsaA van*::pMT675-anti-termination-*rsaA*-ATGtoTAA | This study |
| 2547 | CB15N Δ*rsaA* pMT553::$P_{rsaA}$-FLAG-*rsaA*-full length | This study |
| 2548 | CB15N Δ*rsaA* pMT553:: $P_{rsaA}$-FLAG-*rsaA*-2xTAA2xter-at-560bp | This study |
| 2549 | CB15N Δ*rsaA* pMT553:: $P_{rsaA}$-FLAG-*rsaA*-2xTAA2xter-at-1060bp | This study |
| 2550 | CB15N Δ*rsaA* pMT553:: $P_{rsaA}$-FLAG-*rsaA*-2xTAA2xter-at-2060bp | This study |
| 2551 | CB15N *van*::pMT675-*res-tetAR-res* and pMT687-Pc-γδ-$tnpR^{CCDAS4}$ | This study |
| 2552 | CB15N *van*::pMT675-*res-tetAR-res* and pMT687-Pc-γδ-$tnpR^{ADBD}$ | This study |
| 2553 | CB15N Δ*rsaA van*::pMT675-*res*-$P_{rsaA}$-full-length-*rsaA*-ter-*tetAR-res* and pMT687-Pc-$tnpR^{CCDAS4}$ | This study |
| 2554 | CB15N Δ*rsaA van*::pMT675-*res*-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-60bp-*tetAR-res* and pMT687-Pc-$tnpR^{CCDAS4}$ | This study |
| 2555 | CB15N Δ*rsaA van*::pMT675-*res*-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-560bp-*tetAR-res* and pMT687-Pc-$tnpR^{CCDAS4}$ | This study |
| 2556 | CB15N Δ*rsaA van*::pMT675-*res*-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-1060bp-*tetAR-res* and pMT687-Pc-$tnpR^{CCDAS4}$ | This study |
| 2557 | CB15N Δ*rsaA van*::pMT675-*res*-$P_{rsaA}$-*rsaA*-2xTAA2xter-at-2060bp-*tetAR-res* and pMT687-Pc-$tnpR^{CCDAS4}$ | This study |
| 2558 | CB15N Δ*rsaA van*::pMT675-*res*-$P_{rsaA}$-*rsaA* anti-termination ATGtoTAA-*tetAR-res* and pMT687-Pc-$tnpR^{CCDAS4}$ | This study |
| 2559 | CB15N Δ*rsaA van*::pMT675-*res*-$P_{rsaA}$-*rsaA* ATGtoTAA -*tetAR-res* and pMT687-Pc-$tnpR^{CCDAS4}$ | This study |
| 2560 | CB15N Δ*rsaA van*::pMT675-*res-lacO*$_{(10)}$-*tetAR-res* and *xyl*::$P_{xyl}$-*tetR-cfp-lacI-yfp* and pMT687-Pc-$tnpR^{CCDAS4}$ | This study |
| 2561 | CB15N Δ*rsaA van*::pMT675-*res*-promoterless-*rsaA-tetAR-res* and pMT687-Pc-$tnpR^{CCDAS4}$ | This study |

## APPENDIX TABLE S2. PRIMERS

| Primers | Sequence |
|---|---|
| | |
| **For construction of pVMCS-4-478-*dnaA*** | |
| CA7 | tcgagttttggggagacgaccatatgaccatgaagggcggg |
| CA12 | gctctagaactagtggatcccccgggttagccccgcagcttgcgc |
| | |
| **For insertion of *parS*$^{pMT1}$ or *parS*$^{P1}$ at various genomic positions** | |
| label400-NdeI-F | CATatgacccagaccttcgacctgatc |
| label400-SacI-R | GAGCTCctatcccgccagcgtttccagg |
| label600-NdeI-F | CATATGgtggcgctgaccagcgccgggcc |
| label600-SacI-R | GAGCTCtcacagcgtcacggtgatatcgcc |
| label800-NdeI-F | CATATGttgggacggaatggcgcagggacg |
| label800-SacI-R | GAGCTCctaccagcccacggcgcggcgacg |
| label1400-NdeI-F | CATatgacacaagtcttcccgactcc |
| label1400-SacI-R | GAGCTCctacagatcaaaccgcacccccctg |
| label1600-NdeI-F | CATatggatgaagaaacccgcctgatc |
| label1600-SacI-R | GAGCTCtcaggccttcgcggcgttcagggc |
| label1800-NdeI-F | CATATGctgccgatgacggaggcggcctac |
| label1800-SacI-R | GAGCTCtcatggacgggcgctcccgtgac |
| | |
| **For construction of pJS14-tetracyclineR-P$_{xyl}$-*ctrAD51EΔ3Ω*** | |
| tetARmodJS14F | tcactggatataccaccgttgattcaatcgtcacccttttctcggtc |
| tetARmodJS14R | ccactcatcgcagtactgttgtaatcagcgatcggctcgttgccctg |
| JS14chlRinternaltailF | ttacaacagtactgcgatgagtgg |
| JS14chlRinternaltailR | atcaacggtggtatatccagtg |
| | |
| **For construction of pMT553-Phup1-HU1-GFP** | |
| L553-P-HU-GFP-1 | gaaccacgatgcgaggaaacgcatatgtgttcttggaccttgcaagac |
| L553-P-HU-GFP-2 | gccgttgacggcgtccttcagttgc |
| L553-P-HU-GFP-3 | caactgaaggacgccgtcaacggcaacgttacgcgtcaccggtcggcc |
| L553-P-HU-GFP-4 | tggatcccccgggctgcagctagcttacttgtacagctcgtccatgcc |

| | |
|---|---|
| **Primers for construction of pUC *rsaA*** | |
| rsaA-NdeI-F | CACCATATGttataaagcctcgcgcgttgaccg |
| rsaA-NheI-R | GCTAGCttaggcgagcgtcaggacttcggtg |
| | |
| rrnBT1-100F | caaataaaacgaaaggctcagtc |
| rrnBT1-100R | tcgccagcggcatcagcacc |
| | |
| Gib-rrnB-part1F | ggtcgactctggaggatgatcaaataaaacgaaaggctcagtc |
| Gib-rrnB-part1R | ctatcgtaggagctcttccacagtcgccagcggcatcagcaccttg |
| Gib-rrnB-part2F | ctgtggaagagctcctacgatagcaaataaaacgaaaggctcagtc |
| Gib-rrnB-part2R | gtaaaacgacggccagtgagattcgccagcggcatcagcaccttgtc |
| | |
| pUCTleftrrnB-d-TAA | TAATAAggtcgactctggaggatgatc |
| pUCTrightrrnB | gtaaaacgacggccagtgagattc |
| | |
| rsaA-ter-0F | gcgaatctcactggccgtcgttttacgcctatacgacggcccagttgg |
| rsaA-2stopter-0R | ttgatcatcctccagagtcgaccTTATTAcatgaggattgtctcccaaaaaaaatc |
| rsaA-ter-500F | atctcactggccgtcgttttacaacatcgactacctgaccgccttcg |
| rsaA-2stopter-500R | ttgatcatcctccagagtcgaccTTATTAggcctggcggctcaggaaag |
| rsaA-ter-1kF | atctcactggccgtcgttttacggcgtgacgggtctgaccgccctgaac |
| rsaA-2stopter-1kR | ttgatcatcctccagagtcgaccTTATTAcgaagacgtgttcagggtgatc |
| rsaA-ter-2kF | atctcactggccgtcgttttacggtggttcggtcaacggcggcgacgg |
| rsaA-2stopter-2kR | ttgatcatcctccagagtcgaccTTATTAagcgcccagggtcgccgagctg |
| rsaA-ATGmutTAAF | TTAgaggattgtctcccaaaaaaaatcc |
| rsaA-ATGmutTAAR | gcctatacgacggcccagttggtg |
| | |
| rsaA-insertAT-aroundcir-F | gctcccatgcgcgccactcggtcgcag |
| rsaA-insertAT-aroundcir-R | agcgatggctatagagcaaacgtcatacg |
| amplify-CC-AT-F | cgtttgctctatagccatcgctaaacgatggggccgctgaggcggttc |
| amplify-CC-AT-R | gaccgagtggcgcgcatgggagcggtcatcgccagagcgccgccgcctg |
| | |
| 2xTAA2xter-NheIF | AAAGCTAGCtaataaggtcgactctggaggatg |
| 2xTAA2xter-NheIR | AAAGCTAGCgtaaaacgacggccagtgagattc |
| | |

| | |
|---|---|
| 675cutrsAP-R | acagcatttttctaaccggtacagcaaattc |
| 675cutrsAP-F | taagcctatacgacggcccagttggtgac |
| | |
| **To FLAG tag *rsaA* variants** | |
| FLAG-rsaAF | gacgacgacaaggcctatacgacggcccagttggtgactgcgtac |
| FLAG-rsaAR | gtccttgtagtccatgaggattgtctcccaaaaaaaatcccacac |
| | |
| **For cloning γδ *tnpR*$^{CCDAS4}$ and *res* site** | |
| deltagammaR-NdeIF | CCCATATGatgcgacttttttggttacgcacgg |
| deltagammaR-NheIR | CCGCTAGCttagttgctttcatttattactttatatac |
| deltagamma-resF | gcaaccgtccgaaatattataaattatcgcac |
| deltagamma-resR | agtcgcataaaaatgtatcctaaatcaaatatcgga |
| | |
| GD-res-left-F | cgaaccacgatgcgaggaaacgcaaccgtccgaaatattataaattatc |
| GD-res-left-R | ggttggctagcgggcatatgaaatgtatcctaaatcaaatatcggacaa |
| GD-res-right-F | tttcatatgcccgctagccaaccgtccgaaatattataaattatcgcac |
| GD-res-right-R | ctagtggatcccccgggctgcaaaatgtatcctaaatcaaatatcggac |
| around675R | cgtttcctcgcatcgtggttcggcgacc |
| around675F | tgcagcccgggggatccactagttctagag |
| | |
| deltagammaR-CCDAS4p1 | gattcttccgcgaagttatcgttatccgcgttgctttcatttattactttatatac |
| deltagammaR-CCDAS4p2 | GCTAGCttacgacgcatccgcgtagttttcagattcttccgcgaagttatcgt |
| | |
| DeltagammaR-LcumateF | taactagtagaggaagcttccgcatgcgacttttggttacgcacgggtatc |
| DeltagammaR-LcumateR | gaaaataccgcatcaggcgccatttacgacgcatccgcgtagttttcagattc |
| | |
| pMT687-cir-R | atggcgaatggcgccgcgctgatgtccggcggtg |
| pMT687-cir-F | atggcgcctgatgcggtattttctccttac |
| cumR-p-1 | acatcagcgcggcgccattcgccattcagcgcttgaacttggcgtagcg |
| cumR-p-2 | gcggaagcttcctctactagttac |
| | |
| TetAR-NheIF | aaacGCTAGCtcaatcgtcacccttttctcggtccttc |
| TetAR-NheIR | aaacGCTAGCtcagcgatcggctcgttgccctgcgc |
| | |

| **For cloing *(lacO)*$_{10}$ cassettte** | |
|---|---|
| lac10-array-NdeIF | AAACATATGcaaattgttatccgctcacaattcactttccttaattgttatc |
| lac10-array-NheIR | AAAGCTAGCggaattgtgagcggataacaattgaataaggcactctagcac |
| | |
| **For RT-PCR product 1 to 5** | |
| rsaA-rtPCR-F1 | gtacgcgactcaaaccca |
| rsaA-rtPCR-R1 | gaagaactggtaggtctggatg |
| rsaA-rtPCR-1000F | gaacctgttcaccgcctatc |
| rsaA-rtPCR-1000R | gacttcacccgcaacgaa |
| rsaA-rtPCR-2000F | aacgtcaatggtctgacgac |
| rsaA-rtPCR-2000R | tcgaaccagcgatgttgatg |
| rsaA-rtPCR-3000F | caacgttgcggtgaatgtc |
| rsaA-rtPCR-3000R | acagggtcaggttgaacac |
| ruvA-rtPCR-F | cgagtgaggaagccgtagag |
| ruvA-rtPCR-R | gaccctgttgcacatcgag |

## APPENDIX TABLE S3. Hi-C DATASETS

| Hi-C datasets | Restriction enzyme | Number of qualified reads | GEO number |
|---|---|---|---|
| | | | |
| ML2000 90 minutes after vanillate withdrawal, synchronized | BglII | 8,210,265 | This study, GSE74364 |
| ML2000 150 minutes after vanillate withdrawal, synchronized | BglII | 6,037,764 | This study, GSE74364 |
| ML2000 210 minutes after vanillate withdrawal, synchronized | BglII | 5,158,937 | This study, GSE74364 |
| ML2000 270 minutes after vanillate withdrawal, synchronized | BglII | 3,971,613 | This study, GSE74364 |
| pJS14-$P_{xyl}$-*ctrAD51EΔ3Ω* 0 hr after xylose addition to 0.3%, synchronized | NcoI | 7,712,255 | This study, GSE74364 |
| pJS14-$P_{xyl}$-*ctrAD51EΔ3Ω* 2 hr after xylose addition to 0.3%, synchronized | NcoI | 8,906,455 | This study, GSE74364 |
| pJS14-$P_{xyl}$-*ctrAD51EΔ3Ω* 3 hr after xylose addition to 0.3%, synchronized | NcoI | 9,311,850 | This study, GSE74364 |
| CB15N in PYE, synchronized | BglII | 6,652,977 | Le *et al*, 2013, GSE45966 |
| CB15N in M2 for 90min, synchronized | BglII | 10,942,898 | This study, GSE74364 |
| CB15N + 50 µg/ml chloramphenicol for 30min, synchronized | BglII | 6,875,770 | This study, GSE74364 |
| ML2539, synchronized | BglII | 6,875,846 | This study, GSE74364 |
| ML2540, synchronized | BglII | 5,228,001 | This study, GSE74364 |
| ML2541, synchronized | BglII | 8,672,274 | This study, GSE74364 |
| ML2542, synchronized | BglII | 8,329,099 | This study, GSE74364 |
| ML2543, synchronized | BglII | 7,200,105 | This study, GSE74364 |
| ML2544, synchronized | BglII | 7,871,704 | This study, GSE74364 |
| ML2545, synchronized | BglII | 9,922,661 | This study, GSE74364 |
| ML2546, synchronized | BglII | 3,682,866 | This study, GSE74364 |
| ML2121 (TLS87), synchronized | BglII | 3,376,799 | Le *et al*, 2013, GSE45966 |
| ML2122 (TLS91), synchronized | BglII | 7,708,164 | Le *et al*, 2013, GSE45966 |
| CB15N in M2 for 90min, replicate 2, synchronized | BglII | 5,152,787 | This study, GSE74364 |
| ML2539, replicate 2, synchronized | BglII | 5,419,406 | This study, GSE74364 |
| ML2545, replicate 2, synchronized | BglII | 4,282,233 | This study, GSE74364 |
| ML2546, replicate 2, synchronized | BglII | 5,191,338 | This study, GSE74364 |