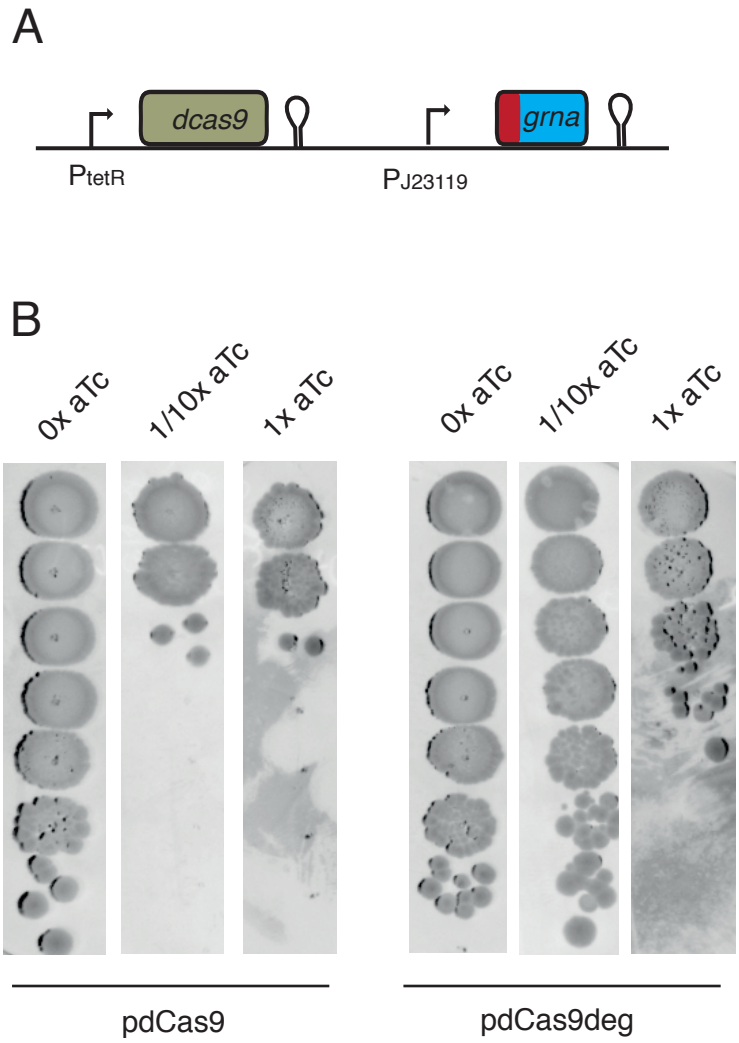
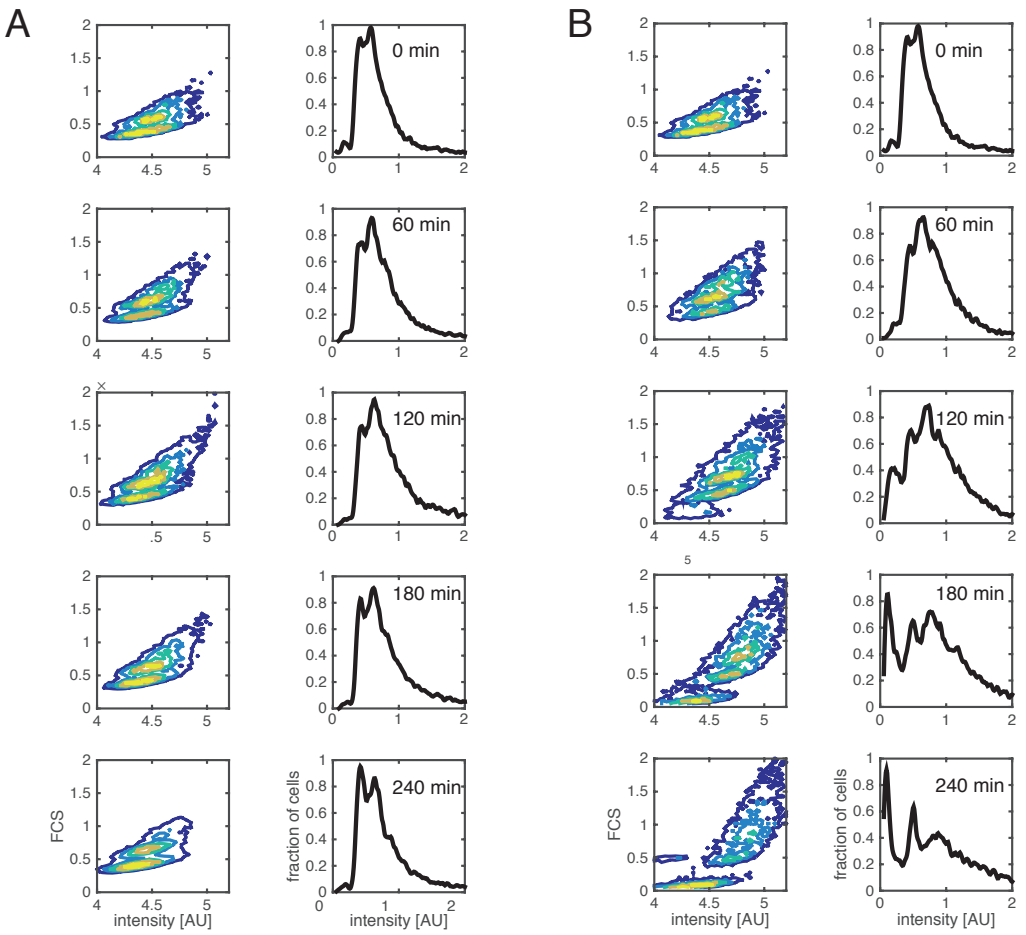
SUPPLEMENTARY MATERIAL

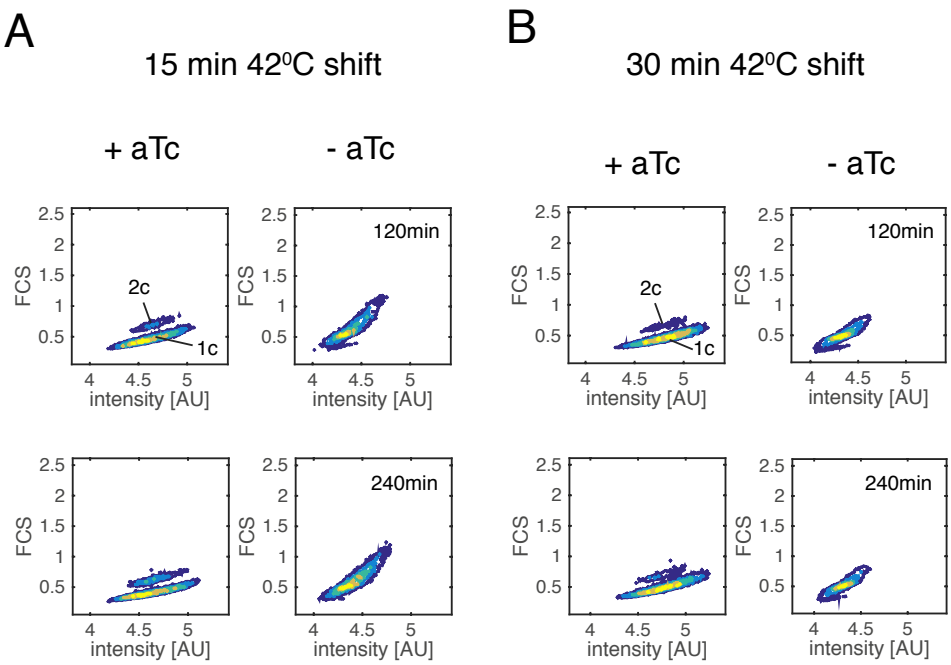
SI to the paper entitled “CRISPR-mediated control of the bacterial initiation of replication”, by Jakub Wiktor, Christian Lesterlin, Dave J. Sherratt, Cees Dekker



Supplementary Figure S1. Creation of the pdCas9deg3 plasmid. (**A**) A pdCas9deg3 plasmid consist of *dCas9deg*-coding gene under the control of an aTc inducible promoter and a *sgRNA*-coding gene under the control of the constitutive promoter with the p15a origin of replication. (**B**) dCas9deg protein is observed to be less effective in inhibiting the replication than dCas9 that is lacking the degradation tag. Serial ten-fold dilutions of liquid bacterial cultures were plated either on the media supplemented with 20ng/ml or 200ng/ml of aTc or lacking aTc. Cells were also co-transformed with pgRNA3.



Supplementary Figure S2. The two-plasmid system does not preserve the cell physiology well. CRISPR/dCas9 system was expressed from two plasmids, pdCas9deg and pgRNA. Cells were grown in M9 glucose at 37°C. Early exponential culture was induced with 200ng/ml of aTc and analyzed with flow cytometer every 60 minutes. DNA was stained with Syto16. (**A)** Uninduced culture maintains a constant DNA/cell concentration throughout the experiment. (**B**) The induced culture shows an anomalous distribution of cell sizes. The increasing population of small, but highly fluorescent cells suggests that overexpression of CRISPR/dCas9deg system binding to the *oriC* has negative effects on the bacterial cells.



Supplementary Figure S3. Short temporal shift of CRISRP/dCas9deg3 arrested cells to high temperature does not promote replication reinitiation. Cells transformed with pdCas9deg3 were grown in M9 media with 0.2% glucose, and arrested by addition of 200ng/ml aTc for 3h before the pulse of temperature shift. Before shifting to 42°C cells were diluted 10x in the fresh M9 media without aTc and analyzed by FACS 2h and 4h after the shift. (**A)** 15 minute incubation in 42°C and subsequent growth in 37°C did not result in the replication reinitiation. Culture induced with aTc showed the arrested phenotype, whereas non-induced cultures showed the replicating cell phenotype. (**B**) Same as in (A), but the cultures were incubated at 42°C for 30 minutes before subsequent growth at 37°C.

Supplementary Figure S4. Detailed genetic map of the origin of replication, and surrounding sequence, with gRNA targets indicated. Constructs targeting top strands are depicted on the top of the sequence; constructs targeting bottom strands are shown as lines at the bottom of the sequence. Red – high-affinity DnaA boxes; green – AT rich region; purple – parts of gene-coding regions flanking the *oriC.*

Table S1. Plasmids

Name	Genotype	Reference
pdCas9	p15a origin, P_{tetA}-*dCas9*	Addgene, (23)
pgRNA	ColE1 origin, P_{J23119}-gRNA	Addgene, (23)
pdCas9deg	p15a origin, P_{tetA}-*dCas9deg (AANDENYALAA)*	This study
pdCa9deg-gRNA3	p15a origin, P_{tetA}-*dCas9deg,* P_{J23119}-gRNA	This study
pgRNA1	CCCTGTGGATAACAAGGATC	This study
pgRNA2	GCACTGCCCTGTGGATAACA	This study
pgRNA3	GCCGGATCCTTGTTATCCACA	This study
pgRNA4	CTACTGTGGATAACTCTGTC	This study
pgRNA5	TTCTATTGTGATCTCTTATT	This study
pgRNA6	TTGAGAAAGACCTGGGATCC	This study
pgRNA7	ATGGATTGAAGCCCGGGCCG	This study
pgRNA8	ATTGTACGCTGTGAACGCGT	This study
pgRNA9	CACGGAACTTCAGTCCCATT	This study
pgRNA10	GTCAGGAAGCTTGGATCAAC	This study
pgRNA11	GAGGCAGAACTCAAAAATTC	This study
pgRNA12	GATCATTAACTGTGAATGAT	This study
pgRNA13	AGCTTATACGGTCCAGGATC	This study
pgRNA14	TTGCGTTTAGTATCCTAAAC	This study
pgRNA15	ATGAGCTGCAACCCGGCGAT	This study
pgRNActrl	CCGCCGTGTCACTTTCGCTTTGG	This study

Table S2. Oligonucleotides (displayed on next page)

Underline in pgRNA oligonucleotides indicate targeted sequence

Name	Sequence (5' – 3')	Purpose
JW096	GGACTAGTATTATACCTAGGACTGAG	pgRNA cloning reverse
JW090	CCACTAGT*CCGCCGTGTCACTTTCGCTT*GTTTTAGAGCTAGAAATAGCAAG	pgRNA ctrl integration
JW092	CCACTAGT*CCCTGTGGATAACAAGGATC*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 1 integration
JW093	CCACTAGT*GCACTGCCCTGTGGATAACA*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 2 integration
JW094	CCACTAGT*GCCGGATCCTTGTTATCCACA*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 3 integration
JW095	CCACTAGT*CTACTGTGGATAACTCTGTC*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 4 integration
JW098	TAACTCGAGTAAGGATCTCCAG	pdCas9 for
JW099	CCCTCGAGTTAAGCAGCCAGAGCGTAGTTTTCGTCGTTAGCAGCGTCACCTC CTAGCTGACTCA	pdCas9 LAA
JW121	TACCTAGGGATATATTCCGCTTCCCTAAAGATCTTTGACAGCTAGCTC	pgRNA for CPEC
JW122	GACCGAGCGTAGCGAGTCGGATCCAGTTCACCGACAAAC	pgRNA rev for CPEC
JW124	GACTCGCTACGCTCGGTC	pdCas9 for CPEC
JW125	GGAAGCGGAATATATCCCTAG	pdCas9 rev CPEC
JW148	CCACTAGT*TTCTATTGTGATCTCTTATT*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 5 integration
JW149	CCACTAGT*TTGAGAAAGACCTGGGATCC*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 6 integration
JW150	CCACTAGT*ATGGATTGAAGCCCGGGCCG*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 7 integration
JW151	CCACTAGT*ATTGTACGCTGTGAACGCGT*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 8 integration
JW154	CCACTAGT*CACGGAACTTCAGTCCCATT*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 9 integration
Jw158	CCACTAGT*GTCAGGAAGCTTGGATCAAC*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 10 integration
Jw159	CCACTAGTGAGGCAGAACTCAAAAATTCGTTTTAGAGCTAGAAATAGCAAG	pgRNA 11 integration
Jw160	CCACTAGT*GATCATTAACTGTGAATGAT*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 12 integration
Jw161	CCACTAGT*AGCTTATACGGTCCAGGATC*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 13 integration
Jw162	CCACTAGT*TTGCGTTTAGTATCCTAAAC*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 14 integration
Jw163	CCACTAGT*ATGAGCTGCAACCCGGCGAT*GTTTTAGAGCTAGAAATAGCAAG	pgRNA 15 integration