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Corresponding author(s): Chenli Liu

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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	The cell counting, population-averaged cellular oriC, and FSC data were collected by software CytExpert2.0 (2.1, 2.2) (Beckman, CytoFLEX (S)). Imaging data were collected using the software NIS-Elements AR 4.50 (Nikon Ti-E Microsystems). The deep sequencing experiments were conducted by BGI, and the data was collected using an Illumina HiSeq 2000 system. The proteomics experiments were conducted by Jingjie PTM Biolabs, and the data were collected using an Orbitrap Fusion Lumos (Thermo). Numerical simulation codes were created in MATLAB R2010a (MathWorks).
Data analysis	CytExpert 2.0 (2.1, 2.2) was used to process the data for cell counting, the population-averaged cellular oriC data, and FSC. The software was upgraded following the manufacturer's suggestion; we confirmed that the data presented in this study were not affected by the version of software used. Image data were processed using MicrobeTracker (0.937), a MATLAB-based package, following the documentation of the software. Python packages including Plotly, Pysam , Biopython, and SKlearn were used for deep sequencing data analysis. Programs including Bowtie2 (version 2.2.9, mode 'very sensitive local') and Samtools (version 1.6) were used for reads alignment based on genome as reference. Then genome was separated into segments and relationship between GC content of each segment and read coverage was first analyzed. ComputeGCBias and CorrectGCBias in DeepTools (3.3.1) were used for computing and correcting GC bias and producing of reads after correction. The MS/MS data were processed as described in the methods by using Maxquant search engine (v.1.5.2.8).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. The deep sequencing data used to characterize the λC and C period have been deposited in NCBI's BioProject though accession code PRJNA615952. Source data for Figs. 1a-d, 2a, 2b, 3a, 3d, 4a, 4c, and Extended Data Figs. 3b-d, 5, 7a, 7b are provided with the paper.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. We used over 30 kinds of growth media in the current study to study bacterial cell cycle regulation. For most growth conditions (26 out of 32 kinds of growth media), we have performed at least three biological independent experiments (n=3-9) to quantify the cell cycle related parameters; for the rest growth conditions (6 out of 32 kinds of growth media), we find the data to follow the same tendency as the other 26 kinds of growth media do. We believe the sample sizes are sufficient because both the kinds of growth media and the range of growth rates spanned is more than in previous studies that are focused on the same or similar topic.
Data exclusions	Triple biological replicates for each growth condition (16 growth conditions in total) were used to quantify the C period by deep sequencing. When plotting as presented in Extended Data Figure 6c, for 6 out of 48 samples, exhibited aberrant fluctuation over the whole chromosome, which was not seen in the other sample. We thus excluded data obtained from these 6 samples in the current study.
Replication	Except for those mentioned in the "Data exclusions" section, all attempts at replication were successful. The exact replication number for each experiment is listed in Extended Data Figure 8.
Randomization	Not applicable. As a non-clinical-related study, we did not take the randomization into the experimental design. However, for many of the experimental data presented in the paper, the data have been cross validated by using data obtained by different authors in different biologically independent experiments.
Blinding	Data collection followed the exact same predetermined protocols throughout the whole study, so no blinding was performed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	To avoid unnecessary loss of cells, no centrifugation was used during the sample preparation process for flow cytometer analysis. Samples for cell counting and FSC characterization were firstly fixed with pre-cooled cell counting buffer (0.9 % NaCl with 0.12 % formaldehyde). The fixed samples were diluted as necessary with staining buffer (0.9% NaCl with 1 μg/ml DAPI) prior to the flow cytometer analysis. Samples for population-averaged cellular oriC number were firstly fixed with pre-cooled 100% ethanol for more than 30 minutes at 4 degrees. The fixed samples were stained with the staining buffer (20 mM Tris·HCl, pH 8.0, 130 mM NaCl, 0.1% Triton X-100, 10 ng/mL DAPI) prior to the flow cytometer analysis. Details of sample preparation are provided in the SI methods.
Instrument	Beckman, CytoFLEX (S)
Software	CytExpert 2.0 (2.1, 2.2). Software was upgraded following the manufacturer's suggestion during the period of the current study.
Cell population abundance	In the experiments for cell counting and FSC characterization, 100 μl cell suspension was analyzed by the flow cytometer. Total cell number counts in the experiments ranged from 50,000 to 200,000. In the experiments for population-averaged cellular oriC characterization, more than 20,000 cells were analyzed for each sample.
Gating strategy	DAPI staining was applied to distinguish bacterial cells from other particles (and/or the electronic noise of the instrument) in cell counting and FSC characterization experiments. The DAPI-positive particles (P1 in Figure S1a) in the DAPI-H/SSC-H plot were deemed as the bacterial cells. In FSC-A/SSC-A plots, signals within P1 formed a distinct and tight population distribution (Figure S1b), while the signals outside P1 presented as scattered points in the plot (Figure S1c). The FSC-A/SSC-A plot of the staining buffer (0.9% NaCl with 1 μg/ml DAPI) alone looks identical to Figure S1c. DAPI-H and FSC-H were used to gate the cells in the experiments to characterize the population-averaged cellular oriC. The staining buffer (20 mM Tris·HCl, pH 8.0, 130 mM NaCl, 0.1% Triton X-100, 10 ng/ml DAPI) was applied to the flow cytometer prior to experimental samples. The newly emerged signals in the experimental samples (P1 in Figure S1d) were deemed as cells. A further gate on DAPI-A/DAPI-H was applied to P1 to ensure that only single cells are used for population-averaged cellular oriC characterization (P2 in Figure S1g). Since no centrifugation was applied during the sample processing procedure, more than 98% of the particles in P1 are single cells. When displaying as a FSC-A/SSC-A plot, signals within P1 formed a distinct tight population in the plot (Figure S1e), while signals outside of P1 formed scattered points in the plot spanning over several order of magnitude (Figure S1f).

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.