nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

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	BGI using the DNBseq platform
Data analysis	Trim Galore Version 0.6.7, Bowtie 2-v2.5.3, Repliscope R package Version 1.1.1, deepTools Version 3.5.4, RELION 3.1, CTFFIND 4.1, EMAN2 v2.9

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The raw sequence data for whole genome sequencing have been deposited in the GEO database under accession number(s) GSE262693.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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 determined sample size. We determined sample sizes mainly based on previous experience and common practice in the field.
Data exclusions	No data exclusion.
Replication	All data was replicated at least twice in independent studies. All attempts of replication was successful.
Randomization	Samples were not allocated to groups.
Blinding	Investigators were not blinded during data acquisition and analysis because because visual inspection is necessary for the methods employed.

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 and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Mcm6 (a gift from Karim Labib, 1;20,000), histone H2B (Abcam, ab1790, 1:2,500), Mcm2 (a gift from Bruce Stillman, 1:10,000), Orc3 (a gift from Bruce Stillman, 1:20,000), Cdc6 (sc6317, Santa Cruz, 1:2000) and Cdt1 (a gift from Stephen P Bell, 1:10,000), GST (Abcam, ab111947, 1:1000)
Validation	All antibodies used in this study have been validated by the manufacturer and previous publications:

Anti-Mcm6, Anti-Orc3, anti-Mcm2 and anti-Cdt1 antibodies are gifts from the indicated sources. Western blots were done to validate the specificity of antibodies towards Mcm6, Mcm2 and Cdt1.
Antibodies against Cdc6 (sc6317), purchased from Santa Cruz, is now discontinuous.
Antibodies against Histone H2B (ab1790) and GST (ab111947) are commercially available in Abcam.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Yeast W303-1a
Authentication	The cell lines were used only for protein purification and functional analyses, and not further authenticated.
Mycoplasma contamination	The cell lines used in this study were not tested for this contamination.
Commonly misidentified lines (See ICLAC register)	No cell line used in this study was commonly misidentified lines.

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	Cells were fixed in 75% of ethanol. After washing with 1xPBS twice, cells were suspended in Propidium iodide (PI) and RNase A, and incubated for 30 minutes at room temperature in the dark.
Instrument	Flow Cytometer and Cell Sorter workstation (BD FACSAriaIII)
Software	FlowJo
Cell population abundance	No flow sorting was applied.
Gating strategy	No gating strategy is applied for the yeast cells.

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