Corresponding author(s): T.D. Halazonetis

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

Describe how sample size was determined.

In this study, each experimental question was tested by high-througput sequencing (more than 80 samples in total), each phenotype was therefore observed at thousands of genomic positions. Furthermore, for each experimental question we performed multiple time points, and, for critical time points, multiple biological replicates. In all cases, the results were highly reproducible as is evident from the correlation coefficient obtained for the scatter plot analyses (Supplementary Information Table 3).

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analyses.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

All attempts at replication were successful. For critical experiments, we performed 4 independent biological replicates (involving high-throughput sequencing) giving virtually identical results. For most experiments, we had at least two independent biological replicates involving high-throughput sequencing.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

This is not relevant to our study as we did not allocate samples into experimental groups.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not relevant in our study, as all procedures and, importantly, the bioinformatic analyses were performed using the same computer codes.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	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 any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	The BWA software was used for sequencing read alignment. A description of custom codes is provided in the Method section and codes used to process and graph the data are available as Supplementary Data.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.	No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	A full description of the antibodies used (Cyclin E (Novocastra, Cat. No. NCL- CYCLINE), α-Actinin (Millipore, Cat. No. 05-384), c-Myc (Cell Signalling, Cat. No. 5605)) is provided in the Method section. For these antibodies, validation statements for their use in WB (Cyclin E and α-Actinin) or IF (c-Myc) using human cells can be found on the manufacturer's websites.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	U2OS cyclin E cells were provided by J. Bartek laboratory, U2OS mycER cells were provided by M. Eilers laboratory and HeLa and hTERT-RPE1 cells were bought from ATCC.
b. Describe the method of cell line authentication used.	U2OS cyclin E cells were authenticated by SKY. For the other cell lines, we verified from the sequencing data we obtained, that the sequence read depth at each genomic position matched that of WGS data publicly available for these cell lines.
c. Report whether the cell lines were tested for mycoplasma contamination.	Yes, cell lines were routinely tested for mycoplasma contamination.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used in this study.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.	No animals were used in this study.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	This study did not involve human research participants.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

- ☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ 2. 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).
- ☒ 3. All plots are contour plots with outliers or pseudocolor plots.
- ☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	U2OS, HeLA and RPE1 cells were harvested and fixed with 90% MetOH. EdU labeling was performed using the Click-it Kit (Invitrogen Cat. No. C-10420) according to the manufacturer's instructions. The genomic DNA was stained with propidium iodide (Sigma, Cat. No. 81845) in combination with RNAse (Roche, Cat. No. 11119915001).
6. Identify the instrument used for data collection.	For flow cytometry analyses: Gallios 8 color/2 laser (Beckman Coulter) For cell sorting: MoFlo Astrios 4 lasers, 16 colors with 1 Yellow/Green laser (Beckman Coulter)
7. Describe the software used to collect and analyze the flow cytometry data.	Data were collected with the Gallios (analysis) and Summit (sorting) softwares and analyzed with the Kaluza software.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	For the REPLIseq experiment, purity of post-sort fractions was assessed by running a small aliquot of each fraction (it was ranging from 98% purity for the early S fractions to 90%purity for the late S fractions).
9. Describe the gating strategy used.	For all experiments, cells were preliminary gated according to FS/SS scatters and PI-FL4 peak/ PI-FL4 area (only cells on the diagonal were considered to remove cell doublets). The gates used for cell sorting and analyses are shown in Supplementary Figure 1.

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

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☐ Initial submission ☐ Revised version ☒ Final submission

ChIP-seq Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data deposition

1. For all ChIP-seq data:

☒ a. Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ b. Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

2. Provide all necessary reviewer access links. *The entry may remain private before publication.*	All data (raw and processed) have been deposited. Called peaks (origins) are already in Supplementary Tables 1 and 2.
3. Provide a list of all files available in the database submission.	The deposited files are listed in Supplementary Tables 6-11.
4. If available, provide a link to an anonymized genome browser session (e.g. UCSC).	Not available.

Methodological details

5. Describe the experimental replicates.	Replicates are descibed in Supplementary Tables 6-11.
6. Describe the sequencing depth for each experiment.	Sequencing depth for each experiment is described in Supplementary Tables 6-11 and Method section.
7. Describe the antibodies used for the ChIP-seq experiments.	A full desciption of the reagents and the protocols that were used is provided in the Method section.
8. Describe the peak calling parameters.	Peak calling was performed using custom codes and can be visualized using datasets and codes provided in the Supplementary Information. The lists of mapped origins (peaks) are provided in Supplementary Tables 1 and 2.
9. Describe the methods used to ensure data quality.	The parameters for peak calling are described in the Method Section of the manuscript.
10. Describe the software used to collect and analyze the ChIP-seq data.	All sequence processing was performed using custom codes that are described in the Method Section of the manuscript and are included as Supplementary Information.