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☐ Initial submission ☐ Revised version ☒ Final submission

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.

Experimental design

1. Sample size

Describe how sample size was determined.

The number of cells analysed was estimated to achieve a 95% confidence level given the expected difference between the treatment group and the controls.

2. Data exclusions

Describe any data exclusions.

No data were excluded in the course of this study.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Each individual experiment consists of 5–10 independent fields of view for analysis with variations in the size and density of cells accounting for the variability in total cell counts for each group. All PWS microscopy measurements were performed at least in triplicate.

4. Randomization

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

Samples were randomly allocated to each group.

5. Blinding

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

Experiments were blinded when assessing the outcome of the treatment to the cells during PWS microscopy.

Note: all studies involving animals and/or human research participants must disclose 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 (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals 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

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.	Code for generating the output for the Monte Carlo and Brownian Dynamics simulations and for the predicted and experimental results of the chromatin packing macromolecular crowding (CPMC) model can be found on GitHub: https://github.com/BiophotonicsNU/macrogenomics Excel was used to test statistical significance between groups for PWS measurements. Mathematica v10 was used to analyze gene expression and to generate figures. Matlab 2015b was used for image acquisition and PWS microscopy analysis.

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 for-profit company.	RNA-seq data analyzed for this manuscript is available in the NCBI GEO database from Lee et al.,2014, accession number SRP040309, and Li et al. 2012, accession number GSE35126. Microarray source data for Figure 3 and Supplementary Figure 5 has been provided as Supplementary Table 1. Normalized Σ values for Figures 4 and 5 as well as Supplementary Figures 1 and 2 have been provided as Supplementary Table 2. All other data supporting the findings of this study are available from the corresponding authors on reasonable request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	No antibodies were used during this study.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	All parental cell lines were purchased from ATCC.
b. Describe the method of cell line authentication used.	No authentication was performed aside from manufacturer authentication provided by ATCC.
c. Report whether the cell lines were tested for mycoplasma contamination.	Hoechst staining was used in combination with live cell microscopy to assess for mycoplasma. No findings consistent with mycoplasma were observed.
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.	N/A

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 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.	No humans were used in this study.

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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.	Cells (from ATCC) were trypsinized and immediately stained with 2µM Caspase-3/7 and 4µM Hoechst 33342 for 30 min. Cells were then centrifuged for 5 minutes at 500xg, washed with PBS, and resuspended in 1mL of fresh media. Mock-stained cells were collected under the same preparation conditions.
6. Identify the instrument used for data collection.	BD LSRII at Northwestern University Flow Cytometry Core
7. Describe the software used to collect and analyze the flow cytometry data.	Analysis of flow cytometry was performed using open source Python software package, FlowCytometeryTools 0.4.5.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	We were not sorting populations of cells based on primary markers. Rather, we were determining the fraction of the population that was apoptotic after a given treatment. For control cells, less than 0.1% of the cells were caspase-3/7 positive.
9. Describe the gating strategy used.	Gates were set for Cas3/7 staining and Hoechst 33342 to minimize false positives from unstained cells (<0.1% of total). Percentage of apoptotic cells was assessed as the ratio of Cas3/7+ cells divided by the population of Hoechst 33342 positive cells. Errorbars represent uncertainty based on ±10% change in gating thresholds.

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