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Corresponding author(s): Tom W. Muir

☐ 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.

> For all biochemical experiments as sample size was chosen as this is standard, and the minimum number of samples/replicates needed to generate statistical parameters.

2. Data exclusions

Describe any data exclusions.

> Three nucleosomes present in the described nucleosome library were removed from the analysis as their unique nucleosome identifier barcodes created an additional PstI site with the surrounding nucleotide sequence. This renders accurate analysis of remodeling rates of these nucleosomes impossible. This and the nucleosomes in question are declared in the methods section.

3. Replication

Describe whether the experimental findings were reliably reproduced.

> All attempts at experimental replication were successful.

4. Randomization

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

> This is not relevant to our study. All data generated were results of specific biochemical conditions, and, thus, "grouping" of resultant data in any other way would be inappropriate.

5. Blinding

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

> Blinding was not relevant to our study as "group allocation" as described was not performed.

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 <u>exact sample size</u> (*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 <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (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.

> The Princeton University installation of GALAXY and a custom script written in R (for simple barcode counting; available upon request) were used to process all sequencing data. GraphPad Prism was used for all rate calculations and plotting bar graphs. Image Studio Lite (LI-COR) was used for all densitometry measurements. All heatmaps were generated in Python using the Seaborn module. Microsoft Excel was routinely used where applicable. All of this information is also stated in the methods section of the manuscript.

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.

> There is no restriction on availability of materials, though all requests should be directed to Tom W. Muir (muir@princeton.edu)

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

> A rabbit polyclonal anti-H3K4me3 antibody (Abcam: ab8580; lot #: GR190237-1) was used to validate nucleosome library integrity.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

> Sf9 cells from Invitrogen were used (Catalog number: 12659017)

b. Describe the method of cell line authentication used.

> Cells are a product prepared by the supplier (see above)

c. Report whether the cell lines were tested for mycoplasma contamination.

> Cells were not 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.

▶ Animals and human research participants

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

> No animals were used.

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

> The study did not involve human research participants.