

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                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                                |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), 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 | All bioinformatic tools used in this study are open source and freely available. Hifiasm was used to generate assemblies from PacBio HiFi data. RepeatMasker was used to identify non-satellite repeats. NCBI BLAST85 was used for sequence similarity searches, StainedGlass was used to create sequence identity heatmaps, HiGlass was used to visualize sequence identity maps, and CENdetectHOR was utilized for HOR detection. RNA sequencing data were aligned using the STAR aligner, while chromatin profiling data were aligned using Bowtie2. Meryl was used for k-mer filtering. PacBio HiFi reads were mapped to T2T and Hifiasm assemblies using minimap2, and DNA methylation calling was performed using modkit. Figure 10 was created with BioRender.com under an academic license, in compliance with the BioRender publication guidelines. Hifiasm assemblies and annotation files for genomic maps of satellite regions in mouse T2T and Hifiasm assemblies can be accessed using the following link.<br><a href="https://github.com/nacpatil/Repeats">https://github.com/nacpatil/Repeats</a> |
| Data analysis   | Hifiasm assemblies and annotation files for genomic maps of satellite regions in mouse T2T and Hifiasm assemblies can be accessed using the following link.<br><a href="https://github.com/nacpatil/Repeats">https://github.com/nacpatil/Repeats</a>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

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](#)

Raw genomics and epigenomics data has been deposited to NCBI SRA under Bioproject number PRJNA1173940 and can be accessed at the following link: <https://dataview.ncbi.nlm.nih.gov/object/PRJNA1173940?reviewer=1frnl4j5o23ukda2nn8l02ovg1>

Assembly sources:

The mhaESC T2T assembly (Liu et al. 2025) was accessed from the following link: [https://github.com/yulab-ql/mhaESC\\_genome/releases](https://github.com/yulab-ql/mhaESC_genome/releases)

The mESC T2T assembly (Francis et al. 2024) was accessed using GenBank accession number: GCA\_964188535.1

Sequencing data:

PacBio HiFi reads for the mhaESC cell line were accessed from NCBI BioProject: PRJNA1097000I

PacBio HiFi reads for the female C57BL/6J mouse were accessed from the NCBI SRA: SRR11606870

Additional resources:

Hifiasm assemblies and annotation files for genomic maps of satellite regions in mouse T2T and Hifiasm assemblies are available at: <https://github.com/nacpatil/Repeats>

## 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                                        | NA |
| Reporting on race, ethnicity, or other socially relevant groupings | NA |
| Population characteristics                                         | NA |
| Recruitment                                                        | NA |
| Ethics oversight                                                   | NA |

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](https://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     | RNA expression data was collected from three different animals to generate three replicates. Data analysis was performed on two different mouse T2T assemblies and three different Hifiasm assemblies. |
| Data exclusions | NA                                                                                                                                                                                                     |
| Replication     | Centromeres and pericentromeres from all chromosomes were analyzed. Two different reference assemblies and three different Hifiasm assemblies were analyzed.                                           |

|               |    |
|---------------|----|
| Randomization | NA |
| Blinding      | NA |

## 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                                           |
|-------------------------------------|-----------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                                 |

### Methods

| n/a                                 | Involved in the study                           |
|-------------------------------------|-------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Antibodies

|                 |                                                                                                                                                                                                                                                                                |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | anti-CENP-A antibody from Cell Signaling Technologies - #C51A7, anti-CENP-B antibody from Santa Cruz - # sc-376392, anti-H3K9me3 antibody from abcam - #ab8898, anti-H3K27me3 antibody from Cell Signaling Technologies - # 9733, and anti-IgG antibody from abcam - # ab46540 |
| Validation      | All antibodies were validated in previous studies.                                                                                                                                                                                                                             |

## Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

|                         |                                                                                   |
|-------------------------|-----------------------------------------------------------------------------------|
| Laboratory animals      | Male C57BL/6J mouse strain                                                        |
| Wild animals            | NA                                                                                |
| Reporting on sex        | We generated data from a male and analyzed publicly available data from a female. |
| Field-collected samples | NA                                                                                |
| Ethics oversight        | Institutional IACUC                                                               |

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

## Plants

|                       |    |
|-----------------------|----|
| Seed stocks           | NA |
| Novel plant genotypes | NA |
| Authentication        | NA |