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	Next Generation Sequencing was conducted on a Illumina NextSeq 550, and initially processed on the preinstalled software from Illumina.
Data analysis	All scientific software and packages used for Repli-seq processing is publically available and free of charge. Most of this is published in scientific journals, which are cited in the methods section. The software used is: FastQC, FastqScreen, Cutadapt, SNPsplit, Bowtie 2, Samtools, BedTools, EaSeq (EaSeq and source code is available at http://easeq.net), R, Microsoft Excel 2016 R-packages: Zoo, Fbasics, Mixtools, Copynumber, NSPsplit, Aneufinder, Beeswarm GitHub code deposits: Scripts for diploid and haplotype-resolved Repli-seq processing were acquired from https://github.com/kuzobuta/scRepliseq-Pipeline and https://github.com/kuzobuta/scRepliseq-Pipeline/tree/master/scripts/haplotype-resolved-analysis.

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

To review GEO accession GSE237400

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	To ensure data was reproducible, sample size was chosen to ensure that data generated was consistent across experiments. Repli-seq data was generated from single embryos and single cells, the total of which is indicated in each figure panel, and sample collection was done at least twice in all core datasets.
Data exclusions	We followed the standard procedure for single cell Repli-Seq protocols (Dileep and Gilbert 2018, Miura et al 2019) by excluding G1 cells with abnormal karyotypes from our analysis, as they can affect the accuracy of the results. Specifically we excluded two single haplotype from a 2-cell embryo (CAST/EiJ haplotype) and 2 mESC cells due to their abnormal karyotypes. Additionally, in the case of zygote embryos, we excluded several embryos from the analysis if there were no apparent reads that mapped to the paternal haplotype, indicating a likely lack of fertilization. A total of 13 zygotes from 3 experiments were excluded for this reason. A total of 5 repeat experiments failed, of which 3 were abandoned due to low oocyte numbers or poor fertilization, 1 due to experimental error and another due to obtaining no amplification during whole genome amplification.
Replication	For EdU incorporation in zygotes, three independent experiments were conducted, each generating multiple independently imaged embryos. similarly, for EdU incorporation in 2-cell stage embryos, three independent experiments were performed, each also generating multiple independently imaged embryos. The core repli-seq data from zygotes and 2- cell embryos, were collected from three and two experiments, respectively, each collection generated many embryos sequenced as a single embryo (numbers is reported in the figure panel). Additionally, control experiments on zygotes and 2-cell stage embryos were conducted, amounting to three further independent experiments. Full details of each experiment can be found in the source data. Each biological state is represented by multiple replicates, which whenever possible are visualized independently to give the reader full insight into the variation in the data.
Randomization	Cells and embryos were allocated at random into experimental groups. Data from different stages were sequenced together in the same runs, and no run consisted of a large overweight of a single stage.

Blinding	Blind testing can be used when items are to be compared without influences from testers' expectations. We believe that this was not relevant for our Repli-seq study as data were analyzed with computational metrics and no human scoring involved.

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

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	F121-9 (CVCL_VC42) - gift from Professor Joost Gribnau (Erasmus MC, Rotterdam, Netherlands)
Authentication	None
Mycoplasma contamination	The cell line was confirmed to be free from Mycoplasma.
Commonly misidentified lines (See ICLAC register)	N/A

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	The study involved Mus musculus. The following numbers, sex, strains and ages of mice were used in this study: C57/BL6N (Janvier Labs) = 48 (female, 4-6 weeks) 3 (male, 10 weeks-8 months) CAST/EiJ (Jackson Laboratory, JAX) =10 (female, 5-8 weeks) 7 (male, 12 weeks- 8 months). Full details of mice used in each experiment can be found in the methods and source data.
Wild animals	The study did not involve wild animals
Reporting on sex	Embryos were selected at random, ensuring no intentional bias regarding the sex of the embryos.
Field-collected samples	Study did not involve field collected samples.
Ethics oversight	All experiments involving animals were performed under the authorization of the Danish Animal Ethical Committee ("Dyreforsøgstilsynet") permission 2021-15-0201-009 and are compliant with all relevant national ethical regulations regarding animal research under the requirement of Directive 2010/63/EU. All animals were exposed to 12 hours of light (6:00 til 18:00) and were provided with water and standard mouse chow ad libitum and cages maintained at 20-24°C and 45-65% humidity under the care of veterinarians.

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Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A