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Reporting Summary

Nature Research 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 Research 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	Detailed in the manuscript
Data analysis	Detailed in the manuscript

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Published available sequencing raw and processed datasets analyzed in this work are available in GSE110354 for ChOR-seq and GSE117274 for SCAR-seq.

Data analyzed in Figure 3 a and b correspond to GSM2988387, GSM2988389 and GSM2988390. Data shown in Figure 3c are: GSM3290321, GSM3290334, GSM3290324, GSM3290344 and GSM3290342. Data used in Figure 3d correspond to the average signal of all relevant replicates (GSE117274). Figure 3d is adapted from (Petryk et al., 2018, PMID: 30115746), as permitted under the AAAS's license to publish.

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	No sample-size calculation was perfeormed. The sample size is determined by the number of reads obtained by the sequencer machine. The differences between samples are corrected by normalization by million of reads (RPM).
Data exclusions	No data exclusion was applied
Replication	In the original ChOR-seq and SCAR-seq studies (Reverón-Gómez N. et al 2018, and Petryk N. et al 2018, respectively) a minimum of two replicates per condition were used showing a high degree of reproducibility. For ChOR-seq analysis done in synchronized Hela S3 cells, although very similar, replicated regions of the genome were not identical in the different replicates due to slight differences in the release after synchronisation. Thus, it is preferable to analyze each replicate separately. In the figure 3 of this work, one representative replicate was shown except for the Figure 3d, which represents the averaged partitioning signal of all replicates and is adapted from (Petryk et al., 2018, PMID: 30115746), as permitted under the AAAS's license to publish.
Randomization	Not applicable. This a protocols paper with no biological conclusion intended
Blinding	Not applicable. This a protocols paper with no biological conclusion intended

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Described in Table 2
Validation	The commercial antibodies were validated by the manufacturers. For H4K20me2 antibody (Diagenode, C15200205) were additionally validated by dot-blot (described in Petryk et al 2018 (PMID: 30115746)).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Human HeLa S3 cells (Cat. No. CCL-2-2; RRID: CVCL_0058); Mouse E14 ES Cells (Laboratories of Kristian Helin and Joshua Brickman; RRID:CVCL_C320); D. melanogaster S2-DRSC (Drosophila Genomics Resource, Center; Stock No. 181)
Authentication	None were authenticated
Mycoplasma contamination	Negative for mycoplasma
Commonly misidentified lines (See ICLAC register)	None used.

ChIP-seq

Data deposition

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

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

Data access links
May remain private before publication.

To review GEO accession GSE110354 (ChOR-seq) go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE110354
To review GEO accession GSE117274 (SCAR-seq) go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi

Files in database submission

In GSE110354:
GSM2988386 H3K27me3_ChIP-seq_Parental_rep1
GSM2988387 H3K27me3_ChOR-seq_T0_rep1
GSM2988388 H3K27me3_ChOR-seq_T4_rep1
GSM2988389 H3K27me3_ChOR-seq_T10_rep1
GSM2988390 H3K27me3_ChOR-seq_T24_rep1
GSM2988391 H3K27me3_ChOR-seq_T0+EZH2i_rep1
GSM2988392 H3K27me3_ChOR-seq_T4+EZH2i_rep1
GSM2988393 H3K27me3_ChOR-seq_T10+EZH2i_rep1
GSM2988394 H3K27me3_ChOR-seq_T24+EZH2i_rep1
GSM2988395 H3_ChIP-seq_Parental_rep1
GSM2988396 H3_ChOR-seq_T0_rep1
GSM2988397 Streptavidin_pull-down_midS_rep1
GSM2988398 H3K27me3_ChIP-seq_Parental_rep2
GSM2988399 H3K27me3_ChOR-seq_T0_rep2
GSM2988400 H3K27me3_ChOR-seq_T4_rep2
GSM2988401 H3K27me3_ChOR-seq_T10_rep2
GSM2988402 H3K27me3_ChOR-seq_T24_rep2
GSM2988403 H3K27me3_ChOR-seq_T0+EZH2i_rep2
GSM2988404 H3K27me3_ChOR-seq_T4+EZH2i_rep2
GSM2988405 H3K27me3_ChOR-seq_T10+EZH2i_rep2
GSM2988406 H3K27me3_ChOR-seq_T24+EZH2i_rep2
GSM2988407 H3_ChIP-seq_Parental_rep2
GSM2988408 H3_ChOR-seq_T0_rep2
GSM2988409 Streptavidin_pull-down_midS_rep2
GSM2988418 Input_biotin_rep1
GSM2988419 H3_ChIP-seq_biotin_Parental_rep1
GSM2988420 H3_ChIP-seq_biotin_Parental_rep2
GSM2988421 H3K27me3_ChIP-seq_biotin_Parental_rep1
GSM2988422 H3K27me3_ChIP-seq_biotin_Parental_rep2
GSM2988423 Streptavidin_pull-down_biotin_midS_rep1
GSM2988424 Streptavidin_pull-down_biotin_midS_rep2
GSM2988425 H3_ChOR-seq_biotin_T0_rep1
GSM2988426 H3_ChOR-seq_biotin_T0_rep2
GSM2988427 H3K27me3_ChOR-seq_biotin_T0_rep1
GSM2988428 H3K27me3_ChOR-seq_biotin_T0_rep2
GSM3227882 H3K4me3_ChIP-seq_Parental_rep1
GSM3227883 H3K4me3_ChIP-seq_Parental_rep2
GSM3227884 H3K4me3_ChOR-seq_T0_rep1
GSM3227885 H3K4me3_ChOR-seq_T0_rep2
GSM3227886 H3K4me3_ChOR-seq_T1_rep1
GSM3227887 H3K4me3_ChOR-seq_T1_rep2
GSM3227888 H3K4me3_ChOR-seq_T6_rep1
GSM3227889 H3K4me3_ChOR-seq_T6_rep2
GSM3227890 H3K4me3_ChOR-seq_T12_rep1
GSM3227891 H3K4me3_ChOR-seq_T12_rep2
GSM3227892 H3K36me3_ChIP-seq_Parental_rep1
GSM3227893 H3K36me3_ChIP-seq_Parental_rep2
GSM3227894 H3K36me3_ChOR-seq_T0_rep1
GSM3227895 H3K36me3_ChOR-seq_T0_rep2
GSM3227896 H3K79me3_ChIP-seq_Parental_rep1
GSM3227897 H3K79me3_ChIP-seq_Parental_rep2
GSM3227898 H3K79me3_ChOR-seq_T0_rep1
GSM3227899 H3K79me3_ChOR-seq_T0_rep2
GSM3227900 INPUT_earlyS_rep1
GSM3227901 INPUT_earlyS_rep2
GSM3227902 Streptavidin_pull-down_earlyS_rep1
GSM3227903 Streptavidin_pull-down_earlyS_rep2

In GSE117274:
GSM3290319 SCAR_seq_mESC_K20me2_r1
GSM3290320 SCAR_seq_mESC_K20me2_r2
GSM3290321 SCAR_seq_mESC_K20me2_r3

GSM3290322 SCAR_seq_mESC_K20me2_parental
GSM3290323 SCAR_seq_mESC_K20me2_MCM2_2A#1_r1
GSM3290324 SCAR_seq_mESC_K20me2_MCM2_2A#1_r2
GSM3290325 SCAR_seq_mESC_K20me2_MCM2_2A#2_r1
GSM3290326 SCAR_seq_mESC_K20me2_MCM2_2A#2_r2
GSM3290327 SCAR_seq_mESC_K36me3_r1
GSM3290328 SCAR_seq_mESC_K36me3_r2
GSM3290329 SCAR_seq_mESC_K36me3_r3
GSM3290330 SCAR_seq_mESC_K36me3_MCM2_2A#1_r1
GSM3290331 SCAR_seq_mESC_K36me3_MCM2_2A#1_r2
GSM3290332 SCAR_seq_mESC_K36me3_MCM2_2A#2_r1
GSM3290333 SCAR_seq_mESC_K36me3_MCM2_2A#2_r2
GSM3290334 SCAR_seq_mESC_K5ac_r1
GSM3290335 SCAR_seq_mESC_K5ac_r2
GSM3290336 SCAR_seq_mESC_K5ac_parental
GSM3290337 SCAR_seq_mESC_input_EdU30min_r1
GSM3290338 SCAR_seq_mESC_input_EdU15min_r2
GSM3290339 SCAR_seq_mESC_input_EdU15min_r3
GSM3290340 SCAR_seq_mESC_input_MCM2_2A#1
GSM3290341 SCAR_seq_mESC_input_MCM2_2A#2
GSM3290342 OK_seq_mESC
GSM3290343 SCAR_seq_mESC_K5ac_MCM2_2A#1_r1
GSM3290344 SCAR_seq_mESC_K5ac_MCM2_2A#1_r2
GSM3290345 SCAR_seq_mESC_input_K5ac_MCM2_2A#1_r1
GSM3290346 SCAR_seq_mESC_K5ac_MCM2_2A#2_r1
GSM3290347 SCAR_seq_mESC_K5ac_MCM2_2A#2_r2
GSM3290348 SCAR_seq_mESC_input_K5ac_MCM2_2A#2_r1

Genome browser session
(e.g. UCSC)

n/a

Methodology

Replicates

At least two replicates of each condition were performed except for the OK-seq data where only one replicate was done.

Sequencing depth

All experiments were single-end reads of 75bp length. Sequencing depth of the ChOR-seq datasets for this work:
GSM2988387 H3K27me3_ChOR-seq_T0_rep1: Total number of reads: 73.3 Millions; Unique mapped (hg19): 41,62Millions; Unique mapped after PCR duplicates (hg19):26.50 millions; Unique mapped (dm3): 16.95 millions. Unique mapped after PCR duplicates (dm3): 10.3 Millions.
GSM2988389 H3K27me3_ChOR-seq_T10_rep1: Total number of reads: 126.17 Millions; Unique mapped (hg19): 86.27 Millions; Unique mapped after PCR duplicates (hg19):53.15 millions; Unique mapped (dm3): 17.01 millions. Unique mapped after PCR duplicates (dm3): 10.29 Millions.
GSM2988390 H3K27me3_ChOR-seq_T24_rep1: Total number of reads: 132.56 Millions; Unique mapped (hg19): 97.73 Millions; Unique mapped after PCR duplicates (hg19): 61.08 millions; Unique mapped (dm3): 11.91 millions. Unique mapped after PCR duplicates (dm3): 7.41 Millions.

Sequencing stats of the SCAR-seq samples (GSE117274) are listed in Table S1 of Petryk et al 2018 (PMID: 30115746)

Antibodies

All antibodies used are described in Table2

Peak calling parameters

Not applicable in this study

Data quality

It is reported in the manuscript

Software

For ChOR-seq, public tools (Galaxy server and SeqMonk) are reported in the manuscript. For SCAR-seq, a link to custom code is provided.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 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).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Described in the manuscript
Instrument	BD FACSCalibur
Software	Data were collected with CellQuest Pro software and analyzed by FlowJo software version 10.7.1 .
Cell population abundance	2565 cells were acquired.
Gating strategy	The FSC/SSC gates defined the single-cell population. The EdU-positive cells reveal AF647 signal above the G1 cells . Gating strategy of EdU-positive cells is described in the Box 1.

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