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	No new code was developed for this study. Code used to analyze loop strength can be found at: https://github.com/jclqrs/Lam_2024_Code/tree/main/Micro-C
Data analysis	Statistical tests, description of medians and percentiles of whiskers for box plots are described in the figure legends. Please see methods section methods for detailed methods. deepTools v3.5.5 was used to count reads within gene bodies for TT-seq differential gene expression. cooltools (v0.7.1) was used to compute cis eigenvector values on 100bk binned Hi-C matrices and the calldots function of cooltools (v0.7.1) was used to identify loops. hic files were converted to cool files using hic2cool (v0.8.3). Loop strength was calculated using cooltools (v0.5.1). Stripenn (1.1.65.22) was used to call stripes. Contact matrices were visualized with coolbox (0.3.8). Pile-up plots to visualize average TADs/Stripes were generated using coolpuppy (1.1.0).

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

Hi-C and TT-seq datasets generated in this study are deposited in the GEO database (GSE256073: token: exiliogsvbmdzaz). External data from prior studies listed here: LDB1, CTCF, RAD21, YY1, and H3K27ac ChIP-seq (GSE254377), LDB1 asynchronous Micro-C (GSE254373), YY1 asynchronous Micro-C (GSE247254), SMC3 asynchronous Hi-C (GSE229179). No new ChIP-seq data was generated in this study. All ChIP-seq data is published in prior reports as indicated.

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	No sample size calculations were performed in this study. Sample sizes were chosen based on current standards in the field. 2 technical western blot replicates were performed in 2 independent clonal lines. At least 2 Hi-C biological replicates were performed in independent clonal lines.
Data exclusions	No data were excluded.
Replication	ChIP experiments were performed in 2 independent replicates. TT-seq experiments were performed in 3 independent replicates. Hi-C experiments were performed in 2 independent replicates for each condition. Replication attempts were successful.
Randomization	Randomization was not performed in this study.
Blinding	Blinding was not necessary for any experiments in this study. Blinding was not applicable to sequencing experiments. Data generation and analysis were conducted using pipelines that did not rely on investigator-dependent outcome assessment.

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

Antibodies

Antibodies used: nipbl antibody for WB: Santa cruz Sc-374625 LOT:D1922. 1:1000 dilution. The manufacture confirmed that this antibody detects Nipbl through WB. In our hands, we find that it detects a nipbl in the control untreated samples while cannot detect corresponding band in auxin treated samples, confirming that it recognizes nipbl.

Validation: anti-NIPBL antibody was used in western blot experiments to confirm NIPBL degradation in NIPBL-AID cells.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): G1E-ER4 cell line was gifted by the Mitchell J Weiss laboratory.

Authentication: This cell line is tested on a regular basis to confirm it can be induced to undergo erythroid differentiation.

Mycoplasma contamination: G1E-ER4 cells were tested to be negative for Mycoplasma.

Commonly misidentified lines (See ICLAC register): N/A

Plants

Seed stocks: *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*

Novel plant genotypes: *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*

Authentication: *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE256073

Files in database submission

GSM8351485_ZH_hic_S0272_nipbl--17_0h.allValidPairs.hic
GSM8351486_ZH_hic_S0267_nipbl--3_0h.allValidPairs.hic
GSM8351487_ZH_hic_S0274_nipbl--17_with_auxin_1h.allValidPairs.hic
GSM8351488_ZH_hic_S0269_nipbl--3_with_auxin_1h.allValidPairs.hic
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GSM8478338_wapl_smc2_M_with_a_no_d_clone9.allValidPairs.hic
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GSM8478342_wapl_smc2_M_no_a_no_d_clone9.allValidPairs.hic
GSM8478343_wapl_smc2_M_with_a_with_d_clone1.allValidPairs.hic
GSM8478344_wapl_smc2_M_no_a_with_d_clone9.allValidPairs.hic
GSM8478345_wapl_smc2_M_with_a_with_d_clone9.allValidPairs.hic
GSM8478346_wapl_smc2_M_no_a_with_d_clone1.allValidPairs.hic
GSM8478347_wapl_smc2_asyn_with_d_clone1.allValidPairs.hic

Genome browser session
(e.g. UCSC)

N/A

Methodology

Replicates

For Hi-C experiments, we performed 2 biological replicates per condition. For TT-seq experiments, we performed 3 biological replicates per condition.

Sequencing depth

Sample total_reads uniquely_mapped uniquely_mapped_percent multimapped READ_PAIRS_EXAMINED READ_PAIR_DUPLICATES PERCENT_DUPLICATION remove_dupliates fwd.bam.reads rev.bam.reads
Ttseq_S0003_nipbl_clone3_no_auxin_45min_1ug 29858262 18565396 62.18 2181478 20746874 15602152 0.75 5144722 2955842 2188880
Ttseq_S0004_nipbl_clone3_with_auxin_45min_1ug 31400584 19355131 61.64 2379002 21734133 16616542 0.76 5117591 2941985 2175606
Ttseq_S0005_nipbl_clone3_no_auxin_4h_1ug 35605924 22120176 62.12 2683901 24804077 18035766 0.72 6768311 3793375 2974936
Ttseq_S0006_nipbl_clone3_with_auxin_4h_1ug 39474350 24025224 60.86 3051725 27076949 20701009 0.76 6375940 3614751 2761189
Ttseq_S0007_nipbl_clone17_no_auxin_45min_5ug 31182257 21426517 68.71 1964922 23391439 12506428 0.53 10885011 5820222 5064789
Ttseq_S0008_nipbl_clone17_with_auxin_45min_5ug 29638120 20625255 69.59 1523194 22148449 11475183 0.51 10673266 5688639 4984627
Ttseq_S0009_nipbl_clone17_no_auxin_4h_5ug 23078170 17250712 74.75 973402 18224114 6245693 0.34 11978421 6275902 5702519
Ttseq_S0010_nipbl_clone17_with_auxin_4h_5ug 33680082 23318038 69.23 1756697 25074735 12585278 0.5 12489457 6612569 5876888
Ttseq_S0011_nipbl_clone3_no_auxin_45min_5ug 36085714 25424590 70.46 1980629 27405219 12297316 0.44 15107903 7981181 7126722
Ttseq_S0012_nipbl_clone3_with_auxin_45min_5ug 36175266 25801524 71.32 1582608 27384132 11352522 0.41 16031610 8441095 7590515
Ttseq_S0013_nipbl_clone3_no_auxin_4h_5ug 40605066 29261338 72.06 2164243 31425581 13078163 0.42 18347418 9605676 8741742
Ttseq_S0014_nipbl_clone3_with_auxin_4h_5ug 32637380 24871033 76.2 1239656 26110689 7375945 0.28 18734744 9740755 8993989

For ChIP-seq

sample total_reads con_1_time con_n_time dis_1_time sin_1_time sin_n_time rate
SFN_ZH_Chip_Rad21_S0408_nipbl_clone3_release_no_auxin 45322008 21350012 11765661 2756122 549373 888847 80.74%
SFN_ZH_Chip_Rad21_S0409_nipbl_clone3_release_with_auxin 63035350 32792637 17258580 2947802 801634 1297515 85.74%
SFN_ZH_Chip_Rad21_S0410_nipbl_clone17_release_no_auxin 63289840 32171003 16856581 3005279 893218 1360140 83.99%
SFN_ZH_Chip_Rad21_S0411_nipbl_clone17_release_with_auxin 54473957 27883986 14580407 2700592 817659 1295768 84.85%

Antibodies

anti-Rad21 abcam, catlog#ab992, lot #:1048461-1. 5ul/IP.
This antibody has been extensively used in the community. The manufacture validated that this antibody recognizes human and mouse Rad21 through WB. In our lab, it is also validated in mouse cells by ChIP-seq as it detects peaks colocalized to CTCF binding sites as expected (Zhang et al. 2019 Nature, Zhao et al. 2025 Nature, Zhao et al. 2024 Nature Genetics, Luan et al. Cell Reports 2021).

Peak calling parameters

ChIP-seq peaks were not re-called in this study. Please see prior ChIP-seq peak calling parameters from prior studies (refer to data availability statement) for previously-published datasets.

Data quality

Sequencing quality was assessed with FastQC.
Correlation between Hi-C replicates was assessed using eigenvector 1 values.
TAD/stripe formation was assessed for each individual replicate and found to be concordant amongst replicates..

Software

The following software was used to process/analyze ChIP-seq data: Trim Galore(0.6.6), Bowtie2(2.3.5.1), SAMtoos(1.9), Picard(2.23.3), deepTools(3.5.5), and bedtools(v2.31.1).