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	Nikon NIS-Elements software (v 4.6) for collecting single-molecule imaging Zeiss Zen Blue imaging software (v 3.1) for collecting confocal and Airyscan imaging
Data analysis	Sequencing analysis: TrimGalore (v 0.6.5) (https://github.com/FelixKrueger/TrimGalore), Bowtie2 (v 2.3.0) (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml), samtools (v 1.9) (http://www.htslib.org/), deepTools (v 2.4.1 & v 3.5.0) (https://github.com/deeptools/deepTools), MACS (v 2.2.6) (https://github.com/macs3-project/MACS), MAnorm2 (v 1.0) (https://github.com/tushiqi/MAnorm2), HiC-Pro (v 2.11.3) (https://github.com/nservant/HiC-Pro), COOLER (v 0.8.10) (https://github.com/open2c/cooler), JUICER (v 1.22.01) (https://github.com/aidenlab/juicer), HiGlass (v 1.11.7) (https://github.com/higlass/higlass), cooltools (v 0.3.2) (https://github.com/open2c/cooltools), 3DChromatin_ReplicateQC (v 1.0.1) (https://github.com/kundajelab/3DChromatin_ReplicateQC), coolpuppy (v 0.9.5) (https://github.com/open2c/coolpuppy), Mustache (v 1.0.1) (https://github.com/ay-lab/mustache), chromosight (v 0.9.8) (https://github.com/koszullab/chromosight), chromHMM (v 1.22) (http://compbio.mit.edu/ChromHMM/), Bedtools (v 2.30.0) (https://bedtools.readthedocs.io/en/latest/index.html), kallisto (0.46.2) (https://github.com/pachterlab/kallisto), DeSeq2 (v 1.30.1) (https://bioconductor.org/packages/release/bioc/html/DESeq2.html), NRSA (v 2.0) (http://bioinfo.vanderbilt.edu/NRSA/), Rsubread (v 1.22.2) (https://bioconductor.org/packages/release/bioc/html/Rsubread.html) Imaging analysis: ImageJ (v 1.53c) (https://imagej.nih.gov/ij/), Spot-on (v 1.0.4) (https://gitlab.com/tjian-darzacq-lab/spot-on-matlab), quot (v 3.0) (https://github.com/alecheckert/quot), SASPT (v 1.0) (https://github.com/alecheckert/saspt), Thunderstorm (v 1.3) (https://github.com/zitmen/thunderstorm) Flow cytometry analysis: FlowJo (v 10.3) (https://www.flowjo.com/)

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

The Micro-C, ChIP-seq, nascent RNA-seq and total RNA-seq data generated in this publication are available in NCBI Gene Expression Omnibus through GEO Series accession number GSE178982. We also reanalyzed data that we previously generated in wild type mESCs (GSE130275). spaSPT raw data are accessible through DOI: 10.5281/zenodo.5035837. The reference genome mm10 and sacCer3 are available through UCSC genome browser (https://hgdownload.soe.ucsc.edu/downloads.html).

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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	1) Confocal/Airyscan imaging is mainly qualitative. We routinely collected at least six regions of interest (ROIs) with two different cell cultures on two separate days; 2) FRAP: We generally collected data from at least 6 cells per cell line per condition per day, and all presented data are from at least two independent replicates on different days; 3) SPT: We recorded movies for six cells per cell line or condition per day, and all data presented are from at least two independent experiments conducted corresponding to at least 12 cells and at least 100,000 localizations. No statistical method was used to predetermine sample size. We chose the sample size as suggested in the previous report (doi: 10.7554/elife.25776).
Data exclusions	1) FRAP: We excluded data if the bleached spot is not detectable by our algorithm, and if the cell drifted away from the focus during acquisition; 2) SPT: We excluded data if the total localization was lower than 20,000 per cell.
Replication	Sequencing data: We generally collected at least 2 biological replicates per condition per day to gain statistical power. For some samples (Micro-C_ΔCTCF_IAA, Micro-C_ΔRAD21_IAA, Micro-C_ΔWAPL_IAA), we performed pilot tests so that the sample sizes will increase to 4. Imaging data: We generally collected at least 2 biological replicates per condition per day. Immunoblotting, biochemical fractionation, flow cytometry experiments were repeated and confirmed at least twice. All attempts at replication were successful.
Randomization	Samples were divided into groups based on genomic perturbations. Each auxin-degradation sample is coupled with untreated control.
Blinding	The study does not involve therapeutic or animal experiments, so blinding was not necessary. The labeling of samples is also required for all computational analyses.

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

Methods

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

Antibodies

Antibodies used

anti-CTCF Novus NBP2-52909
anti-CTCF EMD 07-729
anti-CTCF Abcam ab128873
anti-Halo Promega G921A
anti-RAD21 EMD 05-908
anti-RAD21 Abcam ab154769
anti-RAD21 Abcam ab154769
anti-V5 ThermoFisher R960-25
anti-YY1 Santa Cruz Biotechnology sc-7341
anti-YY1 Abcam ab38422
anti-YY1 Abcam ab109237
anti-YY1 Bethyl Laboratories Inc. A302-778A
anti-YY1 Bethyl Laboratories Inc. A302-779A
anti-RFP / anti-mScarletI Chromotek 6G6
anti-RFP / anti-mScarletI Rockland 600-401-379
anti-WAPL Proteintech 16370-1-AP
anti-HA Abcam ab9110
anti-ACTB Sigma A2228
anti-OCT4 Santa Cruz Biotechnology sc-8628
anti-TBP abcam ab51841
anti-H2B ThermoFisher MA524697
anti-SMC1A Bethyl laboratories A300-055A
anti-SMC3 Bethyl laboratories A300-060A

Validation

CTCF, RAD21, WAPL, YY1, HaloTag, V5, HA, RFP antibodies were validated by WB in cells depleted with the corresponding protein. SMC1A and SMC3 were validated by ChIP-seq, which signals are largely overlapped with RAD21. ACTB, TBP, OCT4, and H2B antibodies are well-validated by various studies and manufacturers.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

JM8.N4 mESC was obtained from the KOMP Repository at UC Davis.

Authentication

JM8.N4 mESCs were authenticated by whole-genome sequencing and morphology.

Mycoplasma contamination

JM8.N4 mESCs were pathogen tested using the IMPACT II test by IDEXX BioResearch (Westbrook, ME). All cells were negative for all pathogens, including Ectromelia, EDIM, LCMV, LDEV, MAV1, MAV2, mCMV, MHV, MNV, MPV, MVM, Mycoplasma pulmonis, Mycoplasma sp., Polyoma, PVM, REO3, Sendai, and TMEV.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified line was 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.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE178982

Files in database submission

RTCC51A_S1_L001_R1_001.fastq.bz2
RTCC51B_S2_L001_R1_001.fastq.bz2
RTCC51C_S3_L001_R1_001.fastq.bz2
RTCC51D_S4_L001_R1_001.fastq.bz2
RTCC51E_S5_L001_R1_001.fastq.bz2
RTCC51F_S6_L001_R1_001.fastq.bz2
RTCC51G_S7_L001_R1_001.fastq.bz2
RTCC51H_S8_L001_R1_001.fastq.bz2
RTCC51I_S9_L001_R1_001.fastq.bz2
RTCC51J_S10_L001_R1_001.fastq.bz2
RTCC51K_S11_L001_R1_001.fastq.bz2
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RTCC52B_S14_L002_R1_001.fastq.bz2
RTCC52C_S15_L002_R1_001.fastq.bz2
RTCC52D_S16_L002_R1_001.fastq.bz2
RTCC52E_S17_L002_R1_001.fastq.bz2
RTCC52F_S18_L002_R1_001.fastq.bz2
RTCC52G_S19_L002_R1_001.fastq.bz2
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RTCC52I_S21_L002_R1_001.fastq.bz2
RTCC52J_S22_L002_R1_001.fastq.bz2
RTCC52K_S23_L002_R1_001.fastq.bz2
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WAPL_degron_auxin_SMC3_IP_merged_coverage.bw
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YY1_degron_ut_SMC1A_IP_merged_coverage.bw
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YY1_degron_auxin_CTCF_IP_merged_coverage.bw
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YY1_degron_ut_RFP_rep9_coverage_spikeIn.bw
YY1_degron_ut_YY1_abcam_IP_coverage_spikeIn.bw
YY1_degron_ut_YY1_bethyl_IP_coverage_spikeIn.bw
YY1_degron_auxin_input_rep8_coverage_spikeIn.bw
YY1_degron_auxin_input_rep9_coverage_spikeIn.bw
YY1_degron_auxin_RFP_IP_rep8_coverage_spikeIn.bw
YY1_degron_auxin_RFP_rep9_coverage_spikeIn.bw
YY1_degron_auxin_YY1_abcam_IP_coverage_spikeIn.bw
YY1_degron_auxin_YY1_bethyl_IP_coverage_spikeIn.bw

Genome browser session
(e.g. UCSC)

no longer applicable

Methodology

Replicates

We performed Spearman's correlation and Jaccard index to assess the reproducibility between samples. Please see Extended Figure 3a-d for the full result and description.

Sequencing depth

Read length: 51 bp
Single-end
File name Replicate Total reads Uniquely mapped reads
RTCC51A_S1_L001_R1_001.fastq.bz2 DCTCF_UT_CTCF_input_Rep1 28390977 21231461
RTCC51B_S2_L001_R1_001.fastq.bz2 DCTCF_UT_CTCF_ChIP_Rep1 32931783 27107390
RTCC51C_S3_L001_R1_001.fastq.bz2 DCTCF_UT_RAD21_ChIP_Rep1 29663143 22152005
RTCC51D_S4_L001_R1_001.fastq.bz2 DCTCF_UT_SMC1A_ChIP_Rep1 31920364 23816575
RTCC51E_S5_L001_R1_001.fastq.bz2 DCTCF_UT_SMC3_ChIP_Rep1 33634052 25358448
RTCC51F_S6_L001_R1_001.fastq.bz2 DCTCF_UT_YY1_ChIP_Rep1 33959459 24136167
RTCC51G_S7_L001_R1_001.fastq.bz2 DCTCF_IAA_YY1_ChIP_Rep1 35069865 26193557
RTCC51H_S8_L001_R1_001.fastq.bz2 DCTCF_IAA_CTCF_input_Rep1 31335397 23944570
RTCC51I_S9_L001_R1_001.fastq.bz2 DCTCF_IAA_CTCF_ChIP_Rep1 29485544 21870596
RTCC51J_S10_L001_R1_001.fastq.bz2 DCTCF_IAA_RAD21_ChIP_Rep1 36571058 26903685
RTCC51K_S11_L001_R1_001.fastq.bz2 DCTCF_IAA_SMC1A_ChIP_Rep1 34239220 25580983
RTCC51L_S12_L001_R1_001.fastq.bz2 DCTCF_IAA_SMC3_ChIP_Rep1 34809582 24676494
RTCC52A_S13_L002_R1_001.fastq.bz2 DRAD21_UT_CTCF_input_Rep1 25144424 18553371
RTCC52B_S14_L002_R1_001.fastq.bz2 DRAD21_UT_CTCF_ChIP_Rep1 35955736 29096124
RTCC52C_S15_L002_R1_001.fastq.bz2 DRAD21_UT_RAD21_ChIP_Rep1 27333608 20517789
RTCC52D_S16_L002_R1_001.fastq.bz2 DRAD21_UT_SMC1A_ChIP_Rep1 29481356 21800492
RTCC52E_S17_L002_R1_001.fastq.bz2 DRAD21_UT_SMC3_ChIP_Rep1 29484547 22039721
RTCC52F_S18_L002_R1_001.fastq.bz2 DRAD21_UT_YY1_ChIP_Rep1 36126503 25657564
RTCC52G_S19_L002_R1_001.fastq.bz2 DRAD21_IAA_YY1_ChIP_Rep1 30035710 22320231
RTCC52H_S20_L002_R1_001.fastq.bz2 DRAD21_IAA_CTCF_input_Rep1 29583069 23915783
RTCC52I_S21_L002_R1_001.fastq.bz2 DRAD21_IAA_CTCF_ChIP_Rep1 40806074 30018724
RTCC52J_S22_L002_R1_001.fastq.bz2 DRAD21_IAA_RAD21_ChIP_Rep1 37048981 27159280
RTCC52K_S23_L002_R1_001.fastq.bz2 DRAD21_IAA_SMC1A_ChIP_Rep1 32725809 24278048
RTCC52L_S24_L002_R1_001.fastq.bz2 DRAD21_IAA_SMC3_ChIP_Rep1 34037754 23713201
RTCC53A_S25_L003_R1_001.fastq.bz2 DWAPL_UT_CTCF_input_Rep1 24384191 18237846
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RTCC53D_S28_L003_R1_001.fastq.bz2 DWAPL_UT_SMC1A_ChIP_Rep1 32636618 24552472
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RTCC53H_S32_L003_R1_001.fastq.bz2 DWAPL_IAA_CTCF_input_Rep1 27408935 22403634
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RTCC54C_S39_L004_R1_001.fastq.bz2 DYY1_UT_RAD21_ChIP_Rep1 30087634 23277925
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RTCC67C_S41_L004_R1_001.fastq.bz2 DYY1_IAA_YY1_ChIP_Rep3 25209642 16904397
RTCC67D_S42_L004_R1_001.fastq.bz2 DYY1_IAA_YY1_ChIP_Rep4 33004641 22032095

Antibodies

anti-CTCF Abcam ab128873
anti-CTCF Abcam ab128873
anti-YY1 Abcam ab38422
anti-YY1 Abcam ab109237
anti-SMC1A Bethyl laboratories A300-055A
anti-SMC3 Bethyl laboratories A300-060A

Peak calling parameters

ChIP-Seq read alignment: Bowtie (-n 2, -m 1)
ChIP-Seq peak calling: MACS2 (--nomodel --extsize 300)

Data quality

Sample_name Peak_number_FDR5% Peak_number_5-fold
CTCF_degron_auxin_CTCF_IP 26931 18438
CTCF_degron_auxin_Rad21_IP 14989 1129
CTCF_degron_auxin_Smc1a_IP 39093 3210

CTCF_degron_auxin_Smc3_IP 15393 777
CTCF_degron_auxin_YY1_IP 56799 16758
CTCF_degron_ut_CTCF_IP 81432 61404
CTCF_degron_ut_Rad21_IP 38762 24735
CTCF_degron_ut_Smc1a_IP 43472 17216
CTCF_degron_ut_Smc3_IP 20194 9213
CTCF_degron_ut_YY1_IP 46354 14594
Rad21_degron_auxin_CTCF_IP 90823 65960
Rad21_degron_auxin_Rad21_IP 11347 2803
Rad21_degron_auxin_Smc1a_IP 9511 762
Rad21_degron_auxin_Smc3_IP 5464 502
Rad21_degron_auxin_YY1_IP 42273 12447
Rad21_degron_ut_CTCF_IP 95139 72386
Rad21_degron_ut_Rad21_IP 43113 28993
Rad21_degron_ut_Smc1a_IP 41091 15957
Rad21_degron_ut_Smc3_IP 38509 21803
Rad21_degron_ut_YY1_IP 48329 16732
WAPL_degron_auxin_CTCF_IP 97574 74792
WAPL_degron_auxin_Rad21_IP 49451 33319
WAPL_degron_auxin_Smc1a_IP 38650 17317
WAPL_degron_auxin_Smc3_IP 45257 26165
WAPL_degron_auxin_YY1_IP 40640 12558
WAPL_degron_ut_CTCF_IP 98200 75332
WAPL_degron_ut_Rad21_IP 43445 29722
WAPL_degron_ut_Smc1a_IP 51296 21539
WAPL_degron_ut_Smc3_IP 36245 20304
WAPL_degron_ut_YY1_IP 40093 13294
YY1_degron_auxin_CTCF_IP 101925 75420
YY1_degron_auxin_Rad21_IP 45114 29887
YY1_degron_auxin_Smc1a_IP 47950 20330
YY1_degron_auxin_Smc3_IP 47098 25773
YY1_degron_auxin_YY1_IP 14627 3756
YY1_degron_ut_CTCF_IP 98775 74506
YY1_degron_ut_Rad21_IP 48854 32391
YY1_degron_ut_Smc1a_IP 51886 24617
YY1_degron_ut_Smc3_IP 46209 28173
YY1_degron_ut_YY1_IP 32203 10210

Software: Bowtie2 (v 2.3.5.1), samtools (v 1.9), deepTools (v 3.5.0), MASC2 (v 2.2.6), MAnorm2 (v 1.0),

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	We measured RFP (mScarletI) intensity for checking YY1 degradation efficiency. YY1-miniIAA7 cells (clone YD39) were treated with 500 uM IAA for time points at 0, 1, 2, 3 hr. Ethanol-treated cells (negative control) were processed with the same procedure.
Instrument	BD Bioscience LSR Fortessa
Software	FlowJo (v10.3), FlowJo LL
Cell population abundance	Cells were detached and dissociated into single cells by trypsin, washed once by culture media, and resuspended into 1 mL of culture media. We typically have >95% of viable cells.
Gating strategy	We gated the main population on the FSC/SSC plot by excluding the apparent populations of cell debris and cell doublets. For RFP (mScarletI) gating, we defined ~99.5% of cells in the untreated cell as RFP-positive cells.

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