

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <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	Data collection described in Methods section. No custom software was used.
Data analysis	Data analysis is described in the Methods section. The division of nuclei and cytoplasmic regions, as well as the quantification of immunofluorescence intensity, were executed using the Fiji built on ImageJ2 2.14.0/1.54f, complemented by statistical analysis conducted through GraphPad 9.5.1 Prism. Raw CUT&Tag reads were trimmed using Trim Galore v.0.6.6 (Babraham Institute). Trimmed reads were aligned to the human hg19 genome using Bowtie v.2.4.4 with the following parameters: “-N 1 -L 25 -X 700 --no-mixed --no-discordant”. Duplicate reads were removed with Picard Tools v2.26.6 (Broad Institute). The reads were then shifted to compensate for the offset in tagmentation site relative to the Tn5 binding site using the alignmentSieve function of deepTools v.3.5.1 with the ‘--ATACshift’ option. Peak calling was performed by macs2 v2.2.6 with a q-value threshold of 0.05. Raw ChIP-Rx reads were trimmed by Trim Galore v0.6.6 (Babraham Institute) and aligned to the human hg19 and mouse mm10 assemblies using Bowtie v2.3.5.1 with default parameters. Low mapping quality reads (MAPQ < 30) and PCR duplicates were removed using SAMtools v1.9 and Picard v2.26.6 (Babraham Institute). The read number for each of the ChIP-Rx sample were then collected using SAMtools v1.9, and the normalization factor was calculated as 1e6/spike-in_count. Normalized bigwig files were generated by deeptools v3.5.0.

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](#)

Data supporting the findings of this study are available within the article and its Supplementary Figures.

The source data underlying Fig. 1,2,3,5, Supplementary Figs. 2-5 are provided as a Source Data file or supplementary data. Additional details on datasets and protocols that support the findings of this study will be made available by the corresponding author upon reasonable request.

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](https://www.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 were predetermined. Sample size was estimated by robustness of phenotype based on our preliminary experiments. Proper negative and when possible positive controls were used for each experiment.
Data exclusions	No data exclusions occurred in this study.
Replication	Key experiments were performed at least 3 times and all replication attempts were successful.
Randomization	Cells were always randomly allocated into control and experimental groups. All samples used in each set of experiments were equal, except the experimental conditions being tested.
Blinding	Blinding was not done since this study relies on the investigator studying differences in cell lines

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

DSS1 Polyclonal antibody Proteintech Cat. # 13639-1-AP (WB, 1:500)

INTS3 Polyclonal antibody Proteintech Cat. # 16620-1-AP (WB, 1:2000; IP, 2ug; ChIP, 2ug)

Anti-INT4 (Anti-INTS4) Bethyl Laboratories Cat. # A301-296-A (WB, 1:500)

INTS5 Polyclonal antibody Proteintech Cat. # 14069-1-AP (WB, 1:2000; IP, 2ug)

DICE1 Antibody (H-6) (Anti-INTS6) Santa Cruz Cat. # sc-376524 (WB, 1:1000)

Anti-INT7 (Anti-INTS7) Bethyl Laboratories Cat. # A300-271-A (WB, 1:2000)

Anti-INTS8 Antibody Atlas Antibodies Cat. # HPA057299 (WB, 1:2000)

INTS9 Antibody Cell Signaling technology Cat. # 13945S (WB, 1:1000)

Anti-INT11 (Anti-INTS11) Bethyl Cat. # A301-274-A (WB, 1:500)

PPP2R1A Polyclonal antibody (Anti-PP2A-A) Proteintech Cat. # 15882-1-AP (WB, 1:1000)

OBFC2B Polyclonal antibody (Anti-SSB1) Proteintech Cat. # 14809-1-AP (WB, 1:1000)

Rpb1 NTD (D8L4Y) Rabbit mAb Cell Signaling technology Cat. # 14958S (WB, 1:1000)

NELFB Rabbit pAb Abclonal Cat. # A12023 (WB, 1:500)

Anti-NELFe[EPR11600] Abcam Cat. # ab170104 (WB, 1:1000)

SUPT5H Polyclonal antibody (Anti-SPT5) Proteintech Cat. # 16511-1-AP (WB, 1:500)

PSMD4 Rabbit pAb Abclonal Cat. # A1061 (WB, 1:500)

PSMD14 Rabbit pAb Abclonal Cat. # A10782 (WB, 1:500)

c-Myc/N-Myc (D3N8F) Rabbit mAb Cell Signaling technology Cat. # 13987S (WB, 1:1000)

Cdc6 Rabbit pAb Abclonal Cat. # A18249 (WB, 1:500)

Vinculin Rabbit mAb Abclonal Cat. # A2752 (WB, 1:5000)

β-Tubulin Mouse mAb Abclonal Cat. # AC021 (WB, 1:5000)

HA tag Polyclonal antibody Proteintech Cat. # 51064-2-AP (WB, 1:3000; IP, 2μg; IF, 1:100; ChIP, 2μg; CUT&Tag, 1μg)

Mouse anti DDDDK-Tag mAb (Anti-Flag) Abclonal Cat. # AE005 (WB, 1:5000; IP, 2ug; IF, 1:100; ChIP, 2ug; CUT&Tag, 1μg)"

HRP Goat Anti-Rabbit IgG(H+L) Abclonal Cat. # AS014 (WB, 1:5000)

HRP Goat Anti-Mouse IgG(H+L) Abclonal Cat. # AS003 (WB, 1:5000)

HRP-conjugated Mouse Anti-Rabbit IgG Light Chain Abclonal Cat. # AS061 (WB, 1:5000)

HRP-conjugated Goat Anti-Mouse IgG Light Chain Abclonal Cat. # AS062 (WB, 1:5000)

Alexa Fluor 488 conjugated goat anti-mouse IgG Yeasen Cat. # 33206ES60 (IF, 1:1000)

Alexa Fluor 488 conjugated goat anti-rabbit IgG Yeasen Cat. # 33106ES60 (IF, 1:1000)

Rhodamine (TRITC) Goat Anti-Mouse IgG Yeasen Cat. # 33209ES60
(IF, 1:1000)
Rhodamine (TRITC) Goat Anti-Rabbit IgG Yeasen Cat. # 33109ES60
(IF, 1:1000)
Mouse anti-rabbit IgG Solarbio Cat. # SPA231
(CUT&Tag, 1:100)
Rabbit anti-mouse IgG Solarbio Cat. # K0034M
(CUT&Tag, 1:100)
Rabbit Control IgG Abclonal Cat. # AC005
(IP, 2ug)
Mouse Control IgG Abclonal Cat. # AC011
(IP, 2ug)

Validation

All these antibodies were commercially obtained and validated by vendors and multiple published studies, see manufacture's website for references.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Cell lines:
DLD1-DSS1-dTAG clone
DLD1-DSS1-dTAG clone-OE-vector
DLD1-DSS1-dTAG clone-OE-DSS1-WT
DLD1-DSS1-dTAG clone-OE-DSS1-W39R
DLD1-DSS1-dTAG clone-OE-INTS7-WT
DLD1-DSS1-dTAG clone-OE-INTS7-Mutant1
DLD1-DSS1-dTAG clone-OE-INTS7-Mutant2
DLD1 INTS11-dTAG clone
DLD1 INTS8-dTAG clone-INTS8-DN211
Above were generated from DLD-1 cell line. DLD-1 cell was a gift from Ali shilatifard lab (Chikago, Northwestern university) who purchased it from ATCC.

HEK 293 Flp-In T-Rex cells (Invitrogen, R78007) were purchased from vendors.

Authentication

Cells were cultured in media recommended by the vendors. We froze down stocks upon receiving the cell lines, and all experiments will be conducted on cells that have been passaged no more than 10 times. All dTAG knock-in cell lines and overexpression cell lines were validated by PCR and WB.

Mycoplasma contamination

All cell lines tested for Mycoplasma contamination every month.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified lines were used.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A

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=GSE297001>

Files in database submission

ChIP_PolIII-NTD_DSS1-dTAG+OE-DSS1-WT-rep1_Batch1
ChIP_PolIII-NTD_DSS1-dTAG+OE-DSS1-WT-rep2_Batch1
ChIP_PolIII-NTD_DSS1-dTAG+OE-DSS1-W39R-rep1_Batch1

ChIP_PolII-NTD_DSS1-dTAG+OE-DSS1-W39R-rep2_Batch1
 ChIP_INTS3-DSS1-dTAG+OE-DSS1-WT_Batch2
 ChIP_INTS3-DSS1-dTAG+OE-DSS1-W39R_Batch2
 ChIP_input-DSS1-dTAG+OE-DSS1-WT_Batch1
 ChIP_input-DSS1-dTAG+OE-DSS1-W39R_Batch1
 ChIP_input-DSS1-dTAG+OE-DSS1-WT_Batch2
 ChIP_input-DSS1-dTAG+OE-DSS1-W39R_Batch2

Genome browser session
 (e.g. [UCSC](#))

no longer applicable

Methodology

Replicates

Two biological replicates for all next-generation sequencing assays.

Sequencing depth

All sequencing reads are paired-end 150bp, listed are the numbers of uniquely and high-quality mapped reads.
 ChIP_INTS3-DSS1-dTAG+OE-DSS1-WT_Batch2, human:55593592, mouse:6330952
 ChIP_INTS3-DSS1-dTAG+OE-DSS1-W39R_Batch2, human:49319314, mouse:4708384
 ChIP_input-DSS1-dTAG+OE-DSS1-WT_Batch2, human:63164720, mouse:6494624
 ChIP_input-DSS1-dTAG+OE-DSS1-W39R_Batch2, human:86825684, mouse:8158796
 ChIP_PolII-NTD_DSS1-dTAG+OE-DSS1-WT-rep1_Batch1, human:45046090, mouse:4906664
 ChIP_PolII-NTD_DSS1-dTAG+OE-DSS1-WT-rep2_Batch1, human:60079064, mouse:6552986
 ChIP_PolII-NTD_DSS1-dTAG+OE-DSS1-W39R-rep1_Batch1, human:40726484, mouse:5901774
 ChIP_PolII-NTD_DSS1-dTAG+OE-DSS1-W39R-rep2_Batch1, human:44560360, mouse:6477264
 ChIP_input-DSS1-dTAG+OE-DSS1-WT_Batch1, human:39836506, mouse:3579592
 ChIP_input-DSS1-dTAG+OE-DSS1-W39R_Batch1, human:30599846, mouse:4018474

Antibodies

INTS3 Polyclonal antibody Proteintech Cat. # 16620-1-AP, 2ug
 INTS5 Polyclonal antibody Proteintech Cat. # 14069-1-AP, 2ug
 Rpb1 NTD (D8L4Y) Rabbit mAb Cell Signaling technology Cat. # 14958S, 2ug
 HA tag Polyclonal antibody Proteintech Cat. # 51064-2-AP, 2ug
 Mouse anti DDDDK-Tag mAb (Anti-Flag) Abclonal Cat. # AE005, 2ug

Peak calling parameters

macs2 callpeak -c \$input_bam -t \$IP_bam -f BAMPE -n \${sample} -g \${macs2_info} --keep-dup all --outdir \${peak_compare_dir} 2> \${peak_log_dir}/\${sample}_compare.log

Data quality

Peaks were called with macs2 v2.2.7.1, and peaks overlapped with blacklist were removed.
 ChIP_PolII-NTD_DSS1-dTAG+OE-DSS1-W39R-rep1_Batch1: 94640 peaks
 ChIP_PolII-NTD_DSS1-dTAG+OE-DSS1-W39R-rep2_Batch1: 99255 peaks
 ChIP_PolII-NTD_DSS1-dTAG+OE-DSS1-WT-rep1_Batch1: 70877 peaks
 ChIP_PolII-NTD_DSS1-dTAG+OE-DSS1-WT-rep2_Batch1: 79123 peaks
 ChIP_INTS3-DSS1-dTAG+OE-DSS1-WT_Batch2: 916 peaks
 ChIP_INTS3-DSS1-dTAG+OE-DSS1-W39R_Batch2: 4071 peaks

Software

ChIP-Rx analyses used Trim Galore v0.6.6, Bowtie v2.4.4, SAMtools v1.12, Picard Tools v2.25.5, MACS2 v2.2.7.1, R package ChIPseeker v1.28.3, deepTools v3.5.1, bedTools v2.30.0