

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	IX-81 wide-field (Olympus), Illumina Nextseq 2000 and HiSeq (Illumina), ChemiDoc (BioRad), timsTOF pro mass spectrometer (Bruker), Typhoon scanner (Cytiva), Axio Imager.A2 microscope (ZEISS)
Data analysis	ScanR, deepTools, BEDtools, TrimGalopre, Fiji/ImageJ, IGG, STAR v2.7.11, Subread, UMItools, CAGEfightR, R(v4.3.2), MaxQuant, macs2 v2.2.9.1, ZEN blue software 1.0.0.0

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](#)

Plasmids used in this study have been deposited in Addgene. Sequencing data generated as part of this study is available under SRA number PRJNA1275866 [https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1275866]. Processed bigwig files are available under GEO accession numbers: GSE335232 [https://

www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE335232, GSE335234 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE335234>] and GSE335230 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE335230>]. Proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers PXD067831 [<https://www.ebi.ac.uk/pride/archive/projects/PXD067831>], PXD067969 [<https://www.ebi.ac.uk/pride/archive/projects/PXD067969>], and PXD070086 [<https://www.ebi.ac.uk/pride/archive/projects/PXD070086>].

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	Sample sizes were chosen base on common pratice for statistaical analysis (triplicates) or for qualitative analysis (duplicates)
Data exclusions	No data exclusion occurred in the study
Replication	Experiments were repeated two or more times. When statistical analysis was needed, a minimum or triplicates were sampled.
Randomization	NA
Blinding	NA

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

anti-FLAG Sigma Cat#F1804; RRID:AB_262044
 RPB1 D8L4Y (total, N-terminal) Cell Signaling Cat#14958S; RRID:AB_2687876
 RPB1 4H8 (raised against S5-P, recognizes multiple phosphorylated forms) Francis Crick Institute Cell Services 4H8
 RPB1, serine 5 phosphorylated Kind gift from Dirk Eick 3E8
 monoclonal anti-NELFCD (D5G6W) Cell signaling Cat#12265,RRID:AB_2797862
 recombinant anti-NELFE Abcam CAT#ab170104,RRID:AB_2827280
 polyclonal anti-NELFA Thermo fisher scientific Cat#A301-910A,RRID:AB_1524101
 polyclonal anti-GFP Abcam Cat#ab290, RRID:AB_2313768
 polyclonal anti-INTS4 Bethyl (Fortis life science) Cat#A301-296A; RRID:AB_937909
 polyclonal anti_CMTR1 Sigma Cat#HPA029980,RRID:AB_10671094
 polyclonal anti-CPSF2 Bethyl (Fortis life science) Cat#A301-583A,RRID:AB_1078866
 Horse Anti-Mouse IgG Antibody (H+L), HRP Vector Laboratories Cat#PI-2000; RRID:AB_2336177
 Goat Anti-rabbit IgG Antibody (H+L), HRP Vector Laboratories Cat#PI-1000; RRID:AB_2336198
 Goat Anti-rat IgG Antibody (H+L), HRP Jackson ImmunoResearch Labs Cat#112-035-003,RRID:AB_2338128
 Tubulin Abcam Cat#ab15246; RRID:AB_301787
 Histone 3 Abcam Cat#ab18521; RRID:AB_732917
 monoclonal anti-poly-ADP-ribose binding reagent Novus Biologicals Cat#MABE1031, RRID:AB_2665467
 anti-phospho-Histone H2AX (Ser139, JBW301) Merck Cat#05-636,RRID:AB_309864
 Goat anti-Mouse IgG, Alexa Fluor™ 594 ThermoFisher Scientific Cat#A11005,RRID:AB_2534073
 Goat anti-Rabbit IgG,Alexa Fluor™ 488 ThermoFisher Scientific Cat#A11008,RRID:AB_143165
 polyclonal anti-PARP Cell Signaling Cat#9542S,RRID:AB_2160739

Validation

All commercial antibodies were validated based on manufacturer indicated on website.

Eukaryotic cell lines

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

Cell line source(s)

T-REx™ HeLa (HeLa) kind gift of Jesper Svejstrup R71407
 MCF7 kind gift of René Villadsen
 HEK293 Flp-In™ T-REx™ (HEK293) ThermoFisher Scientific R78007
 Flp-In T-REx U2OS cells (U2OS) kind gift of Michael Lisby
 Dosophila S2 cells, kind gift of Hector Herranz lab

Authentication

Commercial authentication from cells acquired from ThermoFischer.

Mycoplasma contamination

All cell line were tested negative for mycoplasma contamination

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

NA

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

NA

Wild animals

NA

Reporting on sex

NA

Field-collected samples

NA

Ethics oversight

NA

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

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 <i>May remain private before publication.</i>	SRA: PRJNA1275866, https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA1275866 GEO: GSE335230, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE335230
Files in database submission	Sample Names ChIP_totPolII_NT ChIP_totPolII_1h ChIP_totPolII_treated ChIP_NelfE_NT ChIP_NelfE_1h ChIP_NelfE_treated ChIP_inp_NT ChIP_inp_1h ChIP_inp_treated
Genome browser session (e.g. UCSC)	NA

Methodology

Replicates	1
Sequencing depth	ChIP_totPolII_NT 2674337 ChIP_totPolII_1h 3130419 ChIP_totPolII_treated 1515838 ChIP_NelfE_NT 2000942 ChIP_NelfE_1h 1553845 ChIP_NelfE_treated 1996902 ChIP_inp_NT 1408163 ChIP_inp_1h 1244533 ChIP_inp_treated 1152842
Antibodies	anti-NELFE (abcam, ab170104) anti-RPB1 (D8L4Y, Cell Signaling 14958S)
Peak calling parameters	macs2 (v2.2.9.1) default parameters. Input in each conditions were used as respective input controls -c in macs2.
Data quality	input raw fastq files were trimmed with trim_galore (paired-end mode) with minimum read length filtering > 19 nt. Filtered and trimmed paired end reads were mapped with STAR against human assembly GRCh38 (Ensembl release 108). Genome alignment BAM files were filtered for multi-mapped reads with samtools view function (-q 10).
Software	macs2/v2.2.9.1