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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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

Statistics

For	all s	tatistical 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 <u>availability of computer code</u>							
Data collection	No software was used to perform data collection.						
Data analysis	Custom R code was used to analyze the data and can be made available upon request.						
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/reviewer							

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/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

The sequencing data and processed files are deposited in the GEO database under accession code GSE123980.

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

Sample size	No explicit power analysis was performed. All information regarding replicates is explained in detail in the Materials and Methods section.		
Data exclusions	Criteria for exclusion/inclusion of data, e.g. gene architecture and sequencing depth considerations, are clearly stated in the Material and Methods section. In box plots (TTseq, mNET-seq), outliers were excluded for visualization purposes, i.e. extending more than 1.5-fold the interquartile range from the box.		
Replication	No replication attempts failed. Biological replicates were performed starting from different cell populations. All information regarding replicates is explained in detail in the Materials and Methods section.		
Randomization	Randomization was not relevant to this study.		
Blinding	Blinding was not relevant to this study.		

All studies must disclose on these points even when the disclosure is negative.

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

Methods	M	let	ho	ds
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n/a Involved in the study n/a Involved in the study × × Antibodies ChIP-seq ✗ Eukaryotic cell lines × Flow cytometry X Palaeontology X MRI-based neuroimaging x Animals and other organisms × Human research participants × Clinical data

Antibodies

Antibodies used	anti-Pol II total CTD (unphos+phos) (Biozol, MABI0601; Lot. #15013)
Validation	ELISA data are available in PMID: 25910207.

Eukaryotic cell lines

Policy information about <u>cell lines</u>				
Cell line source(s)	K562 cell line is from DSMZ, ACC-10. Raji B (CDK9as) cell line are from PMID: 28994650.			
Authentication	Both cell lines were authenticated at the DSMZ Identification Service according to standards for STR profiling (ASN-0002).			
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination. Testing was performed using Plasmo Test Mycoplasma Detection Kit (InvivoGen).			
Commonly misidentified lines (See <u>ICLAC</u> register)	No ICLAC listed cell line was used.			