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Last updated by author(s):	Nov 2, 2020

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 our Editorial Policies and the Editorial Policy Checklist.

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Fora	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. <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
X	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 <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

EPU 2.5

Data analysis

MotionCor2 v1.3.1, RELION 3.0, RELION 3.1, WARP 1.0.5, Gautomatch 0.56, CCP-EM v1.4.1, LocScale 0.1, Molrep 11.7.02, Coot 8.9.1, Coot 8.9.2, Buccaneer 1.6.5, MAINMAST 1.0, Phenix.refine 1.16_3549, Phenix program suite 1.18.1, PyMOL 1.9, PyMOL 2.0.6, APBS 1.5, USCF Chimera 1.11.2, CCP4MG 2.10.11, PDBePISA 1.48, HKL2000 712, AutoSol 1.18.1, AutoBuild 1.18.1, GraphPad Prism 7.02, Quantity One software 4.6.3 (Bio-Rad), Clustal Omega 1.2.2, ESPript 3.0, Gctf 1.18, DataAnalysis 5.0, MASCOT v.2.6, 3DFSC 3.0

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 Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. 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

 $Co-ordinates\ and\ structure\ factors\ or\ maps\ have\ been\ deposited\ in\ the\ wwwPDB\ or\ EMDB\ databases.$

Bsu HelD C-terminal domain (X-ray) PDB ID 6VSX (https://www.rcsb.org/structure/6VSX)

Msm HeID-RNAP complex State I (cryoEM) EMD-10996, PDB ID 6YXU (https://www.rcsb.org/structure/6YXU)

 $Msm\ HeID-RNAP\ complex\ State\ \textbf{II}\ (cryoEM)\ EMD-11004,\ PDB\ ID\ 6YYS\ (https://www.rcsb.org/structure/6YYS)$

Msm HeID-RNAP complex State III (cryoEM) EMD-11026, PDB ID 6Z11 (https://www.rcsb.org/structure/6Z11)

<u>Field-spe</u>	ecific reporting			
Please select the o	ne 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 t	the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
Life scier	nces study design			
All studies must dis	sclose on these points even when the disclosure is negative.			
Sample size	For biochemical analyses, sample sizes of at least of 3 were chosen (biological replicates) to provide significant results.			
Data exclusions	No data were excluded.			
Replication	Experiments were performed at least 3x (biological replicates). Reported data show individual replicates, means, and respective SD.			
Randomization	This study does not involve subjects that require randomization.			
Blinding	This study does not involve subjects that require blinding.			
Danastin				
<u> </u>	g for specific materials, systems and methods			
	on from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, ted 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 & exp	perimental systems Methods			
n/a Involved in th				
■ ★ Antibodies				
Eukaryotic Palaeontol	cell lines ogy and archaeology MRI-based neuroimaging			
	and other organisms			
	search participants			
Clinical dat	za			
Dual use re	esearch of concern			
A #11				
<u>Antibodies</u>				
Antibodies used	Purified anti-E.coli RNA pol Sigma 70 antibody (clone name 2G10, Biolegend, cat. no. 663208); Anti-E.coli RNA Polymerase β Antibody (8RB13, Biolegend, cat. no. 663903); RNA polymerase beta antibody (8RB13, GeneTex, cat. no.			
	GTX12087);			
	ANTI-FLAG (clone M2, Sigma, cat. no. F1804); Goat-anti-mouse IgG (IR800, Advansta, WesternBright MCF-IR fluorescent Western blotting kit, cat. no. K-12022-010);			
	Anti-Mouse IgG (whole molecule)-Peroxidase produced in rabbit (Sigma, cat. no. A9044)			
Validation	All antibodies were validated by WB analysis. All reacting protein were of the correct Mw. Moreover, wherever possible, we verified the identity of the target proteins also by mass spectrometry.			
	Data sheets and references:			
	anti-sigma 70, dilution 1:1,000 (reactivity for E. coli, M. smegmatis https://www.biolegend.com/en-gb/products/purified-anti-e-coli- rna-sigma-70antibody-18128, Pánek et al, 2011 doi:10.1093/nar/gkq1186);			
	anti-RNAP β , dilution 1:1,000 (https://www.biolegend.com/en-gb/products/anti-e-coli-rna-polymerase-beta-antibody-10494) and			
	(https://www.genetex.com/Product/Detail/RNA-polymerase-beta-antibody-8RB13/GTX12087; Hnilicová et al, 2014 doi:10.1093/nar/gku793);			
	anti-FLAG, dilution 1:1,000 (https://www.sigmaaldrich.com/catalog/product/sigma/f1804); goat-anti-mouse IgG, dilution 1:10,000 (https://advansta.com/products/WesternBright-MCF-application-note);			
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anti-mouse IgG conjugated with HRP, dilution 1:80,000 (https://www.sigmaaldrich.com/catalog/product/sigma/a9044).