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	Imaging data was collected using VisiView 6.0 by Visitron Systems
Data analysis	Python, Fiji (ImageJ), Matlab 2024b, Graphpad Prism 10, FragPipe, Microsoft Excel. All single-particle tracking processing and data analysis we performed using custom scripts in Python, Fiji, and Matlab. All codes, including a demo dataset, are available on Github at https://github.com/codedbynayem under the GNU general public license. Software used for the proteomics dataset can be found on Github at https://github.com/cutleraging/Haque-2025.

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

Imaging data and original, uncropped western blots are provided in the Source Data file. Raw imaging files have been deposited to BioImage Archive under the accession code S-BIAD3606.
The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (https://www.ebi.ac.uk/pride/login). The project accession is: PXD072625.

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	Not applicable
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

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	All single-particle tracking data were derived from multiple single-cell measurements (n ≈ 18 - 110 cells per condition), acquired across two to four independent imaging sessions (biological replicates). The CRISPR-engineered cells were derived from a polyclonal population, with Supplementary Fig. 6c,d, containing data from a single-cell clone. These sample sizes are consistent with established practice in single-molecule imaging studies. The proteomics and cell growth assays used three biological replicates.
Data exclusions	For all imaging datasets, binding events present within the first two and/or last two frames of the 500-frame acquisition are excluded because accurate dwell-time estimation requires well-defined start and end frames. For events already present in the first two frames, the true start time is unknown; likewise, for events present in the last two frames, the true end time is unknown. These excluded events typically account for <5% of all detected binding events across datasets. For the SHARP analysis, nuclei with extreme outlier hotspot patterns are excluded, as these cells often contain a small number of large, contiguous hotspots localized along the nuclear periphery and likely reflect signal outside the nucleus (e.g., extranuclear artifacts). In order to enhance fits by GRID, some datasets filtered the top 0.05% longest lived binding events, as indicated in the Source Data file.
Replication	Single-particle tracking data were acquired across 2 to 4 independent imaging sessions performed on different days, with each session including at least 18 cells per condition. The cell growth assay was performed with three biological replicates, each comprising three technical replicates; for each technical replicate, three cell counts were obtained and averaged.
Randomization	Not applicable
Blinding	Not applicable

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies: anti-Pol II (A-10) mouse monoclonal (Santa Cruz, sc-17798), anti-TAF1 (D6J8B) rabbit monoclonal (Cell Signaling Technologies, 12781), anti-TBP rabbit polyclonal (Cell Signaling Technologies, 8515S), anti-PCNA (D3H8P) rabbit monoclonal (Cell Signaling Technologies, 13110), anti-SUPT5H polyclonal antibody (Thermo Fisher, A300-868A), anti-Phospho-SPT5 (Thr794 in mouse, Thr798 in human) polyclonal (FabGennix, PSPT5-140AP), anti-TAF7 mouse monoclonal (Invitrogen, 19TA-2C7), anti-Lamin A/C rabbit polyclonal (Cell Signaling Technologies, 2032T), anti-Vinculin (E1E9V) rabbit monoclonal (Cell Signaling Technologies, 13901).

Secondary antibodies: Alexa Fluor-conjugated goat anti-rabbit (Invitrogen, A32735), IRDye-conjugated goat anti-mouse secondary antibody (LI-COR, 926-32210).

Validation

Anti-Pol II: Recommended for use in WB, IP, IF and ELISA. Used in 48 publications.
Anti-TAF1: Recommended for use in WB and IP. Used in 7 publications.
Anti-TAF7: Reccomended for use in WB, IF, and flow cytometry. Used in at least 3 publications.
Anti-TBP: Recommended for use in WB. Used in 83 publications.
Anti-PCNA: Recommended for use in WB, IP, IHC, IF, and Flow Cytometry. Used in 705 publications.
Anti-SUPT5H: Recomended for use in WB (assay-dependent) and IP. Used in at least 6 publications.
Anti-phospho-SPT5: Verified applications: ELISA, IP, and WB.
Anti-Lamin A/C: Recommended for use in WB and IHC. Used in 366 publications.
Anti-Vinculin: Recommended for use in WB, IHC, and Flow Cytometry. Used in 685 publications.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	U20S cells (ATCC, HTB-96)
Authentication	Cell lines were not authenticated.
Mycoplasma contamination	Cell lines were negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	Not applicable

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable

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	U2OS CRISPR-engineered cells (along with parental cells, if applicable) were grown on a 15-cm dish until they reached a confluency of ~70%. Cells were then stained with either 100 nM SNAP-SiR or 100 nM Halo-JF635 for 30 minutes. Cells were washed twice with PBS and detached from the plate using Accutase. PBS was then added once cells completely detached and the entire suspension pelleted and resuspended in FACS sorting buffer (1 mM EDTA, 25 mM HEPES pH 7.5, 1% FBS, 1% Pen/Strep, in cation-free PBS, filter sterilized using 0.2 µm filter) with Accutase and DAPI. Cells were then strained using 40 µm pluriSelect strainer into a 5 mL Polyproplyene tube, which was used for sorting. Cells were collected in a 15 mL falcon tube containing ~5 mL of FACS buffer. Cells were pelleted, resuspended in fresh culture media, and seeded on a culture dish post sorting.
Instrument	Thermo Bigfoot Cell Sorter
Software	FlowJo
Cell population abundance	The percentage of cells within the user-defined gate is indicated in Supplementary Figure 1. A summary of the sorting strategy, along with the corresponding FCS file(s), is available from the corresponding author upon request.
Gating strategy	Gates were set to capture the cell population showing a fluorescence shift relative to the unstained control and/or the stained parental cell line.

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