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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics

all st	atistical 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, t, r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> 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 <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.		
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Software and code

Policy information about availability of computer code		
Data collection	(LSM-980, Zeiss; Structured illumination microscopy(Olympus Corporation),	
Data analysis	Statistics and graphing were performed using Prism 8 (GraphPad) software. All images were assembled using ImageJ 1.51j8 (NIH)	

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

All data are available from the corresponding author on request. Source data are provided with this paper. The original files of all images included in figures have been deposited in Zenodo with the identifier https://zenodo.org/records/13938287.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.
Reporting on race, ethnicity, or other socially relevant groupings	Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.
Population characteristics	Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."
Recruitment	Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.
Ethics oversight	Identify the organization(s) that approved the study protocol.

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 <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

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

Sample size	All sample sizes are listed in detail in the figure legends and main text. The number of cells imaged are consistent with previous live cell imaging studies (and from N≥3 independent experiments).
Data exclusions	The experimental data is authentic and reliable and can be found in the original data file. There is no intentional deletion of selection.
Replication	All data presented were from biological replicates. All attempts at replication were successful.
Randomization	Aside from the strong premise for the proposed research, additional steps were taken to ensure rigor and reproducibility, as follows: 1) scientific questions were addressed using complementary technical approaches to ensure that the findings were robust; 2) for studies involving multiple different experimental conditions in the same cell line, studies were performed on cells originating from the same cell line batch.
Blinding	Whenever possible, experimenters were blinded to condition and examiner to exclude bias. For example, for our preliminary analyses, we asked other researchers who were either involved or not involved in the study to examine blinded samples for biological effects.

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

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n/a Involved in the study n/a Involved in the study **X** Antibodies K ChIP-seq **×** Eukaryotic cell lines **×** Flow cytometry ▼ Palaeontology and archaeology ▼ MRI-based neuroimaging x Animals and other organisms Clinical data × Dual use research of concern X Plants

Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>		
Cell line source(s)	HeLa and HpG2 purchased from Procell Life Science&Technology Co. (Wuhan, China); Further details are provided in the Methods section (Cell treatment and staining).	
Authentication	Cell lines were previously authenticated by cytochrome c oxidase subunit I (COI) and short tandem repeat (STR) testing.	
Mycoplasma contamination	Cells lines were tested negative for mycoplasma contamination.	
Commonly misidentified lines (See <u>ICLAC</u> register)	No commonly misidentified cell lines were used.	

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor
Authentication	was applied. Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

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.
- **X** A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cells from Chinese Cell Bank. HeLa and HpG2 purchased from Procell Life Science&Technology Co. (Wuhan, China)
Instrument	high-throughput acoustic focusing flow cytometer (ATTUNE NXT)
Software	FlowJo.
Cell population abundance	The same cell line was used for each cell experiment in this manuscript, no cells were crossed over, and cells were inoculated evenly into petri dishes.
Gating strategy	Gating strategy is not addressed in this manuscript.

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