

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

Data analysis

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](#)

Source data are provided in the paper, and the larger number, all raw microscope data available from the corresponding author on reasonable request. The raw sequencing data generated in this study are available in the Genome Sequence Archive of The National Genomics Data Center (NGDC) with the accession number HRA000946. Mass spectrometry data have been deposited in ProteomeXchange with the primary accession code PXD031109. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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	Sample size was chosen based on common practice of described experiment or the need to have sufficient statistical power, Experiments were repeated two or more times.
Data exclusions	No data exclusions occurred in this study
Replication	Experiments were repeated two or more times. Experimental findings for each representative image or data have been reliably replicated.
Randomization	This study does not involve randomization of samples/organisms/participants. Because all of our samples/experiments were specific genetically defined cell lines.
Blinding	This study does not require investigators to be blinded to group allocation during data collection and/or analysis, since no subjective rating of the data was involved.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	Antibodies used are described in the Methods section. CycT1 (IF 1:200 WB 1:1000. Santa Cruz Biotech, sc-10750); pan-ADPr (anti-pan-ADP-ribose binding reagent; WB 1:1000. Millipore, MABE1016); pADPr (ADP-ribose-binding reagent; WB:1:1000. Millipore, MABE1031); polyclonal anti-PAR polymer antibody 10H (WB 1:1000. Enzolifesciences, ALX-804-220-R100); γH2AX (IF 1:500. WB 1:1000. Sigma-Aldrich, 05-636); Flag (WB:1:1000. Sigma-Aldrich, F3165); PARP1 (WB 1:1000. Cell signaling technology, #9542); RNA Pol II phospho-Ser5 (WB 1:1000. Millipore, 04-1572); Phospho-ATM (WB:1:1000. Ser1981; HUABIO, ET1705-50); DNA-PKCS (WB 1:1000. phosphor Ser2056; Abbkine, ABP55803).
Validation	All commercial antibodies were also validated by the manufactures as indicated on their web sites. CycT1 (Santa Cruz Biotech, sc-10750). https://antibodypedia.com/gene/1442/CCNT1/antibody/12236/sc-10750 ; pan-ADPr (anti-pan-ADP-ribose binding reagent; Millipore, MABE1016). https://www.merckmillipore.com/CN/zh/product/Anti-pan-ADP-ribose-binding-reagent,MM_NF-MABE1016?ReferrerURL=https%3A%2F%2Fcn.bing.com%2F&bd=1 ; pADPr (ADP-ribose-binding reagent; Millipore, MABE1031). https://www.merckmillipore.com/CN/zh/product/Anti-poly-ADP-ribose-binding-reagent,MM_NF-MABE1031?ReferrerURL=https%3A%2F%2Fcn.bing.com%2F&bd=1 ; polyclonal anti-PAR polymer antibody 10H (Enzolifesciences, ALX-804-220-R100). https://www.enzolifesciences.com/ALX-804-220/poly-adp-ribose-monooclonal-antibody-10h/ ; γH2AX (Sigma-Aldrich, 05-636). https://www.sigmaaldrich.cn/CN/zh/product/mm/05636 ; Flag (Sigma-Aldrich, F3165). https://www.sigmaaldrich.cn/CN/zh/product/sigma/f3165 ;

PARP1 (Cell signaling technology, #9542). <https://www.cellsignal.com/products/primary-antibodies/parp-antibody/9542>;
 RNA Pol II phospho-Ser5 (Millipore, 04-1572). <https://www.sigmaaldrich.cn/CN/zh/product/mm/041572>;
 Phospho-ATM (Ser1981; HUABIO, ET1705-50). <https://www.huabio.com/products/phospho-atm-s1981-antibody-clone-jm93-25-recombinant-monoclonal-et1705-50>;
 DNA-PKCS (phosphor Ser2056; Abbkine, ABP55803). <https://www.abbkine.com/product/dna-pkcs-phospho-ser2056-polyclonal-antibody-abp55803/>.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HeLa and 293T cell were both from ATCC. the DSB reporter cell line was kindly provided by Drs. R. Greenberg, E. Song, D. Yin, D. Xu and their colleagues.
Authentication	HeLa and 293T cells were authenticated by UC Berkeley Cell Culture Facility, the DSB reporter cell line was not authenticated.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	This study did not involve commonly misidentified lines

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>	The raw sequence data have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformatics / Beijing Institute of Genomics, Chinese Academy of Sciences, under accession number HRA000946
Files in database submission	DMSO iCDK9 MNNG
Genome browser session (e.g. UCSC)	no longer applicable

Methodology

Replicates	2
Sequencing depth	Each experiment is > 20M reads, paired-end, read length 150 bp
Antibodies	Anti-RNA Pol II (Millipore 05-623)
Peak calling parameters	no longer applicable
Data quality	FastQC
Software	STAR (v2.7.6), edgeR (v 3.26.5), Bowtie2 (v2.4.1), samtools (v1.11), Picard (v2.19.1)

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 EJ5-GFP cells reconstituted with WT or Mut2 CycT1 were analysis by FACS for the GFP expression accumulated 48 hr after I-SceI expression.
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Instrument	Data were collected on CytoFlex S (Beckman Coulter)
Software	FlowJo
Cell population abundance	At least 10000 gated cells were collected for each group
Gating strategy	Cells were first gated for FSC/SSC, then for transfected and successfully repaired cells as indicated in the figure legends

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