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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

All softwares are commercially or freely available.
1.Western Blot's proteins were visualized with a Tanon 5200SF Imaging System.
2.The MS/MS raw data were processed for protein identification and PTM analysis using PEAKS Studio 8.5.
3.Comet assay' images were captured using an Olympus BX51 fluorescent microscope.
4.Immunofluorescent images were captured under a Nikon confocal microscope.
5.For the laser assay, cells were locally irradiated with a 365-nm pulsed nitrogen UV laser (16 Hz pulse, 41% laser output) generated from a MicroPoint system (Andor).
6.ChIP qPCR and ChIP-re-ChIP were performed using a qTOWER3G Touch Real-Time PCR Detection System.
7.For chromosome aberration assay, the images were obtained using a DragonFly confocal imaging system.
8.For molecular docking, histone H1.4 or H1.4-2ND were predicted by AlphaFold2. GRAMM and PyMol were used for virtual verification.
9.Flow cytometry data was collected using FlowJo CE and analyzed using FlowJo™ v7.6.5 software.
10.For CUT&Tag-seq analysis, raw sequencing data was aligned to the GRhg38 (hg38) genome build using Bowtie2 (v2.3.4.1).
11. All libraries were sequenced by illumina NovoSeq 6000.
The software used for data collection was described in "Method". No special code were used.

Data analysis

All softwares are commercially or freely available.
1.Comet tail moments were measured using the OpenComet plugin in ImageJ.
2.For Microscale thermophoresis, curve fitting was performed by using MO. Affinity Analysis.
3.Image J was used to analyze Immunfluorescence images.
4.For laser assay, the intensity of the irradiation path signal was calculated using ImageJ.
5.PLA foci were automatically quantified using ImageJ software.

6.For MNase, the intensity of each lane was analyzed using ImageJ.
7.Statistical Analysis was done in PRISM 9.
8.Figures were compiled using Adobe Illustrator.

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

Plasmids and primers used are listed in the Supplementary Information. CUT&Tag-seq and ATAC-seq data generated during this study are available at the Gene Expression Omnibus under accession number GSE288071. Source data are provided with this paper.

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	Sex and gender were not considered in this study and this information was not collected as part of our protocol.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, or other social relevant groupings were not considered in this study.
Population characteristics	Human samples were from patients diagnosed with cervical cancer at Shanghai First Maternity and Infant Hospital and Fudan University Shanghai Cancer Center (China). No other population characteristics were considered in the analysis.
Recruitment	Cervical cancer patient samples were obtained at Shanghai First Maternity and Infant Hospital and Fudan University Shanghai Cancer Center after informed consent was obtained. We then selected randomly from the tumor banks. Participants did not receive any compensation. There are no recruitment biases to impact the results of our analyses.
Ethics oversight	These studies using human specimens were approved by the Clinical Research Ethics Committee of Shenzhen University (IACUC, The mechanisms of chromatin remodeling during early stage of DNA damage response, 202400110).

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	In the study, n= at least 3 biological replicates were used for sample size in all cases order to allow statistical analyses. Each biological replicate was defined as an independent culture of cells. Sample sizes were chosen to satisfy statistical power based on previous report (Nat Struct Mol Biol. 2023 .PMID: 37735618). For animal studies, power analyses were determined using a web-based tool (www.biomath.info).
Data exclusions	No data exclusions.
Replication	All the findings were reliably reproduced in multiple biological independent experiments. For all experiments, our data represent at least two independent assays that produce similar results. We also used different assays and readouts to confirm our findings in different way.
Randomization	Cultures and same genotype mice were randomly assigned to either experimental or control groups. For the cell culture experiments, cells were randomly allocated to experimental groups. For all analyses and quantifications, cells were randomly selected. Assigning human samples to experimental groups is not relevant to this study, as the groups are defined by clinical and pathological diagnosis.
Blinding	During sample processing and analysis for MS , qPCR, CUT&tag-seq and ATAC-seq, samples were given a de-identified alphanumeric code. After analysis was completed, samples were unblinded and graphed. The quantification for immunofluorescence assays was performed blindly. In vivo experiments were not blinded during experimentation, although the labels were masked during data analysis.

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
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

All information of antibodies are described in supplementary table 1. Rabbit monoclonal anti-H1.1 (#ab254394, Abcam, 1:1000), Rabbit polyclonal anti-H1.2 (#ab4086, RRID:AB_2117983, Abcam, 1:1000), Rabbit monoclonal anti-H1.3 (#ab183736, Abcam, 1:1000), Rabbit polyclonal anti-H1.4 (#41328S, RRID:AB_2799199, Cell Signaling Technology, 1:1000), Rabbit polyclonal anti-H1.5 (#ab18208, RRID:AB_470263, Abcam, 1:1000), Rabbit polyclonal anti-H3 (#17168-1-AP, RRID:AB_2716755, Proteintech, 1:1000), Mouse monoclonal anti-γH2AX (#80312, RRID:AB_2799949, Cell Signaling Technology, 1:1000), Mouse monoclonal anti-β-actin (#66009-1-Ig, RRID:AB_2687938, Proteintech, 1:1000), Mouse monoclonal anti-FLAG® M2 (#F3165, RRID:AB_259529, Sigma-Aldrich, 1:1000), Rabbit monoclonal anti-pATR (#30632, Cell Signaling Technology, 1:1000), Rabbit polyclonal anti-pNF-κB (#3037, RRID:AB_2341216, Cell Signaling Technology, 1:1000), Rabbit polyclonal anti-53BP1 (#NB100-304, RRID:AB_10003037, Novus Biologicals, 1:1000), Rabbit monoclonal anti-RAD51 (#ab133534, RRID:AB_2722613, Abcam, 1:1000), Rabbit polyclonal anti-CTPS1 (#15914-1-AP, RRID:AB_2086513, Proteintech, 1:1000), Rabbit polyclonal anti-GST (#10000-0-AP, RRID:AB_11042316, Proteintech, 1:1000), Rabbit monoclonal anti-H4 (#13919, RRID:AB_2798345, Cell Signaling Technology, 1:1000), Rabbit monoclonal anti-H2A (#12349, RRID:AB_2687875, Cell Signaling Technology, 1:1000), Rabbit monoclonal anti-H2B (#12364, RRID:AB_2714167, Cell Signaling Technology, 1:1000), Rabbit polyclonal anti-Phospho-(S/T) (#9631, Cell Signaling Technology, RRID:AB_330308, 1:1000), Rabbit polyclonal anti-Acetylated-lysine (#9441, RRID:AB_331805, Cell Signaling Technology, 1:1000), Rabbit polyclonal anti-pan methyl lysine (#ab7315, RRID:AB_305840, Abcam, 1:1000), Rabbit polyclonal anti-PAR (#83732, RRID:AB_2749858, Cell Signaling Technology, 1:1000), Rabbit monoclonal anti-p300 (#86377S, RRID:AB_2800077, Cell Signaling Technology, 1:1000), Rabbit monoclonal anti-CBP (#7389S, RRID:AB_2616020, Cell Signaling Technology, 1:1000), Rabbit monoclonal anti-hMOF (#ab200660, RRID:AB_2891127, Abcam, 1:1000), Rabbit polyclonal anti-PCAF (#3378 , RRID:AB_10679933, Cell Signaling Technology, 1:1000), Rabbit anti-Tip60 (#12058S, RRID:AB_2797811, Cell Signaling Technology, 1:1000), Rabbit polyclonal anti-PARP1 (#9542, RRID:AB_2160739, Cell Signaling Technology, 1:1000), Rabbit polyclonal anti-Ki67 (#ab15580, RRID:AB_443209, Abcam, 1:1000), Normal Rabbit IgG (#2729, RRID: AB_1031062, Santa Cruz Biotechnology, 1:1000).

Validation

The validation data for customed antibodies were provided in the manuscript. The validation of commercial antibodies was according to manufacturers' websites or previous reports, listed in supplementary table 1.Rabbit monoclonal anti-H1.1, which is validated in bioRxiv, 2024.2005.2010.593572 (2024). https://doi.org:10.1101/2024.05.10.593572 Rabbit polyclonal anti-H1.2, which is validated in Mol Cell 77, 475-487 e411 (2020). https://doi.org:10.1016/j.molcel.2019.10.020 Rabbit monoclonal anti-H1.3, which is validated in Int J Biol Sci 18, 2583-2596 (2022). https://doi.org:10.7150/ijbs.71519 Rabbit polyclonal anti-H1.4, which is validated in Nat Commun 14, 4991 (2023). https://doi.org:10.1038/s41467-023-40578-2 Rabbit polyclonal anti-H1.5, which is validated in Cancer Res 82, 2887-2903 (2022). https://doi.org:10.1158/0008-5472.CAN-22-0717 Rabbit polyclonal anti-H3, which is validated in Nature 587, 673-677 (2020). https://doi.org:10.1038/s41586-020-2749-z Mouse monoclonal anti-γH2AX, which is validated in Nat Commun 15, 10596 (2024). https://doi.org:10.1038/s41467-024-54978-5 Mouse monoclonal anti-β-actin, which is validated in Cell 186, 2208-2218 e2215 (2023). https://doi.org:10.1016/j.cell.2023.03.032 Mouse monoclonal anti-FLAG® M2, which is validated in Cell Rep 31, 107549 (2020). https://doi.org:10.1016/j.celrep.2020.107549Rabbit monoclonal anti-pATR, which is validated in Nature 629, 443-449 (2024). https://doi.org:10.1038/s41586-024-07350-y Rabbit polyclonal anti-pNF-κB, which is validated in Nat Commun 14, 1713 (2023). https://doi.org:10.1038/s41467-023-37450-8 Rabbit polyclonal anti-53BP1, which is validated in Cell Rep 42, 113181 (2023). https://doi.org:10.1016/j.celrep.2023.113181 Rabbit monoclonal anti-RAD51, which is validated in Nat Commun 15, 3684 (2024). https://doi.org:10.1038/s41467-024-48149-9 Rabbit polyclonal anti-CTPS1, which is validated in Cancer Res 82, 1013-1024 (2022). https://doi.org:10.1158/0008-5472.CAN-21-1707 Rabbit polyclonal anti-GST, which is validated in Cell 187, 4272-4288 e4220 (2024). https://doi.org:10.1016/j.cell.2024.06.024 Rabbit monoclonal anti-H4, which is validated in Nat Commun 15, 9529 (2024). https://doi.org:10.1038/s41467-024-51847-z Rabbit monoclonal anti-H2A, which is validated in Nat Commun 15, 9244 (2024). https://doi.org:10.1038/s41467-024-53639-x Rabbit monoclonal anti-H2B, which is validated in Nat Commun 14, 4103 (2023). https://doi.org:10.1038/s41467-023-39735-4 Rabbit polyclonal anti-Phospho-(S/T), which is validated in Nat Commun 15, 1785 (2024). https://doi.org:10.1038/s41467-024-46166-2 Rabbit polyclonal anti-Acetylated-lysine, which is validated in Sci Adv 10, eadp7423 (2024). https://doi.org:10.1126/sciadv.adp7423 Rabbit polyclonal anti-pan methyl lysine, which is validated in Cell Discov 7, 37 (2021). https://doi.org:10.1038/s41421-021-00279-wRabbit polyclonal anti-PAR, which is validated in Nat Commun 15, 6641 (2024). https://doi.org:10.1038/s41467-024-50912-x Rabbit monoclonal anti-p300, which is validated in Nat Immunol 25, 1580-1592 (2024). https://doi.org:10.1038/s41590-024-01922-w Rabbit monoclonal anti-CBP, which is validated in Nat Immunol 25, 1580-1592 (2024). https://doi.org:10.1038/s41590-024-01922-w Rabbit monoclonal anti-hMOF, which is validated in Nat Cancer 4, 382-400 (2023). https://doi.org:10.1038/s43018-023-00522-1 Rabbit polyclonal anti-PCAF, which is validated in Sci Adv 10, eadm9449 (2024). https://doi.org:10.1126/sciadv.adm9449 Rabbit anti-Tip60, which is validated in Adv Sci (Weinh) 10, e2206584 (2023). https://

doi.org:10.1002/advs.202206584 Rabbit polyclonal anti-PARP1, which is validated in Commun Biol 8, 4 (2025). https://doi.org:10.1038/s42003-024-07409-6 Rabbit polyclonal anti-Ki67, which is validated in Nat Chem Biol 20, 566-576 (2024). https://doi.org:10.1038/s41589-023-01466-4 Normal Rabbit IgG, which is validated in Nat Commun 16, 212 (2025). https://doi.org:10.1038/s41467-024-55768-9.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HeLa, HCT116, A549, H1975, HepG2, Hepa1-6, NCI-H1299, HCC1937, MCF7, T47D-KBluc, 786-O, A-498, 5637, T24 and HEK293T cells were obtained from the American Type Culture Collection (Manassas, Virginia, USA). AsiSI-ER-U2OS-AID cells were provided by Dr. Gaëlle Legube (Université Paul Sabatier, Toulouse, France); EJ5-U2OS and DR-U2OS cells were provided by Dr. Xingzhi Xu (Shenzhen University, Shenzhen, China); and U2OS-265 reporter cells were provided by Dr. Roger Greenberg (University of Pennsylvania, USA).
Authentication	Cell lines from ATCC are routinely authenticated by cellular morphology assay. Established stable cell lines were authenticated by PCR and wetern blot as shown in figures.
Mycoplasma contamination	All cell lines were tested negative mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	4-week-old BALB/c nude mice were purchased from WuXi AppTec, Guangdong, China. Mice were maintained under SFP conditions in the animal facility of Shenzhen University. The animal room has a controlled temperature (18-23°C), humidity (40-60%), and a 12 light/12 dark cycle.
Wild animals	This study did not involve wild animals.
Reporting on sex	Female
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	The use of animals in this study was approved by the Institutional Animal Care and Use Committee (IACUC) of Shenzhen University, China.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	NA.
Novel plant genotypes	NA.
Authentication	NA.

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 *May remain private before publication.*	The data will be accessible before publication. Gene Expression Omnibus under accession number GSE288071.
Files in database submission	ND; ND_4OHT; CT; CT_4OHT; ac; ac_4OHT; aD; aD_4OHT; A; A_4OHT; R_Pi; R_Pi_4OHT; NC; NC_4OHT; SI; SI_4OHT

Genome browser session (e.g. UCSC)	NA

Methodology

Replicates	CUT&Tag-seq and ATAC-seq samples were prepared as one replicate in each independent experiment.
Sequencing depth	ND, total reads:83638754 ; mapped reads:69445216; reads length: 150bp; paired-end. ND_4OHT, total reads:42723254 ; mapped reads:33936984; reads length: 150bp; paired-end. CT, total reads:42840870 ; mapped reads:34682390; reads length: 150bp; paired-end. CT_4OHT, total reads: 40940896; mapped reads:30074578; reads length: 150bp; paired-end. ac, total reads: 38905586; mapped reads:31263642; reads length: 150bp; paired-end. ac_4OHT, total reads: 37108502; mapped reads:31059092; reads length: 150bp; paired-end. aD, total reads:51580120 ; mapped reads:24507006; reads length: 150bp; paired-end. aD_4OHT, total reads:40205754 ; mapped reads:32056362; reads length: 150bp; paired-end. A, total reads:27629496 ; mapped reads:18820764; reads length: 150bp; paired-end. A_4OHT, total reads:471002 ; mapped reads:465174; reads length: 150bp; paired-end. R_Pi, total reads:1972890 ; mapped reads:10769952; reads length: 150bp; paired-end. R_Pi_4OHT, total reads:53756788 ; mapped reads:33294578; reads length: 150bp; paired-end. NC, total reads:51613294 ; mapped reads:49490948; reads length: 150bp; paired-end. NC_4OHT, total reads:87549828 ; mapped reads:84582728; reads length: 150bp; paired-end. SI, total reads:53402592 ; mapped reads:52786150; reads length: 150bp; paired-end. SI_4OHT, total reads:75508938 ; mapped reads:74137900; reads length: 150bp; paired-end.
Antibodies	Histone modification antibodies were customed; Rabbit polyclonal anti-CTPS1 (#15914-1-AP, RRID:AB_2086513, Proteintech, 1:1000).
Peak calling parameters	NA.
Data quality	Raw data (raw reads) of fastq format were firstly processed using fastp (version 0.19.11, Chen et al., 2018)software. In this step, clean data (clean reads) were obtained by removing reads containing adapter, readscontaining ploy-N and low quality reads from raw data. At the same time, Q20, Q30 and GC content ofthe clean data were calculated. All the downstream analyses were based on the clean data with high quality.
Software	Bowtie2 (v 2.5.4) was applied for alignment to the hg38 human genome reference, using the parameters "--very-sensitive --no-mixed --no-discordant." Samtools was used to convert SAM files to BAM format, applying a filter criterion of a minimum mapping quality (MAPQ) score of 10. Duplicate reads were removed using the Picard tool. The bamCoverage function was then used to transform BAM files into BW format, with normalization applied using the parameter "--normalizeUsing BPM." .The generation of metagene profiles and heatmaps, which display the signals of each histone modification and open chromatin at double-strand breaks, was accomplished using the computeMatrix and plotHeatmap functions of deepTools (v 3.5.5).

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	For the DR and EJ5 assays, reporter cells were washed with PBS, collected by trypsinization, then assayed for GFP expression by flow cytometry.
Instrument	Becton Dickinson FACSCalibur
Software	Data was collected with FlowJo CE and data was analyzed with FlowJo version 7.6.5 software
Cell population abundance	Cell populations for DR and EJ5 were differentiable by GFP expression above 1000 intensity and quantified with FlowJo.
Gating strategy	For GFP analysis in the DR and EJ5 assay, the GFP threshold for gating was established using the positive control samples and set at 1000 intensity. This gating strategy is shown in the supplementary Information. A negative control sample confirmed this gating strategy, before the exact same gate was applied to all experimental samples.

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