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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Sample sizes for DNA fiber experiments and immunofluorescence microscopy in this study were chosen according to published guidelines and to our previous experience.

2. Data exclusions

Describe any data exclusions.

No data exclusion

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experiments were reliably reproduced.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

All cell-based samples in each of the group were included and no method of randomization was applied.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinded experiments were performed for DNA fiber analyses.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	MetaMorph (Molecular Devices), GraphPad Prism, CellProfiler, ImageJ-JACoP, FlowJo (LLC), Multi Gauge V3.2 software (Fuji), MO.Affinity Analysis Software version 2.2.4 (NanoTemper Technologies GmbH)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	All unique materials used are readily available from the authors or from standard commercial sources.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The source and the dilution for all antibodies used in this study are included in the manuscript as an extended data table.

Antibodies used in human cells-

Antibody	Catalog No.	Dilution
Mouse anti-BrdU	clone B44 347580, BD Biosciences	1/100
Rat anti-BrdU	clone BU1/75 ABC117-7513, Eurobio Abcys	1/100
anti-BrdU for ssDNA foci	347580, BD Biosciences	1/20
anti-ssDNA	MAB3868, Millipore	1/250
anti-pCHK1 (S345)	2348, Cell signaling	1/1000
anti-pCHK2 (T68)	2661, Cell signaling	1/1000
anti-SAMHD1	A303-691A, Bethyl	1/5000
anti-g-H2AX (S139)	05-636, Millipore	1/1000
anti-pSAMHD1 (T592)	Cribier et.al. 2013	1/500
anti-HA	Clone3F10, Roche	1/300
anti-actin	A4700, Sigma	1/500
anti-RPA1	Ab79398	1/300
anti-RECQ1	A300-450A, Bethyl	1/5000
anti-cGAS	15102, Cell Signaling Technology	1/1000
anti-IRF3	4302, Cell Signaling Technology	1/1000
anti-TBP	8515, Cell Signaling Technology	1/1000

Antibodies used in Xenopus extracts-

Antibody	Source	Identifier	Dilution
Anti-Xenopus SAMHD1	Biogenes	BioGenes Serum #25130	1/500
Anti-Xenopus Pol a p180 (Mouse monoclonal)	Kolinjivadi et al., 2017	Abmart: clone 13026-1-3/C199	1/1000
Anti-Orc1 (Mouse monoclonal)	Aze et al., 2016	-	1/5000
Anti-xMre11 (Rabbit polyclonal)	kindly provided by Jean Gautier (Aparicio et al., 2016)	-	1/3000
Anti-xRPA70 (Rabbit polyclonal)	kindly provided by Jean Gautier (Aparicio et al., 2016)	-	1/5000
Anti-xCtIP (Mouse monoclonal)	kindly provided by Jean Gautier (Peterson SE et al., 2013)	-	1/30
Anti-xATR	Aze et al., 2016	-	1/1000
Anti-xATM	Aze et al., 2016	-	1/1000
Anti-ATM pSerine 1981	Rockland Immunochemicals	200-301-400	1/1000
Anti-Phospho-Chk1 (Ser345) (133D3)	Cell Signaling	mAb #2348	1/500
Anti-Chk1 (G-4)	Santa Cruz	sc-8408	1/500
Anti-Histone H2B	Millipore	clone 07-371; RRID: AB_310561	1/5000
Anti-Mcm7	Santa Cruz	sc-9966	1/5000
Anti-Rad51 (mouse monoclonal)	Abcam	clone ab123	1/1000
Anti-phospho-Histone H2A.X (Ser139)	Millipore	clone JBW301	1/1000

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Human embryonic kidney (HEK) HEK293T cells (ATCC CRL-3216)
HeLa cells (ATCC CCL-2) were purchased from ATCC
SAMHD1-overexpressing HeLa cells (Clifford et al., 2014)
HL116 cells (Garcin et al., 2014)
STING-KO THP1 cells (Holm et al., 2016)

b. Describe the method of cell line authentication used.

None of the cell lines have been authenticated by Standards for Cell Line Authentification (Almeida et al. 2016). However, HEK293T and HeLa cells have been purchased from ATCC and authenticated by this organization with certificates. SAMHD1-expressing HeLa cells were controlled by the expression level of SAMHD1, growth rate and morphology. HL116 cells were controlled regularly by the measurement of luciferase activity induced by type I interferons and HAT selection. STING-KO THP1 cells were controlled by the expression level of STING and monocytic cell line characteristics.

c. Report whether the cell lines were tested for mycoplasma contamination.

All the cell lines are mycoplasma-free. They have been tested for mycoplasma contamination regularly using MycoAlert Mycoplasma Detection Kit (LONZA).

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used in the study.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Xenopus egg extracts are used in this study. The detailed preparation procedure is included in supplementary information.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.