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Supplementary Information for

SETD2-mediated H3K14 trimethylation promotes ATR activation and stalled replication fork restart in response to DNA replication stress

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This PDF file includes:

SI Methods and Materials

Materials.

Cell culture, transfection and generation of cell lines

HeLa, 293T, LN, HEK293, U2OS and A549 cells and mouse embryonic fibroblasts (MEFs) were grown in Dulbecco's Modified Eagles Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, and maintained in a humidified atmosphere of 5% CO_2 at 37°C. HCT116 cells were grown in McCoy's 5a medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, in a humidified atmosphere of 5% CO2 maintained at 37°C. The cells were sub-cultured by trypsinization every 2 days and seeded at the appropriate confluency.

The HeLa cells expressing either Flag-H3.1 WT or Flag-H3.1K14 mutant (K14R, K14A or K14Q) were transfected with the relevant plasmids. The cells were then cultured in 1 mg/ml G418 (Sigma Aldrich) to select for clones stably expressing either Flag-H3.1 WT or Flag-H3.1K14 mutant. Transient and stable transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol.

HeLa parental cells and SETD2 knockout HeLa cells #1 were obtained from Professor Guo-min Li (Southwestern Texas University)(1).
SETD2 knockout HeLa cells #2 was generated by the CRISPR Cas9 technology. The target sequence of the sgRNA is CACCGACTCTGATCGTCGTCGCTACCAT (first exon of SETD2).

Antibodies

The antibodies used in this study were: anti-Flag (F1804, Sigma-Aldrich, St Louis, MO, USA), anti-G9a (G6919, Sigma-Aldrich), anti-GST (C1303, APPLYGEN, Beijing, China), anti-GAPDH (sc-32233, Santa Cruz Biotechnology), anti-H3 (ab1791, Abcam, Cambridge, UK), anti-H4 (ab10158, Abcam), anti-H3K36me3 (9763S, Cell Signaling Technology, Danvers, MA, USA), anti-H3K9me2 (ab1220, Abcam), anti-H3K9me3 (ab8898, Abcam), anti-H3K79me3 (ab2621, Abcam), anti-H3K4me3 (ab8580, Abcam), anti-RPA32 (ab2175, Abcam), anti-Actin (sc-58673, Santa Cruz Biotechnology), anti-SET7/9 (2495643, EMD Millipore, Billerica, MA, USA), anti-MLL (A300-374A, BETHYL Laboratories), anti-DOT1L (ab64077, Abcam), anti-phospho-CHK1 (Ser345) (2348S, Cell Signaling Technology, Danvers, MA, USA), anti-CHK1 (2360S, Cell Signaling Technology, Danvers, MA, USA), anti-ATRIP (2737S, Cell Signaling Technology, Danvers, MA, USA), anti-TopBP1 (14342S, Cell Signaling Technology, Danvers, MA, USA), anti-SETD2 (23486S, Cell Signaling Technology, Danvers, MA, USA; ab184190, Abcam), anti-RPA70 (2267S, Cell Signaling Technology, Danvers, MA, USA), anti-HP1a (MA1-218, Invitrogen, Carlsbad, CA, USA), anti-phospho-ATR (Thr1989) (ab227851, Abcam), anti-ATR (ab2905, Abcam), anti-phospho-

RPA32 (Ser33) (ab211877, Abcam), anti-SUV39H1 (8729S, Cell Signaling Technology, Danvers, MA, USA), anti-SUV39H2 (ab190870, Abcam), anti-Biotin (sc-53179, Santa Cruz Biotechnology).

Plasmids

The GST-SETD2 SET domain plasmid was provided by Dr. Bing Zhu (Institute of Biophysics, Chinese Academy of Sciences). The SETD2 SET domain fragments were amplified and cloned into GFP vectors. Full-length histone H3.1, H3.1K14 mutant and H3.1K36 mutant were amplified and subcloned into pcDNA3.1, p3xFLAG-CMV-10 and GFP vectors. The full-length human SUV39H1 and the G9a cDNAs were amplified and cloned into pGEX-6P-1. Full-length RPA70 and RPA70 fragments were amplified and cloned into p3xFLAG-CMV-10 and pGEX-6P-1. All mutation constructs were generated with a Mut Express II Fast Mutagenesis Kit (Vazyme Biotech Co., Nanjing, China).

siRNA transfection

All siRNAs were purchased from Shanghai GenePharma Company. siRNAs were transfected using a Lipofectamine 2000 transfection kit (Invitrogene), according to the manufacturer's instructions. The RNAi oligonucleotide sequences were as follows:

Non-specific (CTR) small interfering RNA (siRNA) sense strand: 5'-UUCUCCGAACGUGUCACGU-3';

G9a siRNA sense strand: 5'-CCAUGCUGUCAACUACCAUGG-3';

SUV39H1 siRNA sense strand: 5'-ACCTCTTTGACCTGGACTA-3';

SUV39H2 siRNA sense strand: 5'-CACAGAUUGCUUCUUUCAA-3';

SETD2 siRNA sense strand #1: 5'-GAAACCGUCUCCAGUCUGU-3';

SETD2 siRNA sense strand #2: 5'-UAAAGGAGGUAUAUCGAAU-3';

SET7/9 siRNA sense strand: 5'-GCCTTGTAGGAGAAGTAAA-3';

DOT1L siRNA sense strand: 5'-GCUGGAGCUGAGACUGAAG-3';

MLL siRNA sense strand: 5'-ACGAAAGACTGAATGTAAAUU-3';

Methods.

Drug treatments

Cells were treated with 40 M etoposide (E1383, Sigma-Aldrich) for 6 h, 1 mM doxorubicin (Sigma-Aldrich) for 12 h, ATR inhibitor (NU6027, Selleck) or HU (Sigma-Aldrich) at the indicated doses for 5 h or 24 h. Then, the cells were collected for protein extraction, immunofluorescence or flow cytometry.

Histone acid extraction

Cells were lysed in 1 ml hypotonic lysis buffer (10 mM Tris–HCl pH 8.0, 1 mM KCl, 1.5 mM $MgCl_2$ and 1 mM DTT, 1% protease inhibitors), and the intact nuclei were pelleted by centrifugation at 13,523 × *g* at 4°C for 10 min. The supernatant was discarded and the nuclei were re-suspended in 400 ml 0.2 M sulfuric acid and incubated for at least 30 min at 4°C. The samples were again collected by centrifugation at 13,523 × *g* at 4°C for 10 min, and the supernatant containing the histones was collected. Trichloroacetic acid was added to the histones to a final concentration of 33% and the samples were incubated on ice for 30 min. The histone pellet was collected by centrifugation at 13,523 × *g* at 4°C for 10 min, washed with acetone and then dissolved in ddH2O.

Protein extraction and western blotting

Whole-cell lysate extraction. Equal numbers of harvested cells were washed with PBS by centrifugation at 9,391 × *g* at 4°C for 30 s and the cell pellet was re-suspended in 30 ml of 2× protease inhibitor buffer (per 10^6 cells) containing one cocktail protease inhibitor pellet (Roche Holding AG, Basel, Switzerland) in 3.5 ml PBS. An equal volume of 2× sodium dodecyl sulphate (SDS) loading buffer (950 ml Laemmle buffer + 50 ml 2-mercaptoethanol) was added to the re-suspended cells. The samples were boiled for 10 min with a pulse vortex every 5 min, and then pelleted by centrifugation at 13,523 x *g* at 4°C for 15 min.

GST protein purification. GST-fusion proteins were expressed in the bacterial cell strain BL21 with isopropyl b-d-1-thiogalactopyranoside, purified using glutathione-Sepharose 4B beads (GE Healthcare), and then washed in TEN buffer (20 mM Tris · HCl, pH 7.4, 0.1 mM EDTA, and 100 mM NaCl).

Chromatin protein extraction. Cells were harvested in buffer I (50 mM HEPES pH 7.5, 150 mM NaCl and 1 mM EDTA) supplemented with 0.1% Triton X-100, 1% protease inhibitor cocktail (Roche Holding AG, Switzerland) and 2 mM PMSF and lysed on ice for 3 min. The supernatant was discarded and the pellet was dissolved in buffer I supplemented with 200 mg/mL RNaseA and 1% protease inhibitor cocktail and incubated at room temperature for 30 min. The supernatant was discarded after centrifugation and the pellet was resuspended in buffer I, boiled in an equal volume of 2× SDS/PAGE sample buffer at 100 °C for 5 min and analyzed by western blotting.

Mouse tissue protein extraction. Murine tissues were homogenized in RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.1% SDS) supplemented with 1% protease inhibitor cocktail (Roche) and 1% phosphatase inhibitors (APPLYGEN). Whole cell extracts (WCE) were isolated as previously described (2).

Western blotting was used to evaluate protein levels, as previously described (2), with minor modifications. Namely, equal amounts of protein were size-fractionated on a 6–15% SDS-polyacrylamide gel.

Mono-nucleosome preparation and Immunoprecipitation

Mono-nucleosome extraction assay was carried out as previously described (3). Cells were pelleted and resuspended in lysis buffer (10 mM Tris-HCl (pH8.0), 250 mM sucrose, 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 0.5 mM DTT, 0.5% Triton X-100) and kept on ice for 10 min. The nuclei were collected by centrifugation (3,000 × g for 3 min). For micrococcol nuclease digestion, crude nuclei were resuspended in digestion buffer (10 mM Tris-HCl (pH8.0), 250 mM sucrose, 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 0.5 mM DTT, 1 mM $CaCl_2$) and incubated at 37°C for 80 min with MNase (TaKaRa) at 40 U/10^7 nuclei. Digestion was stopped by adding EDTA to a final concentration of 20 mM and chilling at 4 °C. After centrifugation (10,000 × g for 5 min), the nuclear pellet was resuspended in 5 mM EDTA (10 min, 4 °C). A supernatant fraction containing mono-nucleosomes generated by centrifugation (10,000 × g for 10 min) was subjected to further fractionation with a 24-ml Superose 6 gel filtration column (GE Healthcare) in buffer containing 10 mM Tris HCl (pH8.0), 100 mM KCl, 0.5 mM EDTA, 1 mM DTT, and 10% glycerol. The mono-nucleosome fractions were pooled for subsequent immunoprecipitation using anti-Flag M2 beads.

Immunofluorescence

Cells were washed three times with PBS (0.1% Tween-20) and then incubated for 10 min in pre-extraction buffer (25 mM HEPES 7.4, 50 mM NaCl, 1 mM EDTA, 3 mM $MgCl_2$, 300 mM sucrose and 0.5% TritonX-100) at room temperature. The cells were fixed with 2% formaldehyde (w/v) in PBS for 20 min at room temperature. Following three washes with PBS/0.1% Tween-20, the cells were blocked for 1 h with 5% BSA in PBS/0.1% Tween-20 and then incubated with the appropriate antibodies in blocking solution at 4°C overnight. After washing three times with blocking buffer, the samples were then incubated the appropriate secondary antibodies in blocking solution for 1 h at 4°C in the dark. After three washes with blocking buffer, the samples were embedded in DAPI for 3 min. Images were captured under an Olympus confocal microscope.

Nuclear protein Co-IP assay

Cells were lysed in buffer A (10 mM Hepes, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.15% Nonidet P-40, 1% protein inhibitor mixture, and 1 mM DTT) for 10 min. After centrifugation at 4°C, the deposits were washed twice in PBS and then lysed in buffer B (20 mM Hepes, pH 7.9, 40 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.5% Nonidet P-40, 1% Mixture) for 20 min, shaking for 10 s at 5-min intervals. After centrifugation at 12,000 × g for 10 min at 4°C, the supernatant was diluted with buffer B' (20 mM Hepes, pH 7.9, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1% Mixture) at a volume ratio of 1: 1 (vol/vol) and pre-cleared with protein A or G Sepharose for 2-4 h. After centrifugation at 2,000 × g for 5 min at 4°C, 2 mg of the indicated

antibody was added to the supernatant and incubated at 4°C overnight. After centrifugation at 2,000 × g for 5 min at 4°C, 30 mL of protein G or A Sepharose was added and incubated for a further 2 h at 4°C. The beads were washed with buffer B three times. The precipitated components were analyzed by western blotting.

Biotin-peptide pull-down assay

Peptides [H3 (1-22-biotin) and H3 (28-40-biotin), methylated and unmodified as indicated in figure] were incubated with streptavidin agarose slurry (New England Biolabs, Ipswitch, MA, USA) overnight at 4°C in binding buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 10% Glycerol, 1 mM EDTA, 2 mM $MgCl_2$, 0.5% NP-40). After removing the unbound peptides, 2 mg Flag-RPA70 was added for 1 h and incubated at 4°C. After three washes with washing buffer (50 mM Tris–HCl pH 7.4, 300 mM NaCl, 10% Glycerol, 1 mM EDTA, 2 mM $MgCl_2$ 2, 0.5% NP-40), the beads were boiled at 100°C for 5 min and analyzed by western blotting.

GST pull-down assay

The GST-fusion proteins (“bait” and “prey” proteins) were purified as described in *GST Protein Purification*. For GST pull-down, the GST tag of the prey proteins were cut using HRV 3C protease (Takara), according to the manufacturer’s protocol. The bait and prey proteins were then incubated at 4°C overnight in GST-binding buffer (50 mM Tris · HCl, pH 7.4, 150 mM NaCl, 0.05% Nonidet P-40). The beads were washed three times with GST-binding buffer and boiled in 2× SDS loading buffer, and the proteins were analyzed by western blotting with the indicated antibodies.

iPOND assay

iPOND assay was performed as previously described (4). Briefly, cells were labeled with 10 mM EdU for 20 min and then washed with washing buffer (0.5% bovine serum albumin in PBS). After washing, the cells were incubated for 2 h in media containing 4 mM HU. Then, the cells were fixed with 1% formaldehyde in PBS for 20 min at room temperature. Next, the formaldehyde was quenched with 1.25 M glycine before the cells were harvested, permeabilized with 0.25% Triton in PBS for 30 min, and incubated with click reaction buffer (1 mM biotin azide, 100 mM $CuSO_4$, 20 mg ml-1 sodium L-ascorbate in PBS). After the click reaction, the cells were sonicated in lysis buffer (1% SDS in 50 mM Tris-HCl, pH 8.0) and centrifuged at 14,000 rpm for 10 min. The biotin-labeled lysates were incubated overnight at 4°C with streptavidin beads. After the incubation, the beads were rinsed twice with cold lysis buffer for 5 min each, once with 1 M NaCl, and twice with cold lysis buffer. Finally, the beads were heated at 95 °C in 2× Laemmli buffer for 30 min, loaded onto SDS-PAGE and immunoblotted with the indicated antibodies.

DNA fiber assay

For fork restart experiments, the cells were pulse-labeled with CldU for 30 min followed by a 5 h exposure to 4 mM HU. Then, the medium was refreshed, and the cells were pulse-labeled with IdU for 30 min. At least 200 replication forks were analyzed per experimental condition. The lengths of red or green labeled tracts were measured using ImageJ (National Institutes of Health; https://imagej.nih.gov/ij/) and arbitrary length values were converted into micrometers using the scale bars created by the microscope.

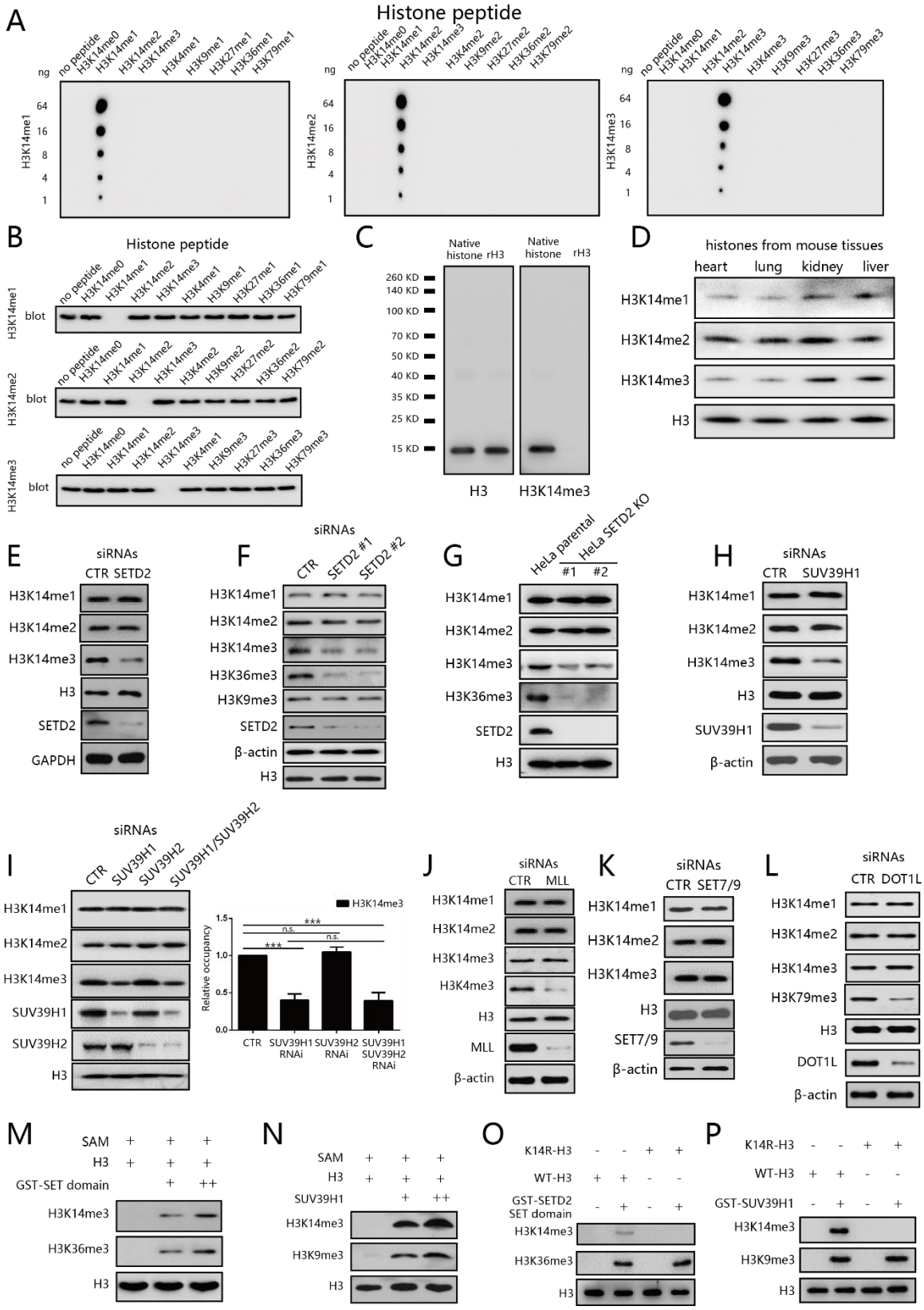
Flow cytometry

Cells were cultured to ~80% confluency and treated with 4 mM HU for 12 h. Then, the cells were washed with PBS twice and released into fresh medium. The cells were collected at indicated times and fixed with 75% ethanol at 4°C under rotation overnight. The samples were then centrifuged at 500 × g for 3 min, and the cells were washed twice with PBS. The cells were incubated with RNase A (250 mg/mL) at 37°C for 30 min and then stained with propidium iodide (5 mg/mL). The cells were analyzed by flow cytometry on a FACSCalibur flow cytometer (BD Bioscience) and the data processed using FlowJo software package..

Colony formation assay

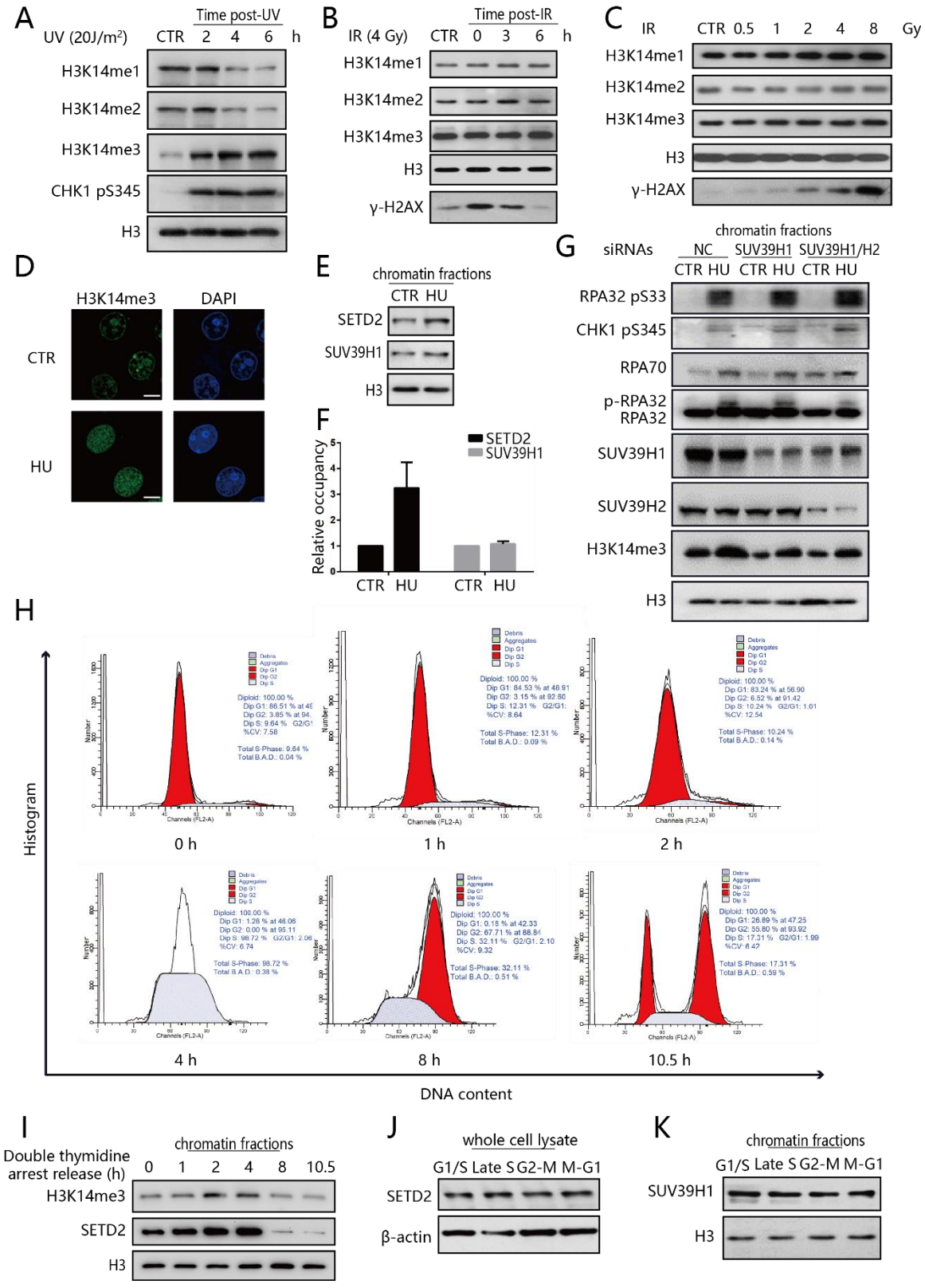
Cells were treated with or without 8 mM hydroxyurea for 2 h, washed four times with PBS and then seeded in 6-cm plates in equal number. After 2 weeks, the cells were fixed with 4% paraformaldehyde and stained with methylene blue to identify colonies. Three independent experiments were performed.

Fig. S1. H3K14 is methylated in mammals by SETD2 and SUV39H1.



(A) A slot blot assay showing the specificity of the H3K14 methylation antibodies.
(B) A peptide competition assay with H3K14 methylation antibodies was performed by western blotting, using a series of H3 peptides (1 μg/ml), either unmodified (H3K14me0) or methylated, as indicated.
(C) Native histones extracted from HeLa cells and recombinant histone H3 (rH3) was analyzed by western blotting. The entire membrane was probed with an H3K14me3 antibody or an H3 antibody as a protein loading control.
(D) H3K14 methylation expression levels in different mouse tissues.
(E) HCT116 cells were transfected with SETD2 siRNAs or a non-specific siRNA (CTR). The cells were collected 72 h after transfection, and the whole cell lysates and histones were extracted for western blotting. GAPDH and H3 served as an internal control for protein loading.
(F) HeLa cells were transfected with two different SETD2 siRNAs or a non-specific siRNA (CTR). The cells were collected 72 h after transfection, and the whole cell lysates and histones were extracted for western blotting. β-actin and H3 served as an internal control for protein loading.
(G) Whole cell lysates of two different SETD2 knockout HeLa cell lines were extracted for western blotting. H3 served as an internal control for protein loading.
(H) HCT116 cells were transfected with SUV39H1 or a non-specific siRNA (CTR). The cells were collected 72 h after transfection, and the whole cell lysates and histones were extracted for western blotting. β-actin and H3 served as an internal control for protein loading.
(I) HeLa cells were transfected with SUV39H1, SUV39H2 or a non-specific siRNA (CTR). The cells were collected 72 h after transfection, and the whole cell lysates and histones were extracted for western blotting. H3 served as an internal control for protein loading (*Left*). Quantification of the band density (*Right*). The band density of H3K14me3 in "CTR" was normalized to 1. Data are shown as means ± SD (n = 3).
(J-L) HeLa cells were transfected with MLL (J), SET7/9 (K) or DOT1L (L) siRNAs or a non-specific siRNA (CTR). The cells were collected 72 h after transfection, and the whole cell lysates and histones were extracted for western blotting. β-actin and H3 served as an internal control for protein loading.
(M) *In vitro* methylation assay using a recombinant SETD2 SET domain as the enzyme. Free recombinant histone H3 was used as the substrate. H3K36me3 served as a positive control for SETD2 enzymatic activity.
(N) *In vitro* methylation assay using a recombinant SUV39H1 as the enzyme. Free recombinant histone H3 was used as the substrate. H3K9me3 served as a positive control for SUV39H1 enzymatic activity.
(O) *In vitro* methylation assay using a recombinant SETD2 SET domain as the enzyme. Purified H3 (WT) or H3 mutant (K14R) proteins were used as the substrate. H3K36me3 served as a positive control for SETD2 enzymatic activity.
(P) *In vitro* methylation assay using a recombinant SUV39H1 as the enzyme. Purified H3 (WT) or H3 mutant (K14R) proteins were used as the substrate. H3K9me3 served as a positive control for SUV39H1 enzymatic activity.

Fig. S2. SETD2-mediated H3K14me3 increases in response to replication stress.



(A) HeLa cells were treated with 20J/m² UV irradiation and then re-cultured under normal

conditions for the indicated time periods. Whole cell lysates were subjected to western blotting. “CTR” indicates the cells without any treatment.

(B) HeLa cells were treated with IR (4Gy) and released for the indicated time periods. Whole cell lysates were subjected to western blotting. “CTR” indicates the cells without any treatment.

(C) HeLa cells were treated with indicated dose of IR irradiation and released for 1 h. Whole cell lysates were subjected to western blotting. “CTR” indicates the cells without any treatment.

(D) HeLa cells were treated with or without 4 mM HU for 5 h. The cells were then fixed and stained with an anti-H3K14me3 antibody (green). The nuclei were counterstained with DAPI (blue). “CTR” indicates the cells without any treatment. Scale bar: 10 μm.

(E) HeLa cells were treated with or without 4 mM HU treatment for 5 h. The chromatin fractions were extracted and analyzed by western blotting. “CTR” indicates the cells without any treatment.

(F) A statistical analysis of (E) was performed by scanning the density of SETD2 and SUV39H1 band of the Western blots. The band density of SETD2 and SUV39H1 in “CTR” was normalized to 1. Data are shown as means ± SD (n = 3).

(G) HeLa cells were transfected with or without SUV39H1 siRNAs, SUV39H2 siRNAs or a non-specific siRNA (NC). The cells were treated with or without 4mM HU for 5 h before harvest. Cells were collected 72 h after transfection, and whole cell lysates were extracted for western blotting. “CTR” indicates the cells without any treatment.

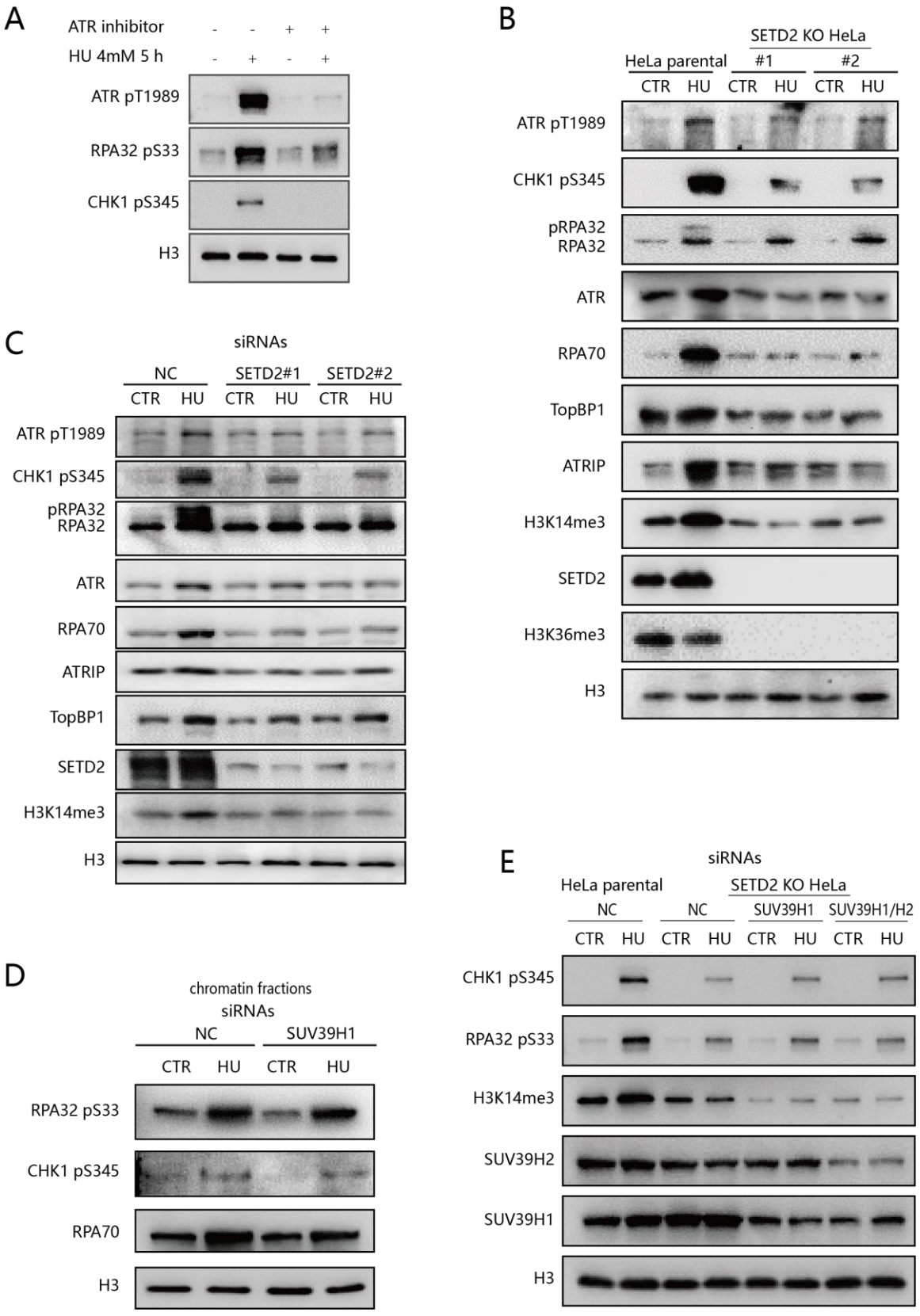
(H) HeLa cells were synchronized at G1/S by double-thymidine arrest and then released into fresh media and collected at the indicated time intervals. Flow cytometry analysis was performed and the histograms show the cell cycle distributions.

(I) HeLa cells were synchronized in G1/S and then released into the cell cycle. Chromatin was extracted at the indicated time points and the abundances of SETD2 and H3K14me3 were monitored by western blotting.

(J) Whole HeLa cell lysates were extracted during the indicated cell cycle phase. The western blot analysis shows the abundance of SETD2 during cell cycle progression. β-actin served as an internal control for protein loading.

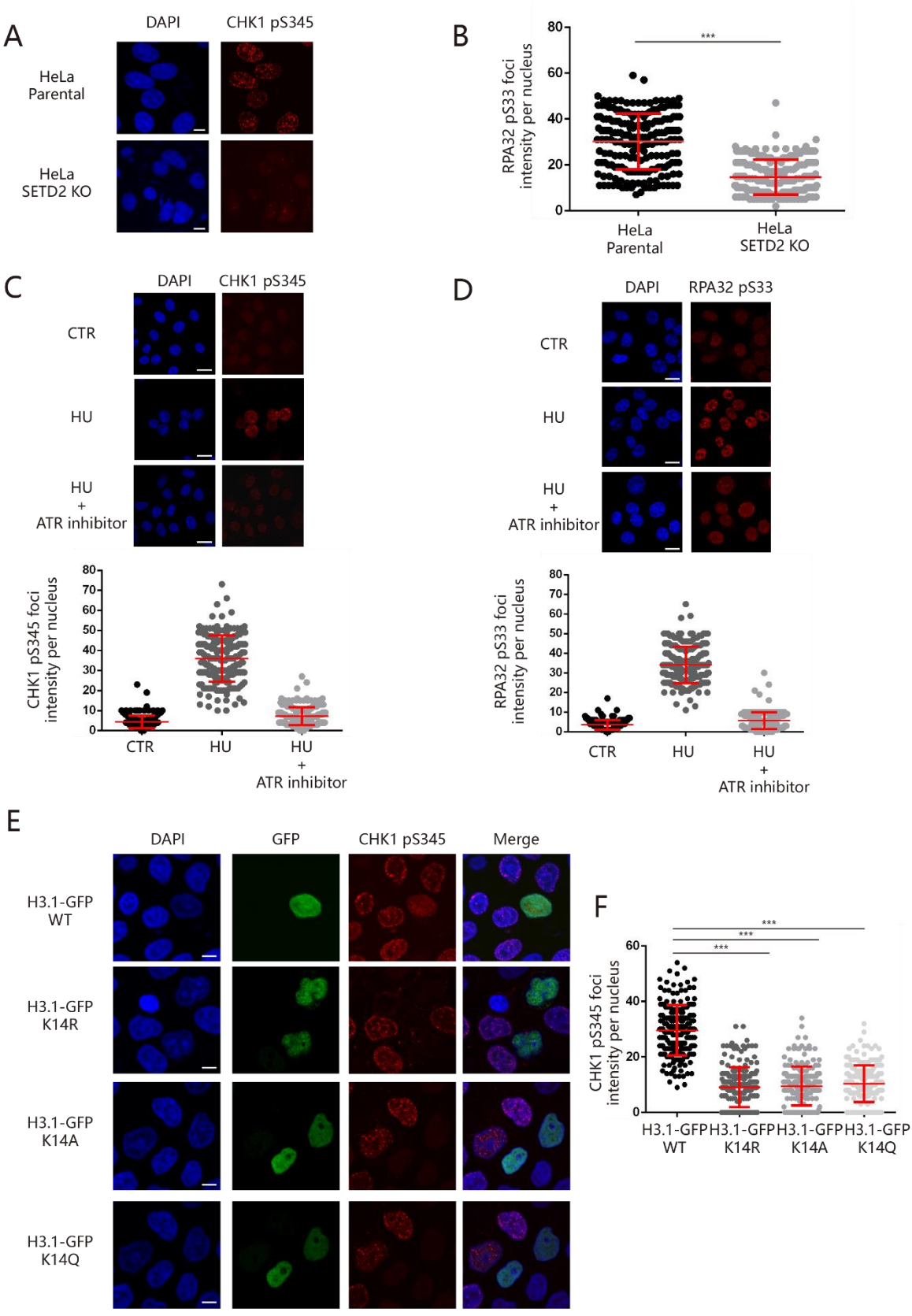
(K) HeLa cells were synchronized in G1/S and then released into the cell cycle. The chromatin fractions were extracted during the indicated cell cycle phase. The western blot analysis shows the chromatin abundance of SUV39H1 during cell cycle progression. H3 served as an internal control for protein loading.

Fig. S3. SETD2 depletion impairs ATR activation.



(A) HeLa cells were treated with or without 4 mM HU and ATR inhibitor (NU6027) for 5 h. The whole cell lysates were extracted for western blotting. "-" indicates the cells without any treatment.
(B) Two different SETD2-KO HeLa cell lines were treated with or without 4mM HU for 5 h before harvest. Chromatin fractions were extracted for western blotting. "CTR" indicates the cells without any treatment.
(C) HeLa cells were transfected with two different SETD2 siRNAs or a non-specific siRNA (NC). The cells were treated with or without 4mM HU for 5 h before harvest. Cells were collected 72 h after transfection, and chromatin fractions were extracted for western blotting. "CTR" indicates the cells without any treatment.
(D) HeLa cells transfected with SUV39H1 siRNAs or a non-specific siRNA (NC). The cells were treated with or without 4mM HU for 5 h before harvest. Cells were collected 72 h after transfection, and chromatin fractions were extracted for western blotting. "CTR" indicates the cells without any treatment.
(E) HeLa parental cells or SETD2-KO HeLa cells were transfected with SUV39H1 siRNAs, SUV39H2 siRNAs or a non-specific siRNA (NC). The cells were treated with or without 4mM HU for 5 h before harvest. Cells were collected 72 h after transfection, and whole cell lysates were extracted for western blotting. "CTR" indicates the cells without any treatment.

Fig. S4. SETD2 depletion and H3K14 mutation impairs ATR activation.



(A) HeLa parental cells or SETD2-KO HeLa cells were treated with 4 mM HU for 5 h. The cells were then fixed and stained with an anti-CHK1 pS345 antibody (red). The nuclei were counterstained with DAPI (blue). Scale bar: 10 μm.

(B) A statistical analysis of (A). The CHK1 pS345 foci per nucleus were counted from a minimum of 200 cells. The data represent the means ± SD. ***P < 0.001 (Student's t-test).

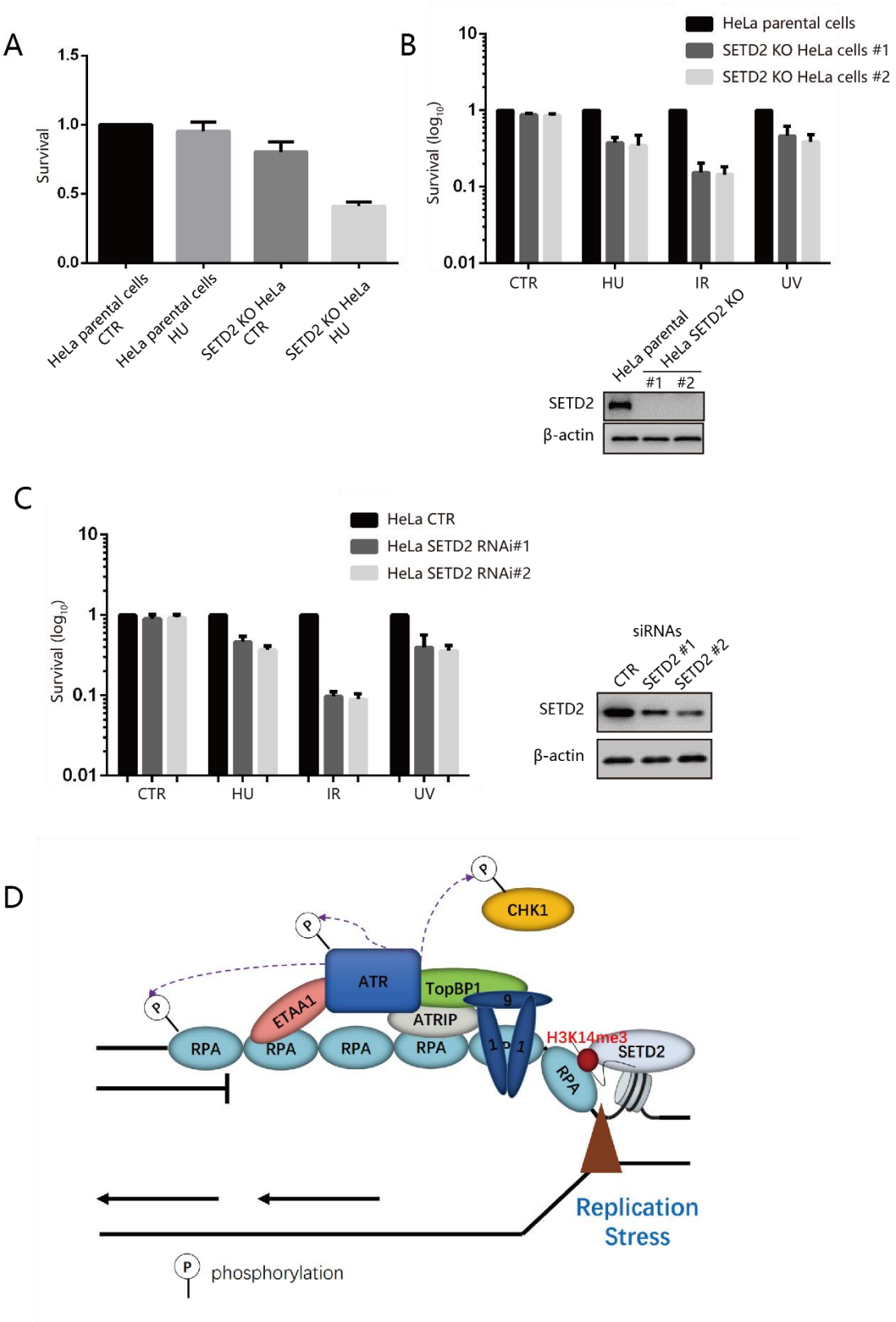
(C) HeLa cells were treated with or without 4 mM HU and ATR inhibitor (NU6027) for 5 h. The cells were then fixed and stained with an anti-CHK1 pS345 antibody (red). The nuclei were counterstained with DAPI (blue) (*Upper*). The CHK1 pS345 foci per nucleus were counted from a minimum of 200 cells (*Lower*). The data represent the means ± SD. Scale bar: 20 μm.

(D) HeLa cells were treated with or without 4 mM HU and ATR inhibitor (NU6027) for 5 h. The cells were then fixed and stained with an anti-RPA32 pS33 antibody (red). The nuclei were counterstained with DAPI (blue) (*Upper*). The RPA32 pS33 foci per nucleus were counted from a minimum of 200 cells (*Lower*). The data represent the means ± SD. Scale bar: 20 μm

(E) HeLa cells were transfected with reconstituted GFP-tagged H3.1WT, H3.1K14R, H3.1K14A or H3.1K14Q for 48 h and then treated with 4 mM HU for 5 h. The cells were then fixed and stained with an anti-CHK1 pS345 antibody (red). The nuclei were counterstained with DAPI (blue). Scale bar: 10 μm.

(F) A statistical analysis of (E). The CHK1 pS345 foci per nucleus were counted from a minimum of 200 cells. The data represent the means ± SD. ***P < 0.001 (Student's t-test).

Fig. S5. SETD2 is required for cells to tolerate replication stress.



(A) HeLa parental cells or SETD2-KO HeLa cells were treated with 8 mM HU for 2 h and analyzed by colony formation assay. The cell survival of HeLa parental cells in "CTR" was normalized to 1. The data represent the means ± SD (n=3). "CTR" indicates the cells without HU treatment.
(B) HeLa parental cells or two different SETD2-KO HeLa cell lines were treated with HU (8mM, 2 h). IR (8 Gy, release 1 h) or UV (20J/ m^2, release 2 h) and analyzed by colony formation assay. The cell survival of HeLa parental cells in was normalized to 1. The data represent the means ± SD (n=3). "CTR" indicates the cells without any treatment.
(C) HeLa cells or SETD2 knockdown HeLa cells were treated with HU (8mM, 2 h), IR (8 Gy, release 1 h) or UV (20J/m^2, release 2 h) and analyzed by colony formation assay. The cell survival of HeLa cells was normalized to 1. The data represent the means ± SD (n=3). "CTR" indicates the cells without any treatment.
(D) Schematic model for SETD2-mediated H3K14me3 promotes ATR activation and replication fork restart in response to replication stress. In response to replication stress, the RPA complex binds to single strand DNA rapidly to activate ATR and downstream signaling pathway. The RPA-ssDNA establishes a platform to recruit numerous factors such as TopBP1, ATRIP and ETAA1 to promotes this process. SETD2 is recruited to chromatin and catalyzes H3K14me3 to facilitates the RPA complex loading to chromatin via a direct interaction between H3K14me3 and RPA70. Thus, this SETD2-H3K14me3-RPA-ATR axis is important for promoting ATR activation and improves cancer-cell survival in response to replication stress.

Dataset S1 legends (separate file).

HeLa cells were treated with or without 4mM HU for 5 h and then nucleic fractions were extracted for pull-down assay with biotin-labeled H3 peptides containing H3K14me3. Proteins identified by mass spectrometry. *Left*: proteins pulled down by H3K14me3 peptide in HeLa cells without any treatment; *Right*: proteins pulled down by H3K14me3 peptide in HeLa cells treated with 4mM HU for 5 h.

SI references

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