SUPPLEMENTARY DATA

Supplementary figures and legends

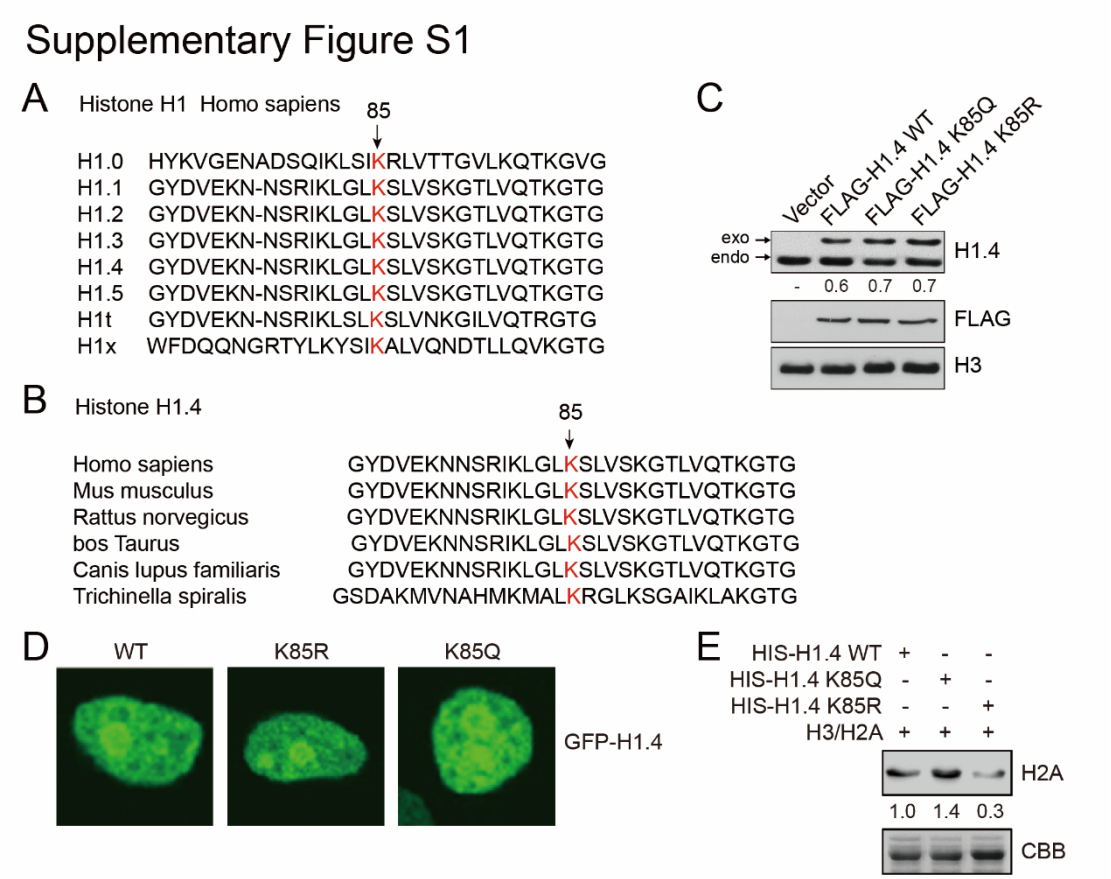

Figure S1. Acetylation-mimic H1K85 mutation leads to chromatin condensation

(**A**, **B**) Alignment of different histone H1 isoform sequences (*Homo sapiens*) surrounding the K85 site or histone H1.4 sequences surrounding the K85 site in different species.

(**C**) HeLa cells were transfected with WT, K85R or K85Q FLAG-H1.4 and chromatin proteins were analyzed by immunoblotting. 'exo' indicates the exogenous H1.4 and 'endo' indicates the endogenous H1.4. The ratio of relative band intensity of the exogenous H1.4 and endogenous H1.4 from three independent immunoblots are shown.

(**D**) HeLa cells were transfected with WT, K85R or K85Q GFP-H1.4 and the subcellular localization of GFP-tagged H1.4 (green) was analyzed by fluorescent microscopy.

(**E**) WT, K85R or K85Q HIS-H1.4 was incubated with recombinant H3 or H2A for *in vitro* binding assay. The relative band intensity from three independent immunoblots are shown and the first lane (HIS-H1.4 WT) was normalized to 1.

Supplementary Figure S2

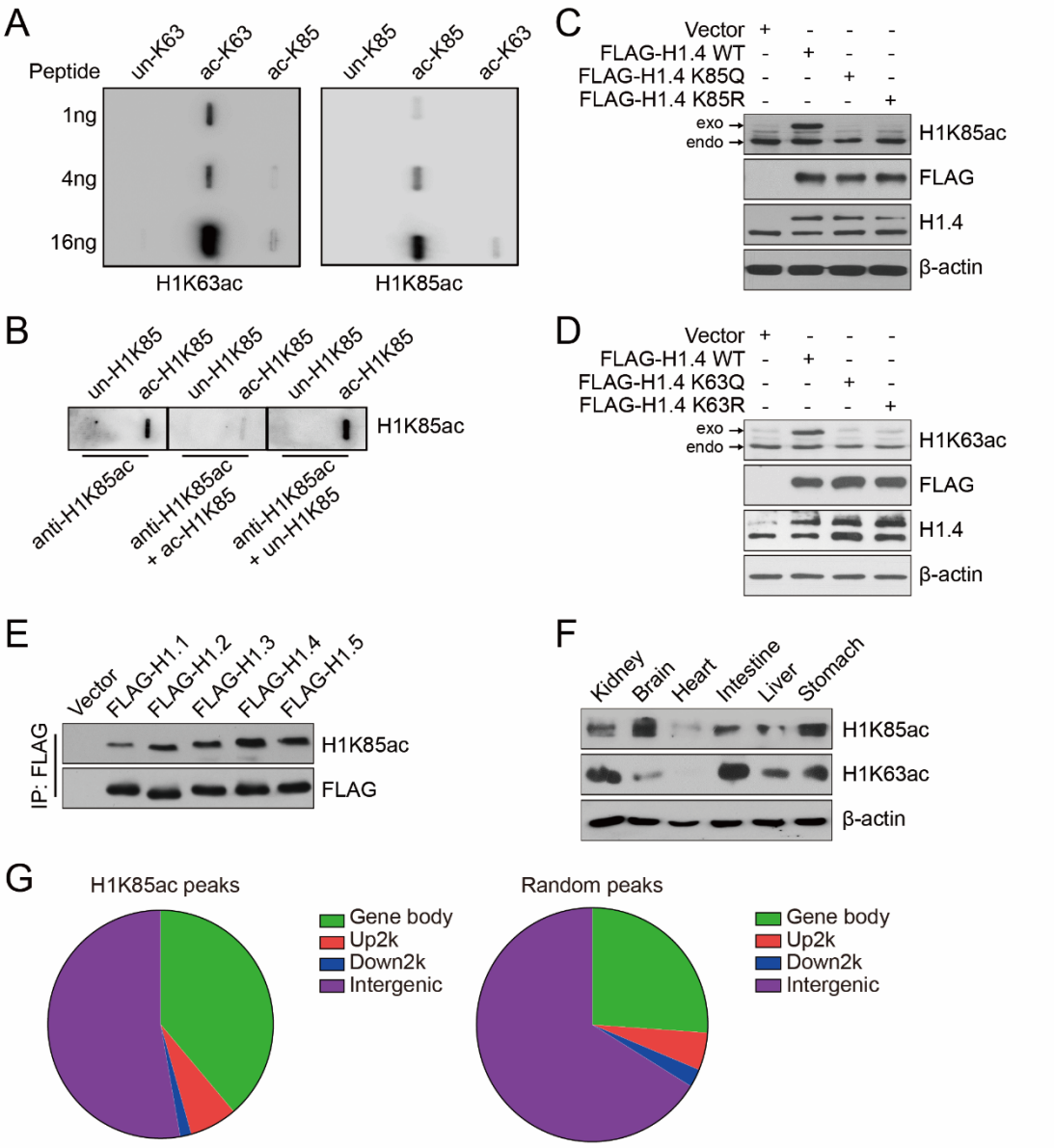

Figure S2. Identification of H1K8ac as a novel modification

(**A**) Slot blot assay for anti-H1K63ac and anti-H1K85ac antibodies. Each antibody was probed against an unmodified peptide and acetylated peptides.

(**B**) Peptide competition assay for H1K85ac.

(**C**, **D**) HCT116 cells were transfected with the indicated plasmids and whole cell extracts were subjected to immunoblotting. 'exo' indicates acetylation of the exogenous H1.4 and 'endo' indicates acetylation of the endogenous H1.4.

(**E**) HCT116 cells were transfected with the indicated plasmids and whole cell extracts were immunoprecipitated with FLAG-conjugated M2 beads and then analyzed by immunoblotting.

(**F**) Proteins from different mouse (normal C57BL/6 mouse, male, 12 weeks of age) tissues were analyzed by immunoblotting.

(**G**) ChIP-sequencing analysis of H1K85ac genomic occupancy patterns.

Supplementary Figure S3

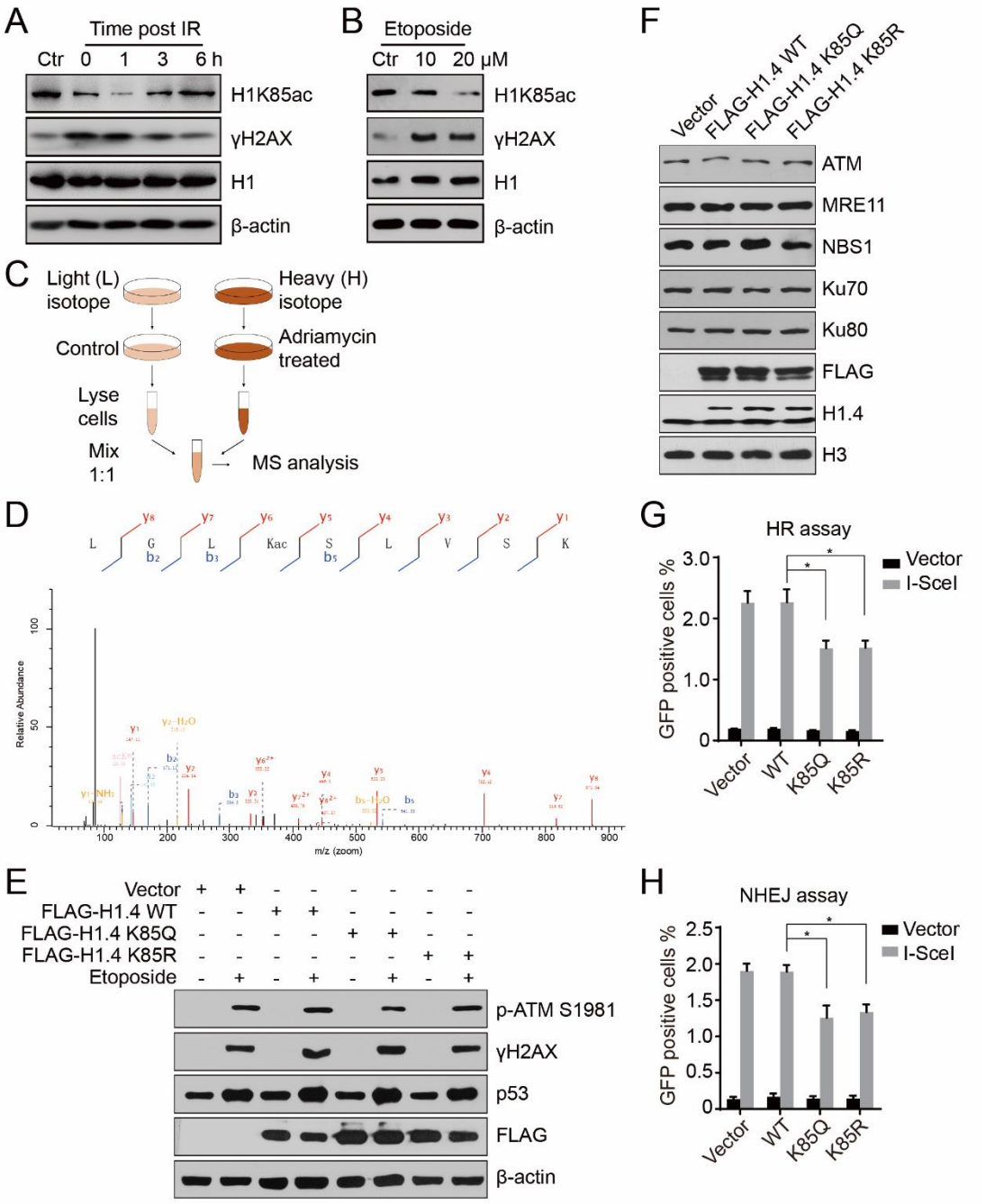

Figure S3. Acetylation of H1K85 is dynamically regulated in response to DNA damage

(**A**) HeLa cells were exposed to 10 Gy IR and released for the indicated times, and whole cell lysates were analyzed by immunoblotting.

(**B**) HeLa cells were treated with etoposide for 12 h (10 μM or 20 μM) and whole cell extracts were analyzed by immunoblotting.

(**C**) Workflow of the SILAC experiments.

(**D**) Peak map of the H1K85ac from the MS experiments.

(**E**) HeLa cells were transfected with the indicated plasmids and treated with or without etoposide for 2 h (40 μM). Whole cell extracts were analyzed by immunoblotting.

(**F**) HeLa cells were transfected with the indicated plasmids and chromatin proteins were analyzed by immunoblotting.

(**G**, **H**) DR-U2OS or pEJ5-U2OS cells were transfected with the indicated plasmids and subjected to HR assay or NHEJ assay, respectively. All data represent the means ± SD.

Supplementary Figure S4

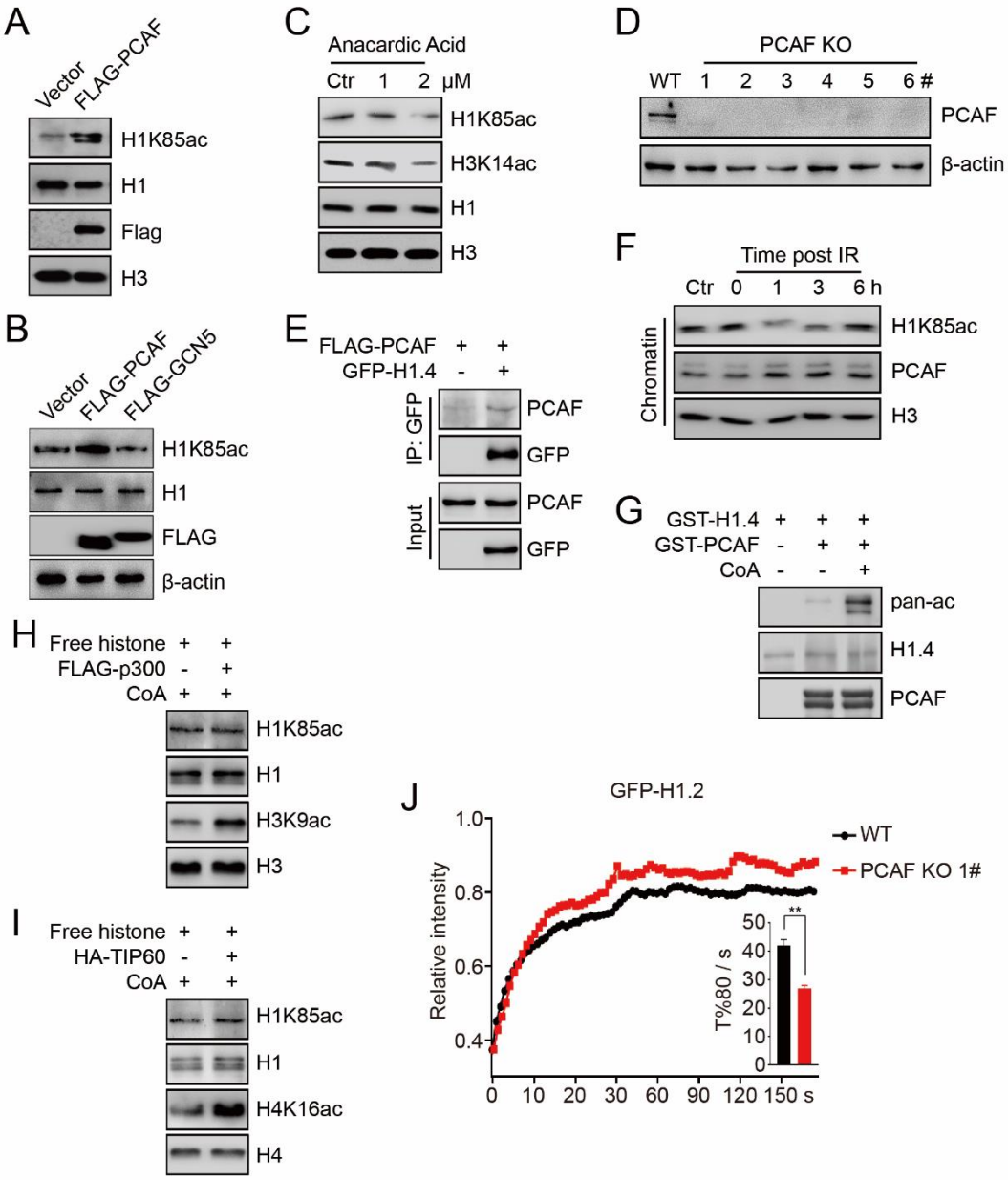

Figure S4. PCAF acetylates H1K85 acetylation *in vivo* and *in vitro*

(**A**) HeLa cells were transfected with the indicated plasmids and histone was analyzed by immunoblotting.

(**B**) HCT116 cells were transfected with the indicated plasmids and cell lysates were analyzed by immunoblotting.

(**C**) HCT116 cells were treated with anacardic acid for 12 h (1 μM or 2 μM) and histone was analyzed by immunoblotting.

(**D**) Selected clones of PCAF KO cells.

(**E**) HeLa cells were transfected with the indicated plasmids and cell extracts were immunoprecipitated using an anti-GFP antibody.

(**F**) HCT116 cells were exposed to 10 Gy IR and released for the indicated times. Chromatin-bound proteins were extracted and analyzed by immunoblotting.

(**G**) GST-H1.4 and GST-PCAF were purified from *E. coli* and subjected to *in vitro* acetylation assay with or without acetyl-CoA. The reaction was then analyzed by immunoblotting.

(**H**, **I**) FLAG-p300 or HA-TIP60 was purified from HEK293T cells and subjected to *in vitro* acetylation assay using free histone as substrates. The reactions were then analyzed by immunoblotting.

(**J**) PCAF KO (1#) and WT cells were transfected with GFP-H1.2 and analyzed by FRAP. All data represent the means ± SD.

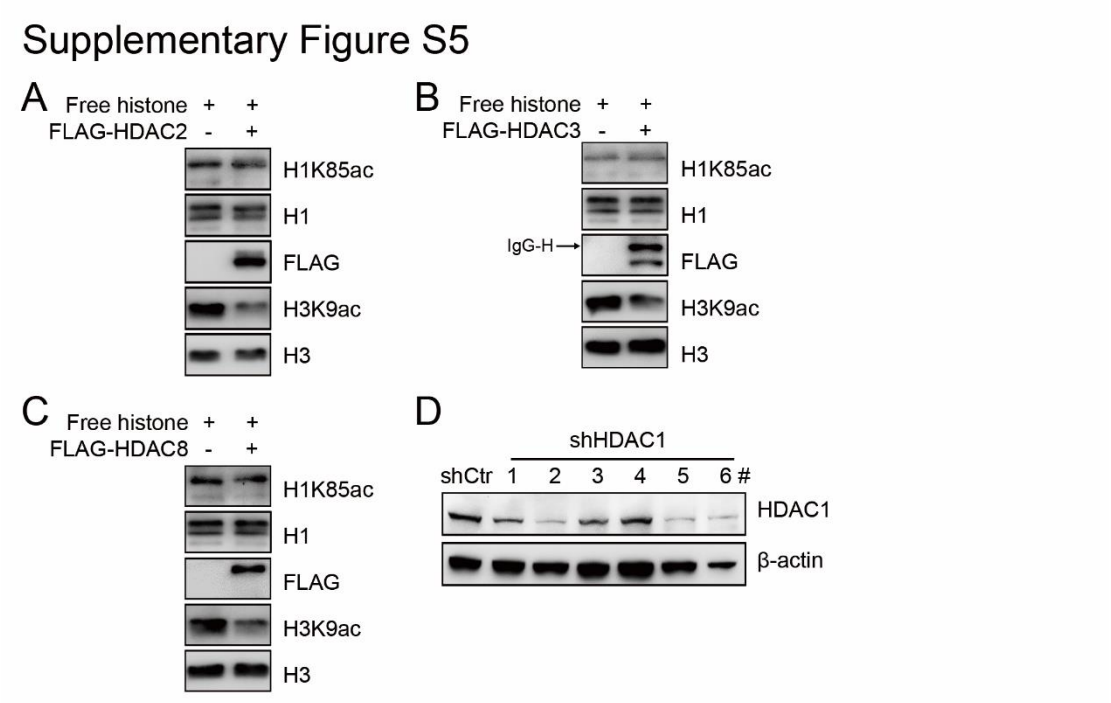

Figure S5. HDAC1 deacetylates H1K85 acetylation *in vivo* and *in vitro*

(**A**-**C**) FLAG-HDAC2, HDAC3 or HDAC8 was purified from HEK293T cells and subjected to *in vitro* deacetylation assay before analysis by immunoblotting. IgG-H indicates the heavy chain of IgG.

(**D**) Selected clones of stable HDAC1 knockdown cells.

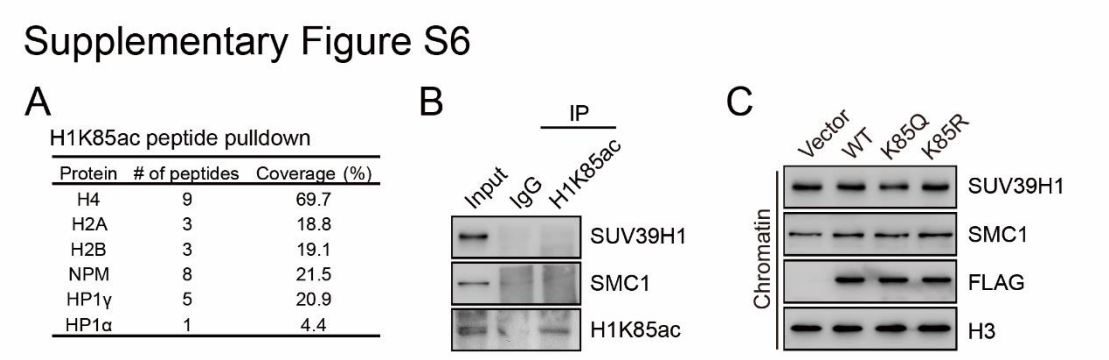

Figure S6. Acetylation of H1K85 promotes the recruitment of heterochromatin protein 1 (HP1)

(**A**) Cell extracts from HCT116 were incubated with biotin-tagged beads or acetylated H1K85 peptide and then subjected to SDS-PAGE electrophoresis. The specific protein bands were silver stained and then analyzed by mass spectrometry.

(**B**) Chromatin-bound proteins extracted from HCT116 cells were immunoprecipitated using IgG or H1K85ac antibodies and analyzed by immunoblotting.

(**C**) WT, K85Q and K85R FLAG-H1.4 or empty-vector alone were transfected into HeLa cells. Chromatin-bound proteins were extracted and analyzed by immunoblotting.

Supplementary Material and Methods

X-ray irradiation

Cells were irradiated using a biological X-ray irradiator RS2000pro Rad Source (Rad Source Technologies) with a radiation output of 160 KV, 25 mA at a dose rate of 4.125 Gy/min for the indicated time.

Whole cell extraction

Equal numbers of harvested cells were washed twice with PBS, and the cell pellets were re-suspended in 2× protease inhibitor buffer (30 μL/10^6 cells) with one protease inhibitor cocktail tablet (Roche) in 3.5 mL PBS. An equal volume of 2× protein sample buffer was added and the samples were boiled at 100 °C for 10 min with vigorous vortex before SDS-PAGE and immunoblotting.

Chromatin fractionation

Cell pellets were suspended in buffer I (50 mM HEPES pH 7.5, 150 mM NaCl and 1 mM EDTA) supplemented with 0.1% Triton X-100 and 1% protease inhibitor cocktail and lysed on ice for 3 min. The supernatant was discarded and the pellet was re-suspended in buffer I containing 200 μg/mL RNaseA at room temperature for 30 min. The pellet was resuspended in buffer I after centrifugation and boiled in an equal volume of 2× protein sample buffer at 100 °C for 5 min before SDS-PAGE and immunoblotting.

GST pull-down assay

GST or GST-tagged plasmids were transformed in *E. coli* BL21 cells (TianGen) and induced using 0.1 mM IPTG at 28 °C for 6 h. The GST or GST-fusion proteins were purified using glutathione-Sepharose 4B beads (GE Healthcare). Recombinant proteins or cell lysates were separately incubated with GST fusion proteins in TEN buffer (10 mM Tris·HCl pH 8.0, 1 mM EDTA, 100 mM NaCl) for 3 h at 4 °C. The beads were washed with TEN buffer and boiled in protein sample buffer before SDS-PAGE and immunoblotting.

Peptide pull-down assay

The following peptides were used: un-H1K85: Biotin-GYDVEKNNSRIKLGLKSLVSKGTLVQ; ac-H1K85: Biotin-GYDVEKNNSRIKLGLK(ac)SLVSKGTLVQ. Peptides were incubated with streptavidin agarose slurry (New England Biolabs) overnight at 4 °C in binding buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 10% Glycerol, 1 mM EDTA, 2 mM MgCl2, 0.5% NP-40). After removal of unbound peptides, 2 μg recombinant histone (New England Biolabs) was added for 1 h incubation at 4 °C. The beads were washed with washing buffer (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 10% Glycerol, 1 mM EDTA, 2 mM MgCl 2, 0.5% NP-40) and boiled in protein sample buffer before SDS-PAGE and immunoblotting.

Peptide competition assay

Equal amounts of peptide were immobilized on a nitrocellulose membrane. After drying and blocking, the nitrocellulose membranes were incubated with specific primary antibodies that had been pre-incubated with an excess of peptide and then the membranes were visualized by

chemiluminescence.

Slot blot assay

An increasing concentration of different peptides were immobilized on the nitrocellulose membranes. The membranes were dried using a vacuum pump and subjected to immunoblotting with the indicated antibodies.

Mass spectrometry and silver stain

HeLa cells were extracted and subjected to electrophoresis using a 4-12% SDS-PAGE gel after a peptide pull-down assay using the indicated peptides or beads. Silver stain was performed using a silver stain kit (Thermo Fisher) following the manufacturer's instructions to detect protein bands. Protein bands were cut and sent to PTM BioLabs for mass spectrometric analysis.

HR assay and NHEJ assay

DR-GFP or pEJ5-GFP U2OS cells were transfected with HA-I-*Sce*I and other indicated plasmids 24 h before HA-I-*Sce*I transfection if necessary. Cells were harvested 48 h after HA-I-*Sce*I transfection and subjected to flow cytometry analysis. The percentage of GFP-positive cells, which indicated HR-mediated or NHEJ-mediated DSB repair efficiency, was determined. The mean values were obtained from 3 independent experiments.