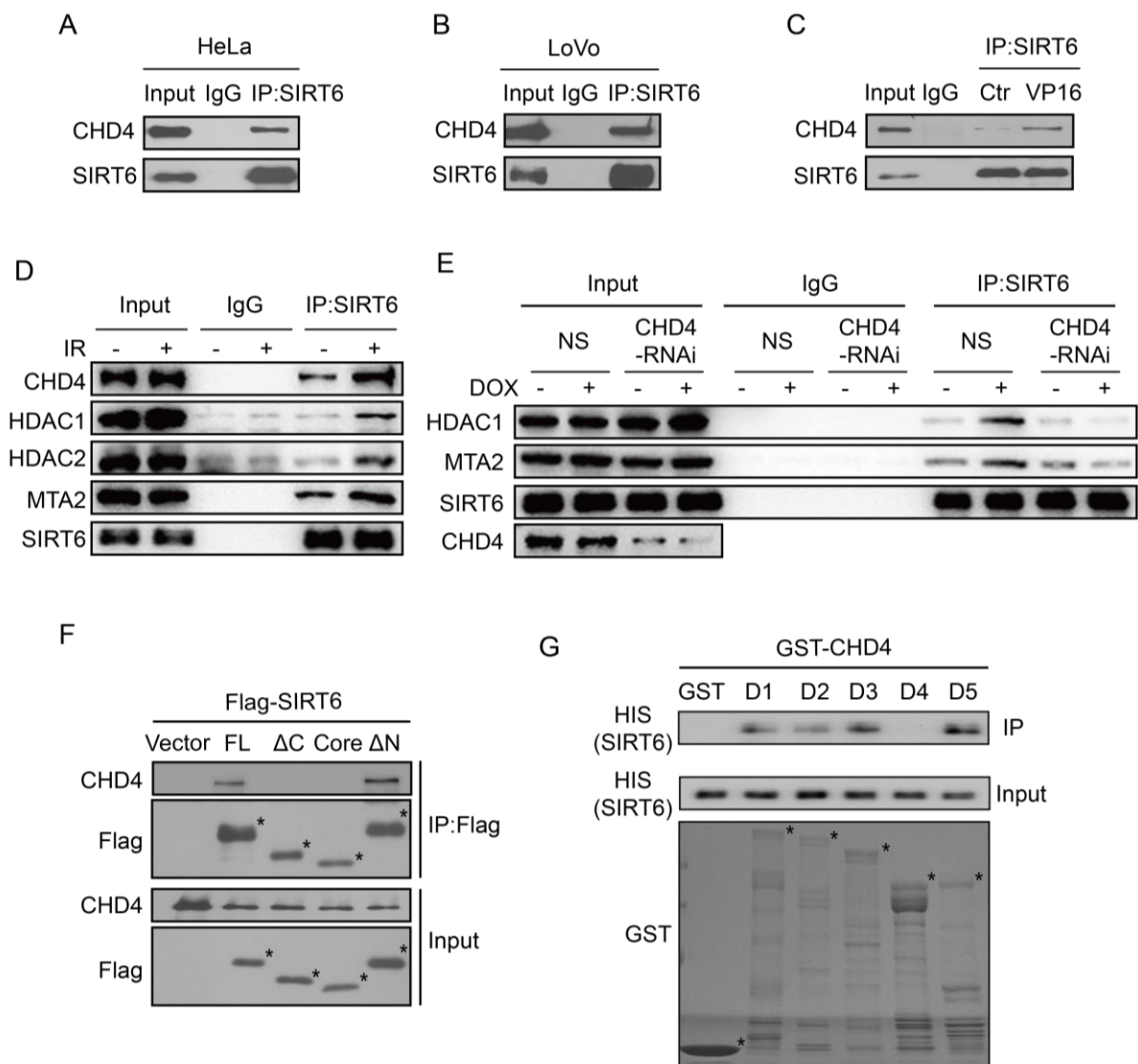


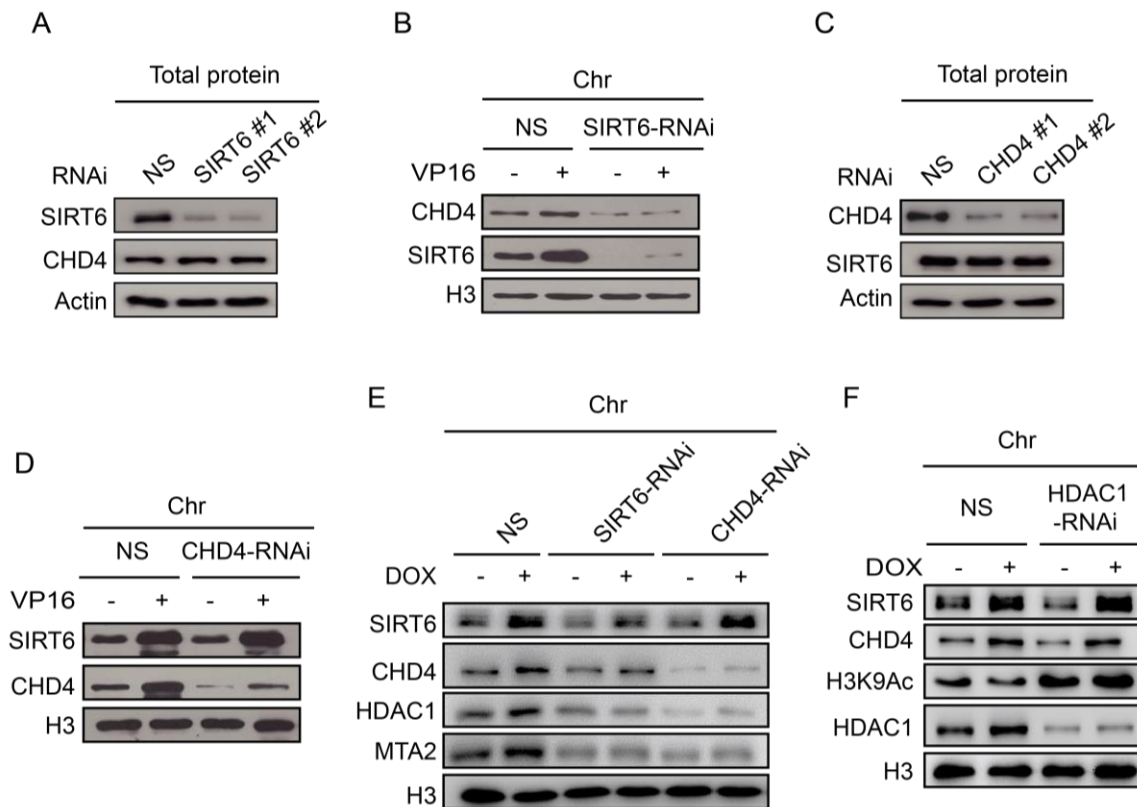
SUPPLEMENTARY DATA

Supplementary Data are available at NAR online.

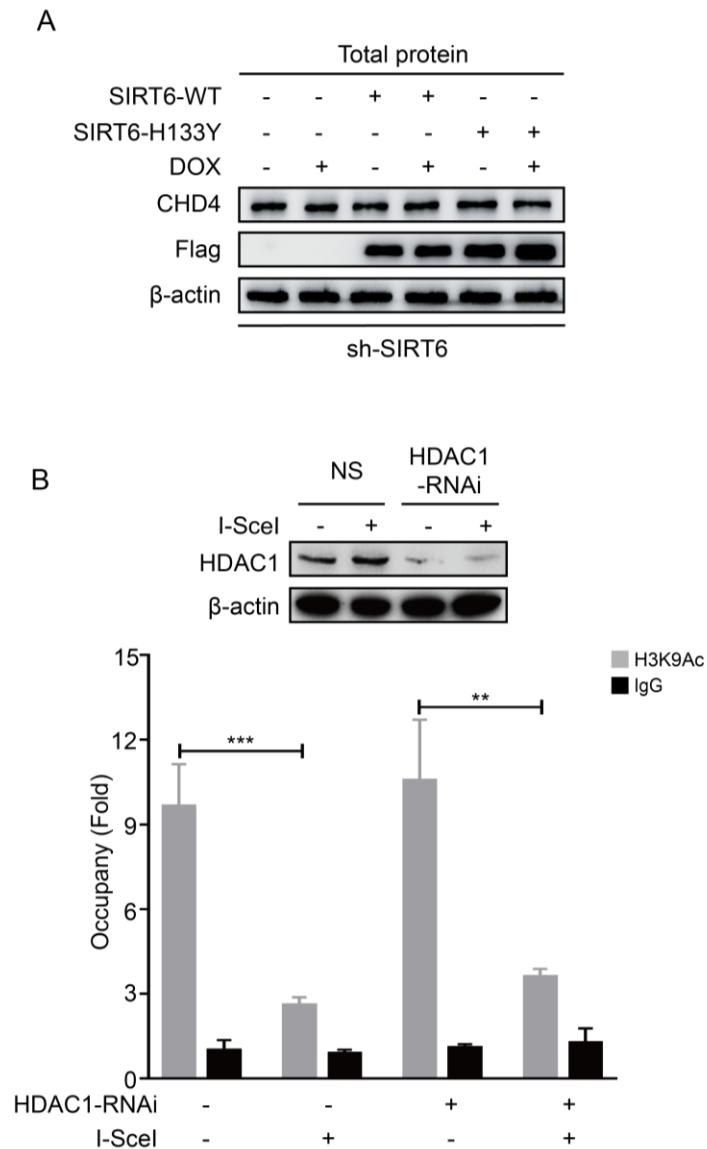


Supplementary Figure 1. The interaction between SIRT6 and CHD4 markedly increases in response to DNA damage. (A-B) Nuclear proteins from HeLa (A) and LoVo (B) cells were extracted and immunoprecipitated using an anti-SIRT6 antibody. Rabbit IgG was used as a negative control. Western blotting was performed with the antibodies indicated. (C) HeLa cells were treated with 40 μ M VP16 for 1 h and then the cell extracts were precipitated with an anti-SIRT6 antibody and analyzed by western blotting. (D) HCT116+ cells were exposed to 10 Gy IR and released for 0.5 h. The cell extracts were then precipitated with an anti-SIRT6 antibody and analyzed by western blotting. Rabbit IgG was used as a negative control. (E) HCT116+ cells were transfected with NS (non-specific) or CHD4 specific siRNAs for 48 h in the presence or absence of 1 μ M DOX. The cell extracts were then precipitated with an anti-SIRT6 antibody and analyzed by western blotting. Rabbit IgG was used as a negative control. (F) Flag-fragments of SIRT6 were infected into HCT116+ cells. Cell extracts were then precipitated with M2 beads and analyzed by western blotting. (G) His-SIRT6 protein was purified

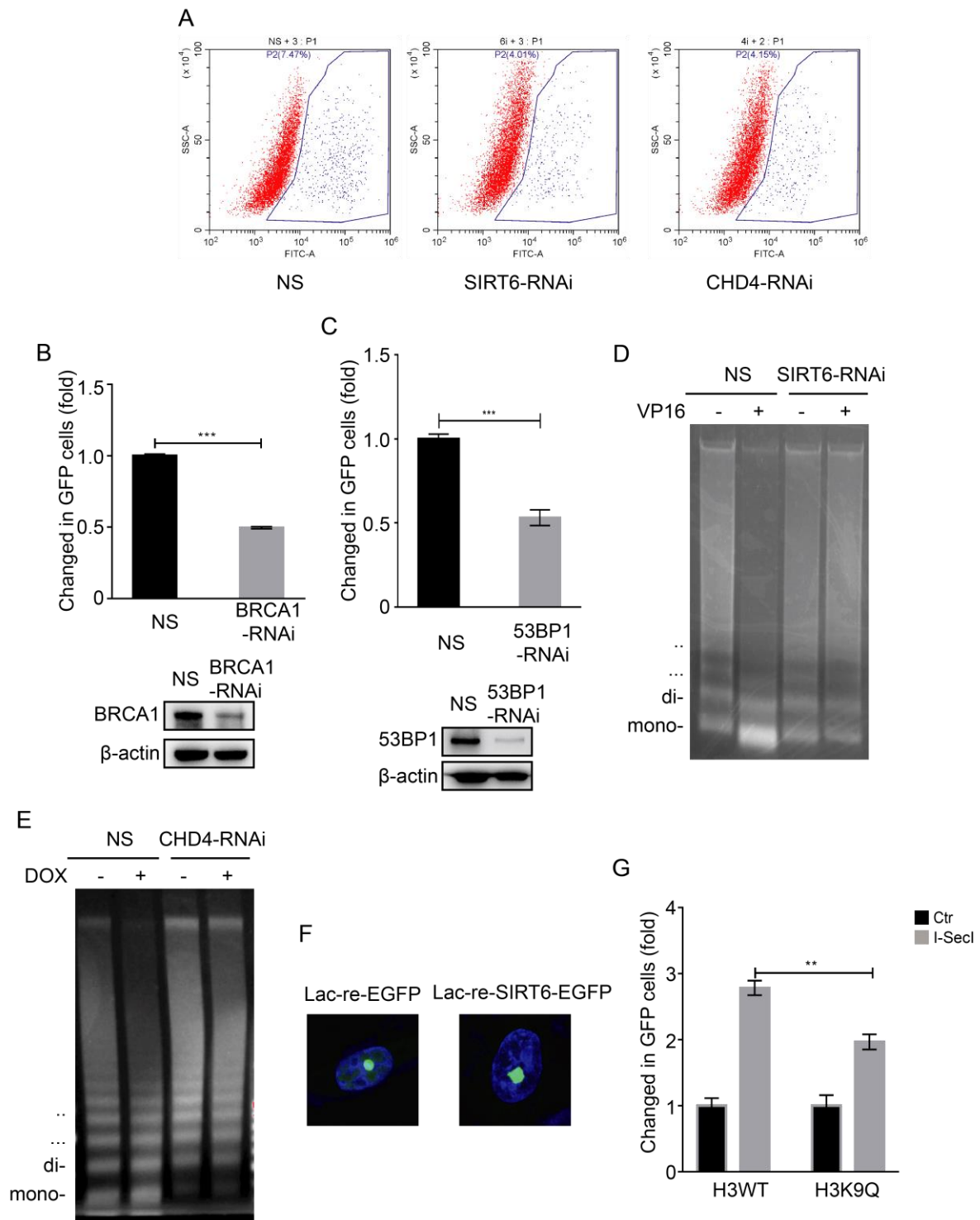
in vitro and incubated with GST fragments of CHD4 fusion proteins before analysis by western blotting.



Supplementary Figure 2. SIRT6 is required to recruit CHD4 to chromatin following DNA damage. (A-B) HeLa cells were transfected with NS (non-specific) or SIRT6 specific siRNA for 48 h in the presence or absence of 40 μ M VP16. Whole cell lysates (A) or chromatin fractions (B) were extracted and analyzed by western blotting. (C-D) HeLa cells were transfected with NS or CHD4 specific siRNA for 48 h in the presence or absence of 40 μ M VP16. Whole cell lysates (C) or chromatin fractions (D) were extracted and analyzed by western blotting. (E) HeLa cells were transfected with NS, SIRT6 or CHD4-specific siRNAs for 48 h in the presence or absence of 1 μ M DOX. Chromatin fractions were extracted and analyzed by western blotting. (F) HeLa cells were transfected with NS or HDAC1 specific siRNA for 48 h in the presence or absence of 1 μ M DOX. Chromatin fractions were extracted and analyzed by western blotting.



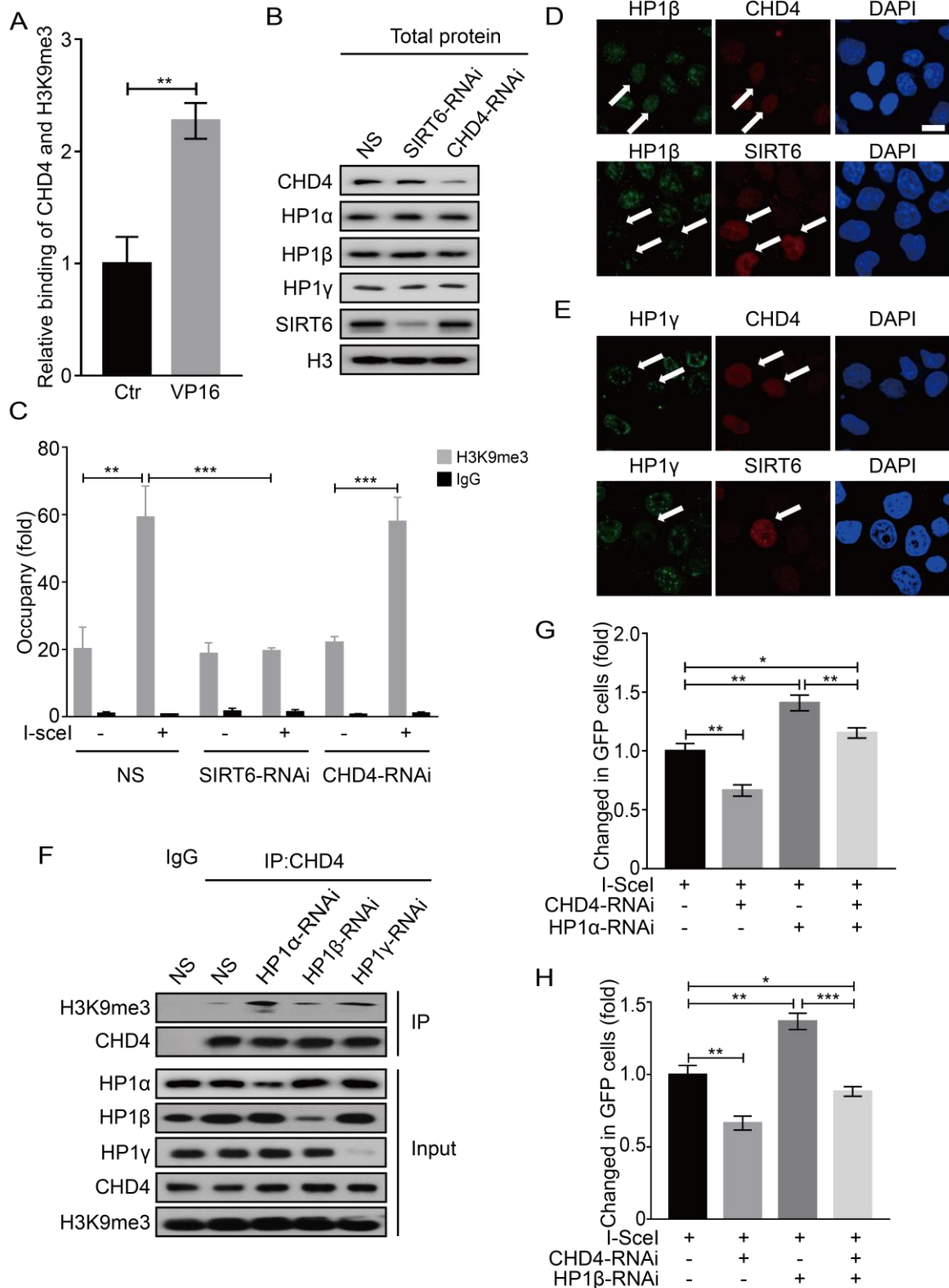
Supplementary Figure 3. The SIRT6 C-terminal domain and SIRT6 enzymatic activity are required to recruit CHD4 to chromatin upon DNA damage. (A) SIRT6-depleted HeLa cells were transfected with an empty plasmid, a plasmid expressing SIRT6 (WT), or a plasmid expressing SIRT6 (H133Y). At 48 h after transfection, the cells were treated with or without 1 μ M DOX for 1 h. Whole cell lysates were extracted and analyzed by western blotting. (B) DR-U2OS cells were transfected with NS (non-specific) or HDAC1 specific siRNA for 48 h; then, the cells were transfected with or without I-SceI expression plasmid for 24 h. ChIP experiments were performed using an anti-H3K9Ac antibody. The data represent the means \pm SEM (n=3, **P < 0.01, ***P < 0.001).



Supplementary Figure 4. SIRT6 promotes chromatin relaxation and DSB repair through CHD4.

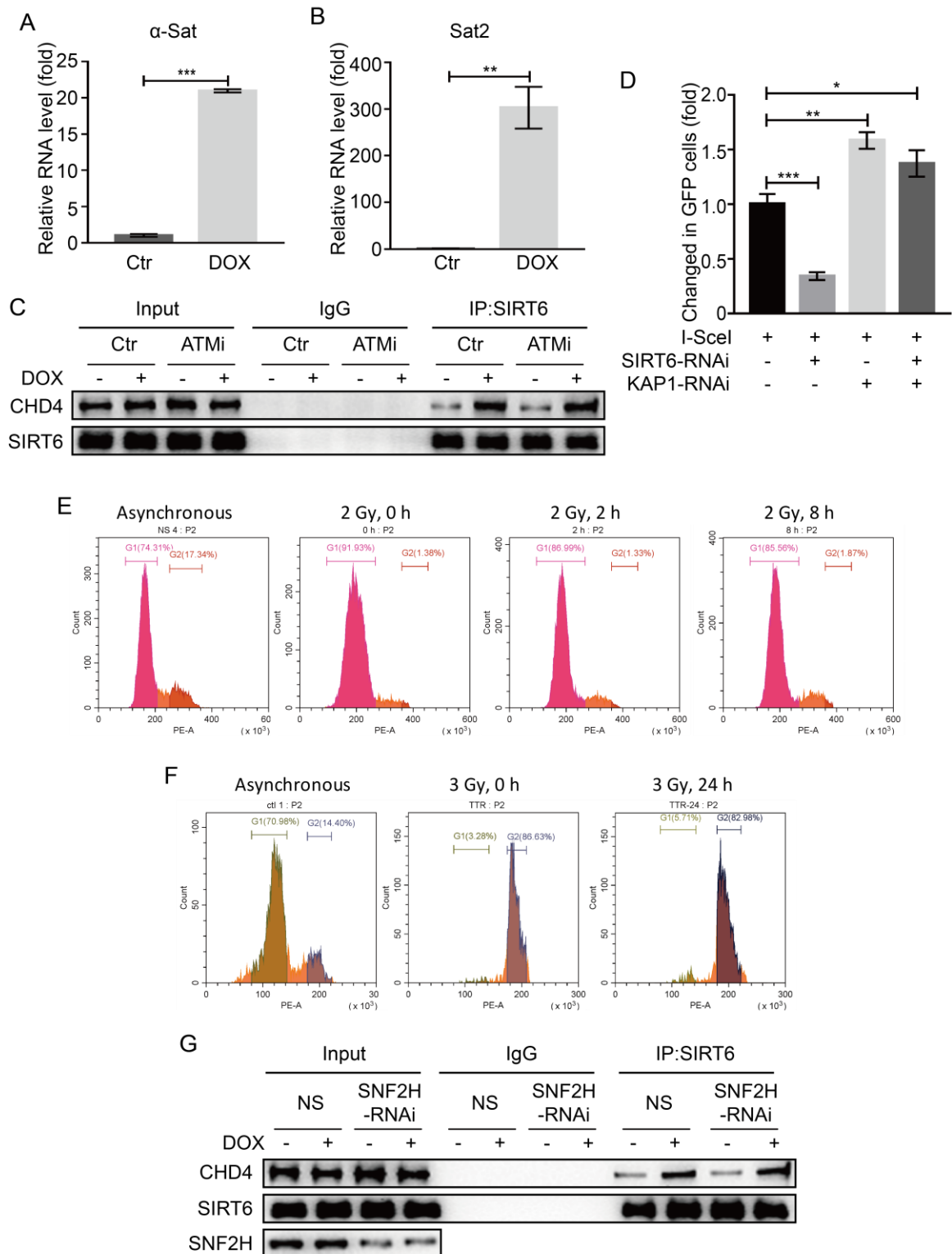
(A) Representative flow cytometry data in the DR-U2OS reporter assay shown in Figure 4 (A). (B-C) NS (non-specific), BRCA1 or 53BP1 specific siRNA were infected into DR-U2OS (B) or EJ5-U2OS (C) cells for 24 h. The cells were then transfected with or without an I-SceI expression plasmid for 48 h. HR (B) and NHEJ (C) efficiency was determined by FACS. The data represent the means \pm s.d. (n=3, ***P < 0.001). (D-E) HeLa cells were transfected with NS or SIRT6 (D) or CHD4 (E) specific siRNA for 48 h with or without 40 μ M VP16 (D) or 1 μ M DOX (E) treatment for 1 h. Nuclei were isolated and

digested with MNase. Different nucleosomal fractions (mono, di, and upper) were separated on a 1.2% agarose gel. (F) AO3_1 cells were transfected with Lac-re-SIRT6-EGFP fusion plasmid or Lac-re-EGFP vectors. After 36 h, the cells were stained with DAPI and observed under a confocal microscope. (G) H3-WT or H3K9Q mutant was transfected into DR-U2OS cells, and then the cells were transfected with I-SceI expression plasmid for 48 h. HR efficiency was determined by FACS. The data represent the means \pm SEM (n=3, **P < 0.01).



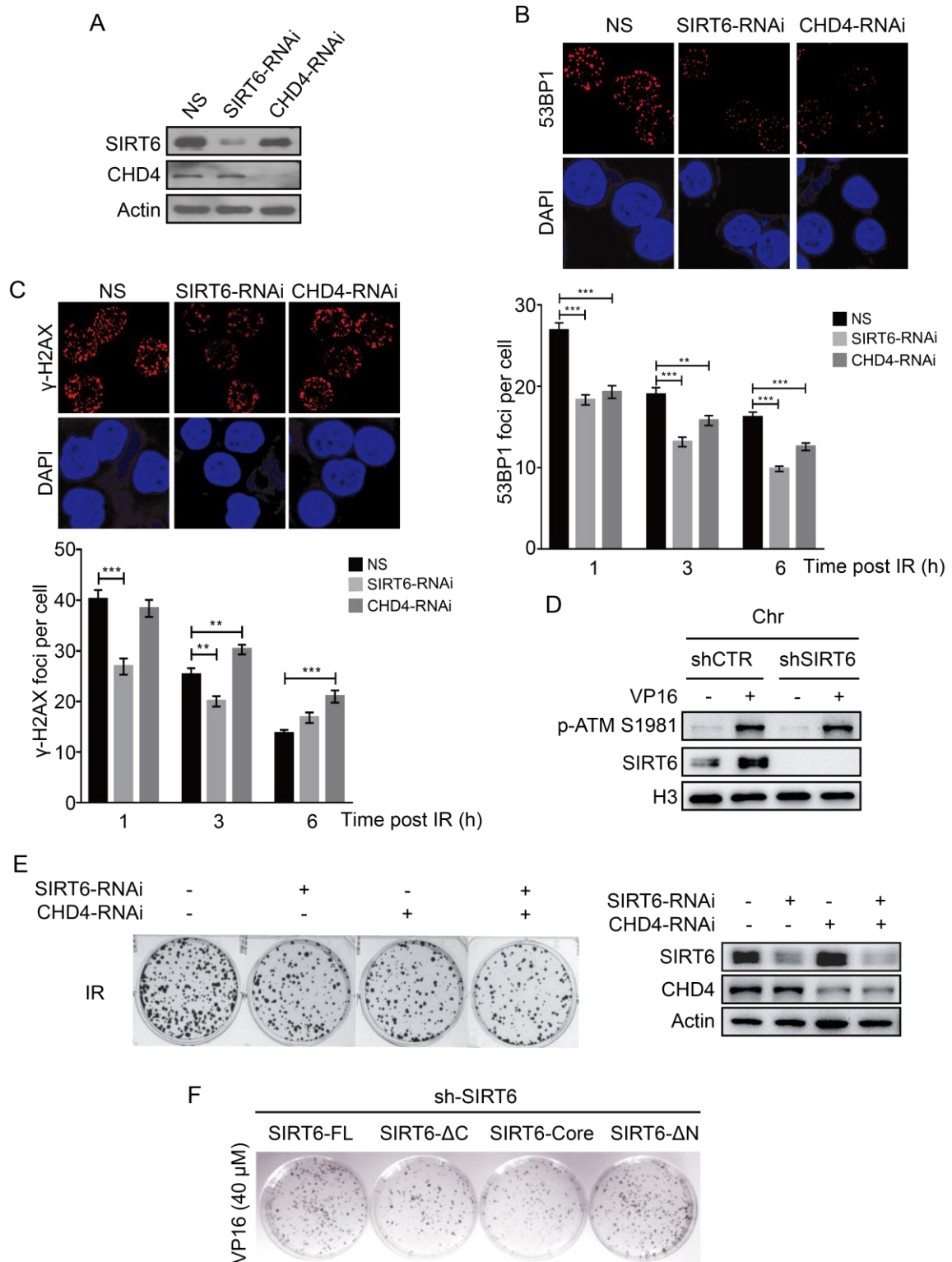
Supplementary Figure 5. CHD4 competes with HP1s for interacting with H3K9me3. (A) Relative binding of CHD4 and H3K9me3 was quantified (from Figure 5 (B)). The data represent the means \pm SEM (n=4, **P < 0.01). (B) The whole cell lysates from Figure 5 (D) were extracted and analyzed by western blotting. (C) DR-U2OS cells were transfected with NS (non-specific), SIRT6 or CHD4 specific siRNA for 48 h, and then the cells were transfected with or without an I-SceI expression plasmid for 24 h. ChIP experiments were performed using an anti-H3K9me3 antibody, and H3K9me3 binding to

the DNA sequences flanking the I-SceI sites was measured. The data represent the means \pm SEM (n=3, **P < 0.01, ***P < 0.001). (D-E) HeLa cells were transfected with CHD4 or SIRT6 specific siRNA for 48 h. Cells were individually stained with CHD4 or SIRT6 (red) and HP1 β (D)/HP1 γ (E) (green) antibodies and DAPI, and observed under a confocal microscope. (F) HCT116+ cells were transfected with NS, HP1 α , HP1 β or HP1 γ specific siRNA for 48 h. The nuclear fraction was immunoprecipitated with an anti-CHD4 antibody and analyzed by western blotting. (G) NS, CHD4 or HP1 α specific siRNA or combined siRNA duplexes targeted to CHD4 and HP1 α were infected into DR-U2OS cells for 24 h. The cells were then transfected with an I-SceI expression plasmid for 48 h. HR efficiency was determined by FACS. The data represent the means \pm s.d. (n=3, *P < 0.05, **P < 0.01). (H) NS, CHD4 or HP1 β specific siRNA or combined siRNA duplexes targeted to CHD4 and HP1 β were infected into DR-U2OS cells for 24 h. The cells were then transfected with an I-SceI expression plasmid for 48 h. HR efficiency was determined by FACS. The data represent the means \pm s.d. (n=3, *P < 0.05, **P < 0.01, ***P < 0.001).



Supplementary Figure 6. SIRT6 and CHD4 enable chromatin relaxation and DSB repair within compacted regions. (A) HeLa cells were treated with or without DOX. *α-Sat* relative expression levels were measured by quantitative real-time PCR. The data represent the means \pm s.d. ($n=3$, $***P < 0.001$). (B) HeLa cells were treated with or without DOX. *Sat2* relative expression levels were measured by real-time PCR. The data represent the means \pm SEM ($n=3$, $**P < 0.01$). (C) HeLa cells were pre-treated for 30 min with 20 μ M ATM inhibitor (ATMi) (Ku55933), followed by treatment with 1

μM DOX. The cell extracts were then precipitated with an anti-SIRT6 antibody and analyzed by western blotting. Rabbit IgG was used as a negative control. (D) NS, SIRT6 or KAP1 specific siRNA or combined siRNA duplexes targeted to SIRT6 and KAP1 were infected into DR-U2OS cells for 24 h. The cells were then transfected with an I-SceI expression plasmid for 48 h. HR efficiency was determined by FACS. The data represent the means \pm s.d. (n=3, *P < 0.05, **P < 0.01, ***P < 0.001). (E) Flow cytometry analysis of cell cycle distributions of A549 cells after synchronization in G1 by serum starvation (from Figure 6E). (F) Flow cytometry analysis of cell cycle distributions of HeLa cells after synchronization in G2 (from Figure 6F). (G) HeLa cells were transfected with NS or SNF2H specific siRNAs for 48 h in the presence or absence of 1 μM DOX. SIRT6 was immunoprecipitated from soluble nucleosome extract, washed with solubilization buffer and analyzed by western blotting. Rabbit IgG was used as a negative control.



Supplementary Figure 7. Depletion of SIRT6 or CHD4 impairs repair protein loading and compromises cell survival. (A) HeLa cells were transfected with NS (non-specific), CHD4 or SIRT6 specific siRNA for 48 h (from Figure 7B-C). Whole cell lysates were extracted and analyzed by western blotting. (B-C) HeLa cells were transfected with NS, CHD4 or SIRT6 specific siRNA for 48 h. Cells were exposed to 10 Gy irradiation (IR), and then fixed at 1, 3 or 6 h post-IR and observed under a confocal microscope. Enumeration of 53BP1 (B) and ̳H2AX (C) foci per cell. The data represent

the means \pm SEM (n=100, **P < 0.01, ***P < 0.001). (D) WT or SIRT6-depleted HeLa cells were treated with or without 40 μ M VP16 for 30 min. Chromatin fractions were then extracted and analyzed by western blotting. (E) HeLa cells stably expressing NS, SIRT6 RNAi or CHD4 RNAi were exposed to 3 Gy IR. The cells were then cultured in a six-well plate for 2 weeks, and clone formation was analyzed by crystal violet staining (from Figure 7F). Whole cell lysates were extracted and analyzed by western blotting. (F) A Flag-SIRT6-FL, Flag-SIRT6- Δ C mutant, Flag-SIRT6-Core or Flag-SIRT6- Δ N mutant was transfected into shSIRT6 HeLa cells. The cells were then treated with 40 μ M VP16 for 2 h and washed free of the drug. The cells were then cultured in a 6-cm plate for 2 weeks and clone formation was analyzed by crystal violet staining.