

## Expanded View Figures

**Figure EV1. CHD1 is co-localized with DNA damage site.**

- A CHD1 is partially co-localized with  $\gamma$ H2AX. PC3 cells were treated with NCS (100 ng/ml) for 2 h and co-immunostained with  $\gamma$ H2AX and CHD1 antibodies. Scale bar, 10  $\mu$ m.
- B 53BP1 recruitment to I-SceI-induced DNA damage sites in U2OS19 ptight13 GFP-LacR cells indicated by white arrows. Scale bar, 10  $\mu$ m.
- C PLA with  $\gamma$ H2AX/CHD1 or  $\gamma$ H2AX/53BP1 in mock or siCHD1 (SmartPool)-transfected PC3 cells after 2 h NCS treatment. Scale bar, 10  $\mu$ m.
- D, E CHD1 is recruited to the chromatin upon DNA double-strand break induction. VCaP cells stably expressing either control (shCont) or CHD1 shRNA (shCHD1) (D) and U2OS cells following transient transfection with either mock or CHD1 siRNA (E) were treated with NCS for the indicated times, and chromatin fractions were immunoblotted with CHD1 and  $\gamma$ H2AX antibodies, respectively. H2B serves as a loading control.
- F PC3 cells were treated with NCS (100 ng/ml) for indicated times, and the total protein lysates were analyzed by Western blotting using the indicated antibodies.

Source data are available online for this figure.

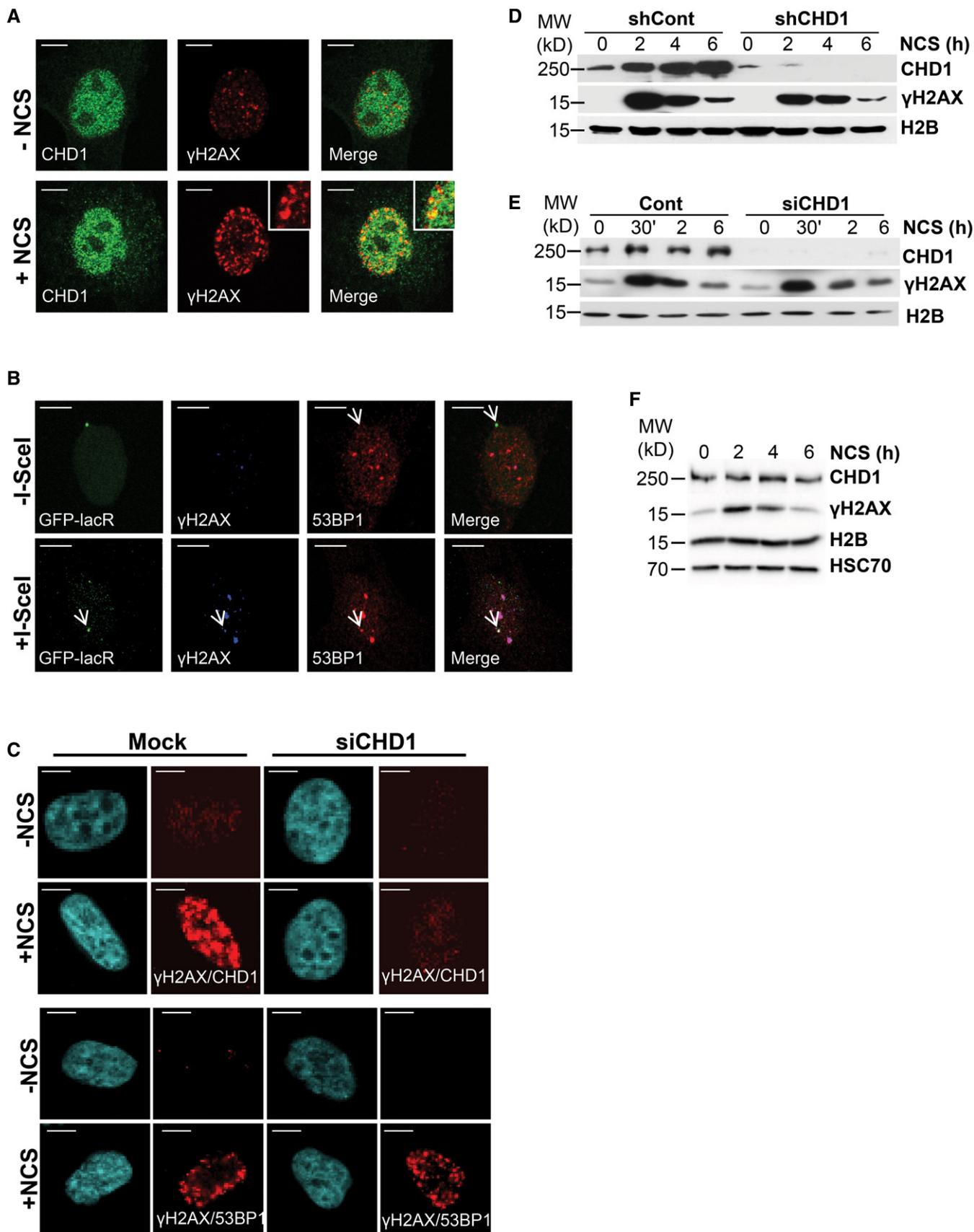
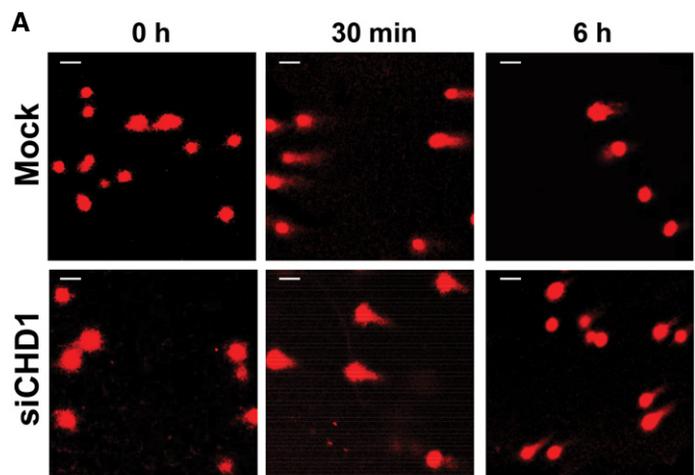


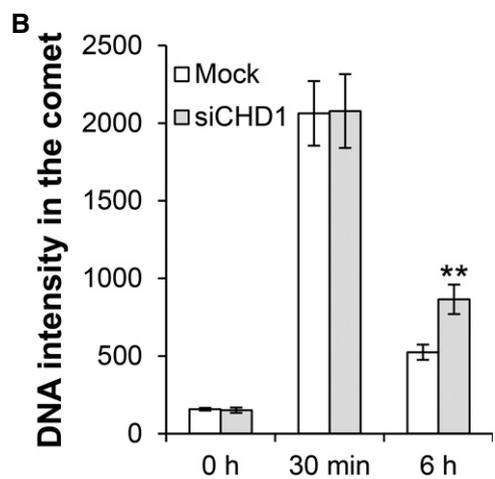
Figure EV1.

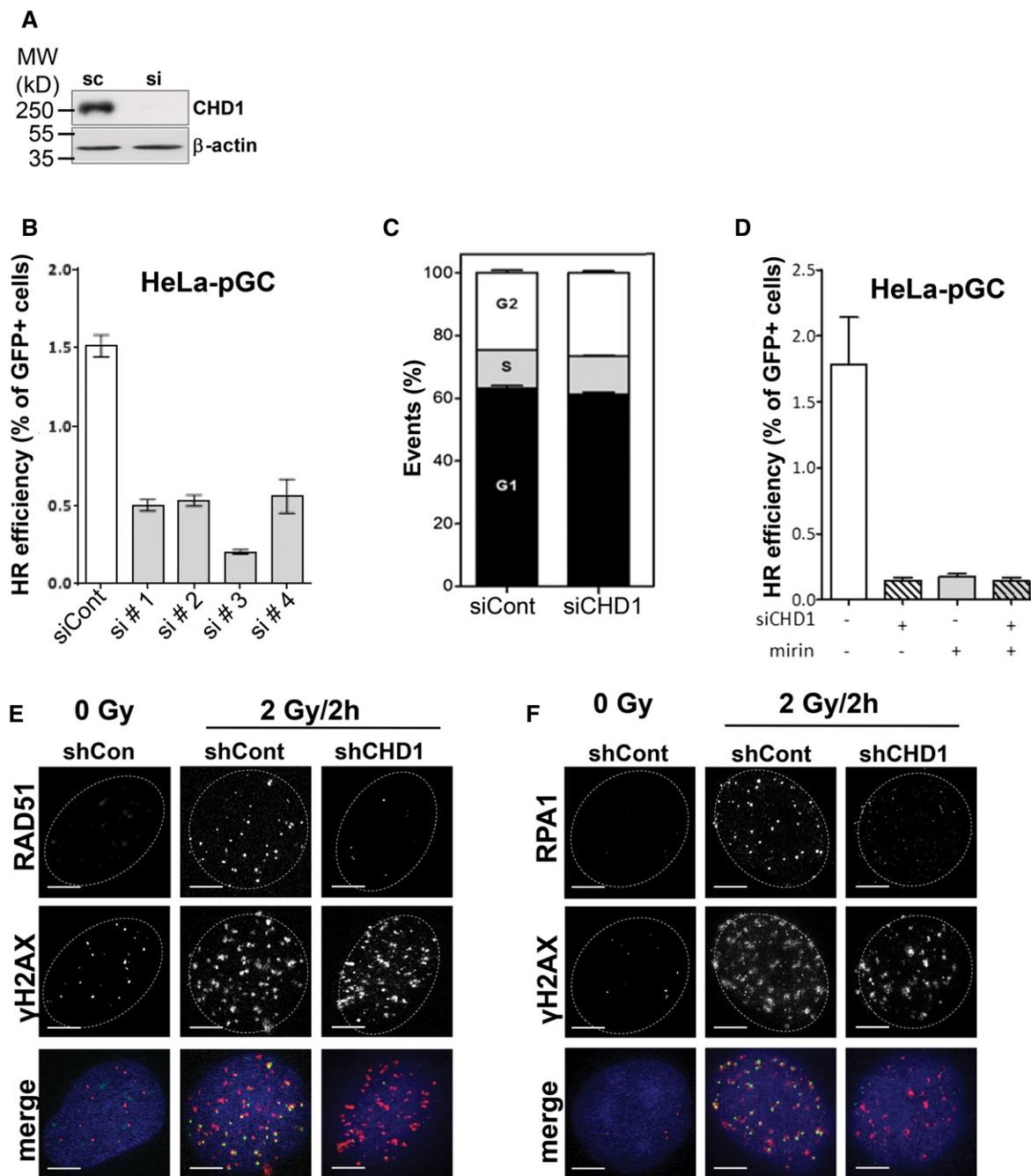


**Figure EV2. CHD1 is required for DSB repair.**

A CHD1-depleted cells show decreased DNA DSB repair efficiency. PC3 cells were either mock transfected or transfected with siCHD1 (SmartPool). After 48 h of transfection, cells were treated with NCS. Neutral comet assay was performed after the indicated times following NCS treatment, and images were taken. Scale bar, 50  $\mu$ m.

B Quantification of comet tail (DNA content) from (A) in control and CHD1-depleted cells is shown. More than 50 cells were counted, and results represent the mean value  $\pm$  SD ( $n = 3$ ).  $P$ -values (\*\* $P = 0.005$ ) were calculated using ANOVA.





**Figure EV3. CHD1 is required for homologous recombination-mediated DNA repair.**

- A HeLa cells were transfected with control or CHD1 siRNA for 48 h, and protein samples were analyzed by Western blotting for CHD1 expression.
- B HeLa cells containing single copy HR (pGC) repair substrates were transfected with either control siRNA or four individual siRNAs targeting CHD1. After 24 h of transfection, DSB was induced by transfecting cells with I-SceI-expressing vector (pCMV-I-SceI-3xNLS). The GFP-positive cells were measured by flow cytometry. Data are represented as mean  $\pm$  SD ( $n = 3$ ).
- C CHD1 depletion did not change the cell cycle profile of HeLa cells. Cells were transfected with control or CHD1 siRNA and 48 h after transfection processed for FACS analysis. Mean values  $\pm$  SD are presented ( $n = 3$ ).
- D HeLa cells containing single copy HR (pGC) repair substrates were either mock transfected or with siRNA targeting CHD1. After 24 h of transfection, cells were treated with mirin and DSB was induced by transfecting cells with I-SceI-expressing vector (pCMV-I-SceI-3xNLS). The GFP-positive cells were measured by flow cytometry. Data are represented as mean  $\pm$  SD ( $n = 3$ ). For flow cytometry, 50,000 cells were counted for each condition.
- E, F CHD1-depleted BHP1 cells show decreased RAD51 and RPA1 foci after DNA damage induction. shCont- or shCHD1-expressing BHP1 cells were irradiated and co-immunostained with  $\gamma$ H2AX and RAD51 or RPA1 antibodies at the indicated time points. Scale bar, 5  $\mu$ m.

**Figure EV4. CHD1 is required for DNA end resection.**

- A Quantification of CHD1/CtIP PLA signal from Fig 4A in control and NCS-treated PC3 cells, mean values  $\pm$  SD are presented ( $n = 3$ ). More than 50 cells were counted for each condition.
- B Similar to Fig EV1D, VCaP cells with shCont or shCHD1 were treated with NCS for different times and chromatin fractions were analyzed by Western blotting.
- C Similar to Fig EV1E, U2OS cells were transfected and treated with NCS for different times and chromatin fractions were analyzed by Western blot analysis.
- D Total protein lysates from shCont and shCHD1 PC3 cells after NCS treatment were analyzed by Western blotting using the indicated antibodies.
- E U2OS19 ptight13 GFP-LacR cells were transfected with either mock or siCHD1. After 48 h of transfection, cells were immunostained for CHD1. Scale bar, 50  $\mu$ m.
- F Total protein lysate from mock or siCHD1 U2OS cells from (E) were analyzed by Western blotting.
- G PC3 cells were transfected with either mock, siCHD1-1, or siCHD1-4. After 48 h of transfection, cells were treated with NCS for the indicated times and chromatin fractions were analyzed by Western blotting.
- H PC3 cells were transfected with either control or CtIP or with four individual CHD1 siRNAs. After 48 h of transfection, cells were irradiated and processed for immunostaining for CenPF and CtIP. Scale bar, 10  $\mu$ m.
- I Quantification of CtIP foci from (H), data are presented as mean  $\pm$  SD ( $n = 3$ ). More than 50 cells were counted per condition.
- J, K Similar to (H) PC3 cells were transfected and immunostained with RPA1 and CenPF antibodies, RPA1 foci were quantified, and mean values  $\pm$  SD are presented in the graph (K) ( $n = 3$ ). More than 50 cells were counted per condition. Scale bar, 10  $\mu$ m.
- L PC3 cells were transfected with either mock or siCHD1 (1 and 4) followed by transfection with empty vector or plasmids expressing ERT2-fusions of Wt-mChd1 or ATPase Mt-mChd1. After 48 h of transfection, cells were treated with 300 ng/ml of 4-OH tamoxifen for 24 h. Whole-cell protein lysates were analyzed by Western blotting using antibodies that recognize mouse and human CHD1, the ERT2 fusion protein, or HSC70 (loading control).
- M Quantification of the fraction of cells with punctate staining after PLA assay for  $\gamma$ H2AX/CtIP from Fig 4E, mean values  $\pm$  SD are presented ( $n = 30$ ,  $**P = 0.007$ , ANOVA).
- N PC3 cells stably expressing ERT2-HA-mChd1 were transfected with either mock or siCHD1 (1 and 4). After 24 h of transfection, cells were treated with either vehicle or 4-OHT for 24 h and whole-cell lysates were analyzed by Western blotting using antibodies specific for hCHD1, the ERT2 fusion protein or HSC70 (loading control).
- O Chromatin fractions from (N) after NCS treatment for the indicated times were analyzed by Western blotting.
- P Quantification of ERT2 (mChd1) and hCHD1 intensity from (O).

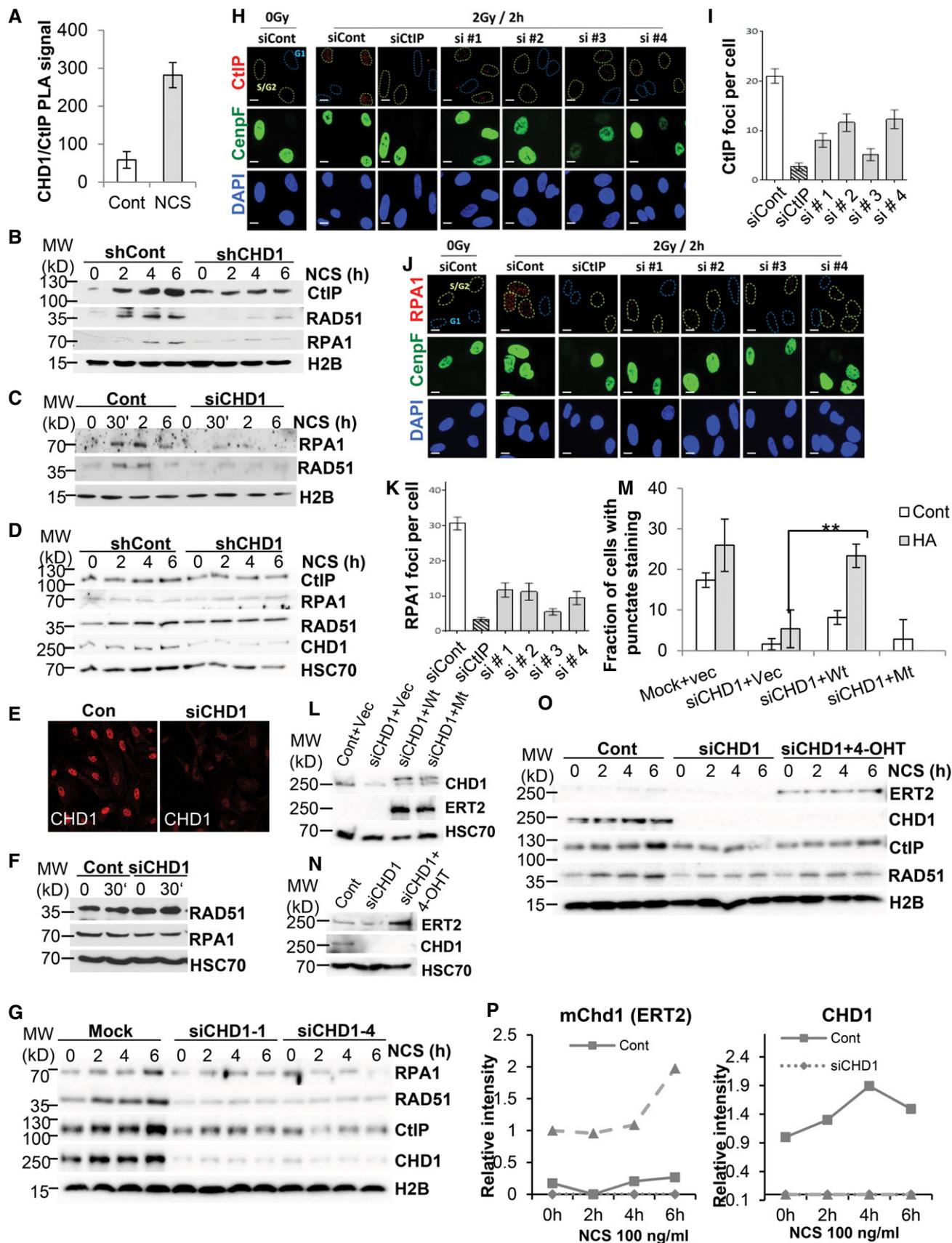


Figure EV4.

**Figure EV5. CHD1 facilitates chromatin opening during HR-mediated repair.**

- A Total protein lysate from cells treated with DMSO, mirin, or veliparib (PARPi) was analyzed by Western blotting.
- B PC3 cells were pretreated with DMSO, mirin (50  $\mu$ M), or veliparib (20  $\mu$ M, PARPi) for 1 h prior to NCS treatment for 2, 4, or 6 h. Chromatin fractions were analyzed by Western blotting for the recruitment of CHD1, CtIP, RAD51, and  $\gamma$ H2AX.
- C, D PC3 (C) and BHP1 (D) cells with stable shCont or shCHD1 expression were transfected with either siCont or siCtIP for 24 h before irradiation with the indicated doses. Surviving fractions were measured by counting colonies after 3 weeks, and mean values  $\pm$  SD are presented ( $n = 3$ ).
- E AsiSI-ER-U2OS cells were transfected either control or CHD1 siRNA. After 48 h of transfection, cells were treated with 4-OHT for the indicated times and the whole-cell protein lysates were immunoblotted with CHD1 and HSC70 antibodies.
- F Cells from (E) were processed for immunostaining with  $\gamma$ H2AX and CHD1. Scale bar, 20  $\mu$ m.
- G, H qPCR analysis from FAIRE DNA from AsiSI-ER-U2OS cells from (E) at sites repaired (H) or not repaired (G) by HR. Mean values  $\pm$  SD are presented in the graphs ( $n = 3$ ).
- I, J CHD1 is required only for chromatin-associated homologous recombination (HR) repair. PC3 cells or BHP1 cells with stable expression of either control or CHD1 shRNA were transfected with I-SceI-linearized pGC pGC repair substrate. After 24 h of transfection, HR efficiency was calculated based on the fraction of GFP-positive cells plotted in the graph, and 50,000 cells were counted per condition. Data are represented as mean  $\pm$  SD from three independent experiments.

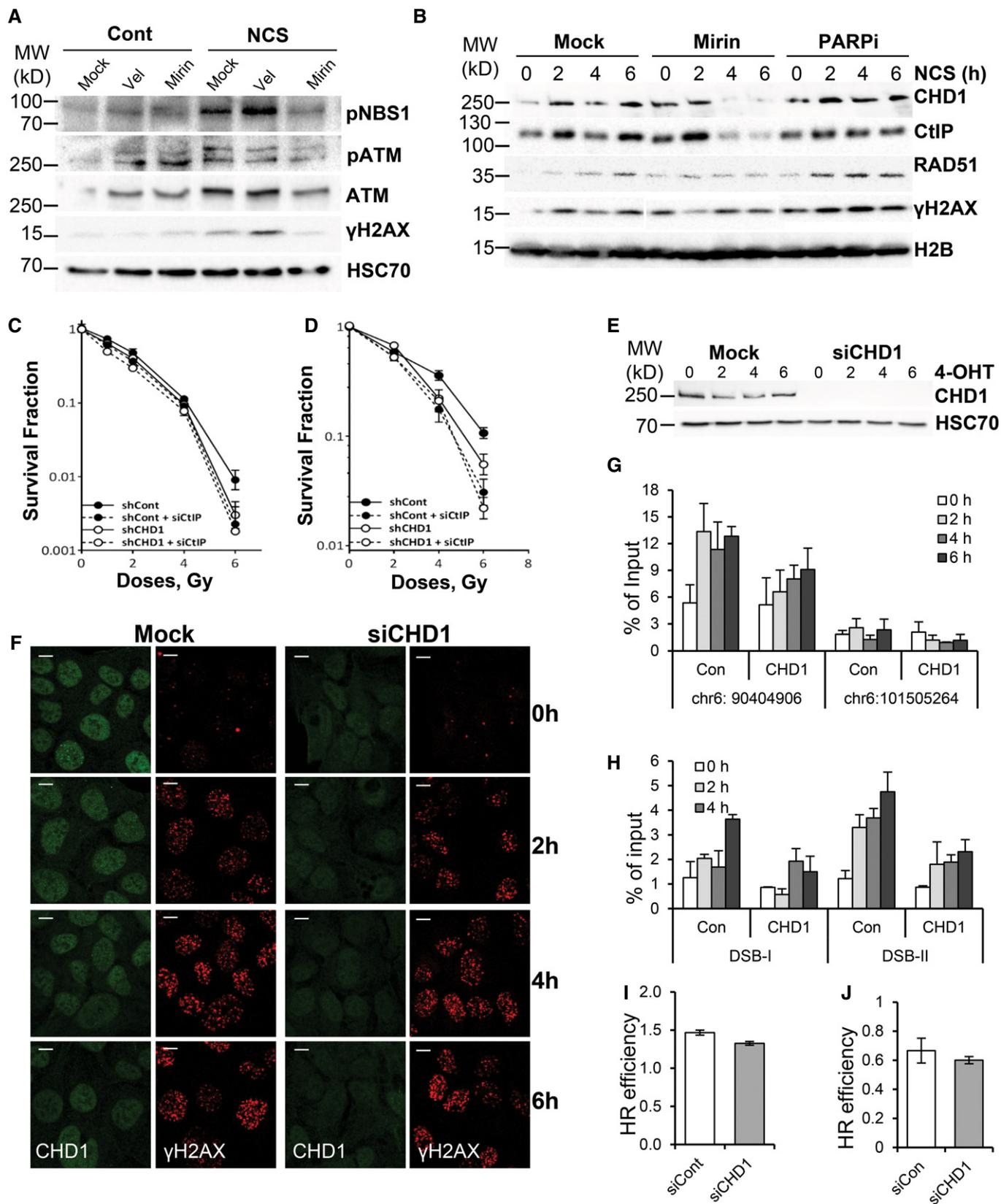
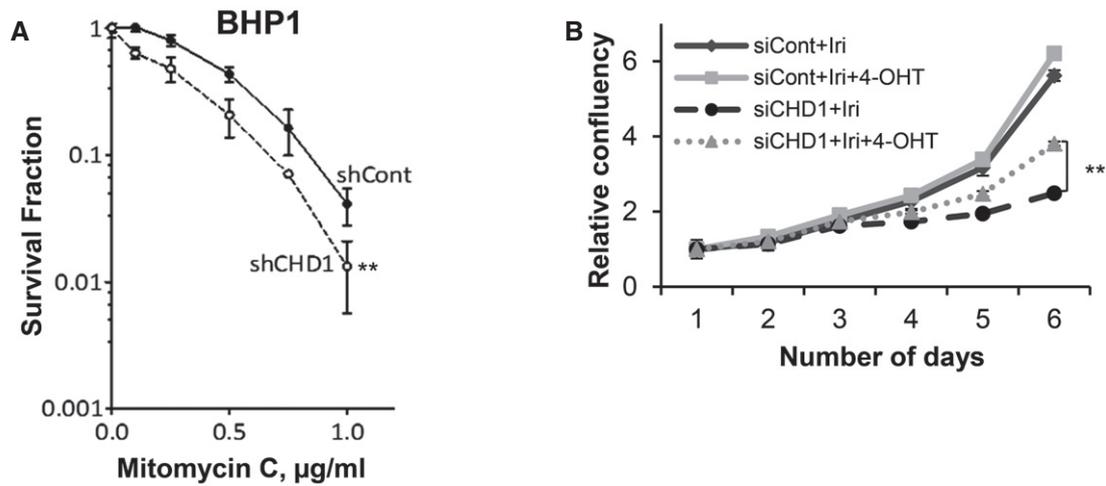


Figure EV5.





**Figure EV6. CHD1 loss increases chemotherapeutic repressiveness.**

- A CHD1-depleted BHP1 cells show hypersensitivity to mitomycin C (MMC) treatment. Control and CHD1-depleted BHP1 cells were treated with the indicated doses of MMC for 4 h, and surviving fractions were measured by counting colonies after 3 weeks. Data are represented as mean  $\pm$  SD ( $n = 3$ ), and  $P$ -value was calculated using ANOVA (\*\* $P = 0.005$ ).
- B Similar to Fig EV40, rescue experiments were performed with cells expressing mChd1-ERT2. Cells were treated as indicated with 0.5  $\mu\text{M}$  of irinotecan and/or 4-OHT, proliferation was measured by Celigo, and the relative confluency was plotted in the graph. Data are represented as mean  $\pm$  SD ( $n = 3$ ). Data were normalized to the plating efficiency.  $P$ -value (\*\* $P = 0.006$ ) was calculated using ANOVA.