Larsen et al., http://www.jcb.org/cgi/content/full/jcb.200912135/DC1

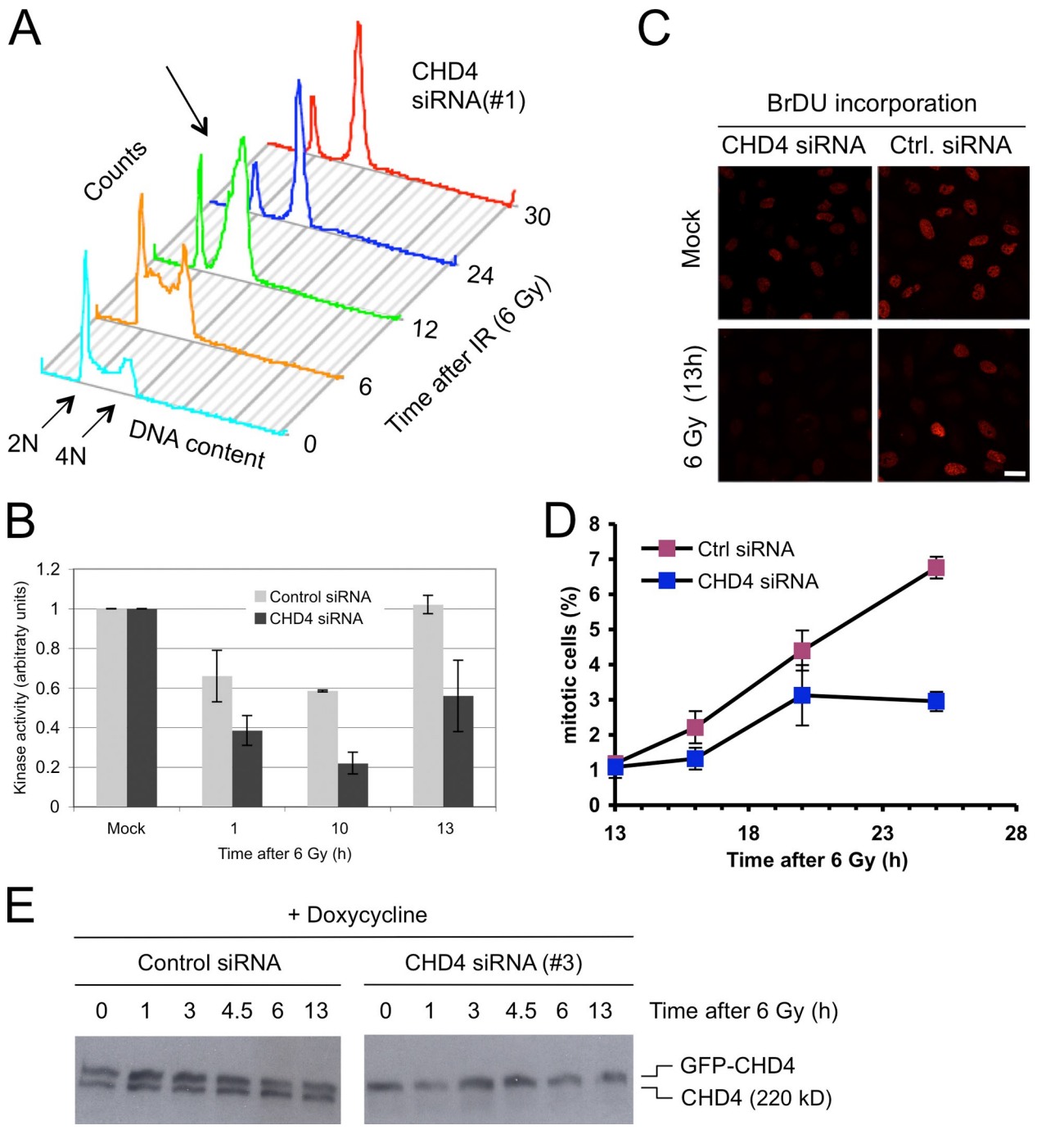

Figure S1. **Down-regulation of CHD4 delays cell cycle progression after IR.** (A) U2OS cells were treated with CHD4 siRNA (#1) for 72 h, irradiated (6 Gy), and analyzed at the indicated time points by flow cytometry. The control DNA profiles of cells treated with nonspecific siRNA are provided in Fig. 2 B. The efficiency of the CHD4 siRNAs is shown in Fig. S3 B. The G1 arrest and S phase delay (the latter manifested as a broader peak above the 4N DNA content) are evident especially at the 12-h time point (arrow). (B) U2OS cells were treated with control or CHD4 siRNAs (SMARTpool) for 72 h, irradiated (6 Gy), and analyzed for the cyclin A–associated kinase activity using histone H1 as a substrate at the indicated time points. The values represent ratios of the phosphate incorporation relative to that measured in nonirradiated cells. (C) U2OS cells were treated with siRNAs as in B, irradiated (6 Gy), incubated for 13 h, pulse labeled for 10 min with BrdU, and analyzed by immunostaining with anti-BrdU antibody. Bar, 10 µm. (D) U2OS cells were treated with siRNAs as in B, irradiated (6 Gy), incubated for 13 h, treated with nocodazole, and the mitotic cells were collected at the indicated time points. Cells were subsequently coimmunostained for the mitotic marker phospho-S^{10} of histone H3 antibody and PI (the total DNA content) and analyzed using flow cytometry. (E) U2OS cell line engineered to conditionally express GFP-CHD4 resistant to siRNA (#3) was treated with control or CHD4 siRNA (#3) as indicated. After 48 h, the transgenes were induced by addition of doxycycline. After additional 24 h, cells were exposed to IR and analyzed at the indicated time points by an antibody to CHD4. Note that the GFP-CHD4 protein resists the siRNA treatment and remains expressed throughout the time course. Error bars indicate SEM.

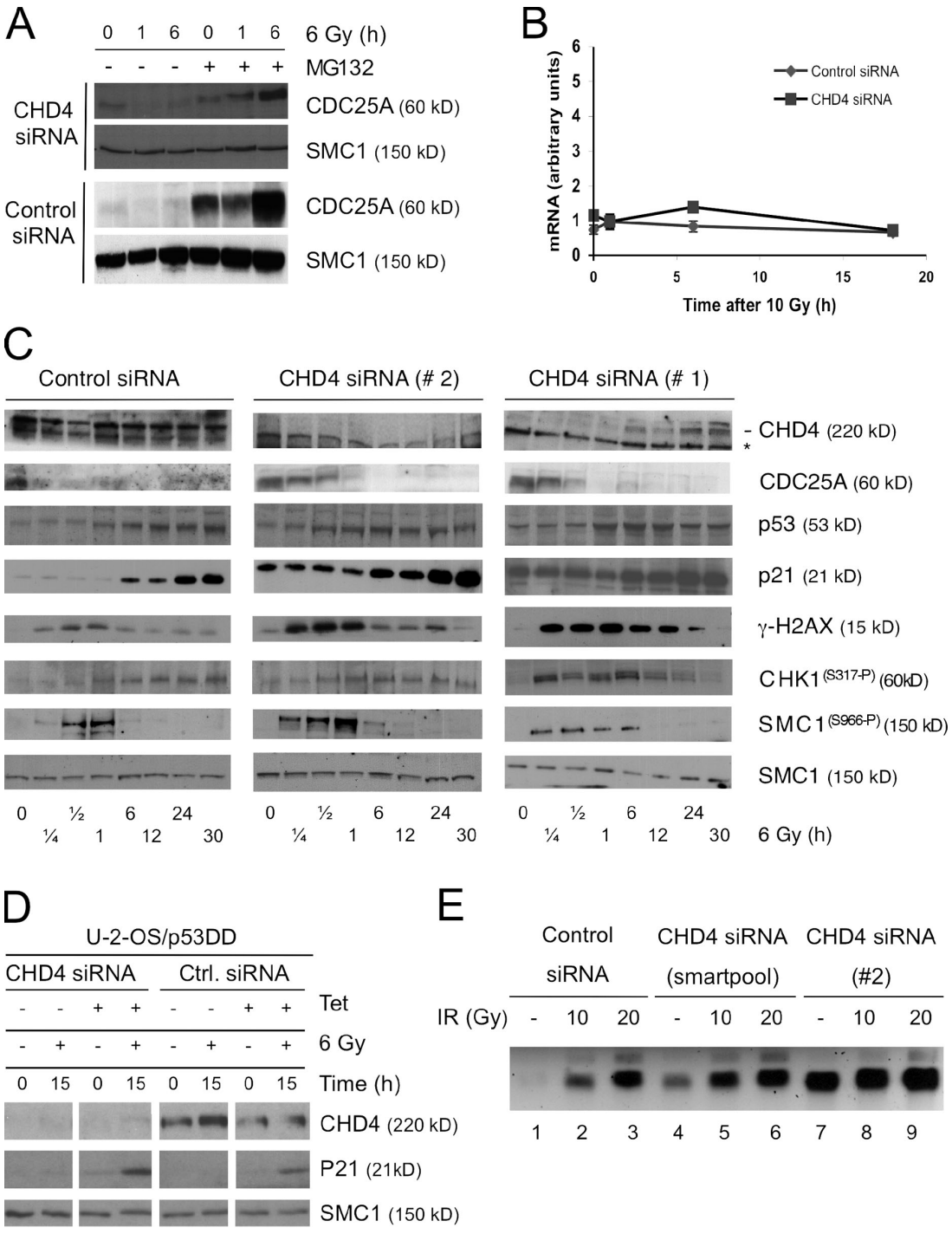

Figure S2. **Impact of CHD4 depletion on checkpoint signaling and cellular response to DNA damage.** (A) U2OS cells were treated with control or CHD4 siRNAs (SMARTpool) as indicated. After 72 h, the cells were treated or not with 5 mM of the proteasome inhibitor MG132 for 30 min, irradiated (6 Gy), and analyzed for the Cdc25A protein levels by immunoblotting. (B) U2OS cells were treated with siRNAs as in A, irradiated (10 Gy), and harvested at the indicated time points. The Cdc25A mRNA was quantified by RT-PCR, and the data are plotted as ratios relative to the housekeeping gene porphobilinogen deaminase. Experiments were performed in duplicate. (C) U2OS cells were transfected with control siRNA or two distinct siRNAs to CHD4 as indicated. After 72 h, cells were irradiated and analyzed by immunoblotting with antibodies at the indicated time points. The efficiency of the CHD4 siRNAs is shown in Fig. S3 B. Asterisk, nonspecific band. (D) U2OS/p53DD cell line engineered to conditionally express the dominant-negative fragment of p53 was treated with control (ctrl) or CHD4 siRNAs (SMARTpool), and 48 h later, induced by removal of tetracycline (Tet) to express the transgene. After an additional 24 h, cell were irradiated and analyzed by immunoblotting at the indicated time points. (A, C, and D) Total SMC1 serves as loading control. (E) U2OS cells were treated with the indicated siRNAs, irradiated, and subjected to the PFGE analysis as in Fig. 3 C. The efficiency of CHD4 siRNAs is shown in Fig. S3 B. Error bars indicate SEM.

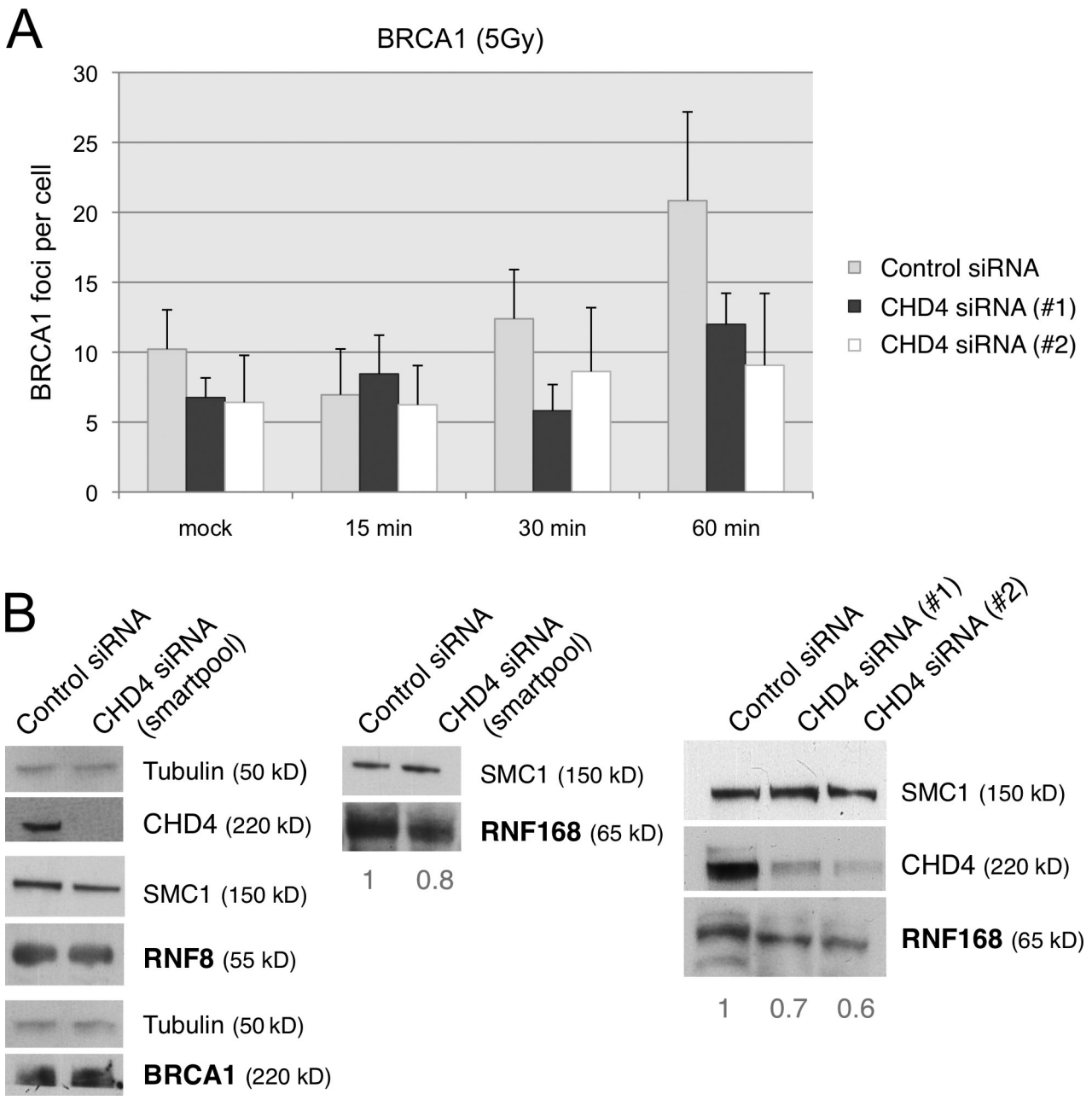

Figure S3. **Impaired BRCA1 retention at IR-induced DSBs in CHD4-deficient cells.** (A) U2OS cells were treated with control siRNA or two distinct CHD4 siRNAs as indicated, incubated for 72 h, irradiated (5 Gy), and immunostained with antibodies to BRCA1 at the indicated time points. The number of IR-induced BRCA1 foci per nucleus was determined as described in Materials and methods. The siRNA efficiencies are shown below the blots in B. Error bars indicate SD. (B) Ablation of CHD4 partially destabilizes RNF168. U2OS cells were treated with the indicated siRNAs for 72 h and analyzed by immunoblotting for accumulation of all chromatin-associated proteins whose recruitment to DSBs was measured in Fig. 4. Note that although BRCA1 and RNF8 levels remained unchanged, the levels of RNF168 decreased to some extent in the absence of CHD4. The relative densities of the RNF168 band, normalized to the values in cells treated with control siRNA, are indicated below the blots. Tubulin and SMC1 serve as loading controls.