Badie et al., http://www.jcb.org/cgi/content/full/jcb.200811079/DC1

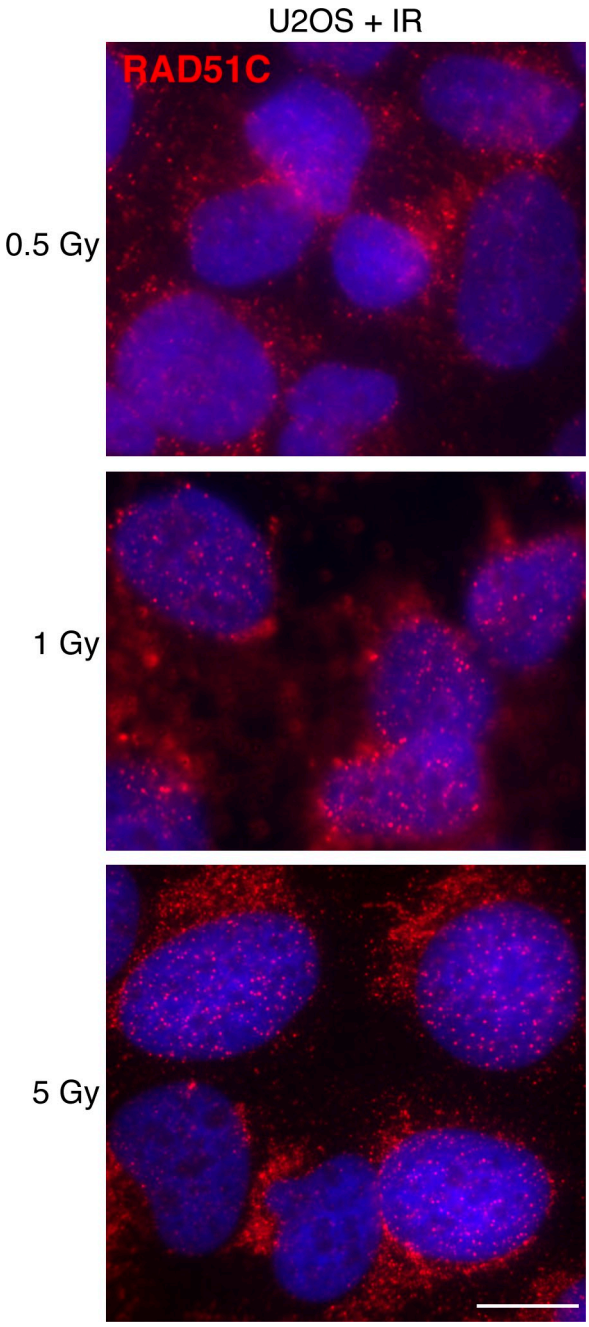

Figure S1. **RAD51C foci formation as a function of irradiation dose.** U2OS cells were treated with the indicated doses of IR, fixed 2 h after irradiation, and stained with anti-RAD51C antibody (red). DNA was stained with DAPI (blue). Bar, 10 µm.

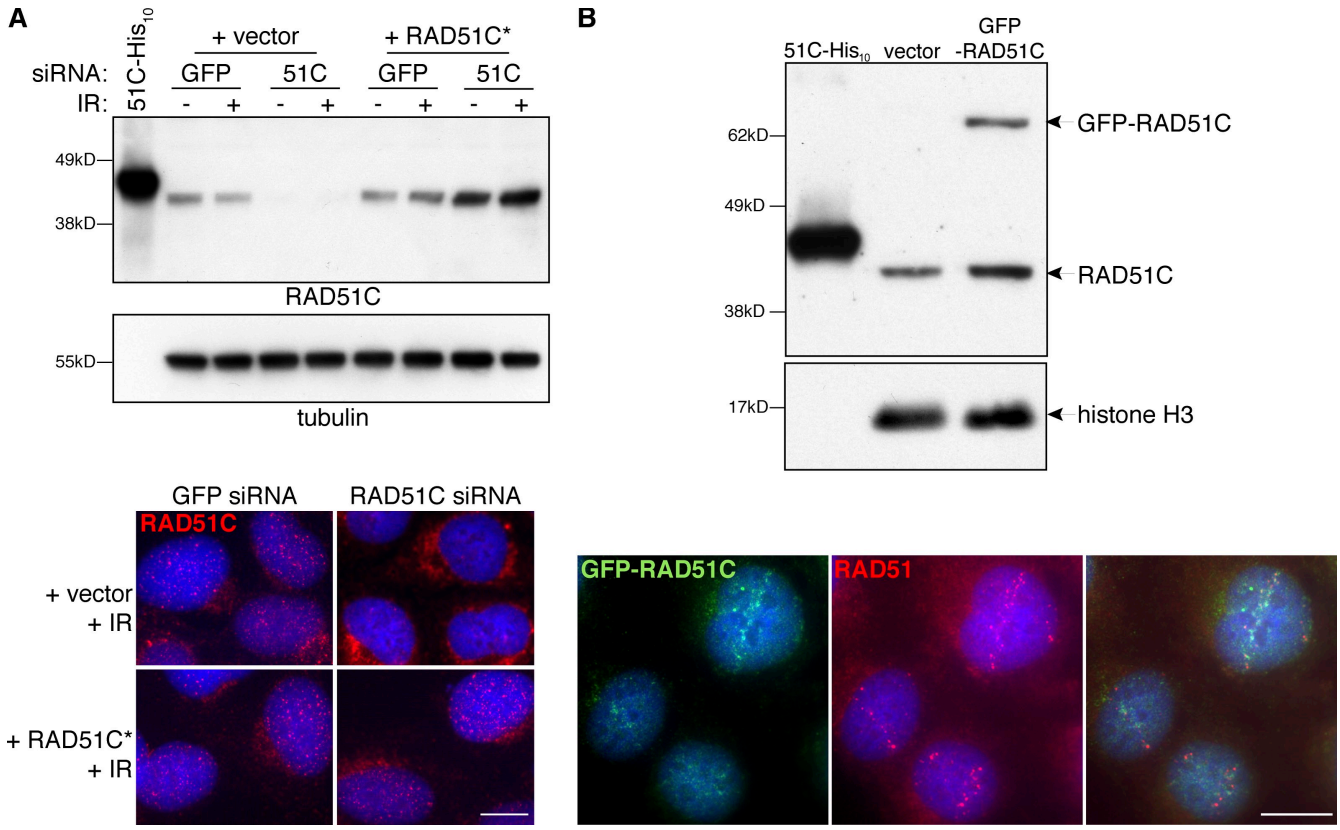

Figure S2. **RAD51C accumulates at sites of DSBs.** (A) U2OS cells stably transfected with the pcDNA3 vector or a construct expressing the human RAD51C protein from an siRNA-resistant cDNA (RAD51C*) were kept under 1 µg/ml puromycin selection for 14 d, and then single-cell clones were derived and tested for their response to siRNA depletion of RAD51C protein. Lysates of cells treated with GFP or RAD51C siRNA were analyzed by immunoblotting with monoclonal anti-RAD51C antibody. Recombinant RAD51C-His_{10} protein migrates more slowly than the endogenous human RAD51C protein. Tubulin was used as a loading control. Control cells or cells expressing the RAD51C* from an siRNA-resistant cDNA construct were irradiated (10 Gy) and fixed 2 h later. Cells were stained with monoclonal RAD51C antibody (red) and DNA with DAPI (blue). (B) U2OS cell lines stably expressing GFP-RAD51C were generated by cotransfecting the expression construct (Rodrigue, A., M. Lafrance, M.-C. Gauthier, D. McDonald, M. Hendzel, S.C. West, M. Jasin, and J.-Y. Masson. 2006. *EMBO J.* 25:222–231) with the pBabe–puro construct as previously described (Bekker-Jensen, S., C. Lukas, R. Kitagawa, F. Melander, M.B. Kastan, J. Bartek, and J. Lukas. 2006. *J. Cell Biol.* 173:195–206). After selection with 1 µg/ml puromycin for 14 d, single-cell clones were derived and tested for expression of the GFP-tagged RAD51C protein. Lysates from U2OS clones transfected with vector or GFP-RAD51C construct were analyzed by immunoblotting with a monoclonal anti-RAD51C antibody. Histone H3 was used as a loading control. Cells expressing GFP-RAD51C were partially irradiated with 1-µm stripes of ultrasoft x rays (~2 Gy averaged over the cell), fixed 1 h later, and stained with a combination of anti-GFP (green) and anti-RAD51 (red) antibodies. Bars, 10 µm.

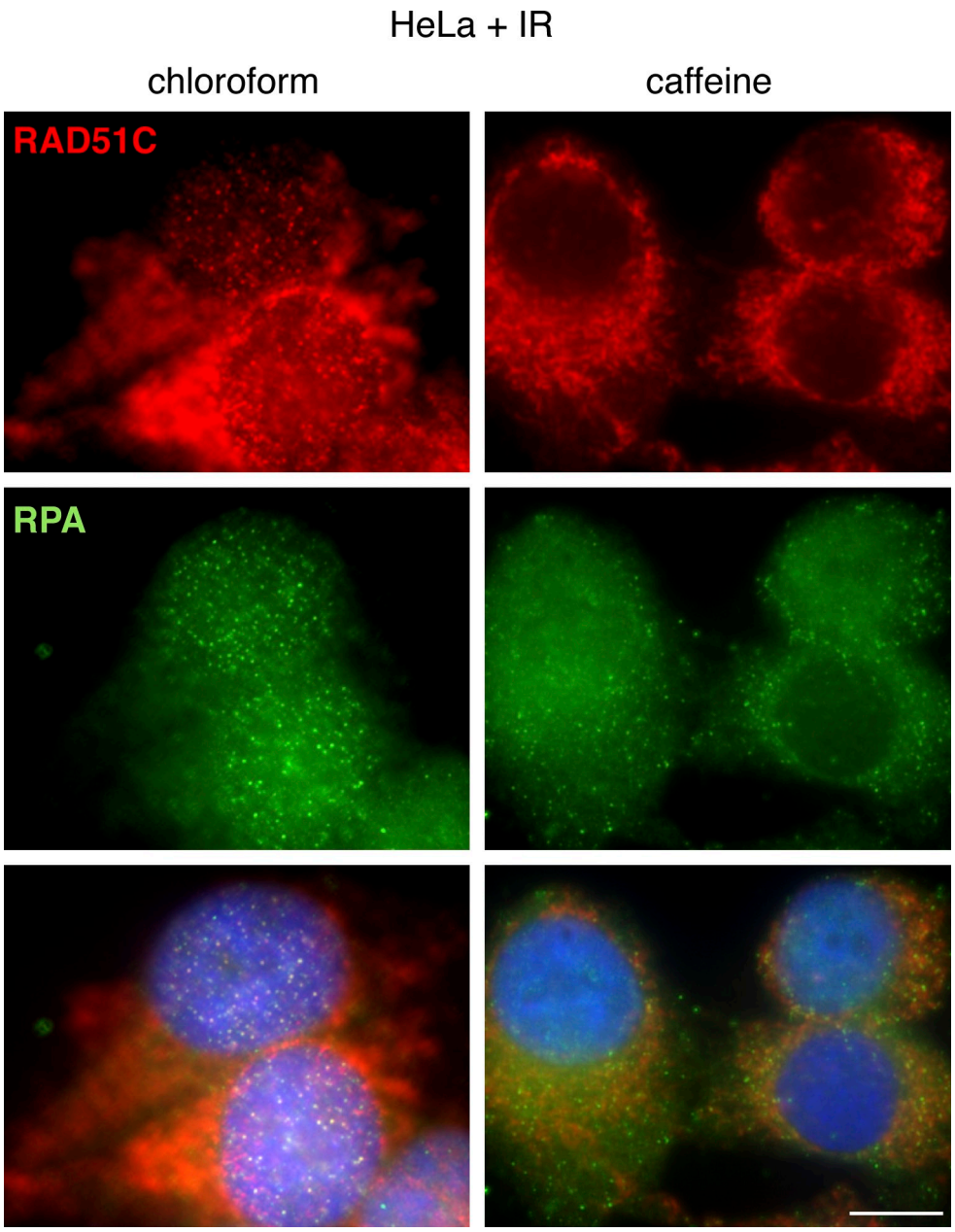

Figure S3. **Caffeine inhibits formation of IR-induced RAD51C foci.** HeLa cells were treated with 5 mM caffeine or solvent (chloroform) for 2 h, irradiated (10 Gy), and fixed 2 h later. Cells were stained with a combination of anti-RAD51C (red) and anti-RPA antibodies (green). DNA was stained with DAPI (blue). Bar, 10 μm.

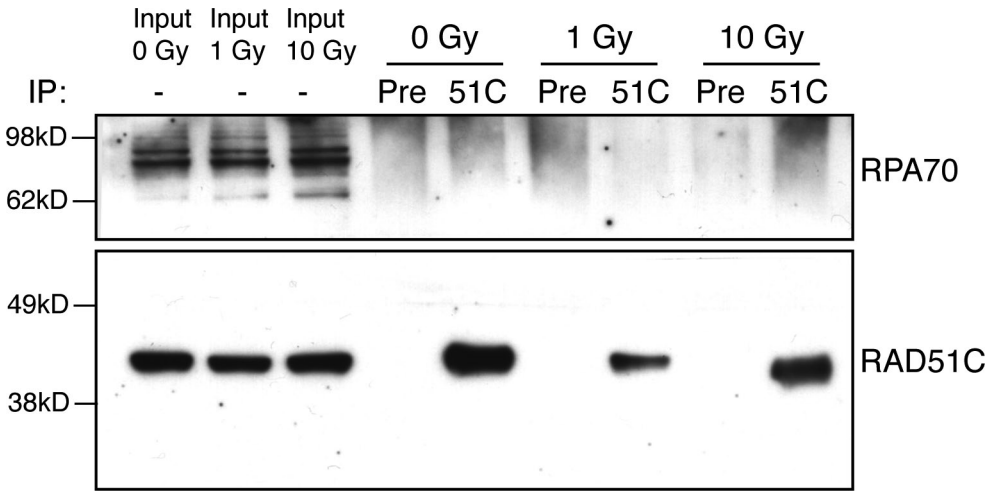

Figure S4. **RAD51C and RPA coimmunoprecipitation analysis.** U2OS cells were exposed to 0, 1, or 10 Gy of IR, and extracts were prepared 2 h later. After incubation with preimmune serum (Pre) or RAD51C (51C) polyclonal antibody crossed-linked to magnetic beads, the immunoprecipitated material and input extract were immunoblotted as indicated. IP, immunoprecipitation.

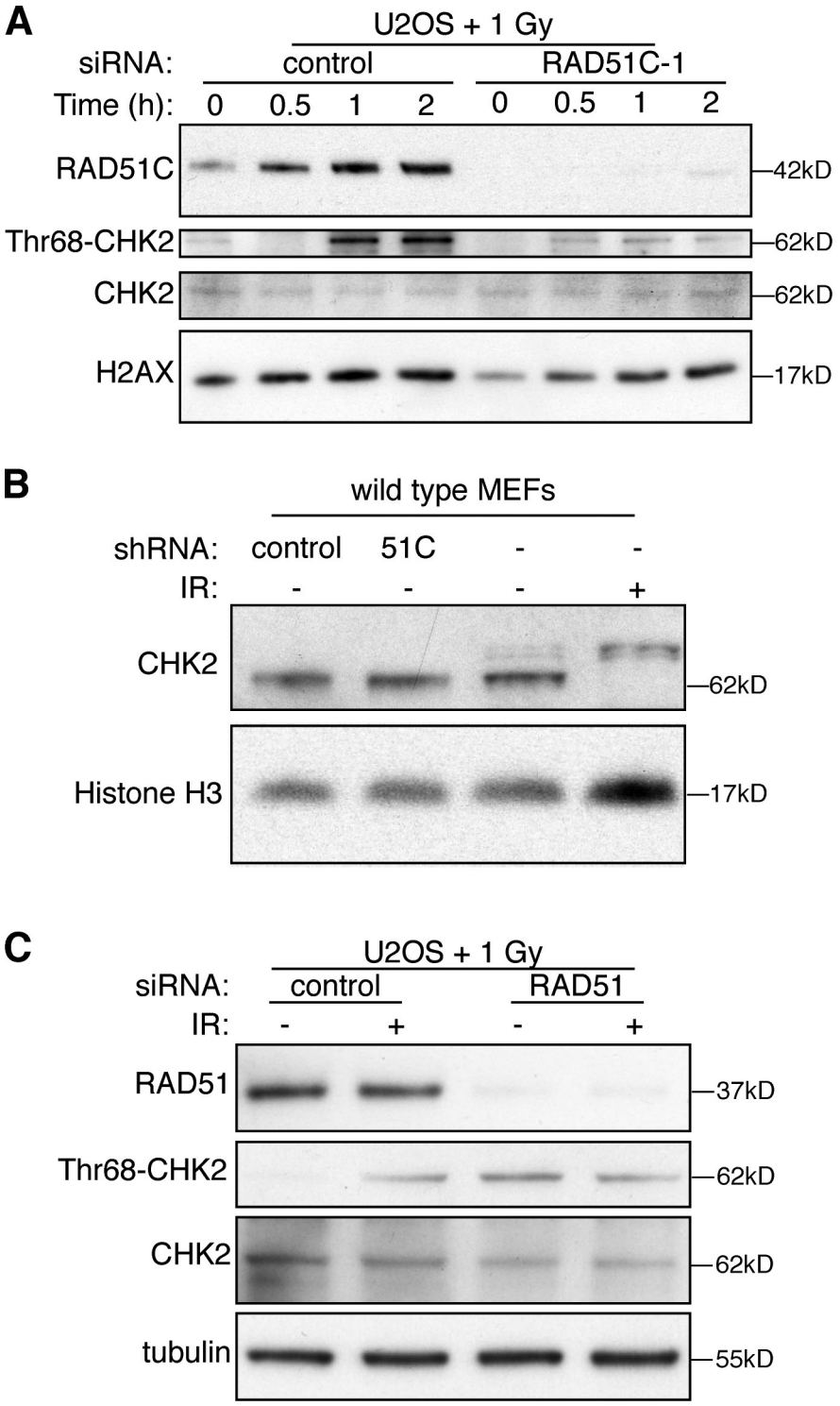

Figure S5. **Analysis of CHK2 phosphorylation in RAD51C- and RAD51-depleted mouse and human cells.** (A) U2OS cells were transfected with a control or a second RAD51C siRNA (RAD51C-1) and irradiated (10 Gy) 48 h after transfection. After 2 h, extracts were prepared and immunoblotted as indicated. (B) Cell lysates prepared from MEFs infected with RAD51C shRNA, as described in Fig. 6 C, or MEFs exposed to 0 and 10 Gy of IR were immunoblotted as shown. Histone H3 serves as a loading control. (C) U2OS cells were transfected with a control or a RAD51 siRNA and irradiated (1 Gy) 48 h after transfection. After 2 h, extracts were prepared and immunoblotted as indicated.