Wang et al., http://www.jcb.org/cgi/content/full/jcb.201010026/DC1

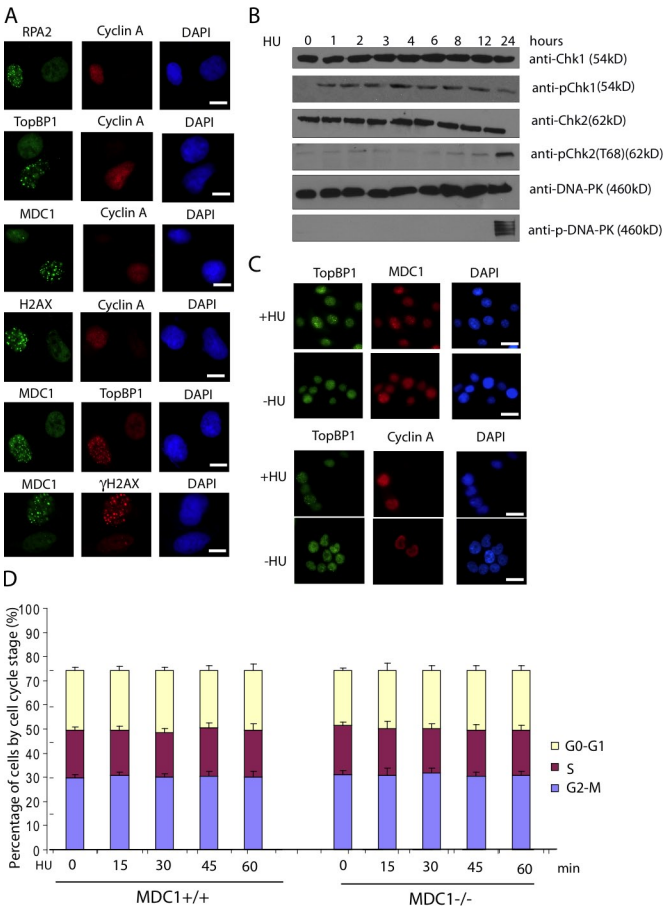

Figure S1. **HU-induced TopBP1 and MDC1 focus formation occurs at stalled replication forks.** (A) HeLa cells were treated with 2 mM HU for 2 h, and immunostaining was conducted using anti–cyclin A and other antibodies as indicated. Bars, 10 µm. (B) HeLa cells were mock-treated or treated with HU for the indicated time periods (1–24 h), and cell lysates were subjected to Western blotting using antibodies as indicated. (C) HeLa cells were treated with 2 mM HU for 2 h, and immunostaining was performed using the indicated antibodies. Bars, 10 µm. (D) MDC1$^{-/-}$ and WT control MEFs were treated with HU as shown in Fig. 4 E. Samples were taken at the indicated time points and analyzed by FACS. Data are presented as mean ± SD (error bars) from three different experiments.

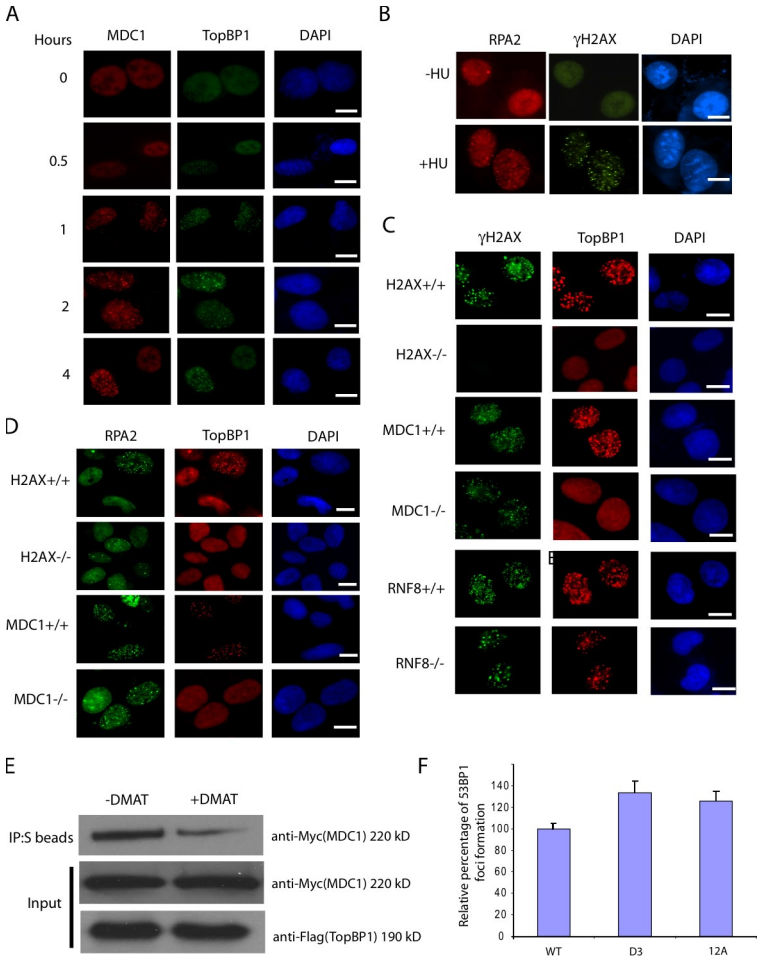

Figure S2. **TopBP1 acts downstream of H2AX and MDC1 in response to replication stress.** (A) U2OS cells were treated without or with 2 mM HU at different time points. Immunostaining experiments were performed with the indicated antibodies. (B) U2OS cells were treated without or with 2 mM HU for 2 h. Immunostaining experiments were performed with the indicated antibodies. (C) Cells deficient in H2AX, MDC1, and RNF8, and their respective WT counterparts were treated with HU, and immunostaining experiments were performed with anti-TopBP1 and anti–γ-H2AX antibodies. (D) Cells deficient in H2AX, MDC1, and their respective WT counterparts were treated with 3 μM aphidicolin for 16 h, and immunostaining experiments were performed with anti-TopBP1 and anti-RPA2 antibodies. (E) Extracts prepared from 293T cells expressing HA-tagged MDC1 were mock-treated or treated with 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole (DMAT). Extracts were then incubated with bacterially expressed and purified GST or GST-BRCT4+5 fusion protein immobilized on glutathione agarose beads for 2 h at 4°C. The complex was subjected to Western blotting using the indicated antibodies. (F) HeLa cells were transfected with FLAG-tagged siRNA-resistant WT, D3 mutant, or 12A mutant of MDC1, and with MDC1 siRNA twice within a 24-h interval. Cells were selected with puromycin for 48 h and then treated with 10 mM HU for 16 h. Immunostaining experiments were performed with the indicated antibodies. The percentages of cells stained positive for 53BP1 foci were determined. Data are presented as mean ± SD (error bars) from three different experiments. Bars, 10 μm.

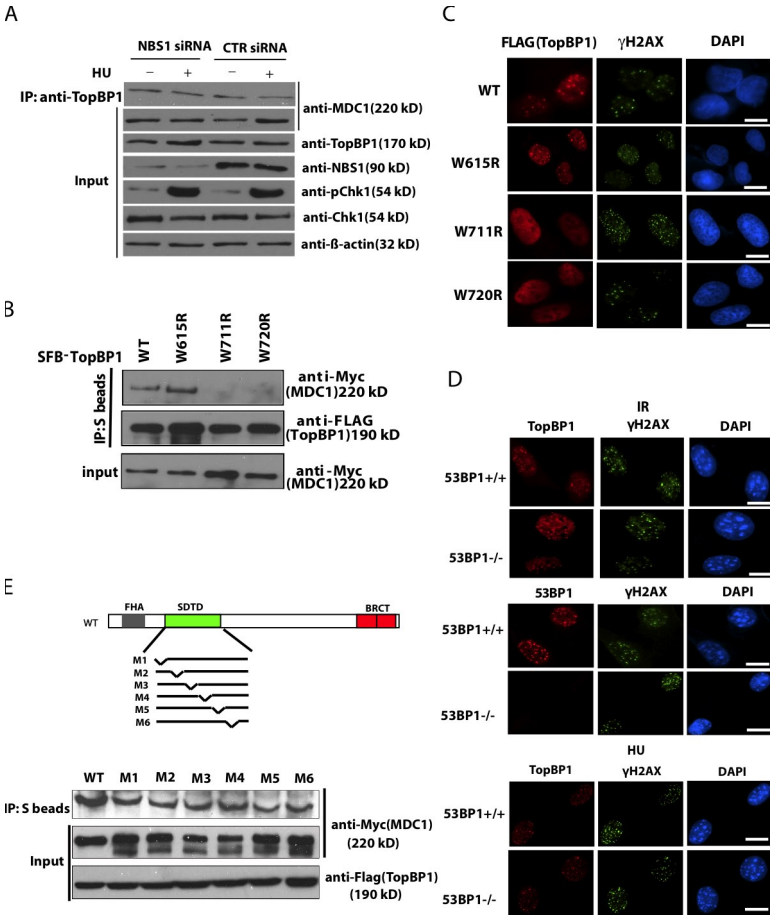

Figure S3. **The phosphorylation-dependent interaction between MDC1 and TopBP1.** (A) 293T cells were transfected with plasmids encoding Myc-tagged MDC1 together with plasmids encoding SFB-tagged WT or mutants of TopBP1. IP reactions were conducted using S protein beads, and then subjected to Western blotting using the indicated antibodies. (B) U2OS cells transfected with plasmids encoding SFB-tagged WT or mutants of TopBP1 were exposed to 2 mM HU for 2 h. Cells were fixed and immunostained with anti-FLAG and anti–γ-H2AX antibodies. (C) 53BP1$^{-/-}$ and WT control cells were treated with HU or IR. Immunostaining was performed with the indicated antibodies. Bars, 10 μm. (D) Cells transfected with control or NBS1-specific siRNA were treated with or without HU for 1 h, and the interactions between TopBP1 and MDC1 were examined by a co-IP assay. Whole-cell extracts were immunoblotted with antibodies as indicated. (E) 293T cells were transfected with plasmids encoding SFB-tagged TopBP1 together with plasmids encoding WT or six small internal-deletion mutants (deleted residues 200–240, 240–280, 280–320, 320–360, 360–400, 400–440) of Myc-tagged MDC1. IP reactions were conducted using S protein beads and then subjected to Western blotting using the indicated antibodies.