Supplemental Figures and Legends:

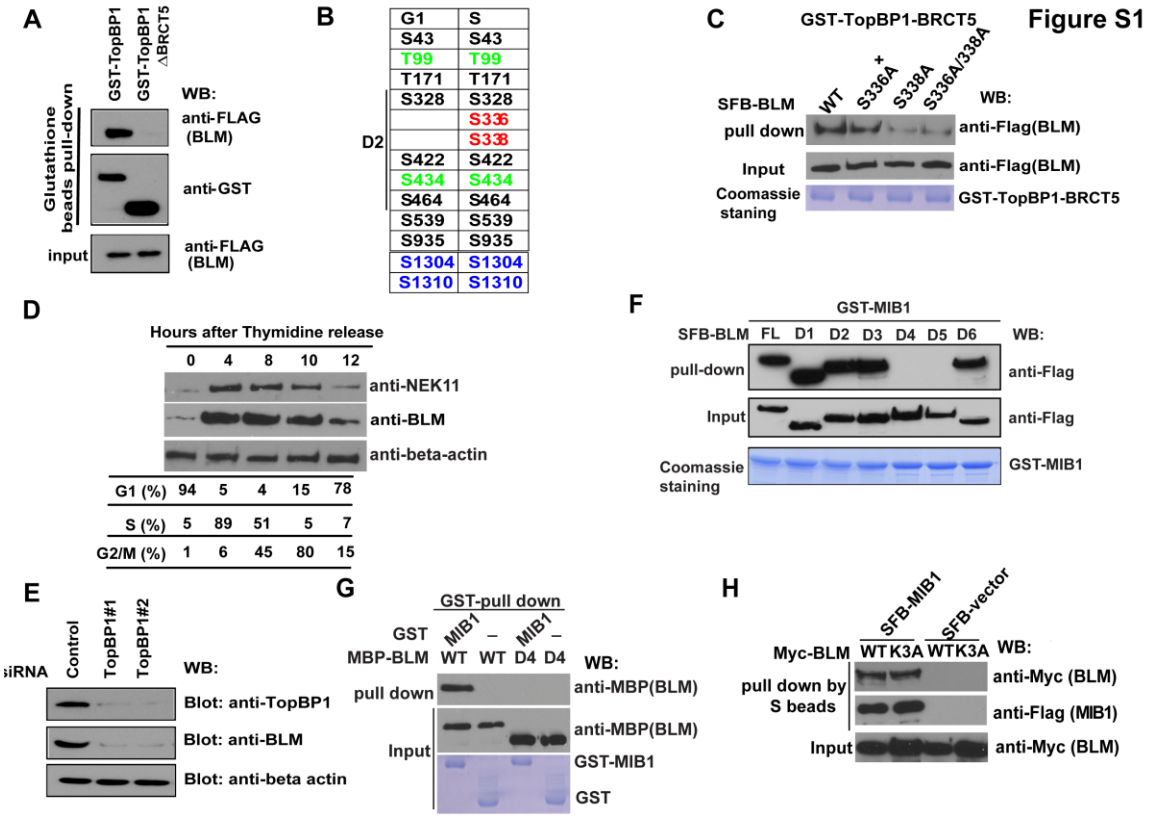

Figure S1, related to Figures 1, 2, 3, and 4.
(**A**) GST-TopBP1, GST-TopBP1-ΔBRCT5, and SFB-BLM were purified from Sf9 cells. GST pull-down assays were performed and immunoblotted with antibodies as indicated.
(**B**) Lists of BLM phosphorylation sites in G1 and S phases identified by two independent mass spectrometry analyses. The red color indicates phosphorylation sites that were only identified in S phase cells, the green color indicates the phosphorylation sites that were previously reported, the blue color indicates the phosphorylation sites only identified once by mass spectrometry analysis.
(**C**) Cell lysates were prepared using 293T cells transfected with constructs encoding SFB-tagged wild-type BLM, S336A, S338A, or S336A/338A BLM mutant. Beads coated with bacterially expressed GST-TopBP1 BRCT5 fusion protein were incubated with cell lysates. Immunoblotting experiments were carried out using indicated antibodies.
(**D**) HeLa cells were synchronized by double thymidine block, and then released in fresh medium without thymidine and collected at the indicated time points. Cell lysates were prepared, immunoprecipitation and immunoblotting experiments were performed using antibodies as indicated. Cell cycle distributions were confirmed by fluorescence-activated cell sorting analysis and summarized in the table.
(**E**) HeLa cells were transfected with additional TopBP1 siRNAs as indicated. Cell lysates were prepared and immunoblotted with indicated antibodies.
(**F**) Mapping the MIB1-binding domain on BLM. Cell lysates were prepared from 293T cells transfected with constructs encoding SFB-tagged wild-type BLM or various BLM deletion mutants. Beads coated with GST-MIB1 fusion protein were incubated with cell lysates and immunoblotting experiments were carried out using indicated antibodies.

(**G**) GST, GST-MIB1, MBP-BLM and MBP-BLM-D4 were expressed and purified from *E. Coli*. GST pull-down assay were performed and blotted with antibodies as indicated.
(**H**) 293T cells were co-transfected with constructs encoding SFB-tagged MIB1 or SFB-vector together with constructs encoding Myc-tagged BLM or Myc-tagged BLM K3A mutant. Co-precipitation assays were performed to confirm the association of MIB1 with the K3A mutant of BLM.

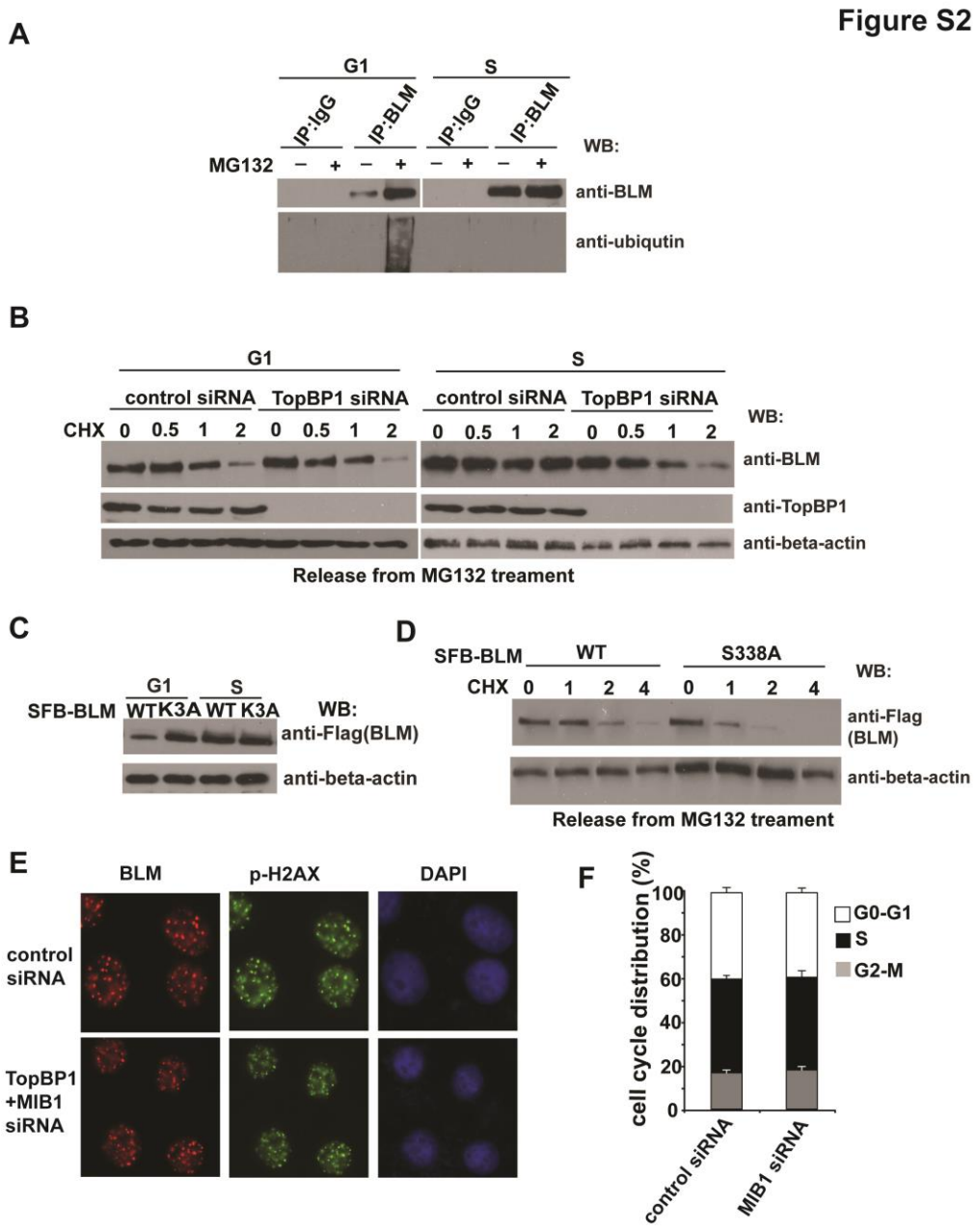

Figure S2, related to Figure 5.
(**A**) Endogenous BLM was ubiquitinated *in vivo*. HeLa cells were synchronized in G1 or S phase and treated with MG132 for 2 hours. Cell lysates were incubated with IgG or

BLM antibody for 12 hours and protein A beads for 1 hour. The immunocomplexes were washed and Western blotting was conducted with anti-BLM or anti-Ubiquitin antibodies.
(**B**) TopBP1 modulates the half-life of BLM in S phase cells, but not in G1 cells. HeLa cells were synchronized in G1 or S phase as described in Materials and Methods. HeLa cells were treated by cycloheximide (CHX) at a concentration (10 μg/ml) for indicated time. To ensure that BLM protein levels were about the same in G1 and S phase cells, cells were pre-treated with MG132 for 2 hours before cycloheximide treatment.
(**C**) HeLa cells stably expressing wild-type BLM and K3A mutant BLM were synchronized in G1 or S phases. BLM protein level was determined by Western blotting.
(D) The half-life of S338A mutant of BLM is shorter than that of wild-type BLM. HeLa cells stably expressing wild-type or the S338A mutant of BLM were treated with MG132 for 2 hours and released in fresh medium contain 10 μg/ml cycloheximide (CHX) for indicated time. Western blotting was carried out using indicated antibodies.
(**E**) Control or TopBP1/MIB1 doubly depleted cells were collected 6 hours after 10 Gy of IR treatment. BLM and γH2AX foci formation were determined by immunostaining using indicated antibodies.
(**F**) HeLa cells and MIB1-depleted HeLa cells were collected. Their cell cycle distributions were determined by FACS analysis and summarized here. The results were the average of three independent experiments and presented as mean ± SD.

Figure S3

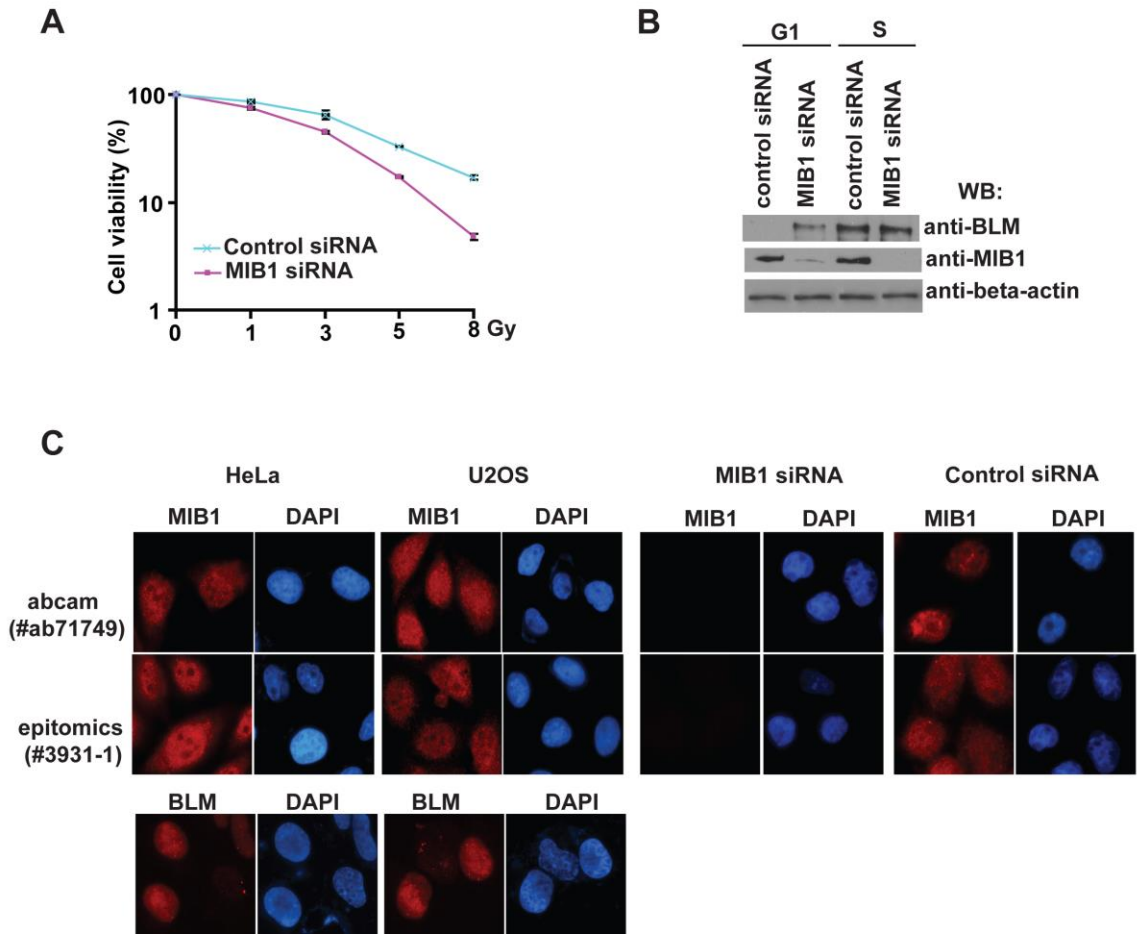

Figure S3, related to Figure 4 and 5.

(**A**) HeLa cells and MIB1-depleted HeLa cells were irradiated by the indicated dose of X-ray. Cell survival following irradiation was measured by clonogenic assay.
(**B**) Control and MIB1-depleted HeLa cells were synchronized in G1 or S phases. BLM protein level was determined by Western blotting.
(**C**) HeLa and U2OS cells were fixed and stained with indicated antibodies. siRNA mediated knock down were performed in HeLa cells. The cells were fixed and stained by two different MIB1 antibodies. BLM staining was also included as a control.