Inventory of Supplemental Information

Supplemental Fig.S1 is related to Figure 1, which further confirmed that the very C-terminal tandem BRCT domains of TopBP1 are required for its interaction with BACH1.

Supplemental Fig.S2 is related to Figure 2, which described that TopBP1-BRCT7&8 specifically binds to pT1133 of BACH1.

Supplemental Fig.S3 is related to Figure 3, which further demonstrated a functional link between BACH1 and TopBP1 on the focus formation of RPA1, TopBP1 or BACH1 following replication stress by performing damage-induced foci formation assay.

Supplemental Fig.S4 is related to Figure 4, which indicates that TopBP1 and BACH1 are positioned at early stage of replication checkpoint control.

BACH1/FANCJ acts with TopBP1 and participates early in DNA replication checkpoint control

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Supplemental Data

Supplemental Fig.S1.

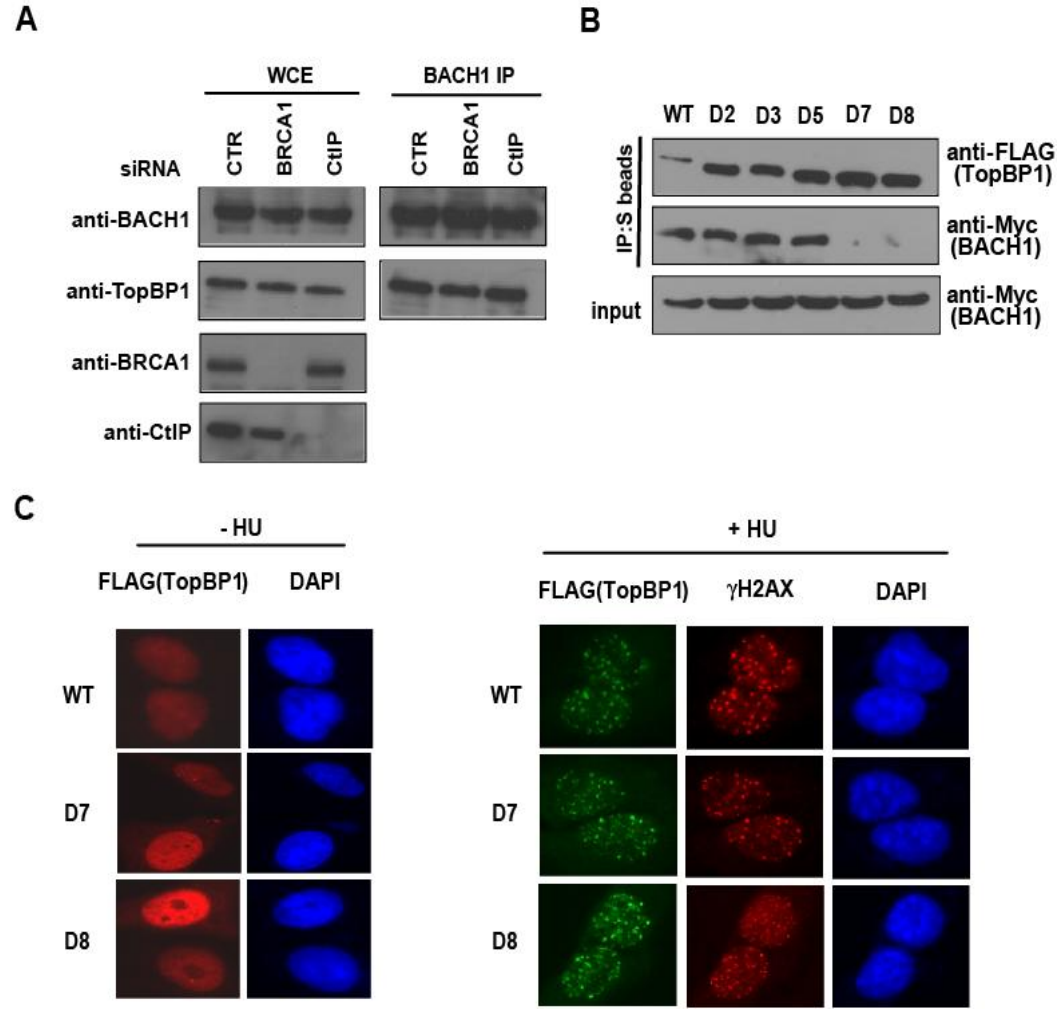

Figure S1. (**A**) The interaction between TopBP1 and BACH1 can take place independently of other proteins such as BRCA1 or CtIP. HeLa cells were transfected with

control siRNA (siCTR), BRCA1 specific siRNAs or CtIP specific siRNAs. Cell lysates were prepared and immunoprecipitation and immunoblotting experiments were performed using antibodies as indicated. (**B**) Mapping of the BACH1-binding regions on TopBP1. Lysates were prepared from cells expressing Myc-tagged BACH1 along with SFB-tagged wild type or deletion mutants of TopBP1. Immunoprecipitation reactions were performed using S-protein beads and then subjected to Western blot analyses using antibodies as indicated. (**C**) Localization of TopBP1 and its deletion mutants. U2OS cells were transfected with plasmids encoding SFB-tagged wild-type or deletion mutants of TopBP1. Immunostaining was carried out with anti-FLAG and anti-γH2AX antibodies 20 hours after cells were treated without or with HU (2mM).

Supplemental Fig.S2.

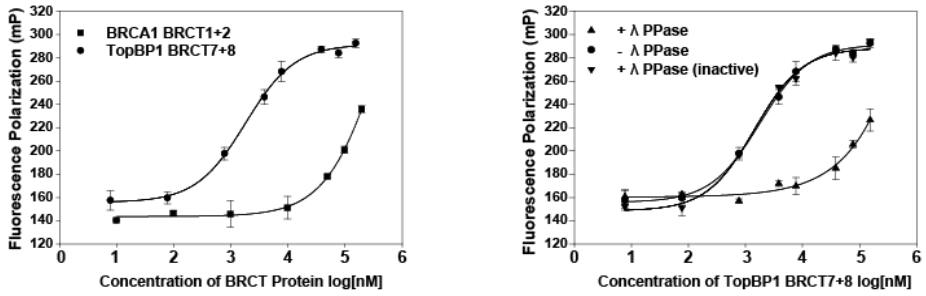

Figure S2. TopBP1-BRCT7&8 specifically binds to pT1133 of BACH1. Fluorescence polarization assay of FITC-labeled BACH1 pThr^{1133}-peptide with BRCA1 BRCT1+2 and TopBP1 BRCT7+8 shows specificity for TopBP1 BRCT7+8. Treatment of the FITC-labeled BACH1 pThr^{1133}-peptide with λ protein phosphatase (λ PPase) markedly reduces its binding to TopBP1 BRCT7+8. Quantification results represent the average of triplicate runs and were presented as mean ± SD.

Supplemental Fig.S3.

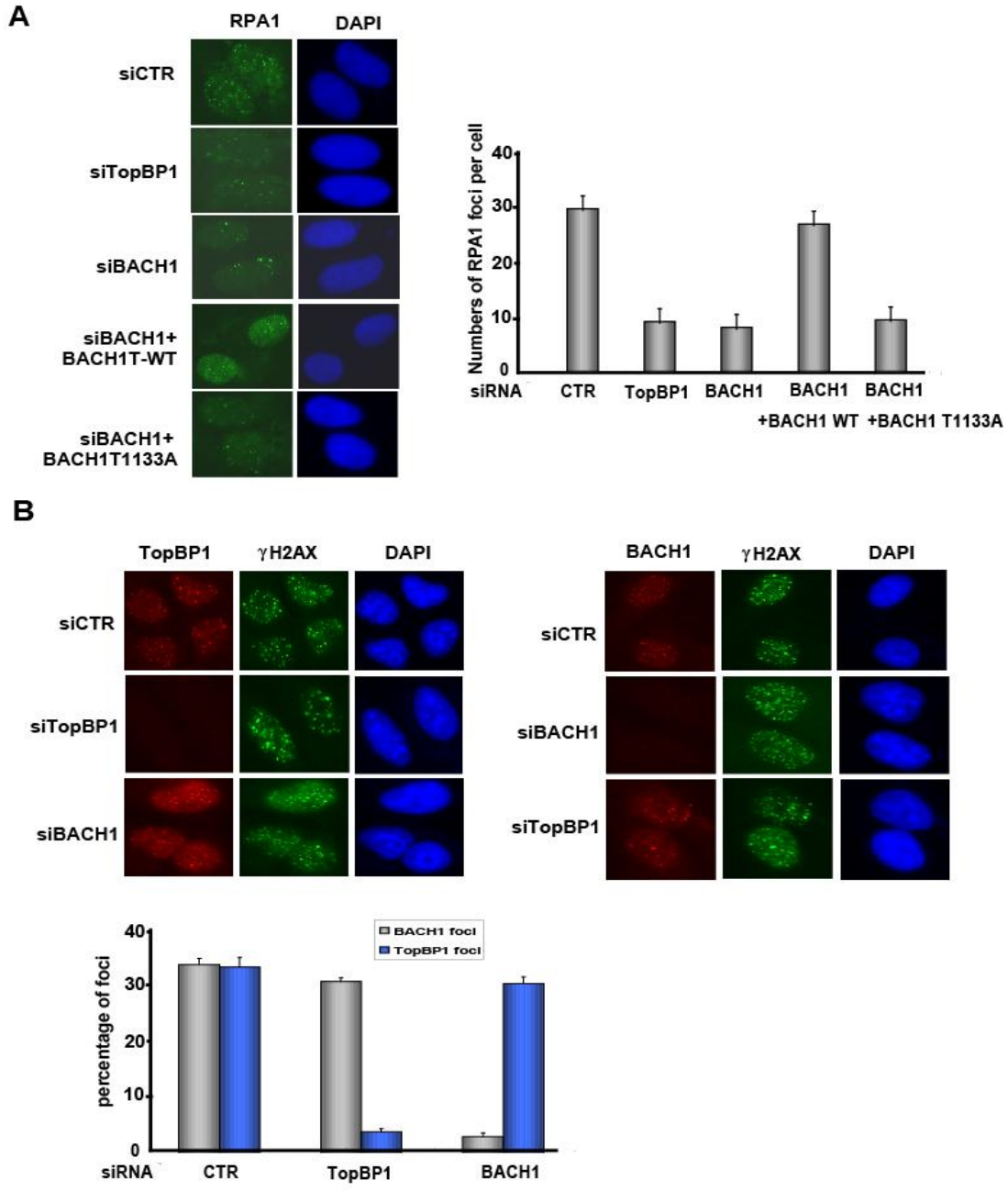

Figure S3. (**A**) The number of HU-induced RPA1 foci reduced dramatically in BACH1 or TopBP1 depleted cells. (Left panel) U2OS cells or U2OS cells stably expressing siRNA resistant WT or T1133A mutant of BACH1 were transfected with control siRNA (siCTR), BACH1 or TopBP1 specific siRNAs. Cells were treated with HU, fixed and immunostained with anti-RPA1 antibody. (Right panel) The numbers of RPA1 foci in

each RPA1 containing cells was counted. Quantification results represent the average of three independent experiments and were presented as mean ± SD. (**B**) Depletion of TopBP1 does not affect the focus formation of BACH1 at stalled replication forks and *vice versa*. (Upper panel) U2OS cells were transfected with control siRNA (siCTR), BACH1 or TopBP1 specific siRNAs. Cells were treated with HU, fixed and immunostained with anti-BACH1 or anti-TopBP1 antibodies together with anti-γH2AX antibodies. (Lower panel) Percentages of cells stained positive for TopBP1 or BACH1 foci were determined. The results were the average of three independent experiments and were presented as mean ± SD.

Supplemental Fig.S4.

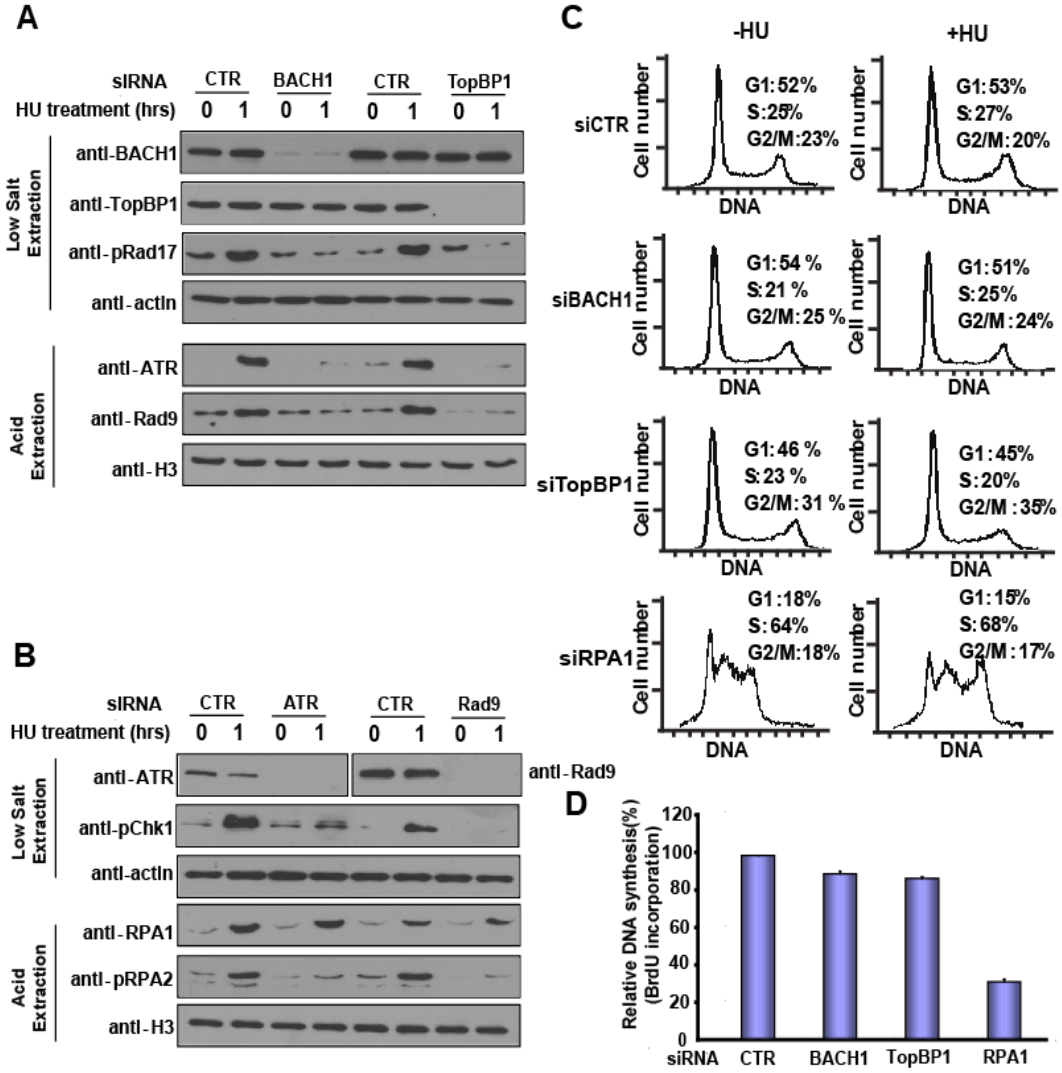

Figure S4. (**A**) TopBP1 and BACH1 are involved in the chromatin loading Rad9 or ATR following replication stress. U2OS cells transfected with control, TopBP1 or BACH1 siRNAs were mock treated or treated with HU (10 mM). Cells were harvested at the indicated timepoints. The soluble and chromatin fractions were prepared and immunoblotted with antibodies as indicated. (**B**) Neither ATR nor Rad9 participates in RPA loading following HU treatment. U2OS cells transfected with control, ATR or Rad9 siRNAs were mock treated or treated with HU (10 mM) and collected one hour later. The soluble and chromatin fractions were prepared and immunoblotted with antibodies as indicated. (**C**) Depletion of TopBP1 or BACH1 does not drastically affect cell cycle distributions or DNA synthesis with or without HU treatment. (**A**) Depletion of TopBP1 or BACH1 does not alter cell cycle distribution. HeLa cells were transfected with control, TopBP1, BACH1 or RPA1 specific siRNAs and then treated with HU (10 mM) for 1 hour. Samples were taken at the indicated time points and analyzed by FACS. (**D**) Depletion of TopBP1 or BACH1 does not noticeably affect DNA synthesis. HeLa cells were transfected with control, TopBP1, BACH1 or RPA1 specific siRNAs. Cells were incubated with 10 μM BrdU for 10 minutes, and subsequently trypsinized and fixed at -20°C. Cells were incubated with mouse monoclonal anti-BrdU antibody (Roche) and BrdU incorporation was analyzed by FACS. To calculate relative rate of DNA synthesis, BrdU-positive cells were gated, and the number of cells was multiplied by their mean BrdU signal intensity. The value obtained from cells with depletion of TopBP1, BACH1 and RPA1 was then divided by the value obtained from cells transfected with control siRNA (which was set as 100%) to obtain relative value of ongoing DNA synthesis. Quantification results represent the average of three independent experiments and were presented as mean ± SD.

Supplemental Experimental Procedures

Mitotic spreads

HeLa cells were transfected with control siRNAs or siRNAs against human TopBP1 or BACH1. 48 hours after the first transfection, 2 mM HU plus nocodazole (200 ng/ml) were added. Cells were harvested for chromosome preparation using standard protocol 6 to 8 hours following Colcemid treatment (50 ng/ml). Cells were incubated for 20 minutes in 0.075 M KCl at 37°C and then fixed by multiple changes of Carnoy fixative (3:1 methanol:acetic acid). Cells were dropped onto slides and stained with Giemsa. Premature chromosome condensation was scored as previously described (Nghiem et al., 2001).

Tandem affinity purification

293T cells stably expressing SFB-TopBP1 were used for tandem affinity purification. The SFB-TopBP1 stable cells were lysed with NETN buffer (see above) on ice for 20 minutes. After removal of cell debris by centrifugation, crude lysates were incubated with streptavidin sepharose beads for 1 hour at 4°C. The bead-bound proteins were washed three times with NETN buffer and eluted with 2 mg/ml biotin (Sigma) for 1 hour twice at 4°C. The eluates were combined and then incubated with S-protein agarose (Novagen) for 1 hour at 4°C. Beads were washed three times with NETN buffer. The proteins bound to S-protein agarose beads were separated by SDS-PAGE and visualized by Coommasie Blue staining. The identities of eluted proteins were revealed by mass spectrometry analysis, performed by the Taplin Biological Mass Spectrometry Facility (Harvard University).

Immunofluorescence staining

Cells grown on coverslips were mock treated or treated with HU (2mM) for 20 hours. Cells were fixed in 3% paraformaldehyde/2% sucrose solution for 10 minutes, and then permeabilized in 0.5% Triton X-100 containing solution for 5 minutes on ice. Cells were incubated with primary antibodies diluted in 5% goat serum at 37^{o}C for 30 minutes.

After washing with PBS twice, cells were incubated either with FITC conjugated or rhodamine conjugated secondary antibodies for 30 minutes at 37^{o}C. Nuclei were counterstained with DAPI, and then mounted onto glass slides with anti-Fade solution. Images were taken with a Nikon Eclipse E800 fluorescence microscope.

Fluorescence Activated Cell Sorting (FACS)

For cell cycle analysis, cells were washed twice with PBS, resuspended in 300 μl of PBS and then fixed with the addition of 700 μl of 100% ethanol. After stored at -20°C overnight, fixed cells were washed and incubated in sodium citrate buffer containing RNAse A for 30 minutes, and then stained with propidium idodide for 30 minutes. Cells were then run on a FACScan and cell cycle analysis was performed.

GST pull-down assay

GST fusion proteins were expressed in *Escherichia coli* and purified as previously described. GST fusion proteins were immobilized on glutathione-Sepharose 4B beads and incubated with lysates prepared from cells transiently transfected with plasmids encoding the indicated proteins. The samples were subjected to SDS-PAGE and analyzed by Western blotting.

Fluorescence Polarization

FP measurements were carried out in a 384-well OptiPlate (Perkin Elmer) using an Envision multilabel plate reader (Perkin Elmer). Polarization values were measured at an excitation wavelength of 480 nm and emission wavelength of 535 nm. Each well consisted of 0.5 μL of 400 nM FITC-labeled BACH1 phospho-peptide (FITC-ESIYF-(phospho-T)-PELYDPEDT-NH_2) and 19.5 μL of BRCT domain protein in assay buffer (10 mM Tris-HCl pH 7.5, 400 mM NaCl and 1mM TCEP). Binding assays were incubated for 15 min at RT prior to taking FP measurements. Data points were obtained from triplicate runs. For λ PPase treatment, 400 units of λ PPase (New England Biolabs) were used to dephosphorylate 200 pmol of FITC-BACH1 phospho-peptide. Inactive λ PPase was heat inactivated at 65°C in 50 mM Na_2EDTA prior to incubation with FITC-phospho-peptide. Both data fitting and K_d values were calculated using SigmaPlot 10.