Supporting Information

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SI Materials and Methods

Cell Culture and Transfection. A549, HEK293T, MCF-7, U2-OS, and HCT116 cells were cultured in DMEM with 10% (vol/vol) FBS. Transfection was conducted with Lipofectamine 2000 (Life Technologies) or X-tremeGENE (Roche) according to the manufacturer's protocols.

Plasmid Construction. To generate GFP-tagged 53BP1 WT or the S28A mutant, we used PCR to amplify the full-length human 53BP1 (WT or the S28A mutant) using the pLPC-Puro-Myc-53BP1 WT (Addgene) and the pFlag-53BP1 S28A (a gift from Daniel Durocher, Lunenfeld-Tanenbaum Research Institute, Toronto) as the template, respectively. Forward and reverse primers are: GTAGACTTCTCGAGCTATGGACCCTACTGGAAGTCAGTTG (forward) and CGCCGCGGTACCTTAGTGAGAAACATAATCGTGT (reverse). The PCR products were inserted into the XhoI/KpnI site of the pEGFP-C1 vector. To construct the Flag-53BP1 WT or S28A mutant, we performed PCR using the same template and the reverse primer; however, the forward primer was CCGCGCCGGCTAGCATGGATTACAAGGATGACGACGATAAGGACTATAAGGACGATGATGACAAGATGGACCCTACTGGAAGTCAGTTGGATTCAG with two Flag tags. The PCR products were inserted into the NheI/KpnI site of the pcDNA3.1(-) plasmid.

To generate Flag-UbcH7 WT, we used PCR to amplify the UbcH7 full-length using the HA-Ube2l3 (Addgene) as the template. Primers are: GCCGGATCCATGGCGGCCAGCAGGAG (forward) and CGGGAATTCTTAGTCCACAGGTCGCTTTTC (reverse). The PCR product was cloned into the BamHI/EcoRI site of the pCMV-3xFlag vector. To mutate the C86 to S, Quick Change mutagenesis (Strategene) was used. The mutation primers are CACTAATTACTGGCAGACTGACCTG and AAGGGGCAGGTCAGTCTGCCAG. To generate RNAi-resistant UbcH7 plasmids, the RNAi targeting site in the UbcH7 coding region of the Flag-UbcH7 WT or C86S mutant were mutated with the Quick Change mutagenesis kit using the following primers: CTTTCCAGCGGAATATCCGTTTAAGCCACCGAAGATCACATT and TCTTCGGTGGCTTAAACGGATATTCCGCTGGAAAGTTGATTTCG.

shRNA Screening. Lentiviral shRNA vectors targeting human E2 and E3 enzymes were purchased from Sigma. To produce virus, HEK293T cells grown in 48-well plates were transfected with shRNA vectors for control or Ub genes and packaging vectors for 2 d. The media that contained virus were used to infect the targeting A549 cells in 48-well plates for 2 d, reseeded into 6-well plates, and incubated in the presence of 1.5 μg/mL puromycin for 10 d. Stable cell pools were collected and used to perform experiments. To assess the DDR, we treated A549 control or Ub gene-depleted stable cells with 2 mM HU for 4 h, collected cell lysates, and loaded equal amount of total proteins onto SDS/PAGE. The membrane was immunoblotted with the specific anti–pS345-Chk1 antibodies, then stripped and reprobed with the anti-Chk1 antibodies. The protein band intensities of both Chk1 and pS345-Chk1 were analyzed using the NIH ImageJ software. The intensity of phosph-Chk1 or total Chk1 in nontreated control cells was set as 1 and the relative intensities of Chk1 and phospho-Chk1 in Ub gene-targeted cell lines were obtained by normalizing to the nontreated control. Finally, the relative phospho-Chk1 intensity was adjusted by the relative intensity of total Chk1. We repeated the screening process two times, and potential positive hits were further screened for at least two more times.

DSB Repair Assays. To measure NHEJ, the pEGFP-C1 vector was cut with the unique restriction enzyme, Bsp1407I, which disrupts the coding region of GFP, and the linearized DNA was gel-purified. A549 control or UbcH7-depleted cells grown in 100-mm dishes were infected or not with sh53BP1 for 24 h, transfected with the cut pEGFP-C1 and pcDNA3.1-mCherry (at a 4:1 ratio) for another 48–72 h. The GFP^+ and $mCherry^+$ cell population was quantitated using FACS analysis and the NHEJ rate is represented as GFP^+/mCherry/4. The NHEJ rate in control cells without sh53BP1 was set as 1.0, and other samples displayed corresponding relative NHEJ rate.

For HR assay, we used the well-established U2-OS DR reporter cell line, which relies on HR to repair DSBs generated by a rare-cutting enzyme, I-SceI, to reconstitute a functional GFP gene (1). In brief, U2-OS DR cells grown in 100-mm dishes were infected with shRNA lentivirus for control or UbcH7 in the presence of absence of sh53BP1 for 24 h, transfected with pCBA-HA-I-SceI vector and pcDNA3.1-mCherry at 4:1 ratio for another 48 h. GFP^+ and $mCherry^+$ cell populations were quantitated and the HR rate is represented as GFP^+/$mCherry^+$/4. The HR rate in control cells without sh53BP1 was set as 1.0, and other samples exhibited relative HR rate.

In Vivo Ubiquitination of 53BP1. A549 control or UbcH7-depleted cells grown in 100-mm dishes were treated with 300 μM CHX in the presence or absence of 25 μM MG132 for 6 h. Cells were harvested by trypsinization followed by wash with PBS once. About 15% (vol/vol) of cells were directly lysed in lysis buffer (50 mM Tris·HCl, pH 7.6, 150 mM NaCl, 10 mM NaF, 1 mM Na3VO4, 1 mM PMSF, 1 mM DTT, 10 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin A, and 1.0% Nonidet P-40) for WCE analysis, and the remaining cells were resuspended in 150 mL PBS in 1.5-mL Eppendorf tube. While vortexing, 8 μL of hot 20% (vol/vol) SDS was added into cell suspension and vortexed hard for 30 s. The cell solution was boiled in heat block until becoming nonviscous. Then 1,350 μL of 1% Triton X-100 lysis buffer was added into each sample followed by vortex and sonication. The supernatant was collected by centrifugation at 15,700 × *g* for 15 min at 4 °C. Equal amount of total proteins were used to perform immunoprecipitation by adding anti-53BP1 antibodies and rotate at 4 °C overnight. In each sample, 30 μL of Protein A/G (1:1) beads was added and kept rotating at 4 °C for 3 h. The beads were collected by centrifugation and washed three times with lysis buffer, resuspended in 30 μL of 2× sample buffer, and loaded onto 6–12.5% (wt/vol) SDS/PAGE gel. The membrane was probed with anti-Ub antibodies and stripped and reprobed with anti-53BP1 antibodies.

Immunoblotting and Antibodies. Immunoblotting was carried out as previously described (2, 3). Anti-Chk1 (DCS-1310), anti-Rad17, anti-Cdc25A (F-6), and anti-ATR (N-19) antibodies were from Santa Cruz Biotechnology. Antiphospho-S345-Chk1 and anti-RPA (4E4) were from Cell Signaling. Anti-MCM7 was from BD Pharminogen. Anti-Ligase IV and anti-PCNA were from Abcam. Anti-Flag (M2) and anti-Ub sera were from Sigma. Anti-UbcH7 (A300-737A) and anti-Rif1 (A300-569A) were from Bethyl Laboratories. Anti-HA (rat, 3F10) was from Roche. Anti-53BP1 (NB100-304) was from Novus Biologicals.

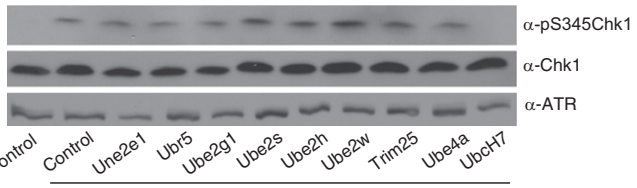
Long-Term Cell Survival Assay. A549 control or UbcH7-depleted cells were treated with different doses of CPT, IR, or UV. After 4 h, 10,000 viable cells from each treatment were seeded into six-well plates in triplicate, and were left growing for 7 d. The cells were washed with Hank's balanced salt solution, stained with 0.2% Crystal violet in 50% (vol/vol) methanol for 30 min at room temperature, and then washed extensively with water. A picture of each plate was taken and viable cells were analyzed using the ImageJ software as well as solubilization of the viable colonies.

Immunofluorescence Staining. A549 control or UbcH7-depleted cells grown on glass coverslips were treated with 5 Gy IR and fixed in 3.7% (vol/vol) formaldehyde for 15 min. Cells are permeabilized and blocked with PBS containing 10% (vol/vol) FBS in 0.4% Triton X-100 for 20 min at room temperature, washed three times with washing buffer (PBS containing 0.2% Triton-100, 0.1% BSA) and then incubated with primary antibodies in PBS containing 0.1% Triton X-100 at 4 °C overnight (dilutions: anti-RPA, 1:100; anti-UbcH7, 1:100; anti-53BP1, 1:200; anti-Ligase IV, 1:50). The samples were washed three times with washing buffer, and incubated with secondary antibodies (Alexa Fluor 594, Life Technologies; 1:200) in PBS containing 0.1% Triton X-100 for 1 h at room temperature, followed by washing three with washing buffer. The slides were mounted with antifade solution (Life Technologies) and visualized using a Leica laser microscope.

qPCR. Total RNA was extracted using the TRIZOL reagent (Life Technologies). RNA was treated with DNase (Life Technologies) and reverse-transcribed into cDNA with the SuperScript III First Strand Synthesis system (Life Technologies). Quantitative RT-PCR was performed using SYBR Green master mix-based platform (Roche Applied Science). β-Actin was used as an internal control, and relative gene expression was calculated as $2^{-\Delta\Delta Ct}$. Primers used are: β-actin-forward: CATGGGCCAGAAGGACTGCT; β-Actin-reverse: CACACGGAGCTCATTGTAGA. 53BP1-forward: CTCCTCCACATGGCCATGTCTTAC. 53BP1-reverse: CCTGAGGAGCCCCCAGTCTGT.

1. Pierce AJ, Johnson RD, Thompson LH, Jasin M (1999) XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes Dev* 13(20):2633–2638.
2. Wang J, Han X, Feng X, Wang Z, Zhang Y (2012) Coupling cellular localization and function of checkpoint kinase 1 (Chk1) in checkpoints and cell viability. *J Biol Chem* 287(30):25501–25509.
3. Wang J, Han X, Zhang Y (2012) Autoregulatory mechanisms of phosphorylation of checkpoint kinase 1. *Cancer Res* 72(15):3786–3794.

A



B

	pChk1 down		pChk1 down		pChk1 down
UbcH7	+++	Trim13	-	Brap	-
Ube2e1	-	Trim21	-	Kcmf1	-
Ube2g1	-	Trim24	-	RNF20	-
Ube2k	+	Trim25	-	RNF8	++
Ube4b	+	Pdzrn3	-	RNF168	++
Ube4a	-	Cbl	+	FEM1B	-
Ube2w	+	Birc2	-	Rchy1	-
Ube2s	-	Ttc3	+	RFWD2	+
Ube2g2	-	Traf5	-	RFWD3	-
Ub2eq1	-	PML	-	AMF1R	-
Ube2l	-	Irak1	-	Ubr5	-
Ube2m	-	Cul1	+	Cdc34	-
Ube3c	+	Cdc20	+	KLHL20	-
Ube2h	+	G2E3	-	Birc3	+
Ube2r2	+	Birc6	-	BAHD1	-
Ube2e2	-	FZR1	-	Stub1	-
Ube2o	-	MACT1	-	TOPOROS	-
Ube2d1	-	WWP1	-	MBI1	-
Ube2w	-	WWP2	-	CHFR	+
Ube2n	-	Ube2t	-		
Ube2q2	-	Ube2z	-		

Fig. S1. Screening of Ub genes in DNA damage-induced Chk1 phosphorylation. (*A*) Representative results of CPT-induced Chk1 phosphorylation in A549 cells stably infected with shRNAs targeting Ub genes. (*B*) Summary of effects of knockdown of E2s and known E3s on Chk1 phosphorylation. The protein band intensities of the phospho-Chk1 and total Chk1 blots in *A* were analyzed by the ImageJ software and the relative intensity of phospho-Chk1 was normalized to that of the total Chk1 in the corresponding sample. Using UbcH7 as a reference, the inhibition in Chk1 phosphorylation by different Ub shRNAs was categorized as no effect (–), weak (+), moderate (++), or strong (+++).

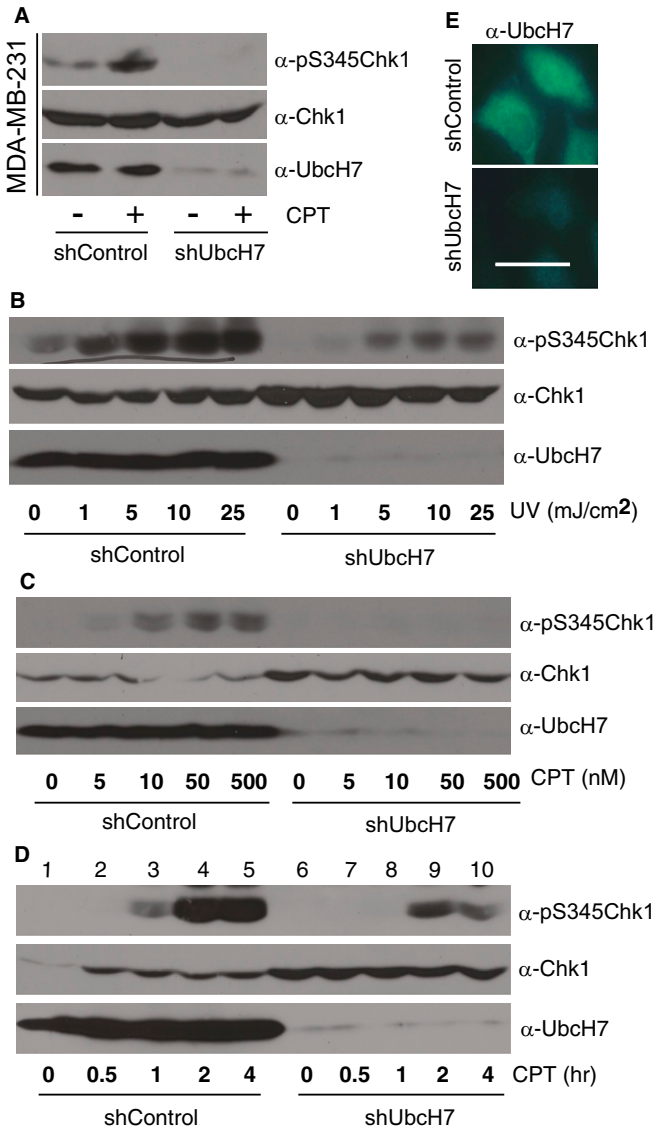


Fig. S2. UbcH7-dependent Chk1 phosphorylation defects. (*A*) MDA-MB-231 cells were infected with lentivirus for control or UbcH7, treated with 500 nM CPT for 4 h, and protein expression was examined. A549 control or UbcH7-depleted cells were treated with increasing doses of UV light (*B*) or CPT (*C*) for 4 h, or 500 nM CPT for indicated time points (*D*) and immunoblotted as in *A*. (*E*) A549 control or UbcH7-depleted cells were grown on glass coverslips, fixed, and immunostained with anti-UbcH7 antibodies. (Scale bar, 10 μm.)

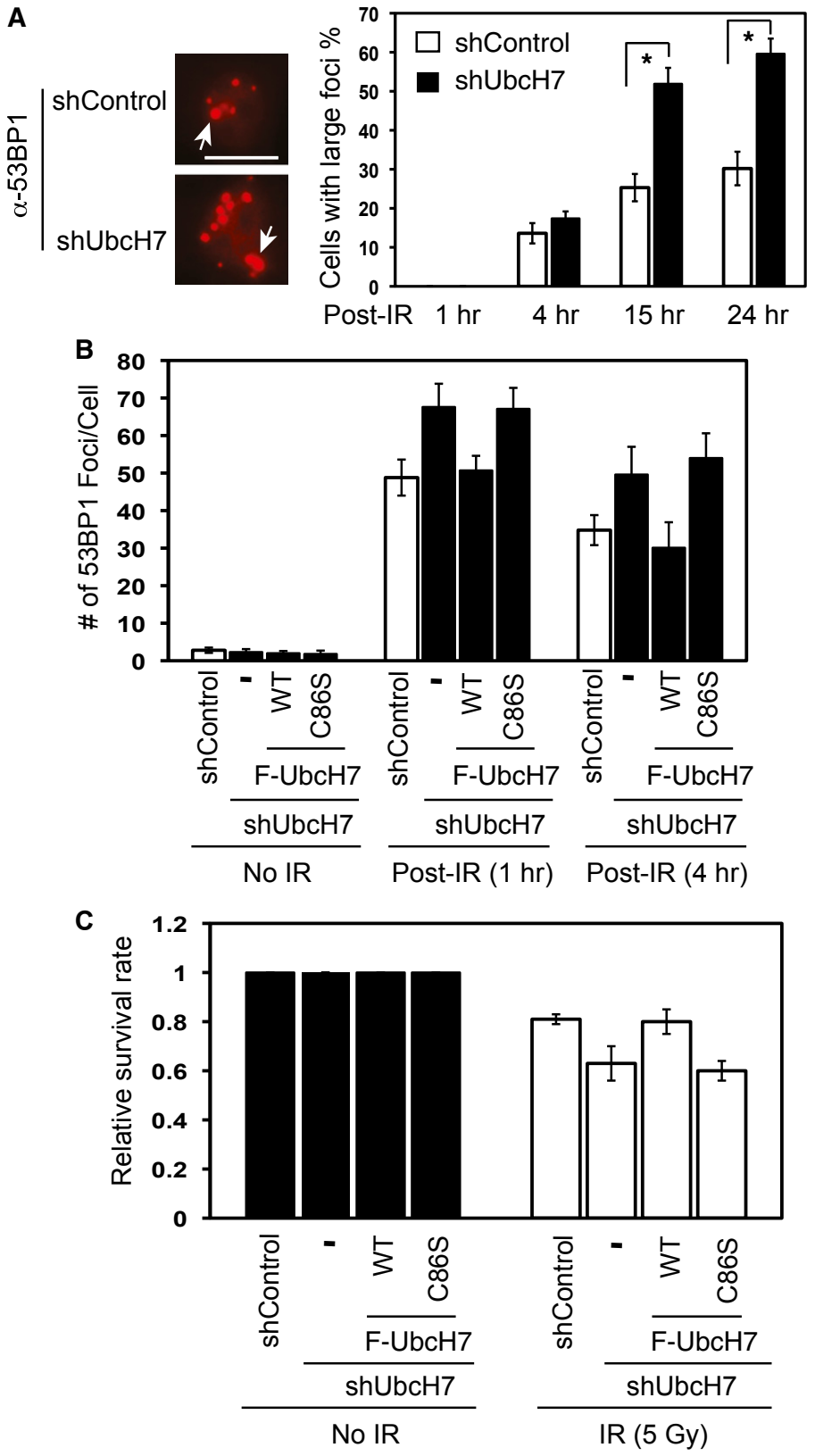


Fig. S3. UbcH7 regulates 53BP1 foci formation and cell sensitivity to DNA damage. (A, *Left*) Representative images of 53BP1 foci in A549 control or UbcH7 depleted cells. Arrow indicates large 53BP1 foci. (Scale bar: 10 μm.) (*Right*) Percentage of cells with large 53BP1 foci after IR. Data represent mean and SD from >50 cells. *$P < 0.05$. (*B*) HEK293T cells grown on glass coverslips were infected with shRNA control or UbcH7 for 24 h, transfected with RNAi-resistant Flag-UbcH7 WT or C86S mutant for an additional 48 h, irradiated with 5 Gy IR and fixed 1 or 4 h post-IR. Cells were stained with anti-53BP1 and anti-Flag antibodies. 53BP1 foci in $Flag^+$ cells were counted. Data represent mean and SD from >50 cells. (*C*) Cells from *B* after IR treatment were replated in six-well plates (10,000 cells per well) in triplicate and grown for 7 d. The viable cell colonies were stained and quantitated as described in *SI Materials and Methods*. Those in nontreated group was set as 1.0, and relative survival rate was normalized within each group.

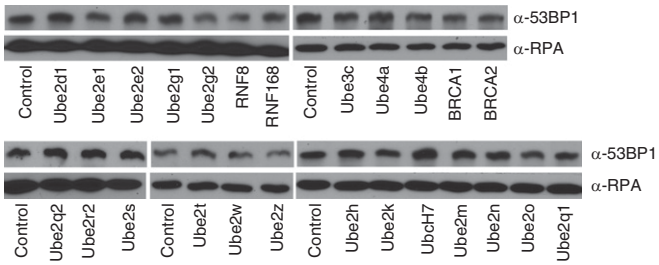


Fig. S4. Effects of E2s on the protein level of 53BP1. A549 control or Ub gene-depleted cells were analyzed with indicated antibodies. UbcH7 was the one whose depletion repeatedly increased the level of 53BP1.

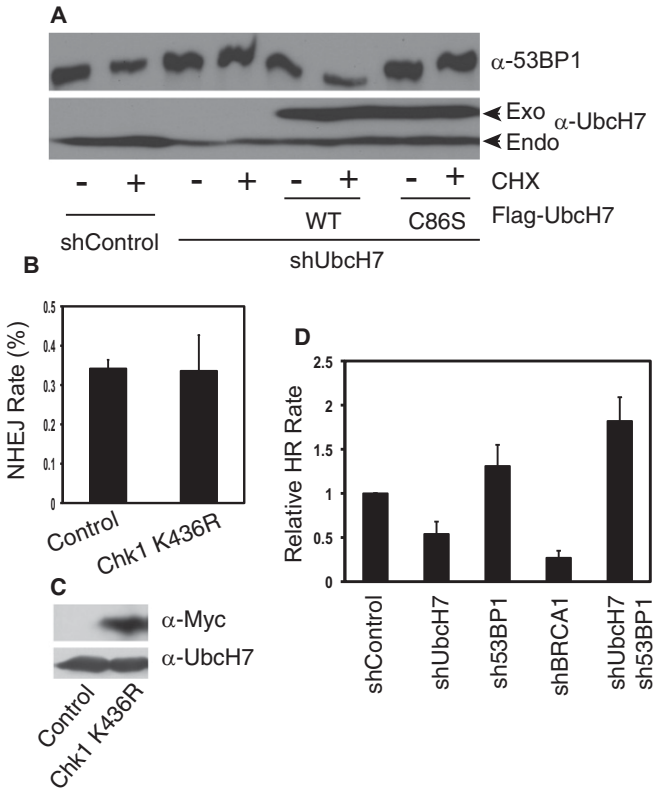


Fig. S5. Effects of UbcH7 on DSB repair. (*A*) HEK293T cells were infected with shRNA control or UbcH7 for 24 h, transfected with RNAi-resistant Flag-UbcH7 WT or C86S mutant for an additional 48 h, treated with 320 μM CHX for 6 h, and immunoblotted with indicated antibodies. (*B*) HEK293T cells were transfected with control or Myc-Chk1 K436R together with the linearized pEGFP and pcDNA3-mCherry for 48 h, and NHEJ was analyzed as described in *SI Materials and Methods*. Data represent mean and SD from three experiments. (*C*) Expression of Myc-Chk1 mutant from cells in *B*. (*D*) U2-OS DR cells were infected with indicated shRNAs for 24 h, transfected with pCBA-HA-I-SceI vector and pcDNA3.1-mCherry at 4:1 ratio for another 48 h, and HR was measured as described in *SI Materials and Methods*.

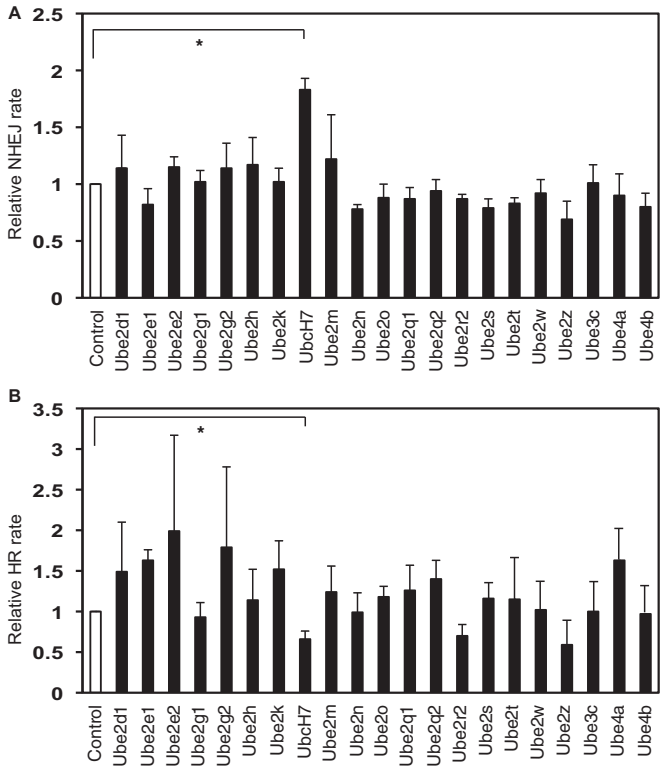


Fig. S6. (*A* and *B*) Effects of E2s on NHEJ or HR. Normal U2-OS or the U2-OS DR cells were infected with the indicated Ub shRNA lentiviral vectors and NHEJ and HR assays were carried out as in *SI Materials and Methods*. *$P < 0.05$.

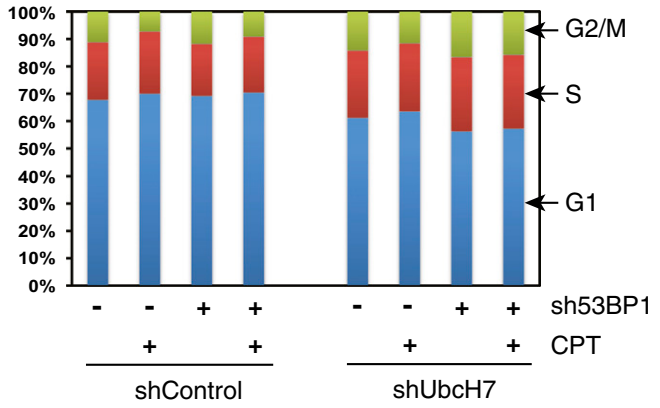


Fig. S7. A549 control or UbcH7-depleted cells were infected with sh53BP1 or not for 72 h, treated with 500 nM CPT for 6 h, and cells were collected for FACS analysis.

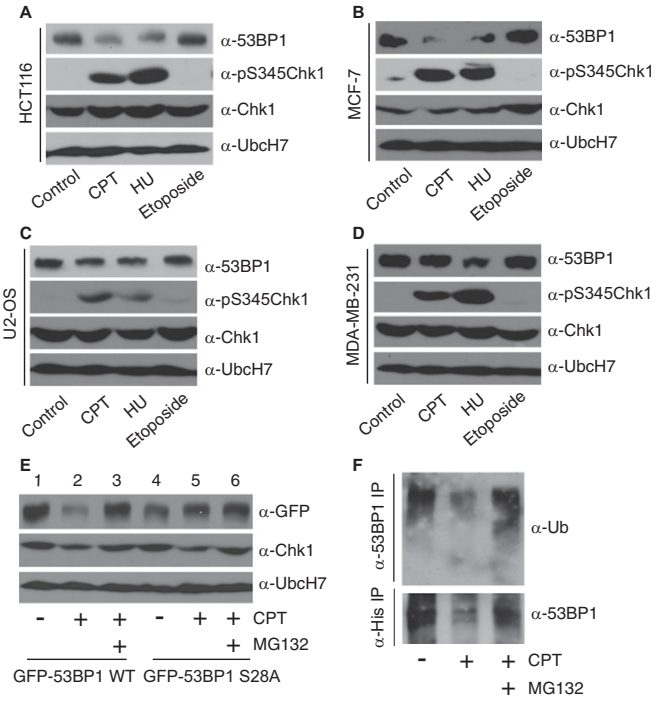


Fig. S8. Replicative stress-induced degradation of 53BP1. HCT-116 (*A*), MCF-7 (*B*), U2-OS (*C*), or MDA-MB-231 (*D*) cells were treated with 500 nM CPT, 2 mM HU, or 500 nM ETO for 4 h, and protein expression was analyzed. (*E*) HEK293T cells were transfected with GFP-53BP1 WT or the S28A mutant for 48 h, treated with 500 nM CPT with or without 25 μM MG132 for 6 h, and protein expression was analyzed with indicated antibodies. (*F*) HEK293T cells were transfected with His-Ub and GFP-53BP1 WT for 48 h, lysed in 1% hot SDS, boiled, and vortexed until completely nonviscous. The lysates were then diluted to 0.1% SDS with lysis buffer and split into pull down by anti-His and anti-53BP1 antibodies, followed by immunoblotting with anti-53BP1 and anti-His antibodies, respectively.

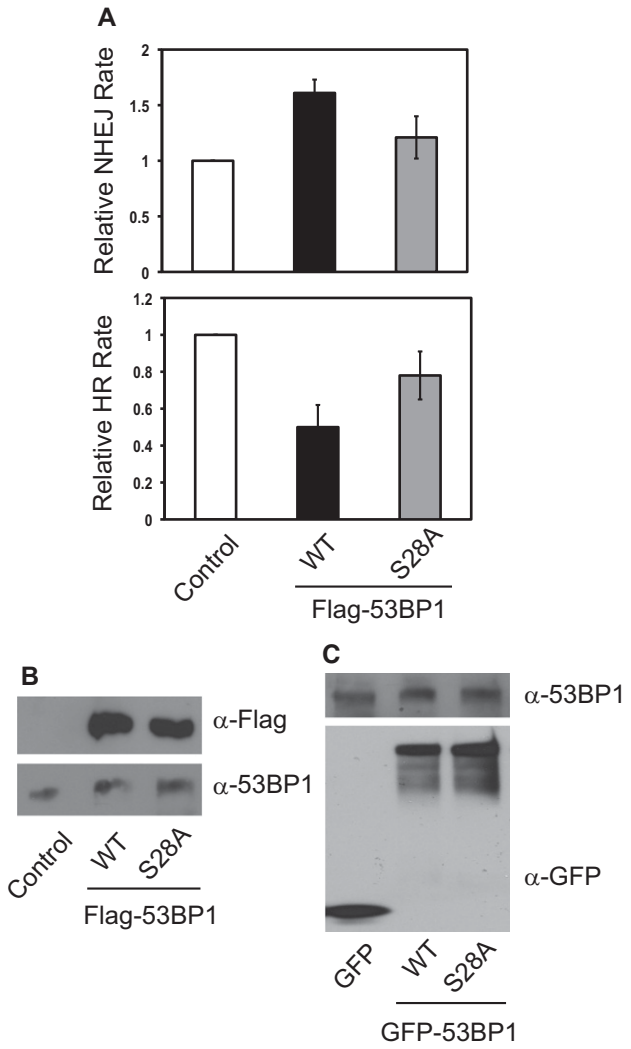


Fig. S9. Effects of 53BP1 expression on DSB repair. (*A*) Normal U2-OS or the U2-OS DR cells were transfected with Flag-53BP1 WT or the S28A mutant and NHEJ and HR assays were carried out as described in *Materials and Methods*. (*B*) Protein expression from samples in *A*. (*C*) Protein expression of 53BP1 from samples in Fig. 6*D*.

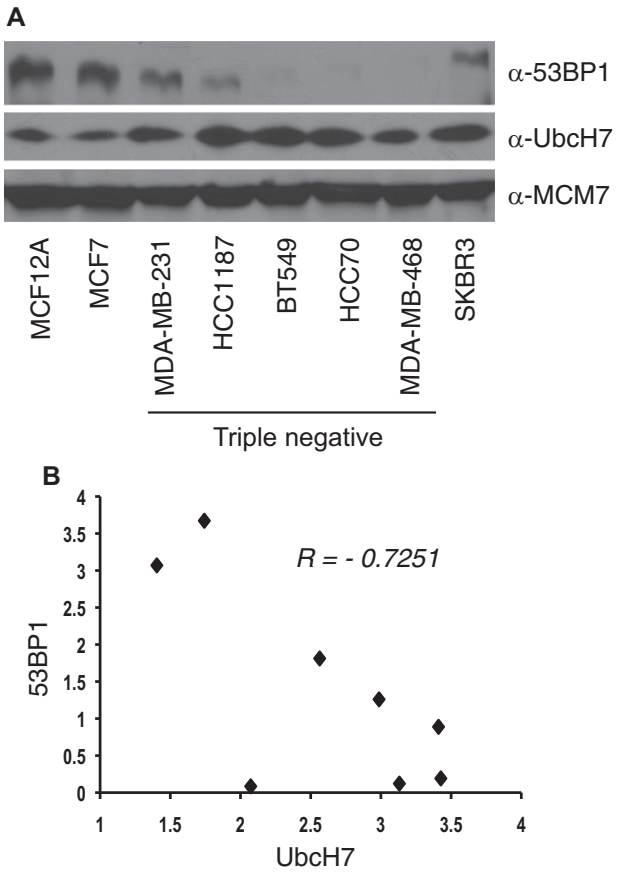


Fig. S10. Expression of UbcH7 and 53BP1 in breast cell lines. (*A*) Equal amounts of total proteins from eight breast cell lines were run on SDS/PAGE and expression of UbcH7 and 53BP1 was analyzed. (*B*) The band intensity in *A* was quantitated by the ImageJ software and analyzed using the Pearson's correlation coefficients, which showed an *R* value of −0.7251.