Supplementary materials and methods

Cell culture and transfection

HEK293T, HeLa, U2OS, and Huh7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, 11995500BT, Thermo Fisher Scientific) supplemented with 10% (volume fraction) fetal bovine serum (FBS, FSD500, ExCell Bio) and 1% (100 mg/L) penicillin/streptomycin (SV30010, Hyclone) at 37 °C in a 5% CO_2 atmosphere. The U2OS DR-GFP and U2OS EJ5-GFP cell lines were kindly provided by Maria JASIN (Memorial Sloan-Kettering Cancer Center) and Dr. Jeremy STARK (Beckman Research Institute of the City of Hope), respectively.

All siRNAs were synthesized by Ribobio (Guangzhou, China) and their sequences are as follows, control (Con): UUCAAUAAAUUCUUGAGGUUU; *FBL#1*: GUCUUCAUUUGUCGAGGAAdTdT; *FBL#2*: CAAUGGUGGAUGUGAUCUUdTdT. Transfections of siRNAs were performed using siRNA duplexes and Lipofectamine 3000 (L3000075, Invitrogen), following the manufacturer's instructions. Plasmid transfections were carried out using Lipofectamine 2000 (11668500, Invitrogen), according to the manufacturer's instructions.

Plasmids

All cDNAs were subcloned into pDONR201 or pDONR221 (Invitrogen) as entry clones, and then transferred to gateway-compatible destination vectors for the expression of N-terminal-tagged fusion proteins. Mutants were generated using the Q5 site-directed mutagenesis kit (NEB, E0552S) and confirmed by sequencing.

Antibodies

Anti-GAPDH (#2118, 1:10 000 dilution (volume ratio, the same below)), anti-Actin (#3700, 1:1000 dilution), anti-RPA2 (#35869, 1:1000 dilution), anti-CHK1 pS317 (#2344, 1:1000 dilution), and anti-CHK2 pT68 (#2661, 1:1000 dilution) antibodies were purchased from Cell Signaling Technology. Anti-HA antibody (H9685, 1:2000 dilution) was purchased from EMD Millipore.

Anti-RAD51 (ab133534, 1:5000 dilution), anti-FBL (ab166630, 1:2000 dilution) and anti-ATM pS1981 (ab81292, 1:5000 dilution) antibodies were purchased from Abcam. Anti-CHK1 (A7653, 1:1000 dilution) and anti-CHK2 (A2145, 1:1000 dilution) antibodies were purchased from ABclonal. Anti-Cyclin B1 (YT119, 1:1000 dilution) antibody was purchased from Immunoway. Anti-ATM (ET1606-20, 1:1000 dilution) antibody was purchased from HUABIO.

Laser micro-irradiation

Cells were subjected to DNA break induction using a pulsed nitrogen laser (365 nm, 20 Hz pulse) in the nucleus. Briefly, cells cultured on 35 mm glass bottom dishes were treated with 10 μmol/L BrdU (B5002, Sigma) for 24 h to sensitize genome DNA and then subject to microirradiation using a Nikon Eclipse Ti-E inverted microscope (60× oil-immersion objective) equipped with a MicroPoint laser ablative system (Photonics Instruments). The laser power output was adjusted to deliver 7 pulses at 30% transmission. Images were acquired and analyzed using MetaMorph microscopy automation software, and Adobe Photoshop CS5 was used for further image processing.

HR and NHEJ reporter assays

U2OS DR-GFP or U2OS EJ5-GFP cells were transfected with indicated siRNAs. 24 h after transfection, 1×10^6 cells were electroporated with 12 μg of the I-*Sce*I expression plasmid pCBASce at 270 V and 975 μF using the BioRad Genepulsar II. 48 h after electroporation, cells were harvested and analyzed by flow cytometry (CytoFlex S, Beckman) to measure the percentage of GFP-positive cells. HR or NHEJ frequencies are presented as the mean±standard deviation (SD) of at least three independent experiments.

Co-immunoprecipitation and western blot

Cells were lysed on ice for 15 min with Nonidet P-40-EDTA-Tris-NaCl (NETN) buffer (20 mmol/L Tris–HCl (pH 8.0), 100 mmol/L NaCl, 1 mmol/L EDTA, 0.5% (volume fraction) Nonidet

P-40) containing 500 U/mL benzonase nuclease (71205, Millipore), 20 mmol/L NaF, and protease inhibitors (1 μg/mL aprotinin and leupeptin). For sonication, the cell extracts were subjected to accumulated sonication of 20 s after cell lysis. The output power of sonication is adjusted to 20%, the sonication stop is 5 s for every 1 s. After centrifugation, the supernatants were incubated with protein A-Sepharose conjugated to 2 μg of the relevant antibodies for 4 h at 4 °C with gentle rocking. The beads-bound proteins were washed three times with NETN buffer and resolved on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Immunoblotting was performed using the corresponding antibodies diluted in universal antibody diluent (WB500D, New Cell & Molecular Biotech).

Immunofluorescence staining

24 h after siRNA transfection, cells were digested and cultured on coverslips for 24 h. Then, cells were treated with 1 μmol/L CPT (C9911, Sigma) for 3 h. Following treatment, cells were pre-extracted with 0.5% (volume fraction) Triton X-100 solution for 5 min and fixed with 3% (30 g/L) paraformaldehyde for 10 min at room temperature. The cells were then incubated with primary antibodies for 30 min, washed three times with phosphate-buffered solution (PBS), and incubated with secondary antibodies at room temperature for 30 min. After staining with DAPI to visualize nuclear DNA for 5 min, images were captured using a fluorescence microscope (Eclipse 80i; Nikon) equipped with a Plan Fluor 60x oil objective lens (NA 0.5-1.25; Nikon) and a camera (CoolSNAP HQ2; PHOTOMETRICS). Foci of RAD51 and RPA2 were analyzed and quantified using ImageJ software. Data were derived from analysis of at least 100 cells in each experiment and are presented as mean±standard deviation (SD) of three independent experiments.

BrdU incorporation assay

HeLa cells were incubated with 100 μmol/L BrdU (B5002, Sigma) for 1 h. Following the incubation, cells were harvested, washed with PBS, and fixed with ice-cold 70% (volume fraction) ethanol overnight at 4 °C. DNA was denatured by incubating cells with 2.5 mol/L HCl for 1 h at

room temperature. After washing three times with PBS, cells were incubated with mouse anti-BrdU antibody (555627, BD Biosciences) diluted 1:1000 (volume ratio) in blocking buffer (PBS+0.1% (volume fraction) Triton X-100+5% (0.05 g/mL) BSA) overnight at room temperature. Cells were then washed three times with blocking buffer containing 500 mmol/L NaCl, followed by incubating with secondary Alexa Fluor 488-conjugated Goat Anti-Mouse IgG H&L antibody (ab150113, Abcam) for 4 h at room temperature. After washing with blocking buffer containing 500 mmol/L NaCl, cells were resuspended in PBS containing propidium iodide (PI, 20 μg/mL) and RNase A (200 μg/mL) at 37 °C for 30 min. The cell cycle analysis was performed using a FACScan flow cytometer (CytoFlex S, Beckman).

Cell survival assay

24 h after siRNA transfection, cells (3×10^2) were seeded onto 35 mm dishes in triplicates. 24 h later, cells were treated with the indicated doses of CPT or Veliparib (also termed ABT-888) and incubated for 24 h. Then, the drug-containing medium was replaced with fresh medium, and the cells were cultured for an additional 14 d at 37°C to allow for colony formation. The resulting colonies were stained with Coomassie blue and counted. The results were normalized to the plating efficiencies of the untreated group.

Chromosomal aberrations assay

HeLa cells transfected with control or *FBL* siRNA were treated with 4 nmol/L CPT for 24 h. Subsequently, cells were incubated with 1 μg/mL colcemid for 4 h. The cells were harvested and resuspended in a hypotonic solution containing 75 mmol/L KCl for 20 min at 37 °C. After fixed in a 3:1 (volume ratio) methanol/acetic acid solution for 30 min, cells were droped onto ice-cold wet slides, air-dried, and stained with a 5% (0.05 g/mL) Giemsa solution for 5 min. Matephase spreads were captured by a light microscope (Nikon) equipped with a Plan Fluor 100× oil objective lens (Nikon) and a camera (CoolSNAP HQ2; PHOTOMETRICS). The number of chromosomal

aberrations was scored in at least 40 metaphases per sample. Statistical analysis of the chromosomal aberration data was performed using a two-tailed t-test.

Recombinant protein purification

RAD51 cDNA was cloned into pCold-MBP vector by MultiF Seamless Assembly Mix (RK21020, ABclonal). FBL cDNA was transferred to gateway (Invitrogen) compatible pDEST15 destination vector for the expression of GST-tagged fusion protein. The plasmids were then transformed into BL21 (DE3). The transformed BL21 were grown at 37 °C until the logarithmic growth phase and then induced with 0.2 mmol/L IPTG at 16°C. 12 h later, cells were harvested and sonicated in lysis buffer (20 mmol/L Tris-HCl, 300 mmol/L NaCl, 1% (volume fraction) Triton X-100, 2 mmol/L DTT, 1 mmol/L PMSF, 1 μg/mL each of leupeptin and aprotinin). The extract was centrifuged at 14,000 g for 30 min. Afterwards, the supernatants were collected and incubated with Glutathione Sepharose (16101, Thermo Fisher Scientific) or Amylose Resin (E8021, NEB) for 4 h at 4 °C, respectively. After incubation, the beads were washed 3 times with washing buffer (20 mmol/L Tris-HCl, 500 mmol/L NaCl, 0.5% (volume fraction) Nonidet P-40, 2 mmol/L DTT, 1 mmol/L PMSF, 1 μg/mL each of leupeptin and aprotinin). The Amylose Resin bound MBP-tagged RAD51 was eluted with 10 mmol/L maltose for subsequent pull-down assay.

In vitro GST pull-down assay

For the GST pull-down assay, 0.5 μg of bacterially purified GST or GST-FBL bound on Glutathione Sepharose and 0.5 μg of purified MBP-RAD51 were mixed in a total volume of 1 mL of NETN buffer (20 mmol/L Tris HCl (pH 8.0), 100 mmol/L NaCl, 1 mmol/L EDTA and 0.5% (volume fraction) Nonidet P-40) supplemented with protease inhibitors. The mixture was rotated at 4°C for 30 min. Then the beads were washed three times with NETN buffer, boiled in 2×SDS loading buffer for 5 min. Subsequently, the resolved proteins were subjected to immunoblotting with the anti-RAD51 antibody or Coomassie blue staining.

Cell proliferation assay

24 h after transfection with control or *FBL* siRNAs, cells (1×10^3) were seeded in 96-well plates and cultured for 1 to 4 d at 37 °C to assess cell proliferation. At the indicated time points, cell viability was determined by incubating them with cell counting kit-8 (CCK-8) reagent (CK04, Dojindo) for 90 min at 37 °C. Optical density (OD) was measured at 450 nm using a multifunctional microplate reader (M20 pro, TECAN).

Bioinformatics analysis using the GEPIA Database

We conducted overall survival (OS) analysis based on gene expression data obtained from the GEPIA database (http://gepia.cancer-pku.cn). The expression level of FBL in cancer patients was assessed using the Log-rank test, and patients were categorized into high and low expression groups based on the median value. Survival analysis was performed using Kaplan-Meier curves. We screened and selected statistically significant (P<0.05) survival plots with the number of patients exceeded 100 from the list of 33 cancers in The Cancer Genome Atlas. The data for liver hepatocellular carcinoma (LIHC), sarcoma (SARC), and skin cutaneous melanoma (SKCM) were selected.

Statistical analysis and reproducibility

All experiments were repeated independently at least three times with similar results. All statistical analyses were carried out using GraphPad Prism 8.0. The data are expressed as the means±SD. Statistical significance between the two groups were determined by the student's two-tailed *t*-test. * P<0.05; ** P<0.01; *** P<0.001; ns, not significant. P<0.05 was considered statistically significant.