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Supplementary Materials for

Repression of BET activity sensitizes homologous recombination–proficient cancers to PARP inhibition

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at
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Table S1. Epigenetic compounds used in drug combination screen (provided as an Excel file).
Table S2. TCGA specimens used in this study (provided as an Excel file).
Table S3. Fusion events of BET genes in TCGA (provided as an Excel file).
Table S4. Mutation frequency of BET genes in TCGA (provided as an Excel file).

Materials and Methods

Drugs

Twenty epigenetic compounds were purchased from commercial vendors (details listed in table S1). Other compounds were obtained from the following vendors: olaparib (Cat No: S1060, Selleckchem), veliparib (Cat No: S1004, Selleckchem), cisplatin (Cat No: S1166, Selleckchem), paclitaxel (Cat No: Y0000698, Sigma), and methyl methanesulfonate (MMS, Cat No: 129925, Sigma). All drugs were dissolved and stored in DMSO. For all in vitro experiments, the final concentration of DMSO was $\leq 0.1\%$.

Cell culture

MDA-MB-231, MDA-MB-468, MDA-MB-361, BT474, HCC1937, OVCAR4, OVCAR8, OVCAR10, A2780, TOV112D, IGROV1, PEO1, UWB1.289, VCaP, and CAPAN1 cells were purchased from the ATCC or DCTD tumor/cell line repositories. Except VCaP and UWB1.289, all cells were cultured in RPMI1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen). VCaP was cultured in high glucose DMEM medium (Thermo Scientific) containing 10% FBS. UWB1.289 cells were cultured in 50%-RPMI1640/50%-MEGM medium containing 3% FBS.

Drug combination screening

MDA-MB-231 cells were used for drug combination screening. Before screening, each drug was arrayed in 96-well plates and serially diluted 2-fold, yielding a concentration equal to IC50 of a given drug in MDA-MB-231. For screening, cells were seeded in 96-well plates at a concentration of 3.0×10^4 cells/mL in 100 µL of medium per well, and then treated with a single drug or with a combination of olaparib and one of the 20 epigenetic drugs for another 72 hours. When testing for synergy of olaparib with epigenetic drugs, the drug combinations were plated serially with constant ratio concentrations in 96-well plates with Eppendorf Xplorer plus Electronic Single Channel Pipette. Cell viability testing was performed using the MTT assay (Sigma). Combination index (CI) and fraction affected (Fa) values were calculated using Compusyn software.

JQ1 and cisplatin or JQ1 and paclitaxel combination

To estimate IC50, cisplatin or paclitaxel was arrayed in 96-well plates and serially diluted 2-fold, yielding a concentration equal to IC50 of cisplatin or paclitaxel in MDA-MB-231. Then, cells were seeded in 96-well plates at a concentration of 3.0×10^4 cells/mL in 100 µL of medium per well, and treated with a single drug or with a combination of JQ1 and cisplatin or paclitaxel for another 72 hours. Cell viability testing was performed using the MTT assay (Sigma). Combination index (CI) and fraction affected (Fa) values were calculated using Compusyn software.

Gene expression array analysis

Gene expression array data of VCaP treated with or without 0.5 µM JQ1 were obtained from GEO dataset (GSE55064) and analyzed by BRB Array tool.

siRNA transfections

Cells were plated 24 hours before transfection at 50% confluence. *BRD2/3/4* siRNA and control siRNA transfections were performed with Lipofectamine RNAiMAX (Cat No: 13778, Invitrogen). The following siRNA oligonucleotides from Sigma were used: *BRD2* siRNA1: SASI_Hs02_00325597; *BRD2* siRNA2: SASI_Hs02_00325595; *BRD3* siRNA1: SASI_Hs01_00086811; *BRD3* siRNA2: SASI_Hs01_00086814; *BRD4* siRNA1: SASI_Hs01_00037409; *BRD4* siRNA2: SASI_Hs01_00037410 and *RAD51* siRNA: SASI_Hs01_00018925. Universal negative control #1 (Cat No: SIC001) was purchased from Sigma. For single

BRD2/3/4 siRNA transfection, each individual siRNA (siRNA against *BRD2*, *BRD3*, or *BRD4*) was used at 20 nM concentration with 40 nM negative control siRNA to make a final concentration of 60 nM of siRNA mixture. For *BRD2/3/4* siRNA combination transfection, each siRNA was used at 20 nM to make a final concentration of 60 nM total of the siRNA mixture.

Colony formation assay

Cells were seeded in 96-well plates at a concentration of 3.0 x 10^4 cells/mL in 100 µL of medium per well, and then treated with DMSO, olaparib, JQ1, or a combination of olaparib and JQ1 for another 5 days. After fixation, colonies were stained with 0.5% crystal violet for 30 min.

Soft agar assay

The bottom layer was prepared with a 0.8% agarose (Cat No: 16550-100, Invitrogen) solution in culture medium in 6-well plates, and the gel was allowed to set for 20 min at room temperature. Cells (5×10^3) were resuspended in 0.4% top agarose solution (in culture medium), then carefully placed on top of the bottom agarose in the 6-well plates. Cells were cultured in normal medium for 3 days and then treated with DMSO, JQ1, olaparib, or combination of JQ1 and olaparib. Medium was changed every 3 days. After 2-4 weeks, cell colonies were stained using crystal violet (Cat No: HT901-8FOZ, Sigma) and counted.

Plasmid construction

For the *RAD51* P-Luc and *BRCA1*-P-Luc luciferase reporters, -1630 ~ +628 bp of *RAD51* promoter and -900 ~ +1 bp of *BRCA1* promoter, respectively, were cloned into the pGL3-basic reporter vector (Promega). To generate the *BRCA1* P-Luc-*BRCA1*- E reporter, AgeI and BstEII restriction sites were first added to the *BRCA1* P-Luc reporter via the Xba I site, then 1341 bp of Enhancer locus 2 were cloned into *BRCA1* P-Luc luciferase reporter via AgeI and BstEII restriction sites.

RNA isolation and qRT-PCR

Total RNA was extracted using TRIzol Reagent (Invitrogen) and reverse transcribed using the High Capacity RNA-to-cDNA Kit (Applied Biosystems). cDNA was quantified by an ABI ViiA 7 System (Applied Biosystems).

Protein isolation and Western blot

Western blot was performed using the following primary antibodies: anti-BRD2 (Cat No: A302-583A, Bethyl); anti-BRD3 (Cat No: A302-368A, Bethyl); anti-BRD4 (Cat No: A301-985A50, Bethyl); anti-BRCA1 (Cat No: sc-6954, Santa Cruz); anti-RAD51 (Cat No: ab213, Abcam); anti-Ku70 (Cat No:MA5-13110, Clone No:N3H10, Thermo); anti-Ku80 (Cat No:MA5-12933, Clone No:111, Thermo); anti-PARP (Cat No:9542, CST); anti-PAR (Cat No:4336-BPC-100, Trevigen); Anti-NBS1 (Cat No:A300-187A, Bethyl); anti-MRE11 (Cat No: GTX70212, Gentex); anti-phospho-H2AX(S139) (Cat No:05-636, Clone No: JBW301, Millipore); anti-MYC (Cat No: 5605, CST); anti-β-Tubulin (Cat No:2128, Clone No:9F3, CST); anti-actin (Cat No: A3854, Sigma), followed by incubation with secondary antibodies conjugated with horseradish peroxidase (HRP, GE Healthcare Life Sciences). Immunoreactive proteins were visualized using the LumiGLO chemiluminescent substrate (Cell Signaling).

Co-immunoprecipitation (Co-IP)

MDA-MB-231 cells were treated with DMSO, 0.5 µM JQ1, 5 µM olaparib, or the combination of 0.5 µM JQ1 and 5 µM olaparib for 1 hour. For Ku80 immunoprecipitation, one 145 mm dish of cells was harvested by trypsinization, washed with PBS twice, and lysed with RIPA buffer containing 1 x protease inhibitor cocktail

(Cat No: 11836153001, Roche) by rotating for 1 hour at 4 ºC. Cell debris were removed by centrifugation, cell lysate was split into two parts (5% of lysate was saved as input), and 8 µg anti-Ku80 (Cat No:MA5-12933, Clone No:111, Thermo) antibody and 8 µg IgG (Cat No: 5415, CST) was used for immunoprecipitation at 4ºC overnight. 30 µl protein G beads (Cat No: P3296, Sigma) were used for pull-down at 4 ºC for 1 hour. Beads were washed with RIPA buffer three times, and bead-bound proteins were lysed with cell lysis buffer (50 mM Tris-Cl, pH 6.8, containing 2% SDS). Lysates and saved inputs were used for Western blot detection of Ku70 (Cat No:MA5-13110, Clone No:N3H10, Thermo), Ku80 (Cat No:MA5-12933, Clone No:111, Thermo), and PAR (Cat No:4336-BPC-100, Trevigen). For PAR immunoprecipitation, all steps were the same as above, except anti-PAR (Cat No:4336-BPC-100, Trevigen) was used instead of anti-Ku80.

Comet assays

Cells were treated with DMSO, JQ1, olaparib, or combination of JQ1 and olaparib for 72 hours. Neutral comet assays with SYBR gold staining (Invitrogen) were performed. The quantification of tail DNA was done using CASP software.

Immunofluorescence staining

Cells seeded on coverslips were treated with DMSO, JQ1, olaparib, or combination of JQ1 and olaparib for 72 hours for γH2AX foci staining or with 10 Gy of irradiation for BRCA1, RAD51, and γH2AX foci staining. Cells were fixed in solution containing 3% paraformaldehyde and 2% sucrose for 10 min at room temperature. Cells were subsequently permeabilized with 0.5% Triton solution for 5 min at 4 °C and then incubated with anti-γH2AX antibody (Cat No:05-636, Clone No: JBW301, Millipore); anti-γH2AX antibody (Cat No: ab81299, Abcam), anti-BRCA1 (Cat No: sc-6954, Santa Cruz), or anti-RAD51 (Cat No: ab213, Abcam) in PBST buffer (PBS plus 0.1% Tween-20, 0.02% NaN_3) overnight at 4 ºC. Cells were then washed three times with PBST and then incubated with goat anti-mouse IgG cross-adsorbed secondary antibody, Alexa Fluor 488 (Cat No: A-11017, Thermo), and/or goat anti-rabbit IgG cross-adsorbed secondary antibody, Alexa Fluor 594 (Cat No: A-11072, Thermo), for 1 hour at room temperature. After four washes with PBST, coverslips were mounted onto glass slides using Vectashield mounting medium containing DAPI (Vector Laboratories) and visualized using an Axiovert 200M inverted microscope (Zeiss).

ssDNA staining

Cells seeded on glass coverslips were cultured for 24 hours in the presence of 5-bromo-2′-deoxyuridine (BrdU; 10 µg/mL; Invitrogen). Cells were treated with DMSO or 0.5 µM JQ1 for 16 hours, followed by treatment with 10 Gy irradiation. Cells were then incubated for another 6 hours and harvested for ssDNA staining. Cells were washed twice with PBS, fixed in methanol for 20 minutes at -20 ºC, and then briefly dipped in acetone at -20 ºC. Cells without BrdU incorporation were used as negative control. Samples were incubated in 70% formamide with 2 x SSC at 80 ºC for 1 minute and then dehydrated in an alcohol series. After fixation, cells were rehydrated in PBS for 5 minutes and incubated in blocking buffer (PBS, 0.02% NaN_3, 0.2% Triton X-100, 0.05% Tween 20, 0.1% bovine serum albumin) for 30 minutes at room temperature. Samples were incubated with the anti-BrdU mouse mAb (Cat No: B2531; sigma) and anti-γH2AX antibody (Cat No: ab81299, Abcam) in blocking buffer overnight at 4 ºC. Cells were next washed three times with PBST and then incubated with secondary antibody for 1 hour at room temperature. After four washes with PBST, coverslips were mounted onto glass slides using Vectashield mounting medium containing DAPI (Vector Laboratories) and visualized using an Axiovert 200M inverted microscope (Zeiss).

Total cellular PARylation and Ku80 PARylation

For total cellular PARylation, MDA-MB-231 and OVCAR10 cells were treated with DMSO, 0.5 µM JQ1, 5 µM olaparib, or the combination of 0.5 µM JQ1 and 5 µM olaparib for 1 hour. Cells were harvested, and whole cell extracts were analyzed for PARylation by anti-PAR antibody (Cat No: 4336-BPC-100, Trevigen). For Ku80 PARylation, MDA-MB-231 cells were treated with DMSO, 0.5 µM JQ1, 5 µM olaparib, or the combination of 0.5 µM JQ1 and 5 µM olaparib for 1 hour. For Ku80 immunoprecipitation, one 145 mm dish of cells was harvested by trypsinization, washed with PBS twice, and lysed with RIPA buffer containing 1 x protease inhibitor cocktail by rotating for 1 hour at 4 ºC. Cell debris were removed by centrifugation, cell lysate was split into two parts (5% of lysate was saved as input), and 8 µg anti-Ku80 (Cat No:MA5-12933, Clone No:111, Thermo) antibody and 8 µg IgG was used for immunoprecipitation at 4 ºC overnight. 30 µl protein G beads were used for pull-down at 4 ºC for 1 hour. Beads were washed with RIPA buffer three times, and bead-bound proteins were lysed with cell lysis buffer (50 mM Tris-Cl, pH 6.8, containing 2% SDS). Lysates and saved inputs were used for Western blot detection of Ku70 (Cat No:MA5-13110, Clone No:N3H10, Thermo), Ku80 (Cat No:MA5-12933, Clone No:111, Thermo), and PAR (Cat No:4336-BPC-100, Trevigen). For PAR immunoprecipitations, all steps were the same as above except anti-PAR (Cat No:4336-BPC-100, Trevigen) was used instead of anti-Ku80.

PARP trapping and chromatin fractionation

MDA-MB-231 cells were treated with DMSO, 0.5 µM JQ1, 5 µM olaparib, or the combination of 0.5 µM JQ1 and 5 µM olaparib with or without 0.01% MMS for 1 hour. Cells were harvested by trypsinization, washed with PBS twice, and split into two parts, one part lysed for whole cell extracts and the other part used for chromatin extraction. For chromatin extraction, cells were resuspended in cytoplasmic extract (CE) buffer (10 mM HEPES, 60 mM KCl, 1 mM EDTA, 0.075% (v/v) IGEPAL, 1 mM DTT, and 1 mM PMSF, adjusted to pH 7.6), and incubated on ice for 5 min. Cell lysates were centrifuged at 300 *g* for 2 min, and the supernatant (cytoplasm fraction) was removed. The remaining pellet (enriched with nuclei) was washed with CE buffer once and then lysed in buffer B (50 mM Tris-HCl, pH 8.0; 500 mM NaCl; 1.5 mM $MgCl_2$; and 0.5% IGEPAL) on ice for 5 min. The nuclei lysate was then centrifuged at 1700 *g* for 4 min, and the supernatant (soluble nuclear fraction) was removed. The final pellet was the chromatin fraction. 25 µg of protein lysate was used for the detection of PARP1 expression by Western blot. Tubulin, which is enriched in the cytoplasmic fraction, was used to indicate the success of chromatin fractionation. Histone H4 expression was used as an internal control.

HR and NHEJ reporter assay

Cells were seeded in 12-well plates at a confluence of 50% one day before transfection. Cells were transfected with 1000 ng I-SceI cut HR or NHEJ reporter plasmid with Lipofectamine 2000 transfection reagent. 6 hours after transfection, cells were treated with DMSO or 0.5 µM JQ1 for 48 hours, and then harvested for FACS analysis by a BD ACCURI C6 PLUS flow cytometer. Cells treated with DMSO were used as a control; other treatments were normalized to DMSO treatment.

ALDH activity assay

ALDH activity was detected using the AldeRed ALDH Detection Assay kit (Millipore, Cat: SCR150) as described by the manufacturer. Briefly, dissociated single cells from MDA-MB-231 and OVCAR10 cell lines were suspended in AldeRed Assay Buffer containing AldeRed 588-A, and incubated for 1 hour at 37 °C. A specific inhibitor of ALDH, diethylaminobenzaldehyde (DEAB), was used as negative control. Flow cytometry data was obtained with a BD ACCURI C6 PLUS flow cytometer.

Cell cycle assay
MDA-MB-231 and OVCAR10 cells were synchronized in G0/G1 phase by serum starvation for 24 hours, and then treated with serial dilutions of JQ1, olaparib, or the combination of JQ1 and olaparib in serum-free conditions for 48 hours. MTT assay was used to calculate cell viability. Cell cycle arrest was confirmed by PI staining and FACS.

***BRD4* overexpression**
For *BRD4* overexpression, MDA-MB-231 cells were seeded in 12-well plates at a confluence of 50% one day before transfection. Cells were transfected with 2 µg empty pcDNA4 (Invitrogen) or pcDNA4-*BRD4* (Cat No: #31351, Addgene) with Lipofectamine 2000 Reagent (Invitrogen). Medium was changed the next day. Cells were selected with 400 µg/ml Zeocin for 5 days, then seeded for the detection of PARPi sensitivity. For cell viability assay, Zeocin selected cells were seeded in 96-well plates at a concentration of 3.0×10^4 cells/mL in 100 µL of medium, then treated with serial dilutions of olaparib for 72 hours. Cell viability testing was performed using the MTT assay (Sigma).

In vivo tumor experiments
For MDA-MB-231 xenograft experiments, MDA-MB-231 cells were implanted into mammary fat pad of female nude mice and grown until tumors reached a size of approximately 30 mm^3. Xenografted mice were randomized and then received vehicle, 50 mg/kg JQ1, 50 mg/kg olaparib, or the combination of both agents, 5 days a week for 3 weeks (n = 12 per group). Caliper measurements were taken every three days starting from the initiation of drug treatment. Tumor volumes were calculated according to the formula: tumor volume [mm^3] = $(1/6) \times \pi \times$ (tumor length) $\times$ (tumor width)^2. Mice were sacrificed, and tumors were harvested for RNA and protein. For OVCAR10 xenograft experiments, OVCAR10 cells were implanted intraperitoneally in mice and grown for 2 weeks. Xenografted mice were randomized and then received vehicle, 50 mg/kg JQ1, 50 mg/kg olaparib, or the combination of both agents, 5 days a week for 4 weeks (n = 11 per group). Mice were sacrificed for measurement of ascites and peritoneal tumor nodules. To monitor the in vivo toxicity, body weight was measured on every drug day during the treatment. Due to the nature of the performed experiments, no randomization and no blinding was used during in vivo drug treatment because it was deemed unfeasible. However, the resulting tumors were analyzed in a blinded manner. All animal procedures were in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Luciferase assays
For drug treatment reporter assay, 500 ng of reporter vector plus 5 ng of the renilla luciferase plasmid were transfected to cells using FuGENE 6 (Roche); cells were then treated with DMSO, JQ1, olaparib, or combination of JQ1 and olaparib for 24 hours. For *BRD2/3/4* siRNA treatment reporter assay, cells transfected with *BRD2/3/4* siRNAs were then transfected with reporter vector and renilla, and cells were harvested 48 hours after transfection. Reporter assays were performed using a dual luciferase reporter assay system (Promega) by Fluoroskan Ascent FL fluorometer (Thermo Fisher Scientific).

Chromatin Immunoprecipitation (ChIP)
3×10^7 MDA-MB-231 cells treated with DMSO or JQ1 were harvested for ChIP experiment. Cells were cross-linked with 1% formaldehyde at room temperature for 10 min, and then neutralized with 125 mM glycine for 5 min. Cells were rinsed with ice-cold PBS twice and scraped into 1 ml of ice-cold PBS. Cells were resuspended in 0.3 ml of lysis buffer and sonicated. After centrifugation, supernatants were collected and diluted in IP dilution buffer followed by immunoclearing with protein A-sepharose for 2 hours at 4 °C. 5 µg anti-BRD2 (Cat

No: A302-583A, Bethyl); anti-BRD3 (Cat No: A302-368A, Bethyl); anti-BRD4 (Cat No: A301-985A50, Bethyl); or control IgG (Cat No: 2729S, CST) was used for immunoprecipitation. After immunoprecipitation, 45 µl protein A-Sepharose was added and incubated for another hour. Precipitates were washed, and DNA was purified after de-crosslinking for real-time PCR. Primers used are listed below:

RAD51 Promoter ChIP F	TTGGCGGGAATTCTGAAAGC
RAD51 Promoter ChIP R	ACGCTCCACTTCTCTACTCG
BRCA1 Promoter ChIP F	GAATGAAGTTGCGGACCCTC
BRCA1 Promoter ChIP R	GCTGTAACACTCACCGCAAA
BRCA1 Enhancer ChIP F	AGGAGATGGGAGAGGGGAAT
BRCA1 Enhancer ChIP R	GTCACATAGCCCCTCACTGT
GAPDH ChIP F	GCTTGCCCTGTCCAGTTAAT
GAPDH ChIP R	TAGCTCAGCTGCACCCTTTA

Analysis of ChIP-seq data

ChIP-seq data sets for MDA-MB-231 cells (H3K27ac: GSM1693018, H3K4me1: GSM1855985, H3K4me3: GSM1904660) and VCaP cells (GSE55064) were obtained from GEO. ChIP-seq data sets for GM12878 cells (H3K27ac: ENCFF000ASJ, H3K4me1: ENCFF000ARZ, H3K4me3: ENCFF000ASF, CTCF: ENCFF000ARJ) were obtained from ENCODE (https://www.encodeproject.org/) online database. BigWig files of ChIP-seq experiments were used for visualization in IGV.

Topologically associating domains (TAD) visualization

BRCA1 TAD in GM12878 cells was obtained from GM12878 Hi-C interaction data and visualized by Interactive Hi-C Data Browser (http://promoter.bx.psu.edu/hi-c/view.php) with resolution of 1 kb.

Quantitative analysis of 3C-qPCR

1 x 10^7 cells treated with or without JQ1 were harvested for this experiment. Cells were crosslinked in 2% formaldehyde/10% FCS/PBS for 10 min, quenched with glycine at a final concentration of 125 mM for 5 min, and centrifuged at 1000 *g* for 10 min at 4 °C. After being lysed in cold lysis buffer (10 mM Tris-HCl, pH 7.5; 10 mM NaCl; 5 mM $MgCl_2$; 0.1 mM EGTA; 1 x complete protease inhibitor) for 10 min on ice, nuclei were centrifuged and digested in 1.2 × restriction enzyme buffer containing 0.3% SDS for 1 hour at 37 °C while shaking at 900 r.p.m. Triton X-100 was added to the reaction and incubated for 1 hour at 37 °C. 400 U of XbaI was used to digest DNA overnight at 37 °C. Nuclei were incubated in 1.6% SDS for 25 min at 65 °C, suspended in ligation buffer containing 1% Triton X-100 for 1 hour at 37 °C, then ligated by 100 U ligase for 4 hours at 16 °C followed by 30 min at room temperature. Reaction was terminated, and de-crosslinking was performed with PK buffer (5 mM EDTA, pH 8.0; 10 mM Tris-HCl, pH 8.0; 0.5% SDS) at 65 °C overnight. DNA was incubated in 300 µg RNase A for 45 min at 37 °C, then purified by the phenol-chloroform method. DNA was dissolved in 150 µl of 10 mM Tris pH 7.5. Bacterial artificial chromosome (BAC) (RP11-948G15 and RP11-242D8) containing *BRCA1* promoter and enhancer loci were used as positive controls. 3C-qPCR was performed with 200 µg DNA and primers listed below. BAC 3C DNA was used to determine the PCR efficiency for each set of primers. *GAPDH* promoter was used as negative control. PCR products from the *BRCA1* promoter and enhancer locus 2 and from the *BRCA1* promoter and enhancer locus 6 were also run in 2% agarose gel. Primers for 3C-qPCR:

Promoter Forward: TTTGGTCGTTGTTGATTTTGGTTTTATG
Enhancer 1 Reverse: AGGTGGGAGGATCACTTGAA
Enhancer 2 Reverse: TCCTTTCCTCATTGGTGTCC

Enhancer 3 Reverse: TCGGGCAAATCTCTCTAAGC
Enhancer 4 Reverse: CTGAATCCAGAGCCCACAGT
Enhancer 5 Reverse: CCTCCAGGAAACAGCAAAAG
Enhancer 6 Reverse: AGACACTGTGCCTGGCATTT
Enhancer 7 Reverse: GCAAGACTCCATCTCGGAAA

Supplemental Figures

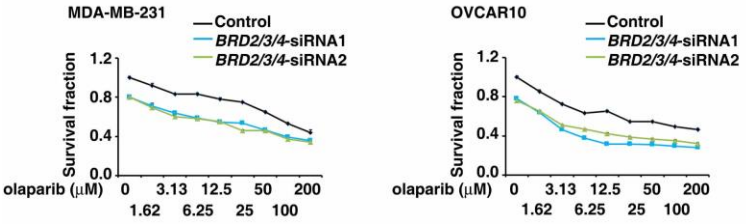

Fig. S1. BET depletion increases PARPi sensitivity.
Pooled siRNAs were used to simultaneously target *BRD2*, *BRD3*, and *BRD4* in cancer cells. Fraction of cells surviving olaparib treatment shown in MDA-MB-231 (left) and OVCAR10 (right) cells treated with control siRNA or *BRD2/3/4* siRNA pools.

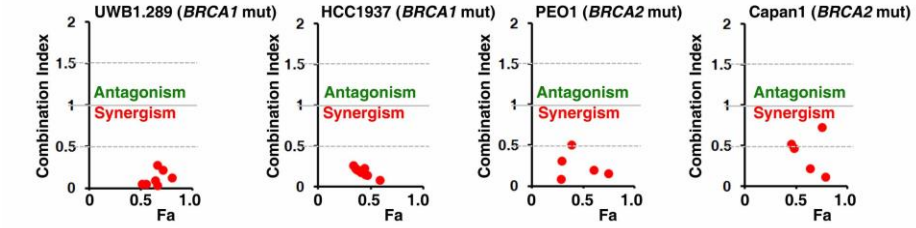

Fig. S2. BETi synergizes with PARPi in HR-deficient cancer cell lines.
Drug combination results (CI) for JQ1 and olaparib were examined in UWB1.289 (*BRCA1* nonsense mutation), HCC1937 (*BRCA1* frame-shift mutation), PEO1 (*BRCA2* nonsense mutation), and CAPAN-1 (*BRCA2* frameshift mutation) cells.

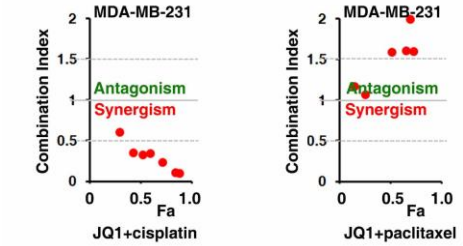

Fig. S3. BETi synergizes with cisplatin but not paclitaxel in MDA-MB-231 cells.
Drug combination results (CI) for JQ1 and cisplatin or paclitaxel were examined in the MDA-MB-231 cell line.

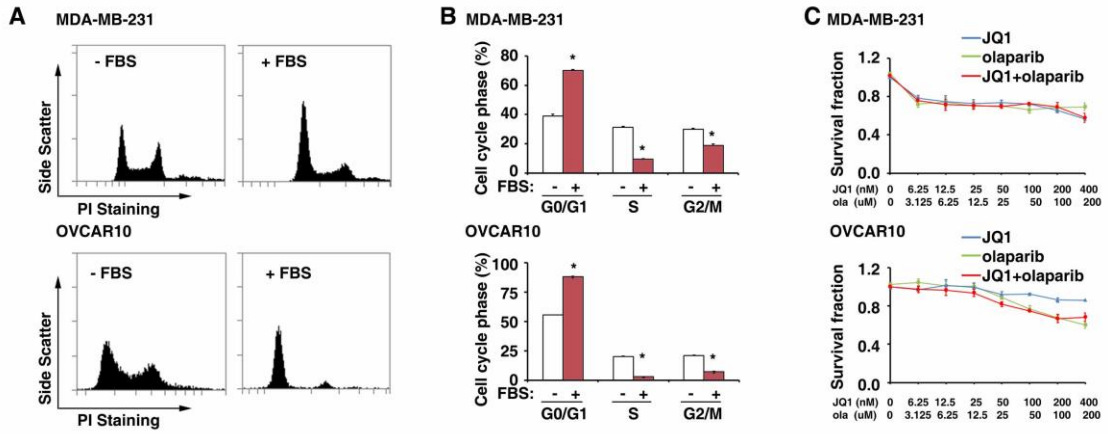

Fig. S4. Combination treatment with BETi and PARPi does not act synergistically in noncycling cancer cells.

A. Cell cycle distribution of MDA-MB-231 (upper) and OVCAR10 (lower) cells with or without serum starvation for 24 hours. **B.** Quantification of G0/G1, S, and G2/M phase cells in MDA-MB-231 (upper) and OVCAR10 (lower) cells with or without serum starvation. **C.** Sensitivity of MDA-MB-231 and OVCAR10 to olaparib alone, JQ1 alone, or olaparib combined with JQ1. Survival fraction was shown for each of these 2 cell lines.

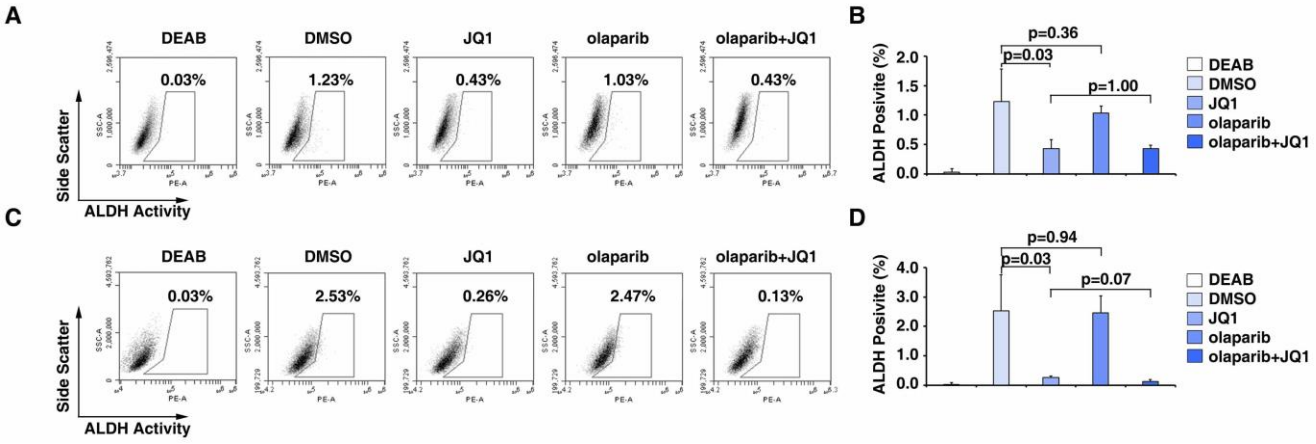

Fig. S5. BET inhibition decreases the ALDH$^+$ tumor-initiating cell population.

A. Representative FACS analysis for ALDH activity in MDA-MB-231 cells. **B.** Quantification of the ALDH$^+$ tumor-initiating cell population in MDA-MB-231 cells. **C.** Representative FACS analysis for ALDH activity in OVCAR10 cells. **D**. Quantification of the ALDH$^+$ tumor-initiating cell population in OVCAR10 cells. MDA-MB-231 and OVCAR10 cells were treated with DMSO, 0.5 μM JQ1, 5 μM olaparib, or the combination of 0.5 μM JQ1 and 5 μM olaparib for 24 hours. N,N-diethylaminobenzaldehyde (DEAB)-treated cells were used as a negative control for ALDH activity.

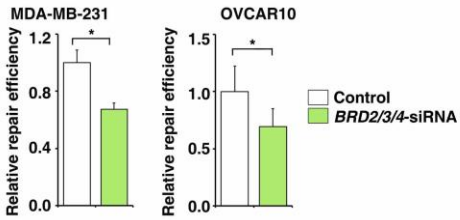

Fig. S6. BET depletion decreases HR.
Pooled siRNAs were used to simultaneously target *BRD2*, *BRD3*, and *BRD4* in cultured cells. Relative HR repair effeciency shown in MDA-MB-231 (left) and OVCAR10 (right) cells treated with *BRD2/3/4* siRNAs pools.

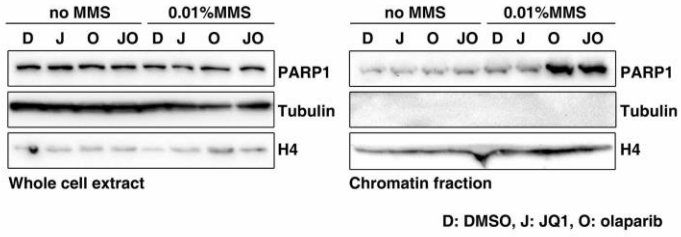

Fig. S7. BET inhibition does not affect PARP trapping.
We analyzed the amount of PARP1 in whole cell extracts and chromatin fractions in MDA-MB-231 cells after treatment with DMSO, 0.5 µM JQ1, 5 µM olaparib, or the combination of 0.5 µM JQ1 and 5 µM olaparib for 1 hour. To increase base damage, we also combined the above treatment conditions with a low dose of the classic alkylating agent methyl methanesulfonate (MMS). The image shows Western blot analysis of PARP1 in whole cell extracts (left) and chromatin fractions (right) of MDA-MB-231 cells treated with DMSO, 0.5 µM JQ1, 5 µM olaparib, or the combination of 0.5 µM JQ1 and 5 µM olaparib with or without 0.01% MMS for 1 hour. Tubulin and histone H4 were used as internal controls.

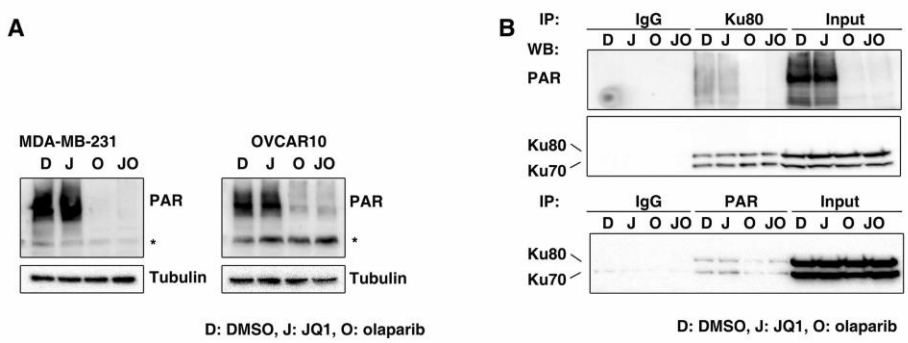

Fig. S8. BET inhibition does not affect total cellular PARylation and Ku80 PARylation.
A. Western blot analysis of total PAR in MDA-MB-231 (left) and OVCAR10 (right) cells treated with DMSO, 0.5 μM JQ1, 5 μM olaparib, or the combination of 0.5 μM JQ1 and 5 μM olaparib for 1 hour. The asterisk indicates a nonspecific band. **B.** Ku80 PARylation after BET inhibition. Upper panel: PAR, Ku70, and Ku80 in anti-Ku80 immunoprecipitates from MDA-MB-231 cells treated with DMSO, 0.5 μM JQ1, 5 μM olaparib, or the combination of 0.5 μM JQ1 and 5 μM olaparib for 1 hour. Lower panel: Ku70, and Ku80 in anti-PAR immunoprecipitates from MDA-MB-231 cells treated with DMSO, 0.5 μM JQ1, 5 μM olaparib, or the combination of 0.5 μM JQ1 and 5 μM olaparib for 1 hour.

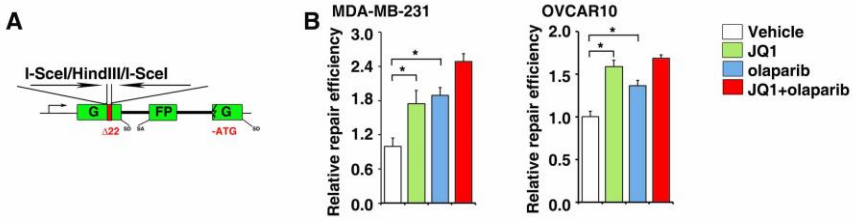

Fig. S9. BET inhibition increases NHEJ repair efficiency.
A. Schematic illustration of the NHEJ reporter assay. **B.** Relative NHEJ repair effeciency in MDA-MB-231 (left) and OVCAR10 (right) cells treated with DMSO, 0.5 μM JQ1, 5 μM olaparib, or the combination of 0.5 μM JQ1 and 5 μM olaparib for 48 hours.

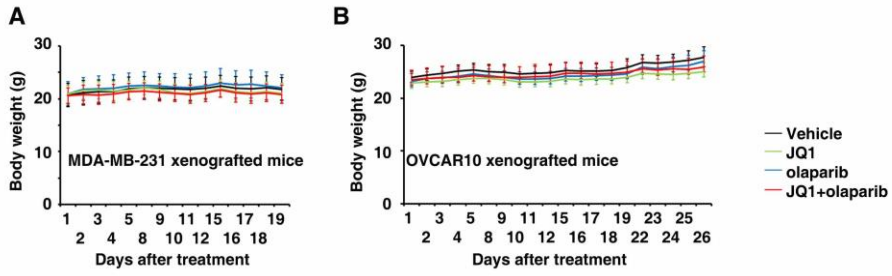

Fig. S10. Combining JQ1 and olaparib does not increase toxicity to mice.
The body weight was measured in four groups of animals treated with vehicle, JQ1, olaparib, and combination of JQ1 and olaparib. A: MDA-MB-231; B: OVCAR10.

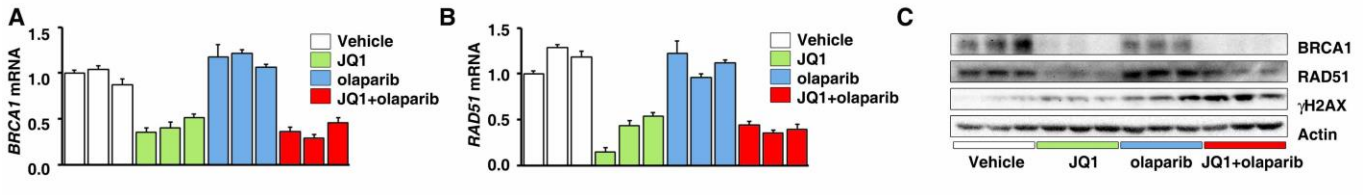

Fig. S11. BET inhibition represses the expression of BRCA1 and RAD51 in vivo.
Tumor specimens were collected from the MDA-MB-231 xenograft model. Total RNA and protein were isolated from frozen tumors. **A** and **B.** *BRCA1* (A) and *RAD51* (B) expression in different groups were detected by qRT-PCR. **C.** Western blots showing the expression of BRCA1, RAD51, and γH2AX. Actin served as a loading control.

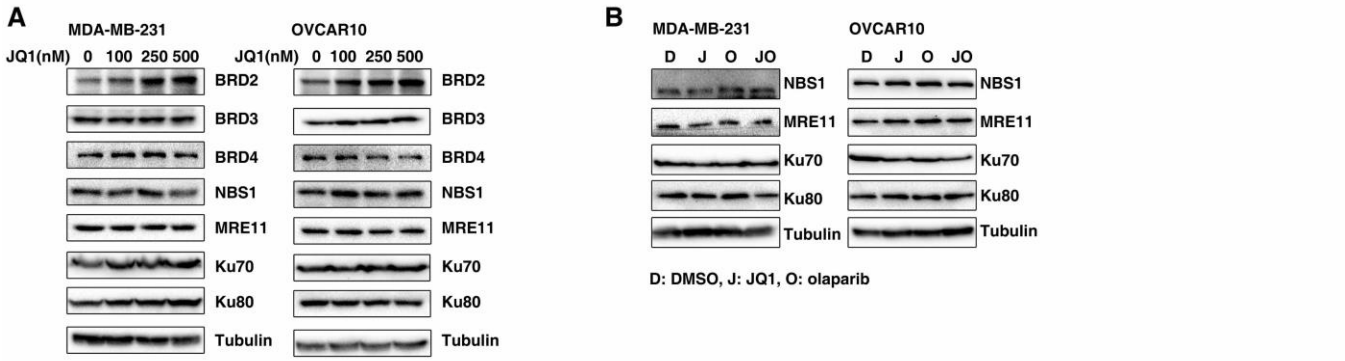

Fig. S12. The effect of BET inhibition on the expression of other proteins was analyzed by Western blot.
A. Western blot analysis of BRD2, BRD3, BRD4, Ku70, Ku80, NBS1, and MRE11 in MDA-MB-231 (left) and OVCAR10 (right) cells treated with different concentrations of JQ1 for 24 hours. Tubulin was used as loading control. **B.** Western blot analysis of Ku70, Ku80, NBS1, and MRE11 in MDA-MB-231 (left) and OVCAR10 (right) cells treated with DMSO, 0.5 μM JQ1, 5 μM olaparib, or the combination of 0.5 μM JQ1 and 5 μM olaparib for 24 hours. Tubulin was used as loading control.

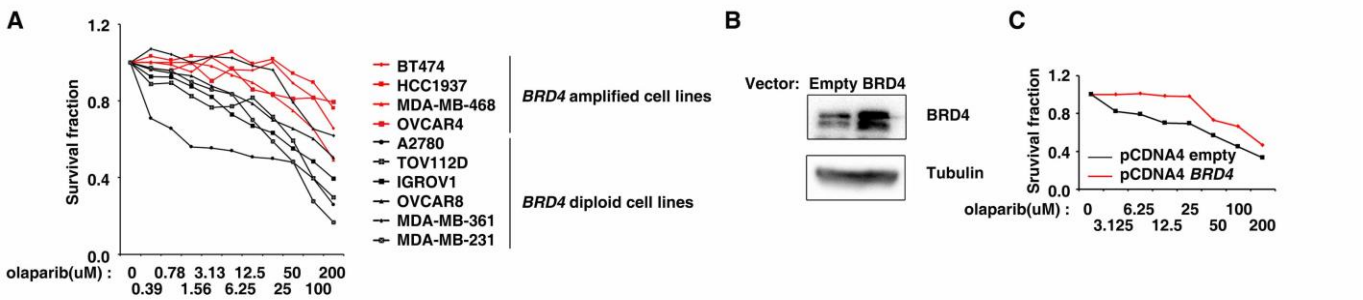

Fig. S13. BRD4 amplification results in PARPi resistance.
A. Fraction of cells surviving olaparib treatment in 10 cell lines with different BRD4 copy numbers. **B.** We overexpressed BRD4 protein in MDA-MB-231 cells by introducing *BRD4* cDNA via pcDNA-*BRD4* vector transfection. Western blots show the overexpression of BRD4 in transfected MDA-MB-231 cells. **C.** Fraction of cells surviving olaparib treatment is shown in MDA-MB-231 cells overexpressing BRD4 and in empty vector control.

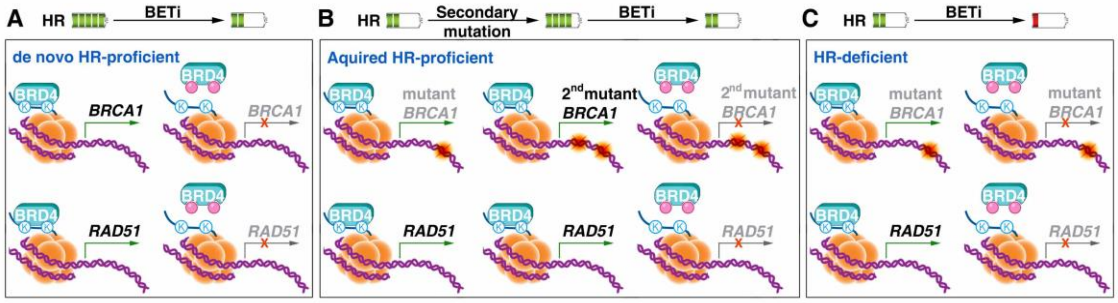

Fig. S14. The study provides a strong rationale for clinical application of PARPi in combination with BETi.
A. BETi may synergize with PARPi to treat de novo HR-proficient cancer that is primarily resistant to PARPi. Repressed BET activity impairs *BRCA1* and *RAD51* expression, subsequently converting HR-proficient tumors to HR-deficient ones. **B.** BETi may re-sensitize cancers with acquired HR proficiency to treatment with PARPi. BET inhibition overcomes PARPi resistance by repressing the expression of secondary *BRCA1* mutations that restores *BRCA1* function or by blocking the expression of *BRCA1* and *RAD51* in the context of other resistance mechanisms such as *53BP/REV7* loss and *BRCA2* reverse mutation. **C.** BETi may enhance response to PARPi HR-deficient cancers such as *BRCA1* or *BRCA2* mutated cancers by further blocking *RAD51* expression.