Synthetic Lethality between PBRM1 Deficiency and PARP Inhibitors: Exploiting G2/M Checkpoint Arrest in Colorectal Cancer

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Abstract

Background: Polybromo 1 (PBRM1), encoding the BAF180 subunit of the polybromo-associated BAF (PBAF) chromatin-remodeling complex, is commonly lost or mutated across malignancies. Despite its prevalence, tailored therapeutics for PBRM1-defective cancers remain limited. PBRM1 loss is associated with elevated replication stress and DNA damage responses, implying dependence on compensatory repair pathways. Given the lack of genotype-matched drugs, exploiting DNA-repair dependencies may provide a precision option for PBRM1-deficient disease. We pursued a synthetic-lethality strategy in colorectal cancer to test whether clinically used PARP inhibitors selectively suppress PBRM1-deficient cells and to define the linked cell-cycle and stress-response mechanisms. We also compared PARP inhibition with broader chromatin-remodeler targeting.

Methods: Isogenic PBRM1$^{-/-}$ HCT116 colorectal carcinoma cells were generated by CRISPR/Cas9 lentiviral editing using sgRNAs cloned into lenti-CRISPR-V2. Knockout was confirmed by Western blotting, Sanger sequencing, and RT-qPCR. A focused compound screen compared four agents PARP inhibitors olaparib and rucaparib, the multi-target chromatin remodeler inhibitor AU-24118, and the SMARCA2/4-targeting degrader AU-1530 using dose-response CCK-8 viability assays and selectivity indices. We then validated our results by 12-day colony-formation assays. Mechanistic analyses measured drug-induced G2/M accumulation by propidium iodide staining and flow cytometry and quantified apoptosis by Annexin V/PI dual staining, with significance assessed by t-test or two-way ANOVA.

Conclusions: PBRM1 loss confers selective hypersensitivity to PARP inhibitors, which intensify DNA-damage signaling, promote G2/M checkpoint arrest, trigger apoptosis, and induce stress-response genes such as CSRNP3. These results indicate that PBRM1 may serve as a predictive

biomarker, supporting its clinical evaluation and the development of biomarker-guided combination approaches to improve patient stratification and help address therapeutic resistance.

Background

PBRM1 is an important tumor suppressor gene that codes for the BAF180 subunit of the PBAF chromatin remodeling complex. It contributes to controlling transcription and maintaining genomic stability(Chabanon et al., 2021; Li & Zou, 2024; Mashtalir et al., 2018; Pawłowski et al., 2013; Varela et al., 2011). Loss-of-function mutations or decreased expression of PBRM1 are commonly identified in malignancies, especially common in clear cell renal cell carcinoma, and also detected in other aggressive cancers(Benusiglio et al., 2015; Carril-Ajuria et al., 2019; Dai et al., 2023; Li & Zou, 2024; Pawłowski et al., 2013). PBRM1 mutations are present in 28% to 55% of clear cell renal cell carcinomas (ccRCC), serving as an early driver event that follows VHL change(Carril-Ajuria et al., 2020; Gad et al., 2021). Other aggressive malignancies also have PBRM1 abnormalities, encompassing 11% to 59% of chordomas, 12% to 23% of cholangiocarcinomas, 7% to 20% of mesotheliomas, 12% of endometrial carcinomas, and 3% of non-small cell lung cancers(Carril-Ajuria et al., 2019; Choudhary; Gad et al., 2021; Nargund et al., 2017). Even though mutations in PBRM1 are common, there are no customized targeted medicines that work specifically for PBRM1-defective malignancies. This is what drives synthetic lethality techniques(Gu et al., 2022; Morel et al., 2017; Zimmer et al., 2023).

Synthetic lethality, which implies that two pathways must be disrupted for a cancer to die, has become a significant technique to attack malignancies that don't have tumor suppressors(Kaelin Jr, 2005; Li et al., 2026; Wu et al., 2025). In PBRM1-deficient circumstances, elevated replication

stress and DNA damage phenotypes engender reliance on compensatory repair mechanisms(Espana-Agusti et al., 2017). Orthogonal functional genetic and small-molecule screens have delineated synthetic lethal interactions between PBRM1 deficiency and DNA repair inhibitors, notably PARP inhibitors, with validation across many in vitro systems models(Chabanon et al., 2021; Concannon et al., 2023; Zimmer et al., 2023). In terms of how they work, cells with PBRM1 defects show signs of replication stress and more DNA damage signaling, which can get worse when PARP is blocked(Ray Chaudhuri & Nussenzweig, 2017). Previous studies reported that olaparib augments 5-fluorouracil cytotoxicity in mismatch repair-deficient colorectal carcinoma cells(de Castro e Gloria et al., 2021). In contrast, the present study evaluates olaparib treatment in PBRM1-deficient HCT116 cells, a model in which PBRM1 deficiency has been associated with G2/M arrest (Wang et al., 2017) and promoted cell death in PBRM1-deficient cells(Fultang et al., 2024).

Our findings demonstrate that PBRM1 knockout cells exhibit selective sensitivity to PARP inhibitors, particularly olaparib and rucaparib, through synthetic lethal interactions. This sensitivity is associated with impaired clonogenic survival(Chabanon et al., 2021), enhanced G2/M cell cycle arrest. In contrast, multi-target chromatin remodeler inhibitors showed limited genotype-specific effects. Collectively, these results show PBRM1 loss as a potential predictive biomarker and support the clinical relevance of PARP inhibitor–based therapies in PBRM1$^{-/-}$ cells.

Materials and Methods

Cell Lines and Culture

The following cell lines, including human HCT116 and 293T and mouse MC38 and B16F10, were obtained from ATCC. HCT116 cells were cultured in McCoy's 5A Media (Gibco) containing 10% FBS and 1% pen/strep. B16F10, MC38, and 293T cells were cultured in DMEM basic media (Gibco) containing 10% FBS and 1% pen/strep. All the cells were incubated in a humidified incubator adjusted with 5% CO_2 at 37°C. All the cells were routinely screened for mycoplasma absence.

sgRNA Oligonucleotide Design, Annealing, and Ligation with Lenti-CRISPR-V2

Designed sgRNAs by using the online tool CHOPCHOP (https://chopchop.cbu.uib.no/) and ordered all oligos from Sangon Biotech (Shanghai). Oligonucleotides annealed with T4 PNK (BioLabs). Meanwhile, digest lenti-CRISPR-V2 plasmids with BsmBI-V (BioLabs) and extract with EasyPure Quick Gel Extraction Kit (TRANSgen Biotech). After that, ligated all annealed sgRNA with digested lenti-CRISPR-V2 plasmids by using T4 DNA Ligase (BioLabs). Competent cell DH5α was transformed with a ligation mixture, and extracted plasmid DNA with the FastPure Plasmid Mini kit (Vazyme), and validated the sequence of the sgRNA expression plasmid by Sanger sequencing. The sequences of oligonucleotides used are included in Table 1.

Table 1: Sequences of oligonucleotides

Oligonucleotides	Sequence (5'-3')

PBRM1-sg2-F	CACCGTCATCCTTATAGTCTCGGA
PBRM1-sg2-R	AAACTCCGAGACTATAAGGATGAC
PBRM1-sg3-F	CACCGCTCTGTGAGCTCTTCATTA
PBRM1-sg3-R	AAACTAATGAAGAGCTCACAGAGC
PBRM1-sg7-F	CACCGGCGAGGAGATCTATATCTT
PBRM1-sg7-R	AAACAAGATATAGATCTCCTCGCC

Lentivirus Preparation and Generation of PBRM1 $^{-/-}$ Cells

PBRM1 gene knockout was performed in HCT116, MC38, and B16F10 cell lines using a CRISPR/Cas9-based gene editing technique. Cells were transfected with the PBRM1 KO constructs using lentiviral transduction. Then the transduced cells were selected by puromycin (Beyotime) for 10 days with 2-8 μM/mL. Prepared single cell KO colonies and isolated polyclonal-KO and monoclonal-KO were verified with Western blotting, Sanger sequencing, and qPCR. To produce lentiviruses, CRISPR V2 plasmid, pMD2.G, and psPAX2 were co-transfected into 293T cells using Lipofectamine 3000 reagent, P3000 reagent, and Opti-MEM (Invitrogen). At 48 h post-transfection, cell supernatants were collected and filtered through a 45 μM strainer. Supernatants were then immediately used for transduction or kept at -80ºC for long-term storage. Then, lentiviruses and polybrene are used to infect cell lines.

Western Blot Analysis

Total protein from cells was harvested with Mammalian Active Protein Extraction Reagent, Protease Inhibitor Cocktail, and Phosphatase Inhibitor Cocktail (MedChemExpress). Proteins were fractionated by 7.5% SDS–PAGE and transferred onto PVDF membrane (Sigma-Aldrich, pore size 0.2 μm). Membranes were incubated with the indicated primary antibodies, anti-PBRM1 (BETHYL, 1:10000) and anti-Actin (Abcam, 1:5000), overnight at 4°C, and further incubated with the corresponding HRP-conjugated secondary antibodies (Abcam, 1:15000) for 1 h at room temperature. Target proteins were detected using ECL Substrate (Biosharp) under an iBright750 Imager (Invitrogen by Thermo Fisher Scientific).

RNA Isolation and RT-qPCR

Total RNA was harvested from cultured cells using Trizol reagent (Invitrogen, USA). RT was then performed with the EasyScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) (TRANSgen Biotech). qPCR was performed using PerfectStart Green qPCR SuperMix (TRANSgen Biotech). The sequences of primers used are included in Table 2.

Table 2: Sequences of qPCR primers

Primer	Sequence (5'-3')
PBRM1-F	CAACTCAGAGGACAGTCGAGCT
PBRM1-R	GCTGCTCAAAGTAGTGGCAACC
BETA ACTIN-F	CACCATTGGCAATGAGCGGTTC
BETA ACTIN-R	AGGTCTTTGCGGATGTCCACGT

Cell Survival Assays

Short-term cell survival assays were performed in 96-well plates. Exponentially growing cells were plated in quintuplicate at a concentration of 1000 cells per well. Drug or vehicle was added 24 hours after seeding, and cells were continuously exposed to the drug for 5 days. The concentrations used ranged from 0 to 6 μM. After the exposure period, cell viability was assessed using the CCK-8 assay (MedChemExpress) on a VARIOSKAN LUX plate reader (ThermoFisher), and survival fractions were calculated relative to the DMSO-treated control.

Colony-formation Assays

Colony-formation assays were performed in 6-well plates. Exponentially growing cells were plated at a concentration of 1000 cells/well. Olaparib, rucaparib, AU-24118, and vehicle (control) were added 24 h after seeding with and cells were exposed to the drug for 12 days, with drug containing media replenishment every 2 days. The concentrations used ranged from 0 to 6 μM. Cells were then fixed with 0.5% crystal violet in methanol for 20 min. Colonies were counted with ImageJ software, and survival fractions were calculated compared to the DMSO-treated control.

Cell Cycle Assays

PBRM1$^{+/+}$ and PBRM1$^{-/-}$ cells grown in 6-well plates were treated with olaparib, rucaparib, and AU-24118 for 24 h, where the concentrations used ranged from 0 to 6 μM. For cell cycle analysis, cells were harvested for propidium iodide (PI) staining (YEASEN). The cell fluorescence was analyzed with a NovoCyte Advanteon VBR (Agilent) flow cytometer.

Flow Cytometer Analysis Annexin V/PI Double Stained

To quantitatively assess treatment-induced apoptosis, flow cytometric analysis was performed using Annexin V and propidium iodide (PI) dual staining. Briefly, PBRM1 wild-type ($PBRM1^{+/+}$) and isogenic PBRM1 knockout ($PBRM1^{-/-}$) cells, derived from HCT116 colorectal cell lines, were harvested at specified time points (24h) following experimental treatments. Cells were then washed with cold phosphate-buffered saline (PBS) and resuspended in a 1X Annexin V binding buffer (BD Biosciences) at a density of approximately 1 x 10^6 cells/mL. Following the manufacturer's protocol (Annexin V-FITC Apoptosis Detection Kit, [BD Biosciences]), cell suspensions were incubated with fluorescein isothiocyanate (FITC)-conjugated Annexin V and PI (YEASEN) for 15 minutes at room temperature in the dark. Data were analyzed using NovoExpress software, with apoptotic rates expressed as the combined percentage of early and late apoptotic cells (total Annexin V^+ population). Statistical comparisons of apoptotic indices between genotypes and treatment conditions were performed to evaluate the functional role of PBRM1 in modulating apoptotic sensitivity.

Statistical Analysis

The statistical significance of differences between control and test groups was assessed using a t-test or two-way ANOVA via GraphPad Prism 6 (GraphPad Software). P-values less than 0.05 have been considered significant. *P < 0.05; **P < 0.01; ***P < 0.001; **** P < 0.0001; ns, not significant.

Results

PARP inhibitors are Synthetic Lethal with PBRM1-Deficient Human Colorectal Carcinoma Cells

To screen and identify the PBRM1 target drug, we first generated PBRM1 knockout (KO) HCT116, MC38, and B16F10 cells by the CRISPR-Cas9 gene editing system(Li et al., 2023). We used PBRM1 isogenic cell pairs, which ensure that the identification effects work in one specific genetic background. As confirmed and verified PBRM1 KO through Western blot (WB), Sanger sequencing, and qPCR analysis, our knockout approach successfully abolished PBRM1 protein **(Figures 1A-C).** We also checked the growth rate of HCT116 isogenic cell pairs through CCK-8 **(Figure 1D).** We used four confirmed HCT116 PBRM1$^{-/-}$ clones (sg2.4, sg3.8, sg3.13, and sg7.3) and two other PBRM1$^{-/-}$ cell lines, MC38 & B16F10 **(Figures 1E-F),** for the synthetic lethal screening. Among the four PBRM1 knockout clones created, the sg7.3 clone was chosen for the synthetic lethal drug screening.

We next used PBRM1-deficient cells to assess potential vulnerabilities using a panel of four drugs. These included the PARP1 inhibitors olaparib and rucaparib, the multi-target inhibitor AU-24118 (against PBRM1, SMARCA2, and SMARCA4), and the SMARCA2/4-targeting PROTAC AU-1530. A CCK-8 assay was used to determine the drug inhibition rate following exposure to concentrations from 0 to 6 μM **(Figure 2),** with B16F10 PBRM1$^{-/-}$ **(Figures 2C, 2F, 2I, 2L)** and MC38 PBRM1$^{-/-}$ **(Figures 2B, 2E, 2H)** showing resistance to four compounds except for AU-1530 sensitivity to MC38 PBRM1$^{-/-}$ **(Figure 2K)** but SI < 2 (Selectivity Index = IC50 WT / IC50 KO). On the other hand, HCT116 PBRM1$^{-/-}$ **(Figures 2A, 2D, and 2G)** shows sensitivity to PARPi (olaparib and rucaparib) and AU-24118 where SI>2 but AU-1530 shows resistance. Taken

202 together, these results demonstrate that PBRM1 deficiency confers selective sensitivity to PARP
203 inhibitors in HCT116 human colorectal carcinoma cells, supporting a synthetic lethal interaction
204 between PBRM1 loss and PARP inhibition.

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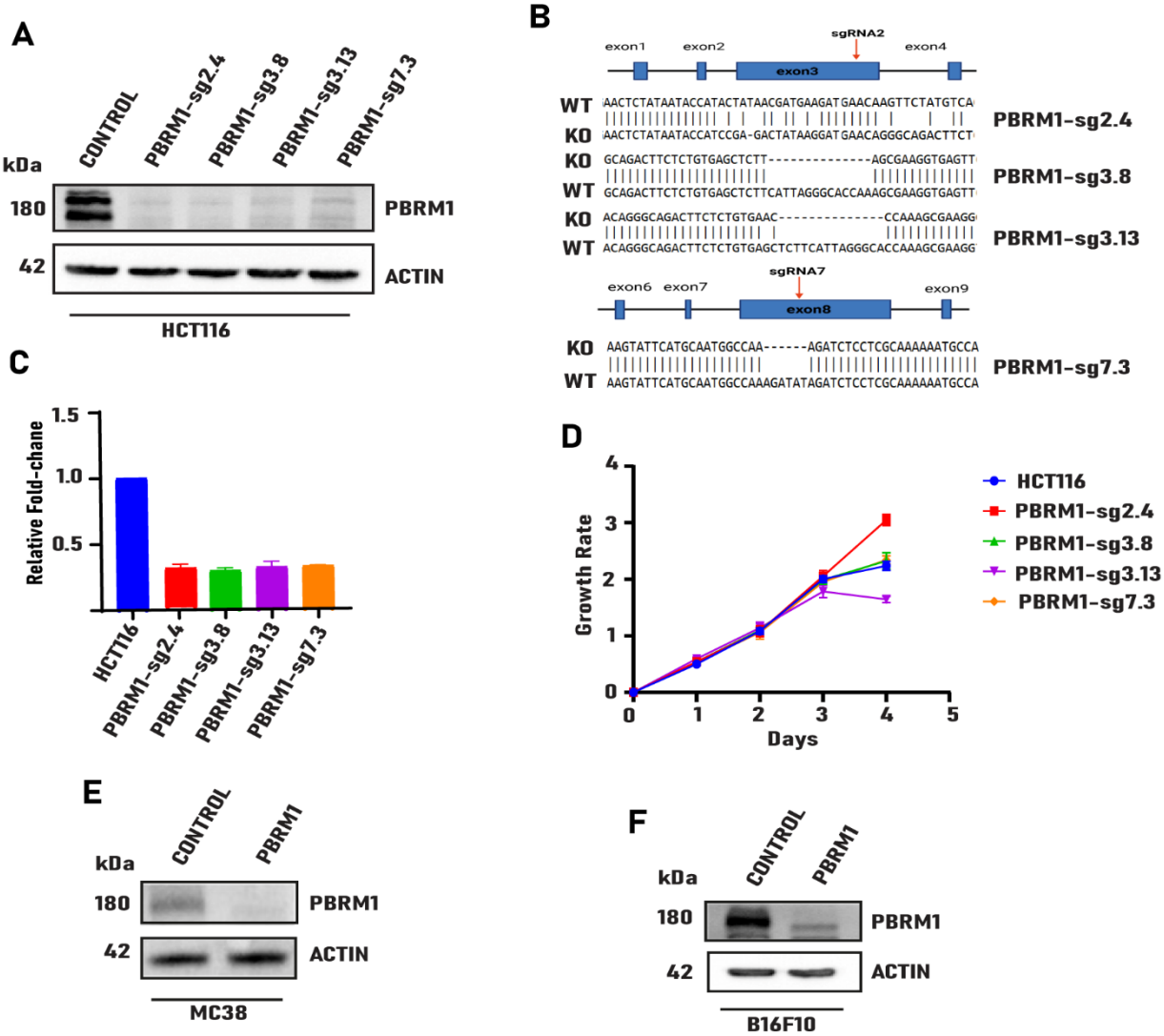

FIGURE 1: Confirmation of PBRM1 knockout. **(A)** The successful knockout of PBRM1 was demonstrated by western blot, which showed significant reduction of protein in the four isogenic clones (sg2.4, sg3.8, sg3.13, sg7.3) compared to WT. **(B)** The Sanger sequencing of the PBRM1 locus in HCT116 PBRM1$^{+/+}$ and PBRM1$^{-/-}$ cells. **(C)** PBRM1 KO was verified with qPCR analysis. **(D)** The growth rate of HCT116 isogenic cell pairs was measured by using CCK-8. **(E-F)** KO confirmation by WB of PBRM1 in the MC38 & B16F10 cell lines.

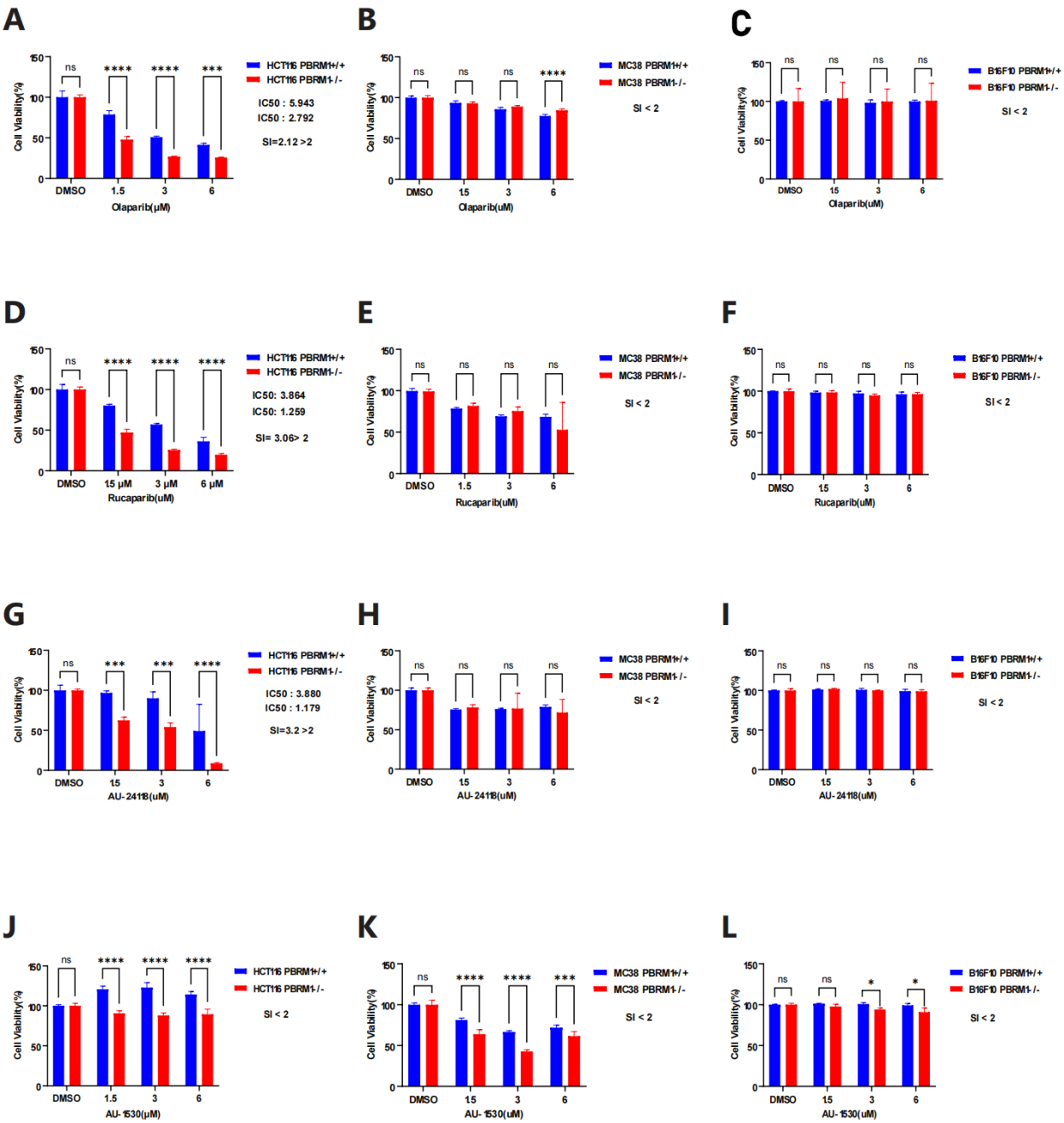

FIGURE 2: HCT116 PBRM1$^{-/-}$ cells show sensitivity to olaparib, rucaparib, and AU-24118 where selectivity index (SI) > 2 (SI = IC50PBRM1$^{+/+}$/IC50PBRM1$^{-/-}$) **(A-C)** Olaparib showing sensitivity to HCT116 PBRM1$^{-/-}$ cells where MC38 PBRM1$^{-/-}$ and B16F10 PBRM1$^{-/-}$ cells show resistance. **(D-F)**

Rucaparib **(G-I)** AU-24118 **(J-L)** AU-1530. Error bars represent s.d. (n = 9) from three independent experiments. Two-way ANOVA *P < 0.05; **P < 0.01; ***P < 0.001; **** P < 0.0001; ns, not significant.

PBRM1 Deficiency Selectively Sensitizes Colorectal Cancer Cells to PARP Inhibition but not to Multi-Target Chromatin Remodeler Inhibition in Clonogenic Survival Assays

Colony formation assays reveal that PBRM1 loss markedly sensitizes HCT116 colorectal cancer cells to PARP inhibitors, but not to the multi-target chromatin remodeler inhibitor AU-24118. Treatment with olaparib **(Figure 3A-B)** resulted in a dose-dependent reduction in clonogenic survival, with PBRM1$^{-/-}$ cells exhibiting near-complete suppression at 6 μM, whereas wild type HCT116 cells remained relatively resistant. Similarly, rucaparib **(Figure 3C-D)** profoundly impaired colony formation in PBRM1-deficient cells across all concentrations, with minimal survival at ≥3 μM. In contrast, the multi-target inhibitor AU-24118, which targets PBRM1, SMARCA2, and SMARCA4 **(Figure 3E-F)**, potently reduced colony formation in both wild-type and knockout cell lines to almost a similar extent, indicating that its efficacy is independent of PBRM1 status. Quantitative analysis of surviving fractions confirms that PBRM1-deficient cells are significantly more sensitive to olaparib and rucaparib, particularly at lower doses, while AU-24118 showed the same activity in both genetic backgrounds. These findings suggest that PBRM1 deficiency specifically confers hypersensitivity to PARP inhibition. This supports the potential of

226 PBRM1 loss as a predictive biomarker for PARP inhibitors while emphasizing distinct mechanistic
227 vulnerabilities.

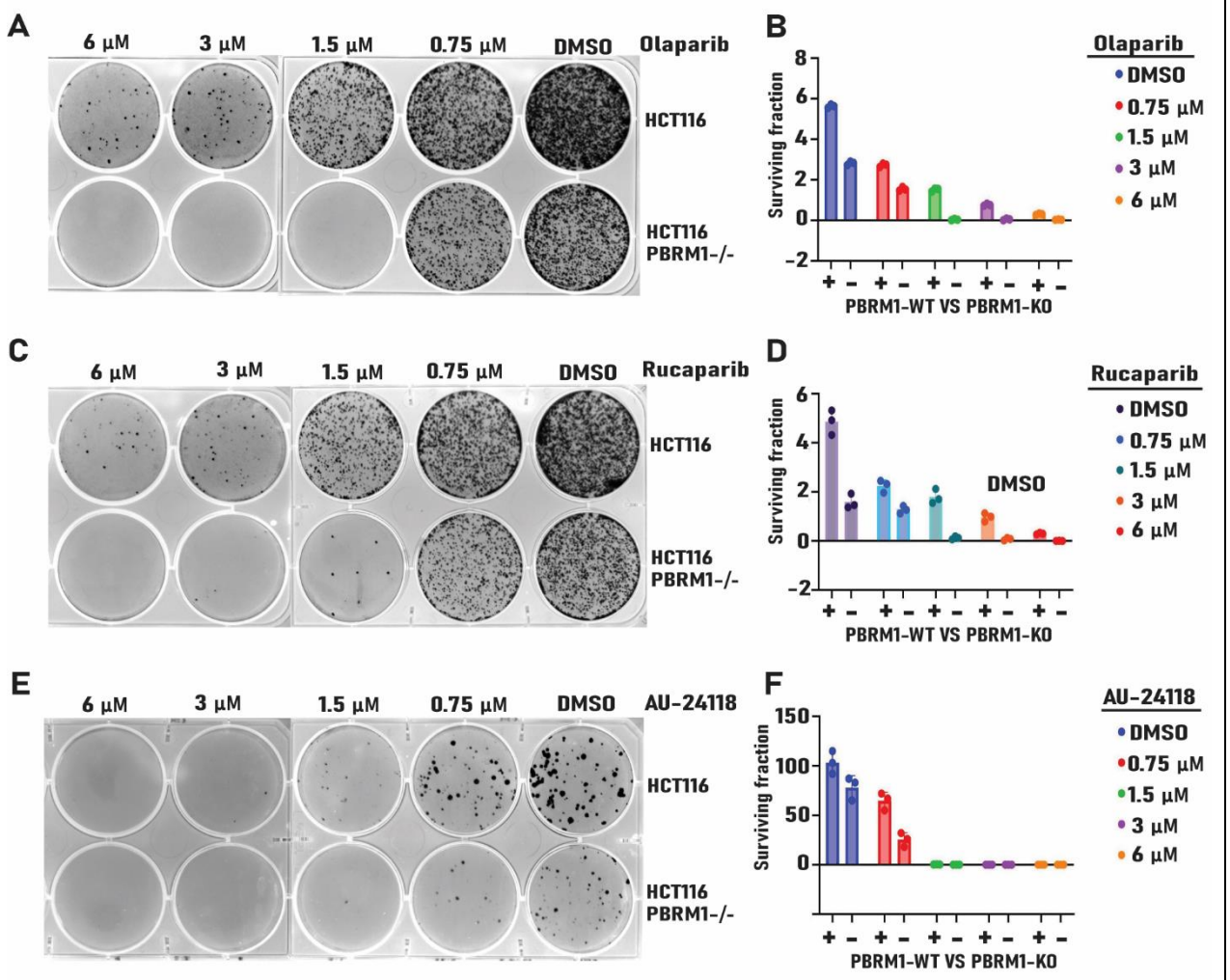

FIGURE 3: PBRM1 deficiency selectively sensitizes colorectal cancer cells to PARP inhibition. **(A-B)** Olaparib treatment reduces colony formation with enhanced sensitivity in PBRM1-deficient HCT116 cells. **(C-D)** Rucaparib impairs clonogenic survival preferentially in PBRM1$^{-/-}$ HCT116 cells. **(E-F)** The multi-target inhibitor AU-24118 suppresses colony formation in both wild-type and PBRM1$^{-/-}$ HCT116 cells. Error bars represent s.d. (n = 3) from three independent experiments.

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PBRM1 Loss Sensitizes Cells to PARP Inhibitor-Induced G2/M Arrest but not to Multi-Target Chromatin Inhibition

Cell cycle analysis reveals that genetic ablation of PBRM1 specifically alters the response to PARP inhibitors, inducing a pronounced G2/M phase arrest(Dale Rein et al., 2015), while the response to a multi-target chromatin remodeler inhibitor remains consistent between genotypes. Treatment with olaparib **(Figures 4A-B)** resulted in a dose-dependent accumulation of PBRM1$^{-/-}$ cells in the G2/M phase, with a corresponding decrease in the G1 and S phase populations, indicating a robust arrest at the G2/M checkpoint. Parental HCT116 cells exhibited a markedly less severe G2/M accumulation under the same treatment. A nearly identical pattern was observed with rucaparib **(Figures 4C-D)**, where PBRM1$^{-/-}$ cells displayed a significant increase in the G2/M fraction, reinforcing that PBRM1 deficiency creates a specific vulnerability to PARP inhibitor-mediated cell cycle disruption. In striking contrast, the multi-target inhibitor AU-24118, which targets PBRM1, SMARCA2, and SMARCA4 **(Figures 4E-F)**, elicited a profoundly different cell cycle outcome. Both wild-type and PBRM1$^{-/-}$ cells responded to AU-24118 in the same way. The quantitative data confirm that the hypersensitivity of PBRM1-deficient cells is uniquely linked to the efficacy of PARP inhibitors in provoking a G2/M arrest, a phenotype classically associated with unrepaired DNA damage and checkpoint activation. Conversely, AU-24118's activity bypasses this specific vulnerability. These results delineate a clear mechanistic divergence: PBRM1 loss selectively predisposes cells to PARP inhibitor toxicity by exacerbating a DNA damage-induced G2/M checkpoint arrest. This underscores PBRM1's functional role in the DNA damage response and validates its loss as a specific biomarker for sensitivity to PARP inhibition.

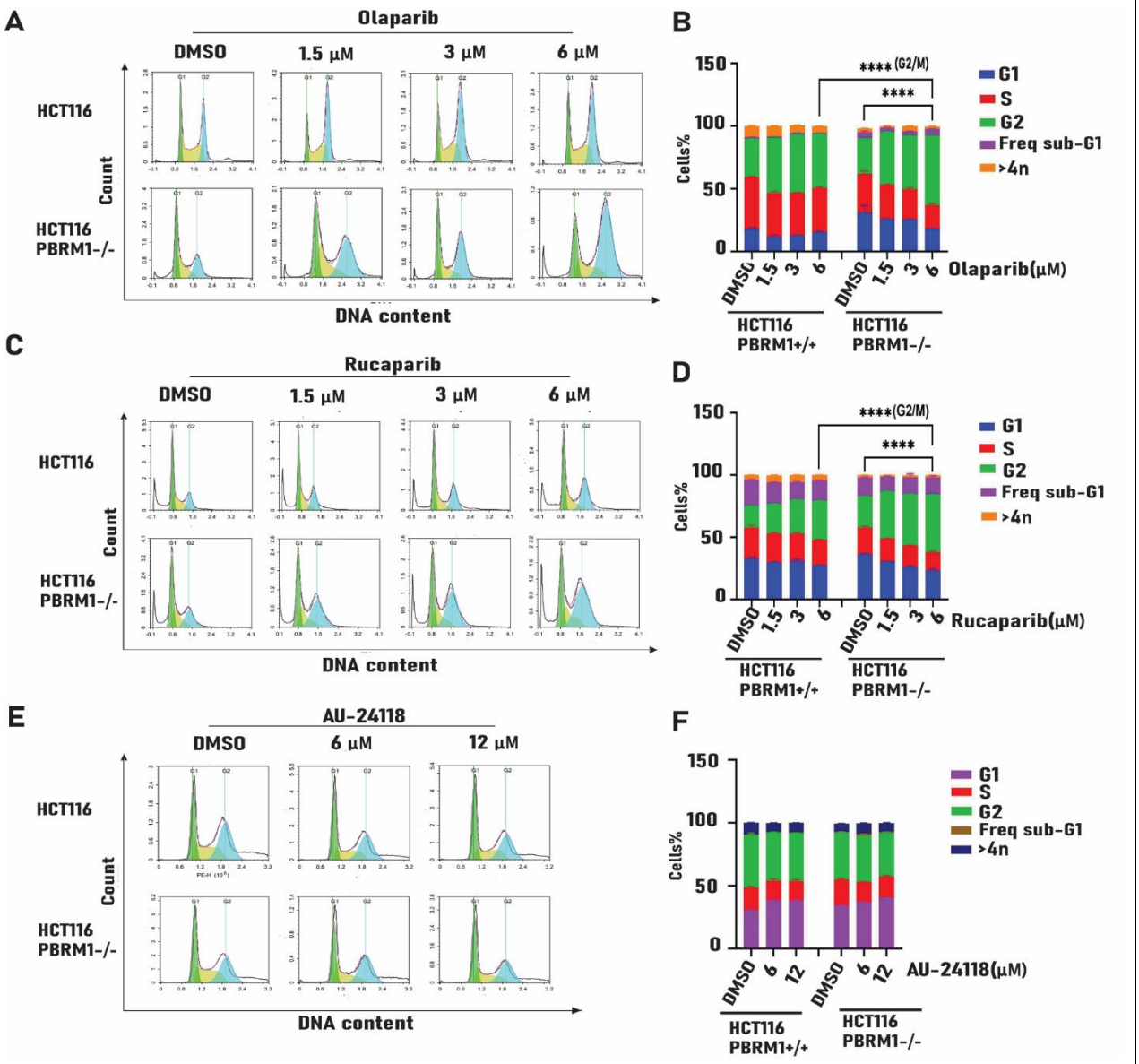

FIGURE 4: PARP inhibitors induce G2/M cell cycle arrest. **(A-B)** Olaparib induces G2/M cell cycle arrest specifically in PBRM1-deficient HCT116 cells. (**C-D)** Rucaparib triggers a pronounced G2/M arrest in PBRM1$^{-/-}$ HCT116 cells. **(E-F)** The multi-target inhibitor AU2-4118 did not respond to both wild-type and PBRM1$^{-/-}$ HCT116. Data are mean ± SD of three independent experiments. ****P < 0.0001, t-test.

Loss of PBRM1 Enhances Apoptotic Sensitivity to Olaparib in HCT116 Cells

Here, our experimental results suggest a link between PBRM1 loss and the enhanced sensitivity to olaparib, supporting the hypothesis of synthetic lethality. To determine how PBRM1 deficiency alters the response of colorectal cancer cells to PARP inhibition, we compared olaparib-treated PBRM1-knockout HCT116 cells with olaparib-treated wild-type HCT116 cells and with DMSO controls. The volcano plot (**Figure 5A)** demonstrates broad differential gene expression, with several genes selectively upregulated in the knockout background, indicating that PBRM1 loss changes the cellular response to PARP inhibition rather than simply reflecting baseline differences. This transcriptional shift is further supported by the enrichment analysis in **Figure 5B**, where pathways related to the mitotic G2/M transition checkpoint, cell-cycle checkpoint signaling, DNA replication, and establishment of mitotic spindle localization are enriched in olaparib-treated PBRM1-KO cells compared with olaparib-treated wild-type cells. These findings are consistent with checkpoint activation and impaired cell-cycle progression, suggesting a G2/M arrest-like state in the PBRM1-deficient background. In **Figure 5C**, gene ontology(GO) biological process analysis of olaparib-treated PBRM1-KO cells relative to DMSO-treated PBRM1-KO controls further illustrates enrichment of DNA repair, DNA damage signaling, DNA integrity checkpoint signaling, DNA replication, double-strand break repair, recombinational repair, and mitotic G2/M transition checkpoint pathways. Similarly, **Figure 5D** confirms enrichment of pathways linked to DNA-dependent DNA replication, negative regulation of cell-cycle progression, and checkpoint control. Together, Figure 5 indicates that olaparib imposes greater DNA replication stress in PBRM1-deficient cells, leading to enhanced checkpoint engagement and defective progression through G2/M.

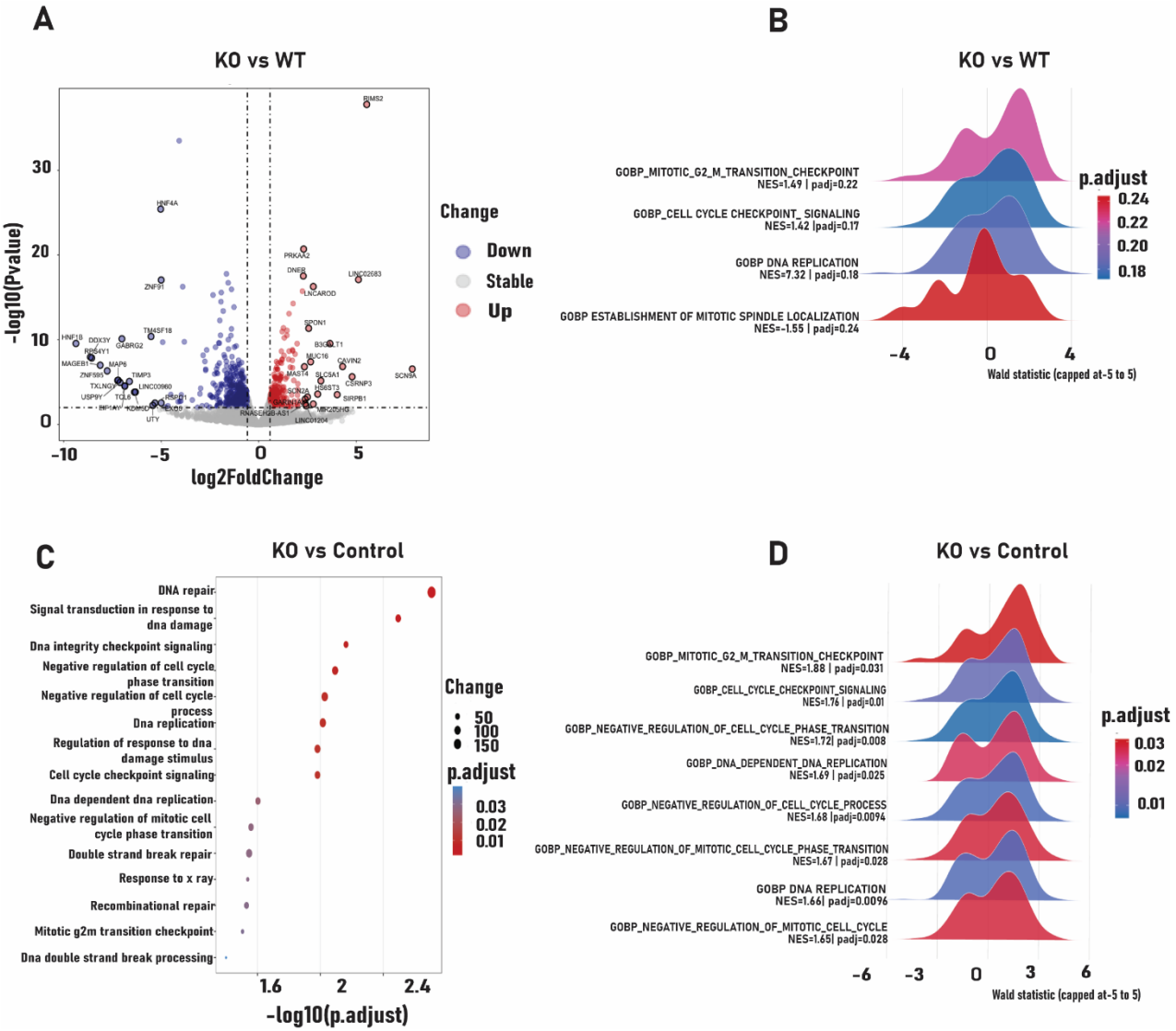

FIGURE 5: PBRM1 loss reshapes the transcriptional response to olaparib in HCT116 cells, with enrichment of cell-cycle, checkpoint, and DNA replication pathways. **(A)** Volcano plot of differentially expressed genes in olaparib-treated PBRM1-knockout HCT116 cells versus olaparib-treated PBRM1-wild-type HCT116 cells. **(B)** GSEA ridge plot showing enrichment of cell-cycle, mitotic checkpoint, and DNA replication pathways in olaparib-treated PBRM1-knockout HCT116 cells compared with olaparib-treated PBRM1-wild-type cells. **(C)** GO biological process enrichment analysis of genes altered in olaparib-treated PBRM1-knockout HCT116 cells relative to DMSO-treated PBRM1-knockout controls. **(D)** Pathway enrichment analysis of olaparib-responsive transcriptional changes in PBRM1-knockout HCT116 cells.

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To further demonstrates the functional consequence of altered stress response and support a model of synthetic lethality between PBRM1 loss and olaparib treatment, we generated heatmap. Olaparib-treated PBRM1-KO cells upregulate PRKAA2, CSRNP3, FAM111B, MANCR, PURPL, DKK1, PIK3R1, CLCA2, and other genes associated with cell-cycle disruption and replication stress **(Figure 6A)**. Transcription factor-level analysis **(Figure 6B)** further reveals enrichment of the CSRNP_N family in the PBRM1-KO group, suggesting activation of a distinct stress-responsive transcriptional program. Morphologically, **Figure 6C** shows that under DMSO both wild-type and PBRM1-KO cells maintain better adherent growth, whereas olaparib treatment leads to increased cell rounding, detachment, and reduced confluence, most prominently in the PBRM1-KO cells, consistent with increased apoptosis. This is confirmed in **Figure 6D**, where flow cytometry reveals a greater apoptotic fraction in olaparib-treated PBRM1-KO cells than in olaparib-treated wild-type cells, especially at higher olaparib concentrations. Finally, **Figure 6E** validates the RNA-seq findings by qPCR, showing significant CSRNP3 upregulation in PBRM1-KO cells following olaparib exposure.

Collectively, these results indicate that PBRM1 loss creates a vulnerability to PARP inhibition, such that olaparib triggers unresolved DNA replication stress, stronger G2/M checkpoint activation, and ultimately enhanced apoptotic cell death, consistent with synthetic lethality in PBRM1-deficient HCT116 cells.

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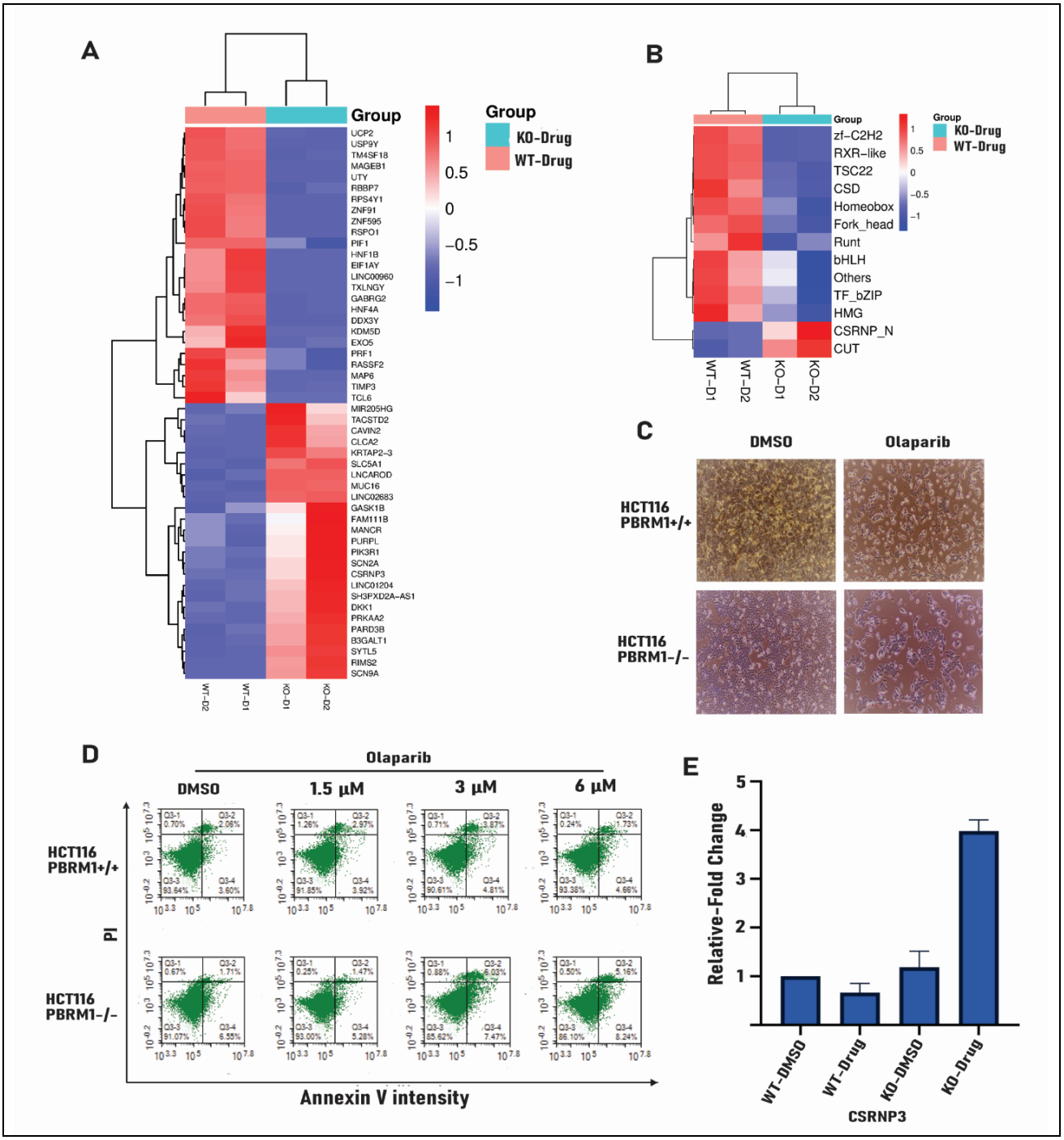

FIGURE 6: Synthetic lethality induced by olaparib in PBRM1-deficient cells. **(A)** Heatmap results showing the upregulation of CSRNP3, CLCA2, and other cell cycle and DNA replication stress-related genes in PBRM1 KO cells following olaparib treatment. **(B)** RNA-seq heatmap showing upregulation of the CSRNP-N transcription factors family in the PBRM1 KO cell treatment group versus the WT group. **(C)** Live cell imaging demonstrating increased apoptosis in PBRM1 KO cells treated with olaparib compared to DMSO-treated controls. **(D)** Flow cytometry analysis reveals increased apoptosis in PBRM1-KO cells treated with olaparib compared to wild-type cells. **(E)** qPCR analysis showing significant upregulation of CSRNP3 in PBRM1 KO cells treated with olaparib compared to other conditions.

Discussion

Our study demonstrates a novel synthetic lethality interaction between PBRM1 deficiency and PARP inhibitors, particularly olaparib and rucaparib, in human colorectal carcinoma cells. PBRM1, a critical component of the SWI/SNF chromatin remodeling complex, is frequently mutated or deleted in various cancers, including clear cell renal cell carcinoma (ccRCC) and other aggressive malignancies(Yao et al., 2023). Although its frequency, treatment approaches aimed at PBRM1-deficient malignancies are yet inadequately explored(Aili et al., 2021).

Our experimental investigations here revealed that the absence of PBRM1 enhances the sensitivity of colorectal cancer cells to PARP inhibitors, indicating a potential strategy for targeted therapy in this specific patient population(Zhang et al., 2025). The synthetic lethality identified between PBRM1 and PARP inhibition in our research is corroborated by the increased susceptibility of PBRM1-deficient HCT116 cells to both olaparib and rucaparib. This was seen in decreased clonogenic survival(Lai et al., 2022), since PBRM1 mutant cells demonstrated nearly total suppression at lower concentrations of PARP inhibitors(Fang et al., 2018). The detected hypersensitivity was corroborated in G2/M cell cycle arrest experiments, wherein PBRM1-deficient cells exhibited a significant accumulation in the G2/M phase, indicative of unrepaired

DNA damage(Feng et al., 2022). The G2/M checkpoint arrest has been previously associated with DNA damage response and PARP inhibition, further corroborating our findings that PBRM1 deficiency intensifies DNA damage signaling(Fernando et al., 2021). The multi-target chromatin remodeler inhibitor AU-24118(He et al., 2024), which also targets PBRM1, did not demonstrate the same genotype-specific effects as the PARP inhibitors. Both wild-type and PBRM1-deficient cells exhibited comparable sensitivity to AU-24118, indicating that its efficacy is independent of PBRM1 status. This distinction underscores the specificity of the synthetic lethality between PBRM1 and PARP inhibitors(Helleday, 2011), indicating that targeting the PBRM1 pathway with PARP inhibitors may represent a more efficacious therapeutic approach than utilizing broad-spectrum chromatin remodeling inhibitors.

Furthermore, our results indicate that PBRM1-deficient cells demonstrate increased apoptotic sensitivity to olaparib, an effect that is absent in wild-type cells. Flow cytometric analysis demonstrated elevated rate of apoptosis in PBRM1 mutant cells subjected to olaparib treatment relative to controls. The RNA-seq analyses indicated the activation of stress response genes, such as CSRNP3, in PBRM1-deficient cells subjected to olaparib treatment, demonstrating the cellular reaction to genotoxic stress. The up-regulation indicates a possible mechanism by which PBRM1-deficient cells exhibit increased susceptibility to DNA damage-induced apoptosis upon treatment with PARP inhibitors. The greater vulnerability to olaparib exemplifies synthetic lethality, wherein the absence of PBRM1 collaborates with olaparib's PARP inhibition to provoke cell death in the lack of DNA repair capability(Gu et al., 2022).

In summary, our findings demonstrate a synthetic lethal interaction between the loss of PBRM1 and olaparib treatment, resulting in enhanced apoptosis and the upregulation of stress response genes, thereby underscoring the therapeutic potential of employing PARP inhibitors such as

olaparib in PBRM1-deficient tumors. The current investigation contributes to the expanding body of information indicating that synthetic lethality may serve as an effective approach for targeting malignancies characterized by specific genetic anomalies, such as PBRM1 mutations(Kaelin Jr, 2005). However, additional investigation is necessary to assess the therapeutic relevance of PARP inhibitors in individuals with PBRM1-deficient malignancies. Clinical trials should focus on assessing the therapeutic efficacy of PARP inhibitors, especially in colorectal and renal malignancies. In addition, the identification of biomarkers, such as CSRNP3, that predict sensitivity to PARP inhibitors could enhance patient stratification and treatment outcomes. It is essential to investigate combination medicines that address both PBRM1 deficiency and alternative DNA repair mechanisms. Integrating PARP inhibition with drugs that intensify replication stress or DNA damage may enhance treatment efficacy. The advancement of precision medicines that exploit genetic vulnerabilities, such as PBRM1 deficiency, presents promising prospects for enhancing treatment outcomes in tumors typically resistant to standard therapies.

Conclusions

In conclusion, our work identifies a novel synthetic-lethal vulnerability in colorectal cancer. Across isogenic and complementary in-vitro models, clinically used PARP inhibitors, especially olaparib and rucaparib, selectively suppressed clonogenic growth of PBRM1-deficient cells, consistent with heightened DNA damage signaling. Mechanistically, PARP inhibition intensified G2/M checkpoint arrest and promoted apoptosis in the absence of PBRM1, accompanied by reactivation of stress-response genes such as CSRNP3. In contrast, multi-target chromatin remodeler inhibition lacked genotype specificity. These findings support PBRM1 as a predictive

biomarker and motivate clinical evaluation plus biomarker-guided combination strategies to improve patient stratification and overcome therapeutic resistance.

Declarations

List of abbreviations

PBRM1	Polybromo 1
PARP	Poly(ADP-ribose) polymerase
SWI/SNF	SWItch/Sucrose non-fermentable
BAF	BRG1/BRM-associated factor
CCK-8	Cell counting kit-8
CSRNP3	Cysteine-serine-rich nuclear protein 3(gene)
VHL	Von Hippel–Lindau (gene)

Consent for publication

Not applicable. The study does not include identifiable individual data.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Z.W conceived and supervised the investigation, as well as manuscript preparation. M.G revised the manuscript. S.C designed oligonucleotide and annealed for PBRM1 knockout cell preparation, Z.X prepared RNA-Seq sample, H.Q analyzed the RNA-Seq results, and M.T.I performed all other experiments and prepared the paper. All authors have read and approved the manuscript for publication.

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