Supplementary Information

PCAT-1, a long noncoding RNA, regulates BRCA2 and controls homologous recombination in cancer

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MATERIALS AND METHODS

Cell lines

All cell lines were obtained from the American Type Culture Collection (Manassas, VA). Cell lines were maintained using standard media and conditions. Du145-derived cell lines were maintained in DMEM supplemented with 10% FBS (Invitrogen) and 1% penicillin-streptomycin (Invitrogen) in a 5% CO_2 cell culture incubator. RWPE-derived cell lines were maintained in KSF (Invitrogen) supplemented with Bovine Pituitary Extract, Epidermal Growth Factor and 1% penicillin-streptomycin in a 5% CO_2 cell culture incubator. LNCAP-derived and PC3-derived were maintained in RPMI 1640 (Invitrogen) supplemented with 10% FBS and 1% penicillin-streptomycin in a 5% CO_2 cell culture incubator.

PC3 cells containing the GFP HR assay construct were generated as described previously (23, 24).

PCAT-1 or control-expressing cell lines were generated by cloning *PCAT-1* or control *LacZ* into the pLenti6 vector (Invitrogen). After confirmation of the insert sequence, lentiviruses were generated at the University of Michigan Vector Core and transfected into RWPE or Du145 cells. Stably-transfected cells were selected using blasticidin (Invitrogen).

For LNCAP cells with stable knockdown of *PCAT-1*, cells were seeded at 50-60% confluency, incubated overnight, and transfected with *PCAT-1* or non-targeting shRNA lentiviral constructs for 48 hours. GFP+ cells were drug-selected using 1 ug/mL puromycin. *PCAT-1* shRNAs were

customed generated by Systems Biosciences using the following sequences: shRNA 1 GCAGAAACACCAAUGGAUAUU; shRNA 2 AUACAUAAGACCAUGGAAAU.

Bisulfite sequencing

Genomic DNA was isolated from cultured cells or tissues using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions. Bisulfite conversion was carried out using an EZ DNA methylation gold kit (Zymo Research) according to the manufacturer's instructions. Briefly, 1.0 ug of genomic DNA from either cultured cells in a 20-μL volume was mixed with 130 μL of CT conversion reagent and was initially incubated for 10 min at 98°C followed by incubation for 2.5 h at 64°C. M-biding buffer (600 μL) was added to the above reaction and DNA was purified using a Zymo spin column. Sequential washes were performed with 100 μL of M-Wash buffer, 200 μL of M-sulphonation buffer, and 200 μL of M-wash buffer was carried out before eluting the DNA in 30 μL of M-elution buffer. Purified DNA (2 μL) was used as template for PCR reactions with primers (Integrated DNA Technologies) and synthesized according to bisulfite-converted DNA sequences for the regions of interest using the Methprimer software. The PCR product was gel-purified and cloned into the pCR4 TOPO TA sequencing vector (Life Technologies). Plasmid DNA isolated from 10 colonies from each sample was sequenced by conventional Sanger Sequencing (University of Michigan DNA Sequencing Core).

Patient samples and xenografts

For the University of Michigan patient samples, prostate tissues were obtained from the radical prostatectomy series and Rapid Autopsy Program at the University of Michigan tissue core. These programs are part of the University of Michigan Prostate Cancer Specialized Program Of

Research Excellence (S.P.O.R.E.). All tissue samples were collected with informed consent under an Institutional Review Board (IRB) approved protocol at the University of Michigan. (SPORE in Prostate Cancer (Tissue/Serum/Urine) Bank Institutional Review Board # 1994-0481). For the Weill Cornell Medical College patient samples, prostate tissues were collected as part of an IRB approved protocol at Weill Cornell Medical College. The samples derived from patients with localized or locally advanced disease that were treated with radical prostatectomy as monotherapy. H&E slides of frozen tissue blocks were examined by the study's pathologists (M.A.R.) to select for high-density cancer foci with <10% stroma or other contaminating noncancerous material. For human prostate cancer xenografts, nucleic acid material was obtained for 20 primary prostate cancer xenografts developed at Dr. Nora Navone's lab. Small pieces of the human prostate cancer samples were implanted subcutaneously in 6- to 8- week old male CB17 SCID mice (Charles River Laboratories). Developing tumors were serially passaged in mouse as the cells did not sustain *in vitro* growth. Written informed consent had been obtained from the patient before sample acquisition and sample processing according to a protocol approved by the University of Texas, MD Anderson Cancer Center IRB.

Whole genome amplification

Genomic DNA from tissue samples was isolated using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions as previously described (25). DNA was quantified by a Nanodrop spectrophotometer (Thermo-Scientific). In cases with limiting amounts of sample material, 50 ng of amplified using the Genomeplex-Complete Whole Genome Amplification Kit (Sigma-Aldrich) according to the manufacturer's instructions. The final whole genome amplified DNA was purified by AMPure XP beads (Beckman-Coulter) and

quantified by a Nanodrop spectrophotometer. 15 ng of gDNA were used for each genotyping PCR assay using High-Fidelity Taq Polymerase (Invitrogen) in a 40-cycle PCR amplification with the following cycling conditions: 94C for 30 seconds, 56C for 30 seconds, 68C for 1 minute).

Cell line treatments

Cells were plated in 6-well plates and allowed to grow for 24 hours. For radiation experiments, after 24 hours, cells were subjected to 2 Gray (Gy), 4 Gy, or 6 Gy of radiation (Philips RT250, Kimtron Medical). RAD51 and γ-H2AX staining was performed 6 hours or 24 hours post-treatment, and RNA was collected 24 hours post-treatment.

For Olaparib or ABT-888 treatments, cells were treated with the indicated doses of drug or DMSO control. RAD51 and γ-H2AX staining was performed 6 hours post-treatment, and RNA was collected 24 hours post-treatment.

For epigenetic drug treatments, RWPE-*LacZ* and RWPE-*PCAT-1* cells were treated with 20uM 5'deoxyazacytidine (Sigma), 500 nM HDAC inhibitor Trichostatin A (TSA) (Sigma), or both 5'deoxyazacytidine and TSA as previously described (15). 5'deoxyazacytidine treatments were performed for 6 days with media and drug re-applied every 48 hours. TSA treatments were performed for 48 hours. DMSO treatments were performed for 6 days.

RNA isolation; cDNA synthesis; and PCR experiments

Total RNA was isolated using Trizol and an RNeasy Kit (Invitrogen) with DNase I digestion according to the manufacturer's instructions. RNA integrity was verified on an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA). cDNA was synthesized from total RNA using Superscript III (Invitrogen) and random primers (Invitrogen). Quantitative Real-time PCR (qPCR) was performed using Power SYBR Green Mastermix (Applied Biosystems, Foster City, CA) on an Applied Biosystems 7900HT Real-Time PCR System.

The relative quantity of the target gene was completed for each sample using the ΔΔCt method by the comparing mean Ct of the gene to the average Ct of the geometric mean of two housekeeping genes, *GAPDH* and *HMBS*. All oligonucleotide primers were synthesized by Integrated DNA Technologies (Coralville, IA). The primer sequences for the transcript analyzed are provided in **Supplementary Table S1**.

Xenograft experiments

Xenografting was performed according to published protocols (26). Five week-old male SCID mice (CB.17. SCID), were purchased from Charles River, Inc. (Charles River Laboratory, Wilmington, MA). 1 x 10^6 Du145-control or Du145-*PCAT-1* stable cells were resuspended in 100µl of saline with 50% Matrigel (BD Biosciences, Becton Drive, NJ) and were implanted subcutaneously into the left and right flank regions of the mice. Mice were anesthetized using a cocktail of xylazine (80-120 mg/kg, IP) and ketamine (10mg/kg, IP) for chemical restraint before tumor implantation. All tumors were staged for two weeks before starting the drug treatment. At the beginning of the third week, mice with tumors (10 tumors per treatment group, average size 150-200 mm3) were treated with Olaparib (100mg/kg, IP twice daily five times per week) or an

equal volume of DMSO control. Olaparib was obtained from Axon Medchem (Groningen, The Netherlands). Growth in tumor volume was recorded weekly by using digital calipers and tumor volumes were calculated using the formula (π/6) (L × W2), where L = length of tumor and W = width. Loss of body weight during the course of the study was also monitored weekly; all changes in mouse weights occurred within tolerable limits as set by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. At the end of the xenograft studies, mice were sacrificed, and mouse xenograft tumors were subsequently harvested, maintained in formalin, and subsequently embedded in paraffin. All procedures involving mice were approved by the UCUCA at the University of Michigan and conform to their relevant regulatory standards.

Immunofluorescence

1 x 10^5cells were plated on sterile coverslips in 12-well plates and cultured for 24 hours. After treated for 6 hour or 24 hour, cells were fixed with 4% paraformaldehyde for 15min at room temperature, and then permeabilized with 0.5% Triton X-100 for 20min. Cells were blocked for 1 hour in blocking buffer (0.5% BSA, 2.5% goat serum and 0.05% Triton X-100 in PBS), and then incubated with primary antibody against Rad51(Santa Cruz sc-8349) at 1:400 or gamma-H2AX (Millipore JBW301) at 1:1,000, followed by secondary antibody Alexa Fluor 488 goat anti-rabbit IgG(Invitrogen A11008) or Alexa Fluor 594 goat anti-mouse IgG(Invitrogen A11005) at 1:2,000. Nuclei were stained with DAPI(4',6-diamidino-2-phenylindole)(Invitrogen P36931) and coverslips were kept in dark overnight. The cells were observed and images were recorded by fluorescent microscope (Olympus 1 X 71), with the software Olympus DP Controller 2002 (Olympus Optical Co. Ltd). A minimum of 100 cells were analyzed on each coverslip and the

number of foci on digital images was determined using the image analysis software package Image J (version 1.42, available from Research Services Branch of NIH). The quantitative results were shown as the average cell fraction with more than 5 or 10 foci of at least three independent experiments.

Luciferase Assays

The indicated cell lines were transfected with full length *BRCA2* luciferase constructs as well as pRL-TK vector as internal control for luciferase activity. Following 2 days of incubation, the cells were lysed and luciferase assays conducted using the dual luciferase assay system (Promega, Madison, WI, USA). Each experiment was performed in quadruplicate.

Immunoblot Analysis

Cells were lysed in RIPA lysis buffer (Sigma, St. Louis, MO) and briefly sonicated for homogenization. Aliquots of each protein extract were boiled in sample buffer, size fractionated by SDS-PAGE at 4C, and transferred onto Polyvinylidene Difluoride membrane (GE Healthcare, Piscataway, NJ). The membrane was then incubated at room temperature for 1-2 hours in blocking buffer [Tris-buffered saline, 0.1% Tween (TBS-T), 5% nonfat dry milk] and incubated at 4C with the appropriate antibody. Following incubation, the blot was washed 4 times with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibody. The blot was then washed 4 times with TBS-T and twice with TBS, and the signals visualized by enhanced chemiluminescence system as described by the manufacturer (GE Healthcare).

The following antibodies were used for immunoblot analysis: BRCA2 (EMD OP95), BRCA1 (Cell Signaling #9025S), XRCC1 (Abcam ab1838), XRCC3 (Abcam ab97390), XRCC4 (GeneTex GTX83406), Ku70 (BD Biosciences #611892), Ku80 (Cell Signaling #2180S), γ-H2AX (Cell Signaling #9718) and B-actin (Sigma A5441).

For immunoblot densitometry, the densitometric scan of the immunoblots was performed using ImageJ. Three replicate experiments were quantified for the final analysis.

I-SceI Homologous Recombination Assay

The assay was established previously (23, 24). PC-3 cells with a single copy of DR-GFP were transfected with empty vector control or *PCAT-1*. *PCAT-1* transfected cells were infected with adenovirus-encoded I-SceI (adeno-I-SceI). Cells were harvested 3 days after infection and subjected to flow cytometry analysis. The GFP-positive cell population was measured. A set of raw data were shown. The mean values were obtained from three independent experiments. The experiment was performed under the control of equal adenovirus infection. Adenovirus infection efficiency was examined in PC-3 cells prior to the HR assays. At MOI of 1000, the infection efficiency was close 100 % using control Adeno-GFP or Adeno-RFP. With the same MOI, we expect the same packaged Adeno-I-SceI expressed in almost all the cells. Each experiment has been performed at least three times. Little variation was observed from three independent experiments, suggesting the consistent adenovirus infection efficiency. Meanwhile, the cell viability was also examined under microscope and by trypan blue staining before adenovirus infection. All the groups showed more than >90% viability.

Chemosensitivity Assays

Five thousand cells were plated in each well of a 96-well plate in sets of ten. Cells were then treated with a single dose of Olaparib or ABT-888 as indicated for 72 hours. WST assays (Roche) were performed according to company protocol. Briefly, 10% WST was added to each well, the plates were incubated at 37C with 5% CO2 for 4 hours and the OD at 450nM was measured.

Cell cycle analysis

1 x 10^6 cells were plated on 12-well plates and cultured for 24 hours. Cells were collected and fixed with ice-cold 70% ethanol at -20C overnight. For analyzing cell cycle, fixed cells were centrifuged at 600g for 6min and washed with ice-cold PBS, resuspended in PBS containing 50ug/ml propidium iodide and 100ug/ml RNAse A (Invitrogen), then incubated at room temperature for at least 20min in dark. Samples were analyzed at the University of Michigan Flow Cytometry Core. Minimum of 10,000 cells were counted for each analysis.

Clonogenic survival assay

1 x 10^5 or 1 x 10^6 cells were plated on 6-well plates or 100mm dish and cultured for 24h. After treated with Radiation for 24 hours or PARP inhibitor (Olaparib or ABT-888) for 72 hours at indicated dose, cells were trypsinized and re-plated at different concentrations on 60mm dishes or Poly-D-Lysine (Sigma P6407) treated 6-well plates in fresh media. After cultured for 7-14 days, cell colonies were fixed ($V_{acetic\ acid}$:$V_{methonal}$=1:3) and stained with crystal violet. Colony number was counted for each condition in at least 3 independent experiments.

siRNA knockdown analysis

1 x 10^5 cells were plated on 6-well plates and allowed to attach overnight in serum-free media. Cells were transfected with siRNAs mixed with Optimem (Invitrogen) and Oligofectamine (Invitrogen) according to standard protocols for a final concentration of siRNA of 10nM. 24 hours post-transfection, media containing 10% FBS was added. RNA was harvested 48 hours later.

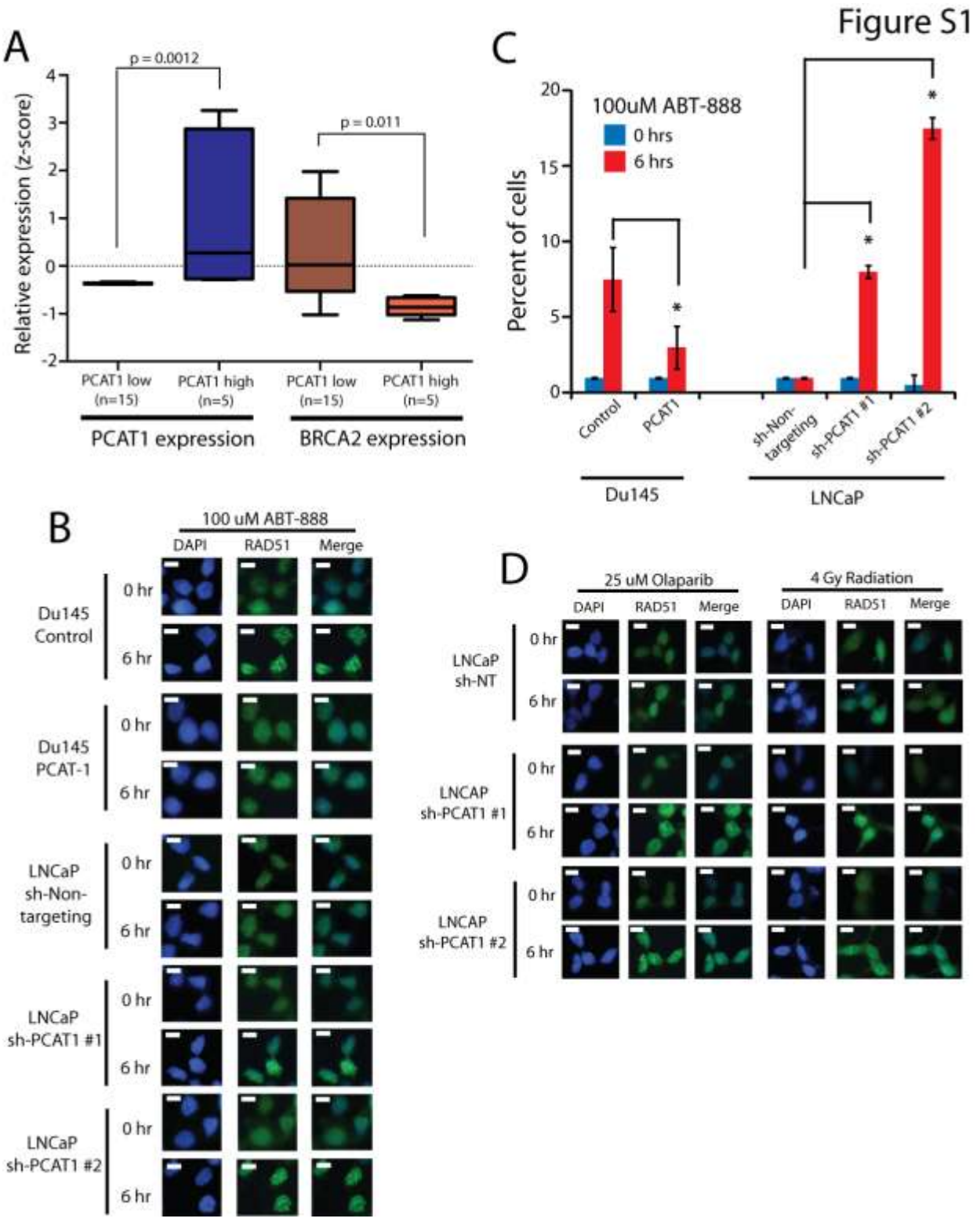
siRNAs used were: STAU1-5: GCAGGGAGUUUGUGAUGCA; STAU1-7: CGAGUAAAGCCUAGAAUCA; STAU1-8: CGGAUGCAGUCCACCUAUA

RNA stability assays

The EU labeling experiment was done according to the protocol of the Click-iT Nascent RNA Capture kit (Invitrogen). Briefly, cells were pulsed with 0.5 mM EU for 1 hour and then were chased for 5 or 10 hours. The total RNA was isolated and used in a copper catalyzed click reaction with azide-modified biotin. After this, the nascent transcripts were captured on streptavidin magnetic beads and cDNA synthesis was performed using Superscript VILO cDNA synthesis kit (Invitrogen) followed by analysis with qRT-PCR.

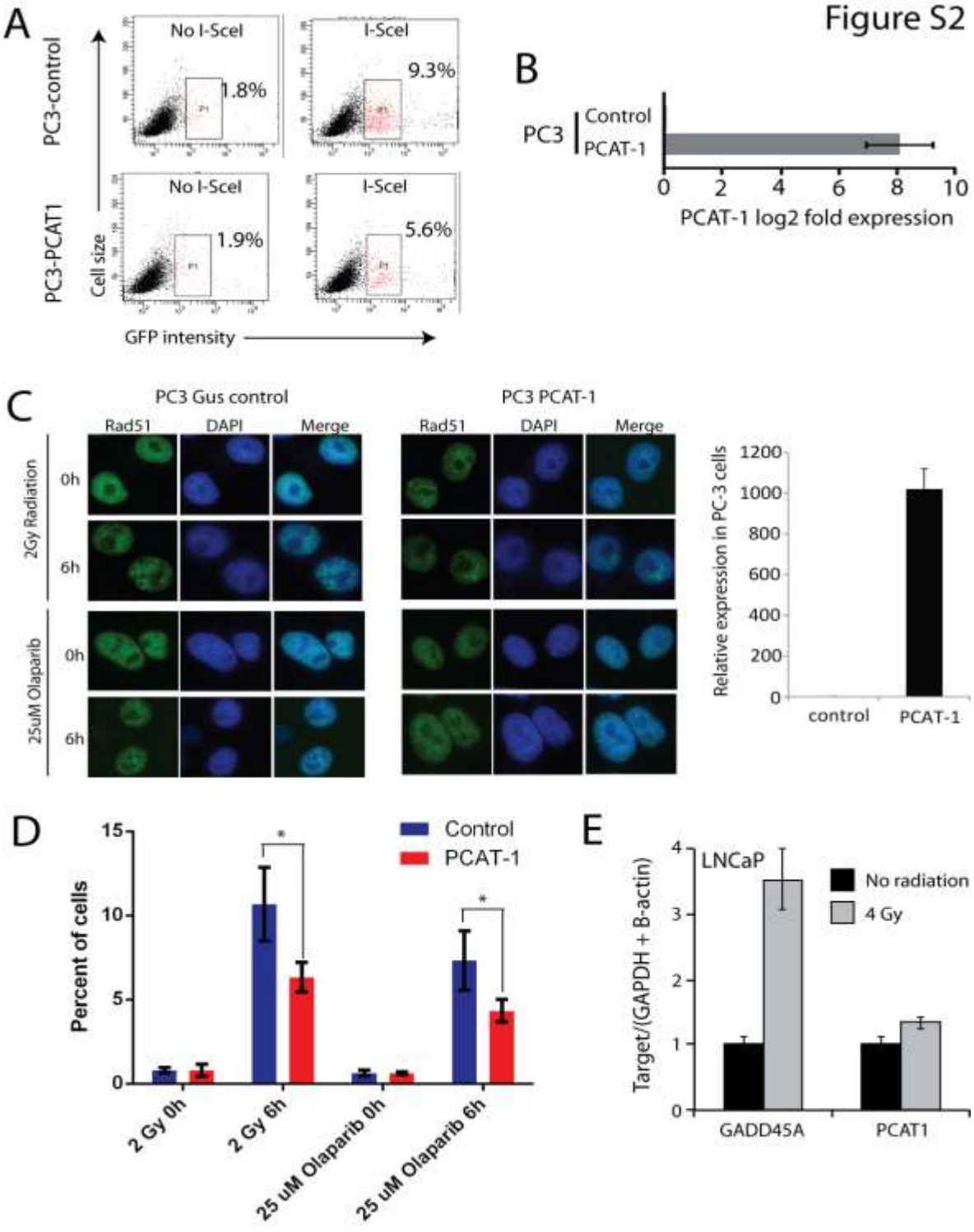
Statistical analyses for experimental studies

All data are presented as means ± standard deviation or S.E.M, as indicated. All experimental assays were performed in duplicate or triplicate. Statistical analyses shown in figures represent Fisher's exact tests or Student's t-tests, as indicated.



Supplementary Figure S1: Effect of PCAT-1 on RAD51 foci formation. A, Expression of PCAT-1 and BRCA2 in a cohort of 20 prostate cancer xenografts derived from prostate cancer patients. Expression is shown as z-scores and stratified by increasing *PCAT-1* expression. P

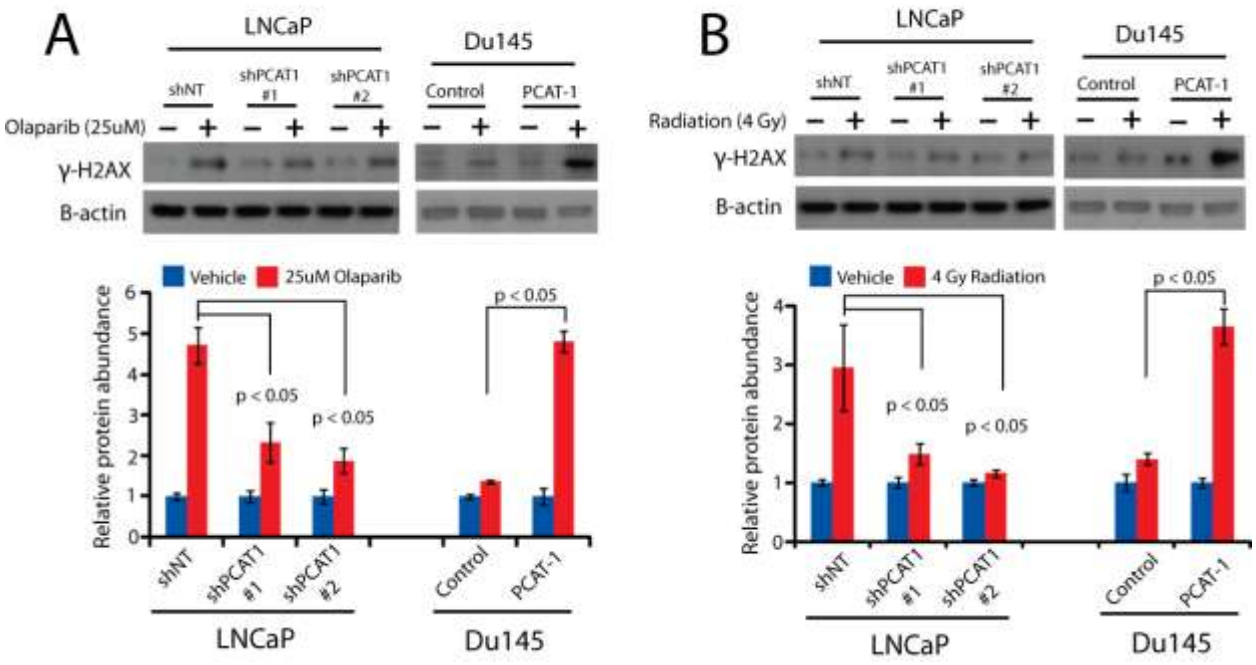
values are determined by a Mann-Whitney U test. **B,** Isogenic Du145-PCAT1 cells or LNCAP shPCAT1 knockdown cells were treated with DMSO control or 100uM ABT-888. After 6 hours, cells were fixed and stained for RAD51 and counterstained with DAPI. The number of RAD51 foci were counted. Immunofluorescence images for RAD51 foci post-ABT-888 treatment. **C**, Quantification of RAD51 foci post-treatment in the respective cell populations. Error bars indicate standard deviation. **D,** Isogenic LNCAP shNT, shPCAT1 #1, and shPCAT1 #2 cells were treated with 4 Gy of ionizing radiation or 25uM of Olaparib. After 6 hours, cells were fixed and stained for RAD51 and counterstained with DAPI. LNCaP cells with PCAT1 knockdown show an enhanced ability to form RAD51 foci when placed under genotoxic stress.



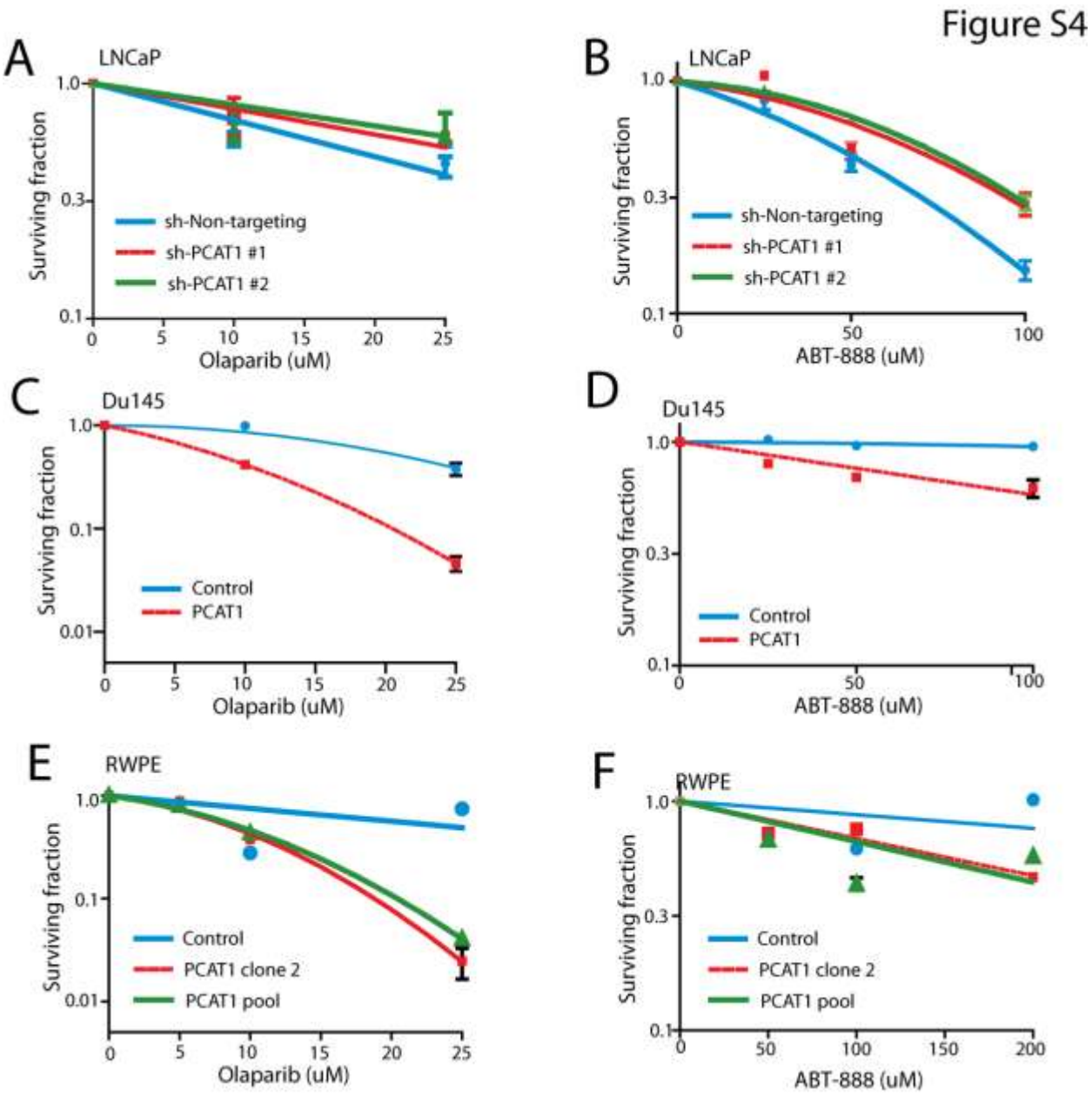
Supplementary Figure S2: PCAT-1 impairs homologous recombination. A, Representative flow cytometry results for PC3-control and PC3-PCAT1 GFP signal intensity following I-SceI-mediated GFP homologous recombination. **B,** qPCR confirmation of PCAT-1 expression in PC3 cells transfected with PCAT1 or empty vector control for the GFP-recombination assays. **C,**

Overexpression of PCAT1 transiently in PC3 cells (quantified on right). PC3-PCAT1 and PC3-control cells were treated with radiation or Olaparib. After 6 hours, cells were fixed and stained for RAD51 and counterstained with DAPI. The number of RAD51 foci were counted. **D,** Quantification of RAD51 foci post-treatment in the respective PC3 cell populations. Cells with >5 RAD51 were counted. Error bars indicate standard deviation. An asterisk (*) indicates $p < 0.05$ by a Student's t-test. **E,** PCAT1 expression is not substantially induced by radiation treatment. LNCaP cells were treated with 4 Gy of radiation and RNA was harvested 24 hours later. qPCR for GADD45A, a positive control known to be induced by radiation, and PCAT1 is shown. Error bars represent S.E.M.

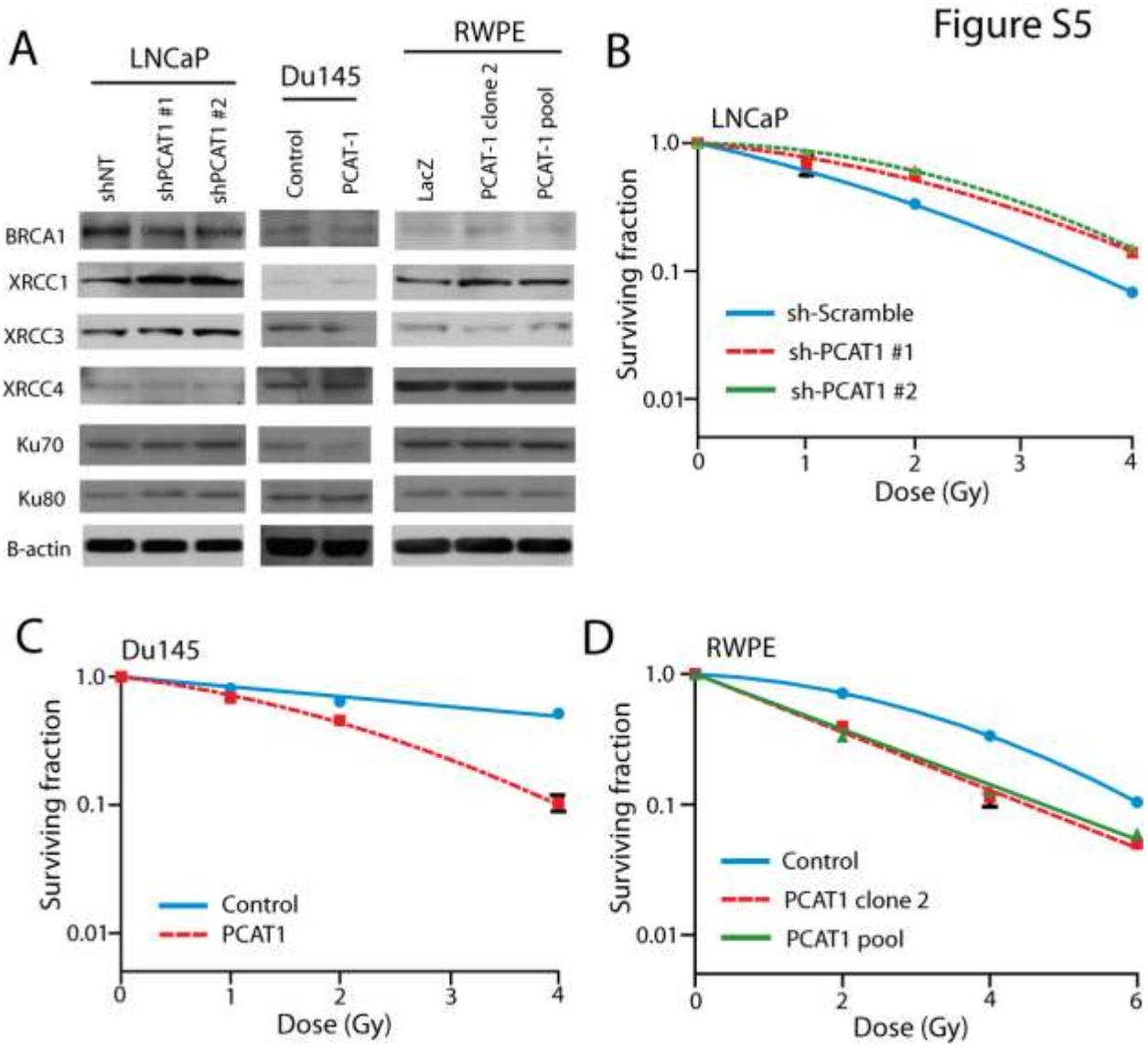
Figure S3



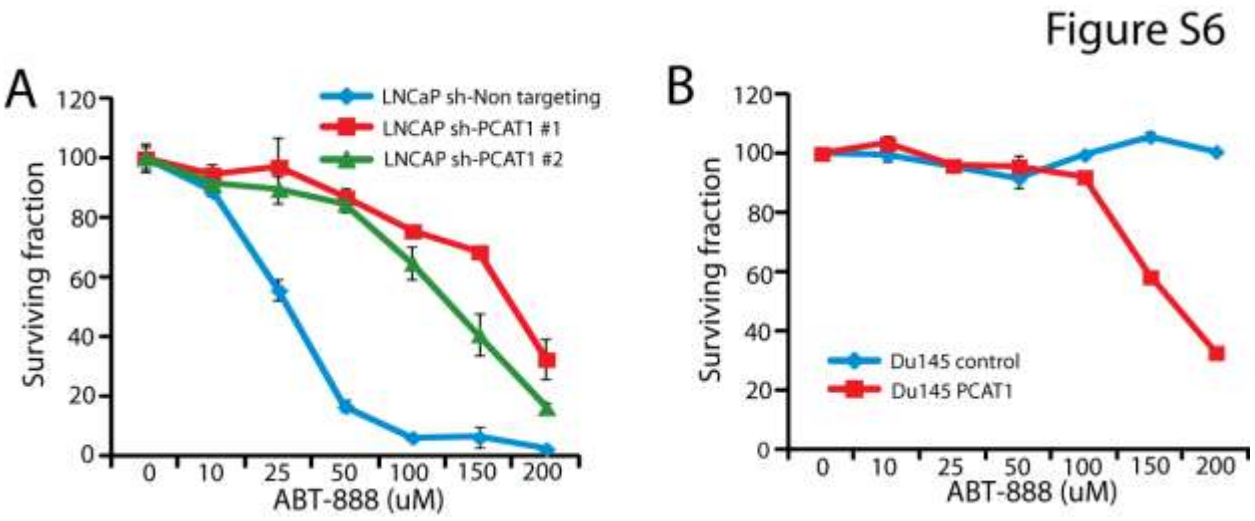
Supplementary Figure S3: PCAT-1 expression alters gamma-H2AX formation. A, γ-H2AX protein abundance in LNCaP shPCAT1 and Du145-PCAT1 cells treated with 25uM Olaparib. Densitometry of three independent western blots is indicated below. **B,** γ-H2AX protein abundance in LNCaP shPCAT1 and Du145-PCAT1 cells treated with 4Gy radiation. Densitometry of three independent western blots is indicated below.



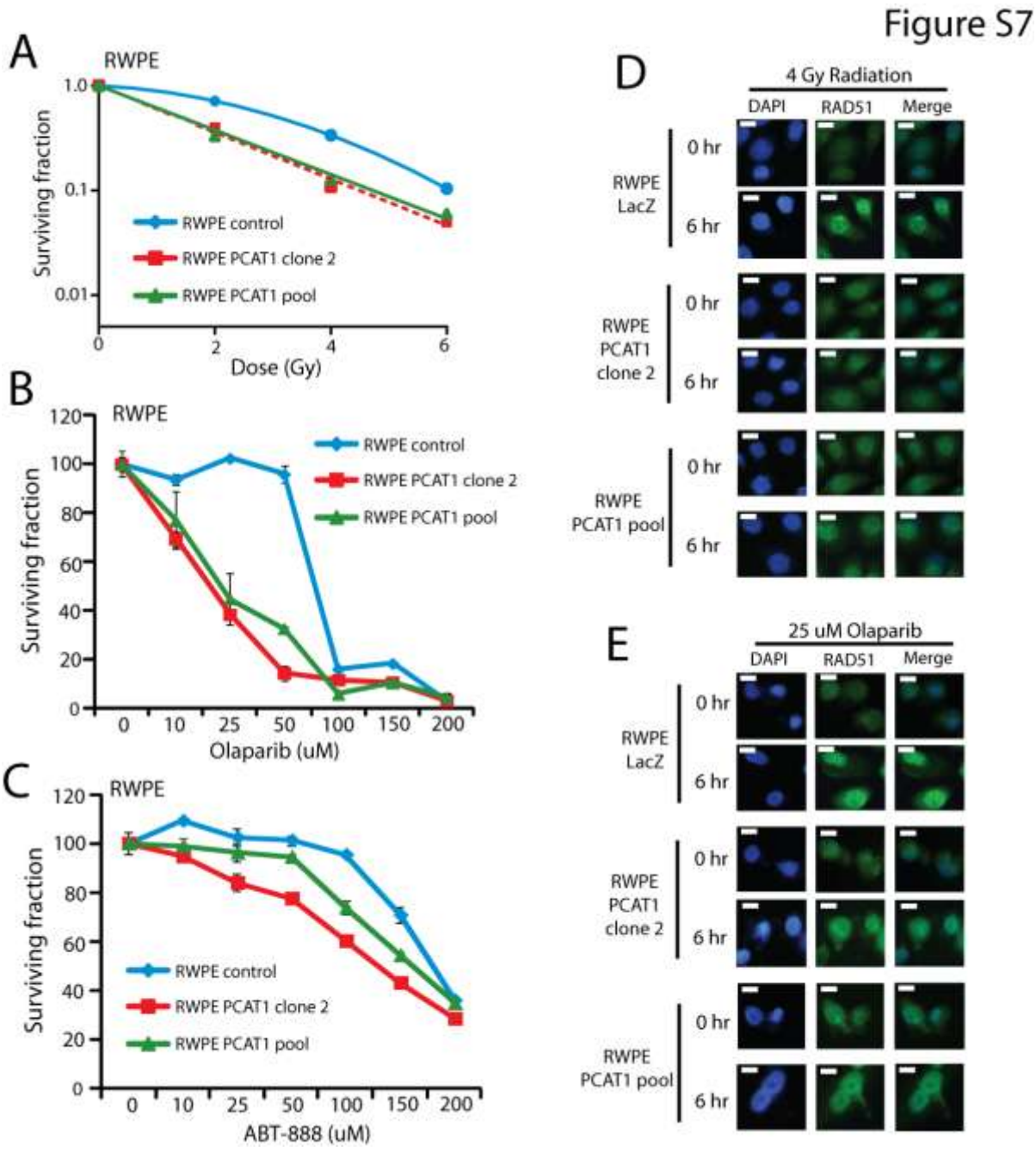
Supplementary Figure S4: PCAT-1 expression effects clonogenic survival when treated with PARP inhibitors Olaparib or ABT-888. A,B, LNCaP PCAT1 knockdown cells show increased resistance to PARP inhibitors Olaparib (**A**) or ABT-88 (**B**) in the clonogenic colony formation assay. **C,D** Du145 PCAT1 overexpressing cells show increased susceptibility to PARP inhibitors Olaparib (**C**) or ABT-888 (**D**) in the clonogenic colony formation assay. **E,F,** RWPE cells overexpressing PCAT1 show increased susceptibility to PARP inhibitors Olaparib (**E**) or ABT-888 (**F**) in the clonogenic colony formation assay.



Supplementary Figure S5: PCAT-1 expression effects clonogenic survival when treated with ionizing radiation. **A,** Immunoblot analyses of key DNA repair proteins (BRCA1, XRCC1, XRCC3, XRCC4, Ku70, Ku80) in isogenic PCAT1 cell lines. PCAT1 does not have a substantial effect on protein abundance of these genes. **B,** LNCaP PCAT1 knockdown cells show increased resistance to radiation in the clonogenic assay. **C,** Du145 PCAT1 overexpressing cells show increased susceptibility to radiation in the clonogenic assay. **D,** RWPE cells overexpressing PCAT1 show increased susceptibility to radiation in the clonogenic assay.

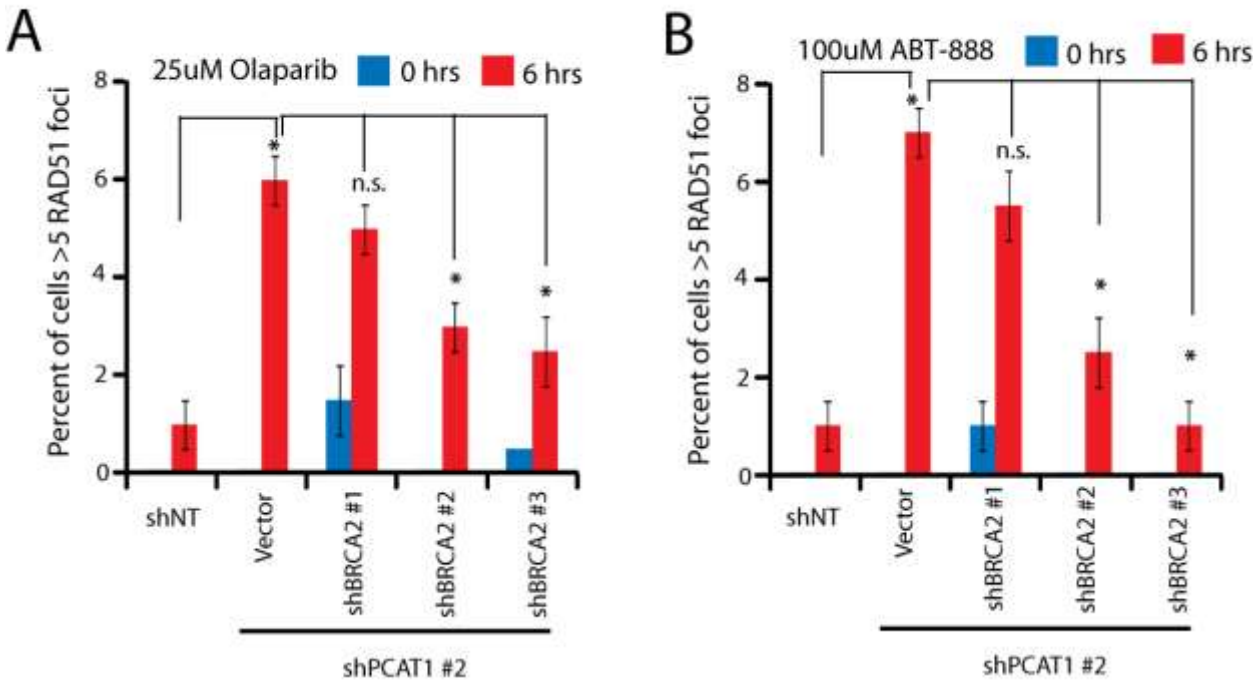


Supplementary Figure S6: Treatment of PCAT-1 isogenic cells with ABT-888. A, Isogenic LNCaP cells with either control knockdown or knockdown of PCAT-1 were treated to increasing doses of ABT-888. **B,** Isogenic Du145 cells with overexpression of PCAT-1 or control were treated to increasing doses of ABT-888.



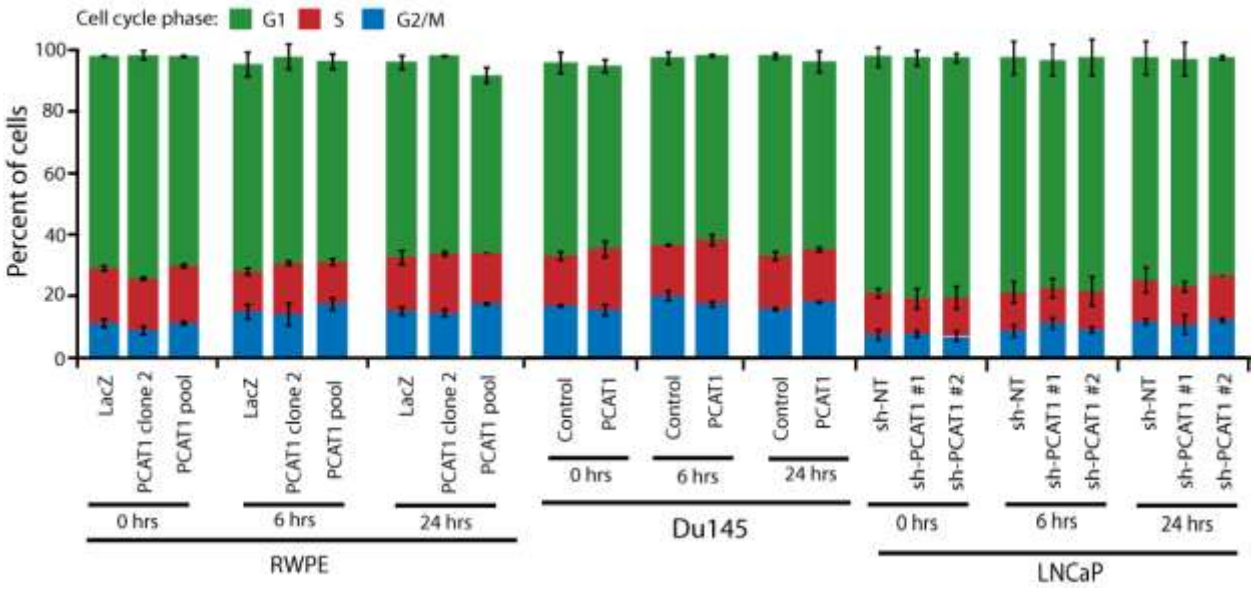
Supplementary Figure S7: PCAT-1 overexpression in RWPE engenders sensitivity to genotoxic stress. A, RWPE cells overexpressing PCAT1 were treated with increasing doses of ionizing radiation and subjected to a clonogenic assay. RWPE-PCAT1 cells show increased cell death following radiation compared to RWPE-LacZ controls. **B, C** RWPE-LacZ and RWPE-PCAT1 cells were treated with increasing doses of Olaparib (**B**) or ABT-888 (**C**). 72 hours later, cell survival was determined using WST. **D,E,** Isogenic RWPE-PCAT-1 cells were treated with 4 Gy of ionizing radiation (**D**) or 25uM of Olaparib (**E**). After 6 hours, cells were fixed and stained for RAD51 and counterstained with DAPI. Error bars indicate standard deviation.

Figure S8



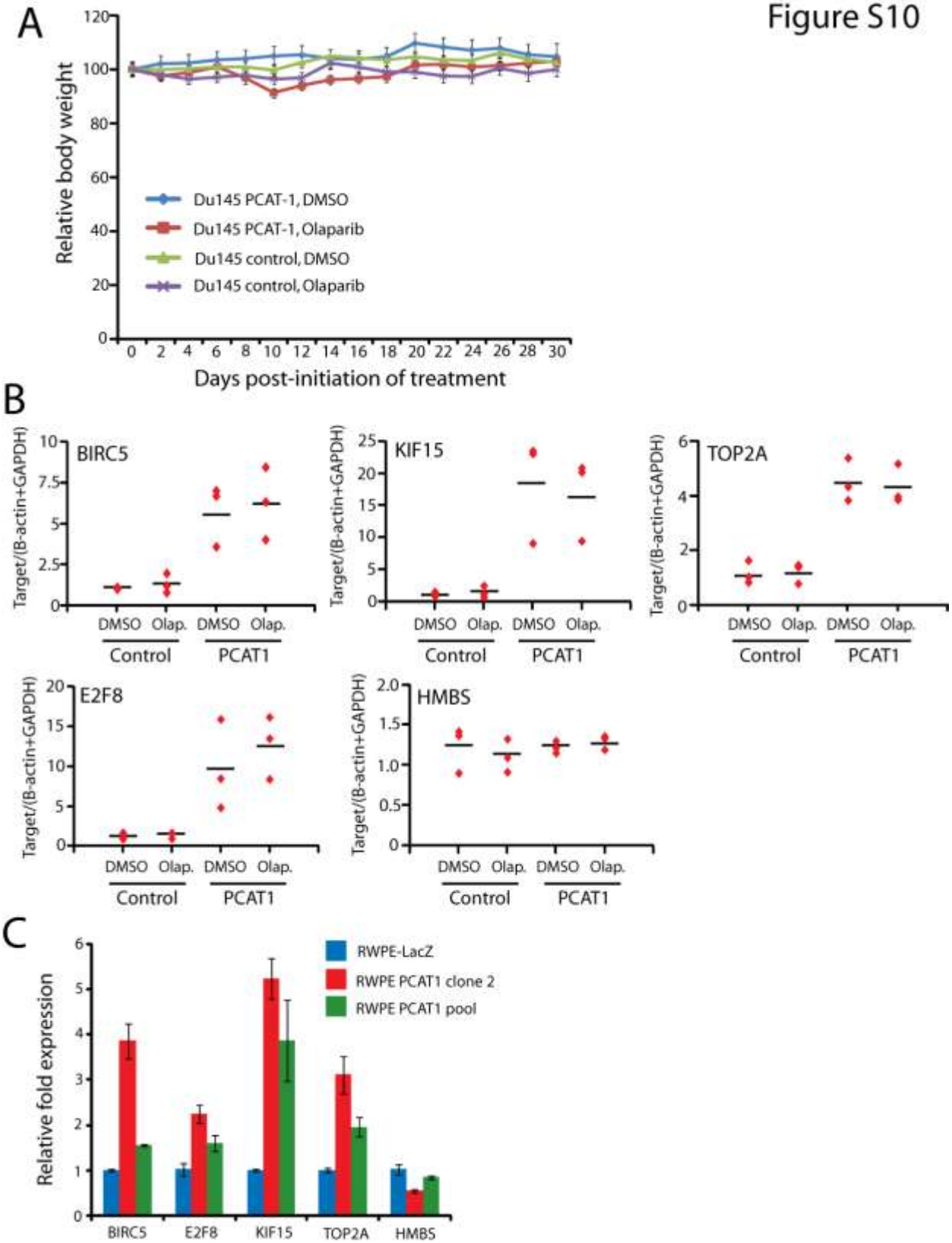
Supplementary Figure 8: BRCA2 knockdown rescues RAD51 foci formation in LNCAP shPCAT1 cells after PARP inhibition. A, B, LNCaP shPCAT1 #2 cells were transfected with three independent shRNAs for BRCA2 or vector control. Cells were treated with 25uM Olaparib (**A**) or 100uM ABT-888 (**B**). 72 hours post-treatment, cells were fixed and stained for RAD51 foci. Quantification of RAD51 foci shows a dose-dependent decrease in RAD51 foci following BRCA2 knockdown. For knockdown efficiency, see **Figure 3D**. Error bars represent standard deviation. An asterisk (*) indicates $p < 0.05$ by Student's t-test.

Figure S9

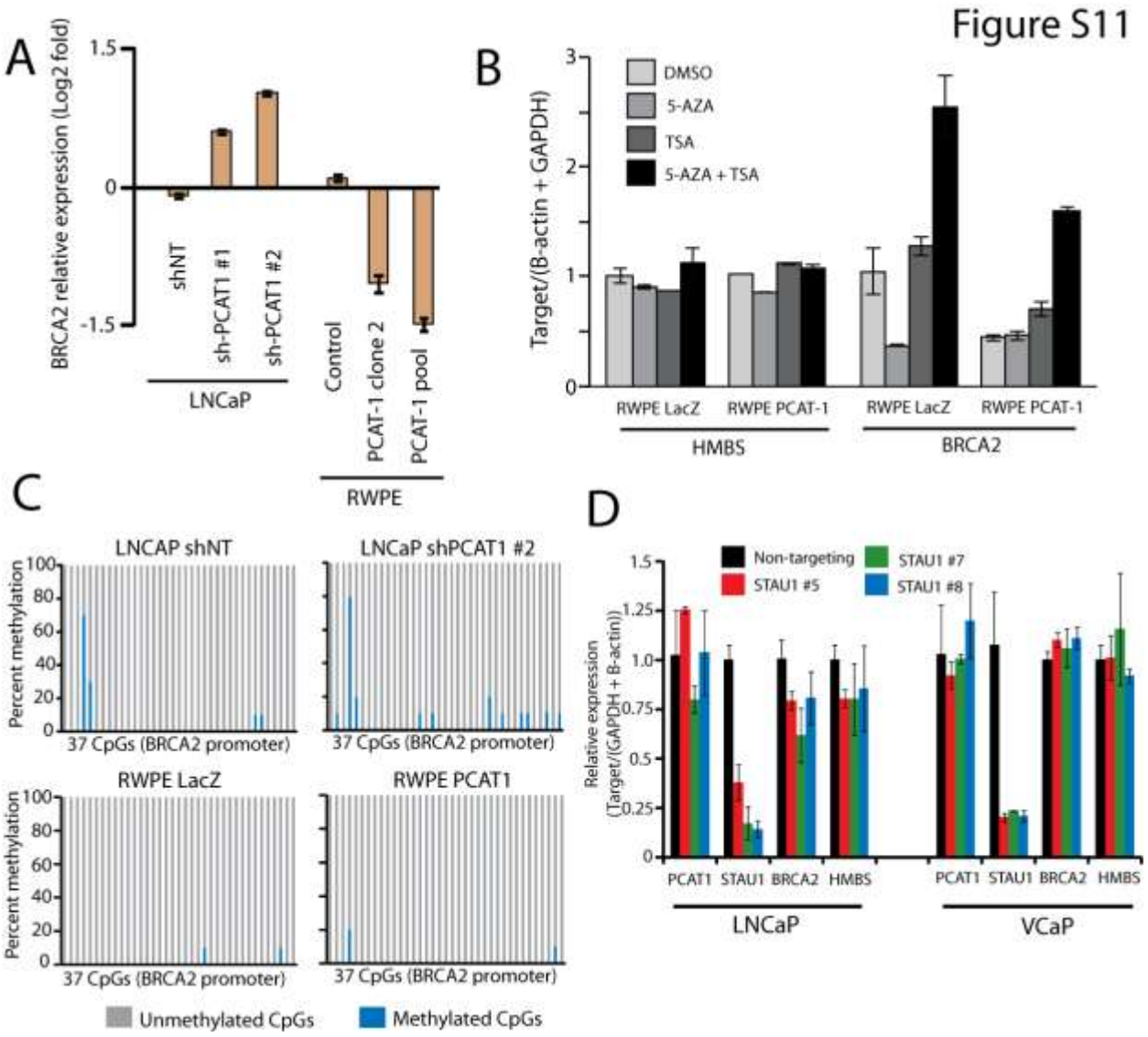


Supplementary Figure 9: PCAT-1 expression does not impact cell cycle distribution Modulation of PCAT1 expression does not impact cell cycle distribution. RWPE, Du145, and LNCaP isogenic cell lines were treated with DMSO and cell cycle was monitored over 24 hrs. Error bars represent S.E.M.

Figure S10

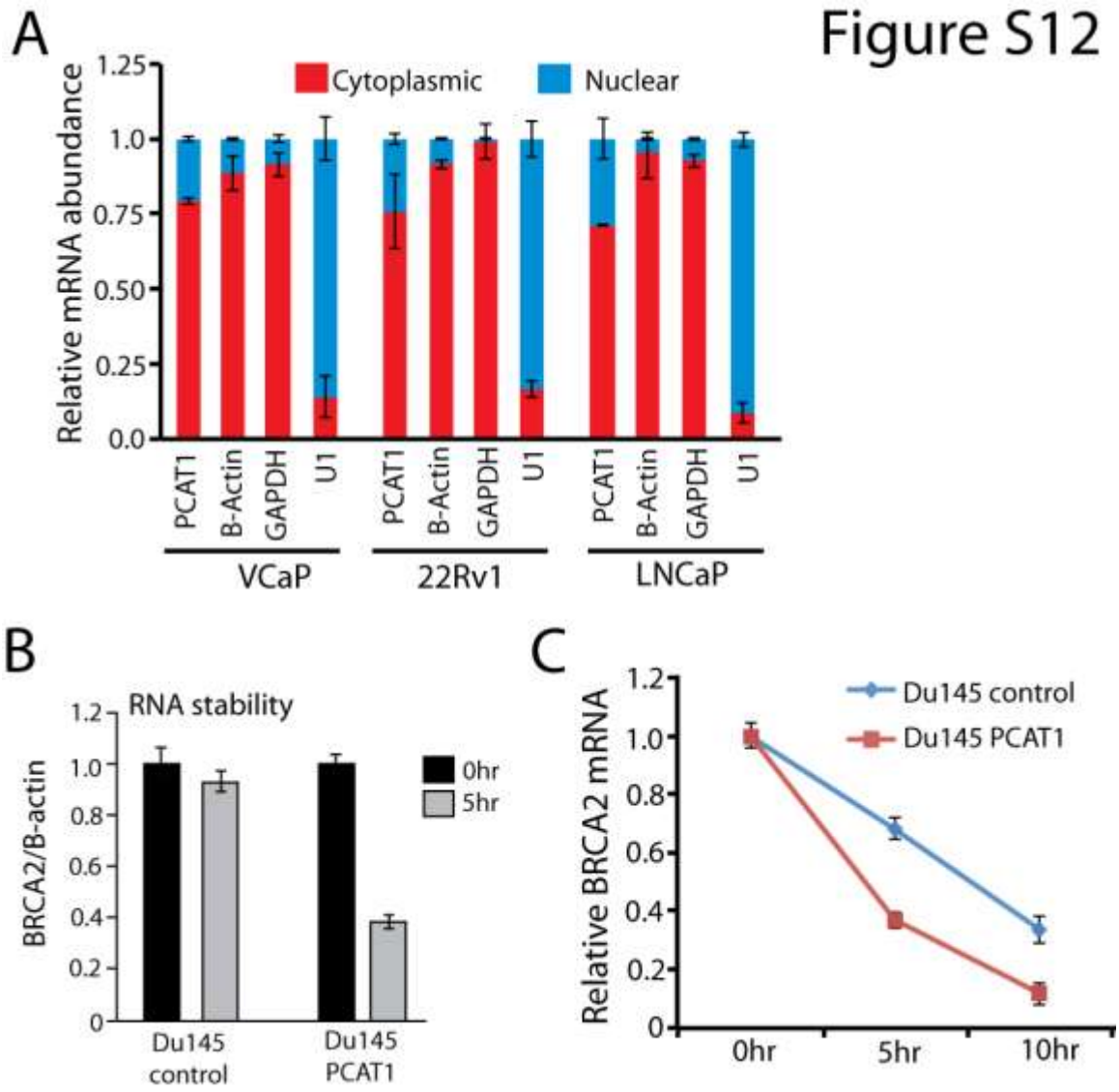

A
Relative body weight
Days post-initiation of treatment
Du145 PCAT-1, DMSO
Du145 PCAT-1, Olaparib
Du145 control, DMSO
Du145 control, Olaparib
B
BIRC5
KIF15
TOP2A
E2F8
HMBS
Target/(B-actin+GAPDH)
DMSO
Olap.
Control
PCAT1
C
Relative fold expression
RWPE-LacZ
RWPE PCAT1 clone 2
RWPE PCAT1 pool
BIRC5
E2F8
KIF15
TOP2A
HMBS

Supplementary Figure S10: Effect of PCAT-1 *in vivo* on Olaparib treatment. **A,** Du145-control and Du145-PCAT-1 xenografts were treated *in vivo* using intra-peritoneal injections of Olaparib or DMSO. Treatment had no effect on mouse body weight over time. **B,** Expression of PCAT-1 target genes (TOP2A, E2F8, BIRC5, and KIF15) are elevated in Du145-*PCAT-1* xenografts compared to Du145-control xenografts in both DMSO-treated and Olaparib-treated mice. HMBS serves as a control. **C,** Four PCAT-1-induced genes (BIRC5, E2F8, KIF15, TOP2A) were measured by qPCR as well as the HMBS control housekeeping gene in the RWPE-LacZ, RWPE-PCAT1 pool, and RWPE-PCAT1 clone 2 isogenic model system. Error bars represent S.E.M.



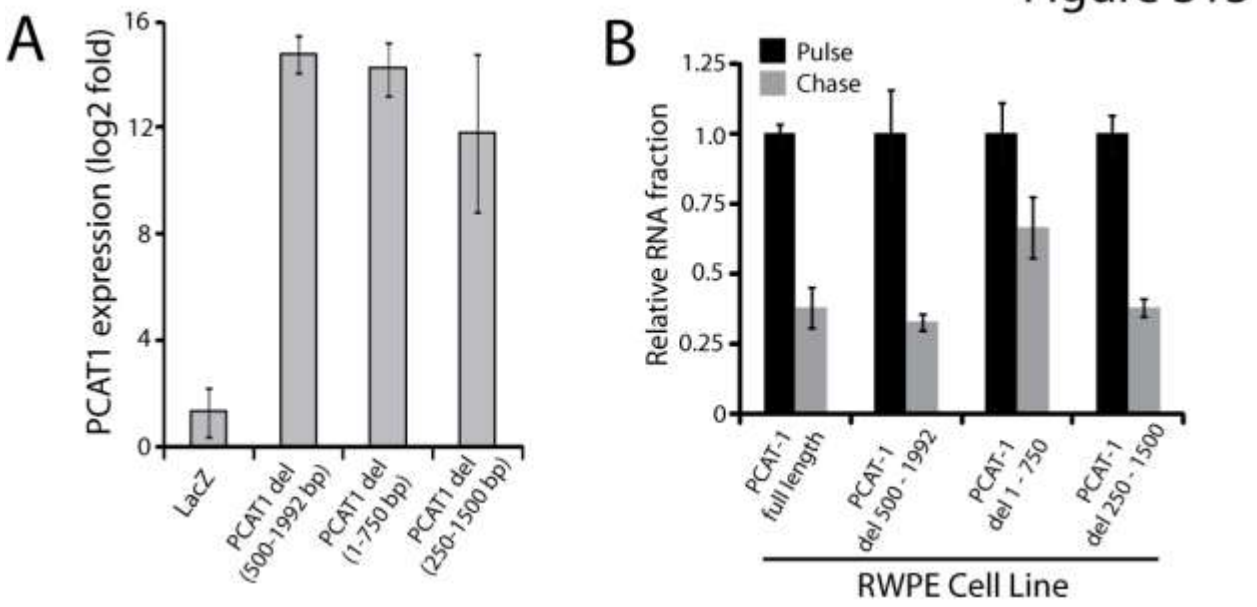
Supplementary Figure S11: PCAT-1 does not operate via an epigenetic or STAU1-based mechanism. A, Expression of BRCA2 mRNA in LNCaP shPCAT1 cells and RWPE PCAT-1 cells. BRCA2 is upregulated upon PCAT-1 knockdown in LNCaP and downregulated upon PCAT-1 overexpression. **B,** RWPE-LacZ and RWPE PCAT-1 pool cells were treated with the demethylating agent, 5-azacytidine, the HDAC inhibitor, TSA, both or control DMSO. RNA was isolated and expression levels of BRCA2 were measured. BRCA2 mRNA levels were unchanged by 5-aza treatment, modestly upregulated by TSA in RWPE-LacZ cells, and modestly upregulated by TSA + 5-aza in both cell lines. BRCA2 regulation by PCAT-1 does not yield sensitivity to epigenetic inhibition pharmacologically. HMBS serves as a control gene unchanged by the treatments. Error bars represent S.E.M. **C,** Bisulfite sequencing of the BRCA2 promoter region in LNCaP cells with stable knockdown of PCAT-1 and RWPE cells with stable overexpression of PCAT-1. **D,** LNCaP and VCaP cells, which endogenously

expression PCAT-1, were transfected with independent siRNAs for STAU1 or non-targeting control. RNA was harvested and qPCR was used to analyze gene expression of STAU1, BRCA2, PCAT-1, and control HMBS. qPCR data were normalized to the average of B-actin + GAPDH. Error bars represent S.E.M.

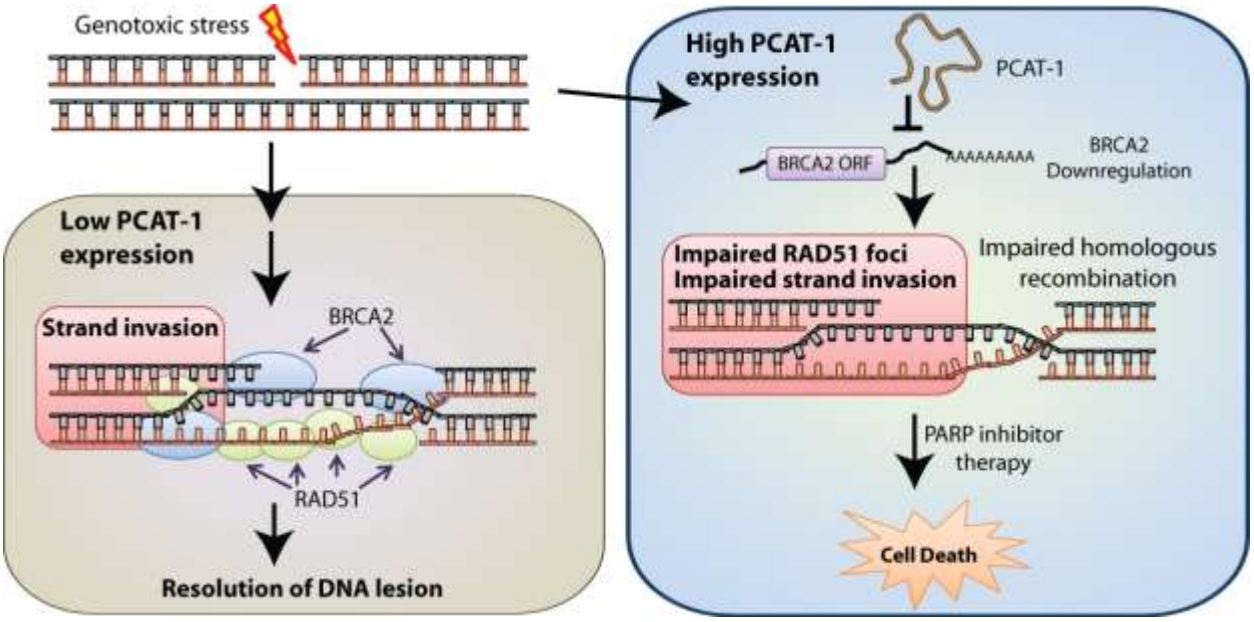


Supplementary Figure 12: PCAT-1 is cytoplasmic and effects target mRNA stability. A, Fractionating of cell lysates into nuclear and cytoplasmic fractions for VCaP, LNCaP, and 22Rv1 cells. qPCR analysis on the separate fractions demonstrates that PCAT-1 is located in the cell cytoplasm. B-actin and GAPDH serve as controls for cytoplasmic RNAs. U1 serves as a control for nuclear RNAs. **B,** mRNA stability of BRCA2 relative to B-actin, determined by uridine incorporation assays. **C,** Abundance of BRCA2 mRNA in Du145-control and Du145-PCAT1 cells.

Figure S13



Supplementary Figure 13: PCAT-1 overexpression in RWPE cells. A, Overexpression of PCAT-1 constructs in RWPE cells. RWPE cells were infected with lentiviruses for various PCAT-1 deletion constructs. Expression was measured by qPCR. Error bars represent S.E.M. **B,** RNA stability assays for PCAT1 deletion constructs. A labeled uridine assay was used to monitor the rate of RNA degradation following a pulse of uridine. The inactive PCAT1 deletion constructs have equivalent RNA stability as the full-length PCAT1 construct.



Supplementary Figure 14: A model of *PCAT-1* function in homologous recombination in prostate cancer.

Table S1: Primer pairs used in this study.

Supplementary Table 1: Primer Pairs used in this study

Primer	Experiment	Forward/Reverse primer	Sequence
BRCA2	qPCR	Forward	CATACAGTTAGCAGCGACAAAAA
BRCA2	qPCR	Reverse	CAAGATGGCTGAAAGTCTGGAT
KIF15	qPCR	Forward	TGCGACTAAAGAAGGAAAATGTC
KIF15	qPCR	Reverse	AAGGTGATGCCTAGGTAGCTGA
BIRC5	qPCR	Forward	CACCGCATCTCTACATTCAAGA
BIRC5	qPCR	Reverse	CAAGTCTGGCTCGTTCTCAGT
TOP2A	qPCR	Forward	TTGTTTCGAAAGCAGTCACAAG
TOP2A	qPCR	Reverse	GTTTCTTTGCCCGTACAGATTT
E2F8	qPCR	Forward	AAATGGACAATCAGTTGCTGTG
E2F8	qPCR	Reverse	CTAATTGTGACCCTTTGGGTGT
GADD45A	qPCR	Forward	GTCGCTACATGGATCAATGGG
GADD45A	qPCR	Reverse	CATTCAGATGCCATCACCGTT
GAPDH	qPCR	Forward	TGCACCACCAACTGCTTAGC
GAPDH	qPCR	Reverse	GGCATGGACTGTGGTCATGAG
B-actin	qPCR	Forward	AAGGCCAACCGCGAGAAG
B-actin	qPCR	Reverse	ACAGCCTGGATAGCAACGTACA
HMBS	qPCR	Forward	GATGGGCAACTGTACCTGACTGGA
HMBS	qPCR	Reverse	TGGGGCCCTCGTGGAATGTTA
PCAT1	qPCR	Forward	TGAGAAGAGAAATCTATTGGAACC
PCAT1	qPCR	Reverse	GGTTTGTCTCCGCTGCTTTA
STAU1	qPCR	Forward	TAACATCTCTTCAGGCCACGTA
STAU1	qPCR	Reverse	GTTCTTGTTGTTTTTGGGGAAG
BRCA2_3'UTR	Molecular cloning	Forward	GCATTTGCAAAGGCGACAATAAATT
BRCA2_3'UTR	Molecular cloning	Reverse	AATCAGTGCCAATTTGAAAGCAAG
BRCA2_3'UTR_Spe1	Molecular cloning	Forward	GACTAGTGCATTTGCAAAGGCGACAATAAATT
BRCA2_3'UTR_HindIII	Molecular cloning	Reverse	GCAAGCTTAATCAGTGCCAATTTGAAAGCAAG
PCAT1_Full_Length	Molecular cloning	Forward	ACACATGGATATTGGATATCTGCAT
PCAT1_Full_Length	Molecular cloning	Reverse	TAGGCTCAAACACACATTTATTCATC
PCAT1_Del_500-1992bp	Molecular cloning	Forward	AGGGAGAAAGGAGATGACGCAAAG
PCAT1_Del_1-750bp	Molecular cloning	Reverse	TTCATTAGCAGTTCCTGGGCCAA
PCAT1_Del_250-1500_bp	Molecular cloning	Forward	GCCAACTGAGGAACCTGAGCAAT
PCAT1_Del_250-1500_bp	Molecular cloning	Reverse	TTGGTGCAAGTTTGGCTTTGGGAAG